ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

The First Fisher Award

We congratulate N. Howell Furman of Princeton as the first recipient of the Fisher Award in Analytical Chemistry. We also congratulate the donor, C. G. Fisher, for providing an original alchemist etching instead of a medal—it will attach to the award a uniqueness and special character.

Dr. Furman's many accomplishments, which brought him the distinction of receiving the first Fisher Award, were reviewed in the August 23, 1948, issue of *Chemical and Engineering News* and, therefore, need not be repeated here.

As the years pass, the Fisher Award will make it possible to recognize appropriately the achievements of present and future leaders in the field of analytical chemistry.

Without detracting one iota from Dr. Furman's achievements but rather accentuating them, we believe the committee charged with selecting the first Fisher award winner must have found it very difficult to make a selection. We have in the analytical field a large number of very distinguished individuals whose contributions have been notable.

Sectional and Divisional Activities

Within the past month we have received notice of the formation of analytical groups within two large sections of the Society. The Boston Microchemical Society has been dissolved and reorganized as the Analytical and Microchemical Division of the Northeastern Section, and a new analytical group has been formed within the framework of the North Jersey Section.

Naturally we are delighted to see the plan first adopted by the Pittsburgh Section extended to other sections of the Society. What has been done by the Pittsburgh, Northeastern, and North Jersey Sections demonstrates that real leadership exists in the field of analytical chemistry.

We believe that the formation of more such groups will also help to strengthen the Division of Analytical and Micro Chemistry. With a strong divisional program of varied activities, the division should have more than a few hundred dues-paying members.

Your personal financial support and active interest are needed to support these very worth-while activities. Send your dollar to R. A. Burdett, secretary, Division of Analytical and Micro Chemistry, Shell Oil Co., Box 262, Wood River, Ill.

Summer Symposium a Success

The first annual summer symposium, jointly sponsored by the Division of Analytical and Micro Chemistry and Analytical Chemistry and held at the Northwestern Technological Institute August 13 and 14, was a distinct success. The use of revolutionary analytical methods and techniques, mostly developed in the Manhattan District project, is still rather restricted in industry. Nevertheless, nearly 200 analytical chemists were attracted to the two-day sessions on "Nucleonics and Analytical Chemistry," to which this symposium was devoted.

Annual symposia conducted separately from regular divisional meetings held during national meetings of the Society provide an opportunity for intensive investigation of special fields. More detailed papers can be presented and more time devoted to floor discussion than is possible at regular division sessions. Furthermore, plenty of opportunity is provided for extensive informal or off-the-record conversations between specialists in any given field, something that is rather difficult to achieve at a national meeting where there may be anywhere from 5000 to 12,000 people in attendance.

The outstanding success of the first symposium can be attributed to the combined efforts of many individuals and organizations. We would like to mention particularly P. J. Elving, chairman of the Division of Analytical and Micro Chemistry, C. J. Rodden of the National Bureau of Standards and general chairman of the symposium, and L. D. Frizzell of Northwestern, who handled all the local arrangements in a highly efficient manner. We are deeply indebted to R. K. Summerbell for the fine hospitality and exceptional facilities of Northwestern, and to W. M. Manning and his associates of the Argonne Laboratories for the very extensive and highly instructive demonstrations. Lastly, we wish to thank the speakers and those who participated in the discussions, for they set a high standard of excellency for future symposia.

Potentiometric Titration of Weak Acids in Anhydrous Ethylenediamine

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A new approach to the problem of titrating phenols and other acids too weak to be titrated in aqueous medium is based on use of an anhydrous, strongly basic solvent such as ethylenediamine and an even more basic titrant. The behavior of phenols or carboxylic acids when titrated in this system is analogous to the behavior of carboxylic acids or mineral acids when titrated in water. With mixtures of phenols and carboxylic acids, two separate end points generally occur, corresponding to the two materials of different acid strength. Dark-colored resins such as Vinsol, which contains both carboxylic and phenolic constituents, give a curve having two points of inflection when titrated in this new system. Resorcinol and salicylic acid give two end points corresponding to each of their two acidic hydrogens. In ethylenediamine, amino acids behave as typical unsubstituted carboxylic acids.

TITRATION of the acidic components of certain resins of commercial importance is complicated by several factors. The dark color of some of these resins makes it impossible to determine the end point visually. This difficulty due to color alone may be overcome by electrometric titration, or the indicator end point may be detected by using a photoelectric device (*12*). However, even the electrometric end point may not be sufficiently sharp to be detected with the desired precision. Titration to an arbitrarily established reading on a glass electrode pH meter is not capable of high precision or accuracy if the sample does not give a sharp inflection in the titration curve. Work in this and other laboratories (*9*) has shown that the poor end point with dark-colored resins is due to the presence in the resin of very weakly acidic components, phenolic in nature, which are too weak in neutral solvents to be susceptible to titration themselves but which obscure the end point given by the stronger carboxylic acids. Hence, neither the total acids nor the stronger acid fraction can be satisfactorily determined. The work reported here is concerned with a practical solution of this problem of titrating such resinous materials containing weakly acidic components.

Although all the possibilities of the proposed method have by no means been explored, it is believed that the results demonstrate the soundness of the principles involved.

The problem of titrating dark-colored resins can be considered to consist of two phases: (1) determination of acids that are too weak to give satisfactory inflections and, therefore, cannot be titrated in the conventional media; and (2) titration of mixtures of acids whose strengths are not greatly different.

According to the Brønsted theory (*3*), the dissociation of an uncharged acid in a solvent is a reaction of the type

$$A + S \rightleftharpoons SH^+ + B^-$$

where A is the acid, S the solvent, SH^+ the solvated proton, and B^- the conjugate base of the acid. If this equilibrium is to be shifted appreciably to the right, the solvent must be more basic than the conjugate base of the acid. Thus, in a basic solvent, weak acids would show a much greater acid strength than in water or alcohols. It has been reported, for example, that in liquid ammonia carboxylic acids behave like strong acids (*6*). Use of a basic solvent, therefore, seemed a logical approach to the titration of very weak acids—e.g., phenol, which cannot be titrated satisfactorily in either aqueous or alcoholic solutions. This principle was applied some years ago by Hall and Conant (*5*) and Hall (*4*) to the analogous case of the titration of very weak bases, using glacial acetic acid as the solvent, but does not appear to have been applied before to the titration of weak acids. In addition to being strongly basic, the solvent should have a small autoprotolysis constant to minimize solvolysis effects. It is evident that the titrant used must be even more basic than the solvent.

The problem of determining each of two acids whose strengths are not greatly different was discussed theoretically by Auerbach and Smolczyk (*1*), who showed that in the titration of a dibasic acid two points of inflection will not appear unless the ratio of the dissociation constants is greater than 16. MacInnes (*10*) points out, however, that if the two inflection points are to be determined experimentally with any accuracy, this ratio must be considerably greater.

In this investigation, it was decided to carry out electrometric titrations in a nonaqueous, basic solvent. It was hoped that a solvent could be chosen of such basicity that carboxylic acids would behave as strong acids while the phenols, having lower

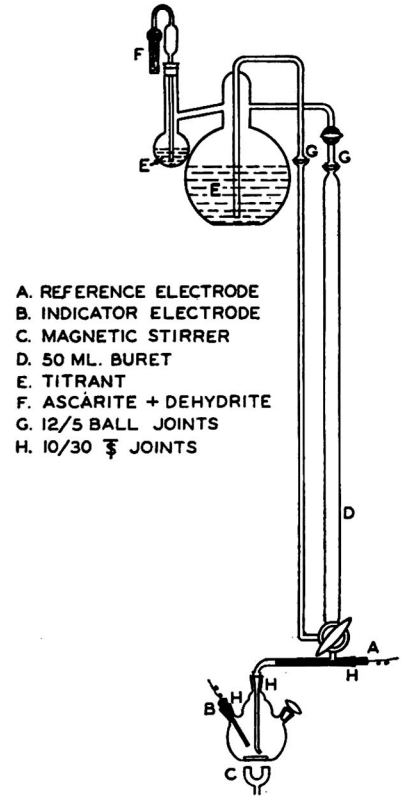

Figure 1. Buret and Electrode Assembly

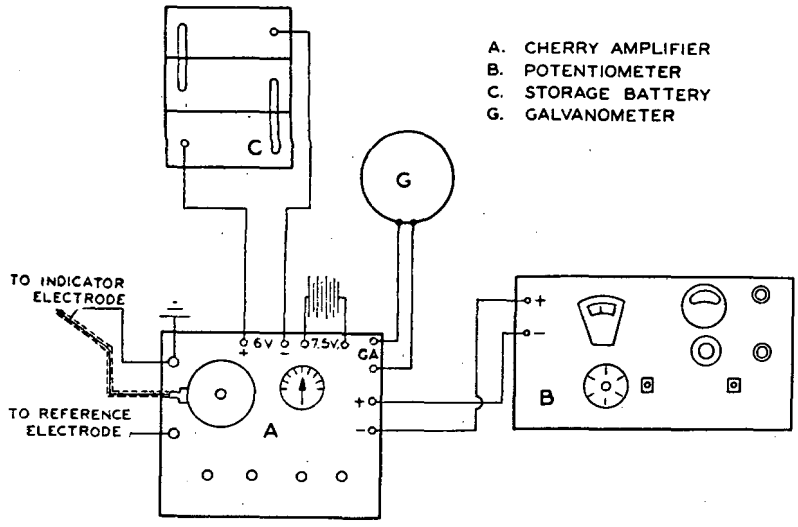

Figure 2. Arrangement of Electrical Apparatus

acid strengths, would behave as weak acids and, hence, two inflection points would be obtained. Ethylenediamine was chosen as the solvent because it may be obtained commercially and is a good solvent for resins. The relatively high dielectric constant, about 16 (*7*), is desirable because ion-pair formation will take place to a lesser extent than in media of lower dielectric constant. Furthermore, it has been frequently observed that electrometric measurements in low dielectric-constant media are difficult to make because of electrode instability and slow attainment of electrode equilibria.

As solvents, other amines including diamylamine, pyridine, and ethanolamine were tried. None of these, however, gave end points as distinct as those obtained in ethylenediamine.

With ethylenediamine as the solvent, the obvious titrant to use would be an ethylenediamine solution of an alkali metal derivative of the ethylenediamine. As preliminary attempts to prepare such a solution were not promising, an ethylenediamine solution of sodium aminoethoxide was chosen.

Several methods of detecting the end point in this system were studied. Various indicators, including phenolphthalein and thymol blue, change color when titrated although the color changes are not considered sufficiently sharp for visual work particularly with dark-colored samples. Phenol gives a very sharp end point if titrated using trinitrobenzene as the indicator and a photometer is used for measuring the color change, preferably at about 450 mμ. The behavior of the indicator is not the same as in water, inasmuch as in ethylenediamine it changes from orange on the acid side to colorless on the alkaline side.

For the weak acid mixtures studied, the potentiometric method of titrating gave the most satisfactory end points of the various methods considered. Although the conductometric method would have been the most convenient electrometric method to use in ethylenediamine, it did not give a satisfactory end point with phenol in this medium. A potentiometric method was, therefore, adopted.

APPARATUS AND ELECTRICAL SYSTEM

The titration flask, buret, and electrode assembly as shown in Figure 1 were designed to exclude atmospheric moisture and carbon dioxide. By using a magnetic stirrer, an opening in the flask for a stirrer shaft is eliminated. Both hydrogen and antimony indicator electrodes were used; the latter was somewhat more convenient.

The antimony electrode consists of a rod prepared by sucking the molten metal into a male 10/30 ⅀ thermometer joint and chipping off the glass below the joint after cooling. The reference electrode is a similarly prepared antimony rod mounted in the buret below the stopcock, a variation of the electrode suggested by Willard and Boldyreff (*14*). Thus, immersed in the titrant, the reference electrode is connected electrically with the solution being titrated through the buret tip. This affords the advantage, not existing in other reference electrodes tried, of a continual flushing of the reference electrode to prevent diffusion. Renewal of the liquid junction occurs at the end of the buret tip, serving as a salt bridge, with addition of each new increment of titrant.

Figure 2 shows the arrangement of electrical apparatus used.

Although the resistance of the system is relatively low, an amplifier is used to increase sensitivity of the measuring system. Amplification is especially desirable when hydrogen and calomel electrodes are used. A Leeds & Northrup Cherry amplifier (Catalog No. 7673), portable potentiometer (Catalog No. 7655), and galvanometer (Catalog No. 2420-C) were used.

The hydrogen–calomel electrode combination was employed for much of the work, although it is less convenient than the antimony electrodes described. Several types of hydrogen electrodes were used, including the type described by Newbery (*11*), coated with Du Pont liquid bright gold No. 4618. After being fired, the gold electrodes were platinized in the usual way. Hydrogen was introduced through a sintered-glass bubbling tube placed under the electrode.

The most practical and serviceable hydrogen electrodes consisted of stainless steel tubes, 10 mm. in diameter and 100 mm. in length, with sintered metal disks at one end. The hydrogen was bubbled through, making contact with the solution at the surface of the disk. These one-piece electrodes were mounted with sealing wax and paraffin in ground-glass joints to fit the titration flask. They were satisfactory as received with a bright surface, no platinizing being necessary.

With the hydrogen-calomel electrode system in ethylenediamine, potential differences as high as 1.6 volts required use of an extra working battery to oppose the output of the amplifier in order to bring the readings below 1.11 volts and within the range of the potentiometer.

A pH meter equipped with a hydrogen or antimony electrode may be used for the e.m.f. measurements, although a potentiometer graduated in millivolts is preferred. The glass electrode itself is unstable in this system, whether filled with the usual aqueous solutions or with a buffer in ethylenediamine. It does register a sharp change in potential at the end point but drifts back immediately in the direction of its original potential.

SOLUTIONS AND REAGENTS

Ethanolamine (Eastman Kodak practical) was distilled three times, as recommended by Kohlrausch and Ypsilanti (*8*), through a 2-foot Vigreux column. Dry ethylenediamine was prepared from the commercial 70% aqueous grade (Carbide and Carbon) by the method of Putnam and Kobe (*13*) using sodium hydroxide and, finally, distilling over sodium. A more recent method of Bromley and Luder (*2*) employs activated alumina and may offer some advantages over the earlier method.

The effect of moisture is important and best results are obtained with anhydrous ethylenediamine prepared by distilling over sodium. As the water concentration increases, sharpness of the end points deteriorates progressively and the system assumes essentially aqueous characteristics. For example, at 20% moisture, phenol gave an end-point break of only about 20 mv., measured with the hydrogen electrode, in comparison with 180 to 200 mv. in 99 to 100% ethylenediamine. Removal of even the last 1% of moisture is advantageous.

The titrant was prepared by dissolving approximately 2.5 grams of sodium, which had been washed successively in ethanol and ethanolamine, in 100 ml. of ethanolamine with cooling, and diluting to 500 ml. with ethylenediamine. Potassium and lithium were also tried but did not offer any apparent advantages. Some excess ethanolamine, 10 to 20% of the total volume, was required in order to obtain a solution that would remain clear on standing. This solution was standardized against benzoic acid by the following procedure, which was also used for titrating the samples.

Titration Procedure. Weigh a sample of 0.1 to 1 gram, transfer to the titration flask, and dissolve in 75 ml. of ethylenediamine, using the magnetic stirrer. Flush out the buret tip and the reference electrode and connect the flask to the buret. Carry out the potentiometric titration in the usual way adding increments of 0.1 to 0.3 ml. of titrant as the end point is approached. Plot the titration curve taking the inflection as the equivalence point.

DISCUSSION

Because carboxylic acids are stronger acids in liquid ammonia than in water, it was expected that an amine solvent should enhance the acidic properties of phenols to such an extent that they might then be readily titrated, perhaps even in the presence of carboxylic acids. This would be analogous to the titration of mixtures of mineral and carboxylic acids in water. Even though ethylenediamine itself is basic, the titrant used is more strongly basic and acts, therefore, as a base in the presence of the diamine.

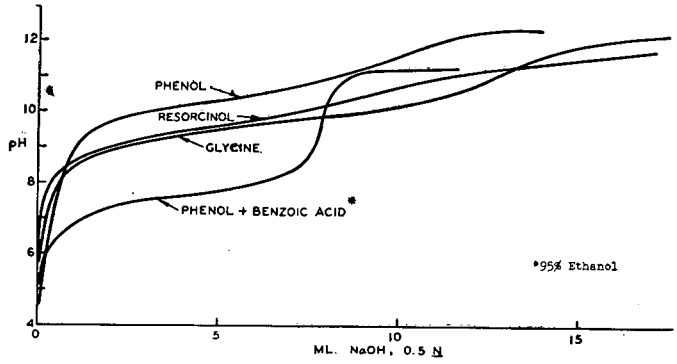

Figure 3. Titration of Weak Acids in Aqueous Medium with Glass-Calomel Electrodes

The over-all reaction involved in the titration of a carboxylic acid in this solvent with sodium aminoethoxide may be represented by the following equations:

$$RCO_2H + H_2N{-}C_2H_4{-}NH_2 \longrightarrow H_2N{-}C_2H_4{-}NH_3^+ + RCO_2^-$$

$$H_2N{-}C_2H_4ONa \longrightarrow Na^+ + H_2N{-}C_2H_4O^-$$

$$\underset{\text{(acid)}}{H_2N{-}C_2H_4{-}NH_3^+} + \underset{\text{(base)}}{H_2N{-}C_2H_4O^-} \longrightarrow H_2N{-}C_2H_4{-}OH + H_2N{-}C_2H_4{-}NH_2$$

The neutralization reaction then merely involves transfer of the proton from the cation to the anion with regeneration of the two free bases.

For purposes of comparison, several titration curves for neutralization reactions in aqueous medium are shown in Figure 3. Although 95% ethanol was used as solvent for the phenol-benzoic acid mixture, this system is essentially aqueous in its behavior. As indicated, it is virtually impossible to titrate unsubstituted phenol in water solution, as there is no appreciable inflection point. A similar curve is obtained with amino acids such as glycine.

Generally speaking, in a mixture containing two sufficiently acidic components of relatively different acid strengths, it is possible to determine the two acids simultaneously by obtaining two separate end points. This situation is illustrated by the titration curves shown in Figure 4 for the mixtures of acetic and hydrochloric acids in aqueous medium with which separate end points are obtained for each of the acids present. When titrated in the presence of each other, the weak acid gives the sharper end point although the reverse is true when the acids are titrated separately.

Figure 5 shows titration curves obtained in the ethylenediamine system. Comparing these with the previous curves obtained in aqueous medium, the striking effect of the basic solvent and the stronger titrant is readily apparent. In this system, phenol behaves like a carboxylic acid in water and the carboxylic acid behaves like a strong mineral acid in water. The possibility of determining phenols and carboxylic acids simultaneously is, therefore, promising. The curve for the phenol–benzoic acid mixture (Figure 6) has two distinct breaks and very nearly the same form as the curve for the mixture of hydrochloric and acetic acids in water shown in Figure 4.

Table I. *Titration of Mixtures of Benzoic Acid and Phenol* in Ethylenediamine

Sample No.	Benzoic Acid, Gram			Phenol, Gram		
	Present	Found	Difference	Present	Found	Difference
1	0.2500	0.247	−0.003	0.2500	0.254	+0.004
		0.247	0.003		0.254	0.004
2	0.5000	0.501	+0.001	0.1000	0.102	+0.002
		0.502	0.002		0.104	0.004
	0.1000	0.101	+0.001	0.5000	0.500	0.000
		0.108	0.008		0.503	+0.003
		0.108	0.008		0.492	−0.008

Results obtained on mixtures of benzoic acid and phenol are listed in Table I. In such mixtures, the changes in e.m.f. for the carboxyl end points amount to about 20 to 100 mv. compared to 100 to 150 mv. for the phenol end points. The magnitude of the breaks appears to be influenced by such factors as composition of the sample, the electrodes used, and dryness of the solvent.

From these and similar data, it appears that best results are obtained when the ratio of carboxyl acidity to phenol acidity is comparatively high, although it is possible to measure total acidity irrespective of their relative proportions.

In ethylenediamine, the difficulty of analyzing samples high in phenolic constituents occurs in the carboxyl end point, which is somewhat obscure in such mixtures. The uncertainty of the first end point then enters into both the phenol and carboxyl results. It does not, however, affect the calculation of total acidity represented by the second end point, which is considerably more distinct than the first. Calculation of either carboxyl or phenol alone involves taking the difference between the two end points, and thereby requires an accurate determination of each. The same situation is encountered in titrating mixtures of hydrochloric and acetic acids in water.

The results reported in Table I are typical of what may be obtained without unusual precautions and do not necessarily repre-

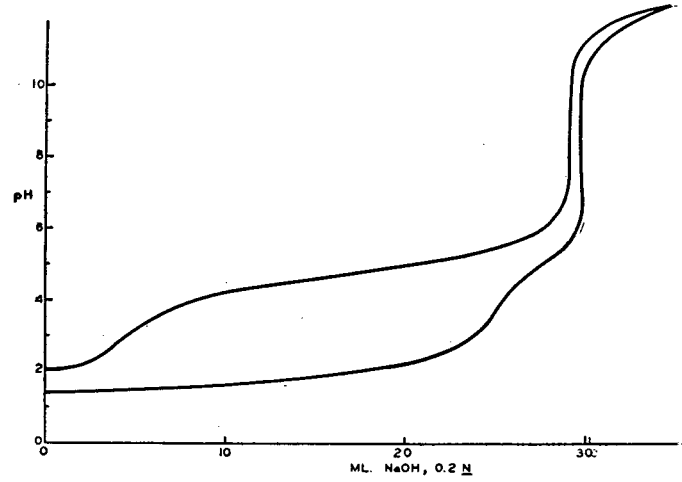

Figure 4. Titration of Hydrochloric and Acetic Acid Mixtures in Water with Sodium Hydroxide

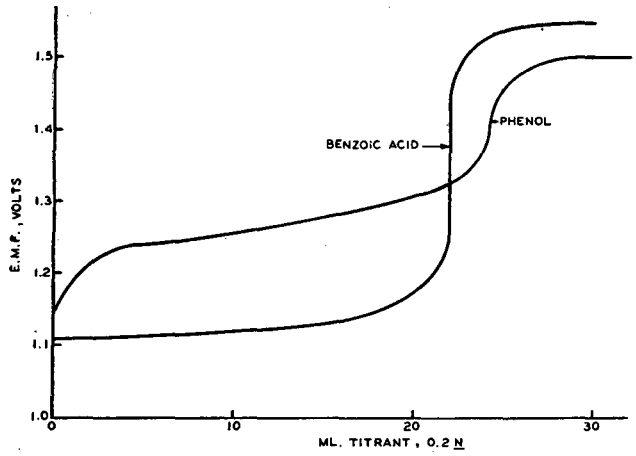

Figure 5. Titration of Phenol and of Benzoic Acid in Ethylenediamine with Hydrogen-Calomel Electrodes

sent the limit of accuracy obtainable. As the primary interest to date has been in basic principles, no effort has been made to exhaust the possibilities of precision and accuracy.

Although the development work has been concerned mainly with the titration of phenol and carboxyl acidity in the presence of each other, the principal objective was to devise means for titrating acids too weak to be titrated by existing methods. Weak acids of this kind occur in certain commercial resinous materials such as Vinsol resin, a paraffin hydrocarbon–insoluble fraction of pine wood resin, whose titration curve in ethylenediamine is given in Figure 6. The general shape of the Vinsol curve is similar to that obtained with the mixture of phenol and benzoic acid. This confirms the results of the other work performed in this laboratory, which indicate the presence of both phenolic and carboxylic acid groups. From the data, it appears that acids of two different strengths are present in approximately equivalent amounts. In alcoholic medium the titration curve for Vinsol shows no sharp inflection.

Another potential application of the method being studied is the titration of amino acids. In aqueous solution these compounds are difficult to titrate because of their amphoteric character. In ethylenediamine, however, the buffering effect of the amino group is completely eliminated and the amino acid can be titrated as readily as any other carboxylic acid. Curves for glycine and ϵ-aminocaproic acid, shown in Figure 7, are of the same general form obtained

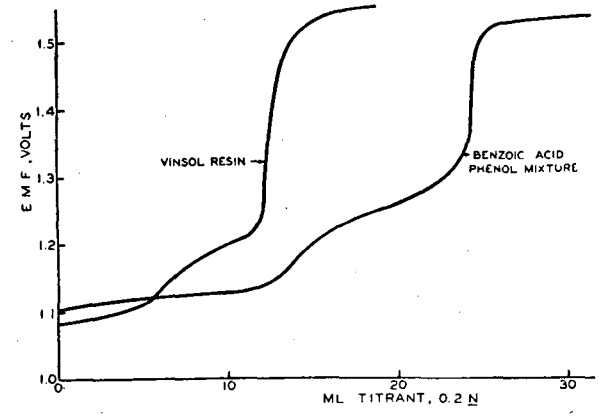

Figure 6. Titration of Vinsol Resin and of Phenol-Benzoic Acid Mixture in Ethylenediamine with Hydrogen-Calomel Electrodes

Table II. Acid Numbers of Various Materials Determined by Titration in Ethylenediamine

Compound	Acid No., Mg. KOH/Gram: Found, First end point	Found, Second end point	Theoretical, First end point	Theoretical, Second end point
Resorcinol	528	1000	510	1019
Salicylic acid	415	810	406	812
Methyl *p*-hydroxybenzoate	374	..	369	..
Ethyl *p*-hydroxybenzoate	353	..	348	..
Vinsol resin	125	257	..	..
Belro resin	176	..	..	..
Glycine	77	..	75.1	..
ϵ-Aminocaproic acid	419	..	427	..
Anthranilic acid	412	..	409	..
p-Aminobenzoic acid	414	..	409	..
Boric acid	873	1829	906	1812
		2788 (third)		2718 (third)

with benzoic acid and rosin. The descending nature of the curves in Figures 7, 8, and 9 is characteristic of the electrode system used.

Several substituted phenols containing more than one acidic hydrogen were also titrated in ethylenediamine (see Figure 8). Resorcinol gives an interesting curve with two breaks, the first much more pronounced than the second, indicating the second

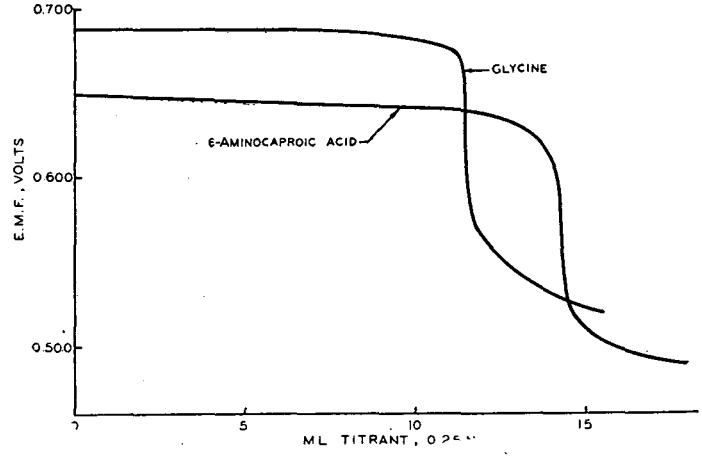

Figure 7. Titration of Amino Acids in Ethylenediamine with Antimony Electrodes

hydrogen to be extremely weakly acidic. No evidence of either acidic hydrogen is found on titrating in aqueous medium. With salicylic acid, the situation is similar to that found with a mixture of phenol and benzoic acid; the e.m.f. change is greater at the second end point and somewhat more abrupt than at the first.

Because of the general interest often expressed among analysts in the possibility of titrating the third hydrogen of phosphoric and boric acids, several titrations were undertaken with these compounds. Phosphoric acid precipitated in ethylenediamine and in ethanolamine and, therefore, could not be titrated. In the case of boric acid, which is much weaker than phosphoric acid, there were three detectable inflections, as shown in Figure 9, close to the calculated values. Although the end points are not sufficiently distinct for accurate results, the curve shows the possible advantages of titrating weak acids in a basic solvent.

Acid numbers obtained in ethylenediamine for various acidic materials are shown in Table II.

Most of the samples listed cannot be titrated satisfactorily by ordinary methods. Results are rounded off to the nearest whole number, as the purity of most of these materials was not determined. In cases where more than one end point was observed, the second (and third) acid numbers indicate total acidity titrated up to those end points.

Although ethylenediamine is somewhat objectionable as a solvent because of its caustic properties and tendency to fume in air, it can be handled conveniently in the apparatus described. It offers obvious advantages over liquid ammonia, which was first considered. Existing methods employing various neutral nonaqueous solvents for the titration of dark-colored resins leave much to be desired, even with the best electrical equipment. In the opinion of the authors, any outstanding progress in the field will probably depend first on a drastic departure from conventional solvent—titrant combinations.

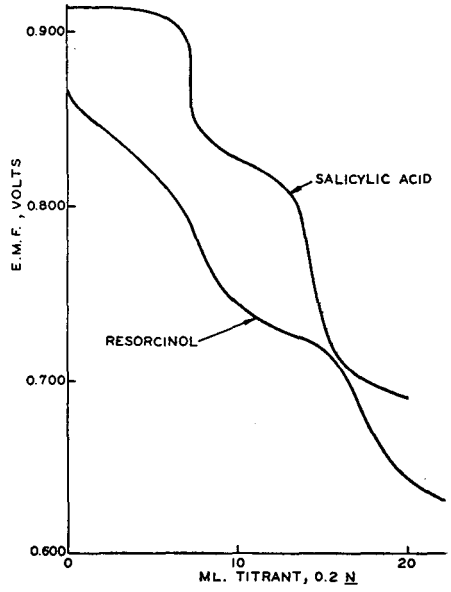

Figure 8. Titrations in Ethylenediamine with Hydrogen-Antimony Electrodes

The most promising approach to the problem would seem to be to work in a basic solvent so as to take advantage of the "newer" concepts of acids and bases.

ACKNOWLEDGMENT

The authors wish to acknowledge the many helpful suggestion made by Martin Kilpatrick, Hercules Powder Company consultant, during the course of this work.

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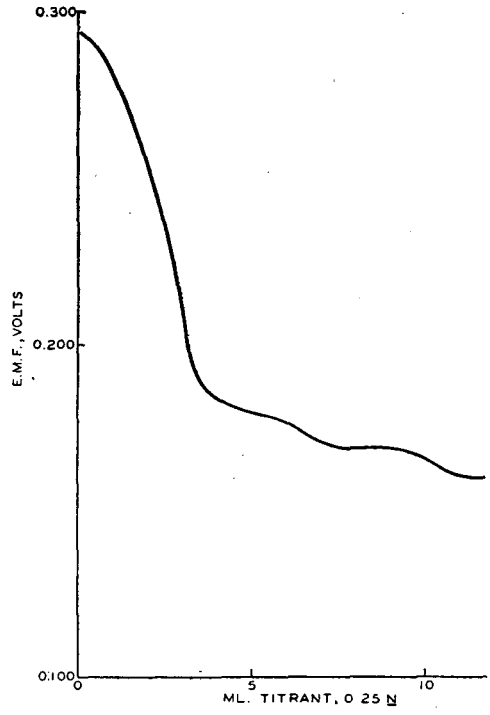

Figure 9. Titration of Boric Acid in Ethylenediamine with Antimony Electrodes

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Organic Quantitative Analysis Using the Geiger Counter X-Ray Spectrometer

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A discussion is presented of the use of the Norelco Geiger counter x-ray spectrometer in the quantitative analysis of organic systems. Complete details covering certain improvements to the spectrometer and in the methods used for analysis are given. In particular, a method for the determination of the crystalline content of sodium penicillin G samples, accurate to ±2.5%, is given. The x-ray results obtained on a series of experimental samples of sodium penicillin are compared with those obtained by ultraviolet and infrared methods.

IN A recentarticle Strong (*17*) presented an interesting survey of the papers published on quantitative analysis during 1946, and showed that 56% of all these papers were concerned with the use of instrumental methods of analysis. One such method which as yet has not received widespread acceptance involves the use of x-ray diffraction.

The usefulness of this method for the qualitative identification of the various crystalline components present in mixtures, either inorganic or organic in nature, has been thoroughly proved, considerable stimulus having been given to this aspect by the 1938 publication of Hanawalt, Rinn, and Frevel (*13*) and the subsequent publication of the A.S.T.M. card file of x-ray diffraction patterns (*1*). . [A review paper covering the field of x-ray diffraction qualitative analysis has recently been published by Smith and Barrett (*16*).]

As a result of a combination of experimental difficulties, however, the use of x-ray diffraction for quantitative analysis has, in general, not yielded results of sufficiently high accuracy and reproducibility to recommend its widespread use. Nevertheless, its potentialities have been recognized and in recent years it has been applied successfully to the quantitative analysis of mineral mixtures, and in particular to the determination of quartz (*2*, *4*, *8*, *12*). These studies employed the usual powder techniques: photographic recording of the diffraction pattern of the sample under consideration and densitometering of selected lines on the processed film. These procedures are rather time-consuming and subject to all the limitations of the photographic method (*4*, *19*). On the other hand, the introduction of the Norelco x-ray diffraction Geiger counter spectrometer (*6*, *11*) manufactured by the North American Phillips Company, New York, N. Y., has made possible quantitative techniques which are considerably more practical. This has been pointed out in recent publications on the determination of quartz in various base constituents (*7*, *14*) and the determination of heavy metal carbides (*15*). This present paper deals exclusively with the application of such a spectrometer to the quantitative analysis of crystalline organic systems.

A wide variety of quantitative analyses which may be investigated profitably with the x-ray method are encountered in connection with the study and evaluation of such organic materials as dyes, pigments, drugs, and intermediates. For example, in addition to the ordinary problems of analysis, it is often necessary to determine specifically such quantitities as the relative amounts of the crystalline and noncrystalline forms of a given chemical species present in a sample, or the relative amounts of two or more polymorphs present. Both in the laboratory and in the plant, problems of these types are plentiful. As an illustration of the former type of analysis, a procedure is described below for the determination of the crystalline sodium penicillin present in experimental and commercial samples of this substance. The details of experiment and procedure are generally applicable and are not restricted to the penicillin problem chosen as the example.

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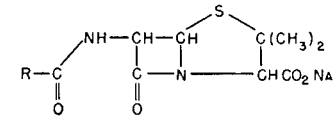
QUALITATIVE ANALYSIS OF PENICILLINS

It is now recognized that in the natural fermentation process used for the production of penicillin, at least five types of penicillin may be produced under certain conditions. The suggested structure of the sodium penicillin molecule (*9*), together with the substituents R which characterize these five types, is shown in Figure 1. As a preliminary to the quantitative analysis, it was first necessary to carry out a complete qualitative study of the x-ray diffraction properties of each of these types of penicillin. With the exception of dihydro F, each of the samples used as a prototype standard was isolated from commercial penicillin especially for this purpose. These standards were obtained through the use of chromatographic columns, and were crystallized as sodium salts. Each was studied by the methods of infrared (*5*) and



TYPE	R	
G	BENZYL	$C_6H_5-CH_2-$
F	Δ^2 PENTENYL	$CH_3CH_2CH=CHCH_2-$
DIHYDRO F	N-AMYL	$CH_3CH_2CH_2CH_2CH_2-$
K	N-HEPTYL	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2-$
X	P-HYDROXYBENZYL	$HO-C_6H_4-CH_2-$

Figure 1. Sodium Penicillin Structure (*9*)

ultraviolet (*10*) absorption spectrometry and with the polarizing microscope, and compared critically with the best samples available from the Food and Drug Administration and the Antibiotics Section of the National Institute of Health. As a result of the combined information furnished by these studies, the relative purity of the samples is believed to be: G and X, as pure as any samples studied, no evidence of any of the other types; F con-

Table I. Values of Interplanar Spacings and Relative Intensities for Five Common Types of Sodium Penicillin

1. Sodium Penicillin G		2. Sodium Penicillin Dihydro F	
d/n, Å.	*I/I*$_0$	*d/n*, Å.	*I/I*$_0$
15.6	1.00	15.6	1.00
6.0	0.2	9.3	0.04
5.8	0.2	7.8	0.04
5.3	0.05	5.7	0.16
5.0	0.1	5.3	0.04
4.8	0.1	5.0	0.16
4.33	0.1	4.7	0.13
4.15	0.1	4.27	0.2
3.64	0.08	3.54	0.04
3.46	0.03	3.32	0.04
3.29	0.03	3.25	0.04
3.13	0.1	3.02	0.05
3.00	0.1	2.83	0.05
2.90	0.03	2.66	0.03
2.78	0.04	2.53	0.04
2.60	0.03	2.40	0.04
2.55	0.03	2.11	0.04
2.41	<0.03		
2.25	<0.03		
2.09	<0.03		

3. Sodium Penicillin K		4. Sodium Penicillin F	
15.6	1.00	15.6	1.00
10.3	0.13	6.5	0.06
8.8	0.06	5.7	0.06
6.5	0.06	5.3	0.06
6.2	0.03	4.9	0.17
5.5	0.12	4.6	0.14
5.2	0.06	4.10	0.04
4.9	0.45	3.95	0.04
4.35	0.23	3.70	0.03
3.80	0.10	3.32	0.04
3.53	0.03	3.22	0.04
3.37	0.03	3.04	0.04
3.21	0.06	2.98	0.04
2.91	0.03	2.85	0.04
2.80	0.06	2.69	0.04
		2.64	0.02
		2.54	0.02
		2.48	0.02
		2.44	0.02

5. Sodium Penicillin X	
12.5	1.00
10.8	0.80
6.7	0.02
6.0	0.10
5.7	0.47
5.4	0.80
5.1	0.47
4.8	0.21
4.5	0.07
4.40	0.07
4.15	0.40
3.93	0.60
3.70	0.13
3.58	0.27
3.44	0.13
3.34	0.40
3.25	0.02
3.17	0.33
3.03	0.13
2.97	0.10
2.91	0.27
2.86	0.02
2.75	0.02
2.63	0.27
2.47	0.13
2.33	0.11
2.28	0.11
2.23	0.11

tained no K, X, or dihydro F, and less than 1% of G; K contained no G, F, or X, contained possibly some small amount of dihydro F, and was by far the purest sample of K studied; dihydro F contained about 2% G and 12% F. In none of the above cases was there any evidence of the presence of appreciable quantitites of amorphous material.

In view of the limited quantities in which certain of these types were available, the qualitative x-ray diffraction patterns shown schematically in Figure 2 were obtained using a G.E. XRD powder camera. To obtain a more accurate value of the spacing of the

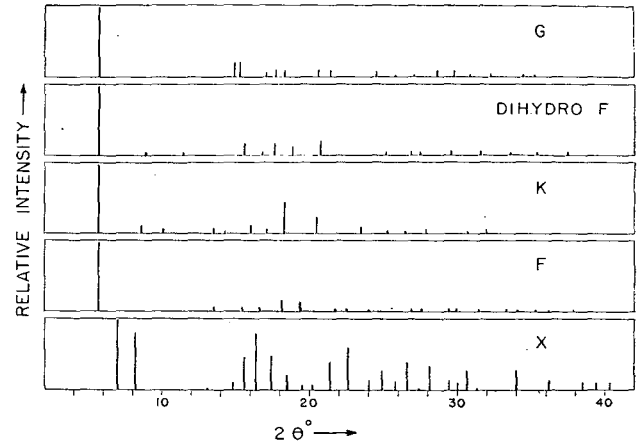


Figure 2. Diffraction Patterns of Five Common Types of Sodium Penicillin

Line positions are plotted according to their 2θ values for CuKα radiation, λ = 1.539 Å.

innermost line, the diffraction patterns of the samples were also recorded using the G.E. flat film cassette with a specimen to film distance of 10.0 cm., and 0.25 mm. (0.010-inch) beam collimating pinholes. With the exception of X, each of these patterns is characterized by the strong line at $2\theta = 5.70°$. Although differences may be seen in the cases of other lines, the fact that this, the only really strong line, is common to four of the five types renders very difficult the possibility of using this method for

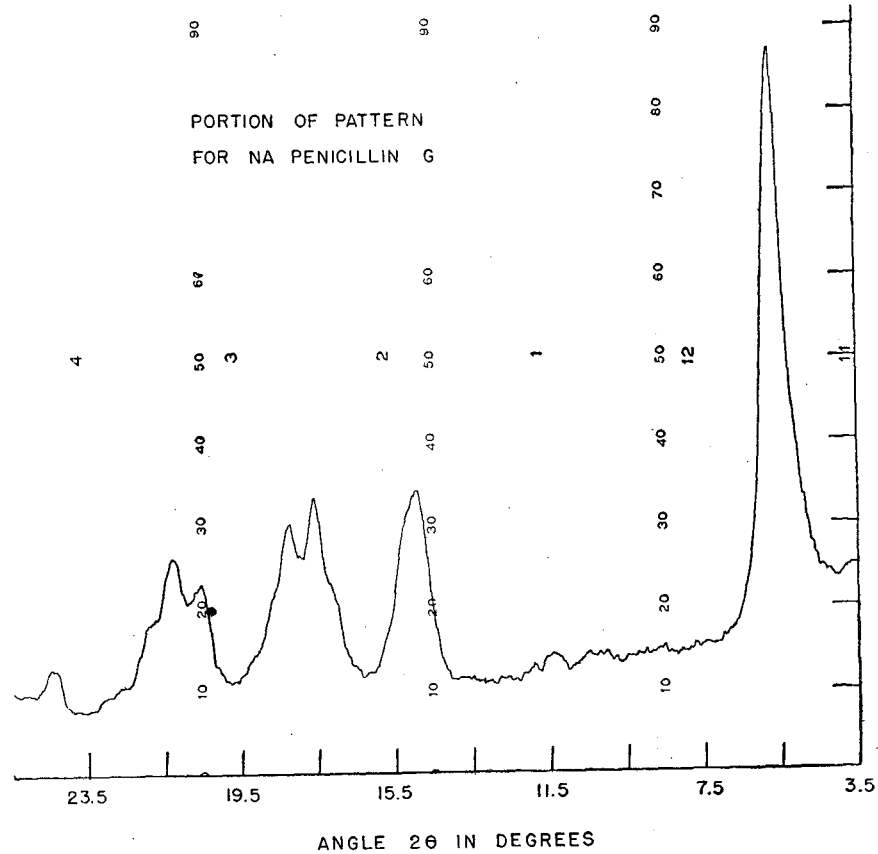


Figure 3. Norelco Pattern of Sodium Penicillin G Cu (Ni Filtered) Radiation

Slits 0.020-0.040-0.020 inch. Brown recorder, 10-millivolt full-scale deflection

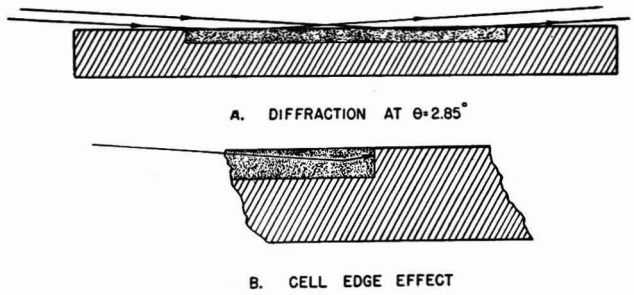


Figure 4. Geometrical Relationship between X-Ray Beam and Sample

the general qualitative analysis of any except highly homogeneous samples. An analytical procedure for sodium penicillin X may easily be based upon either or both of the strong lines at $2\theta = 7.0°$ and $2\theta = 8.2°$.

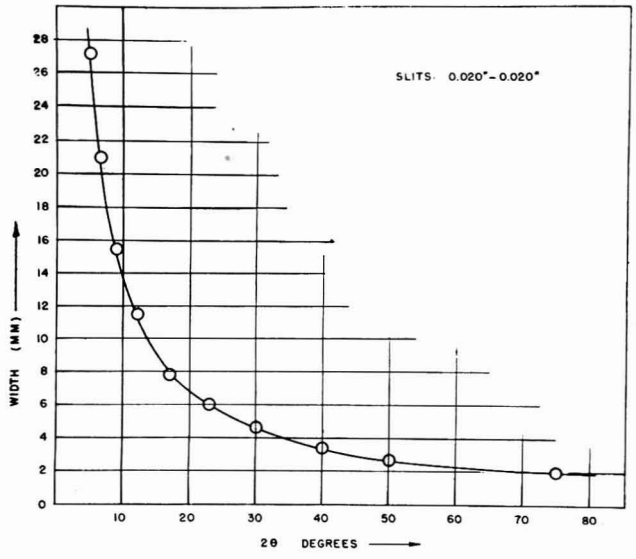


Figure 5. Width of X-Ray Beam Incident upon Sample

Measured Photographically

As a result of the above facts, it was not possible to consider the x-ray diffraction method, as originally intended, as a quantitative method for determining the relative amounts of each type of crystalline penicillin present in an unknown mixture. On the contrary, it was clear that this method could be applied best for the determination of the total amount of crystalline penicillin, other than X, present in the unknown. This determination alone is, however, of considerable practical importance and the failure of this method to differentiate between the various types does not detract from its usefulness. As a result of the widespread use of phenyl derivatives as additives to the fermentation media, and the nature of the chemical processes used in the recovery and crystallization of penicillin, the average commercial sample of crystalline penicillin, unless otherwise noted, is almost wholly G in type. (It is now common practice to add any one of several phenyl derivatives to the fermentation medium in order to enhance the proportion of G penicillin produced.) Accordingly, in the analytical example shown below crystalline sodium penicillin G was used throughout in the determination of the analytical working curve. All results obtained are reported on a percentage basis in terms of crystalline sodium G, realizing that they in reality included the other types if present, exclusive of X. In effect, it was assumed that each of the unknowns was a two-component mixture of crystalline sodium penicillin G and amorphous material.

PROCEDURE FOR QUANTITATIVE ANALYSIS

The methods of x-ray diffraction quantitative analysis, as usually practiced, are essentially empirical. The intensity of a diffraction line (or lines) characteristic of the substance under consideration is determined as a function of the percentage composition of suitable standard mixtures containing known amounts of that substance. As a diluent in making up the calibration standards one uses a substance or substances having, as nearly as possible, the same absorption and scattering characteristics as the base material likely to be encountered in the unknowns. Thus a working curve is obtained which relates the percentage of the substance, in the presence of a given type of diluent, to the intensity of a diffraction line characteristic of the substance.

A portion of the diffraction pattern of crystalline sodium penicillin G, as recorded on the Norelco spectrometer, is shown in Figure 3. As was noted above, the only strong line in the pattern occurs at a relatively low angle with the peak at $2\theta = 5.70°$. The occurrence of diffraction lines, often the most intense ones, at small 2θ values is characteristic of organic compounds as a consequence of the relatively large unit cell dimensions possessed by all but the simplest of such compounds. In this respect organic substances differ from metals and alloys and most inorganic substances. The quantitative work, herein reported, was based upon the line at $2\theta = 5.70°$, as the other lines were not sufficiently intense to afford the necessary precision of measurement.

In order to attain reproducible intensity measurements at the required low angle, it was found necessary to adjust the sample–x-ray beam geometry rather carefully. In Figure 4,*A*, the sample shape requirements are shown for $\theta = 2.85°$, the width of the beam here being defined by the two collimator slits of the

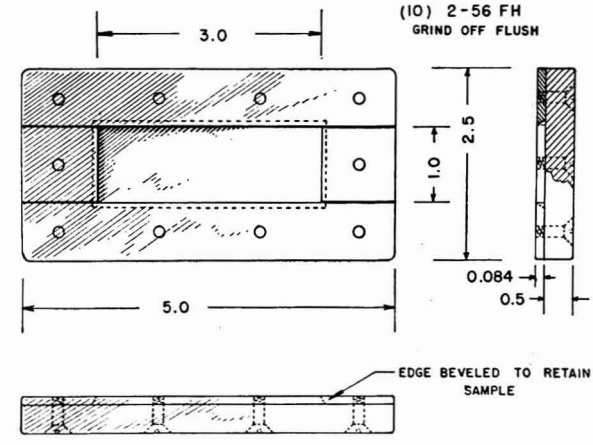


Figure 6. Sample Holder for Low Angle Diffraction

Dimensions in cm.

spectrometer set at 0.5 mm. (0.020 inch), the settings used in the subsequent work. (The Norelco spectrometer used in this work is one of the early models, having two micrometer slits in the beam collimator and a micrometer slit in front of the Geiger tube.) At these settings the beam has an actual width of about 0.9 mm. and a projected width for $\theta = 2.85°$ of 20 mm. When a sample of insufficient width is used, not only is that portion of the beam wasted which does not fall on the sample, but errors may arise due to the fact that the portion of the beam being diffracted near the edge of the sample holder can be trapped by this edge, as is illustrated in Figure 4,*B*. Experience has shown that because of this edge effect, variations in sample packing and relatively

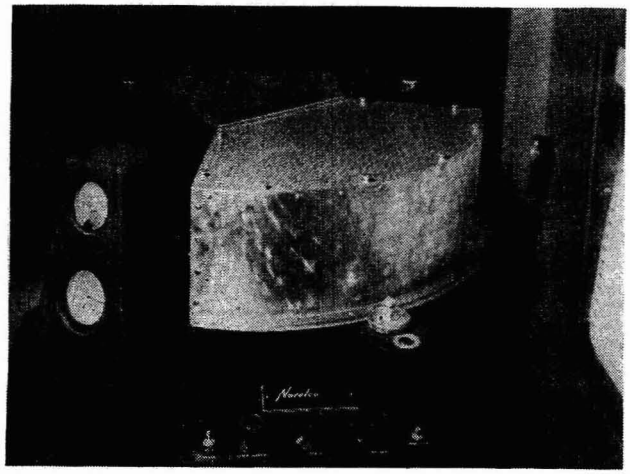


Figure 7. View of Spectrometer Showing Reinforced Scanning Quadrant

small displacements of the sample holder horizontally in the post holder will cause intolerable variations in diffracted intensities. Figure 5 is a plot of the projected width of the beam incident upon the sample, as a function of the angular displacement, 2θ, of the scanning arm, for the collimator slits both set at 0.5 mm. (0.020 inch).

In order to eliminate these effects, the sample holder shown in Figure 6 was designed and used throughout this work.

The holder was made of stainless steel, the front surface (sample side) being ground flat. In use the loaded sample holder is placed in the spectrometer post asymmetrically, so that about one third the sample width is to the left of the vertical axis of rotation, toward the x-ray tube of the instrument. It is also necessary to align the sample holder post so that the sample block will be correctly positioned in the vertical direction, the height of the x-ray beam having been limited to the correct amount, and so that the face of the sample is correctly oriented with respect to the axis of rotation of the spectrometer.

Another source of error in the attainment of reproducible intensity measurements was found to reside in the mechanical instability of the scanning system of the spectrometer as received. It was easy to rock the scanning quadrant in a vertical arc with mechanical pressure exerted by hand. After such a pressure has been released, the instrument does not return to its original position, and these displacements, although very small, are sufficient to change the position of the defining slit in front of the Geiger tube with respect to the diffracted beam and thus cause significant variations in observed intensity. In order to avoid the possibility of any errors due to this effect, the structure of the scanning unit was mechanically strengthened. The whole scanning quadrant was stiffened through the use of aluminum sheets bolted to one another and to the scanning arc and its supporting arms, as indicated in Figures 7 and 8.

After elimination of the mechanical difficulties discussed above, it was found, as reported by other investigators (*7*, *15*), that the limit of the analytical precision obtainable at the present time is determined by the nature of the sample preparation and mounting. In this respect, one particularly wishes to avoid crystal orientation, with its resulting false intensity production, in the same mount. Photomicrographs of the two extreme typical habits assumed by sodium penicillin G, as a function of the method of crystallization, are shown in Figure 9. Samples having the lath-like habit shown in Figure 9,*B*, were rather easy to grind up by hand with a small agate mortar and pestle and gave resulting finely crystalline powders having an average particle size of about 2μ. Samples prepared in this way gave reproducible intensity measurements at $2\theta = 5.70°$ upon successive reloading of the sample holder. On the other hand, material having the habit shown in Figure 9,*A*, was very difficult to process in this way. It was pointed out by Dan McLachlan, Jr., that finely divided carbon black added in small quantity to organic substances as a dispersing agent will prevent the formation of agglomerates having

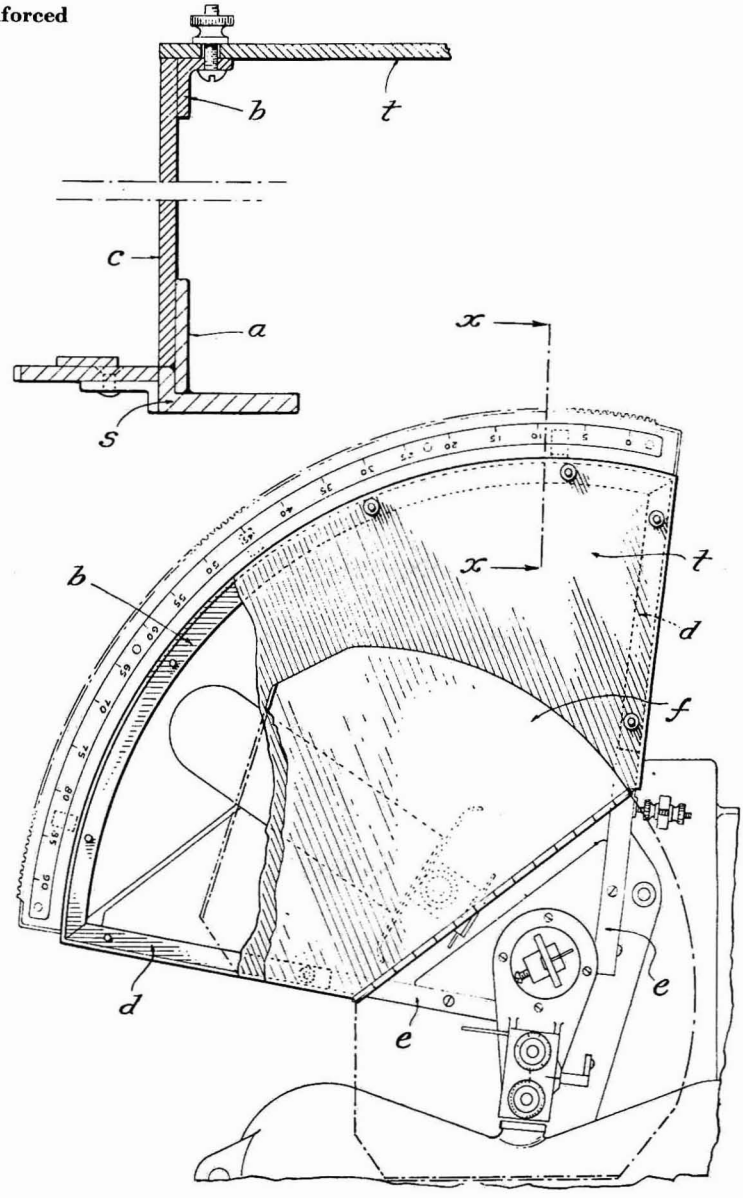


Figure 8. Schematic Diagram Showing Construction Details of Reinforced Scanning Quadrant

Upper drawing represents a cross-section at line *xx* on lower one. An iron strip, *a*, of 3/32 inch thickness is spot-welded along scanning arm *s* of spectrometer; to this is bolted 1/8 inch thick aluminum sheet, *c*; this in turn is bolted to aluminum sheet, *t*, through aluminum angle strip, *b*. In a similar fashion side plates of aluminum, *d*, are bolted through aluminum angle strip to top, *t*, and to supporting arms of arc, *e*. Hinged flap, *f*, shown here folded back

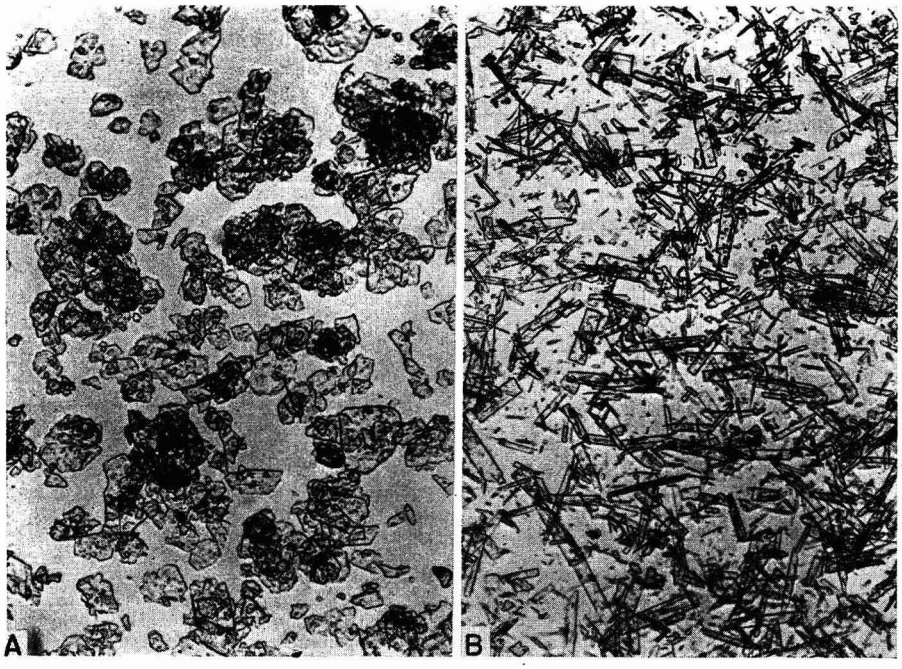


Figure 9. Photomicrographs of Sodium Penicillin G (5)

A. Platelike crystals difficult to grind to desirable particle size
B. Lath-shape crystals easy to grind to desirable particle size

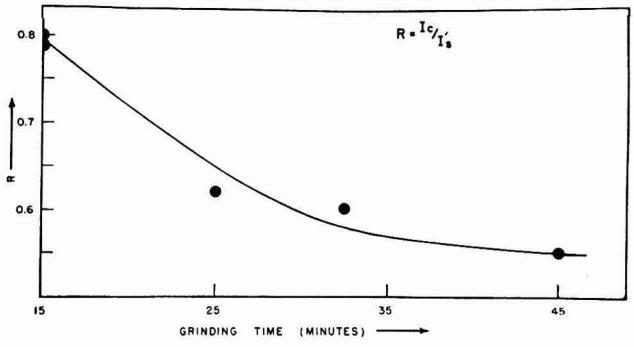


Figure 10. Effect of Continued Grinding with Carbon Black in Bringing about Deorientation with Subsequent Lowering of Intensity Value at $2\theta = 5.70°$

I_c' is peak height of penicillin line at $2\theta = 5.70°$; I_s' is the peak height of the brass standard at $2\theta = 49.13°$

their component crystallites oriented in a preferred way when ground with the substance. The small amount of carbon (1 to 2%) added in this way will contribute only slightly to the general background of the diffraction pattern. Moreover, when added to a white mixture and ground with it, the carbon serves as a visual indicator of the extent to which homogeneity has been achieved. It was found that the addition of 2% of carbon black to samples having the platy habit shown in Figure 9,*A*, helped considerably in reducing the material by grinding to particles of about 2μ, which samples gave reproducible diffraction intensity. Consequently, all samples examined were ground with carbon black present, including those used in the preparation of the working curve. In practice, prior microscopical examination having indicated the sample habit, those samples having a platy structure were repeatedly reground after x-ray spectrometer examination until a constant diffraction intensity value was obtained. A particularly recalcitrant sample gave the deorientation curve shown in Figure 10. Microscopical examination of the samples prepared in this way showed that the spread in particle size distribution was small, the particles being about 2μ in size and showing clean cleavage; few particles were as large as 10μ.

The addition of carbon black to organic systems being ground has been found generally useful in preventing the accumulation of static charge on the material during grinding. Methanol and other polar liquids have been much used as dispersing agents in ball-milling minerals and ores (*3*); there is much advantage in using the inert carbon black in the case of organic systems, especially those incompletely characterized.

As a material for the preparation of the working curve, a well crystallized sample which infrared and ultraviolet absorption study indicated was 100% crystalline sodium penicillin G and having the lathlike crystal habit of Figure 9,*B*, was chosen. Samples containing various known percentages of this material were made up using cornstarch as a diluent. The use of this material as a diluent was based upon the idea that it would be a good approximation, in its x-ray diffraction properties, to the base material actually found in samples of sodium penicillin G to be analyzed, as was pointed out above. Each calibration standard was made by grinding together the appropriate weights of sodium penicillin G and cornstarch with carbon black added to the amount of 2% of the total weight of the mixture. The grinding was done by hand with a small agate mortar and pestle, each sample being ground for about 20 minutes. The weight of each calibration standard made up in this way was about 200 mg. The intensity of the line at $2\theta = 5.70°$ was then measured for each sample of known composition, to furnish the data for the preparation of a working curve.

In order to arrive at reliable intensity measurements, reasonable care must be exercised in filling the sample holder. The method used in this work was to add the sample to the holder in slight excess, press it firmly into the well with a glass microscope slide, and then remove the excess material by sliding the slightly tilted slide along the surface of the holder, so that its forward edge acted as a plow. In this way the surface of the material may be made flush with that of the holder, which is necessary if reproducible intensity values are to be obtained. In early experiments it was found that the surface of penicillin samples not containing carbon charged up in this process and made it more difficult to obtain a smooth surface. With the carbon added, however, this difficulty was not encountered. Repeated reloading of the sample holder with sodium penicillin G samples gave sample weights of 125 ± 10 mg.

In making the intensity measurements, the whole Norelco unit was turned on and allowed to warm up for a period of about an hour before any quantitative measurements were attempted. The sample holder carrying the sample block prepared as indicated above was then set in position in the spectrometer post. With the collimator slits set at 0.5–0.5 mm. (0.020–0.020 inch) and the Geiger tube slit at 0.75 mm. (0.030 inch), the diffracted intensity from the sample was measured by counting at $2\theta = 5.70°$, no nickel filter being used in the counting experiments. This done with the pulse register, using a counting time of 64 seconds and a scaling constant of 64. For each intensity measurement, the counts occurring in three successive 64-second intervals were totaled, the count in each time interval being noted, however. In this way the agreement among the three separate intensity measurements could be observed, while the total count for all three time intervals was used in the calculations.

With the slits set at the values given, for 100% crystalline sodium penicillin G the observed counting rate is about 380 counts per second. For each intensity measurement, therefore, a total of $380 \times 64 \times 3 = 69{,}000$ counts was obtained. For this number of counts, the probable error due to the random production of the counts is of the order of 0.3% (*18*). Several experi-

ments in which repeated intensity measurements were made on an undisturbed penicillin sample were carried out. For ten such measurements with an arithmetic mean of about 69,000 counts an average deviation of about 0.4% was obtained.

In order to take into account any drift in x-ray energy output or in Geiger counter response, it was felt desirable to have some method of standardizing the equipment. A suitable standard sample may be used for this purpose. The authors chose as such a standard sample a block of yellow brass giving a strong line at $2\theta = 49.13°$. Figure 11 shows a portion of the pattern given by the brass block in this 2θ region.

In performing the quantitative analyses the procedure has been followed of measuring the count given at $2\theta = 5.70°$ for the penicillin sample, replacing the sample holder by the brass block, and measuring, in the same fashion, the count given by this at $2\theta = 49.13°$. The time-integrated intensity from the sample I_c' and that from the standard I_s' may then be used to form the ratio $R = I_c'/I_s'$, a quantity essentially independent of any long-period drift of the instrument. In practice, it was found that I_s' could be brought to the same value within 1 to 2% from day to day by slight adjustments of the height of the collimator slit or Geiger tube voltage. This practice obviated the necessity for correcting the intensities I_c' and I_s' for nonlinearity of Geiger counter response (*11*). Repeated determinations of R, in the case of sodium penicillin G, for new mounts of the same sample differ by ±1.5 to ±2.0% of the value of R.

A working curve derived through the use of calibration standards as outlined above is shown in Figure 12. Each measured point on the curve was obtained as the average for the intensity measurements derived from two separate loadings for each sample. Of course, the precision obtained could be increased by averaging the value of R found for a larger number of loadings of the same sample.

The intensity measurements used represent peak heights, and are not corrected for background. The use of this simplified procedure is justified by the fact that preliminary experiments had shown that in the neighborhood of $2\theta = 5.70°$ cornstarch and amorphous sodium penicillin scatter to about the same extent. Moreover, the rate of increase of background with respect to peak height is very small for concentrations down to about 40% crystalline sodium penicillin G, as is evidenced by the linearity of the working curve down to this point.

As a test of the x-ray method a number of commercial samples were assayed and the results obtained compared with those obtained on the same samples by the infrared (*5*) and ultraviolet (*10*) methods. This comparison is shown in Table II, from which it can be seen that an accuracy of approximately ±2.5% may be expected in the range 40 to 100% crystalline content. It is to be noted that the ultraviolet method gives total sodium penicillin G. As seen from the table, most of the samples examined were 100% sodium penicillin G, the percentage of crystalline material being somewhat less in several cases. In general, the agreement between the results obtained by the x-ray and infrared methods for the crystalline sodium penicillin G content of the several samples is very good.

Table II. Analytical Results

Sample	Percentage of Sodium Penicillin G: Total % ultraviolet (*10*)	Crystalline %: X-ray	Crystalline %: Infrared (*5*)
A	101	101	99
B	80	75	75
C	103	98	..
D	100[a]	100[a]	..
E	98	60	60
F	100	99	..
G	100	75	98

[a] Taken as standard.

In the case of sample G, however, there is considerable disagreement. Careful optical microscopical examination of this particular sample showed that it contained a large proportion of extremely small crystals, below 0.2μ in size, occurring singly or in aggregates. It appeared that there was a sufficiently large fraction of this material present to bring about line broadening with consequent lowering of the peak height at $2\theta = 5.70°$. The sample was recrystallized and a recovery equal to the theoretical recovery value for sodium penicillin G was obtained, in agreement with the ultraviolet and infrared results on the original sample. The recrystallized sample did not show the finely divided fraction, and x-ray diffraction analysis of it gave a value of 98% for the crystalline sodium penicillin G content.

The results obtained on sample G demonstrate clearly that the present method is valid for the analysis of organic materials, provided only that the particle size distribution of the unknown is

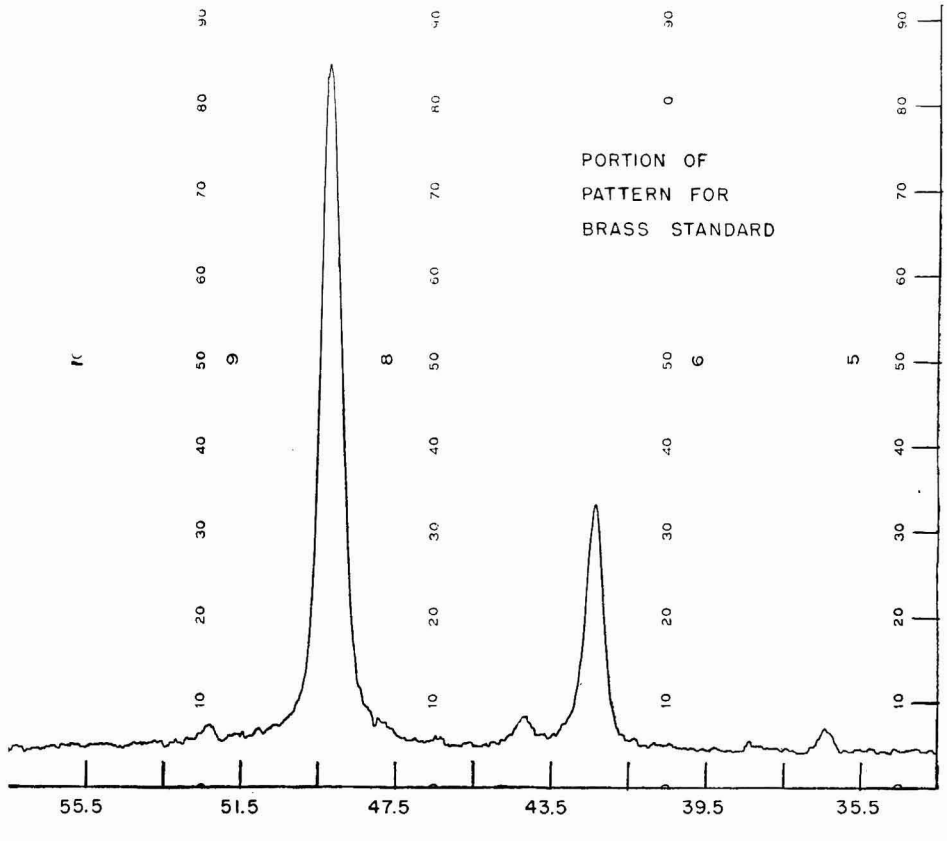


Figure 11. Norelco Pattern for Brass Standard

Cu (Ni filtered) radiation. Slits 0.020–0.040–0.020 inch. Brown recorder, 10-millivolt full-scale deflection

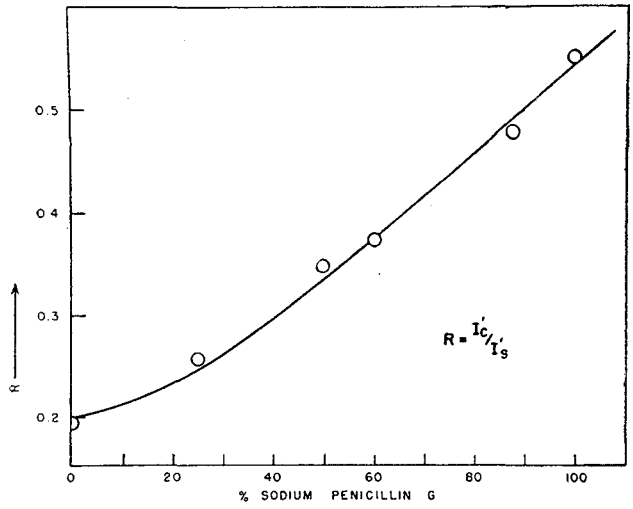


Figure 12. Working Curve for Determination of Crystalline Sodium Penicillin G

essentially the same as that of the standards used in making up the working curve. It is advisable, therefore, that all unknowns be observed microscopically prior to x-ray analysis.

ACKNOWLEDGMENTS

It is a pleasure to record the authors' indebtedness to Dan McLachlan, Jr., E. F. Champaygne, and other members of the staff of these laboratories for much help in this work. The sample of crystalline sodium penicillin dihydro F was furnished through the courtesy of the Chas. Pfizer Company, Inc. The authors are deeply grateful to the Food and Drug Administration and the Antibiotics Section of the National Institute of Health for penicillin samples.

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Electronic Trigger Circuit for Automatic Potentiometric and Photometric Titrations

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A Schmitt trigger in combination with a single-stage pentode preamplifier provides a circuit that can be made to switch abruptly and definitely at a preset level of input potential. The trigger action is reversible, with a small dead zone. A stabilized line-operated instrument is described which is suitable for automatic potentiometric and photometric titrations, with a sensitivity of ±5 mv. and ±2 × 10^{-5} lumen, respectively.

IN A recent paper (*1*) it was shown that precise automatic titrations can be made with a motor-driven syringe type buret, the delivery of which is stopped exactly at the equivalence point by a mercury switch attached to the slide wire of a recording potentiometer. Intermittent action in the vicinity of the end point is obtained by appropriate adjustment of the distance between the indicator electrode and buret tip. A Brown Electronik recording potentiometer was used in the previous investigation.

The present paper describes an amplifier trigger combination which will switch abruptly at a definite predetermined potential and switch back again for a slightly smaller potential and which can thus be used for automatic titrations in place of an expensive recording potentiometer. The new circuit is also suited, without modification, for phototube control, and is therefore very useful in automatic titrations using indicators or other color changes.

The behavior of the circuit is best understood by reference to the simplified schematic of Figure 1.

The twin triode represented by T_1 and T_2 is the trigger circuit essentially as described by Schmitt (*3*). Section T_2 has a relay connected in the anode circuit and the grid of this section is connected to the anode of the first section through a resistor and through another resistor to ground. A common cathode resistor, R_c, is returned to a point 150 volts above ground and the common plate supply is 300 volts above ground. With no signal applied to the input of T_1 this section is nonconducting and section T_2 is fully conducting. This is a steady state and will prevail indefinitely. If the grid of T_1 is made somewhat more positive, the plate potential will decrease and therefore drive the grid of T_2 negative. The decrease of current in section T_2 decreases the voltage drop across R_c and therefore makes the grid of T_1 still more positive. This action is cumulative and during a period of the order of microseconds, T_1 becomes fully conductive and T_2 is cut off.

The full range of current in T_2 between conduction and cutoff can be made much larger than the difference between pull-up and drop-out values of the relay; consequently the switching action is very positive. Both states of equilibrium are stable; at one value of potential on the grid of T_1 the first section is conducting, at a somewhat more negative value it will be cut off and the second section will conduct.

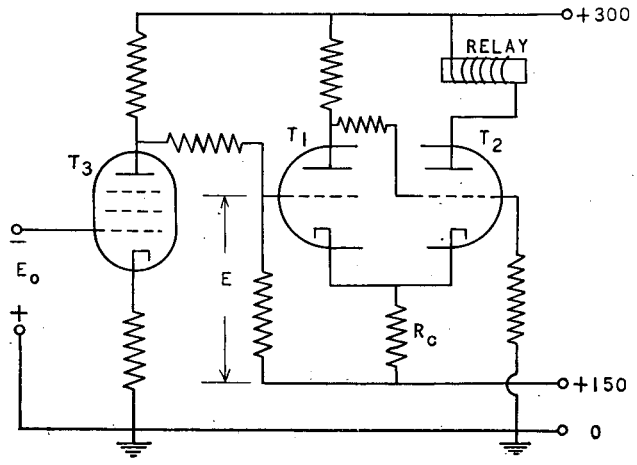

Figure 1. Schematic Circuit

The difference in these two triggering levels becomes progressively smaller as the value of the cathode resistor, R_c, is reduced, and may be made as small as 0.1 volt. Schmitt has shown that if R_c is reduced still more the circuit fails to trigger, but becomes a very sensitive amplifier. In the present application a very conservative dead zone of 0.5 volt was chosen, first of all to preserve the certainty of triggering, and secondly because preliminary amplification of the signal was necessary anyway to apply the circuit to potentiometric titrations.

The function of the pentode amplifier, T_3, is also shown schematically in Figure 1. A slight amount of degeneration in this stage was provided by a cathode resistor.

The plate of the pentode is connected to the grid of T_1 through a resistor and the input potential of the pentode, E_o, which is the sum of a variable bias plus the desired signal voltage, is so adjusted that the plate potential of the pentode is of the order of 150 volts above ground. This is the order of magnitude of the grid potential of T_1 in its untriggered condition. If, now, the grid of the pentode is made slightly more negative, its plate potential rises and initiates switching in the trigger circuit. A change in E_o of only a few millivolts in either direction is sufficient to cause positive reversible trigger action.

The complete circuit is shown in Figure 2. Its similarity with the schematic of Figure 1 requires no further explanation of fundamental behavior. The 300- and 150-volt direct current supplies are regulated by the two VR-150 tubes, T_2 and T_3.

The desired signal potential (0 to 1 volt) at which switching is to occur is set by the coarse and fine potentiometers, R_{12} and R_{13}, and indicated on the voltmeter, V. The auxiliary bias is then adjusted by the coarse, medium, and fine potentiometers, R_{14}, R_{15}, and R_{16}, until the circuit triggers. Triggering is indicated by red and green pilot lamps actuated by one side of the double-pole double-throw relay. If the system is now switched to the electrodes of the titration cell by reversing switch S_1 the circuit will trigger as soon as the cell potential reaches the preset value—i.e., the equivalence point potential.

The second side of the double-pole double-throw relay acts as an on-off switch in series in the circuit of the motor which drives the syringe buret (*1*). Inasmuch as in various titrations the cell potential may either decrease or increase toward the equivalence point potential, switch S_2 is provided so that the syringe motor circuit can be set to open for either direction of potential change.

The input resistance of the circuit is approximately 5 megohms, which is suitable for all potentiometric titrations in which the cell resistance is not larger than a few thousand ohms. The present

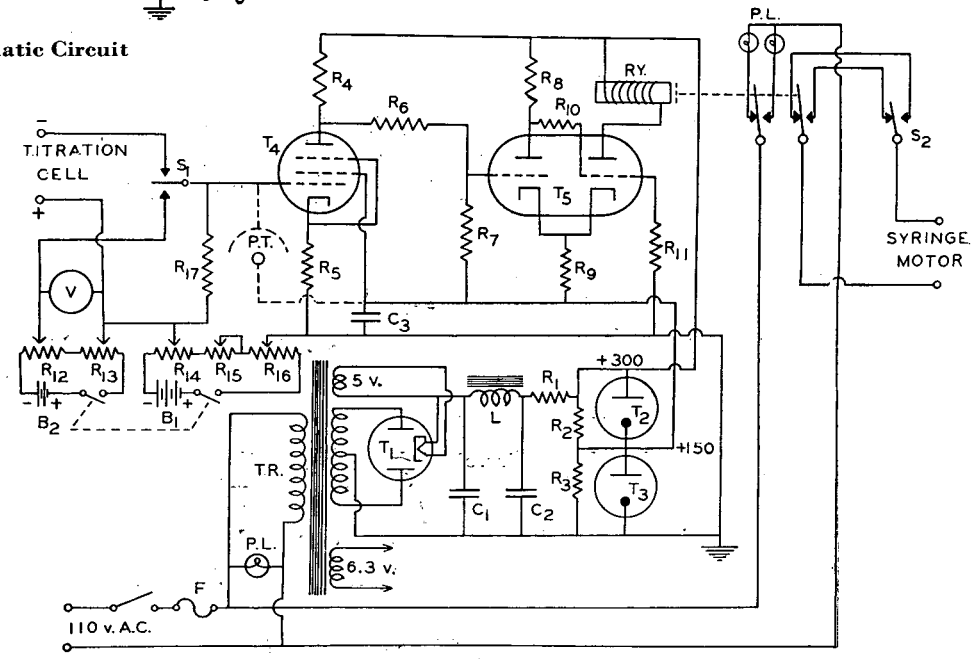

Figure 2. Complete Circuit

R_1.	9000 ohms (10-watt)	R_7.	5 megohms (1-watt)
R_2, R_3.	100,000 ohms (0.5-watt)	R_8.	100,000 ohms (1-watt)
R_4.	1 megohm (0.5-watt)	R_9.	200 ohms (1-watt)
R_5.	4000 ohms (1-watt)	R_{10}.	500,000 ohms (1-watt)
R_6.	100,000 ohms (1-watt)	R_{11}.	2 megohms (0.5-watt)

R_{12}. 100-ohm potentiometers (wire-wound, linear taper)
R_{13}. 1000-ohm potentiometers (wire-wound, linear taper)
R_{14}. 10,000-ohm potentiometer
R_{15}. 100,000-ohm potentiometer
R_{16}. 1-megohm potentiometer
R_{17}. 5 megohms (0.5-watt)
V. Weston voltmeter Model 269 (0 to 1 volt in 0.01-volt divisions and 1000 ohms resistance)
B_1. 22.5-volt dry battery (Burgess 4156)
B_2. 1.5-volt dry battery (Burgess 2F)
T_1. 5Z4 rectifier tube
T_2, T_3. VR150 voltage regulator tubes
T_4. 6SJ7 pentode amplifier (6.3-volt heater connection not shown)
T_5. 6SN7 twin triode (6.3-volt heater connection not shown)
Ry. Double-pole double-throw relay (C. P. Clare and Co., Chicago, No. 40 E. C., 11,300 ohms)
L. Choke (UTC, 30 henrys, 100 ma., 375 ohms d.c.)
TR. Transformer (UTC, 350–0–350, 90 ma.)
C_1, C_2. 8 μf. Aerovox electrolytic capacitors (475 volts d.c.)
C_3. 1 μf. Pyranol capacitor (500 volts d.c.)
F. 2-amp. fuse
S_1, S_2. Telephone type single-pole single-throw switches (General Control Co., Boston, Mass.)
$P.L.$ Pilot lights
$P.T.$ High vacuum phototube (R.C.A. 929)

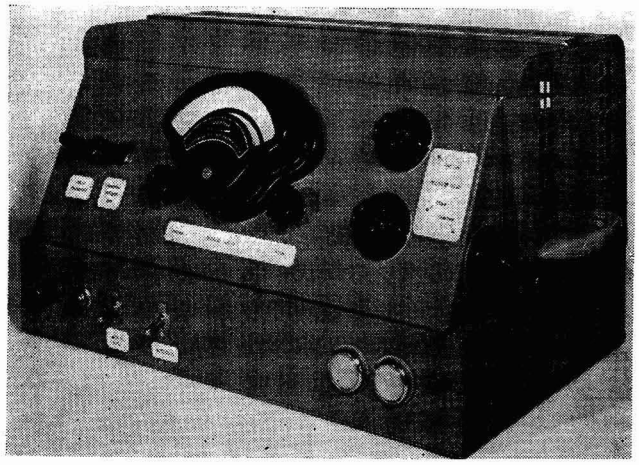

Figure 3. Assembled Instrument

circuit is not applicable with high resistance glass electrodes, and if it is desired to make titrations with the glass electrode a preliminary resistance matching stage may be added.

The instrument was assembled as a portable unit in a radio-type steel cabinet, as shown in Figure 3.

The only external power supply required is a 110-volt, 60-cycle, alternating current line. All wiring, except the leads to the bias batteries and panel controls, was securely anchored to the under side of the chassis, and shielded cable, grounded at several points to the cabinet, was used almost exclusively. Twin-conductor shielded cables (grounded to the cabinet) were used for connection to the electrode system, or phototube, and to the motor of the syringe buret. These precautions rendered the circuit completely immune to body capacity effects and stray fields.

The voltmeter, V, is a high quality instrument (Weston Model 269) with an internal resistance of 1000 ohms, a range from 0 to 1 volt, and 0.01-volt divisions, which permitted readings to ± 0.001 volt. For many purposes a cheaper radio-type meter would probably suffice.

The sensitivity of the instrument—i.e., change in input signal required to actuate the relay—is about ± 3 millivolts. This is more than ample for the great majority of potentiometric titrations. When the instrument was powered from a 110-volt, 60-cycle, Sola constant voltage transformer, rather than the ordinary house line, the dead zone was decreased to less than 2 millivolts. The response time is very short, of the order of a few milliseconds.

The stability of the instrument is very satisfactory. After an initial warm-up period of about 15 minutes, the rate of drift from the preset triggering potential is only about 5 mv. per hour or less. This is entirely negligible for all automatic titrations.

For photometric titrations a high vacuum phototube, $P.T.$, is connected as shown by the dashed lines in Figure 2. An R.C.A. 929 tube was used with an unregulated incandescent lamp source. The instrument is set for photometric titrations with a solution that has the same spectral characteristics as the titrated solution will have at the equivalence point, and potentiometers R_{14}, R_{15}, and R_{16} are adjusted until triggering occurs. No refinements were made to ascertain the limiting precision attainable, but in gross photometric terms the differential sensitivity is extremely high, of the order of $\pm 2 \times 10^{-5}$ lumen. With a properly designed titration cell, and a phototube and filter of optimum characteristics for a particular case, color changes too slight to be perceptible to the eye are sufficient to cause triggering.

The paper of Müller and Partridge (*2*) may be consulted for technical details of automatic photometric titrations. The autotitrator previously described (*1*) is easily adaptable to such titrations, and some typical applications will be described in a later paper.

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Automatic Potentiometric Titration of Iron and Titanium with Chromous Ion

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Automatic potentiometric titrations of Fe(III) and Ti(IV) with chromous ion are described. The automatic titration of ferric ion in sulfuric acid medium with a platinum indicator electrode is precise and accurate to $\pm 0.1\%$. The optimum conditions for the titration of Ti(IV) with chromous ion are the use of a mercury indicator electrode rather than platinum, and of sulfuric acid rather than hydrochloric acid media. Under these conditions the titration is precise and accurate to about $\pm 0.2\%$. Titration curves demonstrate the erroneous behavior of a platinum indicator electrode in solutions whose oxidation potential is below that of the hydrogen–hydrogen ion couple. Very satisfactory determinations of iron and titanium in mixtures of the two can be obtained by titration with chromous ion in solutions containing about 4 N sulfuric acid, using a platinum indicator electrode for the iron titration and a mercury indicator electrode for the subsequent titration of the titanium.

IN A previous paper (*7*) an autotitrator was described which performs potentiometric titrations automatically, with precision and accuracy fully equal to that obtainable in the same titrations by conventional manual techniques. It was shown (*7*) that the instrument is applicable to oxidation-reduction, precipitation, and protolytic titrations, and to slow as well as rapid reactions, so that it is capable of general application.

The primary purpose of the present investigation was to establish optimum conditions for the titrimetric reduction of +4 titanium to the +3 state with chromous ion, and the use of the autotitrator was incidental. However, the autotitrator proved to be ideally suited to titrations involving air-sensitive substances, and it is much more convenient than the usual manual method for establishing the characteristics of potentiometric titration curves as well as for routine titrations.

The only previous study of the titration of +4 titanium with chromous ion is that of Brintzinger and Schieferdecker (*1*), who recommended titration in a very concentrated chloride solution

(mixtures of hydrochloric acid and calcium chloride) with a platinum indicator electrode. The present study has shown that these specifications are most unfavorable; the optimum conditions are the use of a mercury indicator electrode rather than platinum, and titration in solutions acidified with sulfuric acid rather than hydrochloric acid. Steady potentials are established almost instantaneously, the potential change at the equivalence point is greatly increased, and the precision and accuracy are correspondingly much better than under the conditions recommended by Brintzinger and Schieferdecker.

EXPERIMENTAL TECHNIQUE

Standard 0.100 *M* solutions of chromous sulfate in 1 *N* sulfuric acid were prepared directly in the storage flask of the autotitrator (*7*) by the procedure described by Lingane and Pecsok (*8*). The 1-liter storage flask was filled about one third full with amalgamated (1% mercury) mossy zinc, about 500 ml. of a 0.1000 *M* chromic sulfate solution in 1 *N* sulfuric acid were added, and the solution was stored under a slight pressure of hydrogen from a Kipp generator. Complete reduction to chromous ion is obtained after several hours if the flask is shaken frequently to promote contact of the solution with the zinc; a longer time is required if shaking is omitted, or if the volume of solution is large compared to the volume (surface area) of the zinc. Solutions thus prepared are stable for about 3 weeks. On longer standing, the slow reduction of hydrogen ion by the amalgamated zinc finally raises the pH to the point at which hydrolytic precipitation of chromous ion occurs (*8*).

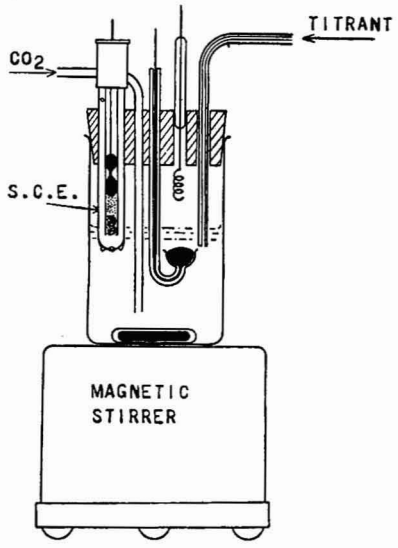

Figure 1. Titration Cell with Mercury Indicator Electrode in Position

The titration vessel is shown in Figure 1. It consists of a 200-ml. tall-form electrolytic beaker, closed by a rubber stopper carrying a gas inlet tube, a saturated calomel reference electrode of the type described by Perley (*10*), the indicator electrode, a hole for introduction of the dispensing tip of the motor-driven hypodermic syringe buret, and a gas outlet hole. The very convenient Perley calomel reference electrode is obtainable from the Leeds & Northrup Co. (Catalog No. 1199-31). A magnetic stirrer was employed (A. H. Thomas Co., Philadelphia, Catalog No. 9235-R). Carbon dioxide, freed from traces of oxygen by passage through a wash bottle containing chromous sulfate solution, was bubbled through the solution for about 10 minutes before and also during a titration, to remove oxygen. All titrations were performed at room temperature.

The delivery tip of the syringe was placed beneath the surface of the solution on a level with the indicator electrode, and about 1 cm. ahead of it with respect to the direction of stirring. The proper relative positioning of the delivery tip and indicator electrode has been discussed in detail (*7*).

The platinum indicator electrode was a small helix of bright platinum wire. The mercury indicator electrode (shown in Figure 1) was held in a shallow cup blown on the short end of J-shaped glass capillary tube. Sufficient mercury is placed in the cup so that the center of the globule is slightly above the cup's rim, and electrical contact is made by a platinum wire inserted down through the stem. To ensure that the indicator electrode will properly anticipate the end point, it should be placed so that the titrant flowing from the delivery tip quickly reaches it.

The initial volume of the solution was adjusted to 100 ml., and the titrations were performed at room temperature.

The previous paper (*7*) should be consulted for a description of the electrical circuit, the technique of recording the titration curves, and other operational details of the autotitrator.

A stock solution of titanic sulfate was prepared in 3.6 *N* sulfuric acid by tenfold dilution of LaMotte 20% titanous sulfate solution. Oxygen was bubbled slowly through the solution for 24 hours to oxidize the titanium completely to the +4 state. In contrast to the very rapid oxidation of chromous ion by oxygen, the corresponding reaction with titanous ion is remarkably slow. Qualitative tests showed that the oxidized solution contained only a negligible trace of iron. The solution was standardized by reducing portions of it with amalgamated zinc in a Jones type reductor in the usual way (*4*). The reduced solution was caught under carbon dioxide in an excess of ferric alum solution, and the resulting ferrous ion was finally titrated with standard ceric solution using *o*-phenanthroline ferrous ion as indicator. The solution was thus found to be 0.1057 ± 0.0002 *M* in respect to Ti(IV).

A standard 0.09000 *M* solution of ferric ion was prepared determinately by dissolving 5.032 grams of Bureau of Standards Iron No. 55b (99.87% iron) in dilute sulfuric acid, oxidizing with hydrogen peroxide (the excess of which was destroyed by prolonged boiling), and finally diluting to exactly 1 liter. A standard 0.02000 *M* solution of ferric chloride in 0.05 *N* hydrochloric acid was prepared similarly.

RESULTS AND DISCUSSION

Titration of Ferric Ion. Figure 2 shows typical automatically recorded curves obtained in the titration of 100 ml. of 0.02 *M* ferric ion in 1.8 *N* sulfuric acid with 0.1 *M* chromous ion. A platinum indicator electrode was used, and the potential values are referred to the saturated calomel electrode. Because the reaction between ferric ion and chromous ion is rapid, the indicator electrode was placed very close to the delivery tip from the syringe. The titrator was set to deliver the chromous solution at a rate of 1.721 ml. per minute.

Curve 1 is a complete curve for the titration, and its charac-

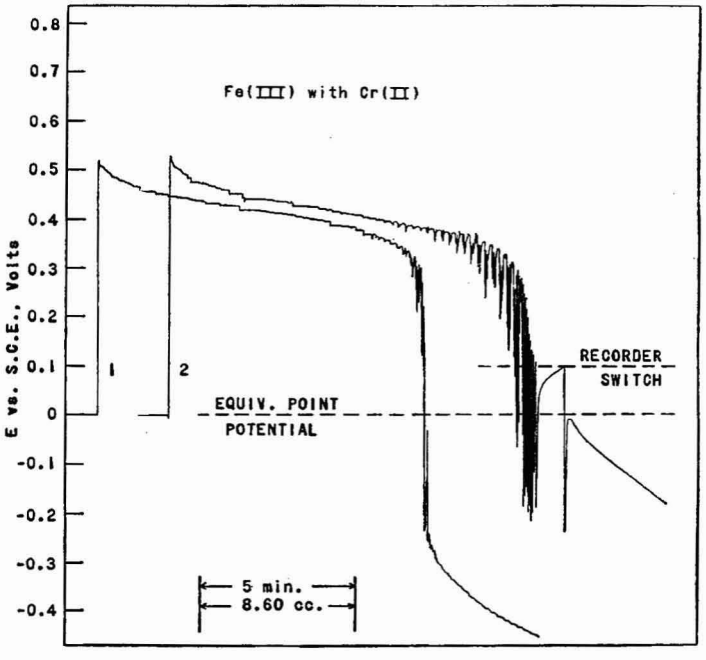

Figure 2. Automatic Titration of Ferric Ion with Chromous Ion

In dilute sulfuric acid with platinum indicator electrode

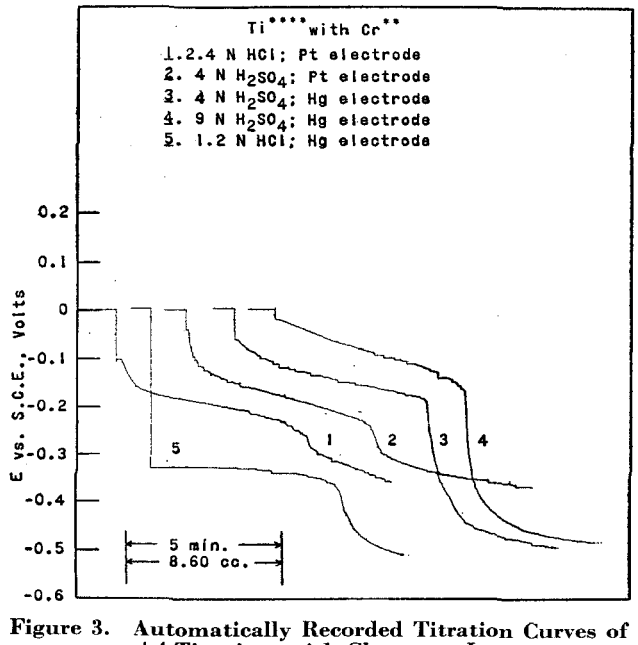

Figure 3. Automatically Recorded Titration Curves of +4 Titanium with Chromous Ion

1. Platinum electrode in 2.4 *N* hydrochloric acid. 2. Platinum electrode in 4 *N* sulfuric acid. 3. Mercury electrode in 4 *N* sulfuric acid. 4. Mercury electrode in 9 *N* sulfuric acid. 5. Mercury electrode in 1.2 *N* hydrochloric acid

teristics agree closely with curves obtained by the conventional manual method (*8*), as well as with the theoretical curves based on the standard potentials of the half-reactions involved. The experimental equivalence point potential is 0.00 volt *vs.* saturated calomel electrode, in good agreement with the theoretical value, −0.05 volt, based on the half-reaction potentials

$$Fe^{+++} + e = Fe^{++};\ E^\circ = +0.53 \text{ volt } vs. \text{ S.C.E.}$$
$$Cr^{+++} + e = Cr^{++};\ E^\circ = -0.64 \text{ volt } vs. \text{ S.C.E.}$$

Curve 2 was obtained by setting the mercury switch in the potentiometer recorder to open the syringe motor circuit at +0.10 volt *vs.* saturated calomel electrode and thus stop delivery of the titrant. By setting the recorder switch slightly ahead of the equivalence point potential overrunning is prevented (*7*). About a dozen small increments of the chromous solution were added in the approach to the final end point; this tentative approach to the end point is a criterion of optimum functioning of the autotitrator. The last increment added corresponded to only 0.02 ml., and it produced a change of potential of about 0.3 volt. The upward potential drift before the last increment was added shows that the end point was not quite reached, and the slow downward drift after the last increment was delivered indicates that the end point was very slightly exceeded. It is evident that the true equivalent volume lies between the last and next to the last increment, and hence the uncertainty is not greater than ±0.01 ml.

In three titrations of 100 ml. of 0.02000 *M* ferric ion the observed counter readings were 3260, 3250, and 3258, corresponding to 19.97 ± 0.02 ml., and in excellent agreement with the theoretical 20.00 ml.

Titration of +4 Titanium. The automatically recorded curves in Figure 3 were obtained in titrations of 10-ml. portions of 0.1057 *M* titanic sulfate solution, in an initial volume of 100 ml., under various conditions at room temperature. The 0.1000 *M* chromous sulfate solution was added at a rate of 1.721 ml. per minute.

Curve 1 was obtained using a platinum indicator electrode and with a solution that was 2.4 *N* in respect to hydrochloric acid; conditions which closely approximate the procedure recommended by Brintzinger and Schieferdecker (*1*). The potential change at the end point is unsatisfactorily small, and the potential values beyond the end point are much larger (more oxidizing) than corresponds to the true potential of the chromic-chromous couple. Curve 2 was also obtained with the platinum indicator electrode but with a solution containing 4 *N* sulfuric acid instead of hydrochloric acid. The potential change at the end point is somewhat more pronounced than in the presence of chloride ion, but it is still small, and the potential values beyond the end point are as abnormally high as in the presence of hydrochloric acid.

Curves 3 and 4 were both obtained with the mercury indicator electrode in sulfuric acid media. For curve 3 the concentration of sulfuric acid was 4 *N* and for curve 4 it was 9 *N*. With the mercury indicator electrode the end point is well marked by a large potential change, and the potentials beyond the end point correspond to the true potential of the chromic-chromous couple.

The unsatisfactory behavior of the platinum electrode arises from the fact that when the oxidation potential of the solution is below the potential of the hydrogen–hydrogen ion couple (−0.24 volt *vs.* the saturated calomel electrode at 1 *M* hydrogen ion concentration) the reaction $2Cr^{++} + 2H^+ = 2Cr^{+++} + H_2$ occurs at the platinum surface. Consequently the actual ratio $(Cr^{++})/(Cr^{+++})$ at the electrode surface becomes much smaller than the ratio in the body of the solution, and the observed potential is correspondingly too oxidizing. In other words, the platinum electrode functions partly as a hydrogen electrode, and the observed potential lies between the potentials of $H_2 - H^+$ and $Cr^{++} - Cr^{+++}$ couples. Because of the large hydrogen overvoltage on mercury no appreciable reduction of hydrogen ion by chromous ion takes place on a mercury electrode, and the latter thus measures the true oxidation potential of the solution. This principle was clearly recognized thirty years ago by Forbes and Richter (*3*), to whom we are indebted for the first reliable measurement of the chromous-chromic potential.

Curve 5 in Figure 3 was obtained with the mercury indicator electrode in a solution that was 1.2 *N* in respect to hydrochloric acid. The fact that the potential up to the end point is much more negative than in sulfuric acid medium is not due to the mercury electrode's functioning as a calomel electrode, because the potential of the half-reaction $Hg_2Cl_2 + 2e = 2Hg + 2\ Cl^-$ in 1.2 *N* hydrochloric acid is slightly positive against the saturated calomel electrode. Furthermore, the surface of the mercury electrode was perfectly bright, whereas the slightest oxidation of the mercury invariably produces a characteristic visible film of calomel. It is reasonable to conclude that the potential values in curve 5 before the end point correspond to the true potential of the titanic-titanous couple, and this potential is much more negative than in sulfuric acid solution because +4 titanium forms a fairly stable chloro complex ion, whereas there is much less complex formation with +3 titanium.

Comparison of curves 1 and 5 shows that a platinum electrode indicates erroneously high (too oxidizing) potentials in a titanic-titanous solution containing a large concentration of hydrochloric acid, doubtless because the titanic-titanous couple in a chloride solution is strongly enough reducing to reduce hydrogen ion on the platinum surface, and thus the platinum electrode functions partially as a hydrogen electrode.

In sulfuric acid solution +4 titanium exists predominantly as titanyl ion, TiO^{++}, and the half-reaction for its reduction is probably

$$TiO^{++} + 2H^+ + e = Ti^{+++} + H_2O$$

From the measurements of Diethelm and Foerster (*2*), and other data quoted by Latimer (*6*), the standard potential of this reaction is close to −0.15 volt *vs.* saturated calomel electrode. The potentials at the half-titrated point in curves 3 and 4 agree well with this value. Because hydrogen ion is a reactant, the potential becomes more oxidizing with increasing hydrogen ion concentration (compare curves 3 and 4 in Figure 3). Kolthoff (*5*) reported that the potential of the titanic-titanous couple

decreases by 61 mv. per unit increase in pH up to about pH = 5, which would correspond to only one hydrogen ion per titanyl ion, but as the measurements were made with a platinum electrode, it is difficult to appraise their significance.

In the simultaneous presence of a large concentration of both hydrogen ion and chloride ion titanyl ion is converted to a chloro complex, probably according to

$$TiO^{++} + 2H^+ + 6Cl^- = TiCl_6^{--} + H_2O$$

From curve 5 in Figure 3 the standard potential of the couple

$$TiCl_6^{--} + e = Ti^{+++} + 6Cl^-$$

is in the neighborhood of −0.33 volt *vs.* the saturated calomel electrode, and thus about 0.18 volt more negative than the reduction potential of titanyl ion.

It is evident that the optimum conditions for the titration of Ti(IV) with chromous ion are the use of a mercury indicator electrode and sulfuric acid solutions. Under these conditions the potential becomes constant immediately after each addition of chromous ion, except right at the end point where 2 to 3 minutes are required, and hence the titration can be completed quickly. By manual titration the equivalence point potential (maximal value of $\Delta E/\Delta V$) was found to be −0.36 ± 0.01 volt *vs.* the saturated calomel electrode in 4 *N* sulfuric acid. The characteristics of the automatically recorded curves agree very well with curves obtained by manual titration (see Figure 4), which reflects the rapid establishment of potential equilibrium with the mercury indicator electrode.

In Figure 4, curve 1 shows the curve of a typical automatic titration of 10 ml. of 0.1057 *M* titanic sulfate in 100 ml. of 4 *N* sulfuric acid with the recorder switch set to stop the addition of the chromous solution at −0.30 volt. Curve 2 is the recorded curve of the entire titration, and curve 3 was obtained by manual titration. In recording these curves the sensitivity of the recording potentiometer was twice as great as for the curves in Figure 3. The fluctuations in potential near the end point are due to variations in the flow of solution over the surface of the mercury indicator electrode and they have no fundamental significance. The incremental addition of chromous solution as the end point was closely approached is clearly shown in curve 1; the last increment amounted to 0.036 ml. The final potential after the titrator stopped is −0.32 volt, which is very close to the true equivalence point potential and indicates that −0.30 volt is an optimum setting of the recorder switch.

In three titrations of 10-ml. portions of the titanic sulfate solution under the same conditions as curve 1 of Figure 4, the observed counter readings were 1717, 1720, and 1719, corresponding to 10.54 ± 0.02 ml. of the 0.1000 *M* chromous sulfate solution, in very good agreement with the theoretical 10.57 ml.

Four titrations of 24.98-ml. portions of the titanic sulfate solution yielded counter readings of 4303, 4319, 4314, and 4315, corresponding to 26.45 ± 0.03 ml. The theoretical equivalent volume is 26.40 ml. These results demonstrate that the automatic titrations are precise and accurate to about ±0.2%, in spite of the fact that the rate of potential change at the end point is relatively small.

Mixtures of Iron and Titanium. The fact that the standard potential of the ferric-ferrous couple is about 0.6 volt greater than that of the titanic-titanous couple renders easy the simultaneous determination of iron and titanium by a single titration with chromous ion in sulfuric acid solutions. These two elements are so commonly associated that such a determination has considerable practical importance.

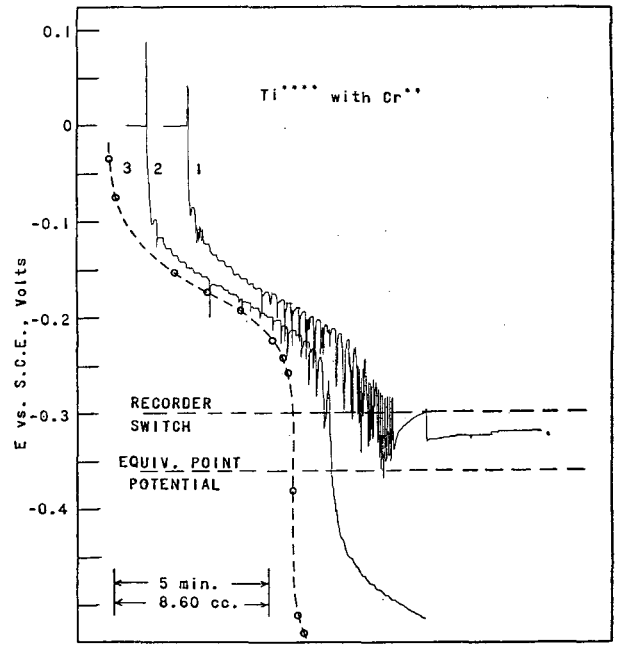

Figure 4. Automatic Titration of +4 Titanium with Chromous Ion in 4 *N* Sulfuric Acid with Mercury Indicator Electrode

Dashed curve obtained by manual titration

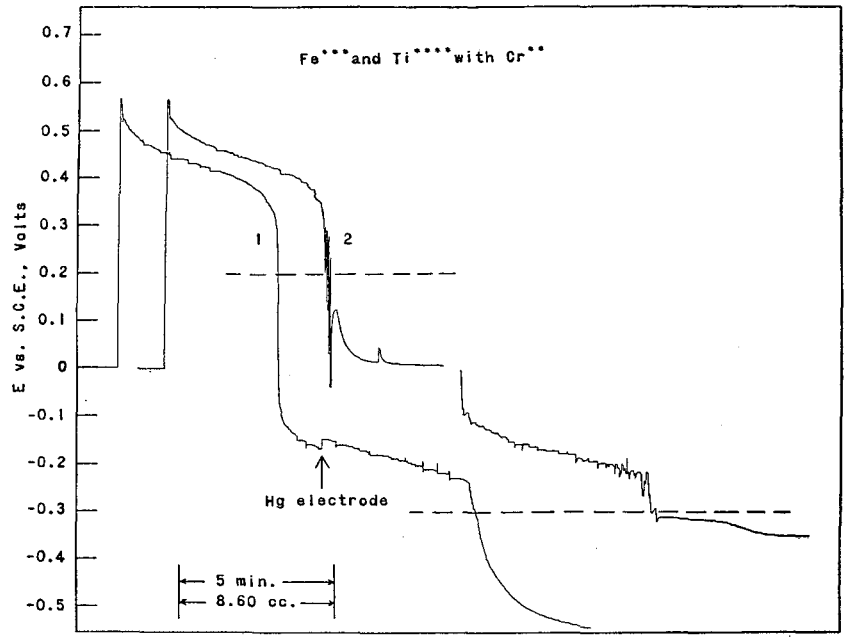

Figure 5. Automatic Titration of Mixture of Ferric Ion and +4 Titanium with Chromous Ion in 4 *N* Sulfuric Acid

Platinum indicator electrode used for titration of iron and mercury electrode employed for subsequent titration of titanium. Horizontal dashed lines indicate settings of recorder switch

A mercury indicator electrode cannot be used in solutions whose oxidation potential is greater (more oxidizing) than that of the mercury-mercurous couple, as otherwise the mercury itself will be oxidized by the oxidant being titrated and the observed potential will be that of the mercury-mercurous couple rather than the true potential of the oxidant-reductant system. In sulfuric acid media the pertinent mercury couple is

$$Hg_2SO_4 + 2e = 2Hg + SO_4^{--};\ E^\circ = +0.373 \text{ volt } vs. \text{ S.C.E.}$$

and in chloride media it is

$$Hg_2Cl_2 + 2e = 2Hg + 2Cl^-;\ E^\circ = +0.026 \text{ volt } vs. \text{ S.C.E.}$$

In practice the upper limit for the use of the mercury electrode is about +0.2 volt in sulfuric acid solutions, and about −0.1 volt in chloride solutions. As the standard potential of the ferric-ferrous couple is +0.53 volt *vs.* saturated calomel electrode titration of iron-titanium mixtures must be performed by using a platinum electrode until the end point for the reduction of the ferric ion has been reached, or passed, and then introducing a mercury indicator electrode for the titration of the titanium.

Curve 1 in Figure 5 is a typical automatic recording of the titration of a solution containing 10 ml. each of 0.09 *M* ferric sulfate and 0.1057 *M* titanic sulfate in 100 ml. of 4 *N* sulfuric acid at room temperature. A platinum indicator electrode was used until the iron end point was slightly passed. At the point indicated by the arrow the titrator was stopped, the mercury electrode was placed in the solution, and the titration was then allowed to continue through the titanium end point.

The electrodes were held in loose holes in the rubber stopper of the titration cell, so that they could be immersed or withdrawn from the solution without dismantling the cell.

Curve 2 in Figure 5 was obtained with the same mixture as curve 1. The recorder switch was first set at +0.20 volt for the titration of the ferric ion. After the titrator had stopped, and the counter reading had been noted, the mercury electrode was introduced (break in the curve), the recorder switch was reset to −0.30 volt, and the titration of the titanium was allowed to proceed.

The titration of the iron required 9.00 ml. of the 0.1000 *M* chromous solution, in exact agreement with theory, and the titration of the titanium required 10.70 ml. compared to the theoretical 10.57 ml. Because the sensitivity of the recorder was decreased to obtain both parts of the titration curve on the same voltage scale, with consequent impairment of the sensitivity of the recorder switch at the relatively small titanium end point, this result does not represent the best obtainable precision in the titanium stage of the titration. A more precise titration of the titanium can be achieved by increasing the sensitivity of the recorder after the iron has been titrated.

It is evident that a small amount of iron can be determined easily in the presence of large amounts of titanium. For the determination of a small amount of titanium in the presence of large quantities of iron, the ferric ion should first be reduced to the ferrous state by sulfur dioxide, and after the excess of the latter has been boiled out, the Ti(IV) may be titrated.

The electronic trigger circuit described by Müller and Lingane (*9*) may be used in these and other automatic titrations in place of the more expensive recording potentiometer, after the characteristics of a titration curve have been established.

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Determination of Trace Impurities in Reference Fuel Grade Iso-octane

By Infrared Absorption Spectroscopy

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A procedure has been developed for using infrared absorption spectroscopy to determine trace quantities of impurities present in reference fuel grade iso-octane of 99.5% purity prepared commercially by continuous fractional distillation of butylene alkylate. The method, which has a time requirement of 2 hours, is useful for fractionating tower control purposes in plant operation for the determination of the relative content of the less volatile and more volatile impurities present with the iso-octane product. The method is accurate to ±0.1% for the total concentrations of low boiling impurities, high boiling impurities, and total impurities.

THE increasing commercial production of pure compounds from petroleum has accentuated the need for precise, yet rapid, control analyses of the finished product. In most cases the product must conform by test to rigorous specifications designed to ensure the quality and purity of the product placed on the commercial market. Examples of products of this type are butadiene for the manufacture of Buna rubber, isobutylene for the manufacture of Butyl rubber, and nitration grade toluene for the manufacture of TNT. In the usual case, these high purity products contain impurities in concentrations of only 0.1 to 2.0%, yet analytical methods must be available for constant check on the concentration of these undesired components.

A problem of this type is encountered in the commercial production of iso-octane of reference fuel grade from commercial butylene alkylate by continuous fractional distillation. In this operation, a freezing point specification is usually imposed upon

tank car shipments of the product. During 1946, the specification was set at a freezing point of −107.49° C., which, based on a freezing point of −107.365° C. for pure 2,2,4-trimethylpentane, corresponds to a purity of 99.50%. (A value of −107.31° C. has also been quoted in the literature for the freezing point of iso-octane; on this basis a freezing point of −107.49° C. corresponds to a purity of 99.25% for iso-octane.) The feed stock to the fractional distillation operations contains approximately 20 components, and the concentration of 2,2,4-trimethylpentane contained therein is of the order of only 18 to 25%. In the separation of a reference fuel grade iso-octane of minimum 99.50% purity, it is necessary to use a minimum of two distillation steps to separate low boiling and high boiling components of the feed from the desired 2,2,4-trimethylpentane. For efficient fractionating column control, it is therefore necessary to know the concentration of low boiling and high boiling impurities in the final product. The specification freezing point test, in addition to being unsatisfactory for control analyses because of the excessive time requirements, can be used only for the determination of total impurities. For this reason, an infrared procedure was developed for the rapid control analysis of reference fuel product for small amounts of low and high boiling impurities.

During the past several years, there have been frequent references in the literature (*1*, *2*, *4*, *7–10*) to methods and techniques employed for the quantitative multicomponent analysis of liquid mixtures by the application of infrared spectroscopy. These methods are nearly always applied, however, to the analysis of mixtures wherein many of the components are present in concentrations of several per cent or greater, and an individual component present in concentrations of 0.2 to 2.0% is considered a minor component in the sample analyzed directly. There are, of course, many references to the use of infrared spectroscopy for the determination of trace impurities, and the advantages of the infrared method for this purpose are well known; however, in these cases, the mixture analyzed has usually not contained more than two or three components for which analysis is desired (*12*). Commercial reference fuel grade iso-octane may contain as many as eight impurity components, totaling 0.50% or less, and determination of these individual components by application of infrared spectroscopy presents a somewhat more difficult problem.

Mixtures of this type have been analyzed successfully by a combination of recognized techniques in infrared spectroscopy, together with careful control of instrumentation. The accuracy of the method is indicated to be of the order of ±0.1% on the high boiling and low boiling impurities, and on the total impurities. Time requirements for the analysis, including calculations, are about 2 hours.

APPARATUS AND EXPERIMENTAL TECHNIQUES

The work described in this article has been carried out on two different infrared instruments. One of these was a Perkin-Elmer Model 12-A equipped with a sodium chloride prism, adapted for the scanning of spectra with a wave-length drive powered with a constant speed motor and equipped with a photoelectric galvanometer amplifier of the type described by McAlister, Matheson, and Sweeney (*9*). This instrument was used for the development of the analysis and most of the work described herein is based on the use of this instrument. Upon reduction of the analysis to routine practice, the control analytical work was performed in the service laboratories using a Beckman infrared spectrophotometer (Model IR 108), manufactured by the National Technical Laboratories, also equipped with a sodium chloride prism, and operated manually with galvanometric measurement of thermocouple signals.

It was found in the early phases of the investigation that control of the source intensity was one of the most important features in the analysis. Short-period requirements for source stability were necessarily of the order of 0.1% of full scale deflection in order to assure accuracy of relative optical density measurements of ±0.001 to ±0.002.

Source stability of this order was approached by the use of a 500-watt Sola voltage regulator, equipped with a 300-watt ballast load, with 200 watts of energy supplied to the Globar. Each measured thermocouple signal at a given spectral position was followed directly by a reference measurement on a rock salt plate with a minimum elapsed time of 5 to 10 seconds. In this manner, all intensity measurements were directly measured against reference intensity, and errors due to fluctuation in the source, or in the measuring, amplification, and recording system were minimized.

Each instrument was housed in an air-conditioned room, and temperature control was maintained to ±1° F. Under these conditions, drift was not a problem. Short-period noises arising in vibrational effects in the galvanometer assembly were variable but were usually of the order of ±0.1% of full scale. Fortunately, the vibrational effect recorded was of a regular periodic character, and it was possible, therefore, to average the fluctuations with an estimated error from this source of somewhat less than ±0.1% of full scale.

Conditions of amplification were adjusted so that the thermocouple signal required for full scale on the 0–10 millivolt Brown Electronik recorder was of the order of 0.5 microvolt. At two spectral positions where measurements of relative optical densities were somewhat more critical, increased accuracy was obtained by using wider slit widths for the measurements of radiant intensity (I) transmitted by the sample and the reference standard than were used for measurements of the incident intensity (I_0), transmitted by the rock salt plate. At 9.87 and 9.95 microns, the slit width used for I measurements was increased for both the reference standard and the sample, so that the I value was increased to approximately three times the value that would have been obtained without change of slit width. In order to calculate the I values that would have been obtained with normal slit width, the observed I values were divided by the ratio of the increased I signal to the normal I signal obtained for the reference standard. This ratio for each of the two wave lengths was obtained from average of a number of determinations on the reference standard.

Sample cells employed for the work were of conventional design, and consisted simply of rock salt plates separated by lead gaskets 1 mm. thick. As all measurements were made in the same sample cell, and calculations were based on differential absorption, a slight amount of fogging of the sample cell windows was not a problem.

Fluctuations of wave-length calibration arising from ±1° F. temperature changes, although detectable, were not a limiting factor in the analysis. During certain periods of the day when outside temperature changed rapidly, a small but noticeable effect on wave-length calibration was observed. Because the major effect of such fluctuations was to change the interfering effect of the 2,2,4-trimethylpentane, this effect was minimized by making reference measurements against a standard sample of reference fuel grade iso-octane at the wave-length drum position at which the given intensity was measured.

Table I. Impurities in Commercial Alkylate

Compound	Boiling Point, ° F.	% of Total Alkylate	Impurity, % of Component Listed
2,3-Dimethylpentane	193.6	2.6	8.1
2-Methylhexane	194.2	0.4	1.2
3-Methylhexane	197.6	0.3	0.9
2,2,4-Trimethylpentane	210.6	18.3	...
2,5-Dimethylhexane	228.4	3.4	10.5
2,4-Dimethylhexane	228.9	3.7	11.5
2,2,3-Trimethylpentane	229.6	1.2	3.7
2,3,4-Trimethylpentane	236.3	11.0	34.1
2,3,3-Trimethylpentane	238.6	9.7	30.0

DEVELOPMENT OF THE METHOD

At the outset of the work, it was apparent that base-line methods (such as those described by Wright, *12* and others, *7*) of detection of individual impurities would not be satisfactory for detection of these minor components because of the weakness of the bands due to these impurities. In fact, scanning of representative samples of reference fuel grade iso-octane shows no absorption bands at the wave-length position corresponding to absorption bands of the impurities, so that qualitative analysis by identification of bands is a virtual impossibility. Furthermore, attempts to increase the intensity of these bands by increasing

sample cell thickness increase background absorption due to the 2,2,4-trimethylpentane at a faster rate than it accentuates the band due to the impurity, so that complete extinction is reached before the absorption band can be detected. There is, however, at each band position a small but definite increment of absorption which cannot be explained by 2,2,4-trimethylpentane. The multicomponent analysis described herein is based on such differences, measured between the actual sample and pure 2,2,4-trimethylpentane. A 1-mm. sample cell was chosen for optimum balance between magnitude of partial optical density due to the impurities and extent of absorption by 2,2,4-trimethylpentane.

Because the impurity components present in the reference fuel grade iso-octane could not be detected by qualitative scanning of the spectrum of the sample for location of bands due to the impurities, an alternative procedure was chosen. Analyses of commercial butylene alkylate have been available in this laboratory for some time and the major impurity components expected in iso-octane prepared from butylene alkylate can therefore be estimated. Data on commercial alkylate composition have been published recently by Heigl, Bell, and White (*7*). For purposes of illustration, Table I shows the relative abundance of various impurity components of commercial alkylate boiling as low as 193.6° F. (2,3-dimethylpentane), and as high as 238.6° F. (2,3,3-trimethylpentane).

Although the distribution of impurities given in Table I is not representative of the distribution in actual samples of reference fuel grade iso-octane, it is apparent that determination of all or nearly all these impurity components is necessary in order that the distribution between high and low boiling impurities can be determined by analysis. In this case, no absorption bands for impurities were available where absorption characteristics were similar, and the absorption effects could be combined for a total impurity analysis as described by Brady (*3*) and Seyfried and Hastings (*11*).

Using as a basis the approximate estimated distribution of impurities available from analyses of commercial alkylate and the total concentration of these impurities as set by specification, spectral positions were selected for the analysis with primary emphasis upon minimum absorption by 2,2,4-trimethylpentane, and secondary emphasis upon interference effects of other impurities. Unfortunately, no spectral position that could conceivably be used with desired accuracy for the determination of 2,5-dimethylhexane was found. This component was therefore not included in the analytical scheme; however, the effect of omission of this component has not proved to be serious, and has been indicated to be within the accuracy of the method. Table II lists the spectral positions selected for the analysis of each impurity.

One of the most accurate ways of determining the composition and total amount of impurities in samples consists of determining the differential absorption between pure 2,2,4-trimethylpentane and the unknown sample at each of the spectral positions shown in Table II, substituting these differential optical density values into simultaneous equations written for the absorption of the impurities at the given spectral positions, and calculating the distribution of impurities according to conventional methods of solution of simultaneous equations. Although this is the essence of the method actually employed, certain modifications were necessary for accurate practicable application of the technique.

Insufficient 2,2,4-trimethylpentane of high purity was available for use for direct comparison in each analysis. For this reason, a set of reference standards was adopted. The primary reference was National Bureau of Standards 2,2,4-trimethylpentane (freezing point −107.39° C., calculated purity 99.88%). The secondary reference employed was Rohm & Haas commercial iso-octane, Batch 36 (freezing point −107.47° C., calculated purity 99.58%). The optical density differences between the primary and secondary references were determined, and the secondary reference was used in all subsequent analytical work; corrections for the difference in optical densities of the primary and secondary standards were made for all samples.

Table II. Spectral Positions

Component	Boiling Range Designation (Relative to Iso-octane)	Wave Length, Microns
2,3-Dimethylpentane	Low boiling	9.87
2-Methylhexane	Low boiling	13.70
3-Methylhexane	Low boiling	13.53
2,4-Dimethylhexane	High boiling	13.01
2,2,3-Trimethylpentane	High boiling	9.23
2,3,4-Trimethylpentane	High boiling	9.63
2,3,3-Trimethylpentane	High boiling	9.95

Because of the rather extreme requirements of the accuracy of determination of differential optical densities at each spectral position, and the necessity for carrying out all measurements on sample and reference in the same cell (to avoid errors due to cell differences that would vary with time), it was necessary that a reference measurement be made on a rock salt plate at a given spectral position within 5 to 10 seconds after measurement on the liquid-filled sample cell.

CALIBRATION AND CALCULATION PROCEDURES

The instrument employed for analysis was calibrated by measurement of the optical density differential between 5% blends of each compound and 2,2,4-trimethylpentane, and the results were computed to a 100% basis for the impurity, assuming Beer's law to apply. Incident intensity or I_0 measurements were based on reference measurements with a rock salt plate. Correction was made for the difference in 2,2,4-trimethylpentane concentrations in the two samples, because, to a slight degree, the measured differential is dependent on the 2,2,4-trimethylpentane concentration.

For actual samples, measured optical density differentials between the secondary reference and the sample were computed, using measurements made on the rock salt plate for reference intensity, or I_0 at each spectral position. These optical density differentials were corrected for difference between the primary and secondary references, and then substituted in simultaneous equations written for the differential absorption at each spectral position.

To a first approximation, the optical density difference at any spectral position is of the conventional form

$$\Delta D_1 = C_1K_{11} + C_2K_{21} + \ldots\ldots C_7K_{71} \quad (1)$$

where C_1, C_2, etc., refer to the concentration of impurities, K_{11} is the calibration coefficient for component 1 at spectral position 1, etc., and ΔD_1 refers to the differential optical density of the sample at spectral position 1. These equations may be arranged for solving by the method of successive approximations so that

$$C_1 = \frac{\Delta D_1}{K_{11}} - C_2\frac{K_{21}}{K_{11}} \ldots\ldots C_7\frac{K_{71}}{K_{11}} \quad (2)$$

In actual practice, equations of form 2 were employed.

After substitution of the optical density in the equations of form 2, these equations were solved by successive approximation to yield values for the concentrations of the several impurities. The sum of the impurities was then subtracted from 1.0000 (or 100.00, on a percentage basis), the volume per cent iso-octane determined by difference, correction applied for the minor difference in 2,2,4-trimethylpentane concentration in the actual sample, and the concentration of individual impurities recalculated. In practice, this second step was not necessary for most cases, as the iso-octane concentration never varied more than a few tenths of 1%, and correction for this effect was made in the initial calculation.

Table III. Distribution of Impurities

Composition of Synthetic	Synthetic 1			Synthetic 2		
	Synthesis	Found	Δ	Synthesis	Found	Δ
2,3-Dimethylpentane	0.31	0.32	+0.01	0.09	0.00	−0.09
2-Methylhexane	0.29	0.22	−0.07	0.08	0.05	−0.03
3-Methylhexane	0.32	0.35	+0.03	0.08	0.16	+0.08
2,2,4-Trimethylpentane	98.00	97.94	−0.06	99.42	99.41	−0.01
2,4-Dimethylhexane	0.28	0.35	+0.07	0.08	0.09	+0.01
2,2,3-Trimethylpentane	0.28	0.24	−0.04	0.08	0.05	−0.03
2,3,4-Trimethylpentane	0.26	0.38	+0.12	0.09	0.11	+0.02
2,3,3-Trimethylpentane	0.26	0.20	−0.06	0.08	0.13	+0.05
Low boiling impurities	0.92	0.89	−0.03	0.25	0.21	−0.04
High boiling impurities	1.08	1.17	+0.09	0.33	0.38	+0.05
Total impurities	2.00	2.06	+0.06	0.58	0.59	+0.01

Table IV. Purity Values

Sample No.	Freezing Point, ° C.	Calculated Purity		Δ
		Freezing point	Infrared method	
1	−107.43	99.74	99.65	−0.09
2	−107.53	99.34	99.40	+0.06
3	−107.49	99.50	99.44	−0.06
4	−107.51	99.43	99.50	+0.07
5	−107.48	99.54	99.54	0.00
6	−107.474	99.56	99.30	−0.26
7	−107.475	99.56	99.47	−0.09
8	−107.474	99.56	99.55	−0.01
9	−107.501	99.46	99.50	+0.04
10	−107.492	99.49	99.48	−0.01
11	−107.47	99.58	99.56	−0.02
12	−107.473	99.57	99.47	−0.10
13	−107.51	99.43	99.27	−0.16
14	−107.467	99.60	99.59	−0.01

Average deviation = 0.07 (all data included)

EVALUATION OF THE METHOD

In order to check on the accuracy of the method for determination of the distribution of high and low boiling impurities in reference fuel iso-octane, two synthetic samples were analyzed by the procedure described above (Table III).

Although the results obtained on these synthetic samples are not considered to be the best obtainable with the method, they illustrate the accuracy obtained under routine conditions. The most serious discrepancies noted are for the 2,3-dimethylpentane content of Synthetic 2, and the 2,3,4-trimethylpentane content of Synthetic 1. That these are probably random errors, however, is indicated by the fact that in each case the corresponding analysis for the same component in the other synthetic is satisfactory.

Further data on the accuracy of the method were obtained by checking the purity data by the infrared method with purity data by the freezing point method on actual samples from plant production. Values shown in Table IV for purity data by the infrared method are corrected for the calculated 0.12% impurities present in the National Bureau of Standards primary reference 2,2,4-trimethylpentane.

Two values for the freezing point of pure 2,2,4-trimethylpentane have been published (*5*, *6*), and the actual purity calculated for samples is dependent upon the value employed. A value of −107.365° C. was employed in this study, as calculation of the purity of National Bureau of Standards 2,2,4-trimethylpentane was based on this value. This has little significance with regard to the accuracy of the infrared method, however, because this method is based on measurement of the difference in purity between National Bureau of Standards 2,2,4-trimethylpentane and the actual sample, corrected for the calculated impurities in the National Bureau of Standards 2,2,4-trimethylpentane. Similarly, computed purity values based on freezing points are based on the value of −107.365° C. for the freezing point of 2,2,4-trimethylpentane. If at some later date it should be shown that the value of the freezing point of 2,2,4-trimethylpentane is different from that used in this study, the fact should in no serious way invalidate the results shown herein for total impurities, as all values are based on the same reference. Although a minor distortion of calculated impurity distribution in actual samples is expected, inasmuch as the distribution of impurities in National Bureau of Standards 2,2,4-trimethylpentane is not known, this effect is not considered serious in view of the small amount of total impurities present in this spectroscopic standard.

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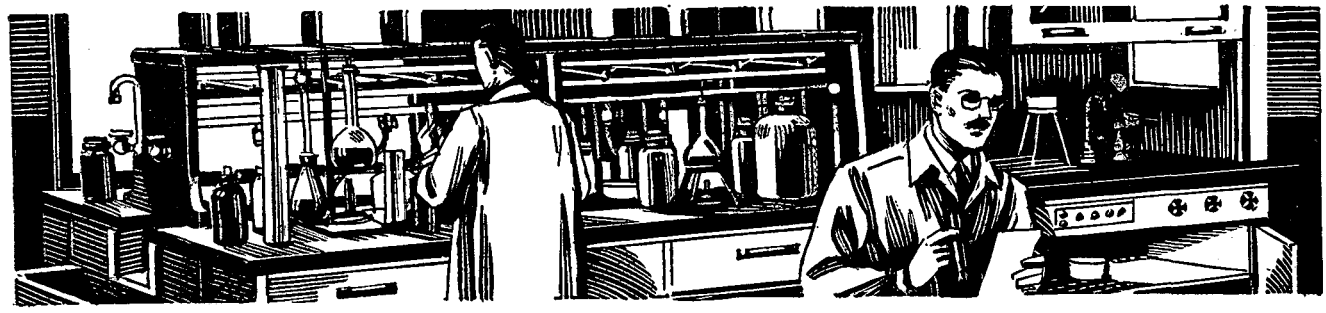

Determination of Olefins in Gasoline

Application of Infrared Spectroscopy

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In many hydrocarbon mixtures it is desirable not only to ascertain the total olefin concentration, but to differentiate among the olefins as to double bond position. This is particularly true in the petroleum industry where the double bond position can be related to gasoline quality. In this paper a method of differentiating alpha-olefins (of the general type R—CH=CH_2 or R_2C=CH_2) from olefins with internal double bonds (of the general type R—CH=CH—R) is discussed. The analytical technique makes use of the characteristic mono-olefin absorption bands in the 9.5 to 11.5-micron region and a silica gel adsorption technique for removing saturate and aromatic interference. Calibration curves are presented for a number of pure olefins at the characteristic wave lengths to demonstrate the absorption similarity of olefins of the same type. The method is applied to a number of refinery products such as catalytically and thermally cracked gasolines and reformed gasoline.

SEVERAL procedures are available for the determination of total olefins in gasolines, and the status of this problem has recently been presented by Kurtz, Mills, Martin, Harvey, and Lipkin (*6*). The value of these data can be increased by differentiation of the olefins as the double bond is shifted toward the center of the molecule. Such information is important in studying improvement in gasoline quality which is reflected in part in an increase in octane rating (*1*).

Infrared spectroscopy has been used for a number of years for the determination of individual olefins (normal, iso, *cis*-, and *trans*-beta) in C_4 hydrocarbon streams (*4*). An extension of the technique to include analysis of higher boiling olefins would provide information hitherto difficult to obtain on olefin types in gasolines. In this extension the determination of each individual olefin would not be required, as the olefin contribution to gasoline quality (aside from the skeletal structure of the molecule) is determined chiefly by the position of the double bond.

Rasmussen and Brattain (*8*, *9*) have tabulated strong band positions for various olefins in the region 10.0 to 12.5 microns. These data for individual mono-olefins indicate a characteristic absorption doublet for alpha-olefins (RCH=CH_2, where R = an alkyl group) at 10.0 and 10.9 microns and a single absorption band near 10.3 microns for olefins with internal double bonds—e.g., RCH=CHR. In the case of alpha-olefins with two alkyl groups on the beta-carbon (R_2C=CH_2) there is a characteristic band at approximately 11.2 microns. For the purposes of this paper it is evident that the size and structure of R cannot be specified in the application of the method to mixtures of olefins such as those in full boiling range (40° to 200° C.) cracked or reformed gasolines. In such mixtures R can be such that the indicated double bond position refers to alkenes, naphthenyl alkenes, or aryl alkenes. It is the purpose of this paper to show that the above general differentiation can be made for synthetic and practical mixtures with a rough correspondence with total olefin content and, in the case of gasolines that have undergone catalytic treatment, a correlation with changes in octane number.

In order to determine whether this differentiation in olefin type absorption exists throughout the gasoline boiling range, infrared spectra were determined for an olefin concentrate from a thermally cracked gasoline, and for distillation cuts therefrom. The concentrate was prepared by a silica gel adsorption technique (*7*) at ice temperature with methyl alcohol as the desorption agent. Unpublished data in these laboratories indicate that there is little, if any, change in the olefin skeletal structure or double bond position upon separation of olefins from other hydrocarbon types by silica gel adsorption at low temperatures. The fraction desorbed from the gel between the saturates and aromatics, as based on bromine number and refractive indices, was called the olefin concentrate. About 85% of the olefins present were recovered. Although it is possible that some olefins are selectively removed in this treatment, it is believed on the basis of experience with other hydrocarbon mixtures that the ratio of olefin types remains essentially unchanged. Table I shows the data on the concentrate preparation.

The olefin concentrate was fractionated in a column of 25 theoretical plates at a 10 to 1 reflux ratio. The distillation cuts were blended as shown in Table II, so that each blend would represent olefins of about the same molecular weight. Infrared spectra of the concentrate and several of the respective blends are shown in Figures 1 to 4. The positions of the absorption bands in the 9.5 to 11.5-micron region indicate that the absorptions fit the tabulated types listed above. Comparison of the individual spectra shows that the strong band locations at 10.0, 10.25, 10.9, and 11.2 microns are similar for the concentrate and the distillation blends. Therefore, for olefins from thermally cracked gasoline the infrared absorption in the 9.5- to 11.5-micron region appears to depend substantially only on the position of the double bond. Thus, a quantitative comparison of the relative concentrations of the

Table I. Silica Gel Separation of a Thermally Cracked Gasoline

Received, Ml.	n_D^{20}	Remarks
11–16	1.4019	Discarded saturate portion
16–18	1.4084	
18–20	1.4099	
20–22	1.4128	
22–24	1.4171	Olefin concentrate blend
24–26	1.4183	
26–28	1.4209	
28–30	1.4229	
30–35	1.4259	
35–40	1.4451	Aromatic concentrate blend
40–42	1.4857	
42–44	1.4951	
44–46	1.4129	Discarded sulfur, aromatics, alcohol

Table II. Properties of Distillation Blends from Olefin Concentrate of Table I

	Bromine No.	d_4^{20}	n_D^{20}	Sulfur, % by Wt.	B.P., °C.
Cuts 2–4	130	0.6825	1.3910	0.004	37–71
Cuts 5–9	131	0.7203	1.4079	0.004	71–99
Cuts 10–16	117	0.7473	1.4209	0.004	99–127
Cuts 17–20	120	0.7666	1.4310	0.009	127–149
Cuts 21–26	101	0.7750	1.4354	0.010	149–171
Residue	73.9	...	...	0.062	171–195

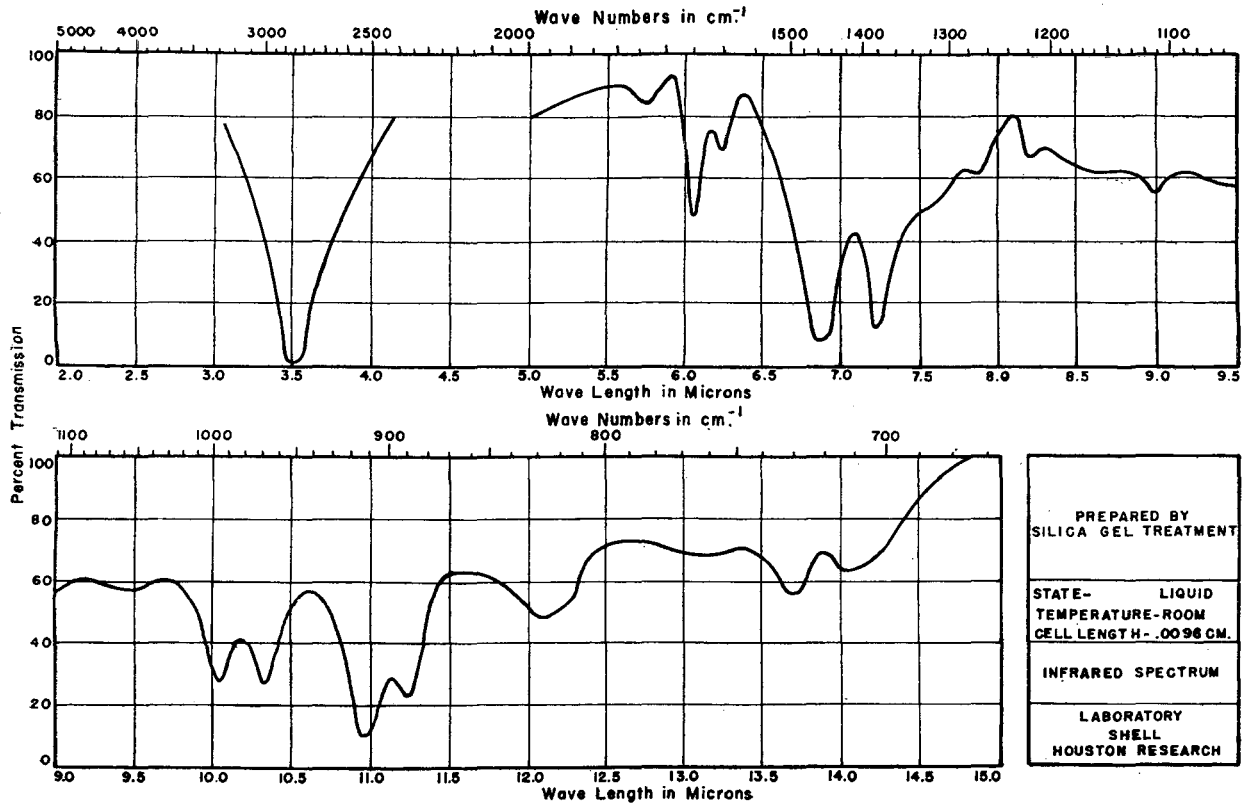

Figure 1. Infrared Spectrum of Olefin Concentrate from Thermally Cracked Gasoline

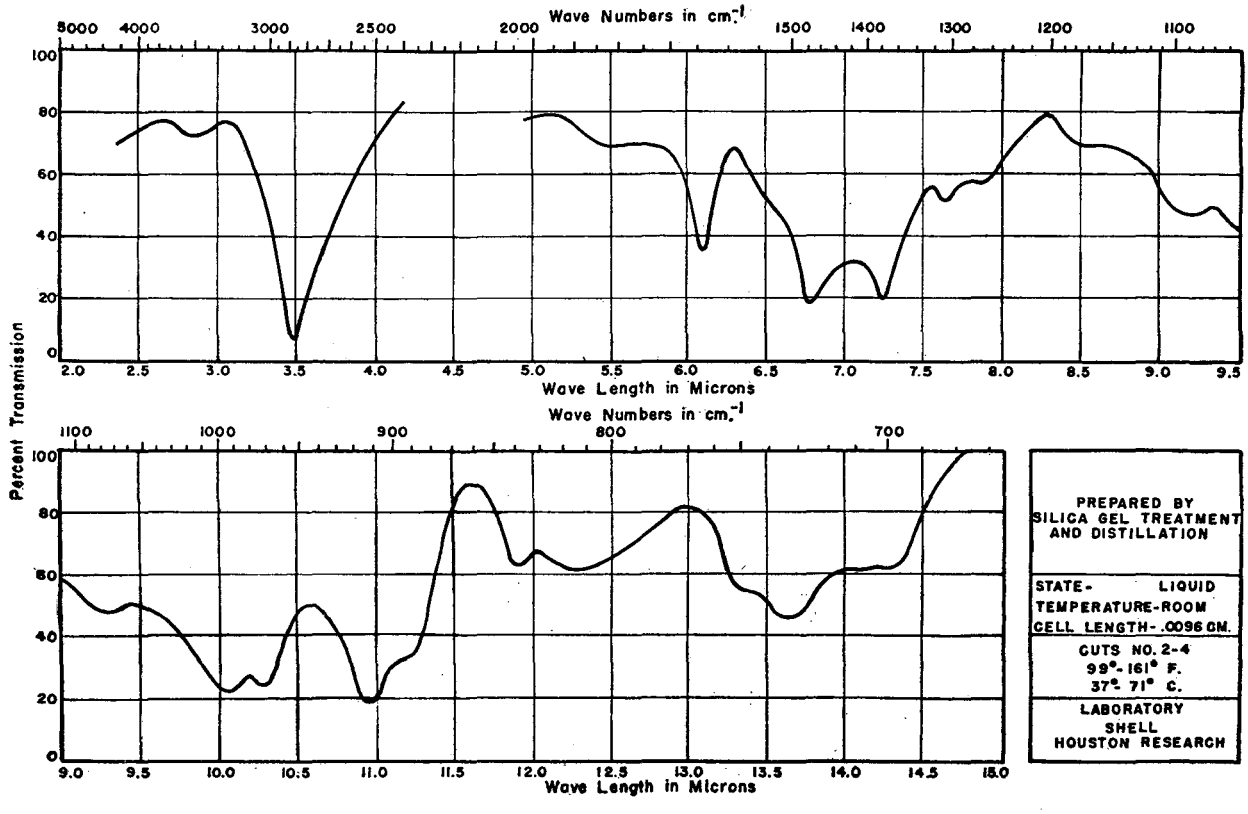

Figure 2. Infrared Spectrum of Olefin Fraction from Thermally Cracked Gasoline

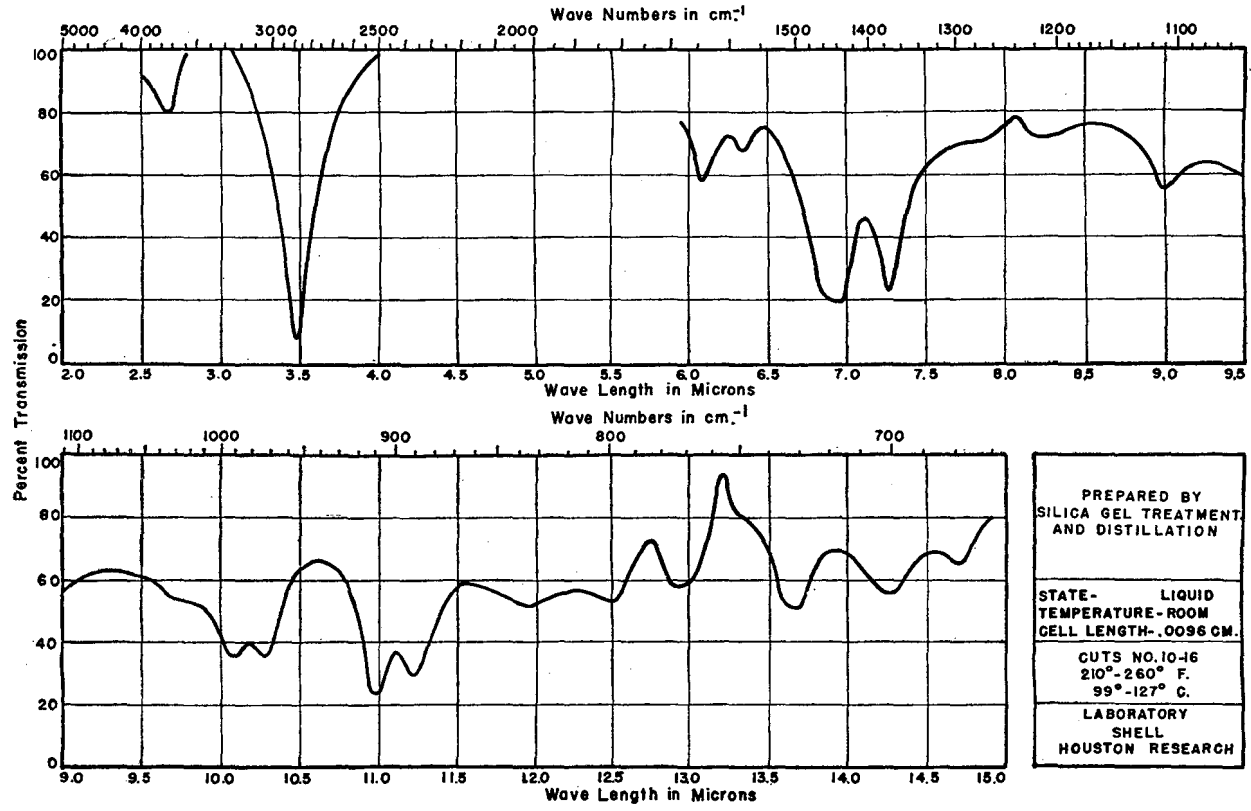

Figure 3. Infrared Spectrum of Olefin Fraction from Thermally Cracked Gasoline

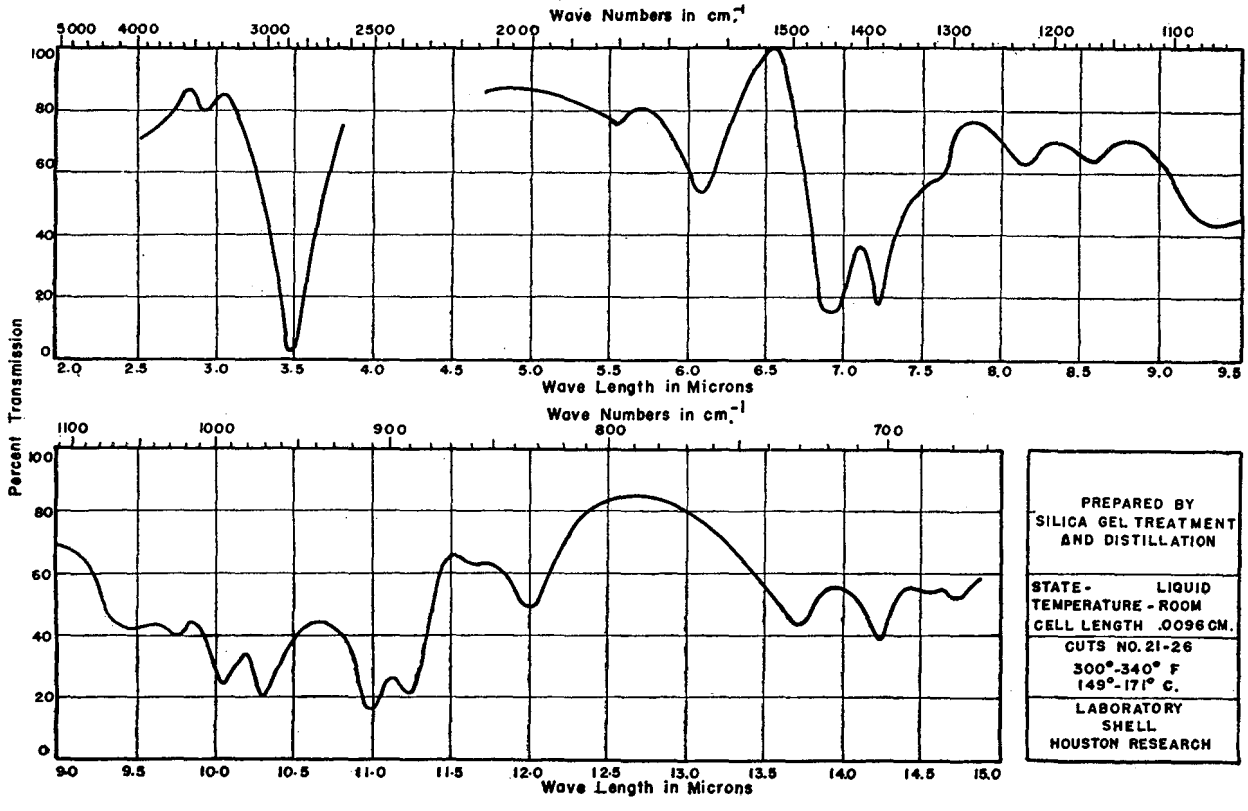

Figure 4. Infrared Spectrum of Olefin Fraction from Thermally Cracked Gasoline

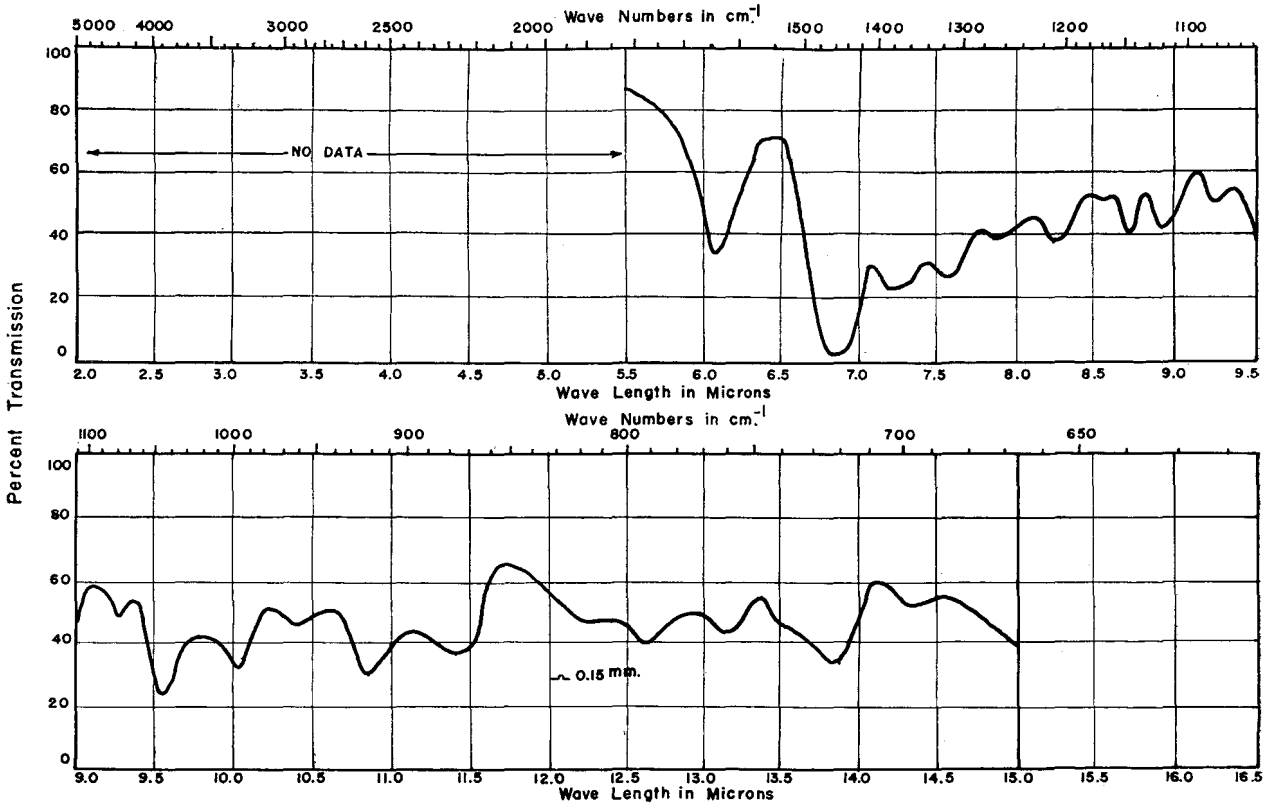

Figure 5. Infrared Spectrum Calculated for Mixture of Nine Cyclopentenes and Cyclohexenes

olefin types listed above can be made if the molecular extinctions of the individual olefins comprising each class are similar, and if interfering absorption can be removed or accounted for.

Diolefins and cyclo-olefins may occur with the noncyclic monoolefins. It is essential, therefore, to consider the interference arising from the possible presence of these compounds. The data of Table III show the absorption maxima of several diolefins (*2*). The infrared and ultraviolet spectra of the blends from the olefin concentrate were compared with the available spectra of diolefins and no diolefin absorption was detected. It is likely that any diolefins that were present in the original gasoline were retained in the aromatic fraction from the silica gel treatment.

Table III. Characteristics Bands of Diolefins

Diolefin	Boiling Point, ° C.	Wave Length, Microns	
1,2-Butadiene	10.3	9.0–10.0	11.0–12.0
1,3-Butadiene	−4.4	9.7	11.0
1,3-Pentadiene	43	10.0	11.0
2-Methyl-1,3-butadiene	34	10.1	11.1

In ascertaining the noninterference of cyclo-olefins the composite cyclo-olefin spectrum shown in Figure 5 was prepared from the spectra of nine pure cyclo-olefins (*2*) by plotting the average of their transmittances at the various wave lengths. This spectrum shows that interference from cyclo-olefins can be expected for both alpha- and internal double bond olefins at 10.0, 10.3, and 10.9 microns. Comparison of the cyclo-olefin spectrum with the spectra of Figures 1 to 4 shows that the bands in the cyclo-olefin spectrum at 8.7, 9.5, 11.4, and 12.5 to 13.5 microns are not observed in the spectra of the olefin concentrate, or distillation blends thereof, from the thermally cracked gasoline. Similar comparisons were obtained with olefin concentrates from thermally reformed and catalytically cracked olefins.

The rather generally low absorption (as compared to noncyclic olefins) of cyclo-olefins indicates that appreciable concentrations of these compounds might be present without contributing significantly to the total infrared spectrum. The marked absence of the strong bands (in the 8.7- to 13.5-micron region) of the composite cyclo-olefin spectrum from the spectra of the olefin concentrates of three cracked gasolines examined by the authors, however, supports their assumption that these concentrates are predominantly noncyclic in character. The data presented on the changes in the infrared spectrum of the gasoline olefins on catalytic treatment also support this view.

If a large number of samples from one source were to be examined on a control basis it would be desirable to omit the silica gel adsorption step in the procedure. This would be possible if the contribution of the material other than olefins could be neglected or determined. To facilitate this type of application for thermal gasoline, and also to evaluate the interference of small amounts of saturates and/or aromatics in the olefin concentrate, a brief study was made of the absorption characteristics

Table IV. Infrared Absorption of Saturates and Aromatic Impurities in Thermal Gasoline Stocks

Band, Microns	Optical Density for 0.0096-Cm. Cell[a]		
	Olefinic concentrate	Aromatics plus saturates[b]	Aromatic concentrate
10.06	0.602	0.098 (no band)	0.398
10.33	0.602	0.170 (no band)	0.301
10.96	0.921	0.122 (no band)	0.398
11.25	0.600	0.114 (no band)	0.602

[a] Measurements made with research spectrophotometer (*3*), and calculated from recorded spectrum.
[b] Obtained by selective hydrotreatment of olefins in thermal gasoline 99% + saturation of olefins with no loss of aromatics.

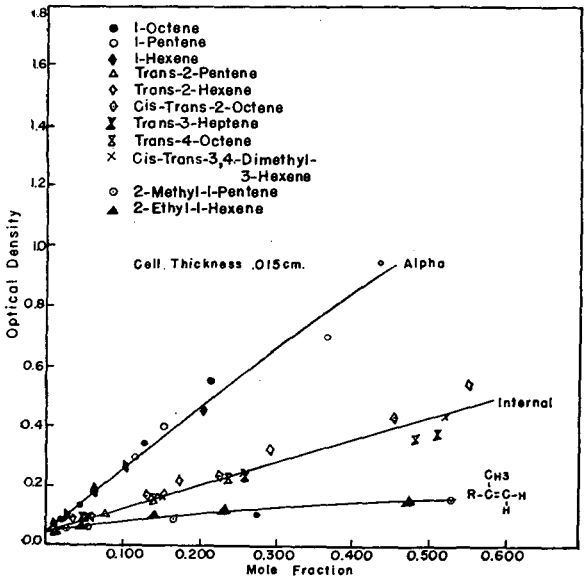

Figure 6. Optical Density *vs.* Mole Fraction of Pure Olefins at 10.07 Microns

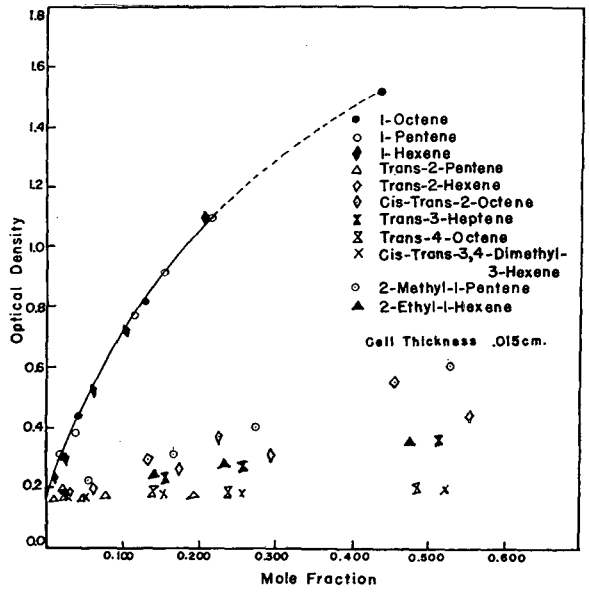

Figure 8. Optical Density *vs.* Mole Fraction of Pure Olefins at 10.99 Microns

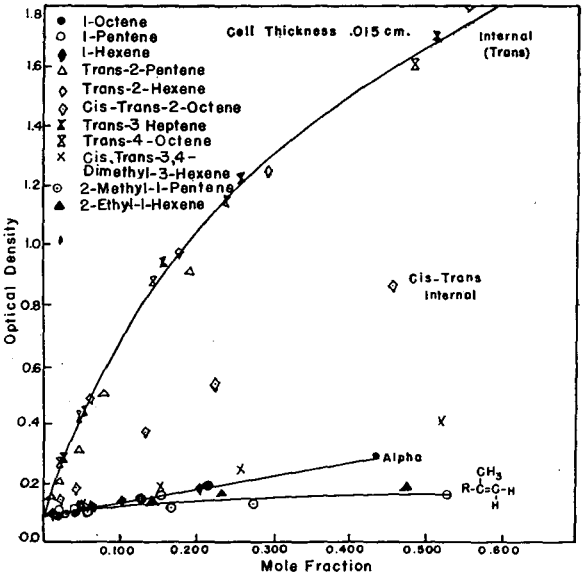

Figure 7. Optical Density *vs.* Mole Fraction of Pure Olefins at 10.25 Microns

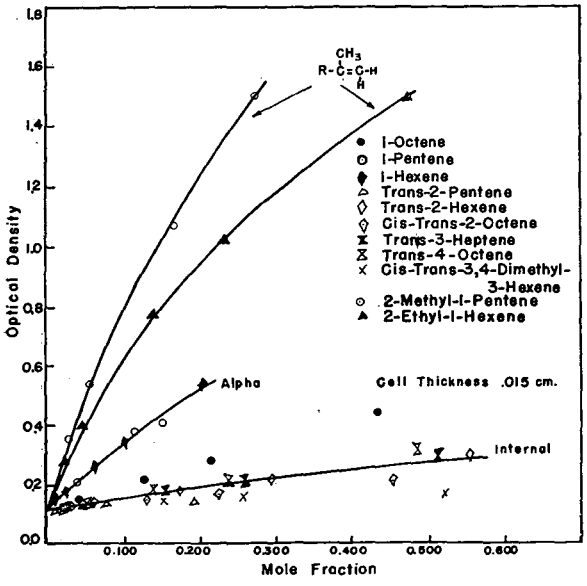

Figure 9. Optical Density *vs.* Mole Fraction of Pure Olefins at 11.25 Microns

of saturate and aromatic portions of the gasoline. The data obtained are shown in Table IV. The effect of the saturates is low and in general represents background absorption while the interference from aromatics could be appreciable. An isomerization process using thermally cracked gasoline (where the diolefin content was below 2 to 4% and the aromatic content below 15% by weight) was successfully controlled, however, over a period of several months by the comparison of olefin types directly from the spectra without preliminary concentration of the olefins by silica gel adsorption. The success of this application shows that under circumstances where the diene and aromatic concentrations are not higher than stated above, the preparation of an olefin concentrate by silica gel adsorption is not necessary.

To differentiate the alpha from the other olefin types in gasolines in an approximately quantitative way, spectrophotometer calibration curves were prepared for blends of pure olefins in *n*-heptane. These curves, presented in Figures 6 to 9, demonstrate that the extinction coefficients are determined almost entirely by whether the location of the double bond is alpha or internal.

APPARATUS AND CALCULATIONS

The spectra of Figures 1 to 4 were obtained using a previously described infrared spectrophotometer (*3*). The calibration curves, Figures 6 to 9, were determined using a Beckman IR-1 spectrophotometer revised to handle liquid samples. The revision entailed the use of a sandwich-type cell with rock salt windows. Transmittances were calculated from a blank run and a sample run.

In measuring the 100% transmittance (blank determination), the most satisfactory procedure has been to use a cell assembly equipped with a single rock salt plate of the same thickness as the sample cell assembly. This is more satisfactory for the blank than an empty cell, because the latter reflects more light at the inner surfaces than a cell full of liquid. This effect is particularly evident as the cell ages. In order to correct optical densities to a

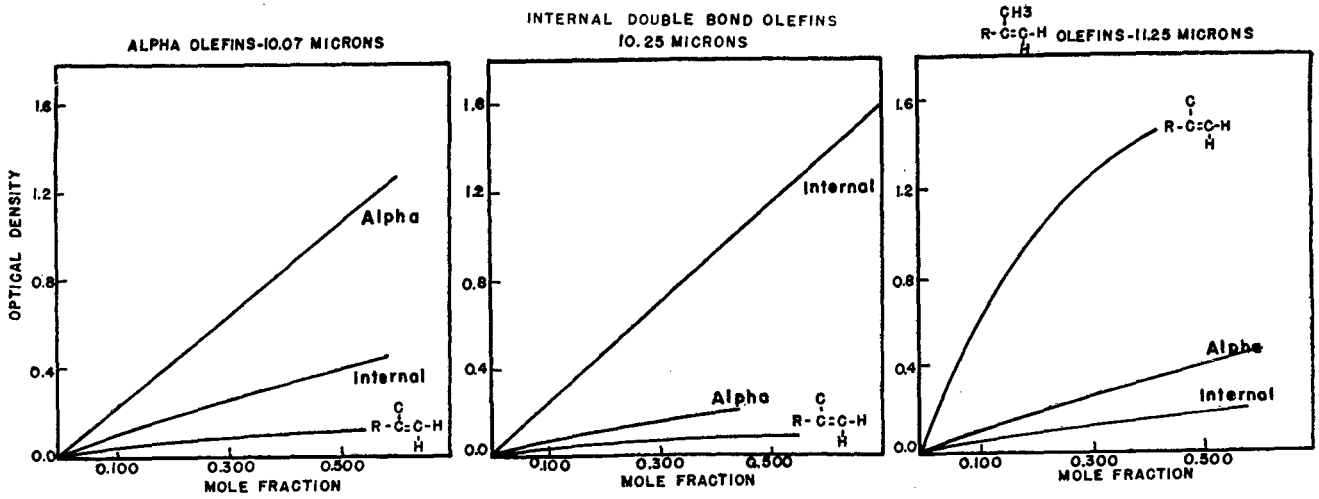

Figure 10. Infrared Calibration Curves for Olefin Types

Curves for internal double bond olefins are those observed for *cis-trans* mixtures concentrated by distillation

standard basis, a daily calibration was made at each analytical wave length with *n*-heptane, and the deviation from the heptane optical density at the time of calibration was used as the correction value.

The original calibrations were corrected for the *n*-heptane optical density contribution by calculation using the method of Fry, Nusbaum, and Randall (*5*). These revised curves are presented in Figure 10; the analytical wave lengths are 10.07, 10.25, and 11.25 microns.

In the case of the internal double bond band at 10.25 microns, the difference in the absorption of the *cis* and *trans* isomers introduces some obscurity into the results. Infrared examination of distillation fractions containing C_8 internal double bond olefin mixtures showed absorption closely approximating that of a *cis-trans* 2-octene mixture obtained by catalytic treatment of 1-octene over a metal catalyst at 495° to 525° C. This absorption was used in the calibrations shown in Figure 10.

The concentration of alpha-olefin of the type R—CH = CH_2 is measured at 10.07 microns. The olefin concentration determined at 11.25 microns, chiefly alpha with branching on the beta-carbon atom, is added to the 10.07 micron value to obtain total alpha-olefins. The concentration of internal double bond olefins is measured at 10.3 microns. The composition of an unknown sample is computed from the infrared measurements by a method

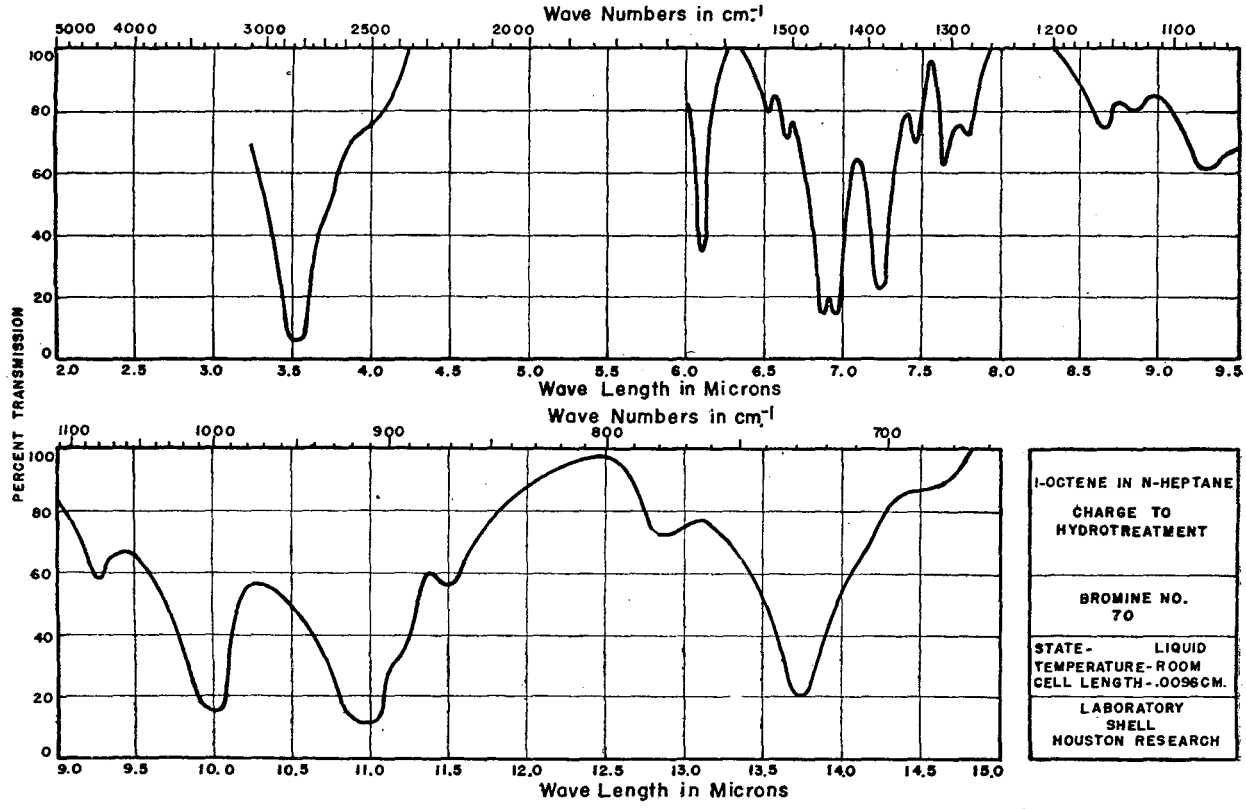

Figure 11. Infrared Sprectum of 50% by Volume, Solution of 1-Octene in *n*-Heptane

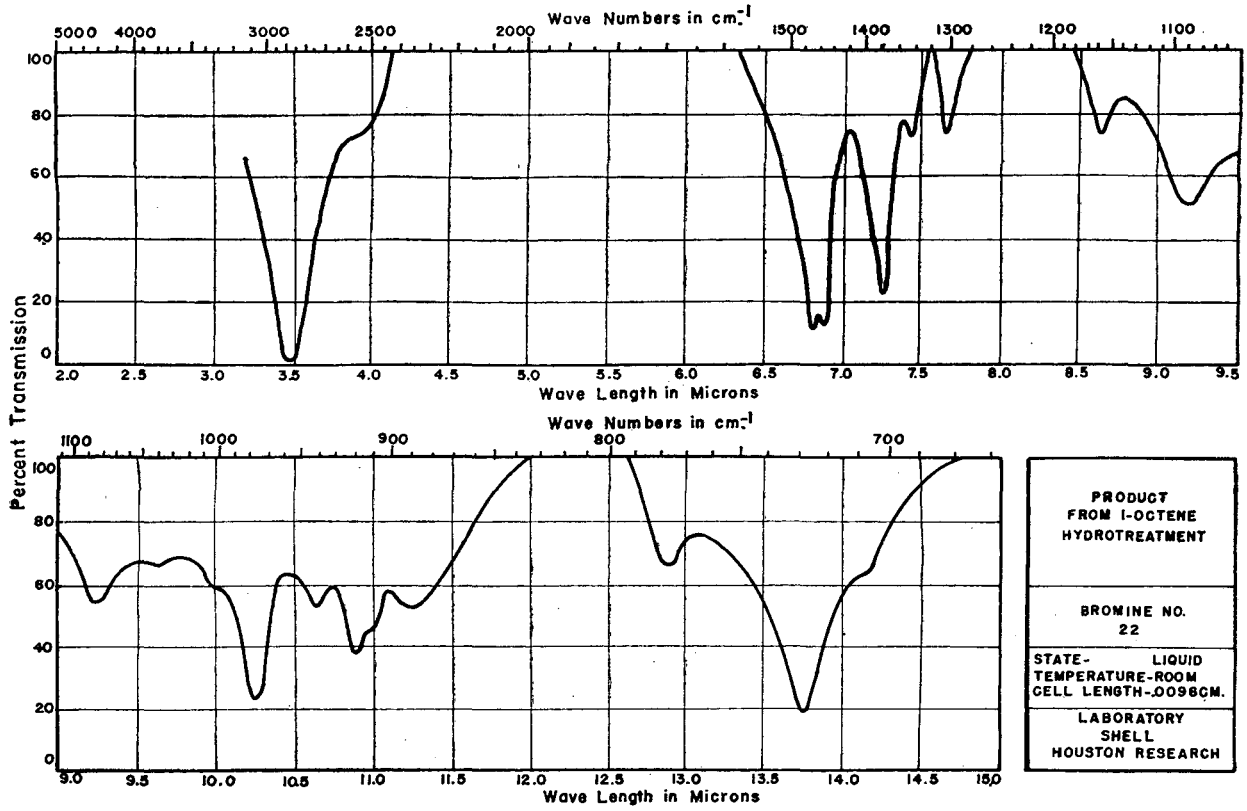

Figure 12. Infrared Spectrum of Product from Hydrotreatment of 1-Octene in *n*-Heptane

of successive approximations from the calibration curves. The results obtained give the ratio of alpha to internal double bond types in the olefin concentrate. In these laboratories these data are converted to the total olefin basis of the gasoline charge to the silica gel treatment. It is recognized that in so doing, any olefin types other than those for which the method was designed, if present, would not be accounted for. Where the reaction studied does not involve a change in the total olefin content of the reactant, only the ratio of olefin types need be determined.

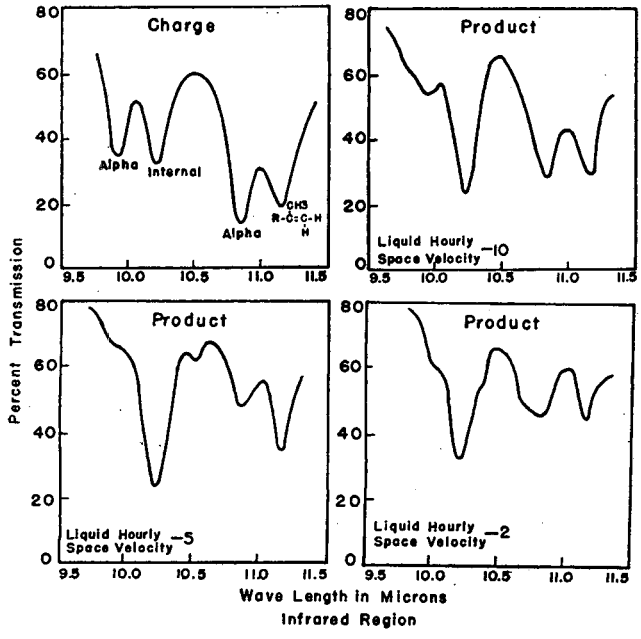

Figure 13. Effect of Hydrotreatment on Thermally Cracked Gasoline

APPLICATIONS OF THE TECHNIQUE

The isomerization of alpha-olefins to internal double bond types can be shown by comparing the spectra of the reactants and products from the catalytic treatment of a 50% by volume solution of 1-octene in *n*-heptane. The spectra are shown in Figures 11 and 12. Whereas the charge shows the characteristic alpha-olefin doublet at 10.0 and 10.9 microns, the product shows a characteristic internal double bond absorption at 10.3 microns. Infrared spectra and physical properties obtained on fractions from an analytical distillation of the product showed that the major olefin product was 2-octene, but that 3- and probably 4-octene were also present.

The technique can also be used to indicate changes in the relative distribution of olefin types in refinery streams.

To illustrate, Figure 13 presents spectra for a component of a thermally cracked gasoline charge (75% by weight olefins) to a hydrotreatment process and the products at three different space velocities. By noting the changes in the alpha and the internal double bond bands it is shown that there is isomerization of the double bond toward the center of the molecule. At the lower space velocities, loss of olefins by hydrogenation is indicated by the decrease in the strengths of all bands. Quality improvement tests confirmed these conclusions, inasmuch as at a space velocity of 10, where about 15 to 20% of olefin hydrogenation occurred and strong isomerization was indicated, there was an octane number increase of 0.5. Had there not been the extensive isomerization indicated by the spectra of Figure 13, a decrease in octane number would have been obtained as a result of the loss of olefins by hydrogenation.

In an additional application, a comparison was made of the relative distribution of types in various refinery products. For this purpose four gasolines were studied: (1) thermally reformed, (2) catalytically cracked, (3) a component of the refinery ther-

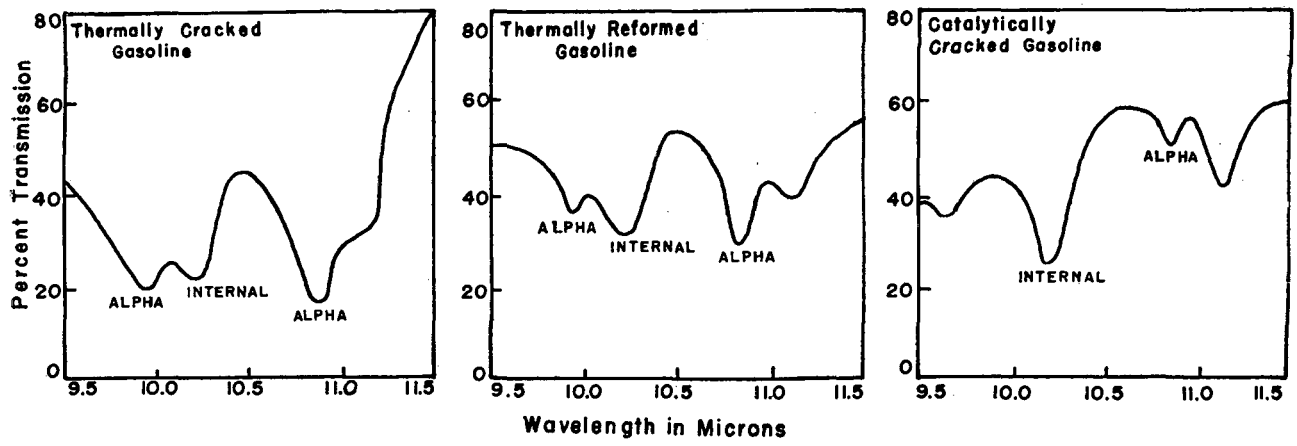

Figure 14. Infrared Spectra of Plant Stream Olefin Concentrates from Silica Gel Adsorption

Table V. Comparison of Olefin Types in Refinery Products[a]

Plant Stream	Alpha	Internal Double Bond
Catalytically cracked gasoline	9	14
Thermally reformed gasoline	13	13
Thermally cracked gasoline	43	32
Thermally cracked gasoline after hydrotreatment	10	50

[a] To enable comparison of relative concentration in stream, ratios are corrected to total olefin basis of stream.

mally cracked gasoline blend, and (4) a hydrotreated product of (3). The spectrum of the olefin concentrate of the hydrotreated product was shown in Figure 13 at a liquid hourly space velocity of 10, and the spectra of the olefin concentrates of the other three gasolines were determined for the region 9.5 to 12.5 microns, and are shown in Figure 14.

Using representative olefin type calibration data as shown in Figure 10, the olefin distributions listed in Table V were obtained. In these analyses the olefin type concentrations were corrected to the total olefin concentration of the original sample as determined by bromine number. The sums of the uncorrected concentrations of the olefin types were from 3 to 12% below the value found by bromine number.

The infrared portion of the presented application required less than 0.5 ml. of sample and approximately 30 minutes of analytical time.

ACKNOWLEDGMENT

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Infrared Analysis of Organic Mixtures

Using C—H Band Structure Resolved by a Lithium Fluoride Prism

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THE present-day availability of lithium fluoride prisms for the various commercially available infrared spectrometers extends the usefulness of infrared spectroscopy very greatly. It is well known (*1*, *9*) that lithium fluoride provides a large increase in dispersion over sodium chloride in the 2 to 5.5μ (5000 to 1820 cm.$^{-1}$) region. This brings out structure characteristics, particularly in the spectra due to the stretching vibrations of the C—H valence bonds. Fox and Martin (*6*) and Rose (*8*) have shown that different types of substitutions in hydrocarbons produce different, recognizable C—H absorption bands. Fox and Martin considered the following groups:

$$=CH_2,\ =\overset{|}{C}-H,\ -\overset{|}{\underset{|}{C}}-H,\ \overset{|}{\underset{|}{C}}H_2,\ \text{and}\ -CH_3$$

The bands due to these groups all lie near 3.4μ and are so closely spaced that they are not satisfactorily resolved in the ordinary instrument that employs a sodium chloride prism.

With a prism of sodium chloride the usual infrared spectrometer does not resolve the structure of the absorption bands due to C—H groups. A lithium fluoride prism which possesses high dispersion in this spectral region resolves these bands very satisfactorily and makes them available for analytical determinations. Such a use of a lithium fluoride prism has been explored and a number of specific analyses have been developed. This method of analysis allows the examination of compounds and mixtures in dilute solutions of carbon tetrachloride as well as the analysis of compounds subject to complex formation such as hydrogen bonding and the analysis of mixtures possessing large differences of intensity of absorption in the sodium chloride region. It makes possible a new approach to systems of very similar isomers; and it extends the usefulness of instruments equipped with such a prism for molecular structure determination work.

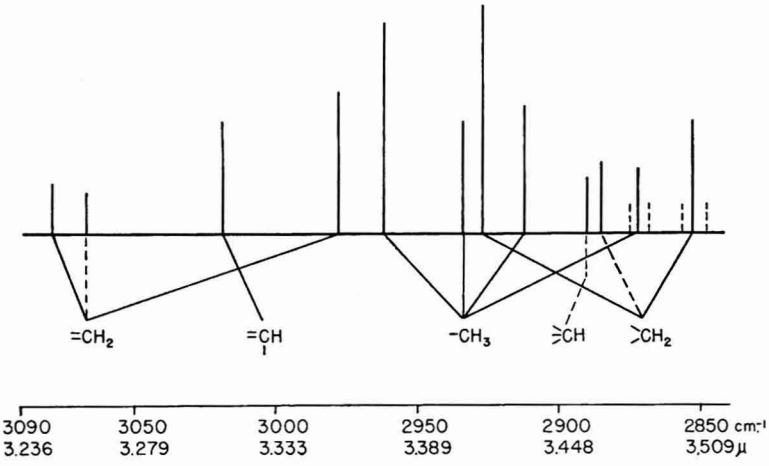

Figure 1. Absorption Frequencies Observable for Different Classes of C—H Groups in Hydrocarbons When Sufficient Resolution Is Achieved with Lithium Fluoride Prism

Data from Fox and Martin (6)

The use of a lithium fluoride prism, however, makes possible the utilization of the C—H structural bands for analytical applications. The present paper reports some of the results obtained in these applications.

Figure 1 presents an absorption band diagram synthesized from the empirical data of Fox and Martin (*6*) for hydrocarbons. This shows the positions and multiplicity of the absorption bands due to the various types of carbon-hydrogen groupings. The relative heights of the lines are some indication of the intensities of the observed absorption bands. However, neither these nor the wave-length positions may be regarded as exact, as variations occur in going from compound to compound. The dashed lines below the base line represent absorption bands observed only in some of the cases studied. The dashed lines above the base line are for the cases where a single band splits into a doublet. Despite these limitations it is evident from the figure that with the satisfactory resolution of these bands, which is possible using a lithium fluoride prism, the scope of applications of infrared spectroscopy may be greatly enlarged. As more data become available in the future it will be possible to determine the specific limitations and generalities of such data as those presented in Figure 1.

It is to be observed in Figure 1 that the absorption bands for the saturated carbon-hydrogen groupings occur at longer wave lengths than do those for the unsaturated groupings. This immediately suggests the use of the C—H bands for the quantitative analysis of mixtures of olefins and paraffins. These systems may be handled, as well as ones containing aromatics and oxygenated compounds. Because of the relatively narrow spectral region and the limited number of bands available, the number of components per sample cannot be as large as can be handled when a sodium chloride prism is used in the longer wave-length region. However, the advantages of the present method lie in the handling of special types of mixtures which often have only a small number of components.

One advantage in the use of the C—H absorption band structure lies in the availability of an excellent solvent for this region. Carbon tetrachloride is very transparent throughout this region and many compounds and systems of compounds are soluble in it. It is so transparent that cells up to several centimeters in length can be used. This makes it possible to examine compounds and mixtures in dilute solutions. Under these conditions the inherent difficulties due to molecular association such as hydrogen bonding may be avoided. Also it makes possible the simultaneous analysis in the same mixture of compounds that may have large differences of intensity of absorption in the longer wave-length regions such as paraffins and some of the polar or unsaturated materials. In some cases, mixtures of homologous compounds which do not have appreciably different spectra in the long wave-length region can be satisfactorily analyzed by this method. A further advantage of the method is a spectrometric one. Because the spectral region used is near the maximum for the radiation curve, the scattered light is very weak. In fact, it is so weak that the authors have detected none at all. This eliminates a correction procedure that must be applied at the longer wave lengths for best results. A further advantage lies in the extension of usage to analytical problems of an instrument equipped with a lithium fluoride prism and otherwise primarily used for molecular structure determination work. The use of the samples in solution also gives greater accuracy, in that it allows a true correction for the absorption, reflection, and scattering of the radiation beam by the cell itself. This is done by comparing the transmittance of the sample in solution with the transmittance of the solvent alone.

The accuracy attainable for a specific mixture depends upon the differences in intensity of absorption that can be utilized. In some cases where unique absorption bands can be found, the average errors are of the order of 0.1 to 0.2% of total sample. Where the wave length and intensity discrimination are not very good, the average errors may be of the order of 1.0% of total sample. High accuracy was not an all-important aim of the present work. Rather, a compromise in reasonable accuracy, rapidity of analyses, and ease of analyses by nontechnically trained personnel was desired. All calibration data and synthetic samples were processed under routine conditions.

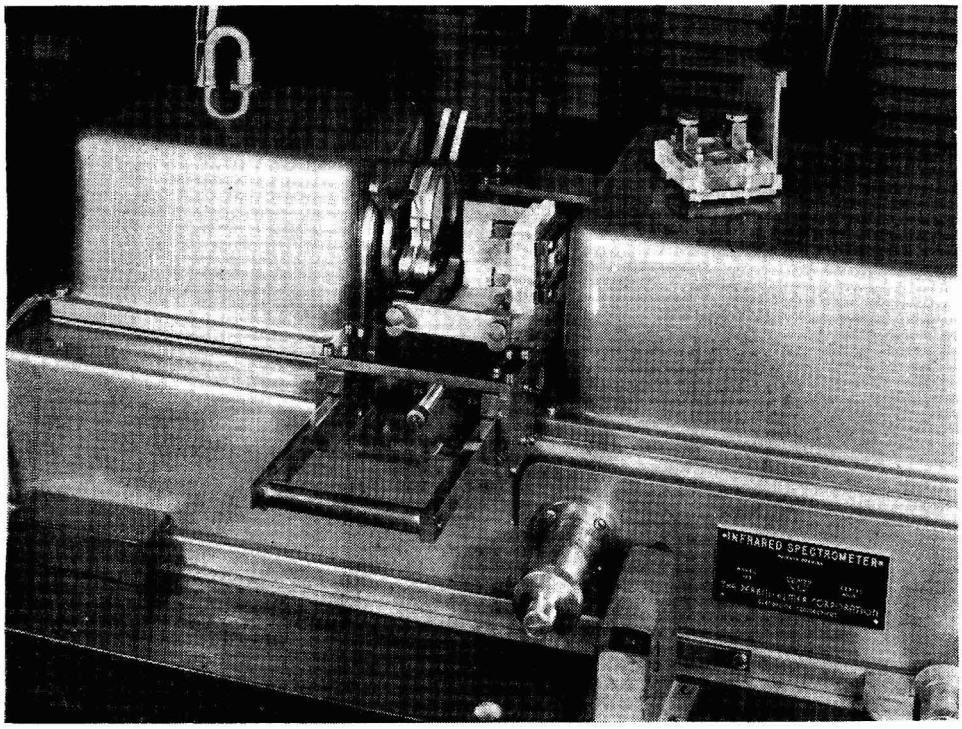

Figure 2. Liquid Absorption Cells and Mounting Arrangements Used in Analytical Work

An assay of the accuracy of the method under routine conditions may be made from the results reported for the synthetic samples below.

EXPERIMENTAL METHODS AND TECHNIQUES

The instrument on which the present work was done was a Perkin-Elmer Model 12B infrared spectrometer. Sodium chloride and lithium fluoride prisms are used in it interchangeably. A cell-in-cell-out arrangement is used wherein the absorption cells are securely clamped to a movable carriage. In Figure 2 may be seen one of the cells clamped in place and another one resting on top of the instrument. The type of cell used has been described (*3*). The carriage arrangement is the same as used in routine gas analysis (*4*). The liquid cells are equipped with a mounting frame which allows them to be held in the movable carriage by means of thumbscrews. The cell employs two needle valves, one fabricated to act as a filling piston when loading the cell. These cells have interchangeable parts and are easily fabricated and repaired. The thickness used most commonly in the present work was 0.006 inch.

For quantitatively measuring the radiation intensities at the various wave lengths, the same system of null measurements (*4*) was used. A high sensitivity galvanometer is used merely as an indicator and the thermocouple signals are balanced out by means of a low voltage obtained from a resistor and potentiometer network. Difficulties due to nonlinearity or change of sensitivity of the high sensitivity galvanometer are thereby eliminated. The instrument is equipped with an Amphenol connector for the thermocouple outlet. With this arrangement either the galvanometer and null system equipment or the automatic recorder may be connected at will. It has been found very convenient to use the automatic recorder to obtain a spectrum of the sample throughout the wave-length region used. This may then be examined to determine what compounds are present or, if the sample is a calibration standard, to determine the most desirable wave lengths to use in the analytical procedure. However, for quantitative measurements of transmittance at specific wave lengths consistently more accurate data are obtained manually, using the null method. The same equipment and general methods have been in use here for several years for the quantitative analysis of multicomponent *mixtures of hydrocarbons*, using a sodium chloride prism and the longer wave-length region.

Tests for the scattered radiation intensity were made by the total absorber method (*2*). For some of these an absorption cell of 0.036-inch thickness was used. Almost any material containing C—H groups is suitable as solute for such tests in the 3.4μ region, provided a strong enough concentration is used.

In the present work all dilutions were made volumetrically in graduated pipets and burets. In certain cases care must be taken to follow a definite procedure in making the calibration blends, diluting the samples, and obtaining the data, in order to avoid trouble caused by differences in evaporation rates. It is well known (*7*) that the high boiling points of the simple alcohols, in comparison with other compounds of similar molecular weights, are due to the hydrogen bonding forces between the molecules. These hydrogen bonding forces, due to the hydroxyl groups, are responsible for molecular association between the molecules and the attendant reduction in vapor pressure. However, in dilute solutions in a solvent such as carbon tetrachloride the relative degree of hydrogen bonding is reduced because of the greater average distance between hydroxylated molecules. Consequently, the alcohol molecules in such a solution will escape much more rapidly, on a relative basis, than from a concentrated solution. This effect is so strong that the same procedure as regards time intervals must be used on the samples to be analyzed and on the calibration blends. Otherwise errors will arise due to loss of some of the material by evaporation. In Figure 3 may be seen the optical density plotted as a function of the number of times analyzed for a 2.5% by volume solution of ethyl alcohol in carbon tetrachloride. This sample was kept in a glass-stoppered bottle and analyzed on successive days. During these tests the concentration of ethyl alcohol changed radically. The

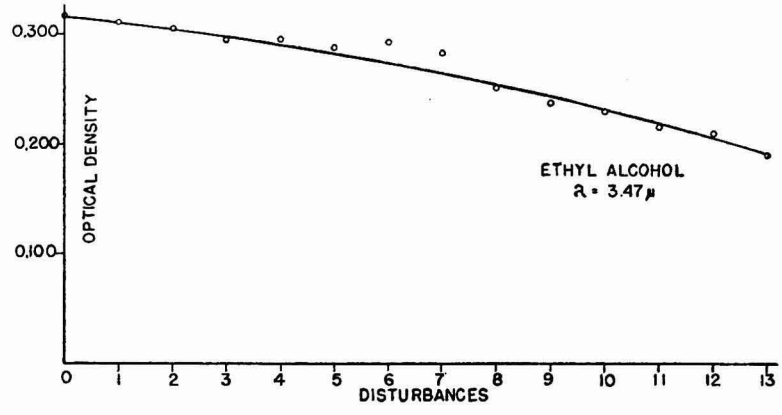

Figure 3. Optical Density of Dilute Solution of Ethyl Alcohol in Carbon Tetrachloride Solution as a Function of Number of Times Analyzed (Disturbances)

authors have found it most advisable to obtain the data for such a solution on the same day as it was prepared.

As Beer's law of absorption is obeyed by the compounds under investigation the calculations and handling of data are straightforward. The absorption, reflection, and scattering effects due to the cell may be eliminated by comparing the transmittance of the sample in solution with the transmittance of the solution alone. Actually this is accomplished by a suitable subtraction of optical densities.

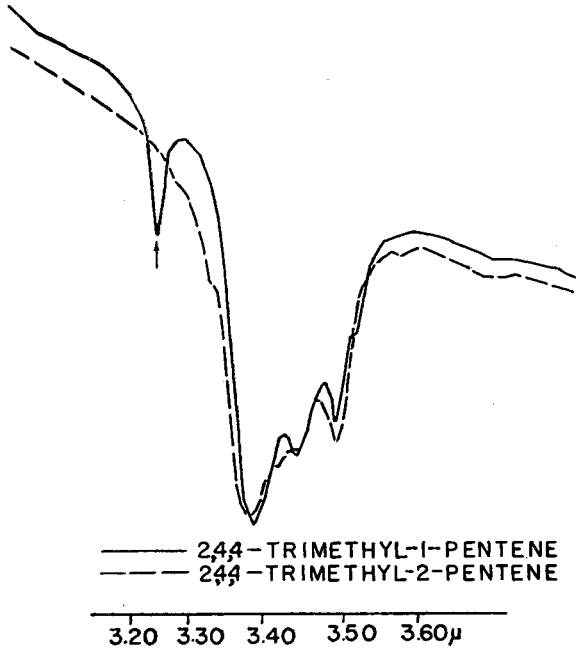

Figure 4. Infrared Absorption Spectra in C—H Region of Isomeric Trimethylpentenes

To see this let us consider the transmittance of a solution of pure compound at wave length λ_i. We have for the solution:

$$D_i = \log (I_0/I) = A_iC + K_i \qquad (1)$$

where D_i is the optical density, I_0 is the incident radiation intensity, I is the transmitted radiation intensity, A_i is the calibration coefficient for the pure compound at wave length λ_i, C is the concentration of the solute, and K_i is an attenuation factor due to the absorption, reflection, and scattering by the cell and absorption by the solvent. Since the concentrations of solute used are small, between 1 and 6% for the present work, the K_i in Equation 1 may be evaluated by obtaining the optical density for the cell filled with solvent only. A subtraction of this value from the D_i of Equation 1 then gives the component of optical density due to the compound. With this method of obtaining the optical density, Beer's law was tested for a number of the compounds in the examples below and found to hold in each case.

With the above method of obtaining true optical densities and with the additivity of optical densities that occurs when Beer's law is obeyed, simple equations of the type below are obtained. Here a three-component system is assumed.

$$\begin{aligned} D_1 &= A_{11}C_1 + A_{12}C_2 + A_{13}C_3 \\ D_2 &= A_{21}C_1 + A_{22}C_2 + A_{23}C_3 \\ D_3 &= A_{31}C_1 + A_{32}C_2 + A_{33}C_3 \end{aligned} \qquad (2)$$

Here D_1, D_2, D_3 refer to the true optical densities of the mixture at the three wave lengths chosen for operation; A_{11} is the calibration coefficient of the first compound at the first wave length, A_{23} is the calibration coefficient of the third compound at the second wave length, etc.; and C_1, C_2, and C_3 are the concentrations of the three compounds. These equations may be readily solved for the concentrations by matrix methods (*2*) or by successive approximations. In the method of successive approximations concentrations C_2 and C_3 are assumed to be zero in the first equation and it is solved directly for C_1. This value is used in the second equation with the assumption that C_3 is zero and a value for C_2 is determined. Next the values of C_1 and C_2 obtained in the first two steps are used in the third equation and it is solved for C_3. The cycle is repeated with the values of C_2 and C_3 from the latter two steps substituted in the first equation and a new value of C_1 is determined. The second equation is then solved for C_2 using the most recent values of C_1 and C_3, etc. Several cycles can be quickly run with a semi-automatic or automatic calculating machine in a few minutes.

A wave-length calibration of the C—H region was prepared from the data of Fox and Martin (*5, 6*). No attempt was made to make a highly precise calibration. In practice the wave lengths for specific analyses are specified in terms of the instrument vernier readings. For that reason specific wave lengths are not given below. For some of the examples the wave-length values may be appraised from the figures. It is believed that these may be relied on to within about 0.005μ or better.

APPLICATION TO SPECIFIC ANALYSES

From the information given in Figure 1 a prediction may often be made as to whether or not a specific mixture can be analyzed. For example, a binary mixture containing two compounds with differences in types of unsaturated groups may generally be analyzed with ease.

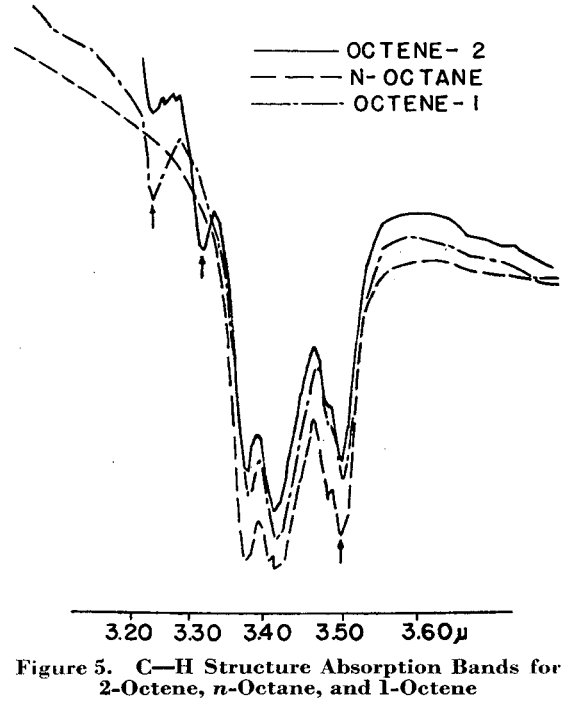

Figure 5. C—H Structure Absorption Bands for 2-Octene, *n*-Octane, and 1-Octene

A specific example of this is a mixture of similarly branched trimethylpentenes. In Figure 4 may be seen the spectra of 2,4,4-trimethyl-1-pentene and 2,4,4-trimethyl-2-pentene. In these curves, which are reproductions of the automatically recorded spectra, the transmitted energy is plotted as a function of wave length. Here, as indicated by the arrow, may be seen the absorption band due to the terminal $=CH_2$ group in one of the compounds. Actually, the difference in absorption is great enough here to permit taking data at this point only. Then instead of having two equations of the type of Equations 2 we have but one plus the equation:

$$C_1 + C_2 = C \qquad (3)$$

where C is the concentration of sample in solution. The results of analyses of two synthetic samples may be seen in Table I.

Table I. Analyses of Synthetic Mixtures of Binary Mixture of Trimethylpentenes

Compound	Synthetic, %	Calculated, %	Difference, %
2,4,4-Trimethyl-1-pentene	50.0	50.4	0.4
2,4,4-Trimethyl-2-pentene	50.0	49.6	−0.4
2,4,4-Trimethyl-1-pentene	30.0	30.6	0.6
2,4,4-Trimethyl-2-pentene	70.0	69.4	−0.6

Table II. Analyses of Synthetic Mixture of Hydrocarbons Containing Paraffins and Unsaturates

Compound	Synthetic, %	Calculated, %	Difference, %
n-Octane	20.0	18.9	−1.1
2-Octene	30.0	31.0	1.0
1-Octene	50.0	50.1	0.1
n-Heptane	50.0	49.9	−0.1
Benzene	50.0	50.1	0.1
n-Heptane	75.0	75.1	0.1
Benzene	25.0	24.9	−0.1

Figure 6. Infrared Absorption Structure in C—H Region for Benzyl Alcohol, *n*-Octanol, and Methyl Ethyl Ketone

Continuing with hydrocarbon systems we see in Figure 5 that satisfactory wave-length discrimination is obtainable for the ternary system of *n*-octane, 2-octene, and 1-octene. The wave lengths used for analysis are indicated by the arrows. At about 3.26μ may be seen the band characteristic of the terminal $=CH_2$ group of 1-octene, at about 3.32μ the band characteristic of the $=CH$ group of 2-octene, and at about 3.50μ the band characteristic of the CH_2 groups, which is stronger for *n*-octane than for the others. The 2-octene shows some of the bands characteristic of the other compounds, because the sample used for recording was not pure.

Although definite differences in spectra are found, the differences in intensity of absorption are not great (Figure 5). Despite this, fair accuracy is obtainable, as may be seen in Table II.

Table II also gives the results for two synthetic samples of the binary system of *n*-heptane and benzene, for which considerably better accuracy is possible.

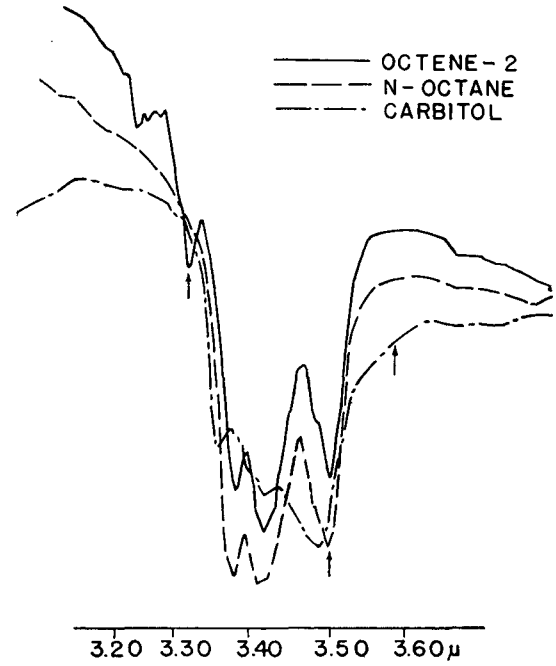

Figure 7. C—H Structure of Absorption Bands for 2-Octene, *n*-Octane, and Carbitol

In the preceding type of examples predictions could be made in advance concerning the success of the method. In other cases, such as the examples discussed below, an idea of the feasibility cannot be reliably formulated until the spectra of the pure compounds are obtained. A number of ternary mixtures of particular interest in the study of azeotropes may be easily analyzed. In general, these systems contain a paraffin, an aromatic or olefin, and an oxygenated compound. Other systems containing all oxygenated compounds may in some cases be analyzed with good accuracy.

In Figure 6 may be seen the spectra in the 3.4μ region of benzyl alcohol, *n*-octanol, and methyl ethyl ketone. Here it is observed that three distinct and unique wave lengths, indicated by arrows, may be chosen for analysis. Table III gives the results on synthetic samples made up to test the accuracy on such a system.

Table III. Analyses of Synthetic Mixtures of the System *n*-Octanol–Methyl Ethyl Ketone–Benzyl Alcohol

Compound	Synthetic, %	Calculated, %	Difference, %
n-Octanol	30.0	30.3	0.3
Methyl ethyl ketone	30.0	29.4	−0.6
Benzyl alcohol	40.0	40.3	0.3
n-Octanol	25.0	24.5	−0.5
Methyl ethyl ketone	50.0	50.5	0.5
Benzyl alcohol	25.0	25.0	0.0

In some cases the spectra of the compounds in the mixtures to be analyzed have such similar spectra that distinctive wave lengths may not be found, but sometimes it is possible to utilize the difference of intensity on the shoulders of the bands for analytical application.

An example of such a case is seen in Figure 7, which is for the system 2-octene–*n*-octane–Carbitol (diethylene glycol monoethyl ether). Here the arrows again illustrate the wave lengths used for analysis. For the Carbitol, utilization is made of its increased absorption in the long wave-length shoulder of its

Table IV. Analyses of Synthetic Samples of Ternaries of *n*-Octane, 2-Octene, Carbitol, and Ethylbenzene

Compound	Synthetic, %	Calculated, %	Difference, %
Carbitol	47.4	47.1	−0.3
n-Octane	18.0	17.6	−0.4
2-Octene	34.6	35.3	0.7
Carbitol	37.5	37.2	−0.3
n-Octane	25.0	25.3	0.3
Ethylbenzene	37.5	37.5	0.0

Table V. Analyses of Synthetic Samples of Ternaries Containing a Paraffin, an Unsaturate, and an Oxygenated Compound

Compound	Synthetic, %	Calculated, %	Difference, %
Methylcyclohexane	25.0	24.6	−0.4
Methyl ethyl ketone	35.0	34.7	−0.3
Toluene	40.0	40.7	0.7
Dichloroethyl ether	70.0	68.7	−1.3
n-Octane	10.0	10.9	0.9
Ethylbenzene	20.0	20.4	0.4
Cyclohexane	30.0	30.7	0.7
Ethyl alcohol	40.0	39.4	−0.6
Benzene	30.0	29.9	−0.1
Hexanol	40.0	40.3	0.3
n-Heptane	20.0	21.0	1.0
Heptenes (mixture)	40.0	38.7	−1.3

Table VI. Analyses of Synthetic Samples of Binary Mixtures of Methyl and Ethyl Alcohol

Compound	Synthetic, %	Calculated, %	Difference, %
Methyl alcohol	51.6	52.0	0.4
Ethyl alcohol	48.4	48.0	−0.4
Methyl alcohol	91.4	92.1	0.7
Ethyl alcohol	8.6	7.9	−0.7

C—H absorption region. Distinct bands near 3.40μ for *n*-octane are available but are not used, because the absorption there is too strong at the concentrations necessary to bring out adequate absorptions at the other wave lengths. The results for this system as well as those for a closely similar system in which the 2-octene is replaced by ethyl benzene may be seen in Table IV. In this latter system the difference of absorption intensity on the long wave-length shoulder was again utilized.

In the preceding discussion a few specific types of analyses found to be practical have been enumerated and the results given on some synthetic mixtures. The practicality of the method for such systems can best be determined after the absorption bands are automatically recorded. In Table V are given a few more results on other systems for which the spectra will not be shown. In Table VI are given the results of tests on synthetic samples of binary mixtures of methyl and ethyl alcohol.

The attainable accuracy depends upon the particular system being analyzed. The method has been found to give satisfactory results for almost all binary and ternary systems investigated. It is also suitable for the analysis of gas mixtures. It considerably augments the usefulness of the absorption spectroscopic techniques in use at this laboratory and is now used as a routine procedure. The time required per sample is approximately the same, except for the dilutions, as for analyses by infrared when a sodium chloride prism and the longer wave lengths are used.

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Magnetic Stabilizer for Direct Current Arcs in Spectroscopy

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IT IS generally recognized that although the direct current arc is a versatile source of excitation, it provides relatively poor reproducibility when used for quantitative spectrochemical analyses. Apparently this defect is due chiefly to wandering of the cathode "spot," which produces both wandering of the arc and fluctuations in amperage with attendant changes in the discharge temperature. Ways of improving reproducibility are available. Hasler and Harvey (*1*), Jeppesen, Eastmond, and Logan (*3*), and others have described electrodes specially shaped for the purpose. Jaycox and Ruehle (*2*) average the effects of wandering of the arc by rotating the lower electrode holding the sample. Mixing the powdered sample with a "spectroscopic buffer" may improve reproducibility. Recently Myers and Brunstetter (*5*) recommended a rotating magnetic field placed near the arc.

The present paper describes an instrument that eliminates almost completely the wandering of a central horizontal slice of the arc. Almost any part could be rendered free of fluctuations, but in the present paper attention is directed only to that part from which the light is dispersed and photographed.

PRINCIPLE OF THE INSTRUMENT

Normally, a direct current arc is surrounded by a cricular magnetic field, depicted by broken circles in Figure 1. By reason of a well-known principle, placing one pole of a horizontal bar magnet near the arc will cause the arc to move sideways in a direction at right angles to the magnet's polar axis. The magnetic field from a north pole moves the arc to the left of the magnet, and from a south pole moves it to the right, when the lower electrode is positive.

Figure 1 shows how the magnetic effect just described was applied to eliminate wandering of the central part of the arc. Figure 2 shows the magnetic stabilizer equipment in position, the two slits of the phototube housing opened wider than usual, in

An instrument is described which eliminates wandering of the central portion of a direct current arc used in spectrochemical analysis. Two phototubes are placed at the two vertical edges of an enlarged image of the arc, and each phototube is connected through a direct-coupled amplifier to a coil of an electromagnet placed behind the arc and with its magnetic axis coincident with the optic axis of the spectrograph. Sideways wandering of the arc illuminates the phototubes unequally, thereby producing a magnetic field about the electromagnet which, acting on the arc current, immediately recenters the arc. With samples of powdered rock, tests for reproducibility showed that greater precision is obtained by using the instrument.

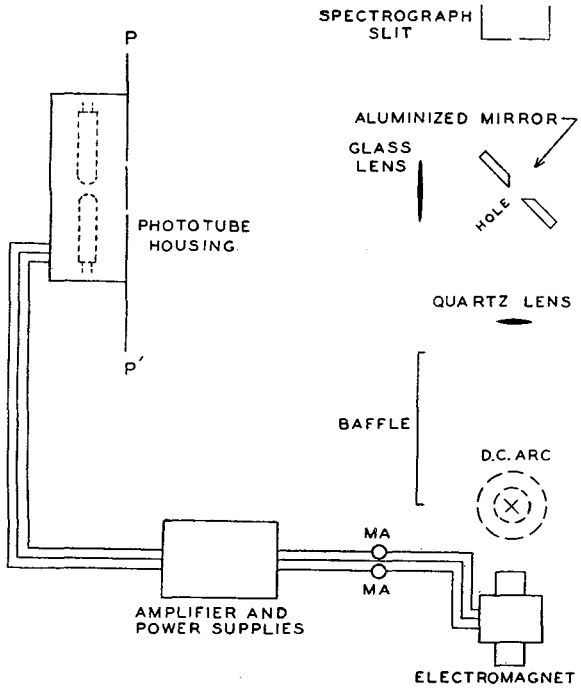

Figure 1. Plan of Stabilizer

order to make them clearly visible. An A.R.L.–Dietert spectrograph was used.

Light from the arc passes through a quartz lens, used to bring the image of the arc to a focus at the grating. An aluminized front-surface mirror with a hole in the center is set between the quartz lens and the slit of the spectrograph. The central part of the beam of light from a properly centered arc passes through the hole in the aluminized mirror and is focused on the grating in the normal way. But light from the peripheral part of the beam is intercepted by the mirror and reflected, passing through the glass convex lens to form an enlarged image of the arc at plane PP' (Figure 1). The optical path for the beam striking plane PP' is 94 cm. in length, but by changing the focal length of the glass lens this can be altered to suit the space available. At PP' is a metal box housing two phototubes set horizontally as shown in Figure 1.

The vertical side, PP', of the box has two adjustable slits, one directly in front of each phototube (Figure 2). Each slit opening is 1.2 cm. high and 0.15 cm. wide, and the slits are 4.9 cm. apart. The height of this phototube housing is adjusted so that the center of its slits, the center of the spectrograph slit, and the center of the grating are in the same horizontal plane. The visible vertical edges of the arc's image, formed on the face of the phototube housing, just completely encompass the two slits. Each slit opening was made 1.2 cm. high, so that all, and almost no more, of that horizontal slice of the arc utilized in producing a spectrogram is also used to illuminate the slits of the phototube housing. Baffles should be installed to shield the slits of the housing from the bright light of the room and of the burning arc.

If the arc wanders sideways, one phototube will be illuminated more than the other. Each phototube is connected to a direct-coupled amplifier. Two amplifiers are required, one for each phototube, and are connected as shown in Figure 3. This circuit has proved to be satisfactory.

To the voltage divider 450 volts direct current are supplied from a conventional full-wave rectifier supplied from 110 volts alternating current, using a 5Y3G rectifier tube and a condenser-input filter system. Matched 6J7G tubes are used. The leads from the the phototubes to the grids of the 6J7G tubes must be shielded.

D and E are two coils forming the electromagnet shown in Figures 1 and 2. The laminated iron core is 7.7 cm. long and 1.7 cm. square in cross section. Each coil was wound in two sections, each section consisting of 7500 turns of No. 39 wire. The coils thus wound in sections were balanced to give the same resistance and distribution of magnetic flux. Equal currents in the two coils produce a resultant magnetic field of zero. The dimensions of the coil including insulation are 4.5 × 5.2 cm. in cross section and 4.5 cm. in length. The electromagnet used was built to specifications by the Hammond Manufacturing Company, Guelph, Ontario. It was mounted with its magnetic axis coinciding with the optic axis of the spectrograph, and with the end nearer the arc 6 cm. from the arc. The end nearer the arc was insulated with asbestos board. A metal support was constructed so that the electromagnet can be swung away from the arc stand to facilitate loading and positioning of the electrodes (see Figure 2).

ADJUSTMENT AND OPERATION OF INSTRUMENT

Phototube Housing. The distance between the slit openings normally should be such that the two slits are just inside the vertical edges of the arc's visible image. Various obvious factors determine the most efficient distance between slits, so that it is necessary for each spectroscopist to find the best distance for his particular set of conditions and type of sample. The writer has analyzed a variety of ores without having to change the distance.

The optimum width of each slit opening will depend on the amount, and wave lengths, of light reaching the phototubes. Naturally, both slits should be of the same width. For a variety of ores the writer has found that a slit opening 0.15 cm. wide is satisfactory for both cup-shaped and "platform" electrodes. If the slit openings are too wide, if the slits are too far apart, if the magnet is too near the arc, or if the amplifier has too high a "gain," the arc appears to fan out into a wide fan-shaped discharge, the image of which extends beyond both slits (even then wandering of the arc is reduced). Therefore, to use a normally shaped arc, it is necessary that each spectroscopist adjust the slits or other variables mentioned, so as just to prevent the fan-shaped arc from forming.

The apparent fanning out of the arc is due to an oscillation which produces a rapid and symmetrical sideways oscillation of the arc. This symmetrical oscillation may improve precision for certain metals, as the entire width of the arc is being surveyed many times a second.

After the switch in the alternating current line is closed, the amplifier is allowed to warm up for 5 minutes. Then resistors R_1 and R_2 are adjusted so that equal currents flow in coils D and E when the arc is not operating. The writer uses a current of 30 milliamperes, which drops to about 23 milliamperes when the arc

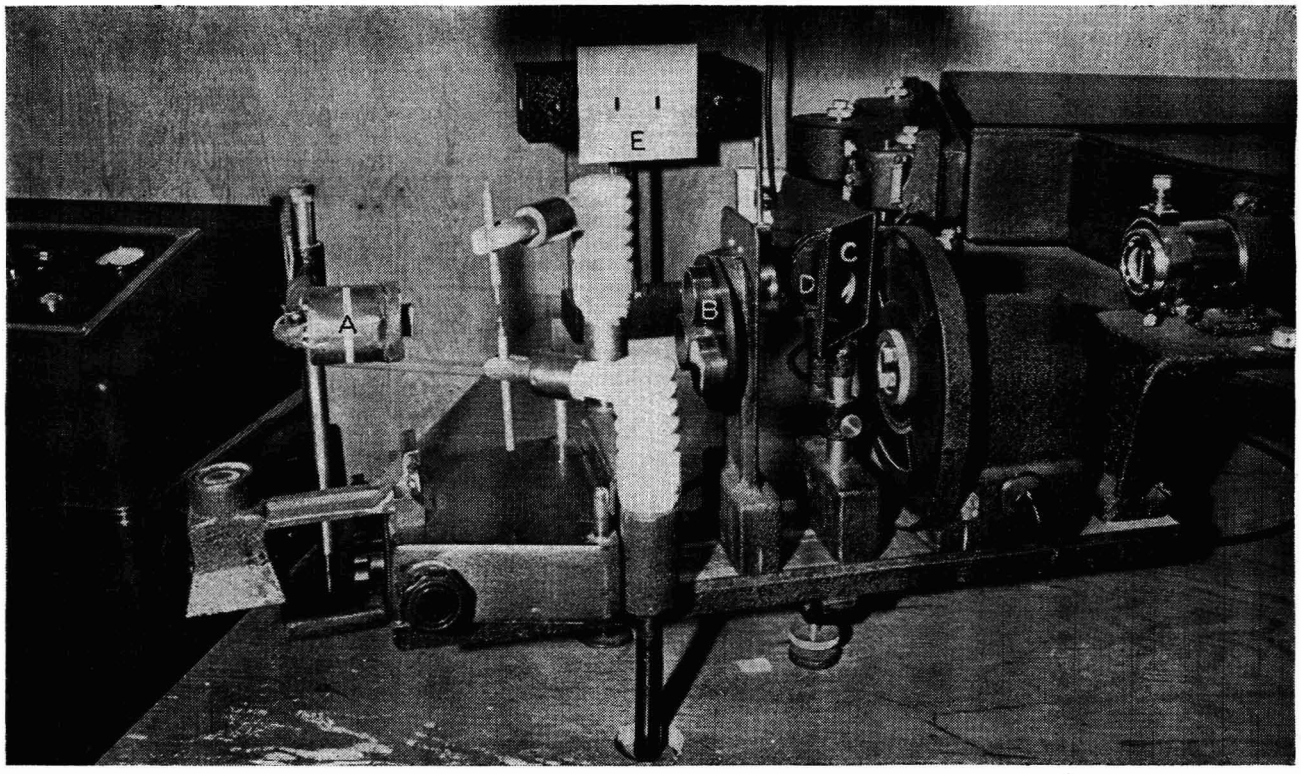

Figure 2. Stabilizer in Position at Spectrograph

A. Electromagnet *B.* Quartz lens *C.* Aluminized mirror *D.* Glass lens *E.* Phototube housing

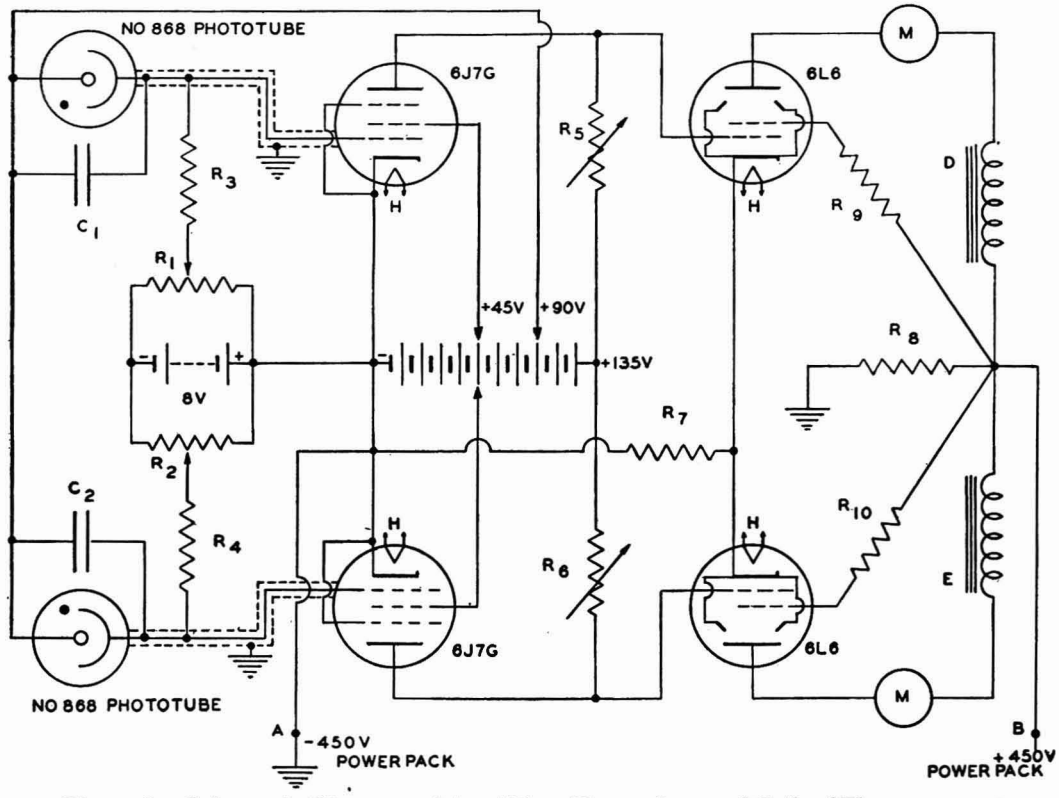

Figure 3. Schematic Diagram of Amplifier, Phototubes, and Coils of Electromagnet

R_1, R_2.	1-megohm potentiometers	R_9, R_{10}.	25,000 ohms
R_3, R_4.	4.7 megohms	D, E.	4000 ohms
R_5, R_6.	250,000 ohms (variable)	C_1, C_2.	Capacitors, 0.0001 mfd.
R_7.	2,000 ohms, 10 watts	M, M.	D.C. milliammeters, 0–50 ma.
R_8.	100,000 ohms		

Table I. Spectral Line Intensities and Variances for Sample A (Limestone)

(28 samples averaged)

Metal	Mg	Mn	Fe
Chemical analysis, %	1.16	0.032	0.11
Wave length of line, Å.	2776.7	2801.1	3020.6
Stabilizer not operating			
Average intensity	14.2	18.2	20.6
Variance	6.47	7.85	11.7
Stabilizer operating			
Average intensity	17.7	21.7	25.2
Variance	3.34	3.84	5.54
F value	1.94	2.04	2.11

Table II. Spectral Line Intensities and Variances for Sample B (Complex Sulfide)

Metal	Cu	Pb	Ti	Ni	Bi	Mn	Ca
Chemical analysis, %	1.49	2.07	0.06	0.02	0.03	0.45	7.69
Wave length of line, Å.	2961.2	2873.3	3372.8	3050.8	3067.7	3442.0	3181.3
Stabilizer not operating[a]							
Average intensity	14.4	11.9	18.7	9.0	10.2	4.37	6.8
Variance	24.4	5.52	19.0	6.81	6.55	1.54	4.65
Stabilizer operating[b]							
Average intensity	19.9	16.5	21.7	11.7	14.3	5.18	10.34
Variance	9.58	3.84	4.12	3.87	5.23	0.570	1.64
F value	2.55	1.44	4.61	1.76	1.25	2.70	2.84

[a] 28 samples averaged.
[b] Originally 28 samples, but one rejected because most of it fell off electrode during first 5 seconds of arcing.

Table III. Spectral Line Intensities and Variances for Sample C (Fluorspar)

(28 samples averaged)

Metal	Pb	Zn	Sn
Chemical analysis, %	0.23	0.35	Trace
Wave length of line, Å.	2873.3	3282.3	3175.0
Stabilizer not operating			
Average intensity	20.0	9.9	2.33
Variance	27.2	8.73	0.124
Stabilizer operating			
Average intensity	14.3	8.29	2.46
Variance	9.35	3.47	0.119
F value	2.91	2.52	1.04

is operating. From time to time, the milliammeter readings should be observed, and if unequal, should be equalized by changing R_1 or R_2; but this adjustment should not be made while the arc is operating.

Experimental Tests for Precision of Analyses. In the writer's laboratory, spectrochemical analyses are confined to powdered ore and mineral samples. Two shapes of graphite electrodes are used: the common cup-shaped electrode and the platform electrode described by Hasler and Harvey (*1*).

To determine whether the magnetic stabilizer improves the precision of quantitative analyses, 56 identical samples were weighed out onto electrodes; 28 were arced with the stabilizer operating, and 28 without use of the stabilizer. Otherwise, each group of 28 was treated in as nearly an identical way as possible, with respect to arcing, processing of film, and obtaining intensities of chosen spectral lines. Standard precautions were taken to render the experimental results as objective as possible. Samples were weighed by an assistant who did not know which were to be used with the magnetic stabilizer operating; electrode spacing and current were not maintained constant by manipulation during arcing. Densitometer readings were taken by another assistant who was not told which spectrograms were obtained using the magnetic stabilizer.

Three different kinds of powdered samples were selected for the tests. Referring to Tables I, II, and III, sample A was a limestone; sample B was a mixture of silicates and sulfides; and sample C was fluorspar, Bureau of Standards sample No. 79. Platform electrodes were used for samples A and B, and cup-shaped electrodes for sample C. Electrodes were not prearced.

For platform electrodes, 10 mg. of sample A, or 8 mg. of a 1 to 1 mixture of sample B and lithium carbonate, were distributed on the platform of the electrode. A small drop of grain alcohol was added, then one drop of a 4% aqueous solution of sucrose, after which the electrodes were dried at about 100° C. For cup-shaped electrodes, 10 mg. of sample C were placed in the cup, and not treated further. The sensitivity of the balance used was 0.03 mg.

The upper and lower electrodes were centered on the optical axis, and were arranged vertically to give an electrode spacing of 1.0 cm. After striking the arc this electrode spacing was allowed to increase through burning. The current of 10 amperes was supplied by an A.R.L.–Dietert rectifier unit supplying 250 volts direct current. Samples A and B were arced until all the sample had vaporized; sample C was arced for 45 seconds, as moving film studies had shown that all the lead, zinc, and tin had vaporized before then.

The arc was brought to a focus at the grating. The spectrograph slit was 0.05 mm. wide. A rotating pie-sector at the Sirk's focus of the grating was adjusted to transmit 7.5% of the incident light for samples A and B, and 25% for sample C.

The slits of the stabilizer's phototube housing were 4.9 cm. apart and 0.15 cm. wide for samples A and B; for sample C the slits were 4.4 cm. apart and 0.08 cm. wide.

Per cent transmittance of spectral lines and adjacent backgrounds were read on an A.R.L.–Dietert densitometer. Then from characteristic curves the intensities of these spectral lines were obtained, and corrected for background in the usual way. These intensities are arbitrary in that 99% transmittance was chosen to represent unit intensity for Table I, 98% for Table II, and 97% for Table III.

Statistics computed from the experimental results are presented in Tables I, II, and III. The arithmetic means of the intensities of several spectral lines are given, and for each arithmetic mean the variance has been computed. Variance is the square of the standard deviation (σ), and an unbiased estimate of the population variance is given by the formula

$$u^2 = \frac{\sum_{\alpha=1}^{N}(x_\alpha - \bar{x})^2}{N-1} \qquad (1)$$

where $x\alpha$ denotes the αth of a series of N measurements (spectral line intensities), and $\bar{x}$ denotes their arithmetic mean. Thus the variance is a measure that shows the amount of dispersion among a set of spectral line intensities.

Given also in the tables are the F values. This statistic is defined by the formula

$$F = \frac{u^2}{v^2} \qquad (2)$$

where u^2 is the variance for a spectral line obtained with the magnetic stabilizer not operating, and v^2 is the variance for the same spectral line obtained with the stabilizer operating.

Each variance reported in Table II is the weighted average of the variances of four sets of seven samples each (six in one case). Hence for the set of 28 samples obtained with the stabilizer not operating there are 24 degrees of freedom, and for the set of 27 obtained with it operating there are 23 degrees of freedom. But first, tests were made for homogeneity within each of the four groups of seven, at the 10% probability level. As all pairs of groups satisfied the test at this level of significance, including even the pair of groups with maximum and minimum variances, it is valid to assume homogeneity of the variance within each set of groups. Consequently it is permissible here to average the variances of the four sets of seven samples, and to apply the F test to decide whether there is a significant difference between the variance obtained with the stabilizer operating and that obtained without it operating (and if there is a difference, to ascribe it to the action of the stabilizer).

In quantitative spectrochemical analysis it is usual to use an internal standard, and to obtain the intensity ratio of two spectral lines, one being of the sought-for metal, and the other of the internal standard. It was therefore of interest to determine whether the use of the magnetic stabilizer improved the precision

of intensity ratios. The spectral line intensities used in the preparation of Tables I, II, and III were available for this purpose. Therefore intensity ratios, variances, and F values were computed for certain metal pairs, and these statistics are given in Tables IV, V, and VI.

In Tables I to VI, variances and F values have been reported to three significant figures. For the averages, two doubtful figures have been retained, these being arrived at by inspection of the probable error expressed to two significant figures.

INTERPRETATION AND DISCUSSION OF RESULTS

The variances given in Tables I to VI make possible comparison of the precisions of intensity measurements of spectral lines obtained with and without the use of the magnetic stabilizer, inasmuch as variance is a measure of the degree of precision of a method. But because these variances are only sample estimates, comparison of two variances is proper only if a method of comparison is used which takes into account their possible fluctuations. For this reason the F values were computed, as these values, when compared with a table of F (*6*), enable a decision to be made as to whether or not the magnetic stabilizer improved precision. A concise description of this sort of statistical analysis has been given by Mandel (*4*).

For 27 and 27 degrees of freedom, a table of F gives
$F = 1.90$ is the 5% critical point
$F = 2.51$ is the 1% critical point
For 27 and 26 degrees of freedom,
$F = 1.92$ is the 5% critical point
$F = 2.54$ is the 1% critical point
For 24 and 23 degrees of freedom,
$F = 2.00$ is the 5% critical point
$F = 2.70$ is the 1% critical point

With this information it is possible to interpret properly the data in Tables I to VI, and the interpretation is given concisely in Table VII. There are indications that the degree to which the stabilizer improves precision depends upon the speed with which the metal vaporizes in the arc, and hence upon the shape of the electrode holding the sample. Thus although the magnetic stabilizer did not produce a significant improvement in precision for the lead of sample B arced on platform electrodes, it did produce a highly significant improvement in precision for the lead of sample C (Table III) arced in 0.6 cm. (0.25 inch) deep crater electrodes.

Table IV. Ratios of Spectral Line Intensities for Sample A (Limestone)

(28 samples)

Metals	Mg/Mn	Mn/Fe
Stabilizer not operating		
Average ratio	0.778	0.891
Variance	0.00358	0.00472
Stabilizer operating		
Average ratio	0.813	0.867
Variance	0.00172	0.00387
F value	2.08	1.22

Table V. Ratios of Spectral Line Intensities for Sample B (Complex Sulfide)

Metals	Ti/Mn	Cu/Ni	Pb/Bi
Stabilizer not operating[a]			
Average ratio	4.45	1.58	1.20
Variance	1.02	0.0354	0.0288
Stabilizer operating[b]			
Average ratio	4.27	1.70	1.169
Variance	0.432	0.0233	0.0142
F value	2.36	1.52	2.03

[a] 28 samples.
[b] 27 samples.

Table VI. Ratios of Spectral Line Intensities for Sample C (Fluorspar)

(28 samples)

Metals	Pb/Zn	Pb/Sn
Stabilizer not operating		
Average ratio	2.11	8.6
Variance	0.322	4.81
Stabilizer operating		
Average ratio	1.79	5.89
Variance	0.256	1.98
F value	1.26	2.43

Table VII. Probability Levels at Which Improvement in Precision of Analyses Gained by Magnetic Stabilizer Is Significant

	Table I			Table II							Table III		
Metal	Mg	Mn	Fe	Cu	Pb	Ti	Ni	Bi	Mn	Ca	Pb	Zn	Sn
Probability level, %	5	5	5	5	—	1	—	—	1	1	1	1	—

	Table IV		Table V			Table VI	
Metal pair	Mg/Mn	Mn/Fe	Ti/Mn	Cu/Ni	Pb/Bi	Pb/Sn	Pb/Zn
Probability level, %	5	—	5	—	5	5	—

Dash indicates no significant improvement in precision at 5% probability level.

SUMMARY

An accessory has been described which, by an electronically controlled magnetic field, practically eliminates the random wandering of a horizontal slice of a direct current arc. It has been proved statistically that this accessory produced an improvement in the precision of quantitative spectrochemical analyses; and it is suggested that the degree of improvement may depend on the shape of electrode used, particularly for the most volatile metals. Examples have been given where the accessory did not produce a statistically significant improvement in precision; these failures were confined to some of the most volatile metals studied. In certain cases the accessory also improved the precision of the intensity ratios of chosen pairs of spectral lines.

Although this paper has considered only the direct current arc, it appears feasible to use the accessory to stabilize an alternating current arc, after first modifying the amplifier slightly and then feeding alternating current instead of direct current to the plates of the 6L6 tubes. A phase-shifting device in the primary of the transformer supplying the alternating current would be required, so that the current in the arc and in the coils of the electromagnet would be in phase.

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Errors in the Use of a Model 18 Perkin-Elmer Flame Photometer for the Determination of Alkali Metals

Interference of Common Metals, Acids, and Solvents

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A model 18 Perkin-Elmer flame photometer is useful for the rapid determination of sodium and potassium in solutions of restricted composition, but its direct use is subject to serious errors which are variously produced by certain common substances possibly present in the test solution. Very large negative errors are caused by appreciable concentrations of phosphate, borate, and oxalate ions, and by very high concentrations of mineral acids; large errors are produced by ammonium, alkali, alkaline earth, and other cations, particularly when present in high concentrations. Occasionally, compensating errors are realized.

THE use of flame photometry (*1*) for the rapid and selective determination of metals, particularly alkali metals, is finding increasing acceptance and is slowly but definitely displacing more troublesome methods in the analysis of many materials, such as plants, biological products, oils, catalysts, and inorganic residues. The most commonly used instrument for this purpose at present is the Model 18 Perkin-Elmer flame photometer (*5*), based on the original design published by Barnes, Richardson, Berry, and Hood (*1*). This instrument photoelectrically measures the color imparted to the flame of a Meker-type gas burner, into the air-intake of which is passed an atomized mist of the test solution under carefully controlled conditions; selective color filters are used to isolate the characteristic light, imparted to the flame by the desired metal, before its intensity is measured photoelectrically. The concentration of the metal in the test solution is determined from calibration curves constructed from results obtained under identical conditions with standard solutions of suitable metal salt content.

A Model 18 Perkin-Elmer flame photometer has been extensively used in these laboratories in research and service analysis of solutions from a variety of sources and containing various concentrations of common salts, acids, and solvents. It was found to be rapid and convenient for the determination of small concentrations of sodium and potassium in aqueous solutions free of interfering substances. It has proved of great value in the checking and qualitative interpretation of alkali metal results obtained by the polarographic method (*6*), which does not distinguish between sodium or potassium. However, this flame photometer has given erroneous results for solutions that contained certain interfering substances.

Possible errors in the direct flame photometric determination of alkali metals in presence of other substances have been pointed out by others (*2–5*). The instruction manual (*5*) supplied with the Model 18 Perkin-Elmer instrument mentions that errors may be caused by light imparted to the gas flame by atoms or molecules other than the element desired but it states that these effects are in general small and gives no useful indication of the order of magnitude of the interference expected from various materials. In describing their improved flame photometer, employing an internal standard technique, Berry, Chappell, and Barnes (*2*) reported that the introduction of certain extraneous ions into the test solution gave serious errors with a flame photometer similar to the Model 18 Perkin-Elmer instrument. They also compared graphically the error produced on the old (*1*) and improved (*2*) flame photometers by changes in physical conditions of the test and by the presence of certain foreign substances in the test solution. Hald (*3*) states that, when a filter-type flame photometer is used, certain foreign substances interfere and that it is necessary to determine the effect of any ion present in non-physiological concentration in order to be able to get accurate results. Recognizing the possible interfering effect of one element on a reading obtained for another element, Berry, Chappell, and Barnes (*2*) and Overman and Davis (*4*) recommend the use of calibration solutions made to contain the same relative concentration of materials expected in the unknown solution. They also state that acid oxidation procedures are not satisfactory means for preparing the sample test solution because the ammonium salts, formed in neutralizing the excess acids, tend to depress the calibration curves for sodium and potassium.

None of the publications gives adequate information concerning the magnitude of the interference in the determination of sodium and potassium by extraneous materials that find their way into the test solution prepared from common materials. Therefore, an investigation was made to determine the nature and quantitative extent of the possible errors produced by common substances found in solutions obtained in the decomposition of a variety of materials. Because it was the only instrument available at the time, the tests were made with a Model 18 Perkin-Elmer flame photometer. Confirming the finding of others (*2, 3, 4*), it was found that accurate results were obtained only on solutions of restricted compositions, when simple salt solutions were used for calibration. For the sake of completeness of data, results are reported in this paper which, in a few cases, are a partial duplication of work previously reported by others (*2, 5*).

EXPERIMENTAL

The procedure used for the tests was based on that published by Perkin-Elmer Corporation in the instruction manual for the instrument (*5*).

The instrument was turned on and allowed to warm up for an hour before taking readings. The burner and air pressure were adjusted, the required filter was put in place, and distilled water was introduced into the atomizer to obtain a zero reading. The sensitivity was then adjusted by introducing a solution containing 100 p.p.m. of the chloride salt of the metal to be determined and varying the control rheostat to give a galvanometer reading of 100. Standard solutions, containing 20, 40, 60, and 80 p.p.m. of the chloride salt of the metal to be determined, were introduced into the atomizer and five readings were recorded for each solution. The average readings were plotted against the concentration of the alkali used.

For each substance tested for interference, a series of solutions was prepared containing several known concentrations of the test substance and, generally, 80 p.p.m. of sodium or potassium. Because acetic acid, ethyl alcohol, and several cations caused off-scale readings when present in high concentrations, the amount of alkali metal was reduced to 40 p.p.m. when testing these materials.

Table I. Error Caused by Sulfuric, Hydrochloric, and Nitric Acids in Determination of Sodium and Potassium by Flame Photometer

Acid Present	Concentration, Moles/Liter	Sodium, P.P.M. Added	Found	Error	Potassium, P.P.M. Added	Found	Error
Sulfuric	1.80	80	49	−31	80	46	−34
	0.90		55	−25		54	−26
	0.18		62	−18		63	−17
	0.09		72	−8		66	−14
	0.02		77	−3		72	−8
Hydrochloric	1.20	80	61	−19	80	60	−20
	0.60		70	−10		62	−18
	0.12		78	−2		67	−13
	0.06		79	−1		77	−3
	0.01		81	+1		80	0
Nitric	1.60	80	56	−24	80	57	−23
	0.80		69	−11		78	−2
	0.16		78	−2		79	−1
	0.08		81	+1		80	0
	0.02		79	−1		80	0

Effect of Sulfuric, Hydrochloric, and Nitric Acids. The effect of sulfuric, hydrochloric, and nitric acids was first determined, as these are often used for the solution of inorganic residues. A series of several concentrations of each of the acids was prepared with 80 p.p.m. of sodium and a similar series with 80 p.p.m. of potassium. Five readings were taken for each solution, the apparent alkali concentration was obtained from the calibration curve, and the average values were recorded. The results obtained are given in Table I. Sulfuric acid caused the greatest errors, nitric acid the least. In each case, the experiments showed that indiscriminate use of the acids in a solution would result in appreciable, unknown errors in the flame photometric determination of the alkali metals.

Effect of Phosphoric, Boric, and Oxalic Acids. Because phosphate, borate, and oxalate ions are often present in solutions analyzed for sodium and potassium, the effect of their acids was also studied. As shown in Table II, phosphoric, boric, and oxalic acids caused much greater errors than equivalent concentrations of sulfuric, hydrochloric, and nitric acids. Two per cent of phosphoric acid caused errors of 50% or more in the determination of both alkalies, whereas as little as 0.01% of the acid caused

Table II. Error Caused by Phosphoric, Boric, and Oxalic Acids in Determination of Sodium and Potassium by Flame Photometer

Acid Present	Concentration, Moles/Liter	Sodium, P.P.M. Added	Found	Error	Potassium, P.P.M. Added	Found	Error
Phosphoric	2.90	80	13	−67	80	4	−76
	1.45		22	−58		6	−74
	0.29		39	−41		23	−57
	0.15		44	−36			
	0.03		54	−26		56	−24
	0.015		59	−21		65	−15
	0.003		62	−18			
	0.001		69	−11		73	−7
Boric	1.60	80	44	−36	80	39	−41
	0.80		53	−27		50	−30
	0.16		59	−21		63	−17
	0.02		64	−16		74	−6
	0.002		79	−1		77	−3
Oxalic	1.10	80	36	−44	80	53	−27
	0.60		56	−24		62	−18
	0.11		59	−21		65	−15
	0.01		62	−18		72	−8
	0.001		68	−12		75	−5

Table III. Cations Tested for Effect on Alkali Determination by Flame Photometer

Cation Tested	Compound	Reagent Used for Solution	Source
Aluminum	Metal	Hydrochloric acid	C.P., Baker's Analyzed
Ammonium	$(NH_4)_2CO_3$	Nitric acid	C.P., Baker's Analyzed
Barium	$Ba(NO_3)_2$	Water	Anal. Reagent, Mallinckrodt
Calcium	CaCO	Nitric acid	Merck, A.C.S.
Cesium	CsCl	Water	C.P., Eimer and Amend
Chromium	CrO_3	Water	C.P., Baker's Analyzed
Cobalt	$CoCl_2.6H_2O$	Water	C.P., Baker's Analyzed
Copper	Metal	Nitric acid	Anal. Reagent, Mallinckrodt
Iron	Metal	Nitric acid	C.P., Baker's Analyzed
Lead	Metal	Nitric acid	C.P., Braun-Knecht-Heimann
Lithium	Li_2CO_3	Hydrochloric acid	C.P., Baker's Analyzed
Magnesium	Metal	Hydrochloric acid	C.P., Braun-Knecht-Heimann
Manganese	$MnCl_2.4H_2O$	Water	C.P., Baker's Analyzed
Molybdenum	MoO_3	Water	Merck, A.C.S.
Nickel	$NiCl_2.6H_2O$	Water	C.P., Baker's Analyzed
Potassium	KCl	Water	Anal. Reagent Mallinckrodt
Rubidium	RbCl	Water	C.P., Eimer and Amend
Sodium	NaCl	Water	Anal. Reagent, Mallinckrodt
Strontium	$SrCO_3$	Hydrochloric acid	C.P., Eimer and Amend
Tellurium	Metal	Nitric acid	C.P., Eimer and Amend
Tungsten	H_2WO_4	Ammonium hydroxide	C.P., B. and A.
Zinc	Metal	Nitric acid	C.P., Baker's Analyzed

Table IV. Effect of Cations in Determination of Sodium by Flame Photometer

Cation Tested	Concentration, P.P.M.	Sodium, P.P.M. Added	Found[a]	Added	Found	Error
Aluminum	10,000	0	0	80	48	−32
	1,000		0		53	−27
	100		0		68	−12
Ammonium	10,000	0	0	80	55	−25
	1,000		0		60	−20
	100		0		78	−2
Barium	10,000	0	5	80	83	+3
	1,000		0		79	−1
	100		0		79	−1
Calcium	10,000	0	10	80	57	−23
	1,000		0		78	−2
	10		0		80	0
Cesium	10,000	0	0	80	78	−2
	1,000		0		80	0
	100		0		79	−1
Chromium	10,000	0	13	80	65	−15
	1,000		0		72	−8
	100		0		78	−2
Cobalt	10,000	0	3	80	55	−25
	1,000		0		80	0
	100		0		80	0
Copper	10,000	0	3	80	59	−21
	1,000		0		68	−12
	100		0		79	−1
Iron	10,000	0	14	80	91	+11
	1,000		0		82	+2
	100		0		78	−2
Lead	10,000	0	3	80	54	−26
	1,000		0		65	−15
	100		0		76	−4
Lithium	10,000	0	47	80	93	+13
	1,000		13		78	−2
	100		0		80	0
Magnesium	10,000	0	0	80	45	−35
	1,000		0		63	−17
	100		0		77	−3
Manganese	10,000	0	0	80	61	−19
	1,000		0		76	−4
	100		0		79	−1
Molybdenum	10,000	0	0	80	77	−3
	1,000		0		79	−1
	100		0		80	0
Nickel	10,000	0	8	80	61	−19
	1,000		5		77	−3
	100		0		80	0
Potassium	10,000	0	0	80	62	−18
	1,000		0		78	−2
	100		0		79	−1
Rubidium	10,000	0	0	80	78	−2
	1,000		0		79	−1
	100		0		75	−5
Strontium	10,000	0	45	40	79	+39
	1,000		11		48	+8
	100		0		40	0
Tellurium	10,000	0	0	80	52	−28
	1,000		0		58	−22
	100		0		79	−1
Tungsten	10,000	0	0	80	65	−15
	1,000		0		79	−1
	100		0		80	0
Zinc	10,000	0	0	80	76	−4
	1,000		0		79	−1
	100		0		80	0

[a] Represents error when no sodium is present.

appreciable errors (14 and 9% for sodium and potassium, respectively). Boric and oxalic acids gave similar but smaller effects.

Effect of Cations. The effect of cations was tested for possible interference in determining sodium or potassium by the flame photometer method. The cations tested are listed in Table III, together with the compounds used, their sources, and the reagents used for preparing the solutions. Wherever possible the original material was dissolved in water, or first dissolved in an appropriate reagent, taken to dryness, and dissolved in water. Each element under test was prepared in two sets of three concentrations, 100, 1000, and 10,000 p.p.m. One set was used as a blank, and known amounts of sodium or potassium were added to the other. In those cases where the cation caused an off-scale reading, a new series was prepared containing a smaller amount of alkali metal. The data for the sodium tests are given in Table IV and for the potassium tests in Table V.

The magnitude and direction of the error caused by the various cations do not appear to be predictable, either from a consideration of the alkali blank readings or from a comparison of the effect of the same cation on sodium and potassium. Some cations, which gave high readings when no alkali was present, caused negative errors in the presence of sodium or potassium. Some cations caused a positive error with sodium and a negative error with potassium, and vice versa.

Effect of Ethyl Alcohol and Acetic Acid. In checking service samples analyzed by the polarographic method (*6*), it was found that a series of solutions containing ethyl alcohol gave very much higher sodium values by the flame photometric method than by the polarographic method. A similar error with methanol has been reported by Berry, Chappell, and Barnes (*2*). This led to an investigation of the effect of alcohol and acetic acid on the flame photometer readings. As shown in Table VI, a positive error was found in all cases where the amount of ethyl alcohol or acetic acid was greater than 1%.

Combined Effect of Phosphoric Acid with Ethyl Alcohol or Acetic Acid. Inasmuch as phosphoric acid caused negative errors and acetic acid and alcohol caused positive errors, tests were made to determine the extent to which these effects would compensate each other and to see if the degree of compensation were predictable from the composition. In general, the results obtained, given in Table VII, for solutions containing phosphoric acid and either alcohol or acetic acid indicate that some compensation does occur, but that the degree of compensation is not predictable.

DISCUSSION

There are several possible explanations for the large errors found in the determination of sodium and potassium by the flame photometer. The shape and temperature of the flame cone, the

Table V. Effect of Cations in Determination of Potassium by Flame Photometer

Cation Tested	Concentration, P.P.M.	Potassium, P.P.M. Added	Found[a]	Added	Found	Error
Aluminum	10,000	0	0	80	41	−39
	1,000		0		64	−16
	100		0		77	−3
Ammonium	10,000	0	0	80	69	−11
	1,000		0		73	−7
	100		0		79	−1
Barium	10,000	0	35	80	95	+15
	1,000		10		86	+6
	100		0		80	0
Calcium	10,000	0	0	80	67	−13
	1,000		0		76	−4
	100		0		81	+1
Cesium	10,000	0	100	40	100	+60
	1,000		48		66	+26
	100		10		52	+12
Chromium	10,000	0	17	80	74	−6
	1,000		0		78	−2
	100		0		80	0
Cobalt	10,000	0	8	80	49	−31
	1,000		0		66	−14
	100		0		73	−7
Copper	10,000	0	0	80	49	−31
	1,000		0		64	−16
	100		0		80	0
Iron	10,000	0	12	80	59	−21
	1,000		5		67	−13
	100		0		80	0
Lead	10,000	0	0	80	45	−35
	1,000		0		78	−2
	100		0		81	+1
Lithium	10,000	0	18	80	52	−28
	1,000		0		80	0
	100		0		80	0
Magnesium	10,000	0	0	80	46	−34
	1,000		0		72	−8
	100		0		80	0
Manganese	10,000	0	0	80	51	−29
	1,000		0		68	−12
	100		0		73	−7
Molybdenum	10,000	0	0	80	34	−46
	1,000		0		62	−18
	100		0		69	−11
Nickel	10,000	0	8	80	50	−30
	1,000		0		60	−20
	100		0		70	−10
Rubidium	10,000	0	> 100	40	> 100	> +60
	1,000		> 100		> 100	> +60
	100		41		58	+18
Sodium	10,000	0	34	80	61	−19
	1,000		11		67	−13
	100		0		80	0
Strontium	10,000	0	7	80	60	−20
	1,000		0		81	+1
	100		0		82	+2
Tellurium	10,000	0	11	80	52	−28
	1,000		0		75	−5
	100		0		81	+1
Tungsten	10,000	0	0	80	61	−19
	1,000		0		80	0
	100		0		80	0
Zinc	10,000	0	0	80	56	−24
	1,000		0		64	−16
	100		0		79	−1

[a] Represents error when no potassium is present.

Table VI. Error Caused by Ethyl Alcohol and Acetic Acid on Determination of Sodium by Flame Photometer

Ethyl Alcohol, Ml./100 Ml. of Solution	Acetic Acid, Moles/Liter	Sodium, P.P.M. Added	Found
20	...	0	0
20	...	80	> 100
20	...	40	53
10	...	80	> 100
10	...	40	52
5	...	80	98
5	...	40	47
1	...	80	81
1	...	40	40
...	3.60	0	0
...	3.60	40	63
...	1.80	40	50
...	0.90	40	48
...	0.18	40	41

Table VII. Combined Effects of Phosphoric Acid and Alcohol (or Acetic Acid) on Determination of Sodium by Flame Photometer

Phosphoric Acid, Moles/Liter	Alcohol Ml./100 Ml. of Solution	Acetic Acid, Moles/Liter	Sodium, P.P.M. Added	Found	Error
0.75	20	0	80	55	−25
0.75	10			49	−31
0.75	0			27	−53
0.15	20	0	80	67	−13
0.15	10			64	−16
0.15	5			56	−24
0.15	0			44	−36
0.015	10	0	80	76	−4
0.015	5			75	−5
0.015	0			59	−21
0.75	0	1.80	40	26	−14
0.15		3.50		38	−2
0.15		1.80		37	−3
0.015		1.80		40	0
0.015		0.90		40	0

size of the droplets of the mist entering the flame, the surface tension and viscosity of the solutions, and the complexing action of the ions on the alkali metal ions are possible contributing factors. The results presented above indicate that a rather large number of commonly encountered cations, anions, and organic compounds may cause serious interference in the determination of sodium or potassium by the flame photometer if present in certain concentrations. This emphasizes the need for careful consideration of the history and probable composition of every sample analyzed by this method.

Berry, Chappell, and Barnes (*2*) report that it is possible to correct the errors caused by foreign molecules by compounding the standard solutions used in calibrating the instrument in such a manner that these standards contain quantities of interfering substances in proportion to those in the solutions to be analyzed. Others, in using this technique, found that the presence of large amounts of foreign salts made the determination impractical (*4*). The authors' experience in using this modification confirms the latter work and indicates that accurate results are obtained only on relatively simple solutions. Generally, complex substances of unknown composition have been accurately analyzed only after removal of the interfering substances.

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Determination of Mannose

Mannans in Hardwoods

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The Hägglund-Bratt method for the determination of mannose and mannans in wood was examined critically, modified, and applied to four hardwoods, all of which were shown to contain small amounts of mannan. In at least one case (aspen), it was shown that mannose units were present in hardwood α-cellulose. Cotton linters, on hydrolysis, gave no mannose.

THE term "mannan," as used by wood chemists, does not necessarily refer to a definite homopolysaccharide like the mannan of vegetable ivory. It simply implies that a hemicellulose (or cellulose) fraction yields appreciable amounts of mannose on acid hydrolysis. Whether or not true homogeneous mannose chains pre-exist in wood is still unknown. There are no convincing experimental data either for or against such a hypothesis. Hess and Lüdtke (*7*) are the only chemists who have claimed the isolation of a pure mannan from spruce sulfite pulp (identical in x-ray diagram and $[\alpha]_D$ with that of vegetable ivory). Whereas they reported that this product yielded only mannose on hydrolysis, they gave no experimental details or yield figures; hence, their work cannot be accepted without repetition and amplification. About 22 years ago, Schorger (*16*), in discussing the mannans in wood, stated that "the relationship of certain hexosans to the 'normal cellulose' is still only a matter of individual opinion." This holds true today. Whether or not mannose units are linked to each other or to some other sugar units is as yet undecided.

Wheeler and Tollens (*23*) were the first to suggest that mannose-yielding components were probably present in wood. Later, Bertrand (*1*) showed the presence of mannan (termed mannocellulose) in the wood of various gymnosperms. Other early investigators who found mannose were Kimoto (*9*) and Storer (*21*). Much later, Sherrard and his co-workers (*18, 20*) found that mannans were present in Cross and Bevan cellulose and in α-cellulose, as well as in sulfite pulp. Cotton, on the other hand, yielded no mannose. An interesting observation of Sherrard's (*19*) was that Cross and Bevan cellulose from white spruce, on long extraction with hot water, gave a polysaccharide which, on hydrolysis, yielded about 30% mannose and 20% pentoses. Unfortunately, this intriguing carbohydrate was not investigated further.

Whereas the occurrence of mannans in the softwood has never been questioned, its presence in the hardwoods has been much less certain. Schorger (*17*) reported its absence from basswood, sugar maple, yellow birch, white ash, and aspen. Heuser and Brötz did not find mannan in *Populus tremula* (*8*), although Fromherz (*3*) and Koch (*11*) had previously reported its presence in this wood. Recently, Thomas (*22*) failed to find mannans in a sample of aspen. Dore (*2*) reported its absence from California live oak (*Q. agrifolia* Nee). Storer (*21*) found mannans in certain hardwoods but absent from others. On the other hand, Nishida, Hamashima, and Fukai (*12*) found mannans in small amounts in all the Japanese hardwoods examined by them. O'Dwyer (*14*) found mannans in the hemicellulose from white oak. Probably these contradictory data are due not so much to the irregularity of occurrence of the mannans in angiosperms as to the methods used in estimating mannose.

A determination of mannan in woods, devised by Schorger (*17*), depends upon the hydrolysis of mannans to mannose by means of 5% hydrochloric acid, followed by neutralization with sodium carbonate, concentration of the solution, and precipitation of the mannose as the phenylhydrazone. Hägglund and Klingstedt (*5*), after making a critical study of Schorger's method, showed that his results were low and that a portion of the mannan in wood was left unhydrolyzed. These findings were confirmed by Nishida, Hamashima, and Fukai (*12*). On the basis of extensive experiments, Nowotnowna (*13*) also indicated that Schorger's method gave low results. However, her own data are open to question, inasmuch as she also used a modified 5% hydrochloric acid in the hydrolysis of wood or cellulose, and there is no assurance that her techniques permitted complete conversion into mannose of the more resistant mannans in wood cellulose. Subsequently, Hägglund and Bratt (*4*) employed a modified mannan analysis (using 72% sulfuric acid in place of 5% hydro-

chloric acid) and their technique, with slight modifications, has been used by many wood analysts.

The practical significance of mannans in wood pulp and their possible influence on sheet properties have been discussed recently (*24*). In certain instances at least, mannans in small amounts have been found in rayon made from coniferous pulps (*6*). The important role played by mannogalactans (like guar mucilage) has been described by Rowland (*15*). There is reason to believe that the configuration of the mannose units in wood may be of some importance in determining the use of chemical wood products. To date, however, the data are so fragmentary that no generalizations can be made.

The present investigation had the following objectives: a critical evaluation of the Hägglund-Bratt method for determining mannans in wood, followed by modifications in the procedure and the qualitative and quantitative assays for mannan in several hardwoods. This work will be followed by a report on the distribution of the mannan of black spruce and slash pine over various hemicellulose fractions and in their respective α-cellulose residues; a study of the effect of progressive acid hydrolysis on the mannan content of slash pine α-cellulose; and an orienting acetolysis study on slash pine α-cellulose.

EXPERIMENTAL

As a result of numerous mannose determinations made on the pure sugar and on various wood pulps, holocellulose, hemicellulose fractions, and α-cellulose residues, the following method for mannan determination was evolved.

Five-gram samples of air-dry wood meal ground to 40- to 60-mesh were used. The sample was treated with 45 ml. of 72% sulfuric acid (specific gravity 1.6338 at 20° C.) previously cooled to 10° to 12° C., and allowed to stand for 10 to 15 minutes at this temperature to ensure complete solution. The mixture was then kept at a temperature of 18° to 22° C., with occasional stirring, for 3 to 4 hours. (In the case of isolated hemicelluloses, the total period was reduced to 1.5 to 2 hours.) The mixture was transferred to a 4-liter beaker covered with a watch glass, diluted with 1500 ml. of water, and heated for 4 hours. Boiling chips were placed in the solution in order to prevent bumping. The water level in the beaker was kept constant by small additions of water from time to time. The beaker was removed from the hot plate and, while the solution was still near the boiling point, its contents were treated very gradually with a thick cream of barium carbonate (containing 135 grams of barium carbonate in water), taking precautions to avoid losses due to frothing. The solution was heated until neutral, but not alkaline, to alkacid paper. Ten to 15 mg. of pyridylmercuric acetate (Pyridose) preservative were then added to the mixture. The contents of the beaker were transferred to a 2-liter graduated cylinder, and the washings used to dilute the solution to the mark. The solution was then cooled to room temperature (usually by allowing the mixture to stand overnight) and sufficient water was added to bring the solution to the mark again. An additional 40 ml. of water were added to compensate for the volume occupied by the barium sulfate precipitate. (A portion of these 40 ml. was used in wetting down the filter papers used in the subsequent filtration.) The solution was filtered on a Büchner funnel into a clean, dry filter flask, using two No. 50 Whatman papers for a filter mat. The precipitate was sucked as dry as possible but was not washed. The volume of the filtrate was measured carefully and designated as volume A (for purposes of calculation).

Sufficient acetic acid was added to the filtrate to render it acid to litmus. The solution was transferred to a large evaporating dish and evaporated on a steam bath to a volume of approximately 200 ml. During evaporation the acidity was checked at least every half hour. The solution was kept acid to litmus (by means of acetic acid) but not sufficiently acid to turn Congo red paper blue. If the solution had been allowed to remain alkaline for even a short time, the determination could no longer have been considered valid.

The solution was then transferred to a 250-ml. Pyrex evaporating dish and concentrated on the steam bath to a volume of approximately 75 ml., maintaining the same check on acidity. The mixture was filtered and washed into a 125-ml. glass-stoppered Erlenmeyer flask. The entire evaporation on the steam bath required about 12 to 14 hours. This evaporation could also be carried out conveniently in vacuo using a water bath at 48° to 50° C. in which to immerse the flask containing the filtrate. However, experiments have shown that the vacuum distillation presents no advantages, and results by the two methods are practically identical.

Inasmuch as the solution requires a mannose concentration of approximately 1% to ensure quantitative precipitation (*13*), about 750 mg. of mannose, accurately weighed, were added to the solution at this point. Subsequently, 20 ml. of phenylhydrazine acetate reagent were added. This reagent was prepared by mixing 2 volumes of pure phenylhydrazine (redistilled, if not pale straw colored), 1 volume of glacial acetic acid, and 3 volumes of water. Both the reagent and solution were cooled before mixing in the precipitation flask to prevent osazone formation. Approximately 0.1 gram of sodium acetate was also introduced into the solution, which was then well mixed by swirling. The total volume was kept at about 100 ml. The flask was stoppered and placed in the refrigerator (at about 3° C.) for 48 hours. During this period, the mixture was shaken occasionally.

The supernatant liquid was decanted through a weighed fritted-glass crucible of medium porosity (Jena 1G3 or Pyrex M). To the precipitate in the flask were added (in several portions) 75 ml. or more of a cold (3°) filtered alcoholic solution saturated with mannose phenylhydrazone. This saturated solution was used to transfer the hydrazone quantitatively from the flask to the crucible. The hydrazone was then washed successively with 5 to 10 ml. of ice water, 5 to 10 ml. of the alcoholic hydrazone solution, and 20 ml. of ether. Air was then aspirated through the filter for several minutes. The precipitate was dried 4 hours at 100° and weighed. (The solution of alcohol saturated with mannose phenylhydrazone is made by saturating boiling 95% ethanol with recrystallized mannose phenylhydrazone. This saturated solution should be allowed to stand at about 3° C. at least 24 hours before use to ensure complete crystallization of the excess phenylhydrazone. The supernatant liquid should be filtered through paper immediately before use.)

The weight of mannan recovered as the mannose phenylhydrazone is calculated as follows:

$$\frac{[\text{Weight of dry precipitate} - (\text{weight of mannose added} \times 1.5)]}{\text{Volume A}/2000} \times 0.6 = \text{weight of mannan}$$

Table I. Mannose Recoveries Using Modified Hägglund-Bratt Procedure

Preliminary Cold 72% Acid Treatment	Heating Period in 3% H_2SO_4	Mannose Taken	Mannose Recovered	Recovery
	Hours	*Mg.*	*Mg.*	%
15 min. at 10–12° followed by 1.75 hours at 18–22°	4	500.8	456.4	91.1
	4	501.7	463.3	92.3
None	4	511.3	503.3	98.4
None	4	505.2	498.3	98.6

The chief points of departure from the original Hägglund-Bratt method may be summarized as follows:

The original suspension in 72% sulfuric acid is not kept in vacuo, and the time of initial treatment with acid is shortened.

The sugar determinations in the hydrolyzates, before and after neutralization with barium carbonate, are eliminated.

Pyridylmercuric acetate is used as a preservative to prevent fermentation of the dilute neutral sugar solutions. This step appears important, especially in summer. Control experiments showed that the preservative did not interfere with either the phenylhydrazone or the sugars.

A larger aliquot portion of the hydrolyzate is taken for the mannose determination.

Larger amounts of mannose are added to increase the mannose content of the hydrolyzate—i.e., 700 to 800 mg. instead of about 500 mg.—so as to bring the final mannose concentration to about 1%.

The mannose phenylhydrazone precipitate is washed, not with 95% alcohol, but with a saturated alcoholic solution of mannose phenylhydrazone, which permits more thorough washing of the precipitate without loss. Such washing serves to remove any co-precipitated phenylglucosazone, as indicated by the complete disappearance of the yellow color.

In the case of an α-cellulose, about 4 grams of air-dry material were used for the determination. When hemicellulose fractions were to be assayed, these were often too small to permit the use of 4-gram samples. In such cases, proportionately smaller amounts of all reagents were employed (with the exception of the mannose added and the final volume of phenylhydrazine reagent). Dilutions with water during the course of the analysis were also cut proportionately. However, here too, the final precipitation was made in a volume of about 100 ml. With the more soluble hemicelluloses, it was found advisable to reduce the initial 72% sulfuric acid digestion to 1.5 to 2.0 hours, so as to lessen the destruction of mannose freed during the treatment.

Recognizing the impossibility of an absolute evaluation of the above method, by making suitable control experiments, runs were made with pure mannose using the foregoing technique (including the addition of "booster" charges of mannose just prior to precipitation). The preliminary acid treatments used and the results are given in Table I.

As a further check on the mannose procedure, an analysis was made of the so-called "mannan A" isolated from vegetable ivory nut shavings by A. P. Yundt. Holocellulose was prepared from these shavings by the usual sodium chlorite procedure, and the product was extracted with hot 5% sodium hydroxide. The alkaline solution was filtered and precipitated with acetic acid, and the resulting crude mannan was redissolved in alkali and reprecipitated with acid, until the product (after washing successively with alcohol and ether) showed a G.E. brightness of 99°+ Analyses by L. O. Bublitz showed 86.2 and 86.8% mannan (without addition of a final "booster" charge). Inasmuch as the usual procedure when applied to pure mannose gave average recoveries of only 91.7%, the mannan values must be considered minimal, and the actual mannan content of Yundt's mannan A may be as high as 94%.

It is evident that the greatest loss in mannose occurs in the first stage of the assay. This is in harmony with the findings of Koch (*10*), who showed that, even at room temperature, 72% sulfuric acid gradually destroyed mannose.

When the method was applied to cotton linters, no mannose was found; only the final "booster" charge of mannose was recovered as shown in Table II.

Thus the results of earlier investigations (*18*) on the absence of mannose units in cotton are confirmed and evidently no mannose is formed during the course of the assay.

Experiments have shown that, whereas glucose appears to aid in the mannose determination, xylose impedes the recovery of mannose. This inhibiting effect is especially noticeable when the mannose concentrations prior to the phenylhydrazone precipitation are low, as shown in Table III. Here the sugars were simply dissolved in water and precipitated directly with the phenylhydrazine reagent.

Table II. Assay of Hydrolyzed Cotton Linters

Wt. of Cotton Linters (Ovendry Basis)	Booster Charge of Mannose Added	Mannose Recovered		Mannose from Hydrolyzed Cotton
Grams	*Mg.*	*Mg.*	%	
3.8092	784.2	775.7	98.9	Nil
3.8202	776.0	770.3	99.3	Nil
3.8298	783.0	768.1	98.1	Nil

In two recent experiments, a similar effect was noted under somewhat different conditions. Approximately 0.45 gram of mannose, 0.45 gram of xylose, and 1.8 grams of glucose were added to 72% sulfuric acid, diluted immediately with water to form a 3% acid solution, heated just to the boiling point, and neutralized directly with barium carbonate. Otherwise, the mannose determination was carried out as above, even to the addition of a small (0.15-gram) booster charge of mannose just prior to the precipitation. The mannose recoveries were 83.0 and 83.1% of the amounts taken. It may be that, in certain instances, the xylan present in a wood is instrumental in lowering mannose yields. This would have a minor effect on the mannan determination in a softwood because of the predominantly large amount of glucose present (due to hydrolysis of the cellulose of the wood).

Table III. Effect of Xylose on Recovery of Mannose from Solutions Containing Other Sugars

Manose Concn. %	Other Sugars %	Mannose Recovery %
0.5	2.0 glucose	99.1
0.5	1.4 glucose 0.6 xylose	93.2
0.2	2.0 glucose	100.1
0.2	1.0 glucose 0.6 xylose	7.0

Whereas the effect of xylan on the mannan content of hardwoods has not been explored, it is highly probable that figures given in Table IV are minimal values, because of the high xylan contents of these woods.

Table IV. Mannan Content of Hardwoods

(On ovendry, unextracted basis)

Wood	Mannan, %
Oregon maple	1.6
Aspen	1.5
Red alder	0.8
Quebracho	0.6

MANNAN IN HARDWOODS

The following hardwoods were tested qualitatively for the presence of mannans: Oregon maple, aspen, red alder, and quebracho. The qualitative procedure was similar to the quantitative method, except that, in each case, the final solution was evaporated to 10 ml., decolorized with Norite, filtered, and then treated directly with sodium acetate and 10 ml. of the phenylhydrazine reagent (without the prior addition of pure mannose). In all cases, the typical mannose phenylhydrazone was obtained. In confirmation, each hydrazone was converted into the typical golden yellow crystalline tetraacetyl anhydro derivative [$C_{12}H_{12}O_4N_2(COCH_3)_4$] described by Wolfrom and Blair (*25*). However, only in the case of the product from aspen was sufficient material obtained by recrystallization from absolute alcohol for a mixed melting point with an authentic sample of the tetraacetyl anhydro derivative prepared from pure mannose (melting point 122.5–123° C.). The mixture melted at 121.5–122.5° C.

Mannan was then determined quantitatively in these same samples. The usual analytical procedure was used, but approximately 1 gram of pure mannose was added to the final solution prior to the addition of the reagent. The results are given in Table IV. It is obvious that these hardwoods all contain mannans.

A sample of aspen α-cellulose prepared by Thomas (*22*) from another lot of aspen wood was shown to contain about 2% mannan—i.e., 1% calculated on the basis of the original wood. Thomas had reported the absence of mannan from this same aspen wood, but inasmuch as, at the time, the mannose techniques lacked the refinements given above and he added no pure mannose to his final solution, it is hardly surprising that he failed to detect mannans in this wood. This example is given to emphasize the importance of ensuring a sufficient mannose concentration in the solution prior to precipitation. It is interesting to note that even the α-cellulose fraction retains mannan.

Data on the distribution of mannans in two typical coniferous pulpwoods will be reported in a subsequent paper.

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Use of a Line Source in Ultraviolet Absorption Spectrophotometric Analyses

Application to Analysis of Mixtures of Aniline, N-Methylaniline, and N,N-Dimethylaniline

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A source of energy with an emission spectrum consisting of discrete lines has certain advantages in spectrophotometric absorption analyses. Such a source when used with a monochromator permits the isolation of monochromatic energy of known and reproducible wave length. Consequently, calibrations made with one spectrophotometer can be applied to any other spectrophotometer using a similar energy source, and thus the laborious calibration of each individual instrument is avoided. A method of this type is described for the analysis of mixtures of aniline, *N*-methylaniline, and *N,N*-dimethylaniline. A mercury arc is used as the energy source.

SPECTROPHOTOMETERS used for analytical determinations customarily use a source of energy whose emission spectrum consists of a continuum. The monochromator isolates a small band of wave lengths in the spectral region desired for the transmittance measurement. This type of source is used principally because it permits measurements at any desired spectral position. This procedure has two disadvantages:

1. The mean wave-length position and the width of the spectral interval cannot be accurately reproduced from one spectrophotometer to another. In fact, considerable care in construction and operation of the spectrophotometer is required for the accurate reproduction of the wave-length position on the same instrument.
2. The energy used for the measurement is not monochromatic. This may cause apparent deviations from Beer's law (*1*) if the transmittance of the absorbing substance changes considerably across the spectral interval isolated by the monochromator.

These disadvantages are particularly important when a transmittance measurement is required on the top or on the side of a sharp absorption peak where small variations in the wave length, and sometimes in the spectral interval, will cause a large change in the observed transmittance. For example, in the method described below, some of the spectral positions must be reproduced to 0.2 Å. Although the wave-length scale of one instrument can be reset with this accuracy, another instrument cannot be set to the same position, within this tolerance, because of very small differences between the wave-length scales. Under these conditions it is impossible to use extinction coefficients determined on one spectrophotometer to calculate accurately the composition of a sample from optical density measurements made on another instrument. Consequently, it is necessary to calibrate independently each spectrophotometer used for an analysis involving wave lengths or spectral band widths which must be reproduced accurately. Calibration of the spectrophotometer is laborious and, in addition, requires pure samples of each of the components to be determined, which are often difficult or expensive to obtain. Spectrophotometric analyses are usually limited, as a result, to applications involving the analysis of a sufficient number of similar samples to justify the expense of obtaining the pure components and calibrating the spectrophotometer.

These difficulties are avoided by using an energy source with an emission spectrum consisting of discrete lines of suitable wave lengths instead of a continuum. The monochromator is then

used only to isolate any desired line for the measurement. This energy is of known and exactly reproducible wave length and is essentially monochromatic, provided the line broadening of the source is not excessive—i.e., a high pressure mercury arc would probably not be satisfactory. Transmittance measurements can be made with any other spectrophotometer at exactly the same wave length if it is equipped with a similar source.

An ideal source for this application should have a sufficiently large number of strong emission lines to permit measurements at the most advantageous spectral positions for a given analysis. However, it is not necessary that an emission line be available which corresponds exactly to the position of the absorption maximum. Usually, a position near the maximum is satisfactory and occasionally may be even preferable. The lines must, of course, be spaced sufficiently far apart to permit isolation of the desired line with the monochromator without including energy from an adjacent line. At present, mercury arcs are about the only commercially available sources with emission lines of satisfactory intensity. Although these arcs are far from ideal because of the limited number of lines, they can be used for some analyses.

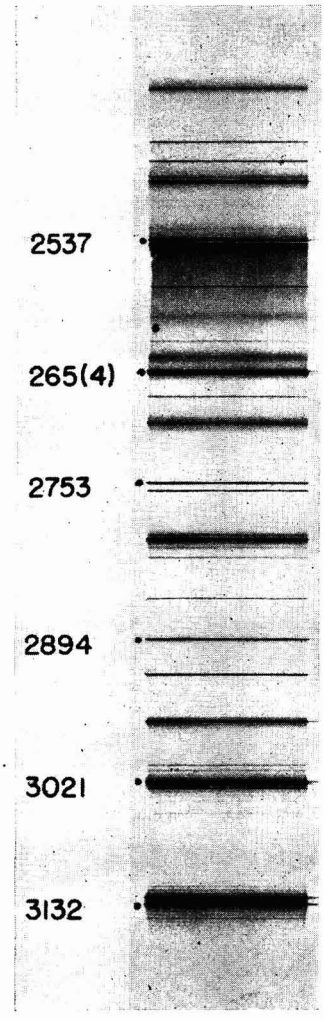

Figure 1. Emission Spectrum of Hanovia Mercury Arc

APPARATUS AND EXPERIMENTAL TECHNIQUE

Two types of medium-pressure mercury arcs have been used as an energy source for a Beckman quartz spectrophotometer (*2*).

1. A G.E. 100-watt, type H-4 mercury arc. A hole about 2.5 cm. (1 inch) in diameter was blown in the side of the glass envelope to permit passage of the short wave-length radiation. The arc was mounted upside down in the clamps in a standard hydrogen-lamp housing for the Beckman spectrophotometer. This lamp gave considerable difficulty due to frequent cracks in one of the electrode seals in the quartz arc tube. (This was observed only for lamps which had a hole blown in the glass jacket.) It was found that this difficulty could be avoided by reducing the voltage to the primary of the transformer required for operation of the arc to 90 to 100 volts.

2. A Hanovia 90-watt arc, type SH. This arc is more compact than the H-4 arc, as it consists of only a quartz arc tube with metal bands around each end for the electrical connections. The arc tube is 12 mm. in diameter; the over-all length is approximately 90 mm. The arc is designed for mounting in metal clips. This arc was mounted in a standard hydrogen-lamp housing in which the metal lamp-mounting plate was replaced by a Transite plate carrying the metal clips for mounting the lamp.

The Hanovia lamp was preferred because of its compactness and slightly better steadiness. However, neither lamp was completely satisfactory in the latter respect. Operation of the Beckman spectrophotometer with the sensitivity control near the end of its range, corresponding to minimum slits, was found to be desirable to reduce the fluctuations of the galvanometer needle. Duplication of each transmittance reading was essential for accurate results.

A photograph of the emission spectrum of a Hanovia mercury arc is shown in Figure 1. The desired emission line is isolated by slowly rotating the wave-length scale to secure maximum photocell response. The 100% adjustment on the Beckman spectrophotometer is made by varying the slit width as a coarse adjustment and then using the sensitivity control as a fine adjustment. No attempt was made to use the same slit width always as the actual value of the slit is unimportant, provided it is sufficiently narrow to eliminate energy from an adjacent line. Slit widths for the stronger mercury lines never exceeded 0.04 mm. and frequently were less than 0.01 mm.

After the wave-length scale has been set to the position of maximum energy, the absorption measurement is made in the usual manner.

METHOD FOR ANALYSIS OF MIXTURES OF ANILINE, *N*-METHYLANILINE, AND *N*,*N*-DIMETHYLANILINE

The absorption spectra of aniline, *N*-methylaniline, and *N*,*N*-dimethylaniline are shown in Figure 2. The extinction coefficients of each of these compounds in iso-octane as determined at a

Table I. Extinction Coefficients of Aniline, *N*-Methylaniline, and *N*,*N*-Dimethylaniline at Various Mercury Lines

Mercury Line, Å.	Extinction Coefficients[a]		
	Aniline	*N*-Methylaniline	*N*,*N*-Dimethylaniline
3132	0.034	1.46	7.80
3021	3.29	15.55	18.2
2894	18.15	19.55	16.55
2753	12.65	9.30	9.68
265(4)	6.49	6.64	33.9
2537	10.75	41.8	122.0

[a] $\frac{\text{Liters}}{\text{grams} \times \text{centimeters.}}$ Temperature 25°C.

Extinction coefficients are probably accurate to ±0.5% of their value, except for aniline at 3132 Å., which may have a considerable greater percentage error because of the low value.

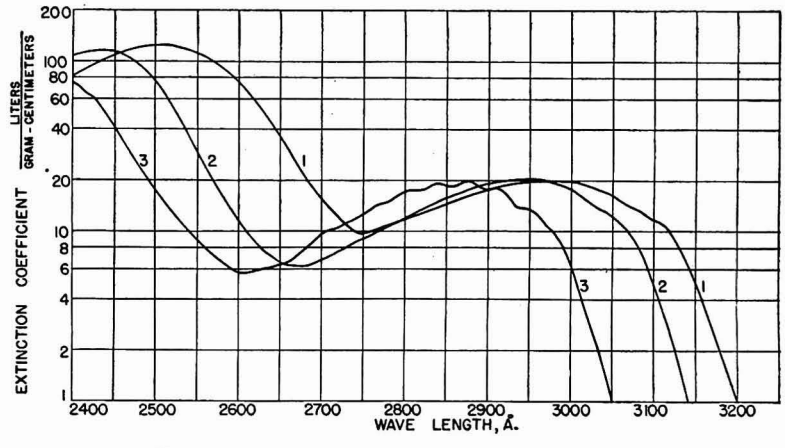

Figure 2. Absorption Spectra in Iso-octane

1. *N*,*N*-Dimethylaniline. 2. *N*-Methylaniline. 3. Aniline

Table II. Analysis of Synthetic Aniline, *N*-Methylaniline, and *N,N*-Dimethylaniline Samples

(Using mercury arc with Beckman spectrophotometers D-274 and D-460. Analysis based on measurement at 3132, 3021, and 2753 Å.)

Sample No.	Aniline					*N*-Methylaniline					*N,N*-Dimethylaniline				
		D-274		D-460			D-274		D-460			D-274		D-460	
	Theory	Found	Error	Found	Error	Theory	Found	Error	Found	Error	Theory	Found	Error	Found	Error
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
1921-33A	100.0	100.0	0.0	99.4	−0.6	0.0	−0.9	−0.9	0.4	+0.4	0.00	0.29	+0.29	0.05	+0.05
1921-33B	100.0	99.9	−0.1	100.1	+0.1	0.0	−0.2	−0.2	0.0	0.0	0.00	0.18	+0.18	0.12	+0.12
1921-34A	0.0	0.0	0.0	0.2	+0.2	100.0	99.5	−0.5	99.3	−0.7	0.00	0.11	+0.11	0.25	+0.25
1921-34B	0.0	0.1	+0.1	0.3	+0.3	100.0	99.6	−0.4	99.4	−0.6	0.00	0.08	+0.08	0.18	+0.18
1921-35A	0.0	−0.1	−0.1	0.4	+0.4	0.0	−0.6	−0.6	−1.7	−1.7	100.0	100.0	0.0	100.8	+0.8
1921-35B	0.0	0.2	+0.2	0.3	+0.3	0.0	−1.3	−1.3	−1.7	−1.7	100.0	100.5	+0.5	100.8	+0.8
1921-36A	33.4	34.4	+1.0	34.3	+0.9	32.8	32.0	−0.8	31.0	−1.8	33.8	34.2	+0.4	34.3	+0.5
1921-36B	34.5	34.1	−0.4	34.3	−0.2	33.2	31.7	−1.5	31.3	−1.9	32.3	33.6	+1.3	34.1	+1.8
1921-37A	46.9	46.4	−0.5	46.4	−0.5	43.2	44.3	+1.1	44.0	+0.8	9.9	9.7	−0.2	9.7	−0.2
1921-37B	49.5	49.2	−0.3	49.9	+0.4	39.9	41.0	+1.1	40.9	+1.0	10.7	10.3	−0.4	10.4	−0.3
1921-38A	45.1	44.8	−0.3	45.0	−0.1	10.0	9.2	−0.8	8.7	−1.3	44.9	45.1	+0.2	45.5	+0.6
1921-38B	46.6	...	...	46.3	−0.3	10.5	...	...	10.7	+0.2	43.0	....	....	42.8	−0.2
1921-39A	12.7	...	...	12.6	−0.1	43.4	...	...	44.4	+1.0	43.8	....	....	44.2	+0.4
1921-39B	14.0	...	...	13.6	−0.4	39.1	...	...	39.5	+0.4	46.9	....	....	47.3	+0.4
Av.			0.3		0.3			0.8		0.9			0.3		0.6

number of strong mercury lines are given in Table I. The 265(4) line is in reality three closely spaced lines which could not be separated with the Beckman spectrophotometer. No difficulty in reproducing measurements was observed at this position. No deviations from Beer's law were observed for any of the lines listed in Table I. However, apparent deviations from Beer's law were observed for some of the very weak mercury lines because of the scattered radiation which becomes of greater significance at the weaker lines.

Mixtures of aniline, *N*-methylaniline, and *N,N*-dimethylaniline are analyzed by measuring the optical densities of suitable dilutions of the sample in iso-octane at 3132, 3021, and 2753 Å. It is frequently necessary to use a different concentration of the sample for the measurement at each wave length in order to secure optical densities in the desired range. The percentage of each component in the sample is then calculated by substituting the extinction coefficients of the sample (optical density for a 1-cm. cell divided by sample concentration in grams per liter) into the following equations:

$$\% \text{ aniline} = 1.86 \times E_1 - 5.80 \times E_2 + 9.41 \times E_3$$
$$\% \text{ } N\text{-methylaniline} = -19.7 \times E_1 + 9.77 \times E_2 - 2.49 \times E_3$$
$$\% \text{ } N,N\text{-dimethylaniline} = 16.5 \times E_1 - 1.80 \times E_2 + 0.425 \times E_3$$

where E_1 = extinction coefficient of the sample at 3132 Å., E_2 = extinction coefficient of the sample at 3021 Å., and E_3 = extinction coefficient of the sample at 2753 Å.

The method was checked by the analysis of a number of synthetic samples (Table II). The optical densities of most of the diluted samples were measured with two Beckman spectrophotometers with serial numbers D-274 and D-460 in order to prove that the results obtained were independent of the spectrophotometer. All the calibration data were determined with spectrophotometer D-460.

DISCUSSION

The close agreement between the results obtained in the analysis of aniline, *N*-methylaniline, and *N,N*-dimethylaniline when using two different spectrophotometers show that the observed optical densities are independent of the spectrophotometer when a line source is used. There is a very low probability of obtaining equally satisfactory agreement using different spectrophotometers in this analysis with a continuous source such as a hydrogen arc.

Table III gives the percentage change in extinction coefficients caused by a 1 Å. shift in the spectral position at each of the wave lengths used in the analysis. These data show that some of the spectral positions must be reproduced to 0.2 Å. to reduce the error in extinction coefficients to 1% of the value. Experience has shown that the wave-length position on a Beckman spectrophotometer can be reset with care to 0.1 to 0.2 Å. in this spectral region, provided the instrument is maintained at a constant temperature. (It has been found that a temperature change of 3° C. will cause a shift in the spectral interval of approximately 0.7 Å.) Consequently, if sufficient precautions are taken, the spectral positions on any given Beckman spectrophotometer can be reset with sufficient accuracy to secure an accuracy of 1% in the extinction coefficients of the components present in this analysis. However, as the wave-length scales of even carefully adjusted instruments may easily differ by 1 Å., it would not be possible to use the same calibration data with different instruments when using a continuous source. It is also probable that there will be small differences in the actual spectral intervals isolated by different instruments for the same setting of the slit scale.

Table III. Percentage Change in Extinction Coefficient Caused by 1 Å. Shift in Spectral Position

Spectral Position, Å.	Change in Extinction Coefficient Caused by 1 Å. Shift in Spectral Position, %		
	Aniline	*N*-Methylaniline	*N,N*-Dimethylaniline
3132	5.0	4.0	1.9
3021	3.6	1.7	0.6
2753	0.7	0.8	0.0

It is believed that the use of a line source would have wide applications if a source of sufficient stability and with a satisfactory number of lines were available. It should then be possible to determine the extinction coefficients of the pure compounds in the research laboratory and apply the method directly in the control laboratory without any calibration with its attendant delay. It should also be possible for workers in this field to develop new methods from published values of the extinction coefficients of the particular compounds in which they are interested without the necessity of redetermining the values for themselves.

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ANALYTICAL METHODS FOR RUTHENIUM

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The distribution of ruthenium losses during the fire assay procedures has been determined. Significant retention of ruthenium by the slag and cupel occurs. Losses of ruthenium as the volatile tetroxide during the fusion and partial cupellation are negligible. A new procedure for parting the button and treating the residues is discussed.

FOR several reasons ruthenium has always been a very difficult element to determine quantitatively (*3*). It occurs in small quantities in difficultly soluble ores and in association with the other platinum metals, from which it is hard to separate quantitatively; it is amphoteric, easily forming acidic oxides; it exists in five common valencies, II, III, IV, VI, and VIII; it is said to possess also the other three I, V, and VII (*16, 18, 19, 31, 33, 37, 38, 40*); it forms the easily lost volatile tetroxide, RuO_4 (*37*); and it avidly forms complex coordination compounds, especially with nitrogen oxides, which cause interference in analytical processes (*5–9, 17, 20, 21, 30, 33, 34, 41*).

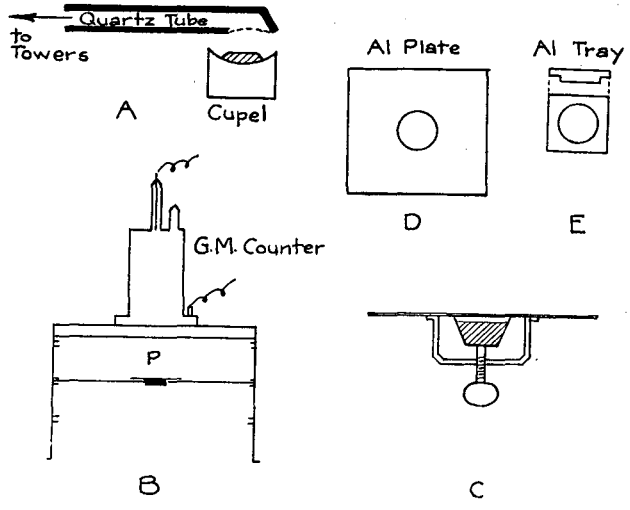

Figure 1. Parts of Apparatus for Counting Samples

Several methods have been proposed for the determination of ruthenium in solutions free of nitrate and interfering metal salts (*1, 10–15, 22, 24, 25, 39*), but the only very satisfactory ones are the hydrolytic precipitation of Gilchrist *et al.* (*12–15*) and the thionalide method of Rogers *et al.* (*39*).

In the method of Gilchrist, an acid chloride solution of ruthenium, obtained by distilling ruthenium tetroxide from sodium bromate–sulfuric acid solution and catching it in sulfur dioxide–hydrochloric acid solution, is treated with sodium bicarbonate until the precipitate which forms suddenly coagulates. Enough sodium bicarbonate is then added to turn bromocresol purple indicator purple. The hydrated oxide thus precipitated is filtered and washed with ammonium chloride, then ignited, reduced in hydrogen, and weighed (*14*).

In the method of Rogers, Beamish, and Russell, the ruthenium is purified by distillation as ruthenium tetroxide from a solution containing sodium bromate and sulfuric acid. The volatile ruthenium tetroxide is caught in ice-cold 3% hydrogen peroxide. This receiver solution is then acidified with hydrochloric acid until it is between 0.2 and 0.5 *N*. Thionalide reagent, dissolved in ethanol, is then added and the precipitate is coagulated by boiling, then filtered, washed, ignited, reduced in hydrogen, and weighed.

A survey of the literature yields few methods of analysis for ruthenium in an ore, and reveals no attempt to prove a method (*2, 3, 5, 11, 27, 28, 29, 35, 36*). The first step in all established methods of analyzing noble metal ores is to concentrate gold, silver, and the platinum metals by fire assay in a lead button (*4*). The lead is then removed by cupellation, and the noble metals are left in a silver bead. Several authors have stated that ruthenium is lost as the volatile tetroxide if the cupellation is carried through to the silver bead stage, and that this loss is avoided if the cupellation is stopped in time to leave a 2- to 5-gram button (*3, 28, 36*). The next step, which is to dissolve or part the bead, is generally done in nitric acid; the residue from this parting is then treated with aqua regia and, if necessary, by fusion (*27, 28*). The last step is the treatment of the solution so obtained and the separation of and analysis for ruthenium.

This fire assay method has been reasonably well tested for gold and almost universally accepted for the platinum metals (*4*). This acceptance, however, has not been based on experimental evidence, but has resulted more or less from the extreme difficulty involved in obtaining evidence. Very few workers have questioned the validity of the assay for the platinum metals and no work whatsoever has been found which checks it. No account was found in the literature of either the completeness of the collection of ruthenium in the button, or of the accuracy of any method of parting or of subsequent analysis. Serious doubts should exist on these points, due to the properties of ruthenium mentioned above, especially its ability to form acidic oxides.

With the aid of a small quantity of radioactive ruthenium made available by the Canadian Atomic Energy Project, the authors have been able to evaluate critically the different steps in the assay for ruthenium, and to develop a new method for parting the button, which allows complete isolation of the ruthenium in the button and its accurate gravimetric determination.

EXPERIMENTAL

Counting Radioactive Samples. A Geiger-Müller counter of the "end-on" beta-ray type was used in this work, associated with a "scale of 128"—that is, an electronic pulse counter which registered one count for each 128 pulses.

In quantitative measurements of radioactivity, the shape of the material and its spatial relationship to the Geiger counter must be fixed and reproducible. The position of maximum sensitivity for a sample would, of course, be at a point just below the aluminum window (*P*, Figure 1,*B*).

It was found that the most convenient and reproducible method of counting samples of 0.5-gram size or less was as follows:

A small sheet of 0.8-mm. ($^1/_{32}$-inch) thick aluminum with a 1.8-mm. (0.72-inch) diameter hole in the center (Figure 1,*D*) is supported in slots in a frame so that the hole comes directly below the counter window and about 5 mm. from it. Small aluminum trays 0.8 mm. ($^1/_{32}$ inch) thick and about 3.1 cm. (1.25 inches) square were die-stamped with a circular depression 1.6 mm. ($^1/_{16}$ inch) deep and of such diameter that the area was 2.0 sq. cm. (Figure 1,*E*). The sample to be counted was weighed, placed in this depression and carefully spread uniformly over it.

It was then covered with a layer of cellulose tape, and the tray was fitted into the hole in the center of the aluminum sheet. This assembly was placed in the slots which fixed it in a reproducible position beneath the counter tube.

In counting solid samples larger than 10 grams in size or in counting liquids, it was found that rather than counting 0.5-gram aliquots as described above, it was better to count 10-gram samples, for by doing so a larger count could be obtained and hence a lower inherent error.

Porcelain crucibles of the 7-ml. size were ground on the top until the edge was in a perfect, flat plane, and a supply of these crucibles was carefully selected for strict uniformity in depth and shape. In counting a sample, 10 grams of the finely ground solid, or 5 ml. of the liquid, were placed in one of these crucibles. A small square of cellophane was placed over it as a cover, and it was clamped into an aluminum holder (Figure 1,*C*) so that the edge of the crucible pressed the cellophane into a thin rubber gasket, which made an air-tight seal. The holder was then placed in the slots and the crucible thus held just below the window of the counter.

In counting by this method it was necessary to calibrate each new type of sample by counting samples of known activity and obtaining a factor of counts known to be present over counts observed, which could subsequently be applied to the count on each unknown. This compensated for "self-absorption". For solutions containing no dissolved heavy metals the factor was found to be 5.2, and for slags containing 15 to 20% of lead, it was 10.0.

All determinations of radioactivity were made by counting first the background, then the sample, then a given sample of aged uranium as a standard. The activity of the original ruthenium used to salt assay charges, etc., was, therefore, determined as a ratio to the constant activity of this uranium sample. The activity of each unknown sample was determined and after applying corrections for the background count, absorption of beta-rays by the sample itself (self-absorption), distance from the counter tube, radioactive decay of the sample activity, and total sample weight, this activity was also expressed as a ratio to the activity of the standard uranium sample. This procedure compensated for day-to-day variation in the counter or its accompanying circuits.

The ruthenium isotope used had a mass number of 110 and half-life of 290 days and had an absorption characteristic which was far from the usual logarithmic form.

In counting radioactive samples the possible error inherent in the counting process is established by theory as plus or minus the square root of a given count and in this report all counts taken, or numbers calculated from these counts, are followed by an expression of this counting error.

Preparation and Analysis of Inactive Standard Solutions. A primary standard containing ruthenium was necessary. Because no ruthenium salt of acceptable purity was commercially available, and the metal itself is difficult to dissolve, it was advisable to synthesize some salt that would be capable of easy analysis and could be readily dissolved and easily handled. Accordingly "ammonium chlororuthenate" was synthesized by the method of Rogers, Beamish, and Russell (*39*) and analyzed by direct ignition in a current of hydrogen. The following results were obtained for the percentage of ruthenium in the salt: 31.26, 31.28, 31.28, and 31.32.

This does not correspond to the theoretical percentage of ruthenium in $(NH_4)_2RuCl_6$, which is 27.96%, but reference to the work of Howe (*23*) and Charonnat (*7*) shows that the compound formed by the method used is $(NH_4)_2RuCl_5.H_2O$, $(NH_4)_2RuCl_5(OH)$, or a mixture of these with ruthenium trichloride or tetrachloride. The product was completely soluble.

METHOD 1. A standard solution of ruthenium was made by dissolving 639.3 mg. of ammonium chlororuthenate in 500.0 ml. of 0.6 *N* hydrochloric acid, which gave a solution containing 10.00 mg. of ruthenium per 25.00 ml. of solution. The acid is necessary to prevent precipitation of hydrated ruthenium oxide.

This solution was analyzed by the thionalide method, giving the results recorded in Table I.

Table I. Thionalide Analysis of Known Ruthenium Solutions

Sample No.	Ruthenium Taken, Mg.	Ruthenium Found, Mg.	Error, Mg.
1	10.00	10.02	+0.02
2	10.00	10.00	0.00
3	9.99	9.99	0.00
4	9.99	9.98	−0.01
5	9.99	9.97	−0.02

The thionalide method was used in subsequent analyses for ruthenium, and for standardizing ruthenium solutions.

METHOD 2. Larger quantities of ruthenium solution were made up by fusing a known amount of metallic ruthenium in sodium peroxide or a mixture of sodium hydroxide—potassium permanganate and distilling the ruthenium twice as ruthenium tetroxide to remove traces of impurity.

METHOD 3. As a result of subsequent findings described below, an adaption of the chlorine-hypochlorite distillation (*26*) provided a simpler way to make pure ruthenium solutions. A known weight of ruthenium metal was placed in a still and a chlorine distillation was performed. The metal was quantitatively dissolved by the sodium hypochlorite and volatilized as ruthenium tetroxide. This avoided the fusions and multiple distillations of the previous method.

Standardization of these solutions by the thionalide method may be performed with great precision.

Standardization of Active Solutions. Solutions containing radioactive ruthenium were made by distilling a roughly known amount of active ruthenium from perchloric acid (*22*) and catching it in ice-cold 3% hydrogen peroxide (*39*), then redistilling the ruthenium from this distillate by the sodium bromate-sulfuric acid method and again catching it in 3% hydrogen peroxide. The second distillation was used to remove any perchloric acid from the product.

The activity of each of these standard solutions was determined by adding to a known volume of the solution a large excess of inactive ruthenium in the form of a known volume of standard solution. A thionalide precipitation was then performed and a known weight of the ruthenium metal produced by igniting, ashing, and reducing. This precipitate was placed on an aluminum tray and counted.

To 1.000 ml. of active solution containing about 40 micrograms of ruthenium, 2.000 ml. of inactive solution containing 12.40 mg. of ruthenium were added.

Weight of Ru counted	8.29 mg.
Weight of Ru taken	12.44 mg.
Activity	191 counts
Volume of active solution taken	1.000 ml.

$$\text{Therefore, activity of solution} = \frac{191}{1.000} \times \frac{12.44}{8.29} = 287 \text{ counts per ml.}$$

Results obtained were:

Active solution 131, 286 ± 2, 286 ± 2, and 285 ± 2 counts per ml.

Testing Thionalide Precipitation with Radioactive Tracer. According to Rogers, Beamish, and Russell (*39*), a chloride solution of ruthenium in 0.2 to 0.5 *N* acid may be quantitatively precipitated by the addition of thionalide (thioglycolic β-aminonaphthalide). The accuracy of this statement was shown by the following method:

To a chloride solution of ruthenium was added a known count of radioactive isotope, also as chloride solution, and the solution was heated and treated with thionalide by the method of Rogers, Beamish, and Russell. After the precipitate coagulated the

mixture was filtered and the filtrate counted. The ratio of the counts in the filtrate to the counts originally added gave a measure of the incompleteness of precipitation. It was found that the filtrate count was 0.00 ± 0.05 when the count added was 500 ± 2. This showed that the thionalide precipitation was, under the recommended conditions, complete to less than 1 part in 10.000 on 6 mg. of ruthenium. This is equivalent to 0.6 microgram left in solution. Outside the recommended acidity range the precipitation was incomplete. This had, of course, been shown gravimetrically (*39*).

FIRE ASSAY PROCEDURE

The fire assays for this work were performed in a Williams and Wilson 25-cycle 15 kv.-amp. Globar type assay furnace, and the cupellations were performed in a small 5-kw. direct current cupellation muffle. Twenty-gram size pots were used throughout.

By melting 15 grams of flux mixture No. 69 in a 25-gram pot and swirling the molten slag around until the inside of the pot was completely wet, then pouring off the excess slag and cooling, an impermeable glaze was obtained over the inside surface of the pot. To 55 grams of flux mixture No. 69 in this pot were slowly added 2 ml. of a solution that contained 6.20 mg. of ruthenium with 523 counts, and the liquid was mixed thoroughly with the solid. Then 45 grams of dry flux mixture 69 were placed on top of the moist mixture and the pot and contents were placed in an oven at 110° C. for 4 hours. The mixture was placed in the assay furnace at reduced temperature (1500° F.) and the silica tube (see Figure 2) was inserted through a previously cut hole in the pot. The suction was turned on to start the operation of the gas-catching train described below, and the temperature was raised at a fairly uniform rate until after about 1.25 hours it was approximately 2100° F. The pot was then removed from the muffle, the lid knocked off, and the melt poured into a small mold and allowed to cool.

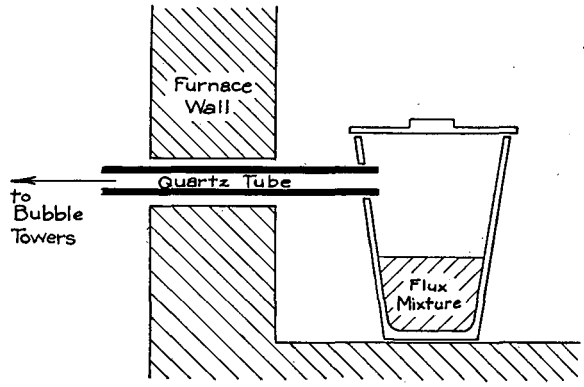

Figure 2. Collecting Gases

The cooled mass was then removed and the slag split off from the button. The slag was ground to pass a No. 45 sieve, rolled to mix, sampled, and counted in 10-gram lots. The button was cupelled on ordinary bone ash cupels with gas-catching apparatus operating, or stored awaiting wet treatment. If cupellation was used the cupel was ground fine, sampled, and counted as with the slag.

In the case of the iron-nail and niter assays, the button usually retained small particles of black slag which would later cause trouble by preventing smooth cupellation or parting. This was easily removed by a "borax wash," which consisted merely of melting down the button with about 30 grams of borax glass in a scorifier, then pouring into the mold. This produced a very clean button. This was especially necessary in the case of the iron-nail assay, where the slag contained much sulfide, which in the parting process is oxidized to sulfate and causes the precipitation of lead sulfate.

Method of Collecting Gases. In order to catch any gases escaping during the fusion, a silica tube 1.25 cm. (0.5 inch) in outside diameter was inserted through a small hole in the muffle wall and through a 1.4-cm. ($^9/_{16}$ inch) hole drilled just below the upper rim of the assay pot. During the fusion a lid was placed on the pot, and the pot was placed so that the end of the silica tube projected just inside the hole (Figure 2). On the other end of the silica tube was an absorption train to which suction was applied. Sufficient cooling of the hot gases was obtained if the silica tube was long enough so that 90 cm. (3 feet) of its length were in open air. Gases being drawn off at about 5 or 10 ml. per second were cooled almost to room temperature in this distance. The receivers each contained about 35 ml. of 3% hydrogen peroxide acidified with 2 ml. of 42% hydrobromic acid. This was kept cool by surrounding the receivers with ice.

The same method was used for collecting the gases over the cupel during cupellation (Figure 1,*A*).

After a fusion or cupellation the contents of the tube and towers were carefully washed into a beaker and saturated with sulfur dioxide and ammonium hydroxide, then slowly evaporated to a few milliliters. The liquid was counted by methods described above.

Composition of Assay Fluxes. The general classifications of ruthenium-bearing ore which might be encountered include oxidizing, reducing, and acidic or basic types. Various fusion compositions were tried, and the following are those which were best representative of the various ore conditions.

NEUTRAL (NONOXIDIZING OR REDUCING) ORES.
Neutral Flux, No. 69.

	Parts by weight
SiO_2	25
Borax glass	10
CaO	5
Na_2CO_3	35
PbO	78
Flour	3.5

Used 100 grams of flux

Very Acid Flux, No. 724.

	Parts by weight
SiO_2	80
Borax glass	16
CaO	20
PbO	312
Flour	14

Used 107 grams of flux

Very Basic Flux, No. 725.

	Parts by weight
SiO_2	40
Borax glass	8
CaO	80
Na_2CO_3	60
PbO	312
Flour	14

Used 130 grams of flux

OXIDIZING ORES. Flux 69 was used with the amount of flour increased to give a 25- to 30-gram button from 90 grams of flux.

REDUCING ORES. A sample of pyrites of reducing power 9.5 (*4*) (on a scale where FeS_2 = 12.0) was picked to represent a reducing ore. Three methods of treatment are possible—preroasting, an oxidizing fusion, or the "iron-nail" assay (*4*).

Preroasted ore can be treated as nonreducing (Flux 69).

Oxidizing Flux, Niter Assay, No. 59.

	Grams
SiO_2	19
Na_2CO_3	25
PbO	54
KNO_3	26

$^1/_2$ assay ton of ore (R.P. 9.5)

Iron-Nail Assay, No. 61.

	Grams
SiO_2	2
Borax glass	25
Na_2CO_3	50
PbO	40
Flour	1

$^1/_2$ assay ton of ore (R.P. 9.5)

Analysis of Button. The button cannot be counted directly, for the presence of the lead reduces the beta-ray emission to so low a figure that accurate counting is impossible. Nor can the lead be removed by cupellation because of the losses involved.

The usual alternative to cupellation is to dissolve the button in nitric acid or aqua regia, and then separate the desired metals.

PARTING WITH NITRIC ACID. When the button containing ruthenium in dilute nitric acid was dissolved, it was found that part of the ruthenium dissolved and part remained as the metal. Attempts to precipitate the dissolved ruthenium completely by thionalide or reduction with zinc were shown by activity counts to be unsuccessful, as the solution always tenaciously held some of the ruthenium.

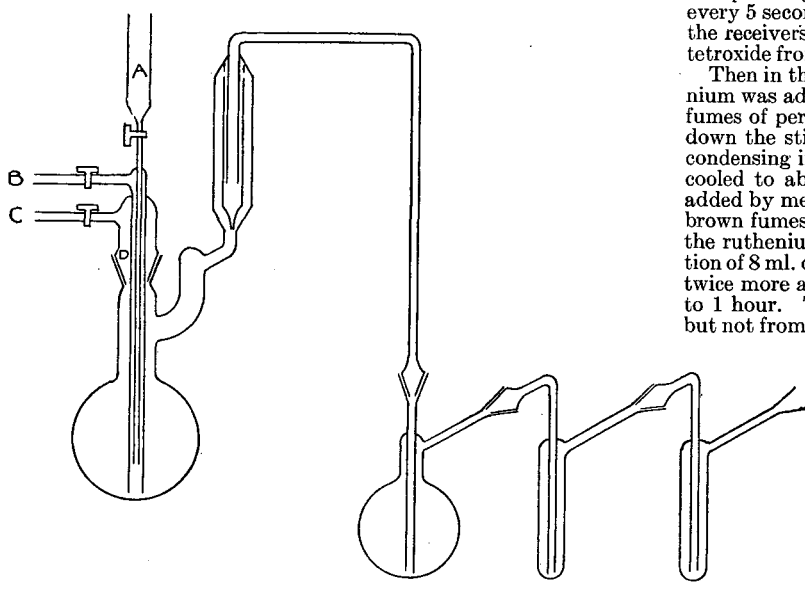

Figure 3. Distillation Apparatus

An attempt was made to find some method of removing the ruthenium from the nitric acid by volatilization of ruthenium tetroxide. The method used was to perform the distillation on a known amount of inactive ruthenium solution containing a known added activity count, then to count the still residue. This was repeated in the presence of nitric acid and lead.

The following distillations were studied and found to be ineffective under these conditions:

Method	Reference
$HClO_4$	(*26*)
$NaBrO_3$–H_2SO_4	(*41*)
NaOCl	(*23*)
$(NH_4)_2S_2O_8$	Unpublished work
$(NH_4)_2Ce(NO_3)_6$	Unpublished work

Complete removal of nitric acid from a ruthenium solution is difficult and involves successive evaporations of acid ruthenium solution. Evidence was obtained which indicated that ruthenium was lost under such conditions.

PARTING WITH PERCHLORIC ACID. An entirely new method of treating the button was devised which does not involve the use of nitric acid. This method shows strong promise of leading to a new and efficient procedure for the separation of the platinum metals. The assay button or the part which remained after partial cupellation was dissolved in perchloric acid, and from this solution without further treatment the portion of the ruthenium which dissolved was distilled and caught in ice-cold 3% hydrogen peroxide. The undissolved portion was filtered off and treated by the use of the chlorine distillation method which was developed as described below. The ruthenium was precipitated directly from these distillates. It is entirely reasonable to expect that osmium would also be volatilized under these conditions, and that the other four platinum metals would be left behind.

Perchloric Acid Distillation. Activity measurements on the liquid still residue showed that this distillation was complete, and this was checked by gravimetric analysis. The check distillations were performed by the following method:

To a clean 250-ml. still (see Figure 3) 50 grams of powdered lead were added, then 75 ml. of 72% perchloric acid. In the first receiver were placed 25 ml. of 3% hydrogen peroxide and 1 ml. of 42% hydrobromic acid, in each of the others 5 ml. of 3% hydrogen peroxide. The still was gently heated until the lead was completely dissolved and effervescence of hydrogen had ceased. The still and contents were then cooled in ice. During this heating and the subsequent distillation nitrogen was passed into the still in two places (see Figure 3), into *B* at the rate of about 1 bubble every 5 seconds, and into *C* at about 2 or 3 bubbles per second in the receivers. Passing nitrogen in at *C* prevents any ruthenium tetroxide from becoming trapped in the blind alley, *D*.

Then in the form of standard solution a known weight of ruthenium was added to the still. The still was heated until the white fumes of perchloric acid disappeared and colorless liquid refluxed down the still walls. Brown ruthenium tetroxide could be seen condensing in the trap and on the still walls. The still was then cooled to about 60° C. and 8 ml. of 36% perchloric acid were added by means of opening *A*. Again heat was applied, until the brown fumes disappeared. This was usually sufficient to drive all the ruthenium tetroxide into the receiver, but the cooling, addition of 8 ml. of perchloric acid, and refuming process were repeated twice more as a precaution. A complete distillation required 0.5 to 1 hour. The receivers were then disconnected from the still but not from each other, and 8 ml. of 42% hydrobromic acid were added to the first receiver (which contained virtually all of the ruthenium). A ground-glass stopper was then placed in the first receiver, and the contents of the receivers were heated to boiling on the hot plate. (Low results were obtained if the liquids were poured into a beaker without this treatment. The hydrobromic acid reduces ruthenium tetroxide, thus preventing its volatilization). The liquid was then placed in a 400-ml. beaker and boiled for 10 minutes to remove bromine and then the ruthenium was precipitated by the thionalide method.

By this method the results recorded in Table II were obtained. They indicate that the perchloric acid distillation is complete in the presence of high proportions of lead.

High results were obtained on this distillation if the amount of perchloric acid added was less than about twice the weight of the lead. This was assumed to be due to volatilization of lead tetrachloride, since the spectrograph showed the presence of lead in the distillate.

Chlorine Treatment of Residue from Parting. On dissolving a lead button containing ruthenium in perchloric acid and performing a perchlorate distillation, part of the ruthenium is unattacked by the acid and remains as ruthenium metal in the still. Two methods of recovering this material were used. In both, the still contents were cooled and diluted to twice their volume with water while being kept cold, then filtered.

In the first method the still residue was filtered through a No. 50 Whatman filter paper on a suction funnel and the filtrate repassed through the same paper until water-clear. Removing the last traces of the finely divided ruthenium from the still required great care. The residue on the filter paper was washed with 50 ml. of hot water to remove all traces of perchloric acid and lead perchlorate (which on subsequent heating can cause an explosion violent enough to knock the material out of the crucible). The paper and residue were then dried and ashed in a 15-ml. silver

Table II. Perchloric Acid Distillation of Ruthenium

Sample No.	Ruthenium Taken, Mg.	Ruthenium Found, Mg.	Error, Mg.
1	8.97	8.99	+0.02
2	8.97	8.91	−0.06
3	8.97	8.93	−0.04
4	8.97	8.88	−0.09
5	1.76	1.76	0.00
6	1.76	1.78	+0.02
7	1.76	1.75	−0.01
8	0.88	0.93	+0.05

crucible at less than 600° C. Ten grams of sodium peroxide were placed in the crucible and the whole was brought slowly to a temperature where the peroxide is molten, at dull red heat. The fusion was allowed to cool, the crucible was placed in a covered 600-ml. beaker, and its contents were dissolved out, using about 150 ml. of water. About 20 grams of sodium hydroxide were dissolved in this solution and it was washed into the original still. The receivers were set up as for the perchloric acid distillation. A chlorine distillation was then performed as follows:

A steady current of nitrogen, sufficient to cause about 2 or 3 bubbles per second in the receivers, was passed into the still at *C*, while chlorine was bubbled in through *B*. This chlorine was immediately absorbed by the sodium hydroxide, forming sodium hypochlorite and hydrochloric acid, and after 10 minutes or so, depending on the rate of passing the chlorine, the temperature rose and a rapid effervescence of very fine bubbles occurred. This may be very rapid, but is easily controlled by turning off the nitrogen and chlorine, and if necessary, placing the finger over the mouth of the last receiver. This raises the pressure in the system, which has a very marked slowing effect on the effervescence, and by judicious release of the escaping gases the effervescence can be allowed to occur without any overflow. After this effervescence subsided, ruthenium tetroxide could be seen condensing in the receiver (but seldom in the trap as in the perchloric acid distillation). The chlorine was passed until it was no longer absorbed, and the liquid was boiled for 15 minutes. As a precaution 10 grams more of sodium hydroxide, dissolved in 10 ml. of water, were added to the still and the distillation procedure was repeated. The receivers were removed, hydrobromic acid was added, and they were treated as described above after the perchloric acid distillation. The whole chlorine distillation required about 0.75 hour.

The second method is based on two discoveries. First, a filter paper may be destroyed by the basic sodium hypochlorite solution at less than 100° C. in much the same way as by hot sulfuric–nitric acid mixtures, but more quickly and cleanly; secondly, the dissolving action of sodium hypochlorite on ruthenium metal, described by Howe (*26*), can be used quantitatively.

Thus, in the second method a filter stick with a No. 42 and a No. 50 Whatman filter paper wrapped around the end was used to remove the liquid from the still while the solid residue was retained. The liquid was caught in a trap, and if not water-clear was replaced in the still and sucked through the filter again.

If some white precipitate of lead sulfate was present, 20 ml. of saturated ammonium acetate solution were added and removed by filtering through the same filter.

The still walls were then washed down with water to remove all the lead perchlorate. The elastic holding the filter paper was removed with a wire hook and the paper pushed off into the still without its ever having been brought out of the still. This minimized the possibility of loss of the fine ruthenium. This method is so superior that the former need be used only for alloys insoluble in sodium hypochlorite. The chlorine distillation was then performed on this residue by adding to the still 150 ml. of water in which were dissolved about 20 grams of sodium hydroxide and bubbling chlorine into the solution; or better, by placing 20 grams of sodium hydroxide in the still, and washing the distillate from the perchloric acid distillation into the still. The chlorine distillation destroys the filter paper, dissolves the metallic ruthenium, and volatilizes all of the ruthenium. Thus, all of the ruthenium from the sample ends up in the distillate from this distillation, and may be precipitated by thionalide.

Although the completeness of the sodium hypochlorite distillation has been proved by activity counts on the residue, the whole method was tested by using a known weight of ruthenium metal with 30 grams of lead. These were placed in the still and run through the whole process. With two samples of ruthenium weighing 10.56 and 11.96 mg. the recoveries were, respectively, 10.56 and 11.97 mg.

Mixing of Isotopes. The radioactive data obtained with certain results gave entirely different recovery values than the gravimetric data. For example, 7.38 mg. of ruthenium isolated from the button gave a count of 370. This was anomalous; the count should have been 470, $\left(\frac{7.38}{8.97} \times 572\right)$, as 8.97 mg. of ruthenium in solution were mixed thoroughly with an active ruthenium solution containing 572 counts and negligible weight of ruthenium and the combined solution was well mixed with flux.

This means that the active and inactive isotopes were not chemically homogeneously mixed. This was true in spite of the fact that the active and inactive solutions were made by the same method, involving identical distillations and subsequent treatments. Information from other workers using radioactive isotopes indicates that this very significant phenomenon has been observed with isotopes of other elements.

This phenomenon sets the boundaries to the application of tracer chemistry, for unless the researcher is sure that it is absent his basic assumption that the tracer and inactive isotopes behave alike is invalid.

To remedy this situation large amounts of active and inactive solutions were mixed and two successive chlorine distillations done on the mixture. The standard solution so formed was used in subsequent determinations. After this was done assays were performed according to the methods shown above, and consistent results were obtained (Tables III to V) with the total activity found equaling that added, within the limits of experimental error.

These procedures were then used as a tool to examine the various types of assay by which ruthenium might be determined. The data are recorded in Tables III to V. In all cases residues from both the perchloric acid and hypochlorite distillations and filtrates from the thionalide precipitation were counted, and in all cases except No. 729, 0.0 counts were obtained. In No. 729,

Table III. Assays for Ruthenium

No.	Flux	Charge: Flux No.	Charge: Grams	Total No. of Equiv.: Of acids	Total No. of Equiv.: Of bases	Button Weight, Grams	Slag Weight, Grams	Time in Muffle, Min.	Temperature: Start, ° F.	Temperature: Finish, ° F.	Remarks
707	Ordinary bisilicate flux	69	100	0.42	0.36	24	70	40	1500	1800	
708	Ordinary bisilicate flux	69	100	0.42	0.36	25	70	50	1700	2000	
714	Ordinary bisilicate flux	69	100	0.42	0.36	28	65	1 hour	1500	2000	Test of borax wash, and of cupellation (partially to 6.5 grams)
716	Ordinary bisilicate flux	69	100	0.42	0.36	26	60	1 hour	1500	2000	Test of partial cupellation 6.5 grams
718	Niter assay on FeS_2	59	100	Approx. 0.62	Approx. 0.62	16	90	1 hour	1500	2000	These buttons were all borax washed to remove sulfide slag
719	Niter assay on FeS_2	59	100	Approx. 0.62	Approx. 0.62	24	85	1 hour	1500	2000	
720	Niter assay on FeS_2	59	100	Approx. 0.62	Approx. 0.62	22	85	1 hour	1500	2000	
727	Very acid flux	724	107	1.73	0.92	34	55	1 hour	1600	2000	
729	Very basic flux	725	130	1.42	2.55	30	85	2 hours	1400	2200	
730	Very basic flux	725	130	1.42	2.55	32	90	3 hours	1400	2400	
732	Very acid flux	724	107	1.73	0.92	30	70	40 min.	1600	2000	
734	Iron nail assay	61	133	Approx. 0.75	Approx. 0.80	42	110	1.25 hours	1600	2100	These slags mostly iron sulfide
735	Iron nail assay	61	132	Approx. 0.75	Approx. 0.80	38	105	1.25 hours	1600	2100	

Table IV. Assays for Ruthenium

(523 ± 3 counts taken)

No.	Counts Found[a] In button	In slag	In assay gases	In borax wash	In cupel	In cupel gases	Total	Remarks
707	380 ±2	144 ±15	0.6 ±2	....	....	.....	525 ±19	
708	472 ±3	57 ±10	4 ±3	....	....	.....	533 ±6	
714	428 ±2	38 ±8	....	7 ± 3	53 ±4	0.0	526 ±17	Assay gases not checked as such
716	428 ±2	53 ±6	....	....	53 ±4	0.0	534 ±12	Other data give 0.05 mg. or less
718	450	55	1 ± 3	20	....	....	526	
718	450 ±2	55 ±3	1 ± 3	20 ±4	....	.....	526 ±12	
719	398 ±2	100 ±3	1 ± 3	13 ±4	....	.....	512 ±12	
720	382 ±2	129 ±3	1–3	11 ±4	....	.....	523 ±12	
727	498 ±2	29 ±7	0.0	....	....	.....	527 ±9	
729	457 ±2	38 ±12	0.0	....	....	.....	495 ±14	38 ±8 counts or 0.41 mg. found in thionalide filtrate
730	490 ±2	34 ±10	....	....	....	.....	524 ±14	
732	436 ±2	117 ±10	....	....	....	.....	553 ±12	No known reason for anomaly
734	358 ±2	12 ±2	0.0	10 ±3	Found on iron nail	Approx. 93	470	Nail counts are rough
735	290 ±2	34 ±15	0.0	11 ±3		188	520	

[a] Counts corrected for decay and absorption and are referred to standard.

Table V. Ruthenium Found

(6.20 mg. of ruthenium taken)

In Button: Gravimetric[a] Mg.	In Button: Radioactivity Mg.	In Slag Mg.	In Assay Gases Mg.	In Borax Wash Mg.	In Cupel Mg.	In Cupel Gases Mg.	Total Mg.
4.66	4.50 ± 0.02	1.71 ±0.18	0.007 ±0.02	....	....	.....	6.38 ±0.20
5.53	5.56 ± 0.04	0.68 ±0.12	0.05 ±0.03	....	....	.....	6.26 ±0.15
4.92	5.04 ± 0.03	0.45 ±0.08	....	0.08 ±0.03	0.63 ±0.04	0.00	6.08 ±0.15
4.91	5.04 ± 0.03	0.63 ±0.08	....	....	0.63 ±0.04	0.00	6.17 ±0.13
5.41	5.36 ± 0.03	0.65 ±0.04	0.01 ±0.03	0.23 ±0.04	....	.....	6.30 ±0.11
4.80	4.82 ± 0.03	1.18 ±0.03	0.01 ±0.03	0.15 ±0.04	....	.....	6.14 ±0.10
4.57	4.62 ± 0.03	1.53 ±0.04	0.01 ±0.03	0.13 ±0.04	....	.....	6.24 ±0.11
6.86	5.82 ± 0.02	0.34 ±0.09	0.00	....	....	.....	6.20 ±0.09
5.39	5.41 ± 0.02	0.45 ±0.13	0.00	....	....	.....	5.84 (+0.41 = 6.25) ±0.13
5.76	5.80 ± 0.02	0.40 ±0.12	....	....	....	.....	6.16 ±0.12
4.18	4.22 ± 0.02	0.14 ±0.13	0.00	0.12 ±0.03	Found on iron nail	Approx. 1.1	5.5
3.27	3.24 ± 0.02	0.40 ±0.16	0.00	0.13 ±0.02		2.2	6.0

[a] All other figures results of radioactivity measurements.

counts on the thionalide filtrate showed an amount of ruthenium which agreed with that missing in the assay (see Tables III to V).

DISCUSSION OF RESULTS

The most important result of this research is the finding that even under the somewhat idealized conditions of this work very serious losses occurred in the assay for ruthenium.

Variable slag losses of about 0.6 mg. occurred during assays containing 8 mg. of ruthenium, and losses on 6-mg. assays were of about the same magnitude. These losses occurred in all types of assay tested, and the variations were so great that no difference could be distinguished among the slag losses for acidic, basic, or other fluxes. Data from the tables seem to indicate that the slag losses were smallest when the assay required a long heating period and a high temperature.

Cupellation losses were shown to be high, even on partial cupellation to 6.5 grams, and the losses were to the cupel, surprisingly not to the air by volatilization. These losses invalidate the inferences made by Lathe (*28*), who analyzed for ruthenium after cupellation to a 3- to 5-gram button, and also those of Peters (*36*), Pardo (*35*), and Lovely (*29*).

The gas losses during fusion and cupellation were found to be negligible, which was a somewhat unexpected result, as was the fact that the slag losses in the niter assay were not high, as might be expected in the presence of nitrates. This latter fact may be due to the absence of water.

The iron-nail assay was shown to be completely useless, because substantial amounts of ruthenium clung to the nail and were thus lost.

CONCLUSION

It has been amply shown that the perchloric acid parting and distillation, the hypochlorite distillation, and the thionalide precipitation are quantitative procedures, and may be used to analyze a lead button for ruthenium.

Serious losses have been found in the conventional methods of analysis for ruthenium in ores. These are not only important in the case of ruthenium, but they also cast doubts on the assay for other platinum metals; for while it has always been assumed that the platinum metals behave like gold during recovery by fusion methods, their chemistry bears far less relationship to that of gold than to that of ruthenium.

This research provides the first data tracing the distribution of any of the platinum metals during the various processes involved in a fire assay. It also provides a new method of parting the button, which, because it does not involve the serious interferences of nitric acid, will lead to a simplified separation of the platinum metals.

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Relationship between Laboratory Abrasion Tests and Service Performance

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The laboratory abrasion resistance test of GR-S commercial recapping compounds, carried out according to the regular A.S.T.M. procedure, gave results that did not agree with road tests. This is due to the formation of a viscous film on the abrasive and on the abraded rubber surface which lubricates the abrasive and leads to a ridiculously low abrasion loss. Extraction of the vulcanized rubber, prior to abrasion, with ethanol-toluene azeotrope prevented the development of this film and brought about excellent correlation with road tests. The purpose of the investigation was to determine the reason for the contamination of abrasive, and it was concluded that softener may not contribute in a major way, and that the main cause is probably depolymerized rubber developed during vulcanization. The theory is advanced that the rubber being abraded on the road is actually extracted rubber because extractable material is probably wiped off the rubber surface onto the road before appreciable abrasion occurs.

IN DEVELOPMENT work on GR-S commercial recapping compounds originated in 1943 by the Directorate of Mechanical Engineering, Department of National Defence, Ottawa, Canada, in which an attempt was made to correlate road performance with physical properties as determined in the laboratory it was found that no relationship whatever existed between the results of road tests carried out under the supervision of that directorate and standard laboratory abrasion resistance tests carried out in the Canadian National Research Council Rubber Laboratory at Ottawa. In the laboratory test the sandpaper in the abrasion machine became coated with a smear of tacky, viscous material which the air jet was unable to remove. Under these conditions the rubber tends to slide over the sandpaper surface with relatively little actual abrasion of the rubber. The effect remains even after a considerable overcure of the sample.

It was felt that the removal of the tacky, viscous material from the vulcanized GR-S by extraction might give more reliable abrasion resistance results, inasmuch as on the road the rubber is constantly coming in contact with a new surface and such viscous material is thus being continally removed as it migrates to the surface of the rubber. From this point of view, then, the tread surface while being abraded on the road may be looked upon as extracted rubber, and may be considered as conforming closely to the extracted laboratory specimen.

METHOD OF TEST

Method of Extraction. Where vulcanized rubber was extracted prior to the abrasion resistance test, this extraction was carried out in standard Soxhlet apparatus, without paper thimble.

The solvent used was ethanol-toluene constant boiling mixture, made from 70 volumes of 95% ethanol and 30 volumes of toluene. The mixture of ethanol and toluene was purified before use by distillation; the liquid boiling at a temperature of approximately 75° C. was retained. The rubber was extracted for 96 hours with this ethanol-toluene azeotrope, followed by a 24-hour extraction with 95% ethanol to remove absorbed solvent from the rubber. The ethanol was changed four times during the 24-hour period. The extracted rubber was then allowed to stand 48 hours under ordinary room conditions before the abrasion resistance test was carried out.

In a few of the earlier tests—i.e., for the recapping compounds—the extraction with azeotrope was followed by a 6-hour extraction with acetone, followed by vacuum drying at room temperature. When the change was later made from acetone and vacuum drying to ethanol and drying under ordinary room conditions, it was mostly a matter of convenience and did not affect any of the conclusions drawn from the results.

The effect of the time of standing after ethanol extraction, before the abrasion resistance test, was investigated and is dealt with below.

Test specimens of various compounds were extracted together in the one Soxhlet apparatus whenever it was more convenient to carry out the extraction in this manner and a separate extraction of each specimen was not considered necessary.

The extraction of rubber vulcanized in the laboratory was, with a few insignificant exceptions, carried out on rubber strips approximately 3.75 × 8.75 × 0.6 cm. (1.5 × 3.5 × 0.25 inch.). After the extraction and period of standing the strips were cut to the proper size, cemented to a rubber backing, and tested in the abrasion machine. Half-way through the extraction with azeotrope the rubber strips were turned end for end in the Soxhlet to ensure uniform extraction throughout the rubber. The size of samples taken from actual tires was, of course, variable and depended upon the contour of the tread, but in every case the sample after extraction was large enough to permit the preparation of a test piece of the specified A.S.T.M. standard dimensions.

Method of Abrasion. Both the Du Pont and Bureau of Standards machines were used in this investigation, and the method outlined in A.S.T.M. Designation D394–40 was followed.

In the Bureau of Standards machine the abrasive used was Garnet E/CL. This abrasive is wrapped around a drum revolving on a horizontal axis, and the rubber test block being abraded rests on the drum under a fixed weight.

In the Du Pont machine two rubber test blocks made from the same sample are held against a disk of abrasive revolving in a vertical plane. The abrasive used was much finer, and was that known as 2/0-100, E2883. By means of a Prony brake system this machine can measure not only the volume loss but also the volume loss per unit of work expended.

A.S.T.M. compound B, unextracted, prepared in the National Research Council Rubber Laboratory, was used as the standard of comparison for obtaining the abrasive index. This compound has the following composition:

No. 1 smoked sheet	100
Zinc oxide	20
Channel carbon black (Micronex)	30
Stearic acid, C.P.	2
Di-*o*-tolylguanidine	1.25
Phenyl-β-naphthylamine	1
Sulfur	3.5

Cure, 60 minutes at 287° F.

This is the compound suggested by Sigler and Holt (*2*) in their description of work on the Bureau of Standards machine.

This compound averaged, during the present investigation, around 200 cc. per horsepower hour, or 3.44 cc. per hour, unextracted, on the du Pont machine. The average of 3.44 cc. per hour was used throughout in calculating all the Du Pont machine abrasive index results. The method of calculating the abrasive index, specified in A.S.T.M. Designation D394–40, is based on the volume loss only—i.e., the volume loss for the standard multiplied by 100, divided by the volume loss for the sample.

On the Bureau of Standards machine the figure used for the standard was that obtained during the individual test concerned, and the standard was run on the same piece of abrasive as the sample being tested.

Changing of Abrasive. Except where otherwise indicated, a fresh piece of abrasive paper was used on the Du Pont machine for the standard as well as for the sample, while on the Bureau of Standards machine both the standard and one sample were run on the one piece of paper. The standard in this latter case was run before and after the test specimen, and the average of the two runs on the standard was used in calculating abrasive index. The question of the changing of the abrasive is discussed below.

Averaging of Results. The figures for abrasion resistance, unextracted, shown in Table I, were the average of two specimens cut from 900/16 tires recapped in the same manner and with the same tread compounds as the tires used in the road tests; the recapping cure in this case was 144 minutes at 307° F. These two specimens were run together on the same piece of sandpaper, as is necessary when the Du Pont machine is used. The figures for the abrasion resistance, extracted, were the average of four specimens, two of which were cut from the tires just mentioned and two of which were press curved 90 minutes at 292° F. in the laboratory from the corresponding unvulcanized camelback compound. These four specimens would represent two runs on the Du Pont machine.

There was a fair correlation between the results of physical tests made on the two different cures, although there was no one laboratory cure that agreed perfectly, for all compounds used, with the 144-minute recapping cure. Lack of sample and help prevented further physical tests to correlate cures. The extracted samples taken from the tires, computed alone, also correlated very well with road tests, but in view of the large error involved in abrasion resistance results, it was thought advisable to average as many test specimens as were available in order to assess the value of the extraction method. When the greater number of samples was averaged, a slightly better correlation was obtained.

For all other results obtained on the Du Pont machine, three pairs of test specimens, run on three different sheets of abrasive, were averaged for each abrasion loss figure in the tables shown herein. With the Bureau of Standards machine each figure shown represents the average of three test specimens, run as indicated, along with the standard, on three different sheets of abrasive.

Cure. Except where otherwise stated, the cure chosen for laboratory devised formulations was a moderate overcure, in order to avoid the possibility that an undercure might increase the contamination effect on the sandpaper.

To have used optimum cures would have gone beyond the capacity of staff and equipment for the large number of stocks tried. There would also be the difficulty of deciding the proper criterion, satisfactory to everyone, upon which to judge optimum cure in a study of abrasion resistance.

On the whole, it was felt that the observed effects were so great as to overshadow any minor variations that would result from choosing a particular degree of cure.

COMMERCIAL RECAPPING COMPOUNDS

Road Test Details. The road test of the recapped tires was carried out by the Directorate of Mechanical Engineering of the Department of National Defence, Ottawa, Canada.

The tires were 900/16 natural rubber tires, all of the same tread design, recapped with GR-S camelback, cured for 90, 144, and 198 minutes at 307° F., and the tread wear mileages shown in Table I are averages for these three cures. Road tests were carried out on two tires at each cure for each of eight different commercial camelback compounds, making a total of 48 tires tested on the road. The mileages given in the table are based upon the mileage at which the tread wore smooth. In cases where the tire had to be removed because of other defects, the mileage necessary to wear the tread smooth was estimated. All the tires were run,

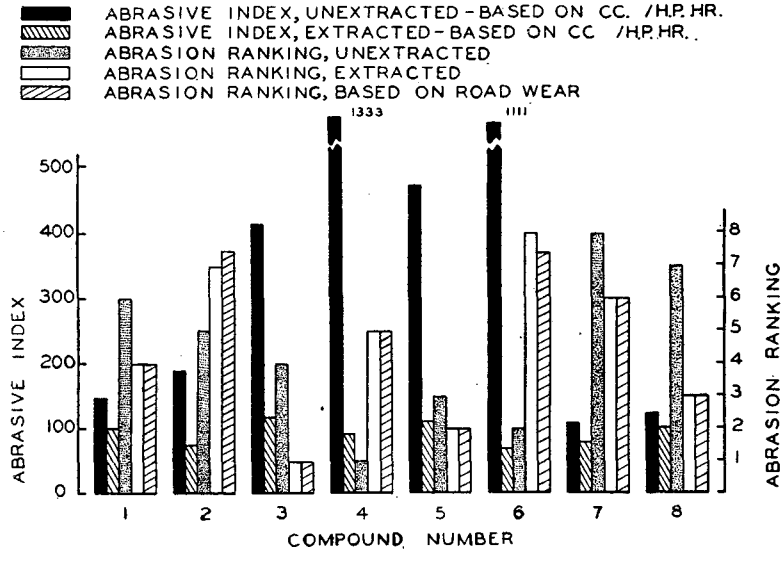

Figure 1. Commercial Recapping Compounds

Du Pont abrader

Table I. Commercial Recapping Compounds on Du Pont Abrasion Machine

	1	2	3	4	5	6	7	8
Specific gravity								
Unextracted	1.17	1.16	1.14	1.16	1.14	1.14	1.16	1.14
Extracted	1.17	1.20	1.20	1.16	1.16	1.14	1.16	1.17
Average horsepower								
Unextracted	0.0155	0.0153	0.0200	0.0187	0.0200	0.0190	0.0159	0.0169
Extracted	0.0133	0.0156	0.0147	0.0163	0.0155	0.0148	0.0152	0.0158
Abrasion loss, cc./h.p. hr.								
Unextracted	136	106	48	15	42	18	170	162
Extracted	199	268	169	218	181	289	253	198
Abrasion loss, cc./hr.								
Unextracted	2.11	1.62	0.96	0.28	0.84	0.34	2.70	2.74
Extracted	2.66	4.18	2.63	3.57	2.81	4.26	3.83	3.12
Abrasive index, based on cc./hr.								
Unextracted	163	212	358	1227	410	1006	127	126
Extracted	129	82	131	96	122	81	90	110
Road mileage, miles	5760	5100	7000	5700	6100	5100	5460	5830
Abrasion ranking								
Based on cc./h.p. hr.								
Unextracted	6	5	4	1	3	2	8	7
Extracted	4	7	1	5	2	8	6	3
Based on cc./hr. (or abrasive index)								
Unextracted	6	5	4	1	3	2	7	8
Extracted	2	7	1	5	3	8	6	4
Based on road wear	4	7, 8	1	5	2	8, 7	6	3
Change in abrasion loss on extraction								
Cc./h.p. hr.	+63	+162	+121	+203	+139	+271	+83	+36
Cc./hr.	+0.55	+2.56	+1.67	+3.29	+1.97	+3.92	+1.13	+0.38

Table II. Commercial Footwear Compounds on Du Pont Abrasion Machine

Sample	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Specific gravity															
Unextracted	1.33	1.33	1.29	1.31	1.25	1.26	1.25	1.36	1.57	1.25	1.32	1.28	1.28	1.24	1.32
Extracted	1.42	1.40	1.39	1.42	1.28	1.33	1.32	1.29	1.48	1.31	1.38	1.36	1.38	1.26	1.34
Average horsepower															
Unextracted	0.0143	0.0136	0.0145	0.0145	0.0138	0.0150	0.0140	0.0136	0.0139	0.0167	0.0139	0.0156	0.0156	0.0167	0.0132
Extracted	0.0129	0.0100	0.0112	0.0100	0.0129	0.0124	0.0118	0.0109	0.0099	0.0133	0.0120	0.0102	0.0105	0.0136	0.0124
Abrasion loss cc. per h.p. hr.															
Unextracted	491	508	389	365	359	380	352	450	406	320	389	345	326	225	483
Extracted	766	646	776	663	386	429	406	626	741	411	437	518	520	428	693
Abrasion loss, cc./hr.															
Unextracted	7.01	6.88	5.66	5.29	4.96	5.69	4.94	6.12	5.64	5.34	5.42	5.37	5.08	3.75	6.38
Extracted	9.86	6.42	8.71	6.63	4.97	5.31	4.81	6.80	7.33	5.48	5.23	5.30	5.48	5.82	8.57
Abrasive index, based on cc./hr.															
Unextracted	49	50	61	65	69	61	70	56	61	64	64	64	68	92	54
Extracted	35	54	40	52	69	65	72	51	47	63	66	65	63	59	40
Abrasion ranking															
Based on cc./h.p. hr.															
Unextracted	14	15	9	7	6	8	5	12	11	2	10	4	3	1	13
Extracted	14	10	15	11	1	5	2	9	13	3	6	7	8	4	12
Based on cc./hr. (or abrasive index)															
Unextracted	15	14	10	5	3	11	2	12	9	6	8	7	4	1	13
Extracted	15	9	14	10	2	5	1	11	12	6	3	4	7	8	13
Change in abrasion loss on extraction															
Cc./h.p. hr.	+275	+138	+387	+298	+27	+49	+54	+176	+335	+91	+48	+173	+194	+203	+210
Cc./hr.	+2.85	−0.46	+3.05	+1.34	+0.01	−0.38	−0.13	+0.68	+1.69	+0.14	−0.19	−0.07	+0.40	+2.07	+2.19

as closely as they could be controlled, under the same conditions, on a specially chosen course consisting of 75% hard surface road and 25% average gravel road.

The test vehicles were 15-cwt. trucks, and the tires were mounted on rear wheel positions only, and were changed from side to side and truck to truck to eliminate inequalities of position, drivers, and trucks. The load per tire was the maximum for the tire—i.e., 3080 ± 50 pounds. The tires were inflated to 45 pounds per square inch, giving 18% deflection at the maximum tire load. The operating speed on the hard surface road was 35 miles per hour and the maximum 40 miles per hour. On the gravel road the operating speed was 30 miles per hour and the maximum 35 miles per hour.

Laboratory *vs.* Road Tests. The results of the laboratory abrasion resistance tests, obtained on the Du Pont machine, and the corresponding road mileage figures for the eight different recapping compounds are shown in Table I. Under "ranking," the best laboratory abrasion resistance and the highest road mileage are ranked first, the poorest laboratory abrasion resistance is ranked eighth, and the intermediate compounds are graded accordingly.

Figure 1 shows these results in bar graph form.

The perfect correlation between the abrasion loss of the extracted samples and the road mileages is probably due, to a certain extent, to coincidence, because the error in abrasion test figures is known to be high, and perfect correlation should not be expected as a general rule. All that these results can show is that by extracting the sample before running the abrasion test one can convert the abrasion test, which formerly was useless in this connection, into a test that may enable one to predict with reasonable accuracy the wearing quality of a GR-S tire compound.

Compound 6 illustrates very well the effect of extraction. Before extraction this compound had a ridiculously low abrasion loss of 18 cc. per horsepower hour, ranked second, and should rank seventh or eighth based upon road performance. After extraction the abrasion loss was raised to 289 and the sample fell into its correct place with respect to road performance.

The complete results of the road test have been reported separately by the Directorate of Mechanical Engineering, Department of National Defence, Ottawa, Canada.

Table III. Laboratory Footwear Compounds

Compound No.	5-1	5-2	5-3	5-4	5-5	5-6	5-7
GR-S	100	67	50	50	72	100	100
Black Diamond reclaim	...	60	91	91	50	....	...
Wyex	40	40	30	30	30	....	0.5
Thermax	90	..	55	...	60	....	....
Pelletex	...	..	...	...	..	5	....
Dixie clay	...	60	...	60	..	130	40
Calcene T	...	..	...	...	..	....	160
Mineral rubber	10	10	...	...	10	....	10
Stearic acid	1	1	1	1	1	....	....
Paraffin	...	..	...	...	..	1	1
Circo light process oil	5	5	10	10	5	....	....
Burnt sienna	...	..	...	...	..	....	5
Litharge	...	..	0.25	0.25	..	....	....
Zinc oxide	5	5	5	5	5	5	5
Agerite powder	1	1	2	2	2	2	2
Thionex	0.5	0.5	0.35	0.35	0.5	....	....
Cumate	...	..	...	...	..	0.75	0.75
Sulfur	2.5	2.5	2.75	2.75	2.5	2.5	2.5
Press cure at 316° F., min.	10	10	10	10	10	15	15
Du Pont abrader Abrasive index (A.S.T.M. standard B)							
Original	75.7	38.0	44.0	36.3	53.2	37.4	24.6
Extracted	72.4	19.5	32.4	17.0	38.5	18.0	18.7
Aged, 24 hr. at 100° C.	89.9	35.5	50.9	35.0	58.9	32.5	....
Bureau of Standards abrader Abrasive index (A.S.T.M. standard B)							
Original	61.4	26.7	35.8	26.3	41.3	25.0	14.6
Extracted	38.5	12.9	21.9	11.8	27.0	14.1	10.1
Aged, 24 hr. at 100° C.	60.4	23.7	36.0	24.3	42.4	32.9	....

COMMERCIAL FOOTWEAR COMPOUNDS

Tests similar to those described for the recapping compounds were carried out on commercial footwear soling compounds at the request of the Canadian Rubber Footwear Conservation Sub-Committee (Table II).

No field tests concurrent with laboratory tests were carried out on these compounds, as was the case with the recapping compounds, but the samples were known by the companies submitting them to give satisfactory abrasion resistance in service. As a matter of fact, the laboratory abrasion resistance results, extracted, are good, and bear out the manufacturers' claims in this respect.

The difference between the results unextracted and extracted is not so great with these footwear compounds as with the recapping compounds, but is appreciable in some instances. In view of the finding with the recapping compounds, it seems reasonable to assume that, at least in those cases where the difference is fairly large—for example, with compounds A, C, D, I, N, and O—the result, extracted, will more nearly approximate what would be obtained in service.

LABORATORY FOOTWEAR TYPE COMPOUNDS

Unaged Samples. In order to investigate the fundamental causes of the serious lack of correlation existing between present standard abrasion resistance tests and service performance, compounds of various types were prepared in the laboratory for study.

The first series so prepared was of the footwear type and Table III shows the results obtained on the Du Pont as well as on the Bureau of Standards abrader with this type of formulation.

These compounds are GR-S only, or GR-S–reclaim, stocks of the type used in heels and soling. In all cases the extraction with ethanol-toluene azeotrope lowered the abrasive index.

Compounds 5-2, 5-4, and 5-6 show a relatively large drop in abrasive index after extraction.

In view of the good correlation of the results on extracted recapping compounds with road wear, it is likely that the results on these three extracted footwear compounds would also agree with actual wearing tests and that the results on the corresponding unextracted compounds are subject to a large error.

Extraction does not rank all the compounds in Table III in exactly the same manner with both machines. This is undoubtedly due to the fact that a number of the compounds have nearly the same abrasion resistance. When there is a difference in abrasion resistance definitely beyond experimental error, the two machines give the same ranking, particularly after extraction—for example, compounds 5-1, 5-5, and 5-3 are ranked respectively first, second, and third in abrasion resistance throughout the table. The other compounds are too close together in abrasion resistance and therefore are not ranked so uniformly.

Aged Samples. Because the accelerated aging test carries on the cure to a certain extent, this treatment of the sample before abrasion might be expected to overcome the effect of undercure. If undercure were the cause of the contamination of the abrasive, a preliminary aging treatment of the sample might be expected to overcome the difficulty.

The results shown in Table III, however, as well as other results not reported herein, show the aging treatment to be ineffective or at best only slightly beneficial in preventing the contamination referred to. The results in Table III show that although some compounds, such as 5-2, 5-4, and 5-6, have their abrasion loss considerably increased by extraction, aging does not produce a commensurate increase, so that, in general, one would not expect the corrective effect produced by extraction to be duplicated by aging.

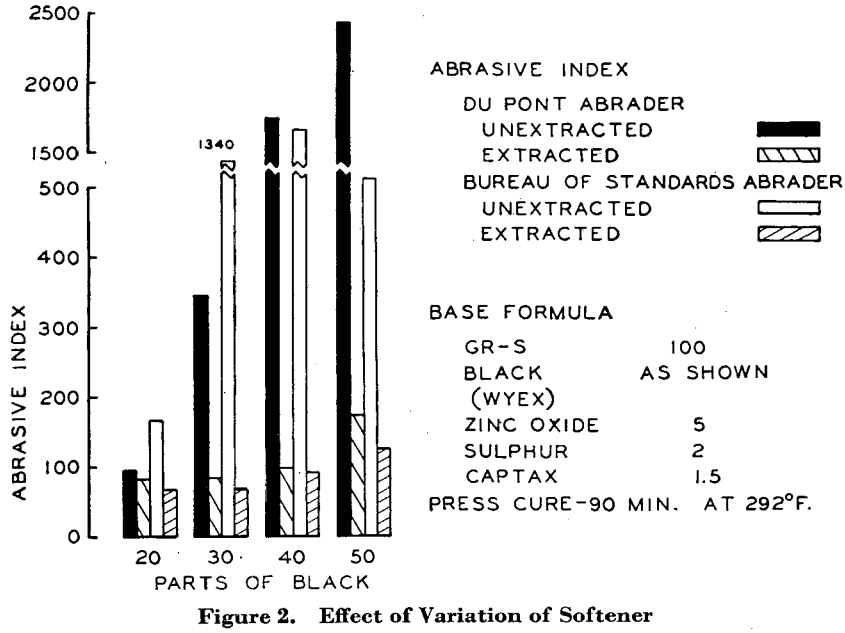

Figure 2. Effect of Variation of Softener

LABORATORY TREAD TYPE COMPOUNDS

Effect of Variation of Softener Content. In Tables IV and V and Figure 2 are shown the results obtained on carbon black compounds in which the quantity of softener has been varied.

Compounds 5-8, 5-9, 5-10, and 5-11, are based upon a simple tread type compound in which the content of B.R.T. No. 7 has been varied from 0 to 10 parts per 100 parts by weight of GR-S. In Table V are shown carbon black compounds Nos. 5-8 (repeated), 5-49, 5-50, and 5-51, containing, respectively, 0, 5, 10, and 20 parts of Circo light process oil.

The abrasive index, unextracted, of all these compounds is ridiculously high, ranging from about 2000 to about 150, the effect being more marked on the Du Pont than on the Bureau of Standards machine. After extraction, however, the results become reasonable and are graded according to softener content, regardless of the nature of the softener; the compound with the highest softener content has the poorest abrasion resistance.

With the unextracted B.R.T. No. 7 compounds on the Du Pont abrader, the relationship of abrasive index to softener content is not regular. As this softener is increased from 0 to 10 parts the abrasive index passes through a maximum at 2.5 parts. With the unextracted oil softener compounds on the Bureau of Standards machine there is an irregularity in that the abrasive index passes through a minimum at 5 parts of oil. However, such anomalies disappear upon extraction.

When not given the preliminary extraction, all these compounds formed a tarry deposit on the abrasive. This deposit was more marked with the Du Pont abrasive than with the coarser Bureau of Standards abrasive, but showed up definitely on both machines.

It is obvious that the conditions that exist during the laboratory abrasion resistance test of the average unextracted GR-S tread compound do not exist when the tire is on the road. The road does not get smeared with a viscous deposit as the sandpaper does, so that the road produces the effect of a continuously dry abrasive. If extractable material leaves the rubber and coats the sandpaper during the laboratory test, such material may also leave the rubber in a tire and be deposited on the road. In the laboratory test the material extracted from the rubber, as it is of a semiliquid consistency, must contain mostly low molecular weight material of a soluble nature and relatively little solid vulcanized rubber compound, and so one of the effects of laboratory abrasion is more or less to remove this soluble material from the rubber. When the tire is on the road, too, it is not unreasonable to expect the removal of soluble material as in the laboratory test, before appreciable abrasion occurs. This extraction on the road should be accompanied by changes in specific gravity, hardness, and other physical properties, such as are shown later to be accompanied by the extraction of the sample with ethanol-toluene azeotrope. In other words, the surprisingly good correlation found between road tests and the abrasion tests of extracted samples may well be due to the possibility that the surface of the tire in contact with the road is extracted rubber. Obviously, any extractable material that migrates to the surface of the rubber will merely be wiped off by the road and thus be extracted from the rubber, as such material is entirely too soft to help the rubber in resisting abrasion. The fact that extracted rubber correlates better than unextracted rubber with the effect of road wear may well be due to a closer resemblance, in composition and properties, of the rubber in contact with the road surface, to extracted rubber than to unextracted rubber. It is felt that there is some justification for this conclusion in the practical results obtained.

The fact that the abrasion resistance is graded according to softener content even after extraction, when the softener is not present, lends support to this conclusion. The effect of softener is simply that of a dilution or a weakening of the rubber compound. This effect becomes inherent in the rubber upon vulcanization, and would remain even after subsequent removal of the softener by the road surface.

Effect of Variation of Carbon Black Content. In Table VI and Figure 3 are shown the results obtained on a series of compounds, without softener—i.e., 5-8, 5-24, 5-23, and 5-22—containing 50, 40, 30, and 20 parts of carbon black, respectively. With the Du Pont machine, there is a gradual decrease in abrasive index as the carbon black content is reduced. Although the ranking was unaffected by extraction it is apparent that, even though the

Table IV. Laboratory Tread Type Compounds

[Effect of variation of softener content (B.R.T. No. 7)]

Compound No.	5-8	5-9	5-10	5-11
GR-S	100	100	100	100
Wyex	50	50	50	50
Zinc oxide	5	5	5	5
B.R.T. No. 7	....	2.5	5	10
Captax	1.5	1.5	1.5	1.5
Sulfur	2	2	2	2
Press cure at 292° F., min.	90	90	90	90
Specific gravity				
Original	1.16	1.16	1.16	1.17
Extracted	1.14	1.15	1.14	1.15
Hardness, Type A, Shore				
Original	67	69	65	67
Extracted	57	58	56	54
Du Pont abrader				
Abrasive index (A.S.T.M. standard B)				
Original	2450	2930	2160	970
Extracted	175	157	140	133
Bureau of Standards abrader				
Abrasive index (A.S.T.M. standard B)				
Original	516	211	194	156
Extracted	128	110	103	84

Table V. Laboratory Tread Type Compounds

[Effect of variation of softener content (process oil)]

Compound No.	5-8	5-49	5-50	5-51
GR-S	100	100	100	100
Wyex	50	50	50	50
Zinc oxide	5	5	5	5
Circo light process oil	....	5	10	20
Captax	1.5	1.5	1.5	1.5
Sulfur	2	2	2	2
Press cure at 292° F., min	90	90	90	90
Specific gravity				
Original	1.16	1.15	1.15	1.13
Extracted	1.14	1.14	1.14	1.14
Du Pont abrader				
Abrasive index (A.S.T.M. standard B)				
Original	2450	1760	1590	895
Extracted	175	126	123	105
Bureau of Standards abrader				
Abrasive index (A.S.T.M. standard B)				
Original	516	318	326	330
Extracted	128	104	94.8	89.6

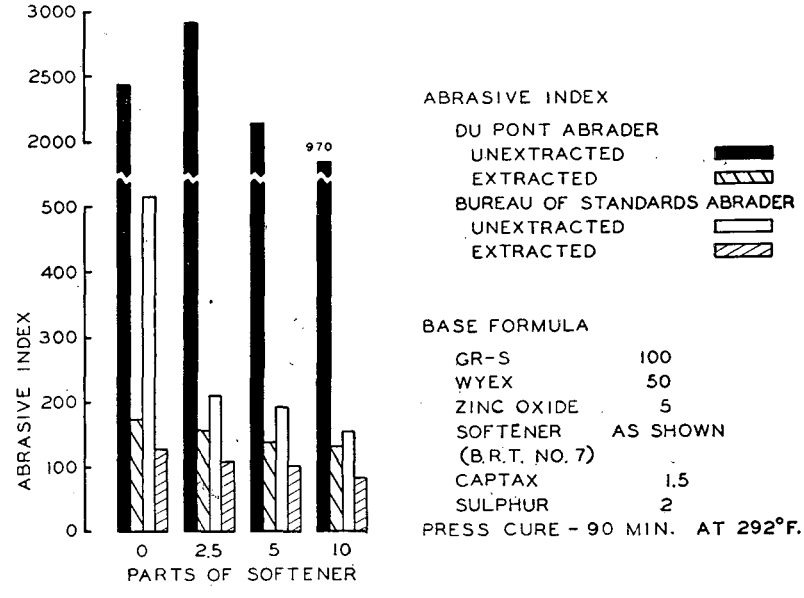

Figure 3. Effect of Quantity of Black

Table VI. Laboratory Tread Type Compounds

(Effect of variation of carbon black content)

Compound No.	5-8	5-24	5-23	5-22
GR-S	100	100	100	100
Wyex	50	40	30	20
Zinc oxide	5	5	5	5
Captax	1.5	1.5	1.5	1.5
Sulfur	2	2	2	2
Press cure at 292° F., min.	90	90	90	90
Specific gravity				
Original	1.16	1.13	1.10	1.07
Extracted	1.14	1.13	1.13	1.07
Du Pont abrader Abrasive index (A.S.T.M. standard B)				
Original	2450	1760	348	96
Extracted	175	100	85	83
Bureau of Standards abrader Abrasive index (A.S.T.M. standard B)				
Original	516	1680	1340	167
Extracted	128	93.7	69.4	69.3

Table VII. Nonblack Compounds

Compound No.	5-12	5-13	5-14	5-15
GR-S	100	100	100	100
Calcene T	202	202	....	....
Silene EF	....	....	64.5	64.5
Zinc oxide	5	5	5	5
Agerite powder	2	2	2	2
Paraffin	1	1	1	1
Cumar MH 2½	....	15	....	15
Cumate	0.75	0.75	0.75	0.75
Sulfur	2.5	2.5	2.5	2.5
Press cure at 316° F., min.	15	15	15	15
Specific gravity				
Original	1.65	1.62	1.25	1.24
Extracted	1.66	1.67	1.25	1.24
Hardness, Type A, Shore				
Original	63	53	65	52
Extracted	72	69	65	60
Du Pont abrader Abrasive index (A.S.T.M. standard B)				
Original	20.9	22.7	52.7	55.4
Extracted	17.5	17.4	49.8	50.9
Bureau of Standards abrader Abrasive index (A.S.T.M. standard B)				
Original	11.4	13.0	32.2	34.9
Extracted	8.2	8.8	28.2	29.8
Modulus at 300% pounds per square inch				
Original	540	320	945	585
Extracted	....	420	985	635
Tensile strength, pounds per square inch				
Original	635	1020	1055	1550
Extracted	575	560	1200	970
Elongation at break, %				
Original	340	575	335	590
Extracted	285	410	355	405

compounds did not contain softener, the figures for the unextracted compounds 5-8, 5-24, and 5-23 are ridiculously high, and become reasonable only after extraction. Compound 5-22 is not changed considerably by extraction, but is lowered in abrasive index just enough to cause it to be ranked correctly after extraction. Compounds 5-23 and 5-22 are, nevertheless, close in abrasive index, extracted (85 and 83, respectively), so that this ranking in the expected order may, to a certain extent, be fortuitous, inasmuch as the experimental error may be greater than the difference obtained.

On the Bureau of Standards machine the abrasive index figures on the unextracted compounds are obviously too high, and are not ranked in order of carbon black content. After extraction, however, these compounds are ranked according to carbon black content, and the results become reasonable. On this machine, also, the results given by compounds 5-23 and 5-22 are close (69.4 and 69.3, respectively) and, as with the figures obtained on the Du Pont machine, the fact that the ranking is as expected may be somewhat fortuitous.

Table VIII. Results with Raw GR-S Types

Compound No.	5-25	5-26	5-27	5-28	5-29
GR-S, Sarnia	100	....	....	....	....
GR-S, Firestone	.....	100	....	....	....
GR-S, Naugatuck	.....	....	100	....	....
GR-S, O.R.D.	.....	....	....	100	....
GR-S, S 2503, Sarnia	.....	....	....	....	100
Wyex	50	50	50	50	50
Zinc oxide	5	5	5	5	5
Captax	1.5	1.5	1.5	1.5	1.5
Sulfur	2	2	2	2	2
Press cure at 292° F., min.	90	90	90	90	90
Specific gravity	1.15	1.16	1.15	1.15	1.15
Hardness, Type A, Shore	61	57	59	61	62
Du Pont abrader Abrasive index (A.S.T.M. standard B) Unextracted	1765	783	2310	1235	2245

NONBLACK COMPOUNDS

In Table VII are shown the results obtained with compounds containing Cumar MH 2½, Calcene T, and Silene EF.

There is a drop in abrasive index with all four compounds after extraction. Before extraction both machines give these four compounds the same ranking. They have the same ranking after abrasion, with the exception that compound 5-12 has an abrasive index greater by 0.1 than compound 5-13 on the Du Pont machine, whereas the Bureau of Standards machine shows 5-12 to be very slightly inferior to 5-13. Here, of course, the two compounds are so close together in abrasion resistance that a small experimental error could reverse the ranking.

There was no visible coating of the abrasive by the four compounds shown in Table VII. The coating may, of course, be present and not be readily observable on account of the fact that the compounds are white. The drop in abrasion resistance of all four compounds on extraction is evidence that the erroneous abrasion resistance results herein described are not necessarily accompanied by visible contamination of the abrasive.

CAUSE OF CONTAMINATION OF ABRASIVE

Effect of Compounding Ingredients. In the tables so far discussed, no particular compounding ingredient appears to stand out as the main cause of the contamination of abrasive.

GR-S carbon black compounds (Tables IV and V), without softener as well as with softener, produce the contamination, so that the particular softeners investigated may be eliminated at least as a main cause. As a matter of fact, one observes an overall tendency for the abrasive index of unextracted specimens to decrease with increase of softener content, so that softener in many cases exerts a preventive effect.

When the carbon black is progressively reduced (Table VI) a decrease in abrasive index, far greater than one would expect as a result of the loss of reinforcement, indicates that carbon black may contribute to the contamination. The nonblack compounds (Table VII), however, also show the effect, as evidenced by the decrease in abrasive index upon extraction, and this leads to the conclusion that even though carbon black may be a contributory factor, it is not the only cause and is probably not the basic cause of the contamination.

What has been said about softener and carbon black can probably also be said about the other softeners and fillers used in the series of compounds so far described. In other words, these ingredients may or may not contribute to the contamination but if they do, they cannot be the basic cause, inasmuch as the effect can be produced without them or, as will be shown later, a compound can be found containing them that does not contaminate the abrasive.

Results with Raw GR-S Types. In view of the failure to find a compounding ingredient on which to lay the blame for the contamination, other possibilities were investigated, such as that

Table IX. Effect of Extraction of GR-S and Carbon Black before Compounding

Compound No.	5-8	5-31	5-32	5-33	5-34
GR-S	100	100	....	....	....
GR-S, extracted	.....	.....	100	100	100
Wyex	50	.....	50	....	....
Wyex, extracted	.....	50	....	50	50
Zinc oxide	5	5	5	5	5
Captax	1.5	1.5	1.5	1.5	1.5
Sulfur	2	2	2	2	2
Stearic acid	.....	.....	....	....	2
Press cure at 292° F., min.	90	90	90	90	90
Specific gravity					
Original	1.16	1.16	1.17	1.15	1.15
Extracted	1.14	1.15	1.14	1.14	1.14
Hardness, Type A, Shore					
Original	67	59	56	52	53
Extracted	57	53	46	46	48
Du Pont abrader					
Abrasive index (A.S.T.M. standard B)					
Original	2450	1460	864	746	950
Extracted	175	106	94	91	133

the contamination might be due to a particular lot or particular type of GR-S.

In Table VIII it will be seen that the coating of the abrasive is produced by GR-S rubber as a class and not by any particular type or lot. Five different types of GR-S are shown to produce varying degrees of contamination of the abrasive. The lowest abrasive index obtained, unextracted, of 783, is still very high.

Extraction before Compounding. In Table IX it will be seen that extraction of either the GR-S or the carbon black before mixing lowers the contamination effect considerably, but still leaves an extremely high abrasive index.

Extraction of the GR-S before mixing (compound 5-32) improves the result somewhat more than extraction of the carbon black (compound 5-31), the lowest abrasive index being given by the extraction of both GR-S and carbon black (compound 5-33). Addition of 2 parts of stearic acid to the compound containing extracted GR-S and extracted carbon black (compound 5-34) increases the contamination effect slightly. This would indicate that stearic acid may be a softener that contributes to the contamination effect, especially as the raw GR-S contains stearic acid and the extraction of this material from the GR-S reduces the contamination. The lowest abrasive index obtained of 746 is still very high. As shown in the table, reasonable results are not obtained with any of these compounds until after the extraction of the vulcanized rubber.

Because the pre-extracted materials also produce the contamination, these results show that neither the raw GR-S nor the carbon black contains soluble material that can be accused of being mainly responsible for the contamination.

The rubber was extracted in an Erlenmeyer flask in sheets 0.3 cm. (0.125 inch) thick with filter paper between the sheets. Freshly distilled ethanol-toluene azeotrope was passed into the flask by continuous flow, so that the rubber was constantly in contact with pure solvent at about 40° C. for a period of 4 days. About 9% of extract was removed from the raw GR-S in this manner, so that the extraction should be considered practically complete.

After a small amount of antioxidant was added by permitting the rubber to absorb it from a 1% solution of the antioxidant in the azeotrope, the rubber was dried in a vacuum for 2 hours at 65° C. Immediately after vacuum drying, 1% of antioxidant was added to the rubber on the mixing mill.

The rubber just before the final compounding operation still retained tenaciously about 10% of residual solvent, but probably lost a considerable amount of this solvent during compounding and vulcanization. The retention of solvent by the rubber in this case should not detract from the value of the conclusions derived from the experiment, because rubber extracted after vulcanization also retains solvent but nevertheless gives results of greatly improved accuracy, and the solvent itself obviously does not contribute to the contamination of abrasive.

Table X. Effect of Temperature of Cure with Straight Captax Acceleration Compound 5-8

Press cure				
Time, min.	326	171	90	47½
Temperature, ° F.	256	274	292	310
Modulus at 300%, pounds per square inch	1590	1770	1945	1940
Tensile strength, pounds per square inch	2875	2655	2620	2515
Elongation at break, %	485	415	385	370
Hardness, Type A, Shore				
Original	59	60	58	61
Extracted	53	53	55	55
Du Pont abrader				
Abrasive index (A.S.T.M. standard B)				
Original	1880	2230	2250	1860
Extracted	164	165	158	168

Table XI. Natural Rubber Compounds

Compound No.	5-38	5-39
Smoked sheet	100	91.1
E.T.A. extract of GR-S compound 5-8	...	8.9
Stearic acid	3	3
Neozone D	1.5	1.5
Zinc oxide	3	3
Wyex	50	50
Sulfur	3	3
Captax	1	1
Press cure at 292° F., min	45	25
Hardness, Type A, Shore		
Original	59	72
Extracted	57	62
Du Pont abrader		
Abrasive index (A.S.T.M. standard B)		
Original	644	169
Extracted	60.3	70.2

Effect of Temperature of Vulcanization. It is not possible to observe the behavior of vulcanizing agents indirectly by omitting them from the compound, as was done with some of the other compounding ingredients, but it was thought that a study of the effect of rate and temperature of cure might yield information of some value.

In a study of the effect of temperature of cure, compound 5-8 was vulcanized at 256°, 274°, 292°, and 310° F. The 90-minute cure at 292° F. was taken as the standard and the equivalent curing times at the other temperatures were calculated from published tables of the variation of cure with temperature for GR-S. Tensile tests were conducted for each cure, following A.S.T.M. procedure. The results of these tests are shown in Table X.

It will be seen that the modulus at 300% for the cures at 256° and 274° F. is lower than for the cures at the higher temperatures. The tensile strength and elongation at break decrease as the curing temperature increases. In spite of these differences, the unextracted abrasive index for all these cures is very high and after extraction is greatly reduced and becomes practically identical for all curing temperatures. Therefore, the temperature at which vulcanization takes place does not account for the extremely high abrasive index obtained for a GR-S tread-type compound with straight Captax acceleration.

Long overcures were also tried with this type of acceleration without affecting the result appreciably.

Natural Rubber Compounds. As shown in Table XI, two natural rubber compounds, 5-38 and 5-39, were studied, the first a tread compound with straight Captax acceleration, and the second this same compound to which the ethanol-toluene extract of GR-S compound 5-8 was added.

A number of sheets of GR-S compound 5-8, 0.23 cm. (3/32 inch) thick, press-cured 90 minutes at 292° F., were cut into strips approximately 0.3 cm. (0.125 inch) wide. These strips were placed in an Erlenmeyer flask and extracted by passing a stream of freshly distilled ethanol-toluene azeotrope into the flask by continuous flow, so that the rubber was constantly in contact with pure azeotrope at about 40° C. for a period of four days. The solution of extract was then vacuum distilled to remove the azeotrope and the residue was dried in a vacuum oven. A master batch was made by adding the total extract to 100 grams of smoked sheet on the rubber mill and the amount of extract was determined by weighing the resulting master batch. In preparing compound 5-39, some of the smoked sheet was replaced by this master batch, so that the proportion of extract in the final compound was equivalent to the proportion found in compound 5-8. Compound 5-38 had the same formula as compound 5-39, except that it contained 100 parts of smoked sheet and no extract.

The abrasive index of 644 for compound 5-38, containing no extract, is extremely high, indicating that natural rubber also contaminates the abrasive as does GR-S. After extraction the result reaches a reasonable level around 60.

The addition of the GR-S extract to the natural rubber compound caused a considerable decrease in the abrasive index, unextracted, as compared with compound 5-38. The figure for compound 5-39, containing the extract, however, is still erroneously high, at 169, and after extraction reaches a more reasonable level, around 70, close to compound 5-38.

It might have been expected that the incorporation of the GR-S extract into the natural rubber compound would increase the contamination of abrasive and raise the abrasive index, unextracted. Such, however, was not the case. The explanation would appear to be that the GR-S extract contains not only contaminants but also accelerator or accelerator decomposition products. This extra acceleration made it advisable to use a 25-minute cure for compound 5-39 as against 45 minutes for compound 5-38, and even at the shorter cure the hardness of compound 5-39 was 13 points higher. One is led to the conclusion that the nature of the acceleration has a profound effect upon the degree of contamination and that this may supersede the effect of other changes in the compound. In this experiment the increased acceleration nullified the effect of extra contaminant added to the compound.

This abrasive index result of about 60 for extracted natural rubber is low in comparison with GR-S, which usually gives an abrasive index well above 100 for the same type of formulation. This may mean one of two things: Either the abrasive resistance of natural rubber tires is considerably poorer than that of the synthetic rubber product, or the laboratory abrasion resistance test is not suitable for comparing natural with synthetic rubber. The authors believe that the latter is the more likely of the two alternatives.

Effect of Acceleration. Table XII shows the results obtained with a number of accelerator combinations in a tread stock base formula.

The profound effect of the nature of the acceleration is at once evident. The abrasive index, unextracted, varies from 792 for an Altax-Tuads combination to 195 for a Beutene–Butyl Zimate–zinc isopropyl xanthate combination. A hexachloro-ethane-litharge combination also shows a low abrasive index, unextracted of 366; but, unfortunately, the rubber becomes too soft and sticky to get a satisfactory result after extraction. At any rate, the abrasion resistance figure given by this combination, of 366, is still ridiculously high. There must have been some contamination of abrasive in this case, and as it was produced in the absence of antioxidant and the usual vulcanizing ingredients, such as sulfur and accelerator, these materials cannot be accused of being the basic contaminants.

In this particular series of tests the various accelerator combinations may be ranked as in Table XIII with respect to their efficiency in reducing contamination, the most efficient being placed first.

The order in which these accelerator combinations stand might, of course, vary with the quantity of accelerator and sulfur and time of cure. The conclusions to be derived from these results, however, would not thereby be affected.

After extraction the results are all reduced to a normal level. That the Beutene–Butyl Zimate–zinc isopropyl xanthate combination, however, is not sensibly changed in abrasive index by extraction, indicates that with this accelerator combination there was no contamination of abrasive. This combination was the most powerful acceleration of all, the cure being only 25 minutes at 230° F.

Table XII. Effect of Acceleration

Compound No.	5-30	5-35	5-37	5-40	5-41	5-42	5-43
GR-S	100	100	100	100	100	100	100
Wyex	50	50	50	50	50	50	50
Zinc oxide	5	..	5	5	5	5	5
Sulfur	2	..	3	2	2	2	2
Captax	0.83	..	..	..	..	..	1
Litharge	..	10	..	..	..	..	..
Hexachloroethane	..	10	..	..	..	..	..
DPG	0.42	..	..	..	..	..	..
Beutene	..	..	1	..	..	..	..
Butyl Zimate	..	..	1.25	..	..	..	..
Zinc isopropyl xanthate	..	..	0.75	..	..	..	..
Altax	..	..	..	1.5	1.5	..	..
Selenac	..	..	..	0.15	..	..	..
Tuads	..	..	..	..	0.15	..	..
Thionex	..	..	..	..	..	0.3	..
Accelerator No. 8	..	..	..	..	..	0.75	..
DOTG	..	..	..	..	..	..	0.25
Press cure							
Time, min.	90	90	25	35	35	25	25
Temperature, ° F.	292	307	230	282	292	292	292
Hardness, Type A, Shore							
Original	64	47	69	62	59	64	60
Extracted	63	..	64	53	51	53	49
Du Pont abrader Abrasive index (A.S.T.M. standard B)							
Original	257	366	195	231	792	239	652
Extracted	183	..	192	130	126	135	115

Inasmuch as contamination of abrasive has been observed in the absence of each and every one of the compounding ingredients used in the compounds studied herein, it may be concluded that no compound ingredient, including even the vulcanizing agents, can be accused of being itself the major contaminant. Moreover all the ingredients in the Beutene–Butyl Zimate–zinc isopropyl xanthate compound just mentioned can be ruled out as possible direct contaminants, as this compound did not contaminate the abrasive. This result also indicates that extraction does not necessarily weaken the resistance of the rubber to abrasion because it shows that when there is no contamination there is no drop in abrasive index as a result of extraction. The drop in abrasive index with extraction that is generally observed, therefore, is apparently due solely to removal of contamination and not to a weakening of the rubber in this respect by the extraction process.

In view, therefore, of the profound effect of the nature of the acceleration, it is felt that there is justification for the belief that the viscous material that contaminates the abrasive is rubber of low molecular weight developed during vulcanization. When a very powerful accelerator combination is used, no molecules of low molecular weight are produced, or if they are, they are joined by cross linkages during vulcanization and rendered solid and insoluble, and the contaminant is thereby eliminated.

Each compounding ingredient, of course, may exert some influence on the contamination, by affecting the vulcanization reaction, by adsorbing the low molecular weight rubber which is the basic cause, by being transferred to the abrasive along with the low molecular weight rubber, or by changing the friction and heat produced during abrasion, thus possibly influencing any depolymerization that may take place at the abrading surface.

Table XIII. Tests with Accelerator Combinations

Accelerator Combination	Abrasive Index: Unextracted	Abrasive Index: Extracted	Contamination[a]
Beutene–Butyl Zimate–zinc isopropyl xanthate	195	192	3
Captax-DPG	257	183	74
Altax-Selenac	231	130	101
Thionex-Accelerator No. 8	239	135	104
Captax-DOTG	652	115	537
Altax-Tuads	792	126	666
Captax	2450	175	2275

[a] Difference between unextracted and extracted.

USE OF A CONTINUOUS STRIP OF ABRASIVE

As an alternative to the preliminary extraction of test specimens, the use of a machine employing a continuously changing abrasive surface might be considered. It would be interesting to know how the results obtained with such a machine as that described by Gavan, Eby, and Schrader (*1*) would compare with those obtained with extracted rubber or with road tests.

It is not certain, however, that the use of a continuous strip of abrasive would eliminate entirely the effect of contamination, as it is believed that the only sure way to do this would be to clean the rubber, whereas the use of the continuous strip would have the effect of cleaning only the abrasive. As the rubber surface is abraded by the continuous strip, contamination could form on the rubber surface first meeting the abrasive. This contamination could be carried by the sandpaper over the surface of the rubber and act as a lubricant before the sandpaper could carry it off. Contaminant continuously forming could produce an error in the result, although this error would undoubtedly not be so great as in a machine where the same abrasive surface comes repeatedly against the sample during the test.

In the absence of comparative data with the continuous strip machine an alternative view may be justified. This view would be that the inability of such a machine to eliminate all the effect of contamination might not be undesirable, because it may develop that the action of the continuous strip machine is a closer approximation to that of the roadway in removing extractable material than is the case when pre-extracted stocks are run on the conventional machines.

CHANGES PRODUCED BY EXTRACTION

Certain observations were made while the present investigation was being carried out on the changes produced in the rubber by extraction.

The extraction itself produces a decrease in hardness. There is also usually an increase (occasionally a decrease) in volume and weight as a result of extraction. Then, upon standing, the weight and volume continually decrease and the hardness increases. The abrasive index, which decreases markedly upon extraction, very slowly rises upon standing after extraction. Figure 4 shows the effect of standing after extraction for compound 5-8 upon volume, hardness, and abrasive index. These trends were observed with about eight GR-S compounds, and so the results obtained might be assumed to be fairly general.

The changes in hardness, volume, and weight upon extraction are undoubtedly the combined result of the absorption of solvent and the loss of soluble extract. Solvent then evaporates from the rubber upon standing, and the result is a weight and volume loss and an increase in hardness. Upon standing, the hardness may increase to the original figure for the unextracted compound and may even become greater than this original figure, all depending upon the nature and quantity of material removed during extraction.

After about 80 days of standing, under ordinary room conditions, after extraction, the weight of the rubber samples becomes practically constant; this indicates that practically all absorbed solvent has left the rubber. Now, the difference between the weight at 80 days and, say, 2 days, represents the weight of solvent retained by the rubber after it has stood 2 days. This retained weight may be as high as 6% and may be accompanied by a swelling as high as 11%. In spite of this there is nothing in the comparative abrasive index figures obtained at the 2-day standing period to indicate that a noticeable error in the correlations has resulted. Since presumably with all compounds there is a tendency for the abrasive index to increase with time of standing after extraction, all the results should be comparable at any fixed period of standing, and so it is felt that the drying period of 2 days adopted for the tests described herein should be satisfactory.

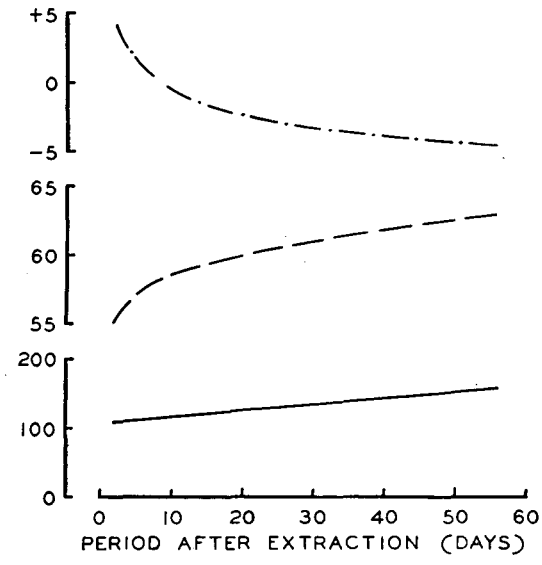

Figure 4. Effect of Standing Time after Extraction

Bureau of Standards Abrader

Compound 5-8	
GR-S	100
Wyex	50
Zinc oxide	5
Captax	1.5
Sulfur	2

Press cure, 90 minutes at 292° F.

Unextracted 503
———Abrasive index
- - - -Hardness, Type A, Shore
—·—·Volume change, %

Referring to Table VII, it will be observed that there is a considerable decrease in tensile strength, upon extraction, with the compounds containing Cumar MH 2½, but not so much change with the compounds containing no Cumar. That the drop in abrasive index does not reflect this drop in tensile strength is shown by compounds 5-13 and 5-15 which contained Cumar and suffered considerably in tensile strength upon extraction, but, on the whole, did not drop appreciably more in abrasive index than the corresponding compounds 5-12 and 5-14, without Cumar, which more or less retained their original tensile strength. This would lead one to discount changes in physical properties, such as tensile strength and hardness which take place upon extraction, in estimating the accuracy of abrasion resistance results obtained after extraction. In any case, it is generally true that any error produced as a result of changes in hardness and tensile strength is negligible compared to the error that is corrected by extraction.

Moreover, as has been pointed out above, rubber abraded on the road is probably extracted rubber and so would have its properties changed in more or less the same way as if the rubber were extracted by a solvent. Consequently, the abrasion resistance on the road should be close to that of extracted rubber.

Table XIV. Effect of Contamination of Abrasive upon Subsequent Determination

Order of Determination	Abrasion Resistance (Revs./0.10 Inch Loss)	Abrasive Index
Experiment 1		
A.S.T.M. standard B, fresh abrasive	382	...
Then 5-8, original, without changing abraisve	2780	708
Then 5-9, original, without changing abrasive	922	234
Then 5-8, original, without changing abrasive	2840	726
Then A.S.T.M. standard B, without changing abrasive	406	...
Experiment 2		
A.S.T.M. standard B, fresh abrasive	454	...
Then 5-9, original, without changing abrasive	917	201
Then 5-8, original, without changing abrasive	2520	549
Then 5-9, original, without changing abrasive	1010	222
Then A.S.T.M. standard B, without changing abrasive	457	...
Experiment 3		
A.S.T.M. standard B, fresh abrasive	432	...
Then 5-8, extracted, without changing abrasive	509	114
Then 5-8, original, without changing abrasive	2870	640
Then 5-8, extracted, without changing abrasive	527	118
Then A.S.T.M. standard B, without changing abrasive	460	...

CHANGING OF ABRASIVE

In an investigation such as this where the degree of contamination of the abrasive is a factor influencing the results, it was thought that the question of changing the abrasive should merit some consideration. The use of a fresh piece of sandpaper for each test is recommended by Williams (*3*) who, in describing the Du Pont machine, claims that this procedure gives greater uniformity. In A.S.T.M. Designation D394-40, method C (U. S. Rubber Co. abrader), a fresh piece of abrasive paper is required for each test. Of late, however, there is a tendency to use the same piece of sandpaper for several tests. Sigler and Holt (*2*), describing tests with the Bureau of Standards machine, used one paper for several specimens. The reporting of the result as an abrasive index where the standard comparison compound is retested at specified stages along with a number of unknowns, on the same piece of sandpaper, is now a standard procedure with many abrasion machine operators. It is claimed that the effect of wear on the sandpaper is not very great after a short initial period of use.

Table XIV shows the results produced with the Bureau of Standards machine when an abrasion resistance test is carried out with sandpaper contaminated from a previous test. In the first experiment the abrasion loss of A.S.T.M. standard compound B was determined. Then compounds 5-8 and 5-9, which were known to contaminate the paper excessively, were run on the same piece of sandpaper, starting with 5-8, followed by 5-9, after which 5-8 was repeated. The standard compound B was then run again on the same piece of sandpaper. In the next series the order was changed slightly, and a second piece of sandpaper used, the order being: compound B, then 5-9, then 5-8, then 5-9, and then compound B. In the third series, with a third piece of sandpaper, the order was: compound B; then 5-8, extracted; then 5-8, unextracted; then 5-8, extracted; then compound B.

The figures show an increase in the number of revolutions per 2.5-mm. (0.10-inch) loss for the standard compound B as a result of running the tests which contaminated the paper in between the two standard samples. The increase is slight, however, and may be partly due to the wearing of the abrasive by the samples previously run. The fact that this change is slight is an indication that even if the paper becomes contaminated this does not too seriously affect the results obtained with a subsequent sample run on the same piece of sandpaper. In abrasion tests such as these it is customary to condition the sample by running it on the abrasion machine for a short period preliminary to the test. This procedure might well also condition the paper, as well as the rubber, by eliminating the previous contamination.

In the first series, with compounds 5-8 and 5-9 on the one piece of paper, there is a slight rise in the abrasive index of 5-8 as a result of running 5-9 in between two samples of 5-8. Similarly, in Experiment 2, there is only a slight rise in the abrasive index of 5-9 as a result of previously running 5-8 on the same piece of abrasive, even though 5-8 excessively contaminates the paper and itself has the impossibly high abrasive index of 549. Again, in Experiment 3, 5-8, extracted, is increased only slightly by previously running a 5-8, unextracted, sample.

On the whole all the samples seem to be unaffected by previous contamination of abrasive except perhaps for a slight improvement in the abrasion resistance result, which is in the same direction as the effect that would be produced by a wearing down of the abrasive. It is possible, however, that the wearing of the abrasive might not be sufficient to explain the observed difference. Sigler and Holt (*2*) state that a number of tests can be carried out without appreciable dulling of the abrasive. The evidence in Table XIV, therefore, points to the possibility that contamination of the abrasive by a previous test might affect a subsequent test, and that a contaminated abrasive should be changed, but only when extreme accuracy is desired.

Thus the running, in this investigation, of both the unextracted standard and the unextracted sample on the same piece of abrasive on the Bureau of Standards machine would not be expected to add appreciably to the very large error observed for unextracted samples. Moreover, as neither the unextracted standard nor the extracted sample produces visible contamination, the running of both on the same piece of abrasive was felt to be justified and not to affect measurably the accuracy of the abrasive index results for extracted samples.

CALCULATION OF DU PONT MACHINE RESULTS

In Tables I and II the results of the abrasion resistance tests on the Du Pont machine have been calculated on the basis of volume loss per horsepower hour, and on the basis of volume loss per hour.

The abrasion loss in cubic centimeters per horsepower hour is increased by extraction. When the abrasion resistance is expressed as the loss in cubic centimeters per hour, however, there may in some cases actually be a reduction in the loss after extraction. The discrepancy is apparently due to the change in horsepower produced by extraction. Extraction has, in every experiment but one, in the tables referred to, decreased the horsepower, and in those cases where the volume loss has been decreased by extraction, the drop in horsepower has been great enough to bring about an increase in the abrasion loss calculated on the basis of cubic centimeters per horsepower hour. However, where the volume loss is decreased or the horsepower is increased as a result of extraction, the decrease or increase, respectively, is of relatively small magnitude.

The decrease in volume loss with extraction was observed particularly with footwear compounds, in which contamination of the abrasive is apparently not a serious factor. The recapping compounds had, on the other hand, an abnormally low volume loss, unextracted, owing to contamination of the paper, so that the prevention of contamination by extraction was enough to cause an increase in volume loss instead of the otherwise possible decrease.

It is obvious from Table I that the figures obtained on the unextracted recapping compounds are meaningless, and that only after extraction can the results be considered comparable with road wear. The ranking is affected but little when the extracted samples are compared either on the volume basis or on the horsepower basis, and this ranking agrees reasonably well with the ranking based on road wear in both cases. The differences in ranking produced by changing the basis of calculation for the footwear compounds are also small and probably within experimental error. That the ranking of the unextracted recapping compounds is different from that of the extracted compounds, indi-

cates that when there is serious contamination extraction introduces a radical change in the results on the abrasion machine.

A comparison of Table I with Table II shows that the percentage change produced by extraction in the abrasive index of the soling compounds is small compared to the change formed for the tread compounds. This may be attributed to the different compositions of the two types of compound.

The fact that the horsepower invariably decreases on extraction is evidence that contamination of the sandpaper does not decrease, but rather increases, the friction between the sandpaper and rubber during the test. Nevertheless, although the friction increases, the wear decreases, presumably because the abrasive particles are covered with a coating that protects the rubber from the cutting action of the abrasive. The effect is that of a lubricating film of such high viscosity that friction is increased instead of being decreased.

Sample 4, Table I, gives an example of the effect of horsepower. Unextracted, with the sandpaper contaminated, the average horsepower during the test was 0.0187, and the wearing effect on the rubber only 0.28 cc. per hour. Extracted, the horsepower was reduced to 0.0163 but the wearing effect increased to 3.57 cc. per hour. Obviously, the 0.0187 horsepower in the first experiment was not all necessary in abrading the sample, as after extraction a much greater volume loss was produced with less horsepower; hence much of the horsepower in the first case was superfluous and associated with the contamination effect. It is not known, of course, just what proportion of the horsepower of 0.0163 was used up solely in comminuting the extracted sample, but this figure can be looked upon as a maximum higher than which it would not be necessary to go in order to comminute the sample. By proportion, if 0.0163 is the maximum for producing an abrasion loss of 3.57 cc. per hour, then the horsepower required to produce a loss of 0.28 cc. per hour should not be greater than

$$\frac{0.0163}{3.57} \times 0.28 = 0.1003 \text{ horsepower}$$

Hence, in the abrasion test of the unextracted sample,

$$0.0187 - 0.0013 = 0.0174 \text{ horsepower}$$

or 93% of the total horsepower was superfluous, and associated with the contamination effect. This amounts to 186 gram-calories per minute, and because such heat would be generated at the surface of the rubber, it would contribute considerably to the heating of the rubber surface before being dissipated.

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An Application of Punch Cards

Filing of Optical Properties of Crystals

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A system is described for recording the optical properties of crystals on punch cards. The system is so designed that a search of the file can be made using the knowledge of any one or a combination of determinative properties. The single punch card file replaces a whole series of individual files based upon one property. It allows for the arrangement of the cards in a definite order with respect to any property. Tables of data can be prepared without card-by-card inspection. The system allows for a greatly increased efficiency in the use of the volume of optical data available for the identification of crystalline substances.

THIS paper is a description of a punch card system for the filing of the optical properties and other physicochemical data on nonopaque crystalline substances. The data on the cards are arranged in such a manner that the card file can be searched for all cards on which is recorded any one or a combination of the following determinative properties:

1. Key Refractive Index (N, N_O, N_E, N_Y, N_1)
2. Optical Character (Isotropic, Uniaxial, Biaxial)
3. Optic Sign (+, −)
4. Other Refractive Indexes (N_X, N_Z, N_1, $N_2 \ldots N_n$)
5. Oblique Extinction
6. Optic Axial Angles ($2E$, $2V$)
7. Extinction Angle
8. Specific Gravity
9. Presence of Functional Groups
10. Presence of Elements Determined by Sodium Fusion (S,X,N,P)
11. Melting Point
12. Presence of Metals
13. Color

Thus the single punch card replaces a whole series of individual files based upon one property.

The card was designed for the methods used by the Microscopy Group of the Stamford Research Laboratories of the American Cyanamid Company. This description is published to bring to the attention of those in the field of optical crystallography the possibilities of punch card techniques in the filing of optical properties and not to recommend a specific system for general use. The punch card system is very flexible and easily adapted to the methods of other laboratories.

HISTORICAL

Although optical and other physical properties of crystalline substances have been reported in the literature, their efficient use for identification has been limited by the lack of an adequate filing system. Tables based upon the knowledge of a refractive index (n, ω, β) have been compiled for artificial minerals (8), organic

compounds (*9*), and natural minerals (*6*, *7*), including tables of birefringence, color, and dispersion.

None of these compilations allows for a systematic search using any one or a combination of the many determinative properties other than those noted. Donnay (*3*, *4*) has described a card system for the compilation of the determinative properties of minerals, which could be searched with the use of any one or a combination of properties. Fairbanks (*5*) described a system for the identification of the ore minerals. These two systems are limited in the number of minerals that can be considered and, therefore, are not applicable to the filing of data for the inorganic and organic substances examined in an industrial laboratory. There are probably over 500,000 organic compounds now known; of these a large proportion is crystalline. A system that allows for an infinite number of compounds is needed.

Recent papers have described the use of punch cards for the systematic filing of technical data. Bailey, Casey, and Cox (*1*) have published a bibliography of these papers and have also presented a general description of punch card techniques (*2*).

The punch card system is unlimited in the number of substances that can be considered. Its use appears feasible for the systematic filing of optical properties and other physicochemical data for use in the identification of crystalline materials, which include organic, inorganic, and mineral substances.

DESCRIPTION OF SYSTEM

Clipping and Sorting Cards. The punch card used in this laboratory is a 5 × 8 inch card as illustrated in Figure 1. In rows around the margin are punched holes, to each of which is assigned a meaning. Whenever this meaning is a function of the properties of the substance described in the card, the paper between the hole and the edge is removed by clipping with a special punch. Thus, the properties of a substance are indicated by the holes that have been clipped. [The blank cards and accessory equipment were obtained from the McBee Company (Keysort), Athens, Ohio. The printing on the face of the card was done by a local printer.]

Sorting and separation are accomplished by the insertion of a needle or needles in the proper hole or holes in a pack of the cards. When the needle or needles are lifted, the cards with the holes clipped fall from the pack of unclipped cards.

Direct assignment of a property to each hole is the simplest method of assigning a meaning. The hole is either clipped or not clipped, according to whether the substance has or does not have the property. Thus, dichotomous selection is made. The 7-4-2-1 field system, described in the literature (*1*, *2*), is used with some modifications to record numerical values. Improved methods of recording numbers have been devised by the card manufacturers since the author set up his filing system, and details of present methods should be obtained from the card manufacturers. At the time the system was devised cards with double rows of holes all the way around were not available. They are now on the market, and the author would advise their use by anyone planning to set up a filing system.

Refractive Indexes. The refractive indexes are clipped using the 7-4-2-1 field system. Because the practical precision of a routine determination of a refractive index of a crystal is ±0.003, only the first two decimal places need be expressed. This allows for a slight range in values.

Key Refractive Indexes. In order to locate a card that has the data for a compound, it is necessary with this system to have a separate alphabetical index file. For this 3 × 5 inch cards are used, on which are recorded key refractive indexes for each substance in the file. This alphabetical file is cross-indexed, so that all synonyms are included. When the key refractive index is determined from the alphabetical index, all cards with this index in common can be removed by hand or by the use of the needles. The cards obtained are then sorted manually for the desired substance. The key indexes for the various types of crystals are listed in Table I. Frequently the principal vibration directions are not easily identified; therefore, the last group of key indexes in Table I is used.

Two punch cards are filed for uniaxial crystals, one with N_O as the key index and the other with N_E. This is done in order to cover the possibility of a crystal that may be uniaxial and elongated parallel to the optic axis and with a cross section too small for the observation of an interference figure. This crystal then falls in the class of crystals of indeterminate orientation showing parallel extinction. The principal vibration direction parallel to the length would correspond to E and not O. The card for this substance would not be found in a search of the key indexes unless N_E is included as a key index. The key index is clipped on the top margin of the card. If the key index is greater than 1.7 this is noted by clipping to the outer hole, and if greater than 2.0 to the inner hole of the set of holes at the extreme top of the left side of the card.

Other Refractive Indexes. The other refractive indexes are clipped on the left margin of the card. The upper group of holes is used for indexes higher than the key index and the lower group for the indexes lower than key index. The <5 and >5 are used to express the third decimal place.

Optic Axial Angles. The optic axial angles, $2E$ and $2V$, are coded in groups to allow for a range in values. The code uses the 7-4-2-1 field system. The first group (code 1) includes all angles between 0° and 20°. The other groups are 10° intervals. Code 9 designates all angles greater than 90°.

Optical Character. Crystalline substances can be divided into three classes according to whether they are isotropic, uniaxial, or

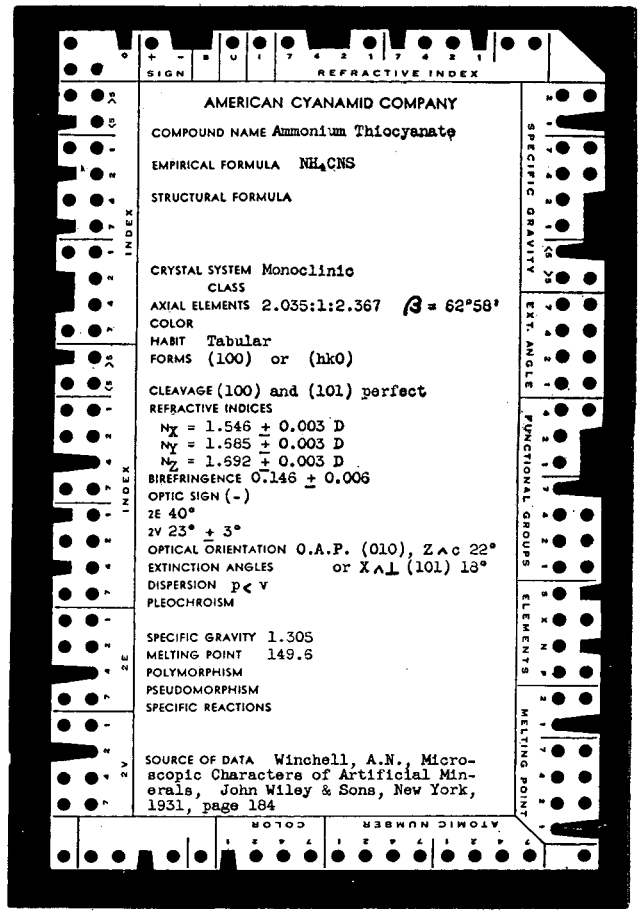

Figure 1. Typical Punch Card

biaxial. In this card system these three classes are designated collectively as optical character. These properties are clipped on the top margin of the card. The letter *I* refers to isotropic, *U* to uniaxial, and *B* to biaxial.

Optic Sign. The optic sign is clipped as illustrated in Figure 1.

Oblique Extinction. If any orientation of the crystal shows oblique extinction, this is noted by clipping the hole corresponding to *O* on the left top of the card.

Extinction Angles. The extinction angles are measured from the trace of the elongation to the principal vibration direction most nearly parallel to this trace. The values of the angles are coded in groups of 5° from 0° to 45°. When data are recorded from the literature, it is sometimes necessary to record the complement of the angle given in order to meet the above definition. (Although provision is not made in this system for recording the sign of elongation, this could be easily handled by designating two holes with a plus and minus sign as is done with the optic sign.)

Specific Gravity. The numerical value of the specific gravity is clipped directly. The card allows for the recording of gravities up to but not including 4.1. Greater values are indicated by clipping only the outer holes marked >5 and <5 and no others.

Functional Groups. The presence of a specific functional group is coded. The groups considered are those determined by chemical tests or by spectroscopy. The presence of only one group can be indicated. The choice of the group to be coded is such that duplication of information as to the presence of *S*, *X*(halogens), *N*, and *P* is avoided. Thus *p*-aminobenzoic acid would be coded by showing the carboxyl group and not the —NH_2 group, for the presence of nitrogen would be indicated under elements.

Elements. The presence of the elements sulfur, halogens, nitrogen, and phosphorus detected by sodium fusion or other chemical tests is indicated directly by clipping the appropriate hole.

Melting Point. The melting point is coded. The range is from 25° to >300° in groups of 10°.

Atomic Number of Elements. The presence of a metallic element is indicated by recording its atomic number. When two or more metallic elements are present, the one most useful in the identification is noted. Thus, the presence of iron is recorded for sodium ferricyanide rather than sodium.

Color. The colors of crystals are coded. The colors noted are red, orange, yellow, green, blue, violet, gray, and brown. Colorless is also noted.

ARRANGEMENT OF CARDS

Because of the nature of the 7-4-2-1 system, it is possible to arrange the cards in increasing numerical order by a mechanical procedure described by Casey *et al.* (*2*). This procedure is of great use in the preparation of tables of properties. The cards are kept in the order of increasing key refractive indexes. Tabs are placed in the file at intervals of 0.05 for convenience in locating a group of cards.

RECORD OF DATA

As is seen in Figure 1, provision is made on the face of the card for recording complete data. Space is provided on the back for additional notes and drawings. These cards serve as a primary file of data.

EXAMPLE OF USE

During the examination of a commercial mixture of crystalline materials, a phase was observed which gave a biaxial negative interference figure and whose N_Y was measured as 1.685 ± 0.003. The group of cards, approximately 80, whose key indexes were between 1.65 and 1.70, were removed from the file and needles were inserted in the appropriate holes corresponding to the above properties. Three cards with these properties in common fell off the needles. The N_Z value was measured and found to be 1.691 ± 0.003. The three cards were then examined and the card for ammonium thiocyanate was found to indicate this index value. The identification was confirmed by a quick check of the other properties listed (Figure 1).

Table I. Key Indexes for Various types of Crystals

Type of Crystal	Key Index
Isotropic	N
Uniaxial	N_O and N_E
Biaxial	N_Y if determinable
Crystals for which principal indexes are not identifiable	
Showing parallel or symmetrical extinction	N_1 for index of ray vibrating in a plane parallel to elongation of crystal
Showing oblique extinction	N_1 for index of ray vibrating in plane corresponding to maximum extinction angle[a]

[a] See discussion of extinction angle.

DISCUSSION

This punch card system has been in use for over a year and a half. At present the data for over 600 compounds are on file and more are being filed daily. It is planned to transfer all usable data from the literature and those determined in this laboratory to the card system.

The system forms a primary file of optical and other physicochemical data and is unlimited in the number of compounds that can be recorded. A search of the file can be made by using any one or a combination of determinative properties. The nature of the punch card system is such that tables of data are easily prepared by routine manipulation of the cards. The certainty of an identification is increased by the knowledge that all data available to the analyst have been searched in a systematic manner. Without this system it is necessary to check all books, periodicals, and laboratory notes by inspection which always leaves the possibility that a set of data has been overlooked.

This description is presented to show what can be done with punch cards in filing physicochemical data, especially optical properties. It is hoped that it will result in the more efficient use of optical data and optical methods in the identification of crystalline substances.

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Determination of Starch in Plant Tissues

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Starch is extracted from a 50- to 250-mg. sample of dried plant tissue with perchloric acid as recommended by Nielsen, precipitated with iodine, and recovered as starch which is hydrolyzed and determined as glucose. The results are independent of the composition of the starch of different species with respect to amylose and amylopectin. Theoretical recoveries of glucose from pure potato starch are obtained; the experimentally determined factor is 0.90 with an uncertainty of ±1.2%. The precision of the method as applied to a variety of plant tissues is approximately 2%.

A COLORIMETRIC method for determining starch in plant tissues was developed in this laboratory 12 years ago (*13*). Revision of this method has become necessary because of two recent developments in the chemistry of starch. The first of these is the observation that plant starches are mixtures of two main components, usually referred to as amylose and amylopectin (*1, 6–8, 15*), which are present in more or less constant ratio in the starch from a given tissue but often in quite different ratios in the starches derived from different plant species. The second is the introduction by Nielsen (*10*) of perchloric acid as an effective solvent with which to extract starch from the plant tissue as a step preliminary to its analytical determination.

The earlier method depended upon measurement with a red filter of the extinction coefficient of a solution of the starch-iodine complex prepared after careful purification of the starch extracted with hydrochloric acid from the sample of tissue. This was compared with the extinction coefficient of a standard solution of potato starch; accordingly, the method is subject to error in those cases where the ratio of the amylose to amylopectin in the specific tissue analyzed differs appreciably from that of potato starch. The failure, in many cases, of the color values to agree with the results of titration of the sugar after hydrolysis of the starch is doubtless attributable to this circumstance. Although correction factors can be determined, as has been suggested by Nielsen and Gleason (*11*), it is not certain that such factors are valid for use in studies of metabolism where samples at different stages of development or of different tissues from the same species are being examined. Moreover, Schoch and Williams (*16*) have shown that the absorption of iodine by starch is repressed by the presence of fatty acid in the preparation. If this should result in variation in the intensity of the color, another source of error is possible.

The revised method consists of extraction of the starch with perchloric acid, precipitation with iodine under conditions that have been shown to be quantitative (*2, 13, 19*), decomposition of the iodine complex, and determination of the sugar produced by hydrolysis of the starch. A maximum of 250 mg. of dry tissue is required; proportionately smaller quantities are sufficient if the starch content is greater than 1%. Once the necessary fundamental values for the starch from a given tissue have been determined, in terms of sugar titration and in comparison with a standard such as a preparation of potato starch, the more rapid colorimetric procedure previously described can be used if desired in a series of determinations on the same tissue.

REAGENTS

Perchloric Acid, 72%, 11.2 to 11.7 *N*, reagent grade.

Iodine–Potassium Iodide. Iodine (7.5 grams) and potassium iodide (7.5 grams) are ground with 150 ml. of water, diluted to 250 ml., and filtered through No. 3 Whatman paper with suction.

Alcoholic Sodium Chloride. Ethanol (350 ml.), water (80 ml.), and 20% aqueous sodium chloride solution (50 ml.) are diluted to 500 ml. with water.

Alcoholic Sodium Hydroxide, 0.25 *N*. Ethanol (350 ml.), water (100 ml.), and 5 *N* sodium hydroxide (25 ml.) are diluted to 500 ml. with water and filtered as above.

Hydrochloric Acid, 0.7 *N*. Sixty milliliters of concentrated hydrochloric acid are diluted to 1000 ml.

Somogyi's Phosphate Sugar Reagent. Prepared as described by Somogyi (*18*) except that standardized potassium iodate solution is included by dissolving 56 grams of anhydrous disodium phosphate and 80 grams of Rochelle salt in about 1 liter of water and adding 200 ml. of 1.00 *N* sodium hydroxide; 160 ml. of 10% copper sulfate pentahydrate are slowly added with stirring and then 360 grams of anhydrous sodium sulfate. When this is dissolved, the solution is transferred to a 2-liter volumetric flask and exactly 200 ml. of 0.1 *N* potassium iodate (3.5760 grams per liter) prepared with quantitative accuracy are added. The mixture is diluted to volume, allowed to stand for several days, and filtered through dry No. 3 Whatman paper in a dry funnel into a dry filter flask, the first 50 ml. of filtrate being discarded. The reagent should be stored at 20° to 25° C.; it is 0.01 *N* with respect to iodate and 5.00 ml. are equivalent to 10 ml. of 0.005 *N* sodium thiosulfate. The effective range for the determination of sugar is from 0.05 to 1.0 mg. of glucose.

Sodium Thiosulfate, 0.005 *N*. Sodium thiosulfate pentahydrate (2.73 grams) is dissolved in water and made to 2 liters. The solution is preserved in a dark colored bottle and standardized daily against 5 ml. of the Somogyi sugar reagent; 1 ml. of 2.5% potassium iodide and 3.0 ml. of 1.5 *N* sulfuric acid are added and the mixture is allowed to stand for 5 minutes before titration.

Starch Indicator, prepared according to Peters and Van Slyke (*12*) as follows: one gram of potato or cornstarch is triturated with cold water and the suspension is poured into 200 ml. of boiling water containing 200 mg. of salicyclic acid. The solution is boiled for a few minutes and allowed to settle and the clear supernatant fluid is decanted for use.

In addition, 2.5% potassium iodide stabilized with a little sodium carbonate, 20% aqueous sodium chloride, 0.05% phenol red indicator solution, 0.5 *N* sodium hydroxide, 0.1 *N* oxalic acid, and 1.5 *N* sulfuric acid are required.

PROCEDURE

Preparation of Perchloric Acid Extract. It is convenient to analyze four samples in duplicate simultaneously.

A 50- to 250-mg. sample (chosen according to expected starch content) of dry powdered tissue and 200 mg. of sharp sand are transferred to a 200 × 25 mm. test tube together with 4 ml. of water. The tube is heated in a boiling water bath for 15 minutes to gelatinize the starch, cooled to room temperature, and placed in a bath at 22° to 25° C., when 3 ml. of perchloric acid are added rapidly with constant agitation. The tissue is then ground against the lower wall of the tube with a stout stirring rod (a rotary motion is used and firm pressure is exerted) for a minute or so at a time over a period of 15 to 20 minutes, each tube of the series with its stirring rod being returned to the bath while another is being treated; about 20 ml. of water are then added so as to wash the rod, the solution is mixed and centrifuged, and the clear solution is decanted into a small beaker and set aside. The residue is again treated with 4 ml. of water and 3 ml. of perchloric acid.

[1] Deceased November 20, 1947.

and ground and extracted as before. The first extract is then returned quantitatively to the tube with the aid of a little water. The rod is rinsed off and the combined extracts are diluted to 50 ml., and are mixed by shaking the stoppered tube vigorously. The tube is finally centrifuged and, if desired, the clear extract is decanted through a plug of dry glass wool in a funnel into another vessel; otherwise aliquots may be removed directly without decantation. Immediate analysis is to be preferred, but the solutions may be stored in the refrigerator for as long as 48 hours if necessary.

Precipitation of Starch–Iodine Complex. An aliquot of the starch extract of from 1 to 10 ml., depending on the starch content, is transferred to a test tube calibrated at 10, 15, and 20 ml. and diluted to 10 ml.; 5 ml. of 20% sodium chloride and 2 ml. of iodine–potassium iodide reagent are added and the solution is mixed. After being allowed to stand for at least 20 minutes, the tube is centrifuged and the supernatant fluid is decanted with extreme care to avoid loss of precipitate. The precipitate is then suspended in 5 ml. of alcoholic sodium chloride wash solution by gently tapping the tube, centrifuged, and the fluid is decanted.

Decomposition of Starch–Iodine Complex. Two milliliters of alcoholic sodium hydroxide are added to the packed precipitate and the tube is gently shaken and tapped until all the blue color is discharged. A stirring rod must not be used and ample time for decomposition of the complex must be allowed. The liberated starch is then centrifuged and washed with 5 ml. of alcoholic sodium chloride as before.

Hydrolysis of the Starch. Two milliliters of 0.7 *N* hydrochloric acid are added to the precipitate, and the tube is covered with a glass bulb, heated for 2.5 hours in a constant-level water bath provided with a cover with holes to accommodate the tubes, and maintained in vigorous ebullition. It is important that holes not occupied by tubes should be covered. The tube is cooled, a few drops of 0.04% phenol red are added, and the solution is neutralized with 0.5 *N* sodium hydroxide; the color is discharged with the necessary amount of 0.1 *N* oxalic acid and the solution is diluted to 10, 15, or 20 ml., according to the starch content, and centrifuged.

Titration of Sugar. A 5-ml. or smaller aliquot of the hydrolyzed starch solution is transferred to a 200 × 25 mm. test tube (if less is taken, water to make up to 5 ml. is added), exactly 5 ml. of the Somogyi phosphate reagent are added, and the tube, together with two or, preferably, three blanks that contain 5 ml. of water and 5 ml. of reagent, is covered with a glass bulb and heated in a vigorously boiling water bath for exactly 15 minutes. For this operation, a rack containing all the tubes of a series of determinations is employed, so that each is heated in the same way. The tube is removed without disturbing its contents and cooled to 25° to 30° C., 1 ml. of 2.5% potassium iodide is carefully added down the wall of the tube without agitation, and then 3 ml. of 1.5 *N* sulfuric acid are added rapidly with simultaneous agitation. After all the cuprous oxide has dissolved, the solution is titrated with 0.005 *N* thiosulfate, starch indicator being added when the titration is nearly completed. The blank solutions are treated similarly.

Calculation. The percentage of starch in the tissue is calculated from the equation $S = 0.90 \times \frac{5000\ VG}{WEA}$ where S is the percentage of starch, W is the weight in milligrams of the sample, E is the volume in milliliters of perchloric acid extract taken, V is the volume in milliliters of the starch hydrolyzate, A is the aliquot of the starch hydrolyzate taken (in milliliters), and G is the number of milligrams of glucose in A. G is the difference between the titration of the unknown solution and that of the blank multiplied by 0.129 and by the factor to convert the actual normality of the thiosulfate solution to 0.005 *N*. The coefficient 0.90 is the theoretical factor to convert glucose to starch. The factor 0.129 is experimentally determined and depends upon a large number of tests with potato starch. In routine calculations of the results of similar determinations, the constants can be conveniently collected into a single constant, due attention being given to the aliquot ratios should these be different.

In carrying out the procedure, special care must be taken in decanting solutions from the precipitates of the iodine complex and from the starch so as to avoid loss of small particles. It is also important that the boiling water bath used to hydrolyze the starch be covered, so that the contents of the tubes are heated to 100° C. for the necessary times. Open baths are not reliable.

After a little experience has been gained with the method, the choice of aliquots to obtain suitable quantities of sugar for the final titration is easily made.

Table I. Comparison of Colorimetric Methods of Nielsen and of Pucher and Vickery for Starch in Various Plant Tissues

(Potato starch as standard)

	Nielsen %	Pucher and Vickery %
Alfalfa stem	4.06	4.01
Alfalfa root	6.09	5.85
Tobacco leaf	1.56	1.53
	3.10	3.06
Maize leaf	2.76	2.96
Rhubarb rhizome	33.8	33.1
Beet root	0.20	0.15

EXPERIMENTAL RESULTS

Table I shows a comparison of the starch content of several plant tissues as determined by the colorimetric methods, respectively, of Nielsen (*10*) and of Pucher and Vickery (*13*). The data are expressed in terms of pure potato starch as a standard of comparison and serve to demonstrate that perchloric acid, as used in the Nielsen method, may be applied generally for the extraction of starch. The agreement between the two methods is excellent; however, in all save one case, the result by the Nielsen method is slightly higher, possibly evidence of the greater effectiveness of perchloric acid as a solvent for starch as compared with the hydrochloric acid used in the other method.

Table II shows the quantities of glucose recovered from potato starch hydrolyzed directly, after extraction with perchloric acid, and after extraction and subsequent precipitation as the iodine complex. Within the limitations of the method, the extraction is clearly quantitative and there is no loss during the precipitation and decomposition of the starch-iodine complex. The potato starch was prepared as described by Pucher and Vickery and the weight taken was corrected for moisture and ash on the assumption that the organic residue was exclusively starch.

Table II. Recovery of Glucose from Potato Starch

	Glucose Found		
Starch Taken	Direct hydrolysis	Extraction with $HClO_4$	Extraction and precipitation with iodine
Mg.	*Mg.*	*Mg.*	*Mg.*
45.5	50.2	51.0	49.4
	50.1	50.9	50.0
	49.8	51.0	50.7
92.2	102.0	103.2	103.2
	103.0	103.2	103.2
	101.5	102.0	103.2
		103.0	101.5
		103.0	103.2
		103.2	102.0

Glucose Factor for Starch. Although many workers have made use of the theoretical factor 0.90 to convert glucose to starch, this magnitude has only rarely been verified experimentally. Notable among early examples mentioned in Walton's bibliography (*20*) are the studies of de Saussure (*14*) in 1815, who obtained the ratio by direct gravimetric analysis, and of Fehling (*5*) in 1849, who used reduction of copper to measure the glucose. Factors in the range from 0.92 to 0.95 have frequently been reported and such compromise values are often employed. Etheredge (*4*), for example, has recently maintained that the theoretical factor 0.90 should be revised upward.

Several uncontrolled variables may contribute to the slightly high values for the ratio that are usually obtained; incomplete hydrolysis of the starch, the presence of moisture in supposedly dry preparations, or the variability of the moisture content with changes in conditions of storage of the sample of starch may be mentioned as well as the possibility of differences in the behavior

Table III. Factor to Convert Glucose to Starch

	No. of Detns.	Maximum	Minimum	Mean[a]
Hydrolysis with 0.7 *N* HCl	6	0.915	0.900	0.906 ± 0.7%
$HClO_4$ extract	29	0.915	0.884	0.900 ± 1.2%
Iodine precipitate	29	0.929	0.893	0.905 ± 1.2%

[a] Uncertainty expressed as coefficient of variation—i.e., standard deviation as a percentage of mean.

Table IV. Analysis of Starches of Different Amylose and Amylopectin Contents Expressed as Percentage of Weight of Preparations

	Organic Solids	Starch: Hydrolysis with 0.7 *N* HCl	Starch: Precipitation with iodine	Starch: Colorimetric, potato starch standard
	%	%	%	%
Potato starch	..	..	..	100
Waxy maize starch[a]	90.3	87.4	88.4	36
Amylose[b]	91.0	90.4	87	187
Amylopectin[b]	90.0	90.0	91	71
Araucaria starch[c] (Brazilian pine)	..	88.6	88.8	73
Arrowroot starch[c]	..	89.5	90.6	67
Apio starch[c] (Arracacia esculenta)	..	88.5	90.6	73
Rice starch[c]	..	85.7	85.4	69
Glycogen (animal)	..	77.1	00.0	3
Lemon pectin	..	29.0	00.0	0

[a] The authors are indebted to R. M. Hixon, Iowa State College, for this sample.
[b] Prepared according to the method of McCready and Hassid (*8*).
[c] From collection of late T. B. Osborne.

of the various sugar reagents employed for the determinations. For example, Somogyi's modified reagent No. 50 (*17*) consistently gave results in this laboratory from 2 to 4% higher than the theoretical and occasionally gave erratic results when applied to the perchloric acid extract. On the other hand, the new Somogyi phosphate reagent was found to give essentially theoretical values, provided moisture determinations were carried out at the same time as the sugar analyses. Illustrative examples are shown in Table III, in which a large number of experiments on potato starch are summarized. The mean value within each group closely approaches the theoretical and the uncertainty (coefficient of variation) is only slightly over 1%. Accordingly, the use of the theoretical factor 0.90 appears to be justified.

Behavior of Starches of Different Amylose and Amylopectin Content. The present method, if it is applied to starches of different origin and different relative amylose and amylopectin content, depends on the assumption that both components are quantitatively precipitated by iodine. Comparison of the data of Clendenning and Wright (*3*), who examined a variety of starches with respect to specific rotation, with those of Steiner and Guthrie (*19*), who likewise observed the specific rotation but interposed the step of precipitation with iodine, would suggest that both components are equally well precipitated. Nevertheless, it seemed desirable to test the point upon samples of known widely divergent composition. Table IV shows the results obtained with several starches as well as with preparations of crude amylose and amylopectin. The mean value for starch in the first seven preparations after hydrolysis with hydrochloric acid was 88.6%, whereas the mean value after precipitation with iodine was 88.8% and it is obvious that complete precipitation occurs.

The last column of Table IV shows the starch content as determined by the colorimetric method of Pucher and Vickery, using potato starch as standard. The wide difference between the results in this column for amylose and amylopectin, as well as the irregular results for the starches of different origin, furnish a demonstration of the fallacy in the earlier colorimetric method, when a single kind of starch is used as the standard.

The results with glycogen of animal origin and with pectin show that error from the presence of either of these substances is entirely avoided by the precipitation with iodine. The analysis of glycogen is of particular importance, inasmuch as Morris and Morris (*9*) have observed the presence in certain varieties of maize of a substance indistinguishable from animal glycogen.

Starch of Plant Tissues. Table V gives the results of determinations of starch in a number of plant tissues. Although Nielsen advocated only a single extraction with perchloric acid, and experiment verified the fact that this is usually sufficient when analyzing preparations of pure starch, the quantitative removal of the starch from dried leaf and root tissues clearly requires two successive extractions. However, a third is not necessary. A few of the samples of Bryophyllum leaf were extracted three times; the second extract gave, as an average 0.73% more starch. In a few cases, a trace of starch representing 0.04% of the tissue was found in the third extract. With the root tissues studied, all starch was removed in two treatments. The first group of experiments with Bryophyllum leaves is presented only in summary; the last experiments in the table show the negligible effect of size of sample on the results. The pairs of duplicate analyses illustrate the precision that is obtained.

Table V. Analyses of Plant Tissue for Starch after Extraction with Perchloric Acid

	One Extraction	Two Extractions
	%	%
Bryophyllum leaf (1943)		
Maximum	6.61	7.24
Minimum	6.21	6.87
Mean	6.40 ± 3[a]	7.05 ± 1.7[b]
Bryophyllum leaf FL	10.18	11.92
	10.18	12.00
Bryophyllum leaf W1	8.38	9.26
	8.38	9.26
Bryophyllum leaf W2	6.00	7.14
	6.00	7.14
Bryophyllum leaf M1	9.36	9.62
	9.26	9.72
Rhubarb rhizome	36.0	39.6
	38.0	40.9
Alfalfa root 7539	6.25	7.52
	6.15	7.37
Alfalfa root 7540	4.80	5.76
	5.00	5.76
Alfalfa tops	0.07	0.08
	0.06	0.08
Tobacco leaf E		
0.1-gram sample	2.02	..
0.2-gram sample	2.10	
0.5-gram sample	2.09	..
Bryophyllum leaf (1943)		
0.1-gram sample	6.50	7.04
0.25-gram sample	6.30	7.14

[a] 27 determinations: uncertainty expressed as coefficient of variation.
[b] 14 determinations.

Effect of Concentration of Perchloric Acid. Nielsen and Gleason showed that the concentration of perchloric acid best suited for extracting starch is 4.8 *N*. This is attained if 3 ml. of 72% perchloric acid are added to a suspension of the tissue in 4 ml. of water. The alternative procedure of adding 4 ml. of 8.5 *N* acid to 3 ml. of water was preferred by Nielsen and Gleason, as it diminishes the effect of momentary high concentrations of the acid. Comparison of the two techniques showed that, with pure starches, identical results were obtained but that, with dried leaf tissues, the use of 72% acid gave consistently higher values. Thus the average of 6 determinations on Bryophyllum leaf with 72% acid was 6.47%, whereas 8.5 *N* acid gave only 5.30%.

Effect of Two Precipitations with Iodine. An examination of the possibility that a second precipitation with iodine might contribute to the purification of the starch indicated that this precaution is not necessary. Table VI shows parallel analyses of several tissues. To obtain the data in the last column, the precipitate with iodine was suspended in 20% sodium chloride and 1 ml. of 0.16 *M* sodium thiosulfate solution was added. After trituration of the precipitate until the color was discharged, the

Table VI. Effect of One and Two Successive Precipitations with Iodine on Determination of Starch

	One Precipitation %	Two Precipitations %
Potato starch	91.0	91.0
Bryophyllum leaf 1	11.74	11.74
Bryophyllum leaf 2	10.40	10.56
Tobacco leaf	0.11	0.11
Tobacco stem	3.73	3.76
Alfalfa root 1	6.90	6.93
Beet tops	0.09	0.09
Beet root	0.15	0.10
Rhubarb leaf	0.55	0.49

solution was diluted to 10 ml. and 1 ml. of 2.3 *N* hydrochloric acid and 2 ml. of iodine reagent were added. The precipitate was centrifuged after 30 minutes and treated according to the usual procedure. The agreement of the results with those obtained after a single precipitation is satisfactory.

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Separation of Aliphatic Alcohols by Chromatographic Adsorption of Their 3,5-Dinitrobenzoates

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Forty pairs of aliphatic 3,5-dinitrobenzoates have been subjected to chromatographic adsorption by Brockmann's fluorescence technique. In this procedure, ultraviolet radiation shows the adsorbed zones as dark bands on a bright fluorescent background. Of these 40 pairs, involving 12 aliphatic alcohols from methyl to hexyl, 23 yielded two zones, 11 gave a single zone of varying composition, and 6 were completely inseparable. The normal primary alkyl dinitrobenzoates from methyl to amyl were easily separated from one another.

CHROMATOGRAPHIC methods have proved of great value in separating small quantities of similar compounds. They have been applied chiefly to naturally occurring colored substances of more or less complex structure. Application to colorless compounds depended on the development of suitable means for observation of the chromatogram. This has been accomplished in several ways: empirically, by examining successive filtrate fractions or by arbitrary sectioning of the extruded chromatogram; by streaking the extruded column with a color-producing reagent; by pretreating the adsorbent or washing the developed column with a reagent that gives a reversible color reaction with the adsorbate; by examining under ultraviolet radiation if the substances fluoresce; by converting the substances to colored or fluorescent derivatives before adsorption; and by introducing a colored substance with adsorptive properties similar to those of the colorless materials.

In a new and elegant procedure for the location of adsorbed bands of colorless and nonfluorescent materials, described by Brockmann and Volpers (*1*), the adsorbents are pretreated with a fluorescent substance so that the entire column becomes fluorescent. Under ultraviolet illumination, bands of adsorbed nonfluorescent material then reveal themselves by a local absence or diminution of fluorescence. By using only radiation absorbed by the substances being separated, such chromatographic procedures can be extended to many colorless substances. Brockmann and Volpers have shown that by using 2537 Å. radiation and quartz adsorption tubes it is possible to locate adsorbed substances that show absorption in this region. They stated that ergosterol and ergosteryl acetate, invisible with 3650 Å. radiation on a fluorescent column, were easily seen when 2537 Å. radiation was used.

The fluorescent material for treating the adsorbent must be chosen with the following essentials in mind: It must not react with the substances being separated, and it must be strongly adsorbed so that it will not be displaced or leached from the column on development or elution. Brockmann and Volpers used morin on alumina, calcium carbonate, and magnesium oxide, berberine on silicic acid, and diphenylfluorindine sulfonic acid on calcium carbonate. Each of these was adsorbed from methanol solution. The following separations on alumina-morin were described: xylene musk, musk ambrette, and musk ketone; ergosteryl and cholesteryl *p*-nitrobenzoates; several aromatic aldehydes; phorone–mesityl oxide; and the *p*-phenylphenacyl esters of acetic and benzoic acids.

Sease (*4*) used fluorescent adsorption columns prepared by mixing fluorescent zinc sulfide with ordinary adsorbents. He separated mixtures of cinnamaldehyde, xanthone, *p*-nitrobenzyl bromide, salicylaldehyde, azoxybenzene, nitrobenzene, and iodoform on silicic acid by this technique.

Table I. Chromatographic Separation of 40 pairs of Dinitrobenzoates Involving 12 Aliphatic Alcohols

	Ethyl	*n*-Propyl	Iso-propyl	*n*-Butyl	Iso-butyl	*sec*-Butyl	*tert*-Butyl	*n*-Amyl	Iso-amyl	2-Pentyl	*n*-Hexyl
Methyl	G										
Ethyl		G	G								
n-Propyl			P	G	G	G	G				
Isopropyl				F	F	F	G				
n-Butyl					O	P	F	G	G	G	G
Isobutyl						P	F	F	F	G	G
sec-Butyl							P	P	P	G	G
tert-butyl								O	P	P	P
n-Amyl									O	P	P
Isoamyl										O	P
2-Pentyl											O

G = good separation. Bands well separated; components recovered with melting point differing by 2° or less from that of original crystalline material.
F = fair separation. Bands close together; components recovered with some contamination, as shown by change in melting point of one or both.
P = poor separation. Single band, but top and bottom halves were distinctly different in composition, as shown by difference in melting points.
O = no separation. Top and bottom halves of band showed no significant difference in melting points.

The commonly used identification derivatives of the alcohols are neither colored nor fluorescent, and until the procedure just described was reported there was little prospect for application of chromatographic procedures to the separation of aliphatic alcohol derivatives. [The method of "flowing chromatography" described by Claesson (*2*), although applicable to the free alcohols, requires considerable elaborate equipment.] Strain (*6*) suggested that alcohols be separated by chromatographic adsorption of the colored esters of keto acid dinitrophenylhydrazones. The small-scale preparation of these compounds is somewhat difficult, however, and data for more than a few members of the series are not available.

Because the dinitrobenzoates, commonly used for the identification of alcohols, show near-ultraviolet absorption, the authors have studied their separation, using the principle described above. These derivatives are easy to prepare and do not require anhydrous alcohols. Moreover, the melting points of a large number are recorded in the literature.

The adsorbents and fluorescent compounds used in preparing preliminary columns for the separation of the 3,5-dinitrobenzoates were as follows: magnesia-morin, alumina-morin, magnesia-uranine, Magnesol-uranine, Magnesol-oxine, Silene EF–oxine, silicic acid–oxine, silicic acid–rhodamine B (C.I. 749), silicic acid–thioflavine T (C.I. 815), and silicic acid–rhodamine 6G (C.I. 752). Of these ten combinations, the latter two were most satisfactory; the silicic acid–rhodamine 6G afforded better visibility under weak ultraviolet radiation, and was used, therefore, for the work reported below.

The 3,5-dinitrobenzoates of 12 aliphatic alcohols, from methyl to *n*-hexyl, were investigated. Of the 66 possible pairs, 40, including all those close together in the series, were subjected to chromatographic adsorption. Of these, only 6 pairs were completely inseparable; 11 yielded a single zone of varying composition; and the remaining 23 separated with different degrees of sharpness into two bands. The adsorption procedure described below has been successfully applied to the separation and identification of various alcohols in volatile apple concentrate.

EXPERIMENTAL

Preparation of Adsorbent. Silicic acid [(Mallinckrodt AR, precipitated), 610 grams] and a diatomaceous filter aid (305 grams) were mixed dry and then dispersed in methanol (2 liters), and a solution of 40 mg. of rhodamine 6G (Calcozine red 6G extra, Color Index #752) in 50 ml. of methanol was added with stirring. (The filter aid should be white, so as not to interfere with the visibility of the fluorescence of the treated adsorbent.) The slurry was filtered on a Büchner funnel, washed on the funnel with 1 liter of methanol, and left on the funnel until dripping ceased. It was then transferred to a pan and dried in a vacuum oven at about 62.5 cm. (25 inches) of mercury and 100° to 120° C. for 18 hours. The quantity of dye is not critical but should be sufficient to give a colored methanol filtrate.

The dried adsorbent was well shaken and stored in a tightly capped bottle. It was pink in daylight and showed a yellow fluorescence under ultraviolet radiation.

Solvents. The dinitrobenzoates were initially adsorbed from hexane (a petroleum ether fraction boiling at 63° to 70° C.). Development was carried out with a 5.0% (by volume) solution of ether in hexane. Ether was used for elution. (The ether was a C.P. diethyl ether, not further purified. Its label stated that ether of that specification normally contained about 2% alcohol and 0.5% water.)

Preparation of Adsorption Column. A glass column 12 mm. in inside diameter, approximately 300 mm. long, and constricted at the lower end, was used. With a cotton plug in the lower end and suction applied, the dry powder was poured in. To obtain a closely packed column, the side of the tube was tapped while the powder was added. The column was filled to a height of 170 to 200 mm., and the top tamped lightly.

Ultraviolet Sources. A Hanovia Inspectolite was used for the work. Subsequently it was found that a 45-cm. (18-inch), 15-watt Sylvania Blacklite fluorescent-type tube was well adapted, as it provided an even radiation over the entire length of the adsorption column.

Procedure. Approximately 20 mg. of each of two alkyl dinitrobenzoates were weighed to the nearest milligram, dissolved in the minimum volume of warm hexane, and poured into the tube with suction applied. [The 3,5-dinitrobenzoates were prepared by the pyridine procedure outlined by Shriner and Fuson (*5*).] After the solution had passed into the column, the developing solvent (5.0% ether in hexane) was added, the vacuum removed, and development carried out under air pressure of about 30 cm. of mercury. The dinitrobenzoates were visible under ultraviolet radiation as dark brown bands on a yellow fluorescent background. Development was continued until the bands were completely separated, whereupon they were either washed through and collected separately or dug out of the column by a long narrow spatula under ultraviolet inspection, and then eluted with ether. When only a single band was obtained, the band was divided in half, and the halves were eluted separately. The filtrates or eluates were evaporated to dryness on a steam bath, and the melting points of the evaporated residues determined. No recrystallization was ordinarily done, as this might have resulted in some fractionation of mixtures.

RESULTS AND DISCUSSION

Table I shows the results obtained by the adsorption of 40 pairs of dinitrobenzoates involving six normal primary alcohols, two branded-chain primary alcohols, three secondary, and one tertiary alcohol. The pairs marked G and F were separated sufficiently to make two zones visible, with a yellow fluorescent band between. In the pairs marked F, there was so little space between the zones that some admixture probably resulted during removal from the column. In practice, sacrifice of yield or a second adsorption would give good separation in these cases. The pairs marked P gave only a single zone on development, but when the band was arbitrarily divided the two parts gave materials of different melting points, showing that a mixture was initially present. Finally, in the pairs marked O, no separation was obtained, as there was no significant difference in the melting points of the material from the upper and lower halves of the zone.

The blank spaces in the upper right part of Table I represent combinations not investigated, but as the derivatives are listed in the table in order of decreasing strength of adsorption, each of these 26 pairs should be completely separable without difficulty.

Table II shows typical examples of each of the four degrees of separation obtained, with a description of the developed column in each case and the weight, melting point, and identity of the fractions obtained.

When mixtures of the dinitrobenzoates of alcohols of a homologous series are separated by this procedure, they wash through in

the order of molecular weight, the heaviest passing through first. The derivatives of the first five normal primary alcohols can be completely separated from their mixture, five bands appearing. A mixture of *n*-amyl and *n*-hexyl derivatives gives a single band, which may be resolved by arbitrary division and readsorption.

When mixtures of dinitrobenzoates with the same number of carbon atoms are separated, the secondary alcohols wash through below the primary, and the tertiary (judging from *tert*-butyl, the only tertiary alcohol studied) below the secondary. Such separations are poor, however; no actual separation into bands was obtained on the column.

Table II. Description of Chromatograms of Typical Mixtures of Alkyl Dinitrobenzoates Showing Different Degrees of Separation

Degree of Separation (Table I)	Original Mixture	Column after Development	Material from Upper Zone[a]	Material from Lower Zone[a]
Good (G)	26 mg. methyl, m.p., 106° 21 mg. ethyl, m.p., 91–3°	94 mm. yellow 29 mm. brown 30 mm. yellow 19 mm. brown	23 mg. methyl, m.p. 107°	18 mg. ethyl, m.p. 92°
Fair (F)	22 mg. isobutyl, 86–9° 24 mg. isoamyl, 61–3°	162 mm. yellow 23 mm. brown 2 mm. yellow 23 mm. brown	24 mg. isobutyl, m.p. 79–80°	20 mg. isoamyl, m.p. 56–7°
Poor (P)	22 mg. *n*-propyl, 74–8° 22 mg. isopropyl, 121–3°	166 mm. yellow 42 mm. brown[b]	19 mg.[c], m.p. 83–7°	21 mg.[d], m.p. 100–2°
None (O)	21 mg. *n*-amyl, 44° 21 mg. isoamyl, 61–3°	159 mm. yellow 32 mm. brown[b]	18 mg., m.p. 39–40°	22 mg., m.p. 40–3°

[a] Melting points of recovered materials are in all cases those of evaporated residues, not recrystallized.
[b] Single zones divided in half before elution.
[c] By mixed melting point found to be mostly *n*-propyl derivative.
[d] By mixed melting point found to be mostly isopropyl derivative.

Primary derivatives containing the same number of carbon atoms—e.g., *n*- and isobutyl, *n*- and isoamyl—cannot be separated by this method.

Secondary or tertiary derivatives of one group may overlap with the primary derivatives of the next higher group and thus prevent separation—for example, *tert*-butyl cannot be separated from *n*-amyl or isoamyl; 2-pentyl cannot be separated from *n*-hexyl.

In the work reported above, individual dinitrobenzoates were prepared and then mixed. As a check on the applicability of the procedure to the separation of alcohols for identification purposes, the following experiment was done.

Methyl, ethyl, *n*-propyl, *n*-butyl, and *n*-amyl alcohols were mixed, and the mixture was reacted with dinitrobenzoyl chloride in the presence of a small amount of pyridine. About 100 mg. of the liquid product were washed with petroleum ether, which was then adsorbed on the column. The reaction product was adsorbed without preliminary crystallization in order to be certain of the presence of all five alcohol derivatives in the adsorbed solution. After development, five well defined zones were obtained; the melting points of the materials from the zones confirmed the separation.

Any dinitrobenzoic acid in the samples is very strongly adsorbed at the top of the column and easily separable from any of the derivatives examined.

The visibility of the adsorbed bands under ultraviolet radiation is excellent. On columns of the size used above, bands containing about 1 mg. of derivative can be discerned. Visibility of faint bands is considerably increased if the column is examined from above with the line of sight making an angle of about 30° with the side of the tube. The stronger bands are also visible in daylight, showing as reddish pink bands on the salmon-pink background, but the visibility in daylight is not good enough to allow elimination of ultraviolet examination. This visible color varies with the particular batch of adsorbent used.

VARIATION IN ADSORBENTS

The work reported above was done entirely with one lot of silicic acid (Mallinckrodt AR, control RXN1). In order to survey the effect of source of adsorbent, four other silicic acids were tested. In each case a solution containing methyl, ethyl, *n*-propyl, *n*-butyl, and *n*-amyl dinitrobenzoates was adsorbed and developed. Such a mixture was separated into five bands by the Mallinckrodt preparation (control RXN1).

Mallinckrodt AR (control REY) separated the test mixture into four bands, the lowest containing butyl and amyl derivatives, which were poorly separated. (Control RXN1 had been shipped and stored in a fiberboard drum; control REY had been shipped and stored in a glass jar. When the latter material was exposed in a thin layer to the laboratory atmosphere for 24 hours before treatment with the rhodamine 6G, it gave five bands in this test.)

Merck reagent grade (control 357) separated the test mixture into five bands.

Baker C.P. powdered (control 31,546) separated the test mixture into five bands, but the separation between the butyl and amyl bands was faint. It was necessary to grind this absorption to 150-mesh before use. Silicic acid was prepared from sodium silicate solution by the procedure described by Ramsey and Patterson (*3*). A column made up of 150-mesh material separated the test mixture into four bands.

REGENERATION OF ADSORBENT

When the adsorbed zones had been washed through the column, it could be prepared for re-use by washing with hexane. When the zones were mechanically removed from the column before elution, the adsorbent after elution was saved and regenerated by washing with methanol and drying as in the original preparation of the adsorbent. The activity of regenerated adsorbent was fully equal to that of the original material.

There was no tendency for the fluorescent dye to leach from the column when washed with hexane, the developing solution, or the ether eluant. More active eluants might cause some dye to appear in the filtrate. It was not necessary to use more active eluants to remove the dinitrobenzoates. In one case, ether eluted 97.3% of the adsorbed methyl dinitrobenzoate (the most strongly adsorbed dinitrobenzoate studied) from the column.

No evidence of decomposition of the adsorbed dinitrobenzoates by the ultraviolet radiation was obtained, but as a precaution the columns were shielded from the source except when observations were being made.

ACKNOWLEDGMENT

The authors are indebted to J. T. Scanlan for supplying several of the dyes used in this investigation.

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RECEIVED March 26, 1948. The mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

Use of Lyophilization in Determination of Moisture Content of Dehydrated Vegetables

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A new reference method for the determination of moisture content of dehydrated vegetables involves addition of a large amount of water to a weighed sample of vegetable; freezing and drying in the frozen state (lyophilization); and completion of the drying in a vacuum oven or vacuum desiccator in the presence of an efficient water adsorbent. The last step can be completed in a relatively short time at, or slightly above, room temperature, because of a marked increase in drying rate brought about by lyophilization. Data presented for white and sweet potatoes, beets, and carrots show that the lyophilized materials can be dried unambiguously to constant weight and that the loss in weight may be taken as a measure of the moisture content. As the final dry weight is virtually independent, within wide limits, of the temperature of drying, the new method obviates the necessity of careful control of drying temperature.

IN A recent publication from this laboratory (*2*) it was shown that the determination of water in dehydrated vegetables by heating in a vacuum oven at elevated temperatures requires calibration to establish the proper time and temperature of drying for different vegetables. The calibration is necessary because there is usually no clear-cut end point observed in the drying and there is no simple way of distinguishing whether the progressive, slow loss of weight of the sample on prolonged drying is due to thermal decomposition or to slow removal of adsorbed water.

Two methods of calibration were described (*2*). One is the primary reference method, which consists of drying to constant weight over magnesium perchlorate in evacuated desiccators at room temperature. Because this method is exceedingly slow, requiring about 6 months for the samples to reach constant weight, another more rapid, secondary reference method was devised. It involves a so-called "redrying procedure," which consists of determination of time necessary to remove a known amount of water that was previously added to an essentially dry sample. The time required to carry through the redrying procedure amounts to several weeks, and it is evident therefore that a still more rapid method is highly desirable.

The factor responsible for the slowness of the two methods is the rate of diffusion of water through the tissues at temperatures low enough to prevent thermal decomposition. The fact that finer grinding, up to a practicable limit, does not increase the speed of drying sufficiently has been demonstrated (*2*). However, the authors have obtained a very marked increase in the drying rate with samples of dehydrated vegetable first saturated with water to form a slurry, then frozen, and subsequently dried in vacuo in the frozen state (*1*) (lyophilized) to a low moisture content.

The increase in the drying rate may be attributed to two factors. One is the increase in the volume of the lyophilized tissue, for when water is added to dehydrated vegetables the tissue swells and on subsequent drying only very little shrinkage occurs (*3*). The other factor is the increase in porosity caused by leaching of soluble materials from within the tissue. The leached materials, principally sugars, appear after lyophilization as a distinct, loosely packed, porous layer.

The effectiveness of lyophilization is demonstrated by the fact that the speed of drying of diced carrots (approximately 0.5-cm. cubes) was much faster than that of the same carrots, unlyophilized but ground to pass a 40-mesh sieve. It also has been found that the lyophilized materials can be dried rapidly to constant weight in a vacuum oven at suitable temperatures, and that the loss in weight is the same as in drying in a desiccator at room temperature. Thus the procedure may serve as a basis for a rapid reference method for moisture content. The results of measurements on sweet potatoes, white potatoes, beets, and carrots are described in this paper.

EXPERIMENTAL

Materials. The dehydrated vegetables used had been prepared from raw materials which were diced, blanched, and dried with hot air to a moisture content of about 7% in the pilot plant of this laboratory.

Procedure. The experiments consisted of determinations of loss of weight as a function of time, at various temperatures, on lyophilized and unlyophilized samples of the several vegetables under study. The operation involved successive dryings and weighings of the same sample at various time intervals.

Drying at elevated temperatures was conducted in a vacuum oven at pressures of 0.05 to 0.15 mm. of mercury. A trap cooled in solid carbon dioxide was maintained between the oven and the oil pump. Drying at room temperature was done over anhydrous magnesium perchlorate in evacuated desiccators. All measurements were made in duplicate on dehydrated vegetables ground to pass a 40-mesh sieve, on samples weighing approximately 2

Table I. Results of Vacuum-Oven Drying Experiments on Lyophilized and Unlyophilized Vegetables

Temperature, °C.	Loss in Weight (%) at Various Times (Hours)[a]								
	0	3	6	22	38	60	100	140	180
	Sweet Potatoes, Not Lyophilized								
70	0	5.40	6.27	7.57	7.89	8.07	8.25	8.33	..
60	0	4.54	5.28	6.78	7.16	7.45	7.77	7.88	8.00
	Sweet Potatoes, Lyophilized								
70	5.05	7.97	7.98	8.27	8.28	8.27	8.27	8.25	..
60	8.00	8.01	7.88	8.19	8.23	8.23	8.25	8.24	8.21
	White Potatoes, Not Lyophilized								
70	0	6.56	7.28	8.33	8.54	8.68	8.80	8.86	..
60	0	5.93	6.61	8.01	8.25	8.47	8.67	8.76	8.84
	White Potatoes, Lyophilized								
70	5.05	8.30	8.33	8.75	8.77	8.76	8.77	8.77	..
60	8.71	8.57	8.29	8.82	8.85	8.84	8.87	8.87	8.82
	Beets, Not Lyophilized								
70	0	3.84	4.66	6.00	6.35	6.58	6.80	6.89	..
60	0	3.13	3.82	5.28	5.76	6.07	6.40	6.56	6.71
	Beets, Lyophilized								
70	5.54	6.50	6.52	6.75	6.82	6.82	6.84	6.84	..
60	6.10	6.59	6.27	6.83	6.87	6.90	6.95	6.95	6.92
	Carrots, Not Lyophilized								
70	0	5.01	5.80	7.09	7.40	7.59	7.78	7.89	..
60	0	3.99	4.74	6.26	6.67	6.95	7.22	7.35	7.43
	Carrots, Lyophilized								
70	5.90	7.18	7.23	7.56	7.63	7.67	7.76	7.82	..
60	6.42	7.12	7.09	7.37	7.44	7.45	7.50	7.53	7.51

[a] Results represent averages of two samples. Values for lyophilized samples are expressed as percentages of original weight before lyophilization. Values at zero time are losses that occurred during lyophilization.

Table II. Drying of Lyophilized Vegetables in Vacuum Desiccators over Magnesium Perchlorate, at Room Temperature

Time, Days	Loss in Weight at Various Times, %[a] Sweet potatoes	White potatoes	Beets	Carrots
0	5.05	7.96	5.43	5.55
1	8.14	8.66	6.14	6.38
4	8.18	8.69	6.36	6.77
11	8.22	8.70	6.52	6.96
19	8.24	8.70	6.57	7.05
27	8.23	8.68	6.58	7.08
35	8.22	8.69	6.61	7.15
43	8.23	8.68	6.62	7.15
50	8.26	8.70	6.63	7.18
64	8.22	8.70	6.65	7.23
78	8.23	8.67	6.67	7.20
99	8.23	8.66	6.67	7.10
164	8.23	8.65	6.71	7.18
214	8.22	8.66	6.70	7.20

[a] Percentages of original weight of samples before lyophilization.

grams each. The differences in the measured losses of weight among duplicates were usually not greater than 0.03%. Reproducibility for replicates not dried at the same time is estimated to be only ±0.1%. Other details of the oven-drying operations and the precautions taken were the same as described previously (*2*).

Lyophilization. The lyophilization procedure was carried out in the following manner. Approximately 2-gram samples of ground vegetables were weighed into cylindrical, ground-glass-stoppered weighing bottles, 4 cm. in diameter and 5.5 cm. high, 15 to 20 ml. of water saturated with toluene were added to each bottle, and the mixture was allowed to stand overnight in a cold room at about 5° C. to allow time for rehydration. Toluene and low temperature were required to inhibit microbiological activity. The rehydrated samples (in the form of a slurry) were frozen and cooled to about −70° C. by immersion in a tray containing a slurry of solid carbon dioxide in ethyl alcohol. They were then quickly transferred to a lyophilization apparatus where they remained for about 20 hours (overnight) and dried to a moisture level of about 2 to 3%.

This apparatus consisted of a Pyrex desiccator (25 cm. in diameter) connected by glass tubing and ground-glass joints to a 1-liter 3-necked flask which served as a trap for water vapor when immersed in a gallon Dewar flask filled with a mixture of solid carbon dioxide in alcohol. Two spherical ground-glass joints (one horizontal and one vertical) were included in the connecting tube to enable the operator to loosen the desiccator lid and either to slide away or attach the bottom part of the desiccator without disengaging any connections. The connecting tube also contained a side tube attached to a vacuum gage. One neck of the flask served as a connection to a vacuum pump (mechanical, oil type) and another as an air inlet through a stopcock.

About ten sample bottles of materials to be lyophilized were placed on a porcelain plate in the desiccator. After evacuation of the apparatus, the plate was heated by a 60-watt lamp placed about 20 cm. (8 inches) below the bottom of the desiccator. With this arrangement, the temperature of the samples was between 25° and 30° C. and the pressure in the system was about 10 microns, at the end of the drying. The temperature was read through the wall of the desiccator on a short mercury thermometer which was embedded inside a duplicate sample of one of the vegetables.

RESULTS

The results of the vacuum-oven drying experiments at elevated temperatures on lyophilized and unlyophilized materials are shown in Table I. The samples dried at 60° C. were kept in the lyophilizing apparatus a few hours longer than those dried at 70° C. and for that reason the former contained very little residual moisture before oven drying (at zero time). These nearly dry samples, when heated in the oven for only a 3-hour period (see results in Table I for 3 and 6 hours of drying), either did not lose any weight or actually gained some weight. They were, of course, very hygroscopic and picked up some moisture when first placed in the oven. Evidently a 3-hour drying period was not sufficient to remove the regained water. The results for lyophilized materials dried in vacuum desiccators at room temperature are shown in Table II.

The data for sweet potatoes are also shown graphically in Figure 1. It is apparent that unlyophilized sweet potatoes undergo continuous weight loss over the whole drying period and there is no clear indication that drying is complete in 140 to 180 hours. After 140 hours there is still a considerable difference (0.6%) between the losses in weight at 70° C. and at 60° C. It can only be inferred that the two drying curves might become asymptotic after a much longer drying time.

The curves for lyophilized samples show that constant weight is reached unambiguously in 38 and 22 hours, at 60° and at 70°, respectively. Furthermore, the final loss in weight at 70° (8.3%) is the same within experimental error as that at 60° (8.2%) and that at room temperature (8.2%) (cf. Table II).

From this agreement and the fact that the loss in weight in a desiccator at room temperature is taken to be the moisture content of the material (*2*), it follows that vacuum-oven drying of lyophilized sweet potatoes at 60° or 70° C. may be used as a rapid secondary reference method for moisture determination.

Similar conclusions may be drawn from data for beets and white potatoes. As with sweet potatoes, constant weight was attained only with the lyophilized samples. The agreement in the loss of weight at 70°, 60°, and room temperature was found to be within about 0.2% or better.

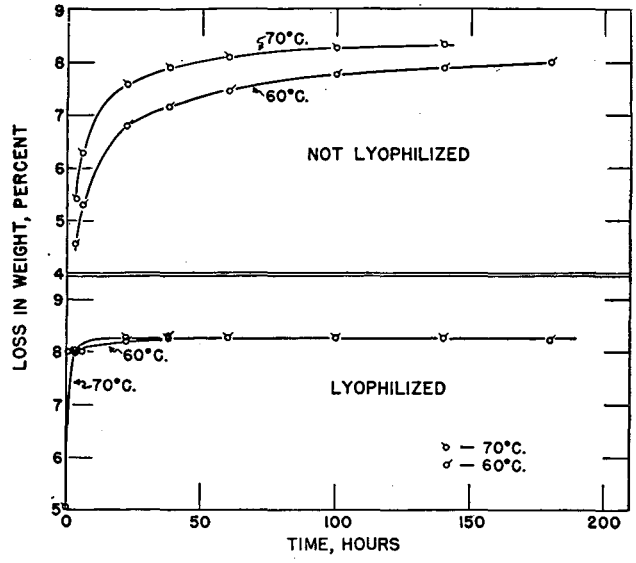

Figure 1. Drying Curves for Lyophilized and Unlyophilized Sweet Potatoes at 60° and 70° C.

From the fact that constant weight is reached with some lyophilized materials dried at 70° C., one might assume that decomposition rate at 70° is negligibly small and that the slow change observed in unlyophilized vegetables at 70° C. is entirely due to slow diffusion of water. If that were true, it would follow that unlyophilized would eventually reach the same constant weight as lyophilized materials. It appears from data in Table I, however, that the eventual loss of weight in unlyophilized would be greater than in lyophilized samples. This observation can be interpreted only on the assumption that measurable decomposition does occur when moisture is present in the vegetable, but is negligible for materials in the dry state. Thus some decomposition would be expected to occur in the drying of unlyophilized vegetables because they remain in the moist state for a long period at 70°. Further support for this explanation is afforded by the observation (unpublished data) that an increase in the measured moisture content occurs when dehydrated vegetables are stored at elevated temperatures.

Carrots differed from the other vegetables in some respects. Constant weight was not reached in drying of lyophilized samples at 70° but was attained at 60° C. Failure to reach constant

weight at 70° C. was probably caused by thermal decomposition, which in this case is apparently appreciable even in the dry state. The final loss in weight for carrots at 60° C. was, however, greater by about 0.3% than the value obtained in desiccator drying at room temperature. This discrepancy exceeds only slightly the estimated experimental error for replicates (±0.1%) but if it is real, it might be attributed to loss of volatile substances other than water. The other vegetables are probably subject to the same error, as evidenced by somewhat lower losses of weight at room temperature than at 60° or 70° C., but the magnitude of this effect is much smaller and is not readily distinguishable from the experimental error.

From the behavior observed with carrots it appears that materials containing appreciable quantities of nonaqueous volatile substances can be dried only by the desiccator method. That this method is not prohibitively long with lyophilized materials is shown by the data in Table II. The actual time necessary to remove all but the last 0.1% of the weight was less than 4 days for white and sweet potatoes and about 30 days for beets and carrots. With higher temperatures (40° to 50° C.) the time can probably be greatly shortened without appreciable error from thermal decomposition, as shown by the data for the vacuum-oven experiments. In contrast, the drying time for unlyophilized vegetables, under similar conditions, was found to be 6 months or more (*2*).

From the moisture values obtained in lyophilization experiments and from the drying experiments on unlyophilized materials, it is possible to establish the drying times at 70° C. that would be required in a routine vacuum-oven moisture determination on unlyophilized vegetables. For example, if the moisture content of sweet potatoes is taken to be 8.2%, the drying time at 70° C. for unlyophilized samples, ground through a 40-mesh sieve, is found from the corresponding drying data in Table I to be about 100 hours. Similarly, the time is 100 hours for white potatoes and beets, and 30 hours for carrots.

It is of interest to compare these calibrations with those obtained previously by the primary reference method and redrying procedure, although it is not possible to make the comparison rigorous because different materials were used in the two tests. The drying times found previously were: beets and sweet potatoes, 100 to 120 hours (unpublished data); carrots, 29 to 35 hours (*2*); white potatoes, 43 to 67 hours (*2*). The agreement with the lyophilization method is good, as the moisture values obtained with either calibration would not differ in any case by more than 0.2%. These drying times are excessively long for routine work, and to shorten the time it would be necessary to employ higher temperatures.

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Detection of Chlordan (Octachloro-4,7-methano-tetrahydroindane) in Insecticide Oil Sprays

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Chlordan, under the influence of heat, pyridine, and alcoholic alkali, reacts with ethylene glycol monoethyl ether to give an intense red color that distinguishes it from other common ingredients of insecticide oil sprays, and is a quantitative indication of the amount present. During dehalogenation with sodium and isopropyl alcohol, a characteristic odor and temporary darkening occur which serve as confirmatory evidence.

REPRESENTATIVES of the companies making the product and of the various federal departments concerned have agreed upon the name chlordane for a new insecticidal ingredient whose chemical structure is said to be represented by 1,2,4,5,6,7,-8,8-octachloro-4,7-methano-3a,4,7,7a-tetrahydroindane (*1*, *4*). It has previously been distributed in technical forms under the name Velsicol 1068 (Velsicol Corporation, 330 East Grand Ave., Chicago 11, Ill.), and also advertised under the name Octa-Klor (Julius Hyman & Co., Denver, Colo.). The purified material is stated to be a colorless, viscous liquid boiling at 175° C. under 2-mm. pressure (*3*).

A 20% concentrate of the product was furnished the United States Department of Agriculture by the Velsicol Corporation. Most of the concentrate, which is of the nature of a solvent for the chlordan, distilled between 95° and 110° C. under 2-mm. pressure, and the color test described below was negative on this fraction at a 10% concentration. The viscous oil, which is reported to be the highly active fraction of the commercial concentrate (*3*), and to which the 20% claim and now the name chlordan refer, distilled at 175° under 2-mm. pressure. This fraction was found to contain the portion that gives the color reaction in the proposed test, and was positive in a 0.2% concentration. A specimen of the commercial 90% concentrate of chlordan (Velsicol 1068) was later tested and gave an intensity of color approximately equivalent to the latter fraction.

Concentrations hereafter expressed are in terms of chlordan; the quantities are divided by 5 whenever the commercial 20% concentrate was used.

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DETECTION OF CHLORDAN IN MINERAL OIL SOLUTIONS

Reagents. Cellosolve-Pyridine Solution. Mix 10 ml. of pyridine with 40 ml. of Cellosolve (ethylene glycol monoethyl ether).

Alcoholic Potassium Hydroxide Solution. An approximately 1 *N* solution of potassium hydroxide in 95% ethyl alcohol, sufficiently fresh to be colorless.

Color Test. In a test tube, mix 1 ml. of the sample oil, 2 ml. of the Cellosolve-pyridine solution, and 1 ml. of the alcoholic potassium hydroxide solution. Heat in a boiling water bath with occasional agitation for 5 minutes. A 1-ml. sample of 0.2% chlordan in a deodorized kerosene base gives a wine-red color of considerable strength, and 1% gives an intense dark red color. Very weak colors should be regarded as possibly due to other substances.

QUANTITATIVE APPLICATIONS

The colors are soluble and stable in numerous solvents. Suitable solutions for photometric comparisons can be obtained by

Table I. Effects of Various Substances

Concentrations are per cent by weight in commercial deodorized kerosene as a medium. Thanite and Toxaphene are of the Hercules Powder Co., Wilmington, Del., the Lethanes of the Rohm & Haas Co., Philadelphia, Pa., the Velsicol products of the Velsicol Corporation, Chicago, Ill., DDT (Santobane) of the Monsanto Chemical Co., St. Louis, Mo., and benzene hexachloride (hexachlorocyclohexane) of the Hooker Electrochemical Co., Niagara Falls, N. Y. Parenthetical figures refer to an approximate comparison with the color intensity of a 0.2% chlordan standard.

Sample	Color Obtained
0.2% chlordan	Positive, wine red (1.0)
5% DDT, technical	Pale yellow, white precipitate
Saturated hexachlorocyclohexane	Pale yellow, white precipitate
5% Toxaphene	Light brown (0.8)
0.5% Toxaphene	Pale straw-color
20% *p*-dichlorobenzene	Very pale yellow
30% *o*-dichlorobenzene	Pale yellow-green
5% Lethane 384	Light yellow
5% Lethane 60	Light yellow
7% Thanite	Weak yellow-orange (0.5)
30% Velsicol AR-60	Pale brown
30% Velsicol AR-50 Special	Weak red (0.3)
30% pine oil	Pale yellow
Official test insecticide, 1945 (pyrethrins solution)	Pale brown
Saturated rotenone	Orange-yellow (0.1)
Saturated dinitroanisole	Yellow-green
5% dinitroanisole mixture, inhomogeneous	Orange immediately, later brown-red (1.5)
10% nitrobenzene	Weak yellow
10% *o*-cresol	Colorless
10% *m*-cresol	Colorless
10% *p*-cresol	Colorless
12% phenol	Colorless
5% thymol	Colorless

proceeding with weighed samples and standards according to the qualitative method, and then diluting the resulting colors with anhydrous isopropyl alcohol to color densities suitable for the particular apparatus used. A simple filter photometer did not resolve any complexities in the absorption pattern, as there was only a gradually accelerated rise of the absorption toward the ultraviolet. Readings in the region of 460- to 530-millimicron wave length were satisfactory. In the absence of Thanite (isobornyl thiocyanoacetate), blanks encountered have been so pale as to be of little concern, but for a perfect blank correction it would be desirable to substitute for the Cellosolve of the blank a reagent that would not give the specific color with the chlordan of the sample and yet would have the same degree of participation in effects on any inherent coloring of the sample and on possible interferences. Butyl Cellosolve was found to perform the first two functions in the blank and be partially satisfactory for the third. In trial of these principles, applied as a photometric procedure, 1.81% was obtained on a commercial sample claiming 1.80% Velsicol 1068 (chlordan), and 1.59 and 2.40% for petroleum oil solutions which were actually 1.60 and 2.39%, respectively. When the last figures are dropped, the agreement is perfect, and the qualitative method is therefore a suitable basis from which a quantitative procedure can be adapted when the need arises.

DISCUSSION

When the quantities of sample and reagents in the method were specified, it was assumed that the critical concentrations of chlordan to be detected in finished insecticides would be of the order of 0.4 to 2%. These proportions gave strong red colors, that from 1% being of such strength as to appear nearly black and to require dilution to observe its true color. Other substances (Table I) occasionally met in insecticide spray oils will give relatively weak reddish colors, some approaching that of a 0.2% standard. It is therefore advised that excessive sample quantities be avoided and that the analyst become familiar with the hue and density of color given by a 0.2% standard and not ordinarily report as definitely positive the weak red colors that are only a fraction of this strength. Where certain interferences are excluded and conditions controlled by standards, the method detects as little as 0.1%. Although this sensitivity exceeds that necessary for the analysis of commercial insecticides, it is not expected to be satisfactory for spray-residue analysis.

Substances like dinitroanisole and rotenone resins are known to give red colors under certain alkaline conditions, but their low solubility in common mineral oil distillates apparently prevents their use in interfering concentrations in the usual type of oil spray. Thanite appears to be the most serious of the interferences, but its color is relatively weaker, of a different hue (yellow-orange), and of a slowly precipitating character. In addition, the color due to Thanite may be distinguished by its changing to a greenish-yellow when diluted with 2 to 3 volumes of acetone. However, because Thanite both competes for the alkali and gives an interfering color, special care is necessary in judging results in its presence. Phenolic substances were entirely negative when pure, but some color may be expected from old polymerized specimens.

In adapting the test to extracts and residues of insecticides in general, it will be desirable to ensure low concentrations of such substances as dinitroanisole and rotenone resins. Exclusion tests and restriction of sample size are useful in accomplishing this. As these substances are of low solubility in solvents of the petroleum distillate type and chlordan is readily soluble, it should suffice to make a solution in such a solvent at room temperature and at a proper dilution for the test and then use the readily soluble portion. Large proportions of readily saponifiable substances may be expected to interfere by competing for the alkali in the test, and special adaptations for them may be required.

In the formation of the color, Cellosolve is a reacting ingredient. Ethylene glycol, diethylene glycol, and ethylene glycol monomethyl ether are capable of substituting for it under similar heating conditions to give weaker colors, but glycerol, dioxane, and many glycol derivatives did not give the color. Pyridine is not strictly essential, but it considerably intensified the color and thereby allows a much safer margin over possible background colors.

SUPPLEMENTARY QUALITATIVE INFORMATION

During the determination of chlorine by methods that involve dehalogenation with sodium in boiling isopropyl alcohol, as described by Donovan (*2*), the presence of chlordan becomes evident when the mixture turns temporarily gray during the first 5 to 15 minutes of the refluxing, and a strong odor of a disagreeable naphthalenic character is developed. The gray color fades on further refluxing, becoming colorless in 2 hours, but the odor persists even through dilute peroxide and nitric acid treatments of the methods to which reference has been made. The odor closely resembles that of crude methylnaphthalene fractions which are sometimes used in insecticides; but if the odor is absent in the sample and appears during the sodium treatment, in the author's opinion this is useful qualitative evidence of either chlordan or of Toxaphene (Synthetic 3956, technical), which gives similar reactions. These characteristics are readily perceptible when a sample representing 40 mg. of chlordan is refluxed with 25 ml. of isopropyl alcohol and 2.5 grams of sodium. A 100-mg. sample of Toxaphene, under similar conditions, showed perceptible but much less graying and the development of an odor of the same type in considerable strength. When these supplementary indications are detected, the possibility of either chlordan or Toxaphene must be considered, but the color test then can distinguish between the two; hence a combination of these tests may be useful for the detection of Toxaphene.

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Color Reactions of Thiophene Compounds with Ceric Nitrate Alcohol Reagent

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With the exception of 2-nitrothiophene and 2-thiophenecarboxylic acid, thiophene derivatives containing hydrogen or an acetyl group in the α- position give color reactions with ceric nitrate alcohol reagent. Derivatives containing halogen or *tert*-butyl groups in both the 2- and 5- positions give no reaction. Of the fifty compounds tested, a few show positional specificity of color, but in general this test cannot be used to distinguish individual thiophene derivatives.

WHEN attempts were made to use the ceric nitrate test for alcohols (*1*, *2*) in the thiophene series, brilliant color reactions were noted. A general investigation of the thiophene compounds was then undertaken. The colors obtained are listed in Table I.

On the basis of the experimental data it appears that the results of this test depend upon the groups present in the α-position. Alkyl and halothiophenes with a free α- position give positive tests, whereas 2,5-di-*tert*-butylthiophene and 2,5-dichlorothiophene do not. On the other hand, 2-acetyl-5-chlorothiophene and 2-acetyl-5-*tert*-butylthiophene both give color reactions. No color changes were observed with 2-nitrothiophene or 2-thiophenecarboxylic acid. These results indicate that, except for the nitro and carboxyl compounds, derivatives containing a hydrogen or an acetyl group in the α- position give color tests. Although the di-(5-methyl-2-thienyl)methane derivatives give a color reaction, it has not been established whether the positive reaction is due to the α-methyl group attached to the thiophene or to the active hydrogen of the methane moiety.

The test can be used to distinguish between 2- and 3-methylthiophenes, as the former gives a bright purple precipitate with the reagent and the latter gives a deep blue precipitate.

Another interesting differentiation can be drawn in colors produced with 2-acetyl-3-methylthiophene and with 2-acetyl-4-methylthiophene. Agitation of the two-phase system produces a bright red color in both cases; but before agitation the former gives no color, and the latter gives a transient blue color. This blue color can be detected in mixtures containing 1 part of 2-acetyl-4-methylthiophene in 10 parts of the 3-methyl derivative.

Furan, like thiophene, gives a brown precipitate with the reagent and 2-acetylfuran, like the thiophene analog, is colored red.

Table I. Color Reaction of Thiophene Compounds with Ceric Nitrate

Compound	Color in Organic Layer	Color Change in Inorganic Layer
Thiophene	None to light brown	Brown ppt.
Alkyl thiophenes		
2-Methyl-	Brown	Bright purple ppt.
3-Methyl-	Brown	Deep blue ppt.
2-*tert*-Butyl-	Red[a]	Colorless
2-5-Di-(*tert*-butyl)-	None	.....
3,4-Dimethyl-	Brown to red	Deep blue ppt.
Di-(5-methyl-2-thienyl)-phenylmethane	Deep red	Yellow
Di-(5-methyl-2-thienyl)-methane	Deep red	Red ppt.
Tri-(5-methyl-2-thienyl)-methane	Bright orange	Yellow
2-*tert*-Amyl-	Red	Colorless
1-(2-Thienyl)-(1,1,3,3-tetramethylbutane)	Yellow	Yellow
2-Pinyl-[b]	Yellow[c]	Colorless
2-Benzyl-	Brown	Blue ppt.
Alkylene thiophene		
2-(α-Methylvinyl)-thiophene	Blue ⟶ brown	Colorless
Acyl thiophenes		
2-Acetyl-	Red	Colorless
2-Acetyl-3-methyl-	Red	Colorless
2-Acetyl-4-methyl-	Blue ⟶ red	Colorless
2-Acetyl-5-methyl-	Red	Colorless
2-Acetyl-5-*tert*-butyl-	Deep red	Colorless
2-Acetyl-5-chloro-	Orange[a]	Light yellow
3-Acetyl-2,5-dichloro-	None	None
3-Acetyl-2,5-di-*tert*-butyl-	None	None
2-Propanoyl-	Red ⟶ deep brown	Colorless
2-Butanoyl-	Orange	Colorless
2-(2-Ethylbutanoyl)-	Light yellow	Yellow
2-Benzoyl-	Light yellow	Yellow
2-Thenoyl-	Yellow to tan	Colorless
2-(2-Thenoyl)-3(4)-methyl-	Green	Colorless
Halothiophenes		
2-Chloro-	Light red[a]	Yellow
2-Bromo-	Brown	Yellow
2,3-Dichloro-	Orange	Yellow
2,4-Dichloro-	Light yellow	Yellow
2,5-Dichloro-	None	None
2,5-Dibromo-	None	None
3,4-Dichloro-	Light yellow	Yellow
2,3,4-Trichloro-	Light yellow	Yellow
2,3,4,5-Tetrachloro-	None	.....
2,2,3,4,5,5-Hexachlorothiolane	None	.....
2,2,3,4,5,5-Hexachloro-3-thiolene	None	.....
Thiophenecarbinols		
2-Thiophenecarbinol	Deep purple	.....
2-(2-Thienyl)-ethanol	Brown	.....
2-(4-Methyl-2-thienyl)-ethanol	Deep red ⟶ brown	.....
2-(5-Chloro-2-thienyl)-ethanol	Deep red	.....
Miscellaneous[d]		
2-Thenylamine[e]	Brown ppt.[f]	Brown ppt.[f]
Di-(2-thenyl) amine	Brown ppt.[f]	Brown ppt.[f]
Di-(5-methyl-2-thenyl)amine	White ppt.[f]	.....
2-Thiophenealdehyde	Red[f]	Colorless
5-Methyl-2-thiophenealdehyde	Deep red[f]	Colorless
2-Thiophenecarboxylic acid	None[a]	None
Ethyl 2-thiophenecarboxylate	Light green ⟶ blue[g]	
2-Nitrothiophene	None	.....
3-Thiophenethiol	Brown	Brown ppt.

[a] On warming.
[b] Reaction product of thiophene and α-pinene received from George C. Johnson, this laboratory.
[c] Red on warming.
[d] (*1*) reports color reactions with aromatic amines and phenols.
[e] 2-Aminomethylthiophene.
[f] Heat of reaction.
[g] Colors aqueous layer of reagent yellow. On warming aqueous layer turns red, yellow, and colorless within a few seconds. Final organic layer color is pink.

Pyrrole produces a deep black precipitate with the reagent.

No attempt has been made to study the chemistry involved, and only visual observations are recorded. Although the color transformations normally occurred in the organic layer, in the case of 2- and 3-methylthiophene the color appeared in the aqueous layer and a precipitate was formed. Fumes of 3-methylthiophene will coat the surface of the reagent standing in a beaker with a blue film. Color transformations in the organic layer may be due to solubility therein of some of the reduced stages of the ceric ion. In order to determine that the cerium compound instead of the nitrate ion in the nitric acid of the reagent was responsible for the color changes noted, 2- and 3-methylthiophene were treated in a similar manner with nitric acid (1 part of fuming acid to 3 parts of water). An oxidizing reaction set in, turning both samples dark brown, and in a short time the organic compounds were almost completely oxidized to carbon dioxide and sulfur dioxide. A 10% lead nitrate solution failed to produce any color reaction with either 2- or 3-methylthiophene.

In general, diluents should be avoided if possible, because they cause variations in sensitivity and color reactions. The test should not be run with dioxane solvent according to the recommended procedure for the alcohol test for water-insoluble compounds (*2*). If dioxane is mixed with the reagent and 2- or 3-methylthiophene is added no color is produced; but if the reverse order is followed and the ceric nitrate is added last, a red color is obtained with 2-methylthiophene and a fleeting blue color changing to red is observed with 3-methylthiophene. In neither case is a precipitate formed. In the case of 2-acetyl-4-methylthiophene in dioxane solution, no color change is noted at all. This latter compound is extremely sensitive to the reagent alone; the blue color is noted in benzene solution, but upon shaking, the color changes to light yellow rather than deep red.

TEST PROCEDURE

Equal volumes, 0.1 to 1.0 ml., of ceric nitrate–alcohol reagent prepared according to the method of Duke and Smith (*1*) and the thiophene derivative to be tested are placed in a small test tube. Normally the color change takes place at the interface and spreads rapidly through the organic layer. Agitation usually is necessary to bring out the final color stage.

If the samples to be tested are solids melting below 100°, the test tube containing the reactants is agitated in boiling water or a steam bath. Water-insoluble samples melting above that temperature have not been tested. Presumably benzene can be used as a diluent, as it does not interfere with tests on 3-methylthiophene, which can easily be detected in dilutions of 1 to 10 in benzene. The color of the precipitate changes to brown in the lower concentrations.

Unless otherwise indicated in the table, the colors were stable for a matter of minutes but no study of color stabilities was made.

PURITY OF CHEMICALS USED

Thiophene, 2- and 3-methylthiophene, and the acetyl- and methylacetylthiophenes were of about 99.8% purity as determined by infrared or mass spectrograms. Other samples were of general laboratory purity, most of the liquids being fractionated through 10- to 12-plate columns. Crystalline compounds were of accurate melting point purity. Samples of 2,4- and 3,4-dichlorothiophene were contaminated with each other to the extent of 5 to 10%.

LITERATURE CITED

(1) Duke and Smith, IND. ENG. CHEM., ANAL. ED., 12, 201 (1940).
(2) Shriner and Fuson, "Identification of Organic Compounds," p. 52, New York, John Wiley & Sons, 1945.

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Replica Studies of Dyed Nylon

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The crystal size of dyes and pigments, when applied to textile fibers, is almost invariably below the limit of light microscopical resolution. This paper describes an electron microscopical investigation of dyed nylon; an actual research problem is discussed. The electron microscope specimens consist of a thin silica replica holding the original dye crystals which have been "transplanted" from the nylon fiber surface to the silica. This silica replica–dye crystal combination is interpreted as though the original dyed nylon fiber were being examined. The technique probably produced no serious artifacts, and the micrographs provide information concerning the size, shape, and distribution of the dye crystals on the original fiber. These specimens can also be used for electron diffraction. These electron diffraction patterns were used to identify the three dyestuffs, based on a comparison with the diffraction patterns exhibited by the authentic (bottled) dyestuffs. The ability to "fingerprint" a dyestuff on a fiber using only a few milligrams of cloth and a few grams of dyestuff makes this type of specimen preparation very interesting. The application of this technique to other synthetic textile fibers is discussed briefly.

TEXTILE dyers and dyestuff chemists have had an almost insatiable desire to learn something about the distribution of dyestuffs on textile fibers. A tremendous amount of effort, both practical and theoretical, has been expended toward the solution of problems dealing with this subject. Light microscopy has furnished some information because the web views and cross sections of fibers exhibit the "gross" distribution of the dye (color) on and within the fiber. However, because of the limited resolving power of the light microscope, it is not possible to gain information concerning the specific details of the fiber surface or of the dye crystals themselves. The size of these dye crystals in dyeings of natural as well as synthetic textile fibers is almost invariably well below the limit of light microscopical resolution.

Royer *et al.* (*1*), in a series of publications, have described the usefulness of light microscopy to the dye technologist. Kern (*3*) has published electron micrographs illustrating replicas of a variety of undyed fibers. His replicas were prepared by means of the low pressure plastic molding technique.

This paper demonstrates the use of a somewhat new replica technique for gaining information on the specific details of the surface of dyed nylon fibers. The microstructural properties revealed by this technique are interpreted in terms of an actual research problem.

GENERAL DISCUSSION

This investigation was an outgrowth of a problem dealing with the effects of a postdyeing steaming treatment on a series of dyed

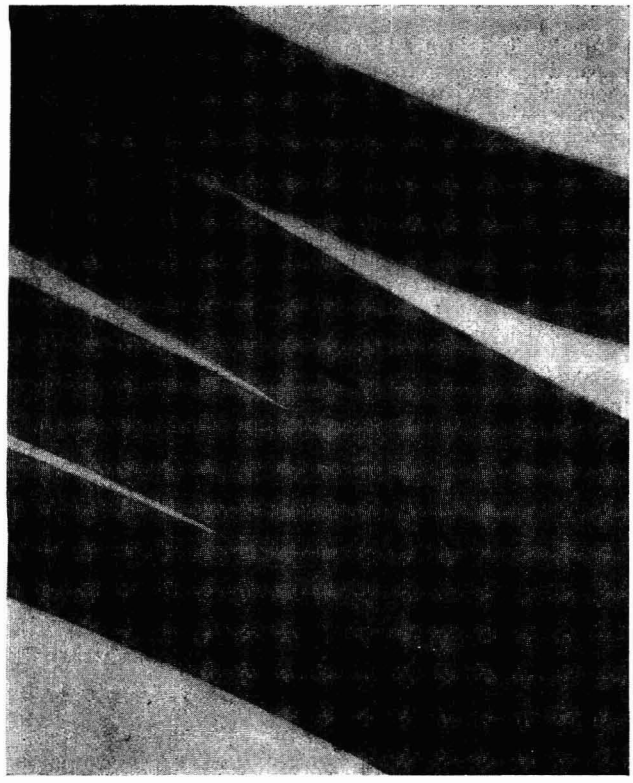

Figure 1. Photomicrograph of Original Yellow Dyeing, 750×

nylon cloths. The use of this and similar post dyeing treatments (metallization, acid aging, vat aging, "cottage" aging, etc.) is common practice, used primarily to cause dye penetration into the fiber, or to effec at more uniform dye distribution (leveling). Sometimes the results are satisfactory; however, the nylon dyeings under discussion in this paper suffered deleterious effects due to a postdyeing steaming at 10 pounds' pressure for 2 hours. The effects were the same in all cases, irrespective of the initial dyeing technique. After steaming these nylon dyeings exhibited (1) loss in tinctorial strength, (2) loss in fastness to crocking (mechanical ruboff onto white cotton), and (3) increase in fastness to ultraviolet light (exposure to a carbon arc in a fadeometer). It was presumed that the effect of steaming was physical in nature, inasmuch as the dyes are chemically stable to steam.

The problem was at first attacked by means of two physical approaches—light microscopy and spectrophotometry. (Spectrophotometry did not appear to be informative, and will not be discussed further.) Cross sections and web views of the dyed fibers (original and steamed) were examined in the light microscope. Although the crystalline detail could not be clearly resolved, there was good evidence to show that the steaming treatment caused a migration of dyestuff from within the nylon to the surface of the fibers, with an accompanying increase in the size of the dye crystals (aggregates?). This increase in crystal size was observed on the samples dyed with Indanthrene Golden Yellow IGK, Indanthrene Brilliant Pink IR, and Indanthrene Brown IRRD. [Some of the details on the steamed samples were known to be single crystals because they appeared to be elongated "rods" that exhibited a uniform polarization color throughout, and they became completely extinct (oblique) between crossed Nicols of a polarizing microscope. However, a great deal of the detail appeared to be aggregates (poorly resolved).] This paper deals with the original and steamed samples (total of 6) of these three dyeings, referred to as the yellow, pink, and brown dyeings, respectively. Permission to describe the technique with which these experimental dyeings were made was not granted. *However, the dyeing procedure* is of no significance in the present discussion.

The photomicrographs illustrated in Figures 1 and 2 show the increase in crystal size on the yellow dyeing due to the steaming process.

A 4-mm. microscope objective was used in order to gain high resolution. The depth of focus under these conditions is very narrow (±0.1 micron), and it is impossible to photograph the fiber and crystals with sharp focus. Obviously not all the crystals on the fiber surface are even indicated, but by "optically sectioning" with the vernier control of the light microscope, it is possible to see more surface detail. In fact, some of the loosely adhering crystals floated off from the nylon fiber when it was mounted in a fluid resin on a microscope slide. It was, therefore, not surprising that these steamed samples showed poor fastness to crocking. The brown dyeing after steaming resembled the yellow dyeing (after steaming). On the other hand, the detail on the steamed pink sample was considerably smaller and less detail could be resolved. All three original dyeings exhibited a rather continuously and uniformly dyed surface. A few "specks," especially on the brown and yellow dyeings, could be seen on the original samples. The electron microscopical study later indicated that these specks were probably large aggregates.

The length of some of the longest crystals on the steamed yellow and brown dyeings was measured by means of a calibrated eyepiece micrometer. A few single crystals were found to be 17 microns long (later confirmed by the electron microscope study). These long crystals were, however, the exception; most of them were on the order of a few microns or less. As the original dyeings are probably typical synthetic fiber dyeings, this electron microscopical study should be of interest to the dye technologist working in this field.

It is difficult to explain how the treatment with steam at 10 pounds' pressure for 2 hours could cause the profound increase in the average crystal size. The effect is not merely an aggregation of the original small and rather poorly developed dye crys-

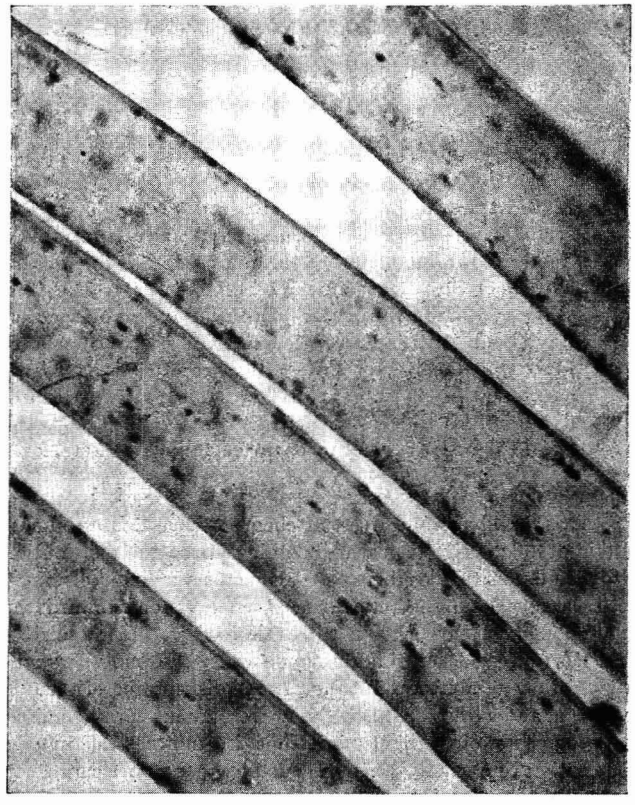

Figure 2. Photomicrograph of Steamed Yellow Dyeing, 750×

tals. Practically all of the original dyestuff recrystallized, in a way similar to the "ripening" of photographic silver halide crystals, or the "digestion" of precipitates before filtration in quantitative chemical analysis. The vapor pressures of the three dyes are relatively low, so that at approximately 100° C. (steam) and under normal atmospheric pressure there is no recognizable tendency for the dyes to sublime. Furthermore, the temperature at the target position (about 7 cm. above the heating coil) of the vacuum chamber used to prepare the replicas was raised to about 90° C. for 15 seconds. This was done five times, allowing for some cooling between heating cycles. No dyestuff sublimed from the nylon in the 0.1- to 0.01-micron pressure range. (Traces of dyestuffs can be detected because of their strong hiding power.) At no time during the preparation of the specimens used in preparing this paper was there any evidence of sublimation of the dyestuff from the nylon.

It is possible, although there is no evidence to substantiate it, that the nylon acted as a "solvent" for the dyestuffs at the elevated temperature in the presence of steam. The dyestuffs would then crystallize (on cooling) from the nylon mother liquor. It is not, however, the purpose of this paper to explain the mechanism of this crystal growth. In any case the electron micrographs (Figures 7 to 20) show that the three dyestuffs did recrystallize and concentrate on the fiber surface.

The three "gross" effects due to the steaming treatment were evaluated in the dye testing laboratory.

The loss in tinctorial strength was obvious in all three cases; in each dyeing the steamed sample was lighter in "shade" than the original sample. Assuming no loss in dyestuff, this may be interpreted as meaning that the hiding power of the dyestuff was weaker in the steamed samples than on the original samples. The relative fastness to crocking was determined by means of a standard crocking test, which involves placing a piece of white cotton cloth in a machine that causes the dyed cloth to rub over the cotton. Some of the dyestuff is rubbed onto the white cotton cloth. The steamed nylon dyeings lost more dyestuff, so that they exhibit poorer fastness to crocking than the original dyeings.

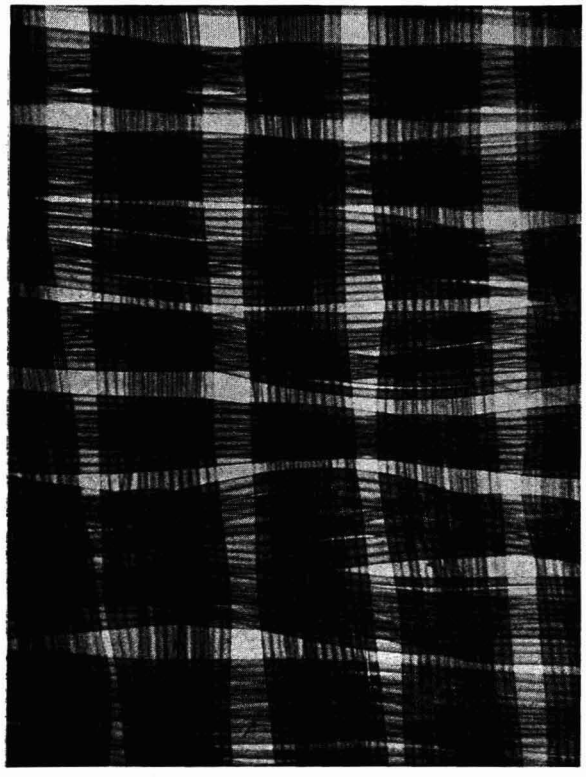

Figure 3. Photomicrograph of Original Brown Nylon Dyeing

Sample used to prepare combination silica replica–dye crystal specimen 45×

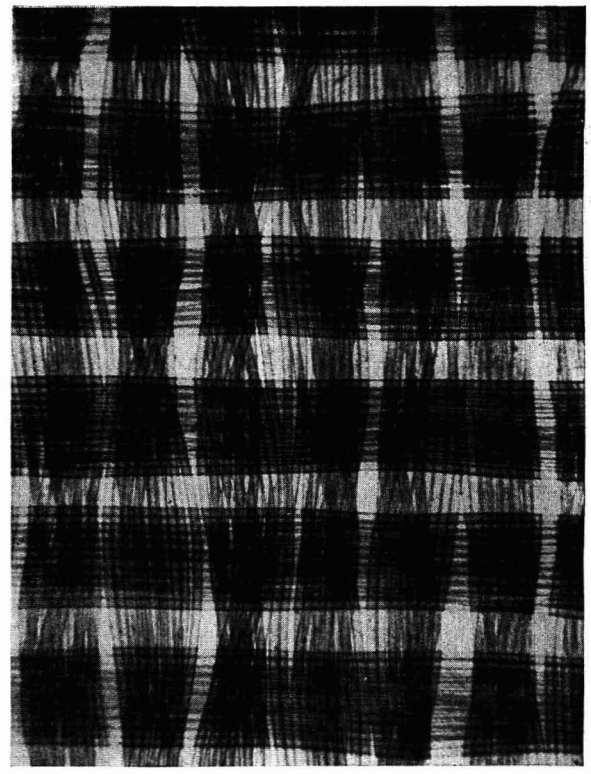

Figure 4. Photomicrograph of Steamed Brown Nylon Dyeing

Loss in color strength and poor resolution of surface dye crystals are illustrated 45×

The relative fastness to ultraviolet light was determined by exposing small swatches (half of which were protected from the ultraviolet light) to a carbon arc for 20 hours in an ordinary fadeometer. The color strength (or shade) of the exposed area compared with that of the unexposed (original) area is used to evaluate the fastness to ultraviolet light. The steamed yellow nylon dyeing turned brownish, indicating a decomposition of the dyestuff. The original yellow dyeing underwent a similar but greater change. Both the steamed pink and brown dyeings exhibited a very slight loss in tinctorial strength, whereas their original dyeings experienced a profound loss in color strength. The decomposition products on the exposed pink and brown dyeings are possibly colorless. The exposed yellow dyeings changed their color shade, presumably as a result of the formation of colored decomposition products. In all three cases, the steamed dyeings exhibited a stronger fastness to ultraviolet light.

EXPERIMENTAL

Soon after this investigation was begun it was realized that the usual replica techniques, utilizing thin films of organic materials (Formvar, polystyrene, methyl methacrylate, etc.) or silica, provided information that was not always complete. The three dyes under discussion and similar dyestuffs are somewhat soluble in organic solvents, thereby precluding the formation of a resin replica free from artifacts.

These dyed nylon fibers (especially the steamed samples) exhibit fine fiber surface structure along with very coarse structure (large dye crystals), so that the topography includes a wide range of surface elevations. Ordinary silica replicas (unshadowed) were prepared by condensing silica vapor directly on the nylon fibers. The fiber and dyestuff were then dissolved. Later work proved that these silica replicas were, for some unknown reason, incomplete because the small dye crystals were not always indicated.

Final recourse was made to a specimen that consists of a very

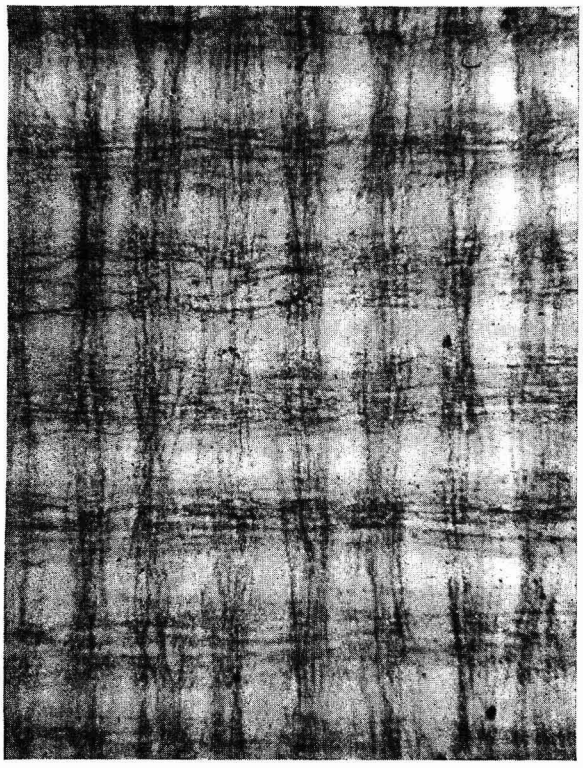

Figure 5. Photomicrograph of Combination Silica Replica-Dye Crystal Electron Microscope Specimen Prepared from Steamed Brown Dyeing, 45×

Figure 6. Electron Micrograph of Silica Replica of Steamed Pink Dyeing

Silica shadow cast sublimed at angle of 70° 7500×

Figure 7. Electron Micrograph of Silica Replica-Dye Crystal Specimen from Original Yellow Dyeing, 7500×

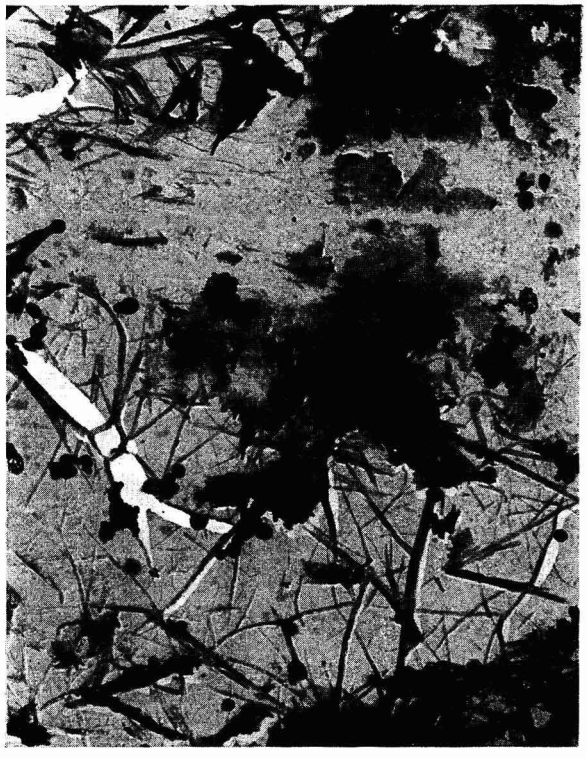

Figure 8. Electron Micrograph of Silica Replica-Dye Crystal Specimen from Original Yellow Dyeing, 7500×

Figure 9. Electron Micrograph of Silica Replica–Dye Crystal Specimen from Steamed Yellow Dyeing, 7500×

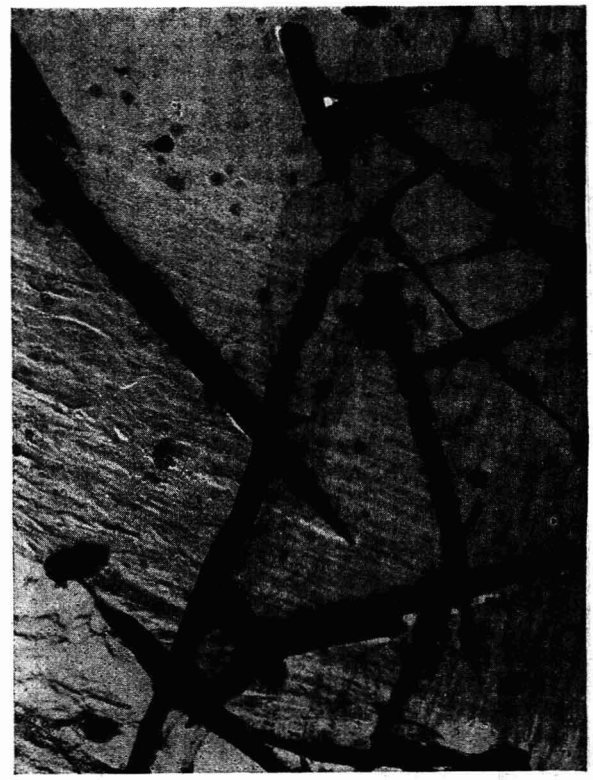

Figure 10. Electron Micrograph of Silica Replica–Dye Crystal Specimen from Steamed Yellow Dyeing, 7500×

thin film (100 to 150 Å.) of sublimed silica that retains the original dye crystals which have been "transplanted" from the nylon in such a way that this combination of silica and dye crystals accurately represents the original dyed nylon. When this specimen is examined in the electron microscope it is interpreted as though it were the dyed fiber. Consequently, this technique provides information concerning the shape, size, and orientation of the dye crystals that are on the nylon fiber surface. The nature of the fiber surface itself, especially as a function of the conditions of dyeing (temperature, solvents, pH, etc.) could also be studied by this technique. This combination of silica replica and dye crystals is very stable to the electron beam and lends itself to electron microscopy because of its high contrast.

Inasmuch as the silica replica and "shadow casting" techniques are now well established and have been adequately described in the literature (*2*, *5*) it is unnecessary to describe the apparatus or the details of the procedure for preparing these electron microscope specimens. All the dyeings had been made on 2.5-ounce nylon cloth. The fibers are essentially round and are on an average about 20 microns in diameter. The following outline gives the salient features of the technique used to prepare the combination silica replica–dye crystal specimens.

A thin film (100 to 150 A.) of silica is sublimed directly onto a small swatch of the dyed nylon cloth. This target is tilted 20° from the normal position (directly above the quartz) so that the dye crystals are shadowed with silica at an angle of 70°. Four 15-second heating cycles are used in order to keep the temperature in the vacuum chamber as low as possible. For some unknown reason the four short sublimations produce better replicas on the "rough" steamed samples than one heating period of equivalent time.

In the earlier work the nylon cloth was caused to adhere to the target holder by means of polystyrene. Treatment with ethylene dichloride (before the acid) was then necessary to remove this resin. Because these dyestuffs dissolved in this organic solvent, the use of polystyrene was omitted (see Figure 6). The nylon cloth was sufficiently inflexible so that it did not sag in the target holder.

After the silica is scored with a scalpel to give a crosshatch appearance, the nylon-silica combination is immersed in concentrated hydrochloric acid for about 30 minutes. The nylon dissolves completely but the insoluble dye crystals adhere to the silica.

The small individual replicas are transferred to fresh acid with an acid-insoluble stainless steel screen. A final wash with distilled water is used to remove the acid.

The replica is then picked up on a specimen screen and is ready for examination in the microscope.

Because there are no strong shearing forces but only the mild solvent action of the acid, it seems plausible to assume that the silica replica holds the dye crystals with the same distribution and orientation that these crystals enjoyed on the nylon fibers. Based on a comparison of the color strength exhibited by the specimen and the original nylon cloth, it seems safe to say that virtually all of the dye crystals are transplanted to the silica. The silica (replica) seems to form a tightly fitting film around the base of the larger crystals. All the dye crystals must be firmly attached to the silica because they remain fixed during exposure to the electron beam and during the transfer through the acid and water baths.

Figures 3 and 4 are low magnification (40× instrument magnification) photomicrographs of the nylon cloth. The weaker tinctorial strength and the speckled appearance due to the larger crystals on the fiber surface are illustrated in Figure 4. It is surprising that the condensing silica vapor forms a continuous film over the entire target surface, because the openings between the warp and filler strands are large. However, reference to Figure 5 shows that the silica replica is continuous; the transfer of dye crystals is also illustrated. (This photomicrograph was not made of the same field shown in Figure 4). [Because the object contrast is poor and the detail is not resolvable with a 5× (32-mm.) microscope objective, Figure 5 does not appear to be in

Figure 11. Electron Micrograph of Silica Replica-Dye Crystal Specimen from Original Brown Dyeing, 7500×

Figure 12. Electron Micrograph of Silica Replica-Dye Crystal Specimen from Original Brown Dyeing, 7500×

Figure 13. Electron Micrograph of Silica Replica-Dye Crystal Specimen from Original Brown Dyeing, 7500×

This would appear as a poorly resolved speck in a light microscope

Figure 14. Electron Micrograph of Silica Replica-Dye Crystal Specimen from Steamed Brown Dyeing, 7500×

Figure 15. Electron Micrograph of Silica Replica–Dye Crystal Specimen from Steamed Brown Dyeing, 7500×

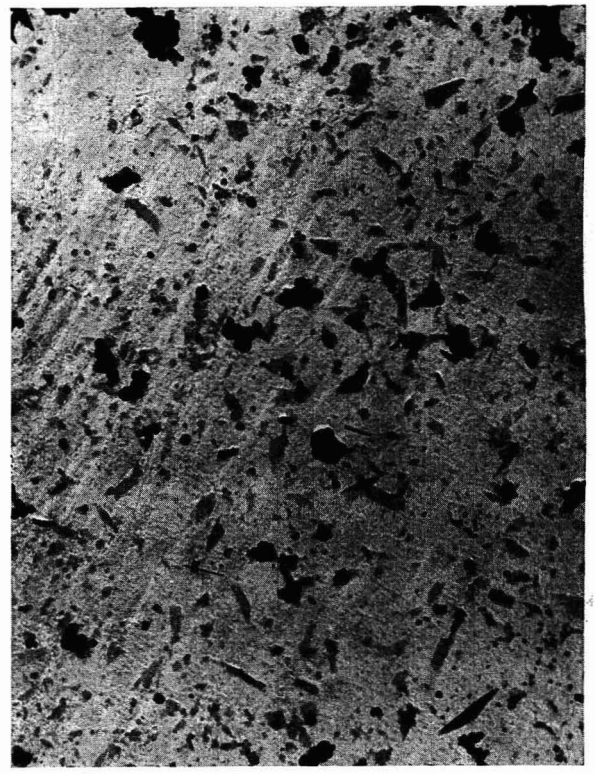

Figure 16. Electron Micrograph of Silica Replica–Dye Crystal Specimen from Original Pink Dyeing, 7500×

focus. However, it is in focus and it illustrates the appearance of a typical electron microscope specimen.]

DISCUSSION OF ELECTRON MICROGRAPHS

All the electron micrographs were taken with an RCA Model EMU instrument, a self-biased electron gun, and a 2-mil platinum objective aperture. Only the top half of the projector pole piece was used; the projector control knob was set on tap 10, which produced an instrument magnification of about 3000× (calibrated). This magnification was adequate for the resolution of all the specimen detail, and it covered a relatively large field, so that the micrographs include a rather large number of dye crystals.

All the electron micrographs show a few opaque spheres, which were observed only in studies of both dyed and undyed nylon. They are not caused by the chemical action of the hydrochloric acid during the preparation of the specimen. It is plausible, therefore, to assume that these opaque spheres may represent some material that oozes out of the nylon during the evaporation of the silica in high vacuum. They do not detract from the significance of the micrographs and must be completely disregarded.

Portions of the surface of the nylon fibers are striated and also show a pebbled structure. These striations may have been caused by mechanical imperfections in the nozzles from which the nylon was extruded during its manufacture. They are always parallel to the long direction of the fiber, but they are not continuous throughout the fiber length. These effects are illustrated in Figure 6 and some of the other electron micrographs.

Figure 6 illustrates a silica replica of the steamed pink dyeing. The specimen was made by the earlier technique in which ethylene dichloride was used to dissolve the resin supporting the nylon cloth during the evaporation of the silica. The three dyestuffs dissolve in this organic solvent, and only the silica remains. This micrograph is illustrated because, even though other dyestuffs might be soluble in hydrochloric acid, the resultant silica replica is useful. Consequently, this specimen technique might be applied with some success to synthetic fibers that are soluble in organic solvents, and possibly dyed with compounds that are soluble in acid or organic solvents. (Acetone is a good solvent for the synthetic cellulose ester fibers.) A wide application of the technique is indicated, although modifications might be necessary, depending on the nature of the sample.

The remaining electron micrographs (Figures 7 to 20) illustrate the combination silica–dye crystal specimens, resulting from the transplanting of the dyestuff. Based on a comparison of a large number of micrographs of both types of specimens, it is certain that the combination type is more free from artifacts (more complete) than the pure silica type, and the micrographs are more pleasing.

The "shadow casting" effect enhances the contrast and has a tendency to add a three-dimensional effect, especially to those crystals that are not lying flat. The spheres are on the same side of the silica as the dye crystals but they are not shadow cast. It would also seem that some of the condensing silica molecules stick and do not migrate after they strike the target. If this were not the case, the shadow effect would not be so pronounced.

It would be impractical to present all the micrographs necessary to illustrate all the different fields observed on the specimens. A sufficient number of typical micrographs are shown to enable the reader to evaluate the results and to correlate them with the initial problem.

Figures 7 and 8 illustrate the original Indanthrene Golden Yellow IGK dyeing. The crystals are essentially very thin blades, rather well dispersed. Some of the crystals show a porous structure. The resolution of the smallest crystals is not very good because their contrast is poor. Most organic compounds of this

Figure 17. Electron Micrograph of Silica Replica–Dye Crystal Specimen from Original Pink Dyeing, 7500×

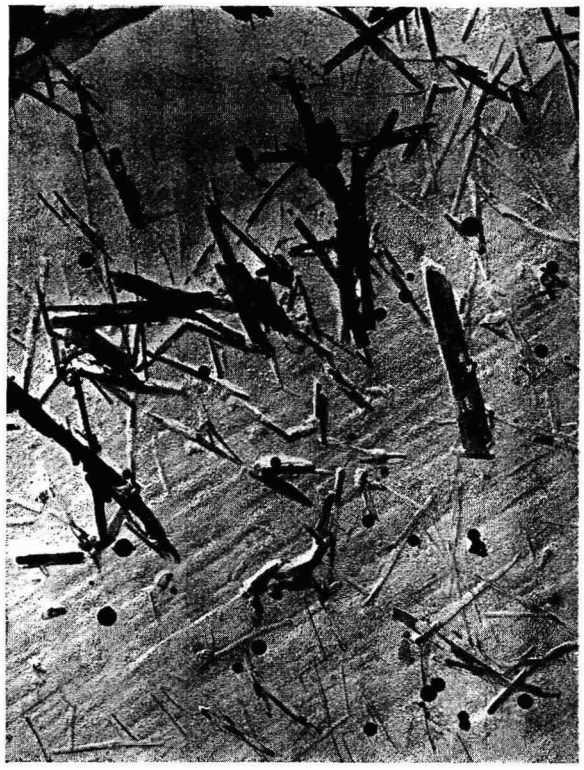

Figure 18. Electron Micrograph of Silica Replica–Dye Crystal Specimen from Steamed Pink Dyeing, 7500×

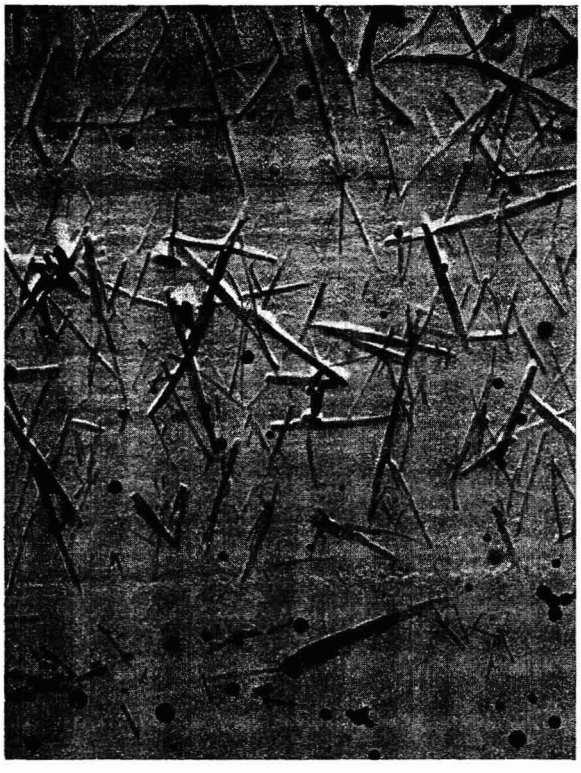

Figure 19. Electron Micrograph of Silica Replica–Dye Crystal Specimen from Steamed Pink Dyeing, 7500×

Figure 20. Electron Micrograph of Silica Replica–Dye Crystal Specimen from Steamed Pink Dyeing, 7500×

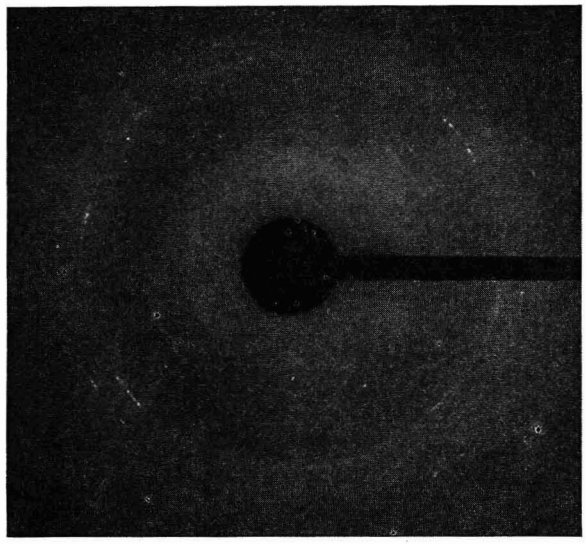

Figure 21. Electron Diffraction Pattern of Yellow Dye

Silica replica-dye crystal combination illustrated in Figures 9 and 10

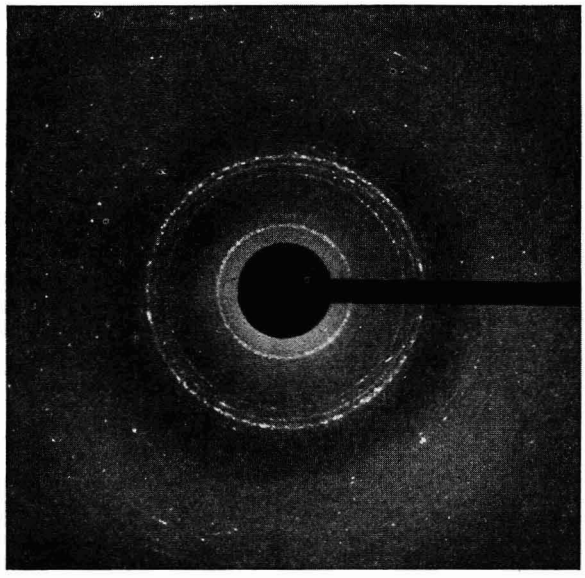

Figure 22. Electron Diffraction Pattern of Brown Dye

Specimen illustrated in Figures 14 and 15

type are relatively poor electron scatterers, especially when they contain no metals.

The steamed yellow dyeing is shown in Figures 9 and 10. The profound increase in crystal size is immediately obvious. The crystal shape is similar to that of the original crystals. Figure 10 is interesting because it shows the striations of two nylon fibers at approximately right angles. Reference to Figure 3 indicates that this particular field represents the junction of a "warp" and "filler" fiber. Naturally, the specimen presents a rather severe depth of field, so that not all portions of the electron microscope image could possibly be in sharp focus. In this case (Figure 10) the electron microscope was focused on the large dye crystal in the center of the field. The silica replica of the fiber striations is, therefore, out of focus. In any case these micrographs are illustrated not for their esthetic appeal but for their scientific value.

At 3000× (instrument magnification) the 2-inch square frame on the photographic plate is equal to 17 microns. The images of a few of the crystals on the steamed yellow dyeing were as long as the exposure frame. This checked with the earlier light microscopical investigation in which it had been learned that a few of

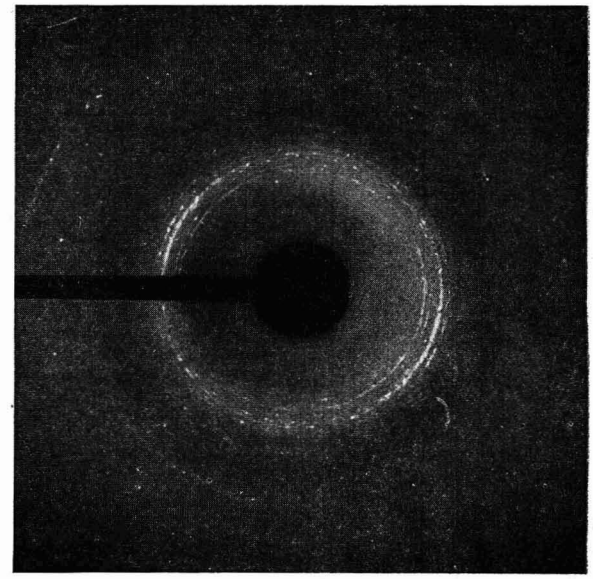

Figure 23. Electron Diffraction Pattern of Pink Dye

Specimen illustrated in Figures 18, 19, and 20

these crystals were about 17 microns long. The nylon fibers are approximately 20 microns in diameter. At an instrument magnification of 3000×, the 2-inch square photographic negative covers about 0.85 of the width of a single nylon fiber.

The original brown dyeing is illustrated in Figures 11 and 12, which are typical fields. However, the field exhibited in Figure 13 was also observed occasionally and has been interpreted as representing the poorly resolved specks, visible in the light microscope. The crystals are overlapping and relatively large. It is somewhat surprising that this mass of crystals was so completely transplanted from the nylon. Figure 12 is a good example of the tremendous crystal size range of all three original dyeings, and especially of the brown dyeing. The smallest crystals, having no specific shape, are on the order of 50 Å.; some of the lath-shaped crystals are approximately 1.5 microns long.

Reference to Figures 14 and 15 shows that many of the crystals on the steam brown dyeing are not much larger than those on the original dyeing. However, they appear to be better formed (elongated plates) than the original small crystals, and presumably they recrystallized just as the larger crystals did. Although the larger dye crystals are not terminated with ends of any apparent crystallographic significance, these large elongated thin plates certainly appear to have been recrystallized. Again the average crystal size on the steamed brown dyeing is much greater than on the original dyeing.

Figures 16 and 17 illustrate the original pink dyeing. This sample is probably very characteristic of the average synthetic fiber dyeing. Care must be taken to avoid interpreting the clusters of the small black spheres (impurity) as being a group of dye crystals. In general, the crystals are very small, somewhat aggregated, and of no significant geometrical shape. The crystals (aggregates) are rather well distributed over the fiber surface and cover a good portion of the fiber surface.

The most profound change in the shape and size of the dye crystals due to the postdyeing steaming treatment is illustrated in Figures 18, 19, and 20. The tremendous size increase is obvious and is again significant in the interpretation of these observations.

The crystals of the pink dye, after steaming, consist mostly of long laths. The ends may be square or terminated by one or more faces possibly forming a definite crystallographic angle, unlike the crystals of the other steamed samples. There is little tendency to aggregate. On the other hand, these crystals are rather uniformly distributed over the nylon surface, usually lying on the broadest crystal face (preferred orientation). There is, however, no relationship between the long direction of the crystals and the long direction of the fibers; many of the crystals are oriented transverse to the long axis of the fiber. Figure 18 shows an incomplete transfer of the dyestuff from the nylon to the silica. How-

ever, good resolution and the details of the nylon fiber surface are illustrated. Figures 18, 19, and 20 are very characteristic of this dyeing and are similar to a large number of electron micrographs made from a considerable number of specimens prepared from the steamed pink dyeing.

ELECTRON DIFFRACTION

Fortunately, the specimens used to prepare the electron micrographs (Figures 7 to 20) lent themselves to electron diffraction. The silica replica–dye crystal combination prepared from the steamed dyeings gave sharper diffraction patterns than those prepared from the original dyeings.

Although this electron diffraction investigation is part of a separate unfinished program, a brief evaluation is in order at this time. The significance of this technique from the standpoint of identification of small amounts of dyestuffs is apparent.

Figures 21, 22, and 23 are primary magnification type electron diffraction patterns. This high dispersion diffraction technique was first described by Simard *et al.* (*4*). Although their paper describes the use of a modified RCA Model EMB instrument, a complete description of the modified EMU instrument would be redundant. In brief, the specimen was placed in the conventional position (specimen chamber), a 1-mil bronze condenser aperture was used, the objective and projector lens pole pieces were removed, and the projector control was set on tap 5 to enlarge the diffraction rings to a convenient size. The electron microscope modified in this way to serve as an electron diffraction camera was calibrated using A.S.T.M. data for zinc oxide, magnesium oxide, or gold.

The interplanar spacings and intensity ratios measured on the patterns of the yellow, brown, and pink dyeings were checked against similar data measured on patterns of the three original authentic dyestuffs. The measurements were in good agreement. The technique appears interesting for fingerprinting minute amounts of dyestuff on a fiber of this type. A few milligrams of each of the three nylon cloths dyed with a few microns of dyestuff adequately served as a sample for the identification of the three dyes under discussion. Some difficulty might arise in those cases where several different dyes were used to prepare a certain shade; this is common practice. The resultant electron diffraction pattern would naturally be more complex.

The electron diffraction lines were in all cases discontinuous. Some preferred orientation is indicated (see Figure 23). The rather large crystal size may have accounted for the broken lines. The silica film presumably introduces a moderate diffuseness to the patterns, especially noticeable on the yellow sample (Figure 21) where the silica replica was known to be thicker than in the other cases.

At the time of this writing no further work had been carried out on this diffraction technique. Nevertheless its simplicity and potentialities justified its brief discussion.

CONCLUSIONS

The electron microscopical investigation showed that the post-dyeing steaming treatment had the following two effects on the three nylon dyeings:

Some of the dyestuff migrated from within the fiber to the fiber surface. (This confirmed a light microscopical examination of cross sections of these fibers, before and after steaming.)

The steaming caused a profound increase in the average size of the crystals on the nylon surface, as the result of a recrystallization of the original dye crystals. This explains the appearance of the many specks (poor resolution) on the steamed dyeings when examined in a light microscope.

These two observations can be used to account for the three modifications in the qualities of dyeings prescribed above. The correlation of the observations with the initial problem is summarized as follows:

The hiding power of a given mass of dyestuff decreases with increasing crystal (particle) size. Consequently, the steamed dyeings exhibit a weaker tinctorial strength than the original dyeings because of the larger average size of the dye crystals on the fiber surface.

The larger dye crystals on the steamed samples would be expected to be more readily rubbed off during a mechanical treatment (crocking), than the smaller ones on the original dyeings.

For a given mass of material, a large number of small particles exposes more surface area than a relatively small number of large particles. The photochemical effect of the ultraviolet light is thought to be largely a surface effect. For this reason, the steamed dyeings exhibited a greater fastness (less decomposition) to ultraviolet.

Inasmuch as the results of this investigation were significant in the solution of the initial problem, similar studies may prove helpful in correlating the microstructural detail of the dyed fiber surface with qualities of the dyeing that can be observed with the naked eye.

The preparation of the combination silica–dye crystal replica is relatively simple and should be applicable to other synthetic fibers. Similar studies of dyed wool and cotton may prove more difficult because of the greater insolubility of these fibers.

Since the termination of this investigation the authors have made a brief study of a dyed cellulose acetate rayon. This work is unfinished and was carried out solely for academic reasons. It is, however, safe to say that this technique is promising for similar studies on dyed cellulose acetate rayon. This fiber is readily dissolved from the silica by means of acetone. Because the dyestuff happened to be soluble in organic solvents and the usual inorganic acids, the final specimen consisted only of a silica replica of the dye crystals and the fiber surface. The nature of the final specimen and the modifications of the technique will be predetermined by the solubility of the dyestuff and the fiber.

It is hoped that the electron diffraction program can be continued. The ability to identify a dyestuff on a fiber, utilizing a few microns of dyestuff on a few milligrams of cloth, precludes the usual rather tedious procedure of extracting relatively large amounts of the dyestuff which might then be identified by other chemical or physical analyses. Having authentic samples of the yellow, brown, and pink dyes at their disposal the authors were able to identify the three dyes by means of their electron diffraction patterns, exhibited by the silica–dye crystal specimens. The dye technologist often suspects that the dyestuff on the fiber is different, chemically or physically (polymorphism), from the original bottled material. The electron diffraction technique described in this paper could conceivably aid in the investigation of such problems.

ACKNOWLEDGMENT

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CORRECTION. In the report of the Symposium on Electron and Light Microscopy [ANAL. CHEM., **20**, 686 (1948)] the abstract of the paper "Crystal Optics on Microscopic Views" by O'Brien and Donnay states that details of this paper can be obtained [ANAL. CHEM., **17**, 593 (1945)]. This is incorrect, in that the paper presented has never been published. The reference cited does, however, deal with related studies by the authors on this topic. The paper presented in the symposium emphasized determination of optical constants from extinction directions and refractive indices measured on microscopic views of the crystal.

Semimicrodetermination of Fluorine in Volatile Organic Compounds

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A simple, rapid semimicromethod for determination of fluorine in volatile organic compounds has been developed. There is no interference from hydrogen in the sample, which is passed at a constant rate with moist oxygen through a platinum tube at 1100° C. The resultant hydrogen fluoride is absorbed in water in an absorber constructed of saran tubing, the chlorine and carbon dioxide being largely carried off. To the hydrofluoric acid solution phenolphthalein is added, and the solution is neutralized and added to a solution of titanium sulfate, sulfuric acid, and hydrogen peroxide. The bleaching effect of the fluoride on the brown color of the acidic peroxidized titanium solution is measured in a colorimeter of the split-path type. The optimum ratio of the number of equivalents of oxygen to the equivalents of oxidizable carbon and hydrogen in the gas passed through the platinum tube was at least 40 to 1, whereas a slight excess of hydrogen (as water) over fluorine sufficed. The effect of temperature on the solution at the time of the colorimetric measurement was appreciable.

THE identification of fluorocarbons may be accomplished by either physical or chemical means. Many physical methods depend upon the separation of the pure compound, or an azeotrope of it, with a Podbielniak column (*5, 6, 21*), followed by a determination of the melting point, boiling point, refractive index, or liquid density. Gas density and melting point lowering may also be used to characterize fluorocarbons. Physical methods applicable to impure samples are measurements of infrared absorption and specific inductive capacity.

The use of chemical means of analyzing fluorocarbons involves the choice of methods, first, for decomposition of the sample, and secondly, for determination of fluoride in the decomposed sample. For determining fluoride in the decomposed sample, colorimetric procedures recommend themselves from the standpoint of simplicity and speed.

Such colorimetric methods for fluoride determination may be classified into three groups: those utilizing the tendency of zirconium to form complex fluorides in solutions; the Steiger-Merwin procedure, in which the brown color of the ion present in acidic peroxidized titanium solutions is bleached by fluoride; and the determination based on the bleaching effect of fluoride on ferric thiocyanate.

A review of the literature indicated that there were no rapid accurate semimicromethods for the determination of organic fluoride with which hydrogen does not interfere and which do not involve the use of expensive and specialized equipment or an excessive amount of manipulation. It was felt that the development of such a method would be very desirable. Although the item of expense has not been eliminated in the procedure described, the amount of manipulation has been reduced and a technique developed that permits analysis of volatile organic fluorine compounds in a flowing system, a distinct advantage in industrial operations.

Two methods of decomposition were tried: decomposition of the sample in the presence of oxygen and water vapor in a hot platinum tube and decomposition by passage through a hot silica tube. The silica tube decomposition method was tried first with fluorochloro compounds, using nitrogen as the carrier gas, but was abandoned because of the interference of silicate and fluosilicate in the direct analysis of the absorbed decomposition products. (The use of oxygen as the carrier gas in place of nitrogen offers some promise for the silica tube method and further study of decomposition under these circumstances is contemplated.)

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THERMAL DECOMPOSITION IN PLATINUM TUBE

A nickel tube packed with scrap platinum was eaten through by the reaction products of trichlorotrifluoroethane, water, and oxygen at 1100° C., and therefore a platinum tube was employed, as shown in Figure 1. Various types of absorbers were tried before the saran tubing absorber shown was decided upon as the most efficient for absorption of hydrogen fluoride. Nonaq stopcock grease was used throughout to avoid loss of sample by absorption in the lubricant. The flow of wet oxygen through the tube was 3 ml. per second. Six inches (15 cm.) of the foot-long 0.25-inch platinum tube were heated to 1100° C., the highest temperature obtainable with a simple Nichrome-wound resistance furnace (*32*). Under these conditions it was found that, whereas the number of equivalents of hydrogen (as water) needed to be only slightly greater than the number of equivalents of fluorine, the number of equivalents of oxygen must be about forty times the number of equivalents of oxidizable carbon and hydrogen to obtain satisfactory results. From this it can be shown that only if the sample being analyzed contained carbon tetrafluoride, hexafluoroethane, octafluoropropane, or CF_3X would it be advantageous to use ammonium hydroxide instead of water for saturation of the oxygen, as suggested by Bockemüller (*4*). Passage of the products of decomposition through water absorbs any hydrogen fluoride, while carbon dioxide and any chlorine present largely pass through the acidic solution. The inch-long flames about an inch above the absorber serve to eliminate frothing in the absorption solution. To the solution thus obtained, phenolphthalein was added; the solution was neutralized and added to a solution of titanium sulfate, hydrogen peroxide, and sulfuric acid. The bleaching effect of the fluoride was determined with a Klett-Summerson colorimeter—a single-path instrument being inadequate for the purpose—using a Corning No. 554 filter and a calibration curve prepared with known amounts of sodium fluoride.

PREPARATION OF SOLUTIONS

Titanium Sulfate–Sulfuric Acid. About 2.5 grams of titanium powder (obtained from Metal Hydrides, Inc., Beverly, Mass.) were dissolved with gentle heating in 300 ml. of a mixture of equal volumes of concentrated sulfuric acid and distilled water. The blue solution was added slowly to 2100 ml. of the sulfuric acid solution and shaken; oxygen was admitted occasionally, until the blue titanous sulfate was oxidized. The entire solution was drawn through a fritted-glass filter.

Sodium Fluoride. One milliliter of 47% reagent hydrofluoric acid was added to 2.1 grams of sodium fluoride and 100 ml. of distilled water in a platinum dish. The solution was evaporated slowly until the volume was about 25 ml., then cooled. The recrystallized sodium fluoride was removed and ignited in a covered platinum crucible for an hour. (Sodium fluoride was fused in the platinum crucible prior to its use for this ignition until a white melt was obtained.) About 0.86 gram of the product, carefully weighed, was dissolved in 1 liter of distilled water. An aliquot was titrated with acid, using phenolphthalein as indicator, and the equivalent weight of alkali was deducted from the sodium fluoride

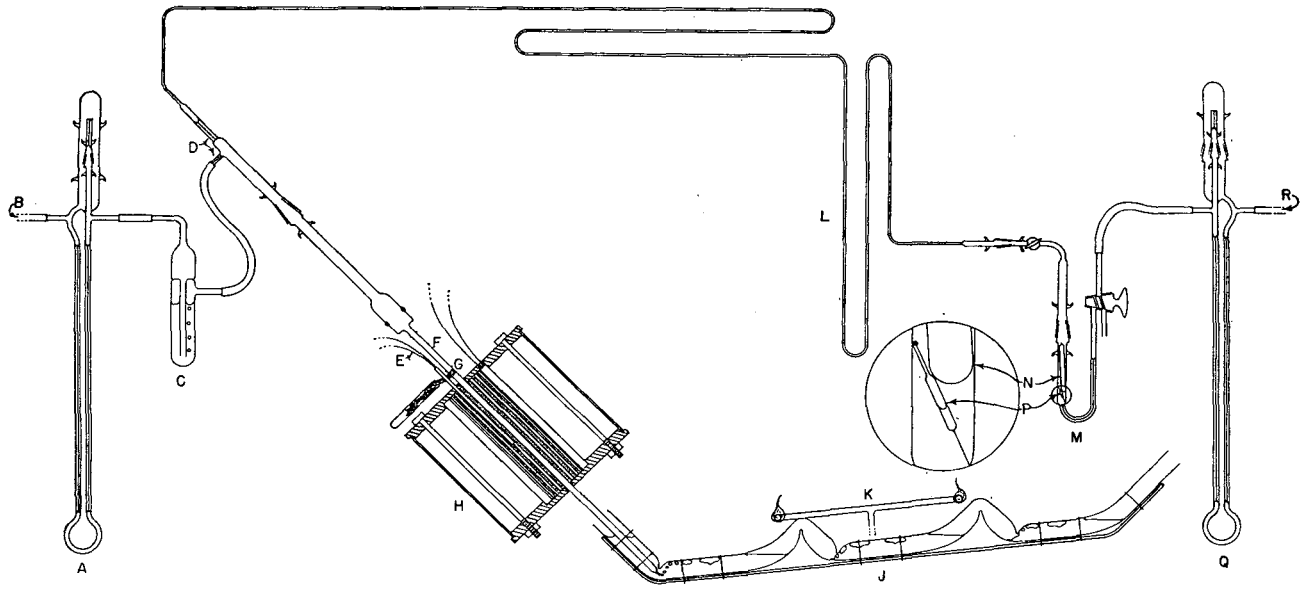


Figure 1. Apparatus for Determination of Fluorine in Volatile Organic Substances

A. Oxygen capillary flowmeter
B. Oxygen at constant pressure
C. Water bubbler
D. Capillaries
E. Thermocouple leads
F. Nickel tube silver-soldered to housekeeper seal and platinum tube
G. Wet cloth wick
H. Electric furnace at 1100° C.
J. Saran tubing absorber wired to bent iron strip
K. Small cool gas flames
L. Flexible small-diameter glass tubing
M. Semimicro sample holder
N. Glass-encased lead hammer
P. Semimicro sample
Q. Nitrogen capillary flowmeter
R. Nitrogen at constant pressure

sample weight. (Although it is not known whether the sodium fluoride hydrolyzed slightly to yield sodium hydroxide on ignition, or whether the end product was sodium carbonate, deduction of a weight corresponding to half sodium hydroxide and half sodium carbonate, left a maximum uncertainty with regard to the amount of sodium fluoride of 0.11% of the concentration itself.)

CALIBRATION OF COLORIMETER

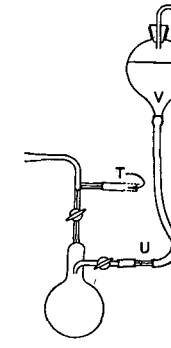
Using 10 ml. of the titanium sulfate–sulfuric acid solution and 5 ml. of 30% hydrogen peroxide, solutions containing known amounts of sodium fluoride were prepared and diluted to 100 ml. A 0.1° thermometer and the colorimeter tube were rinsed several times with the solution to be measured. Another colorimeter tube was filled with distilled water and used to balance the instrument to avoid errors due to insufficient rinsing of the colorimeter tubes. The fluoride solution with the thermometer in it was placed in the colorimeter, the temperature observed, the thermometer raised, and the galvanometer balanced immediately. The heating effect of the colorimeter itself raised the standard solution's temperature slowly. Measurements were made for each of the standard fluoride solutions and the results plotted as shown in Figure 2, by using 40 × 50 cm. coordinate paper graduated in millimeters. The temperature of the solution in the colorimeter is critical, as is shown by the displacement of the curves in the figure.

PREPARATION OF NITROGEN FLOW PLOT

In order to achieve the optimum rate of introduction of the sample being analyzed into the oxygen stream, a plot of NQ against the boiling point of the compound was prepared, where Q is the maximum permissible flow of nitrogen through the sample and N is the maximum possible number of equivalents of oxidizable carbon and hydrogen per mole of the lowest boiling constituent of the sample. This plot was developed, assuming that the compounds obey Trouton's rule, $H_m/T = 22$, where T is the boiling point in degrees Kelvin, and using the modified form of the Clapeyron equation,

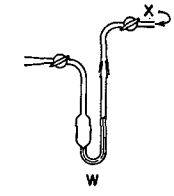
$$2.3 \log (p/p') = \frac{H_m}{R} \frac{(T - T')}{TT'}$$

where p is the vapor pressure at the absolute temperature, T. T' was taken as 300° K., as high a room temperature as



S. Macro gas sample weighing bulb
T. Nitrogen flow
U. Fine capillary
V. Mercury
W. Macro liquid sample weighing tube
X. Regulated nitrogen flow

might ordinarily be expected; p is 1 atmosphere. p' was calculated for each boiling point.



If n moles of nitrogen having a volume v and a pressure p are saturated by a volatile liquid of vapor pressure p' and the total pressure is kept equal to p, giving the saturated nitrogen a volume v', so that the partial pressure of nitrogen is p'', then, allowing n' to be the number of moles of volatile liquid vaporized, it may be shown that:

$$n' = np'/(p - p')$$

Hence the flow of carbon plus hydrogen into the oxygen stream is $NQp'/(1 - p')$ equivalents per second. Equating this to 1/40 of the number of equivalents of oxygen per second in the flow of 3 ml. per second at 300° K., NQ was calculated and the results were plotted (Figure 3).

Experiments have shown that the use of water for saturation of the oxygen will supply adequate hydrogen for all but samples containing carbon tetrafluoride, hexafluoroethane, octafluoropropane, and CF_3X, where X contains nothing that will consume oxygen. If any of these substances are contained in the sample, the rate of introduction of sample must be reduced to obtain enough hydrogen for conversion of fluorine to hydrogen fluoride.

ANALYTICAL PROCEDURE

A sample containing at most about 15 mg. of fluorine was weighed into the conventional type of microsample tube, made from wafer-thin Pyrex tubing drawn to 2-mm. diameter from 25-mm. tubing. With a small amount of experience adjustment of

sample weight was unnecessary. The sample was placed in the sample tube holder which was detached from the system (stopcocks being closed), and the sample tube was broken by allowing the glass-encased lead weight to shatter it. The nitrogen flow was adjusted by consulting the plot of boiling point against nitrogen flow and the sample tube holder was again attached to the system. Oxygen flow was checked to ensure a flow of 3 ml. per second, the furnace temperature was checked at 1100° C., and the sample tube holder's stopcocks were opened, allowing the sample to be carried over.

In less than 15 minutes the samples were completely vaporized. After 30 minutes, the sample tube holder was blackened with a cool flame from a torch. After 50 minutes the solutions of the reaction products were removed, phenolphthalein was added, and the solutions were neutralized in a plastic beaker (unattacked by fluoride) with 0.25 *N* sodium hydroxide and 0.05 *N* hydrochloric acid. They were then added to 10 ml. of the titanium sulfate–sulfuric acid solution and 5 ml. of 30% hydrogen peroxide, diluted to 100 ml., and compared as were the sodium fluoride solutions above. The calibration plot gave the fluoride content of the solution 1 hour from the time the weighed sample was received. Analytical results are shown in Table I.

DISCUSSION

Developed to give a constant indication of the amount of fluorocarbon in a flowing system, the method outlined allows for the analysis of the sample while it is being collected, a feature that is absent in other methods. Inasmuch as the development of the method was done largely with macrosamples of trichlorotrifluoroethane, and no considerable amount of attack of the platinum tube by chlorine was noted even with the large number of macroscale chlorofluorocarbon samples employed, it is seen that the attack of platinum on the semimicro scale is negligible. The larger liquid and gas samples were weighed in the devices shown in Figure 1; the gases were displaced into the nitrogen stream with a constant flow of mercury, secured by means of a constant head above a fine capillary. Results of the macro work are shown in Table II. (The large errors in these preliminary results are now known to be due at least in part to the variation

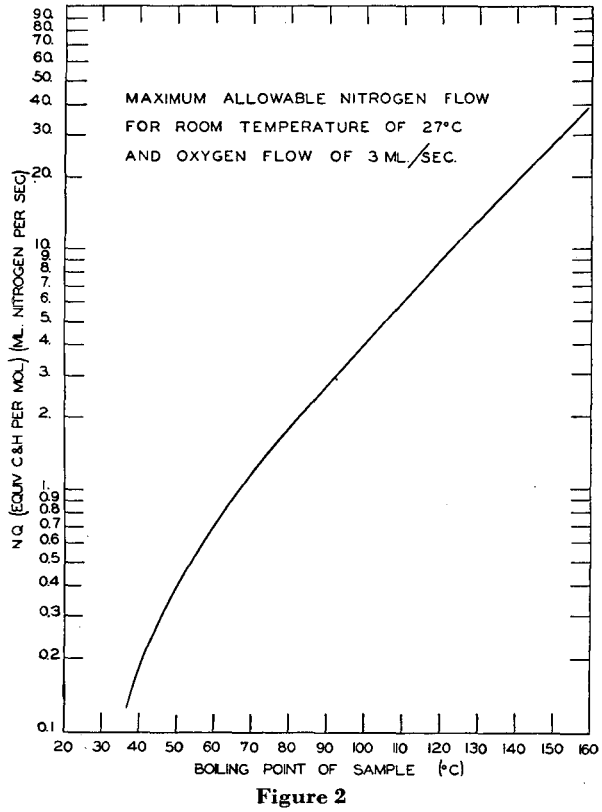


Figure 2

Table I. Semimicro Experimental Results

Compound	No. of Detns.	Per Cent Fluorine Calcd.	Per Cent Fluorine Found (av.)
$C_6H_5CF_3$	2	39.01	38.70 ± 0.44
C_6H_5F	2	19.77	19.59 ± 0.02
C_7F_{16}	2	78.34	78.33 ± 0.08
C_8F_{18}	2	75.98	76.34 ± 0.05
$C_2Cl_2F_2$	2	30.42	30.44 ± 0.07

Table II. Macro Experimental Results

Compound	No. of Detns.	Per Cent Fluorine Calcd.	Per Cent Fluorine Found (av.)
$C_6H_5CF_3$	2	39.01	38.92 ± 0.56
C_6H_5F	3	19.77	19.84 ± 0.09
C_7F_{16}	2	78.34	76.35 ± 0.75
C_8F_{18}	2	75.98	75.30 ± 0.15
$C_2Cl_3F_3$	7	30.42	30.68 ± 0.51
$CF_3COOC_2H_5$	2	40.12	39.52 ± 0.08
CCl_2F_2	3	31.43	31.23 ± 0.63
CCl_4	2	0.	0.015 ± 0.013

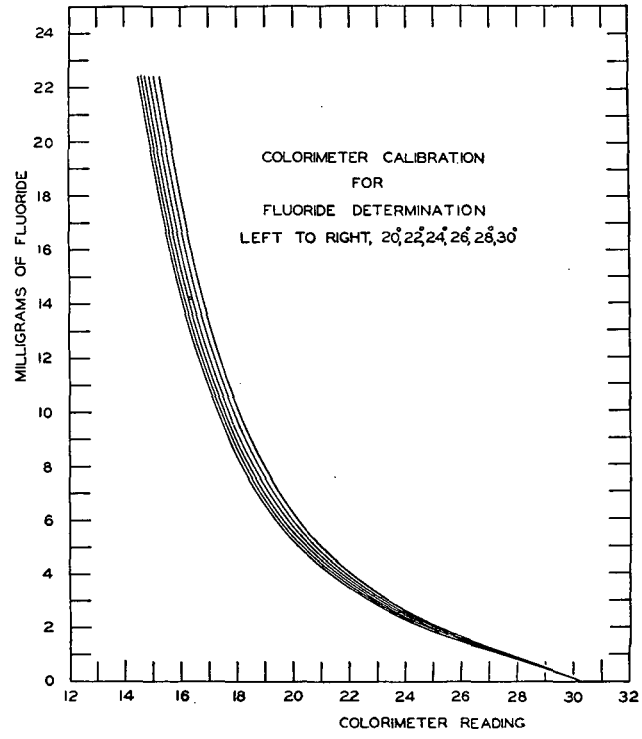


Figure 3

of the temperature of the absorption solution from the temperature of calibration of the colorimeter. Errors arising from this source may be avoided as described above for the semimicrodetermination.)

The analysis of semimicro gas samples has not yet been worked out. Investigation of other possible interfering constituents and the analysis of volatile inorganic fluorides are planned in future studies.

ACKNOWLEDGMENT

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Volumetric Microdetermination of Arsenic and Iron

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The microtitration of ferrous, arsenite, and oxalate ions using 0.001 *N* Ce(IV) solutions in 2 *F* perchloric acid is described. The same titration using 5-nitro-1,10-phenanthroline ferrous ion as redox indicator has been successful.

THE volumetric microdetermination of oxalate and the indirect determination of calcium as applied to blood plasma have been described by Salomon, Gabrio, and Smith (*3*), whose procedure involved the titration of very small amounts of oxalic acid in a 2 *F* perchloric acid solution. The titrating reagent was a 2 *F* perchloric acid solution of perchloratoceric acid, and the ferrous sulfate complex of 5-nitro-1,10-phenanthroline (nitroferroin) served as indicator. A similar volumetric microdetermination of oxalic acid has been described by Ellis (*1*) and Kirk and Tompkins (*2*), who employed the ferrous sulfate complex of 1,10-phenanthroline (ferroin) as redox indicator. As indicated by Salomon, Gabrio, and Smith (*3*), the indicator transition potential is much more favorable when nitroferroin rather than ferroin (nitroferroin 1.25 volts, ferroin 1.06 volts) is employed, and a more precise determination is practicable. Ten micrograms of oxalic acid can be determined by the newer procedure (*3*) with an accuracy of ±0.05 microgram (±0.5%).

The present paper shows that the same degree of accuracy results from an extension of the process to include the volumetric microtitration of iron and of arsenic. The determination of iron by the procedure described compares in accuracy with the best existing colorimetric methods for amounts of iron from 100 to 500 micrograms. Comparable accuracy is attained in the case of arsenic.

POTENTIOMETRIC TITRATION OF FERROUS IRON, OXALIC ACID, AND SODIUM ARSENITE WITH PERCHLORATOCERIC ACID

The titration of 0.001 *N* solutions of ferrous, oxalic, and arsenite ions in 2 *F* perchloric acid solutions by oxidation using 0.001 *N* solutions of perchloratoceric acid in the same acid medium will serve to define the reaction constants involved. The data for the constants of these three potentiometric titrations are given in Table I and the titrations are reproduced graphically in Figure 1.

The data of Table I and graphical representation in Figure 1 indicate that the vertical break in potential at the equivalence point for all three titrations covers the range 0.95 to 1.5 volts. Here the slope of the graph representing the vertical break is at its maximum at approximately 1.23 volts. At this point the ratio of change in potential to oxidant addition following the addition of the smallest increment of oxidant is greatest. Potentiometric determinations of oxalate, arsenite, and ferrous ions such as those shown in Table I, in which the reacting solutions are 0.001 *N* or less, are very time-consuming; equilibrium conditions are slowly attained and such conditions are not acceptable for routine work. The procedures described below are equally precise without any of the disadvantages of the potentiometric determinations.

TITRATION OF FERROUS IRON, OXALIC ACID, AND SODIUM ARSENITE BY PERCHLORATOCERIC ACID

The selection of a redox indicator for the titrations, the electrochemical characteristics for which are shown in Table I and Figure 1, requires that such an indicator have a transition potential of 1.23 volts. The ferrous nitrophenanthrolinium ion was selected because it has an oxidation potential of 1.23 volts in solutions which are 2 *F* in acid. Its color change at the transition point is from red to a faint blue.

Reagents. ARSENIOUS ACID. Primary standard arsenious oxide (National Bureau of Standards sample 83a) was weighed accurately, transferred to a 250-ml. beaker, and dissolved in approximately 10 ml. of conductivity water after the addition of a few pellets of sodium hydroxide. This solution was transferred to an accurately weighed 1-liter flask with ground-glass stopper. Enough 72% perchloric acid and conductivity water to give a free acid concentration of 2 *F* after dilution to approximately 1000 ml. were added, the flask and contents were again weighed, and the calculation was made of the weight of arsenic trioxide per gram of solution. Vacuum corrections were applied to all weighings. The solution prepared following these directions contained 0.04350 mg. of arsenic trioxide per gram of solution and samples from it were weighed into titration beakers from a weight buret.

Table I. Titration Constants for Potentiometric Titration of Ferrous, Oxalate, and Arsenite Ions

(0.001 *N* solution of Ce (IV) in 2 *F* perchloric acid as oxidant. Potential break at equivalence point = 600 millivolts]

Electrode Reaction	Molal Oxidation Potential *Eo, Volts*	Formal Oxidation Potential *Eo', Volts*
$Fe^{+++} + e^- = Fe^{++}$	+0.771	+0.75
$2CO_2 + 2H^+ + 2e^- = H_2C_2O_4 + 2H_2O$	−0.49	+0.88
$H_3AsO_4 + 2H^+ + 2e^- = HAsO_2 + H_2O$	+0.559	+0.84
$Ce^{++++} + e^- = Ce^{+++}$	.....	+1.71

FERRIC PERCHLORATE SOLUTION. Iron wire (99.858% iron) was accurately weighed and dissolved in hot perchloric acid in a 250-ml. beaker. The resulting solution was transferred to a weighed 1-liter glass-stoppered flask. Enough perchloric acid diluted with conductivity water to give approximately 1000 ml. of final solution 2 *F* in perchloric acid was added and the weight of flask and contents was again determined. The solution prepared for use in the present work contained 0.05010 mg. of iron per gram of solution. As in the previous case, samples of this solution were transferred into titration beakers from a weight buret.

SODIUM OXALATE SOLUTION. Bureau of Standards sodium oxalate (primary standard 40e) was employed. Concentrated 72% perchloric acid was diluted with conductivity water to a concentration of 2 *F* and a volume of approximately 1000 ml. This solution was transferred to a weighed glass-stoppered flask into which had been transferred an accurately weighed sample of the pure sodium oxalate. The weight of this solution was then determined. This acidified solution of oxalic acid is stable over extended periods of time (*3*) and as prepared for the present work contained 0.062187 mg. of sodium oxalate per gram of solution. Sampling by use of weight burets was again applied.

PERCHLORIC ACID, 72%. The vacuum-distilled commercial reagent manufactured by the G. Frederick Smith Chemical Company was employed.

5-NITRO-(1,10)-PHENANTHROLINE FERROUS SULFATE (NITROFERROIN). A commercial source of supply of this reagent (0.025 *M* solution) was diluted with water to form a 0.0005 *M* solution.

1,10-PHENANTHROLINE MONOHYDRATE. C.P. white crystalline material purchased in routine trade channels.

OSMIC ACID CATALYST. Enough osmic acid was dissolved in 0.1 *M* sulfuric acid to make an approximately 0.01 *M* solution. One drop of this catalyst serves for each titration of arsenite by the perchloratocerate ion.

PERCHLORATOCERIC ACID. This reagent was supplied as a stock item from the G. Frederick Smith Chemical Company in the form of an approximately 1 *N* solution of $H_2Ce(ClO_4)_6$ in 6 *M* perchloric acid. Sufficient of this stock solution was diluted by addition of conductivity water and perchloric acid to prepare an approximately 0.001 *N* solution which was 2 *F* in perchloric acid. Such solutions when kept in the dark and preferably at 40° C. are sufficiently stable to use after 72 hours' storage without detectable alteration in oxidation value (*3*).

EXPERIMENTAL PROCEDURES

Standardization of Approximately 0.001 *N* Perchloratoceric Acid. The procedure duplicated that described by Salomon, Gabrio, and Smith (*3*) in the microdetermination of calcium, except for the use of weight burets (*4*) for the sampling (volume burets for titrations).

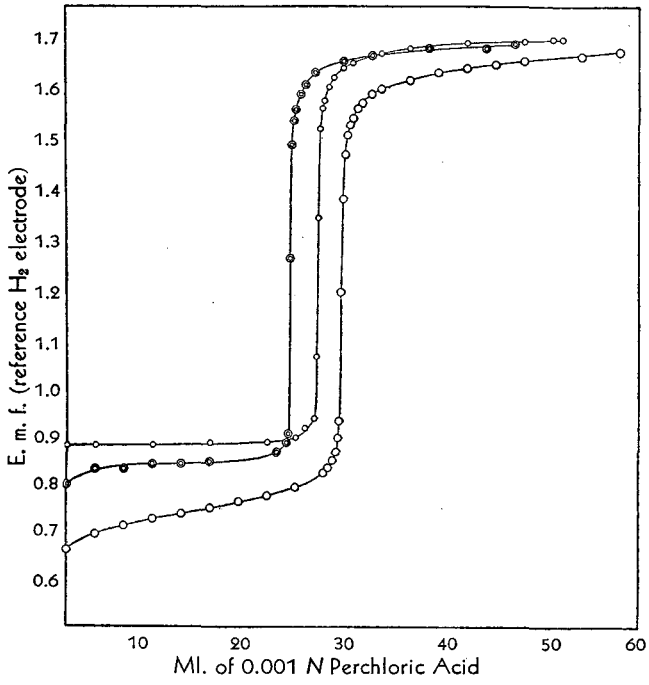

Figure 1. Potentiometric Titration of Ferrous Iron (Lower), Arsenious Acid (Middle), and Oxalic Acid (Top) in 2 *F* Perchloric Acid Solution

Perchloratoceric acid in 2 *F* perchloric acid as oxidant

Table II. Standardization of Approximately 0.001 *N* Perchloratoceric Acid

Solution No. 1			Solution No. 2		
$Na_2C_2O_4$ taken	$H_2Ce(ClO_4)_6$ required	Normality found	$Na_2C_2O_4$ taken	$H_2Ce(ClO_4)_6$ required	Normality found
G.	*Ml.*	*N*	*G.*	*Ml.*	*N*
5.535	6.41	0.0008014	4.425	5.16	0.0007959
5.545	6.43	0.0008004	4.425	5.18	0.0007929
5.546	6.42	0.0008017	4.425	5.17	0.0007944
		Av. 0.0008011			0.0007943

Table III. Determination of Arsenic by Titration with Perchloratoceric Acid (Solution 2) in 2 *F* Perchloric Acid

(Nitroferroin as indicator)

Arsenite Solution	As_2O_3 Taken	Cerate Required	As_2O_3 Found	Error
G.	*Mg.*	*Ml.*	*Mg.*	*Mg.*
5.550	0.2414	6.125	0.2406	−0.0008
5.550	0.2414	6.12	0.2404	−0.0010
5.540	0.2410	6.10	0.2397	−0.0013
5.540	0.2410	6.12	0.2404	−0.0006
6.184	0.2690	6.74	0.2648	−0.0042
4.103	0.1785	4.51	0.1772	−0.0013
4.238	0.1844	4.675	0.1837	−0.0007
3.720	0.1618	4.12	0.1619	+0.0001
3.518	0.1531	3.915	0.1538	+0.0007
			Av.	0.0012
			% error	0.57

Portions of the standard oxalate solution were weighed into 30-ml. beakers and 3 drops of 0.0005 *M* nitroferroin were added as indicator. The sample solutions were stirred with a magnetic stirrer and titrated, using additions of the 0.001 *N* solution of perchloratoceric acid to be standardized at room temperature. The equivalence point potential was sharply indicated by the elimination of the pink indicator color at the point of the addition of the first slight excess of the cerate solution. As all solutions to be titrated in connection with this work were of the same relative normality, and the same amount of indicator was employed in each titration, no indicator correction was necessary. An automatic filling microburet and reagent reservoir were used in all titrations. The buret was of 10-ml. capacity, intended to be read with accuracy of ±0.01 ml. The results are shown in Table II.

Titrimetric Determination of Arsenic Following Perchloratocerate Oxidation. Weighed samples of arsenite solution were taken as described in the standardization of the cerate solution and the same technique of titration was followed (Table III).

Titrimetric Determination of Iron Following Perchloratocerate Oxidation. Weighed samples of the ferric perchlorate were reduced using a micro-Jones reductor, the effluent from which was received in a 30-ml. beaker. This solution of known ferrous iron content was then titrated as in previous cases by use of perchloratoceric acid in 2 *F* perchloric acid, using nitroferroin as indicator and employing magnetic stirring. The reduction of ferric perchlorate to ferrous perchlorate in the Jones reductor does not cause reduction of the perchlorate ion, but a blank correction of 0.10 ml. of the cerate solution due to the "hydrogen

Table IV. Determination of Iron by Titration with Perchloratoceric Acid (Solution 1) in 2 *F* Perchloric Acid

(After reduction with zinc and using nitroferroin as indicator)

$Fe(ClO_4)_3$ Solution	Fe Taken	$H_2Ce(ClO_4)_6$ Required	Fe Found	Error Fe
Grams	*Mg.*	*Ml.*	*Mg.*	*Mg.*
5.545	0.2778	6.23	0.2775	−0.0003
5.545	0.2778	6.22	0.2771	−0.0007
3.921	0.1965	4.46	0.1987	+0.0022
5.553	0.2782	6.24	0.2780	−0.0002
5.848	0.2930	6.59	0.2935	+0.0005
5.077	0.2544	5.77	0.2570	+0.0016
4.412	0.2211	5.00	0.2227	+0.0016
6.259	0.3136	7.08	0.3153	+0.0017
			Av.	0.0011
			% error	0.42

peroxide" error of the reductor was applied as determined by the passage of comparable volumes of 2 *F* perchloric acid through the micro-Jones reductor and subsequent addition of indicator and perchloratocerate solution. The results from these titrations are given in Table IV.

Colorimetric Determination of Iron in Ferric Perchlorate Solution. For the purpose of comparing the accuracy of this type iron determination following perchloratocerate oxidation, a colorimetric determination of iron by the 1,10-phenanthroline reaction was carried out. The preparation of the solutions of iron for spectrophotometric analysis was as follows:

Samples of the standard iron solution (0.05010 mg. per gram of solution) were weighed out of the weight buret into 100-ml. beakers. A small excess of sulfuric acid was added and the solutions were evaporated to fumes of sulfuric acid to remove perchloric acid. The solutions thus obtained were diluted with conductivity water and the ferric iron was reduced by the addition of hydroxylamine hydrochloride. The solutions were then neutralized (Congo red paper) by the addition of dilute ammonia and the ferroin reaction was produced by the addition of 5 ml. of 0.33% aqueous solution of 1,10-phenanthroline. The solutions thus obtained were transferred to 100-ml. volumetric flasks and diluted to the mark with conductivity water. Spectrophotometric transmittance curves for these solutions were obtained using the G.E. recording spectrophotometer and the per cent transmittancy was read at the point of maximum absorption wave length 512 mμ. From a previously accurately determined calibration curve the weight of iron present was determined. A blank correction to account for traces of iron in the reagents employed was applied. The results are given in Table V.

Table V. Spectrophotometric Determination of Iron in Ferric Perchlorate Reference Solution

Iron Taken *Mg.*	Transmittance %	Iron Found *Mg.*	Error Fe *Mg.*
0.239	33.9	0.236	−0.003
0.339	21.3	0.340	+0.001
0.149	49.7	0.151	+0.002
0.245	32.4	0.244	−0.001
0.246	32.1	0.247	+0.001
0.458	12.7	0.456	−0.002
0.249	31.3	0.251	+0.002
		Av.	0.0017
		% error =	0.61

Examination of Tables IV and V shows that the colorimetric determination of iron and the volumetric determination by the perchloratocerate titrational procedure are of comparable accuracy and precision.

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Quantitative Determination of Hemicellulose Constituents by Fermentation

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The D-xylose and L-arabinose in hydrolyzates of hemicelluloses from agricultural residues can be quantitatively determined by fermenting solutions of the uronic acid-free sugars with *H. suaveolens* (NRRL No. 838) and *C. guilliermondii* (NRRL No. 488). *H. suaveolens* selectively utilizes D-xylose and *C. guilliermondii* utilizes both sugars completely. By determination of copper reducing values of unfermented and fermented samples percentage of each sugar may be calculated.

IN THE course of investigations of the hemicelluloses found in agricultural residues, it was desired to determine quantitatively the amounts of xylose and arabinose present in their hydrolyzates. Methods had previously been reported for the determination of D-xylose and L-arabinose by the use of bacteria which selectively utilized these sugars (*1*) and for the estimation of D-galactose and D-xylose by the use of yeasts (*6*, *7*). Because the bacterial fermentations required special media and longer times for complete utilization of the sugars, the yeast method was chosen for further investigation and adaptation to the problem.

Wise and Appling (*7*) found that *Hansenula suaveolens* (NRRL No. 838) would selectively ferment D-xylose in the presence of L-arabinose and certain other sugars. However, in a study of the fermentation of wood-sugar stillage, Kurth and Cheldelin (*2*) claim that *H. suaveolens* (NRRL No. 838) and *Candida lipolytica* (NRRL No. 1094) utilize arabinose, though more slowly than xylose. They obtained evidence for this conclusion by fermentation of wood-hydrolyzate stillage to which D-arabinose had been added. Studies in this laboratory have confirmed the specificity of both yeasts for D-xylose and have shown, contrary to the findings of Kurth and Cheldelin, that neither the D- nor L-forms of arabinose were utilized (Table I).

Because of the appreciable difference in the reducing values of D-arabinose and D-xylose, Kurth and Cheldelin's calculation of all reducing values as xylose led them to erroneous conclusions. Analysis of these sugars with use of Somogyi's reagent (*5*), which contains D-tartrate, shows that D-arabinose reduces only 70% as much copper as does D-xylose. This is in accord with the work of Richtmyer and Hudson (*4*), who showed that the optical isomers of some sugars have different reducing values when analyzed with Fehling's solutions containing optically active tartrates.

In an effort to establish a direct method for the determination of L-arabinose, several yeasts were tested on L-arabinose and D-xylose and known mixtures of the two. Although some yeasts utilized L-arabinose more rapidly than D-xylose, none was completely selective for L-arabinose. Three yeasts, *Candida krusoides* (NRRL No. 305), *Candida guilliermondii* (NRRL No. 488), and *Debaryomyces matruchoti* (NRRL No. 833), completely utilized L-arabinose, D-xylose, or mixtures of the two in 48 hours. Because *C. guilliermondii* gave complete fermentation of both pentoses in 24 hours it was selected as being the most useful for the present work.

As the specificity of *H. suaveolens* (NRRL No. 838) for xylose has been confirmed and *C. guilliermondii* (NRRL No. 488) found to utilize both D-xylose and L-arabinose completely, it is believed that both sugars can be quantitatively determined by fermenting separate portions of a mixture of these sugars with these two

yeasts. The decrease in reducing value effected by *H. suaveolens* is calculated as D-xylose, and the additional decrease brought about by *C. guilliermondii* is calculated as L-arabinose.

Hemicelluloses isolated from cereal straws and corncobs have been found to consist essentially of xylan. Careful examination of their hydrolysis products indicates the presence of L-arabinose and D-glucuronic acid in addition to the expected D-xylose. Fermentation of such hydrolyzates with *Saccharomyces carlsbergensis* (NRRL No. 379), a selective glucose fermenter, shows only slight utilization of reducing sugars corresponding to the apparent slight attack by this yeast on D-xylose, as noted by Wise and Appling (*7*). It was concluded, therefore, that the authors' hemicellulose hydrolyzates contained no glucose. Hence, removal of uronic acid constituents, as their alcohol-insoluble barium salts, should leave an essentially pure mixture of D-xylose and L-arabinose which could then be analyzed by the selective fermentation method proposed above. The uronic acid content of the original hemicellulose is determined by other methods.

The selective fermentation procedure as applied to hemicellulose hydrolyzates from corncobs and wheat straw is demonstrated by the results shown in Table II.

EXPERIMENTAL

Isolation of Hemicelluloses. CORNCOB XYLAN, SAMPLE 1. Ground corncobs (4-mesh) were extracted at room temperature with 8% sodium hydroxide solution. To the dark-colored extract was added an equal volume of 95% ethanol, and the precipitated "xylan" was separated by decantation and filtration. It was then reslurried successively with the following solutions (filtered between treatments): alcohol 50%; sodium hydroxide 2% in 50% alcohol; alcohol 50 to 66% (three times); alcohol 95%; absolute alcohol; and dry ether. Nearly all color was removed by this treatment. The air-dried product was nearly white. Analysis (moisture-free basis): ash 5.01%; lignin 0.79%; pentosans 94.3% (furfural 65.7%).

CORNCOB XYLAN, SAMPLE 2. The hemicellulose sample was extracted and precipitated by the procedure described above except that the xylan precipitate was reslurried one additional time with 2% alkali in 50% alcohol and then acidified to pH 2.5 with hydrochloric acid before the final dehydration. A white product was obtained. Analysis (moisture-free basis): ash 0.80%; lignin 0.92%; pentosans 98.1% (furfural 68.3%).

Table I. Utilization of D- and L-Arabinose and D-Xylose by *H. suaveolens* (NRRL No. 838) and *C. lipolytica* (NRRL No. 1094)

Substrate	Weight, Mg.	Yeast No.	Hours	Pentose Recovered, Mg.
D-arabinose	100	838	24	97 D-arabinose
D-arabinose	100	838	72	97 D-arabinose
L-arabinose	100	838	24	100 L-arabinose
L-arabinose	100	838	72	100 L-arabinose
D-xylose + L-arabinose	50 + 51	838	48	49 L-arabinose
D-xylose + L-arabinose	50 + 51	1094	72	50 L-arabinose
D-xylose	100	838	24	1 D-xylose
D-xylose	100	838	72	1 D-xylose

Table II. Determination of Pentose Sugars by Fermentation of Hydrolyzed Hemicelluloses

Hemicellulose	Before Fermentation	Reducing Values as Ml. of 0.005 *N* Thiosulfate: After Fermentation by NRRL 838	After Fermentation by NRRL 488	Difference Due to Xylose	Difference Due to Arabinose	Xylose, Mg.	Arabinose Mg.	Arabinose %
Corncob, sample 1	795	116	40	679	76	88	11	11.1[a]
Corncob, sample 2	867	95	39	772	56	100	8	7.4
Wheat straw	691	85	38	606	47	78	7	8.2

[a] A diphenylhydrazone value (*3*, *8*) of 12.4% was found for this sample.

WHEAT STRAW XYLAN. Wheat straw xylan was extracted by circulating 5% sodium hydroxide solution through the straw at room temperature for 25 hours. The hemicelluloses in the filtered extract were precipitated by excess alcohol. The precipitate was reslurried four times with dilute alkali (about 2%) in 50% alcohol and washed repeatedly with 60% alcohol. The color was much less readily removed than from the cob products. The precipitate was finally acidified with acetic acid and dehydrated as above. The product was light tan. Analysis (moisture-free basis): ash 11.5%; lignin 4%; pentosan 82.4% (furfural 57.4%).

Hydrolysis of Hemicelluloses. One-gram samples were dissolved in 45 ml. of 3% sulfuric acid (0.62 *N*) and refluxed for 2 hours. The cooled solutions were filtered, neutralized with barium hydroxide solution to approximately pH 7.0, and filtered to remove the barium sulfate. The filtrates were evaporated to dryness under reduced pressure, and the residues extracted repeatedly with hot absolute alcohol to dissolve the sugars. The alcoholic extracts were evaporated to dryness in vacuo, the residues dissolved in water, and aliquots of these solutions used for the fermentation tests. This procedure gives sugar solutions free of uronic acids.

Fermentation Procedure. The fermentation procedure used for both yeasts was essentially the same as that described by Wise and Appling (*7*).

The baker's yeast extract used as nutrient was prepared by mixing 150 grams of starch-free baker's yeast with water and making up to 1-liter volume. This mixture was autoclaved at 15 pounds' pressure for 30 minutes, cooled, and filtered.

The cultures of both *H. suaveolens* (NRRL No. 838) and *C. guilliermondii* (NRRL No. 488) were prepared by the procedure recommended by Wise and Appling (*6*, *7*). The yeast suspensions were made up to approximately 35,000,000 cells per ml. by photometer.

Samples of sugar solutions, 25 ml., containing 50 to 100 mg. of total reducing sugars, were placed in 300-ml. Erlenmeyer flasks and 15 ml. of the baker's yeast extract were added. This mixture was sterilized in an autoclave at 15 pounds' pressure for 15 minutes. After cooling, the sterile media were inoculated with 10 ml. of the standardized suspension of either NRRL No. 838 or NRRL No. 488. The flasks were shaken on a reciprocating platform shaker at 30° C. for 24 hours. The fermented samples were diluted to a volume of 100 ml. and centrifuged. The supernatant liquor was decanted from the yeast and aliquot samples taken for analysis. All fermentations were conducte.. in duplicate with uninoculated controls.

Reducing Sugars. All sugar analyses were made by the procedure of Somogyi (*5*), with the modification of heating the samples for 15 minutes, a time sufficiently long for the slower reacting arabinose. The following reducing values were found (mg. of sugar per ml. of 0.005 *N* sodium thiosulfate): D-xylose (Eastman Kodak Co. α_D + 190) 0.129; D-glucose (Bureau of Standards) 0.130: L-arabinose (Eastman Kodak Co.; α_D + 102.30) 0.146; and D-arabinose (Eastman Kodak Co.; α_D −103.50) 0.178.

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RECEIVED March 1, 1948.

NOTES ON ANALYTICAL PROCEDURES . . .

Control of Throughput, Temperature, and Purity of Solvent in Soxhlet Extraction

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THE value of the conventional Soxhlet extractor lies mainly in the small volume of solvent used, together with automatic functioning at the boiling point of the solvent. In addition, the extract may be easily recovered or the solvent treated continuously by a nonvolatile agent placed in the reboiler flask.

In the purification and study of aluminum soaps (*1*) it became important to combine the advantages of the conventional unit with complete protection from moisture causing hydrolysis, with better throughput control for small samples, with frequent extraction at temperatures other than the boiling point, with occasional recovery of extract, and with continuous drying of the solvent. For these purposes the units shown in Figures 1 to 3 were designed and proved very useful.

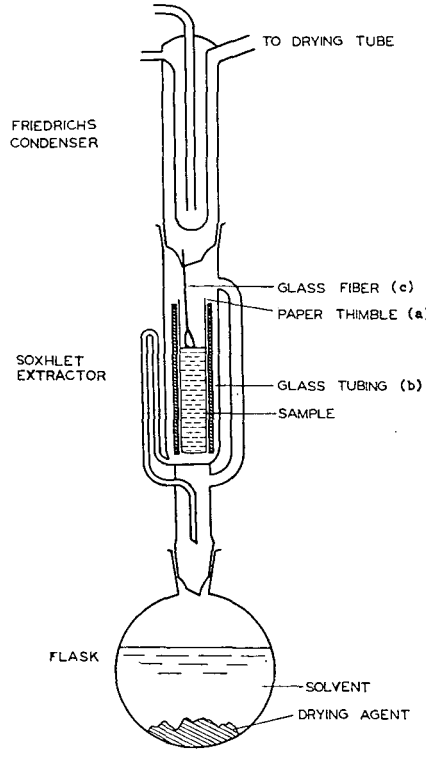

Figure 1. Conventional Soxhlet Apparatus with Special Sample Container

Control of Throughput. In the conventional Soxhlet apparatus, especially when small samples are used, the time of contact between sample and solvent may be easily determined, but the composition and volume of this solvent are variable and indefinite.

To reduce these uncertainties small samples of soap are placed in a filter paper thimble (*a*, Figure 1) enclosed in a section of glass tubing, *b*, high enough to stand above the level of liquid. The total reflux is led into the thimble by means of a glass fiber, *c*. The solvent passes through the soap column, flowing out from the bottom of the tube, and then rises up on its outside until the level is high enough to start the siphon. Thus the total reflux passes through the soap once and once only.

If a small thimble and a large Soxhlet extractor are used, most of the solvent is available for siphoning when the siphon starts instead of being in the thimble and having to filter through before siphoning. The use of the confining tube assures constant renewal of the extracting liquid in the immediate vicinity of the soap, as the holdup of the soap column is of the order of 5 ml. only.

The rate of reflux may be held constant by adjusting the heating rate of the boiler flask; the total time of extraction then determines the extracting volume.

Temperature Control. In the conventional Soxhlet extractor the actual temperature of extraction is within 2° C. of the boiling point of the pure solvent. It is even closer to it in the above modification.

To control this temperature in the range below the boiling point it was necessary to introduce a cooler (*d*, Figure 2) between the reflux condenser, *e*, and the extractor, and to surround the

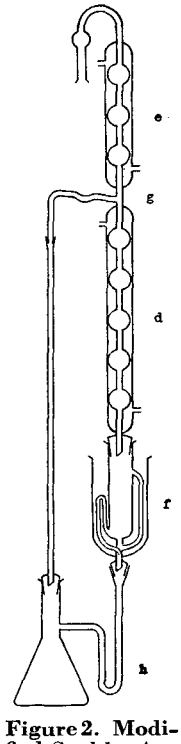

Figure 2. Modified Soxhlet Apparatus for Operation at Controlled Temperatures

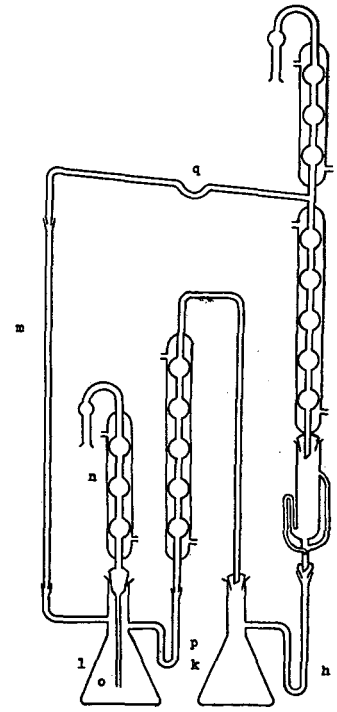

Figure 3. Modified Soxhlet Apparatus

For operation at controlled temperatures and for independent recovery and treatment of solvent

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extractor itself with a small thermostat, *f*. The vapors of the solvent were led between the condenser and cooler by tube *g*. In order to assure the right direction of flow of vapor, a gooseneck trap, *h*, was provided between the extractor and the boiling flask. To allow proper siphoning, the normal vapor duct of the extractor had to be retained to equalize the gas pressure on both ends of the siphon. In this way extraction was readily conducted at room temperature or at 0° C. with solvents such as acetone or iso-octane boiling at much higher temperatures, and the effect of varying temperatures of extraction for a given solvent was studied.

Assembly is facilitated when ball and socket joints, or two ground joints at right angles, are inserted in the vertical tube above the boiling flask.

Independent Recovery and Treatment. In both above modifications as well as in the conventional model, the extract may be recovered in the boiling flask or a treating agent for the solvent, such as a drying agent—for example, Drierite, calcium sulfate—may be placed therein. To accomplish both these objectives simultaneously and still retain the temperature control and automatic operation, the unit of Figure 3 was designed.

It is a modification of the unit of Figure 2 in which the previously dried solvent is freed of extract in a 500-ml. boiler flask, *k*, its vapor is condensed and led into a 500-ml. treating flask, *l*, where it meets the drying agent, and pure solvent is finally distilled therefrom through tube *m* into the condenser, cooler, and extractor. Any amount of solvent between 250 and 500 ml. may be used.

Automatic operation requires that distilling rates from the two flasks, *k* and *l*, be the same so that neither can lack liquid. This is accomplished by heating *l* at a definitely faster rate than *k*, which ensures an adequate supply of liquid in *k*. The level in *l* is prevented from falling low by condenser *n* which refluxes most of the vapor as soon as the level of liquid falls below the end of the tube, *o*, and is inactive when *o* dips in the liquid.

Gooseneck traps (*h*, *p*, and *q*) direct the stream of vapor in the proper direction.

This apparatus performed very satisfactorily. At the time of building these units, ball and socket joints were not available, but it is presumed that their use would greatly simplify both the assembly and the operation.

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RECEIVED July 12, 1946. Study conducted under contract OEMsr-1057 between Stanford University and the Office of Emergency Management, recommended by Division 11.3 of the National Defense Research Council, and supervised by J. W. McBain.

CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

THE description covered this month, β-pyridinesulfonic acid, was completed from data obtained during work on an industrial research project at the Armour Research Foundation. Samples of the pure compound were obtained through the courtesy of Edmond T. Tisza of the Pyridium Corporation.

10. Beta-Pyridinesulfonic Acid

Crystals of β-pyridinesulfonic acid are readily obtained from water either on a microscope slide or macroscopically. There is no evidence of polymorphism during crystallization from solution or the melt.

CRYSTAL MORPHOLOGY (determined and checked by W. C. McCrone, V. Gilpin, and P. T. Cheng).

Crystal System. Orthorhombic.

Form and Habit. Prismatic rods elongated parallel to *c*; showing the forms: prism {310}; orthopinacoid {010}; and bipyramid {111}. When recrystallized rapidly on a microscope slide the crystals often show combinations of macrodomes {101}, {201}, and {301} (see Figure 1).

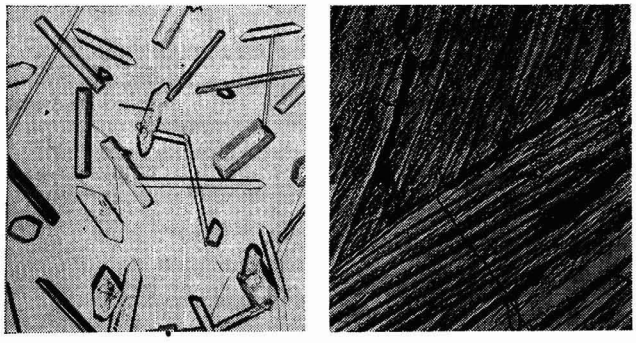

Figure 1. β-Pyridinesulfonic Acid

Left. **Crystals from water on microscope slide**
Right. **Fusion preparation showing characteristic transverse cracks**

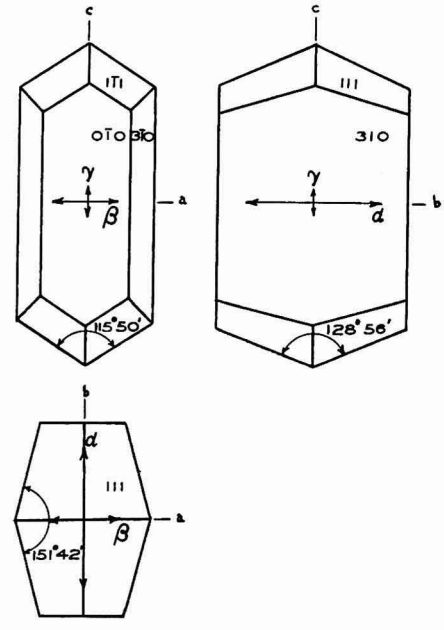

Figure 2. Orthographic Projection of Typical Crystal of β-Pyridinesulfonic Acid

Axial Ratio. $a:b:c = 0.757:1:0.478$.

Interfacial Angles (Polar). $310 \wedge \bar{3}10 = 28°\,18'$; oil $\wedge\, 0\bar{1}1 = 51°\,4'$; $101 \wedge \bar{1}01 = 64°\,10'$; $201 \wedge \bar{2}01 = 103°\,12'$; and $301 \wedge \bar{3}01 = 124°\,18'$.

X-RAY DIFFRACTION DATA (determined by J. Whitney, I. Corvin, and M. Tull).

Cell Dimensions. $a = 11.41$ Å., $b = 15.08$ Å., $c = 7.20$ Å.

Formula Weights per Cell. 8.

Formula Weight. 159.

Density. 1.718 (buoyancy method); 1.715 (x-ray).

Principal Lines

d	I/I_1	d	I/I_1
7.49	0.08	2.621	0.02
5.65	0.04	2.583	0.08
5.33	0.33	2.526	0.05
5.18	0.19	2.481	0.02
4.73	0.08	2.356	0.02
4.279	0.16	2.311	0.09
3.848	1.00	2.247	0.02
3.746	0.73	2.211	0.06
3.591	0.35	2.179	0.05
3.328	0.13	2.116	0.08
3.175	0.05	2.060	0.06
3.040	0.17	2.011	0.03
2.972	0.10	1.959	0.04
2.868	0.05	1.930	0.03
2.805	0.12	1.874	0.03
2.672	0.02	1.826	0.04
		1.786	0.07

OPTICAL PROPERTIES (determined and checked by V. Gilpin, P. T. Cheng, and W. C. McCrone).

Refractive Indices (*5893 Å.; 25° C.*). $\alpha = 1.534 \pm 0.002$. $\beta = 1.670 \pm 0.002$. $\gamma = 1.74 \pm 0.01$.

Optic Axial Angles (5893 Å.; 25° C.). $2V = 67°$. $2E = 112°$.

Dispersion. $r > v$, very slight.

Optic Axial Plane. 100.

Sign of Double Refraction. Negative.

Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.645$. R (calcd.) = 40.5. R (obsd.) = 33.6.

FUSION DATA (determined by W. C. McCrone).

β-Pyridinesulfonic acid melts at 356–357° C. with slight decomposition. There is a very slight sublimation at the melting point, and on supercooling solidification is very rapid, with a needlelike crystal front. Transverse shrinkage cracks are typical (Figure 1).

Correspondence

Noninterference of Pectinous Substances in Aconitic Acid Method and of Aconitic Acid in Uronic Acid Method

SIR: Inasmuch as galacturonic acid is decarboxylated in the potassium acetate–acetic acid reagent for aconitic acid (*6*), it was expected that the "uronic acids" of Browne and Phillips (*2*) would interfere when the method for aconitic acid was applied to sugarhouse materials. However, no interference of this nature has yet been found (*1*). It has been found that polyuronic acids and derivatives, such as pectic acid and pectin, are insoluble in the reagent and are not decarboxylated by it. It was desirable to test the method with a uronic acid derivative which is soluble in acetic acid and basically similar in structure to pectic acid.

It is generally accepted that pectic acid is constituted as a chain of galacturonic acid units, each linked by glycosidic union through its C-1 position to the C-4 position of the next unit. The last unit of each chain has no attachment on its C-1 position, but functions through its C-4 position as an aglucon to the preceding unit. Thus each of the units but the last one may be considered as functioning as the carbohydrate residue in a glycoside of a uronic acid. The simplest substance of this constitution, methylgalacturonide dihydrate, melting point 112° C., was prepared by the method of Morell and Link (*5*). It is easily soluble in the potassium acetate–acetic acid reagent, but during 3 hours in the reagent boiling under reflux it gave no carbon dioxide. When similarly tested with the aqueous hydrochloric acid reagent for uronic acids (*4, 7*) it underwent extensive decarboxylation. The evidence therefore indicates that only the free galacturonic acid is decarboxylated in the aconitic acid method, and that the potassium acetate–acetic acid reagent, being practically anhydrous, is unable to hydrolyze soluble uronides to the free uronic acid.

Experiments showed that galacturonic acid in the boiling reagent for aconitic acid yielded furfural simultaneously with the carbon dioxide, but that the methylgalacturonide, pectin, and pectic acid produced no furfural. It is well known that decarboxylation of uronic acids and polyuronides in mineral acids (*4, 7*) is always accompanied by production of furfural and reductic acid by interdependent reactions that have been discussed by Isbell (*3*). From all known evidence it may be assumed that uronic acids and their derivatives are decarboxylated by chemical agents only when it is possible for them to yield furfural or reductic acid. Neither of these compounds can be formed from any of the galacturonic acid units in pectic acid and polyuronides until the uronic acid units have been freed by hydrolysis from attachments at both positions C-1 and C-4. The aqueous mineral acids used in the uronic acid method (*4, 7*) can do this, but the practically anhydrous acetic acid reagent for aconitic acid cannot. Thus, polyuronides present in plants, whether soluble or insoluble, will not interfere in the aconitic acid method.

Aconitic acid is not decarboxylated in strongly acidic solutions and does not interfere in the uronic acid methods (*4, 7*). Therefore the carbon dioxide obtained from sugar cane products by Browne and Phillips (*2*) originated from material other than aconitic acid.

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Identification of Crystalline Progesterone with 2,4-Dinitrophenylhydrazine

SIR: In a recent issue of this journal, Klein *et al.* [ANAL. CHEM., **20**, 174 (1948)] described a gravimetric procedure for the determination of progesterone which was based on the reaction of the hormone with excess 2,4-dinitrophenylhydrazine. To the product was assigned the structure of a 3,5-pyrazolyl-20-dinitrophenylhydrazone, since prolonged heating of the compound with ethanolic hydrogen chloride removed only one hydrazine group, the presumable pyrazoline ring being unaffected. Actually, such evidence for pyrazoline formation of Δ^4-3-ketosteroids is unacceptable as Anchel and Schoenheimer [*J. Biol. Chem.*, **114**, 539 (1936)] showed that hydrazones of α,β-unsaturated steroid ketones were cleaved only with difficulty unless pyruvic acid was used. The same workers noted that pyrazolines were not attacked under those conditions.

Reaction of progesterone with excess dinitrophenylhydrazine under nitrogen in either acetic acid or ethanol–hydrochloric acid solution gave a product with melting point 276–281° C. (dec., uncorrected), described by Klein and co-workers. When treated in chloroform solution with pyruvic acid in the presence of 4 *N* hydrogen bromide in acetic acid at 50° C. for 3 hours [Mattox, V. R., and Kendall, E. C., *J. Am. Chem. Soc.*, **70**, 882 (1948)], there was obtained 60% of progesterone, thus proving that the product was the bisdinitrophenylhydrazone of progesterone. The ultraviolet absorption spectrum of the orange-red bisdinitrophenylhydrazone in chloroform solution showed a maximum at 383 mμ (log E_m = 4.72), closely resembling that of other monodinitrophenylhydrazones of Δ^4-3-ketosteroids (maximum at 387 to 390 mμ), while the yellow dinitrophenylhydrazone (melting point 218–220° C.) of a 20-ketosteroid such as 3(β)-acetoxy-20-ketoallopregnane exhibited a maximum at 370 mμ (log E_m = 4.33). The influence of the latter grouping is probably responsible

for the slight hypsochromic shift in the spectrum of the bisdinitrophenylhydrazone of progesterone.

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SIR: We are pleased to see that Dr. Djerassi has resolved the doubt concerning the pyrazoline formulation of the bisdinitrophenylhydrazine derivative of progesterone. Our doubts were predicated not only on the known resistance to hydrolysis of such uncyclized derivatives, but also upon the generally accepted rule that pyrazolines normally form only in alkaline medium (Gilman, Henry, "Organic Chemistry," p. 678, New York, John Wiley & Son, 1943). For these reasons our pyrazoline formulation was suggested originally only in a footnote. However, during editing it was incorporated in the text proper, thus giving the suggestion an emphasis not intended.

We were unaware of the work of Anchel and Schoenheimer. That of Mattox and Kendall appeared almost simultaneously with ours. However, in July 1947, we attempted to cleave the derivative by use of pyruvic acid (10 grams) in boiling 50% alcohol (180 ml.). Although we recovered only a fraction of the starting material, no other identifiable product could be isolated. Apparently our conditions were not adequate.

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DANIEL KLEIN
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BOOK REVIEWS

Soil Analysis. *C. R. Johnson.* 16 pages. Northwest Drug and Chemical Products Co., 3106 Southeast Fiftieth Ave., Portland 6, Ore., 1948. Price, 50 cents.

This booklet, which bears the subtitle "Photometric Determination of Available Plant Nutrients in Soil Extracts," is a preprint of a chapter from a book describing a method of adapting fertilizers to crops and soils, on which the author is now working. The methods are not original but present one or two novel features.

Analytical Methods for Aluminum Alloys. 103 pages. Aluminum Research Institute, Chicago, Ill., 1948. Price $1 in United States; $1.25, foreign.

The Aluminum Research Institute provides in this book a convenient handbook-type compilation of practical analytical methods for the analysis of aluminum alloys. The work represents a revision and expansion of the methods issued first in 1932 and revised in 1939. For the most part they are dependable routine methods especially suited to foundry control work. Because of the short-cuts and simplifications employed in the interests of speed and economy, the methods are not generally suitable for precise umpire-type analysis. However, the adequacy of the procedures in the industrial applications for which they were intended has been proved in the laboratories of the members of Aluminum Research Institute.

The book contains a brief section devoted to the sampling of aluminum casting alloys, a section devoted to conventional chemical procedures, and a section on instrumental methods. The section on chemical methods contains procedures for determining ten elements, presented with little discussion and only one reference to the literature. The material on instrumental methods is divided in two parts, photometric and spectrographic. Photometric methods are given in considerable detail for eight different elements with frequent references to pertinent literature. Spectrographic procedures are briefly discussed in broad outline and three specific methods are indicated by tables giving sufficient detail for applying the methods to the particular types of equipment specified.

J. R. CHURCHILL

Laboratory Specialties

"Laboratory Specialties," a 40-page book, presents under one cover for the first time the instrument, apparatus, chemical, and furniture items designed by and available from Fisher Scientific Co., 717 Forbes St., Pittsburgh, Pa., and Eimer & Amend, 635 Greenwich St., New York 14, N. Y. Profusely illustrated, the book pictures 268 laboratory innovations and describes more than 300 equipment items that have been developed to aid laboratory work.

First Congress of the International Union of Crystallography

RALPH H. MULLER, *Contributing Editor*

THIS truly international assembly was held at Harvard University July 28 to Aug. 3, during which nearly one hundred papers, lectures, and addresses were delivered. More than half the contributions were from distinguished foreign scientists representing a dozen countries.

Separate sessions, some of a full day's duration, dealt with instruments and measurements, alloy phase structures, proteins and related structures, organic structures, inorganic and mineral structures, ferro-electrics, random and deformed structures, morphology, twinning, crystal synthesis, and new developments in structure determinations.

The thorough treatment of x-ray absorption in quantitative diffraction analysis by Alexander and Klug is of primary importance for analysts. They presented a convenient and useful mathematical summary of the three important cases arising in quantitative analysis, based upon the respective values of the linear mass absorption coefficients, weight fraction, and densities: (1) A mixture of n components in which the absorbing power of the unknown equals that of the matrix, when the intensity of the diffracted ray is proportional to concentration. (2) A binary mixture where unknown and diluent have differing absorbing powers. These require calibration curves from synthetic mixtures. (3) A mixture of n components with unlike values of absorption. In this general case an internal standard is added in known amount and intensity ratios are measured. The paper is to appear soon in ANALYTICAL CHEMISTRY.

Parrish and Hammacher reported on numerous improvements in the Geiger counter spectrometer technique arising from the

use of increased intensity, mica windows, increased pressure in the counter, line-focus parallel to the sample axis of rotation, and Soller slits. In a detailed discussion of the errors arising from automatic scanning, they emphasized the importance of time-constant in the integrating circuit in affecting the final resolution and amplitude.

The paper of Birks on crystal counters for gamma rays elicited great interest and discussion. Cadmium sulfide and other crystals were mentioned in passing, but the diamond is of principal interest because good specimens promise to excel the Geiger counter. The factors that decide whether a diamond will prove to be a good counter remain a mystery. Discouragingly enough, what is known with the greatest certainty is that good diamond counters are rare. Some of the ensuing arguments about Class I and II diamonds were somewhat resolved by the spirited and well-illustrated remarks of Sir C. V. Raman on the basis of their internal birefringence patterns.

The Thursday evening lecture by Shull on neutron diffraction outlined the technique by which a stream of neutrons is collimated from a pile-source and reflected from a rock-salt crystal and, after passing, the sample is absorbed in a boron fluoride counter. Despite the present limitations, primarily those of low final intensity, many important problems can be solved. Numerous examples illustrated cases in which the alternative x-ray diffraction is useless and cases for which the converse is true. Interesting observations on hydrogen-bearing compounds were described, including a study of ice, which favors the structure proposed by Pauling.

The titles, authors, and institutions with which they are affiliated are as follows:

Basic Aspects of X-Ray Absorption in the Quantitative Diffraction Analysis of Powder Mixtures. LEROY ALEXANDER AND H. P. KLUG, Mellon Institute, Pittsburgh, Pa.

Effect of Crystal and Particle Size on Powder Diffraction Intensities. A. W. WILCHINSKY, Esso Laboratories, Baton Rouge, La.

Geiger Counter Spectrometer. W. PARRISH AND E. A. HAMMACHER, Philips Laboratories, Irvington on Hudson, N. Y.

XRD-3 Unit and the SPG X-Ray Detector. J. RANFTL, G. E. X-Ray Corp., Milwaukee, Wis.

SPG Spectrogoniometer and the SPG X-Ray Spectrometer. H. W. PICKETT, G. E. X-Ray Corp., Milwaukee, Wis.

An Evaluation of Quantitative Procedures for Estimation of Intensities of Diffracted Electrons. S. H. BAUER, Cornell University, Ithaca, N. Y.

Crystal Counters. L. S. BIRKS, Naval Research Laboratories, Washington, D. C.

Method for Studying Extremely Low Angle Scattering of Monochromatic X-Rays. K. BANERJEE, Indian Association for Cultivation of Sciences, Calcutta, India.

Practice of X-Ray Diffraction in an Electrical Research Laboratory. H. P. ROOKSBY, General Electric Co., Wembley, England.

A Microbeam X-Ray Technique. J. N. KELLAR AND P. B. HIRSCH, Cambridge University, Cambridge, England.

Infrared Microscope. R. J. BAILLY, Washington University, St. Louis, Mo.

Structures of the Phases in the System U-Si. W. ZACHARIASEN, University of Chicago, Chicago, Ill.

Bridge Bonds in Metallic Hydrides. R. E. RUNDLE, Iowa State College, Ames, Iowa.

Iron-Nickel-Aluminum Alloys. A. J. BRADLEY, W. Jessop and Sons, Ltd., Sheffield, England.

The Technical Importance of the Sigma Fe-Cr Phase. D. A. OLIVER, Wm. Jessop and Sons, Ltd., Sheffield, England.

Recent Work on Interstitial Fe-C-N Alloys. K. H. JACK, Cambridge University, Cambridge, England.

Structure of Co_2Al_9. MRS. A. M. B. DOUGLAS, Cambridge University, Cambridge, England.

The Large Unit Cells of Protein Fibers. R. S. BEAR, Massachusetts Institute of Technology, Cambridge, Mass.

Studies of Amino Acids. J. DONOHUE, K. N. TRUEBLOOD, AND R. B. COREY, California Institute of Technology, Pasadena, Calif.

The Crystal Structure of Glycylglycine. E. W. HUGHES, California Institute of Technology, Pasadena, Calif.

X-Ray Diffraction Study of Lysozyme Chloride. K. J. PALMER, Western Regional Laboratory, Albany, Calif.

Protein Structure in the Light of Pseudosymmetry and Twinning. DOROTHY WRINCH, Smith College, Northampton, Mass.

Recent British Work on the Structure of Crystalline Proteins. Lecture by J. D. BERNAL, Birkbeck College, London, England.

Electron Microscope Study of the Structure of Crystals. Lecture by R. W. G. WYCKOFF, National Institute of Health, Bethesda, Md.

Recent Progress in Neutron Diffraction. Lecture by C. G. SHULL, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Crystal Structure of *p*-Nitraniline. S. C. ABRAHAMS, The University, Glasgow, Scotland.

Recent Work on Complex Organic Compounds. MRS. D. HODGKIN, University Museum, Oxford, England.

Structures of Pyrimidines and Purines. J. M. BROOMHEAD, C. J. B. CLEWS, AND W. COCHRAN, Cambridge University, Cambridge, England.

Some Ordered and Disordered Structures of Crystalline Molecular Compounds. H. M. POWELL, University Museum, Oxford, England.

The Structural Principles in Certain Hydrogen-Bonded Crystals. A. F. WELLS, Imperial Chemical Industries, Ltd., Manchester, England.

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Optical and X-Ray Examination of $BaTiO_3$ and Related Structures. H. F. KAY AND R. G. RHODES, Cambridge University, Cambridge, England.

Single Domain Barium Titanate Crystals. B. T. MATTHIAS AND G. D. DANIELSON, Bell Telephone Laboratories, Murray Hill, N. J.

Formation of Domains in Barium Titanate. P. W. FORSBERGH, JR., Massachusetts Institute of Technology, Cambridge, Mass.

The Dielectric Properties and X-Ray Investigations of Mixed Crystals of Potassium and Ammonium Dihydrogen Phosphates. I. NITTA, R. KIRIYAMA, AND M. HAISA, Osaka University, Japan. (By title)

X-Ray Examination of the Structure of Cold Rolled and Annealed Copper and Brass. T. L. RICHARDS, Kynoch Works, Birmingham, England.

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X-Ray Diffraction by Bent Crystals. H. EKSTEIN, Armour Research Foundation, Chicago, Ill.

Effect of Surface Treatment on the Texture of Crystalline Materials. J. N. KELLAR, P. B. HIRSCH, AND R. C. EVANS, Cambridge University, Cambridge, England.

Study of the Alloy $AuCu_3$. H. LIPSON, College of Technology, Manchester, England.

Local Order in Some Binary Alloy Systems. J. W. FITZWILLIAM, The Texas Co., Beacon, N. Y.

Sharp Extra Spots on Laue Photographs. K. BANERJEE, Indian Association for Cultivation of Science, Calcutta, India.

Calculations of the Line Profile of Reflections from Random Layer Lattices. A. J. C. WILSON, University College, Cardiff, Wales.

Diffraction in Disordered Crystals. Structure of Uranyl Fluoride. W. ZACHARIASEN, University of Chicago, Chicago, Ill.

Randomness in Layer Structures. W. F. BRADLEY, State Geological Survey, Urbana, Ill.

Periodic Lattice Distortions. R. PEPINSKY, Alabama Polytechnic Institute, Auburn, Ala.

Molecular Rotation in $NaNO_3$ and NaCN. L. A. SIEGEL, Massachusetts Institute of Technology, Cambridge, Mass.

Polymorphism and Anion Rotational Oscillation of Alkaline Earth Carbonates. J. J. LANDER, Bell Telephone Laboratories, Murray Hill, N. J.

The Rotation of CN Radicals and the Phase Transition in NaCN and KCN. T. NAGAMIYA AND T. MATSUHARA, Osaka University, Japan. (By title)

X-Ray Investigations on Some Plastic Crystals. I. NITTA, T. WATANABE, AND T. ODA, Osaka University, Japan. (By title)

Structure Irregularities in the Crystal of Aniline Hydrobromide. I. NITTA, T. WATANABE, AND I. TAGUCHI, Osaka University, Japan. (By title)

Significance of the Space Group Deduced from Crystal Morphology. J. D. H. DONNAY, The Johns Hopkins University, Baltimore, Md.

Pseudosymmetry and the Donnay-Harker Law. J. GARRIDO, Madrid.

Growth of Synthetic Quartz Crystals. D. HALE, Brush Development Co., Cleveland, Ohio.

Detwinning Quartz. W. PARRISH, Philips Laboratories, Irvington-on-Hudson, N. Y.

Growth and Twinning of Quartz. W. A. WOOSTER, N. WOOSTER, AND L. A. THOMAS, Cambridge University, and General Electric Co., Wembley, England.

Motion Picture Demonstration of Thermal Action upon Models of Atomic Aggregates during Crystal Growth. D. McLACHLAN, JR., AND R. WOOLEY, University of Utah, Salt Lake City, Utah.

Electron Micrograph Study of External Form of Crystals of Carbonyl Nickel. C. J. CALBICK, Bell Telephone Laboratories, Murray Hill, N. J.

Bond Energy and Elasticity Constants in Ion Lattices of the Sodium Chloride Type. I. WALLER, Institute of Mathematics and Physics, Uppsala, Sweden.

Relation between the Crystal Forms of Diamond and Their Internal Birefringence Patterns. C. V. RAMAN, Bangalore, India.

A Theory of Thermal Variation of the Refractive Indices of Crystals. G. N. RAMACHANDRAN, temporarily Cambridge University, Cambridge, England.

Boundary Migration. W. C. MCCRONE, Armour Research Foundation, Chicago, Ill.

New Methods of Morphological Analysis and Their Application to Phenomena of Crystal Synthesis. A. J. REIS, Rutgers University, New Brunswick, N. J.

Symmetry Considerations Applied to Debye-Scherrer Patterns. R. FAIVRE, Laboratoire du Pr. Chaudron, Vitry C.N.R.S., France.

Struttura della Cobaltite. E. ONORATO, University of Rome, Rome, Italy.

La Struttura dell' Eritrosiderite. A. BELLANCA, University of Rome, Rome, Italy.

Struttura della Teepleite. M. FORNASERI, University of Rome, Rome, Italy.

Isomorphism of Sr_2 with Hg_2. G. CAROBBI, University of Florence, Florence, Italy.

Prehistory of X-Ray Analysis. Address by P. P. EWALD.

Address by M. VON LAUE.

Aids to Analysis of Crystal Structure. P. J. G. DE VOS, C. J. B. CLEWS, AND W. COCHRAN, Cambridge University, Cambridge, England.

Electronic Computations for Crystal Structure Analysis. R. PEPINSKY, Alabama Polytechnic Institute, Auburn, Ala.

Phases of Fourier Coefficients from X-Ray Data. J. S. KASPER, General Electric Co., Schenectady, N. Y.

Relation between the Fourier Method and Steepest Descents. A. D. BOOTH, Birkbeck College, London, England.

Ambiguities in the Diffraction Analysis of Structure. A. L. PATTERSON, Bryn Mawr College, Bryn Mawr, Pa.

Patterson Transforms of Fiber Diagrams. C. H. MACGILLAVRY, Amsterdam, Holland.

Some Principles and Results of Multidimensional Lattice Theory. C. HERMANN, The University, Marburg, Germany.

An attractive exhibit of models and equipment was well housed in a wing of the Institute of Geographical Exploration. X-ray diffraction apparatus, cameras, densitometers, crystal goniometers, and crystal models were on display with extensive data, charts, and descriptive literature.

North Jersey Analytical Group

A new Sub-Analytical Group within the North Jersey Section of the AMERICAN CHEMICAL SOCIETY has been formed, with the following officers: chairman, Frank A. Meier, American Platinum Works; secretary, Al Steyermark, Hoffmann-LaRoche, Inc., Nutley, N. J.; treasurer, H. E. Zschiegner, Platinum Chemicals; Program Committee, William Rieman III, chairman, Rutgers University, William Seaman, Calco Chemical Division, American Cyanamid Co., and W. O. Baker, Bell Telephone Laboratories.

The group plans to hold four meetings during the coming season, beginning Wednesday, October 27, at the Newark Athletic Club, Newark, N. J., when Beverly L. Clarke, Merck & Co., Inc., will speak on "The Analytical Chemist in Industry."

The Analyst's Calendar

Pittsburgh Analytical Symposium

The Fourth Annual Analytical Symposium, sponsored by the Analytical Division of the Pittsburgh Section, AMERICAN CHEMICAL SOCIETY, will be held at the Hotel William Penn, Pittsburgh, January 20 and 21, 1949, under the chairmanship of D. P. Bartell, Allegheny Ludlum Steel Corp., Brackenridge, Pa. An exposition presenting new analytical tools will be one of the innovations of the symposium. Companies desiring to exhibit new developments in apparatus should write at once to Henry Freiser, University of Pittsburgh, Pittsburgh 13, Pa., as floor space will be assigned in the order in which applications are received.

All analytical chemists are invited to attend and papers on any phase of analytical chemistry will be welcomed. Titles of papers with a statement of the approximate time required for presentation should be submitted to R. G. Russell, Gulf Research & Development Co., Box 2038, Pittsburgh 30, Pa., before November 1. A 200-word abstract is required not later than November 30, 1948.

The program will be printed in ANALYTICAL CHEMISTRY.

Fourth Annual Analytical Symposium. Hotel William Penn, Pittsburgh, Pa., January 20 and 21, 1949.
Second Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., March 9 to 12, 1949.
Second Annual Summer Symposium on Analytical Chemistry. Wesleyan University, Middletown, Conn., June 1949.

AIDS FOR THE ANALYST.....

Filling Capillaries for X-Ray Analysis. George Gibons and E. J. Bicek, Illinois Institute of Technology, Chicago 16, Ill.

POWDERED substances that are to be studied by x-ray diffraction are frequently loaded in small glass capillaries of approximately 0.025-inch (0.6-mm.) outside diameter, by repeatedly pressing an open end of the capillary into a layer of the powder on a glass slide or in the agate mortar in which the sample was ground. When the filling must be carried out in a dry box or an inert atmosphere, the operation may be difficult or impossible.

Described in this paper is a modification of the filter-stick technique by which capillaries may be filled with powdered materials for x-ray analysis. The technique is applicable to loading directly from the sample bottle, in partial vacuum, under pressure, or in any desired atmosphere. The sample may also be removed directly from suspension in either liquid or gas. The sample must be in the state of subdivision desired for the analysis and must not pack immediately to an impervious layer.

Figure 1 shows one possible form of the capillary holder—the straight form, which is commonly used for dry solids. A bent modification connected to a flask permits capillary loading directly from liquid suspension with recovery of the filtrate as well.

A, the "handle" of the device, consists of a 10- to 15-cm. length of capillary tubing having an inner bore of about 1 mm. The rubber tubing, *B*, protrudes about 5 mm. beyond the end of the capillary to make a cup for the filter paper disk, *C*, and the short capillary, *D*. *D* is the same diameter as *A* and has cemented on one end a disk of a fairly soft natural or synthetic rubber. A disk punched from Gooch rubber tubing proved satisfactory; for some applications, special elastomers such as Tygon, neoprene, Buna-N, or Thiokol may be necessary. The disk, which is approximately 1 mm. thick and has been perforated in the center with a needle, may be cemented to the glass with a Bakelite, glyptal, or similar adhesive.

Further improvement can also be obtained by using two such disks, one cemented at either end of *D*. Although much less convenient, it is also possible to seal the sample capillary in *D* with a temporary wax or cement joint.

To assemble the parts, the sample capillary is pushed from the left end through the perforation in the rubber disk, *E*. The rubber around the advancing capillary expands slightly in the direction of motion. When the force is removed the relaxing rubber returns the capillary slightly. This condition ensures good contact between the sample capillary and the filter paper and produces a seal that tends to tighten rather than open when a vacuum is applied. The filter paper is dropped into the cup made by the main capillary and its attached rubber tubing. The short capillary holding the sample capillary is then inserted in the cup.

To collect the sample, the left end of *A* is connected to vacuum and the powdered sample is probed with the capillary. With most materials the filling is rapid and the building up of the column of powder can be observed through the side. The vacuum is broken before the capillary is removed to avoid expulsion of the sample in the capillary as it is separated from the filter paper.

Materials vary widely in their response to this technique. Some pack densely against the filter paper and rapidly cut down the flow of air; however, enough sample is usually collected to serve for the production of a diffraction pattern. A few materials pack so loosely that tamping with a fine rod is necessary if a sample of suitable density for x-ray work is to be obtained. The wire-gage drill used for measuring the internal diameter of the capillaries makes a satisfactory tamp rod when mounted in a suitable handle.

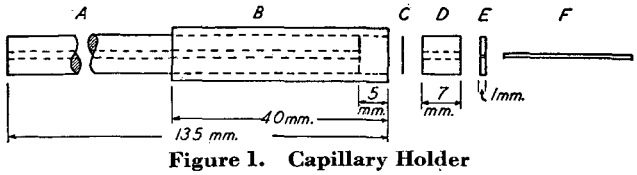

Figure 1. Capillary Holder

***A*, *D*. Capillary tubing, 7-mm. diameter, 1-mm. bore. *B*. Rubber tubing, 7-mm. inside diameter. *C*. Filter paper disk. *E*. Rubber disk, 7-mm. diameter. *F*. X-ray sample capillary**

Most crystalline powders pack densely during loading and show very little decrease in volume on tamping. Great caution must be exercised in packing, because even a very light force on the rod results in tremendous pressures inside the capillary. The No. 78 drill rod recommended in the A.S.T.M.–Hanawalt method for checking the internal diameters of the capillaries has an end area of approximately 0.0008 square inch. One-ounce force on such a rod produces a pressure of 5 atmospheres on the inner walls of the capillary. The capillaries burst or split with amazing ease if this is not kept in mind. This same precaution must be observed if the capillary is sealed by pushing the open ends into wax.

When the sample is to be collected under a special atmosphere, the device may be passed through a stopper and sealed with a flexible joint. A second opening in the stopper is connected to the gas supply.

When the sample is to be removed from suspension in a liquid, the handle is made considerably longer and bent twice to form an inverted U. One arm is inserted through a stopper into the trap which serves to collect the liquid. The trap, in turn, is attached to the vacuum line. The other arm of the inverted U bears the rubber tubing, filter paper, and capillary.

Electrical Thermostatic Control of Gas-Heated Baths. Leland C. Clark, Jr., and Frederick Hooven, Fels Research Institute for the Study of Human Development, Antioch College, Yellow Springs, Ohio

IT IS frequently necessary to maintain a rackful of test tubes or a large flask at a constant temperature for a given time in the laboratory. Open baths are often difficult to heat by conventional electrical methods, particularly if high temperatures are to be maintained, and an auxiliary heater must be employed to bring the bath to the desired temperature. Aside from its inconvenience, such equipment is expensive to set up and maintain.

The large range of heat (the equivalent of 50 to 2700 watts) available from an ordinary Bunsen burner, coupled with its low cost and ready availability, makes this type of heating highly desirable. Such heating methods were widely used before the advent of electrical heaters, but the mechanical thermostats used at that time left much to be desired.

The electronic relays used to operate electrical heaters can readily be adapted to regulate a Bunsen flame by an ordinary solenoid type gas valve. A highly effective device is supplied for regulating the gas pilot on oil burners. The valve in use here (solenoid gas pilot valve, Type V-446 A, Minneapolis-Honeywell Regulator Co.) operates on 110 volts, 60 cycles, consumes only 8 watts, snaps "open" when the solenoid is energized, and is provided with a small by-pass valve to maintain a low pilot flame. Because the electrical load is only 8 watts there is very little wear on the relay and no danger of arcing. As these valves can also be adjusted to turn the gas completely off, they may be used with electrical timing devices to shut off flames under Kjeldahl digesters, extractors, etc., at a given time.