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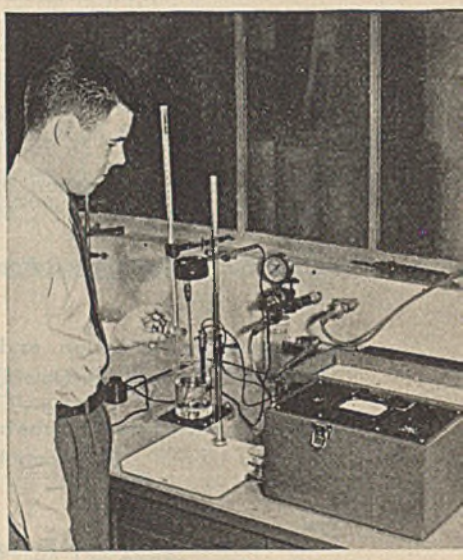

L&N pH Indicator No. 7662 being used for titration; note how long leads of electrode permit them to reach beaker at convenient distance from Indicator.

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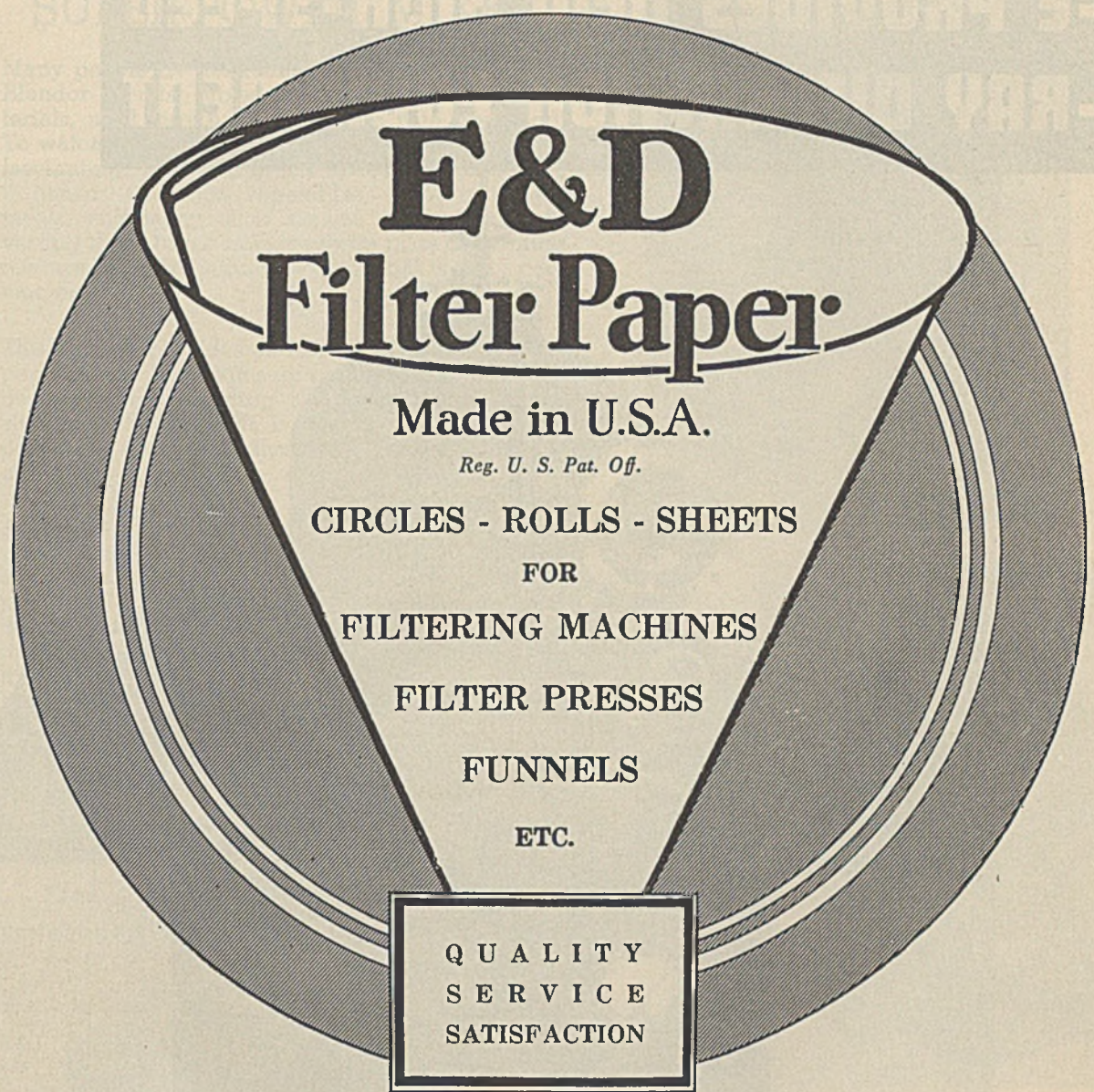

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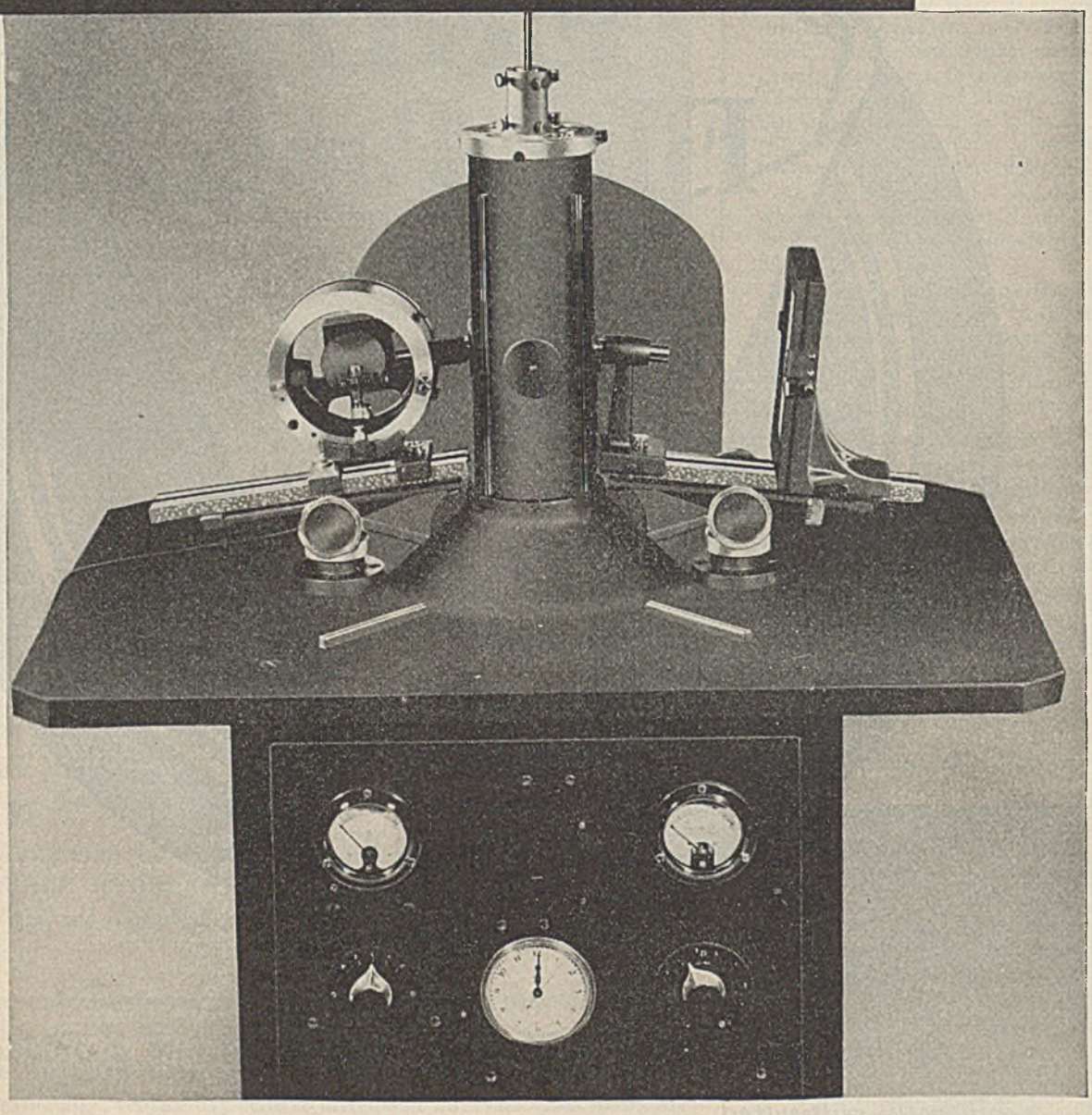

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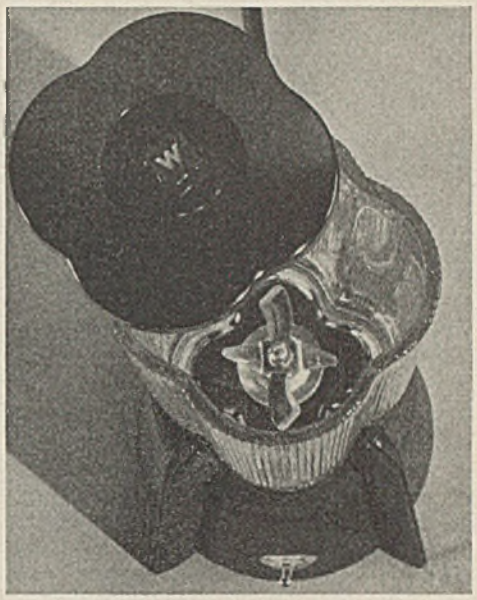

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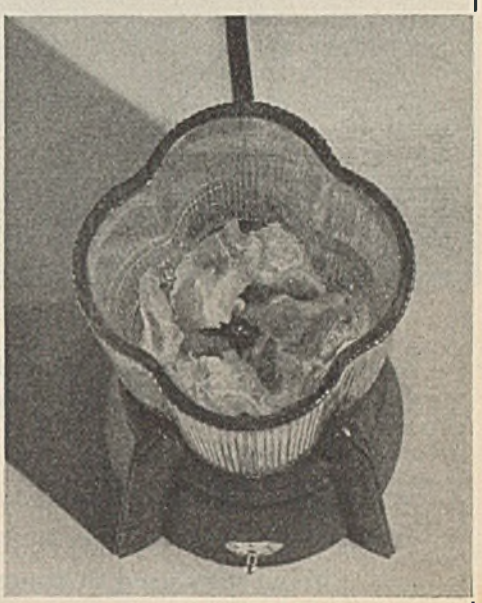

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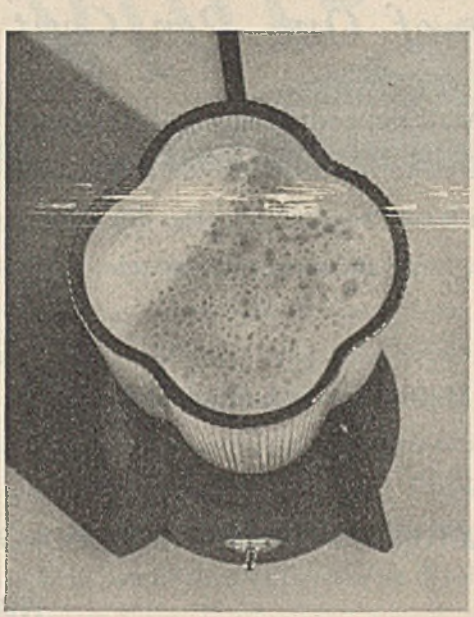
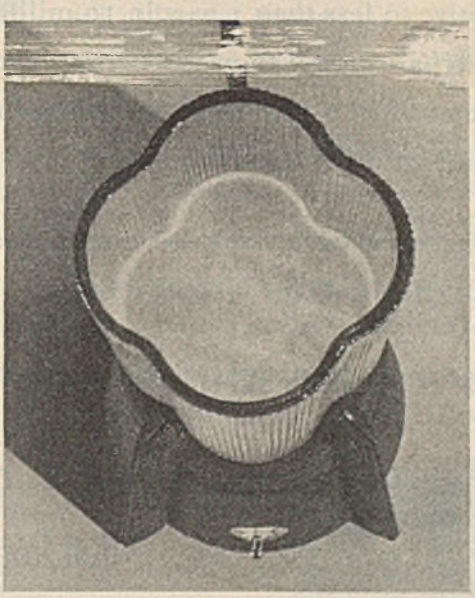
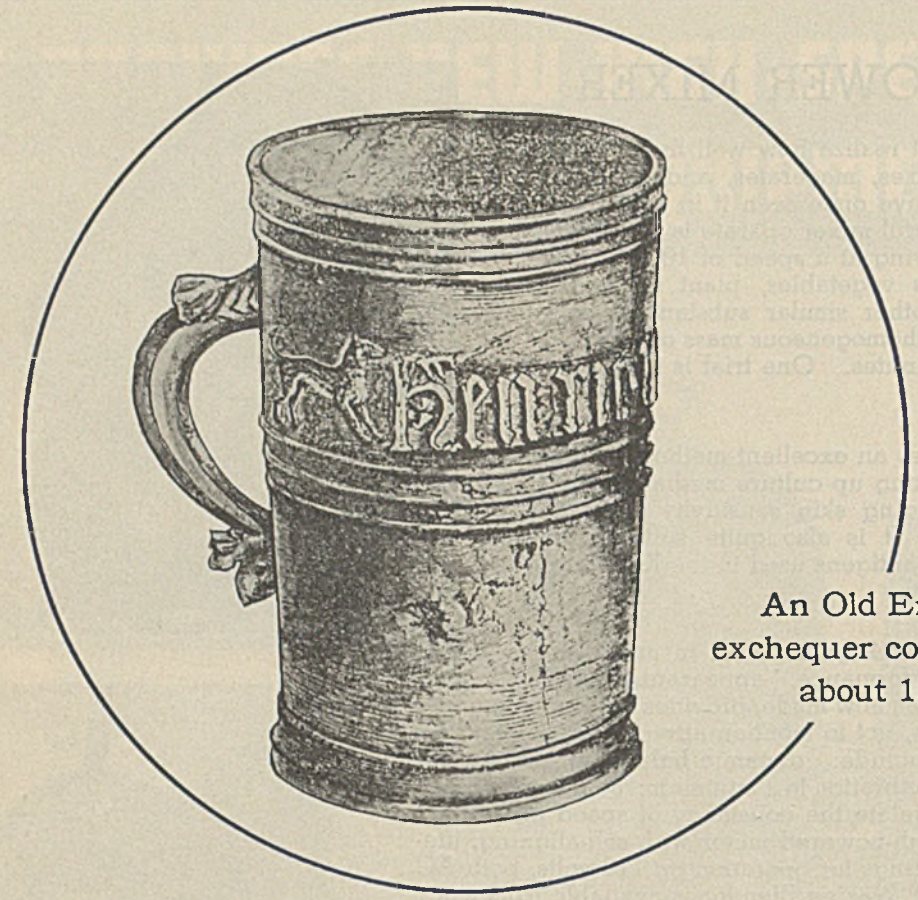




The two lower illustrations show the foaming mixture after eight minutes of operation and with the foam removed. In the resultant mixture it will be noticed that the meat particles have been finely divided and uniformly dispersed throughout the liquid.

The two upper illustrations show top views of a Waring Blendor with its cutting knives before and after the receptacle has been half-filled with water and several chunks of raw beef.



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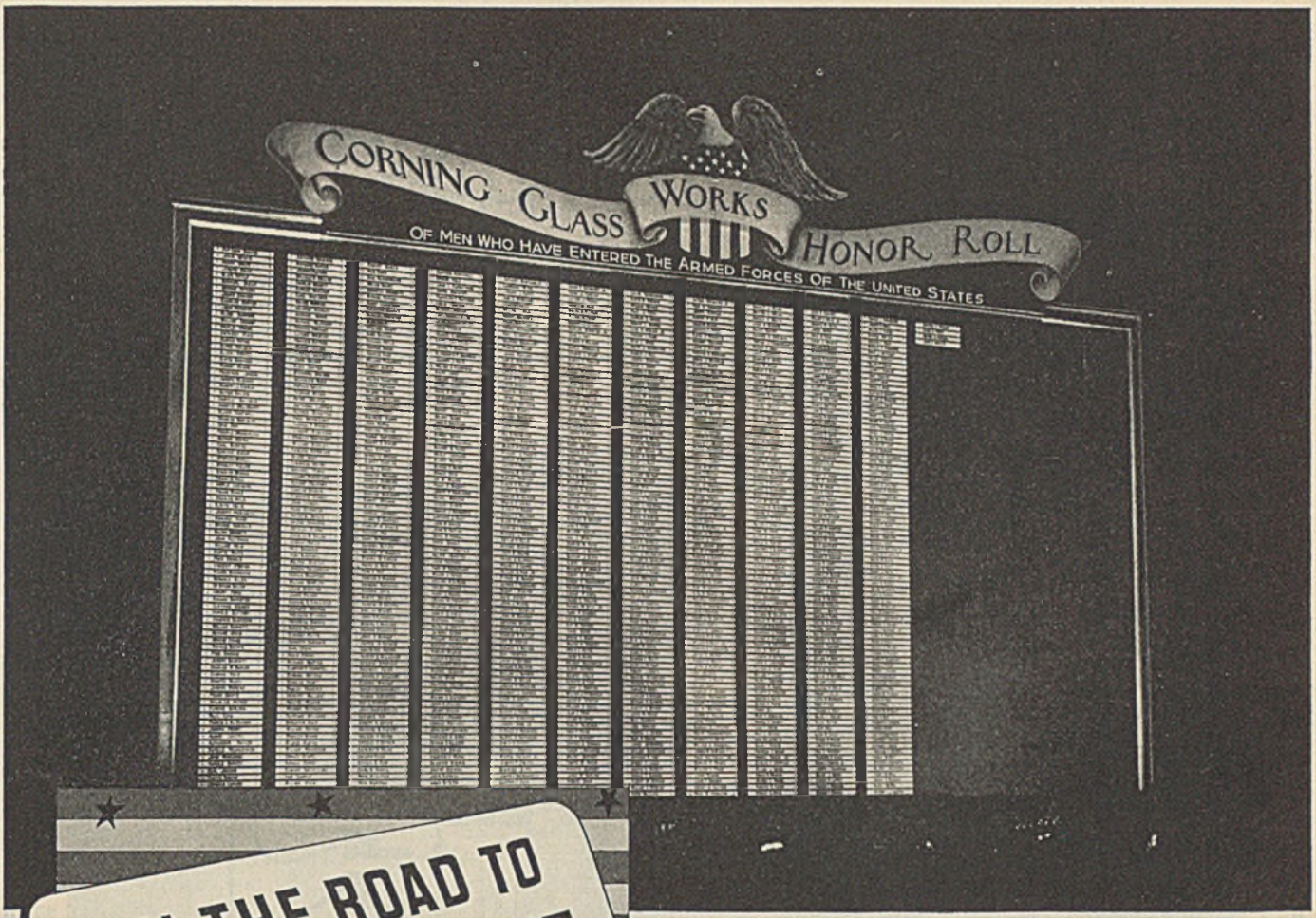

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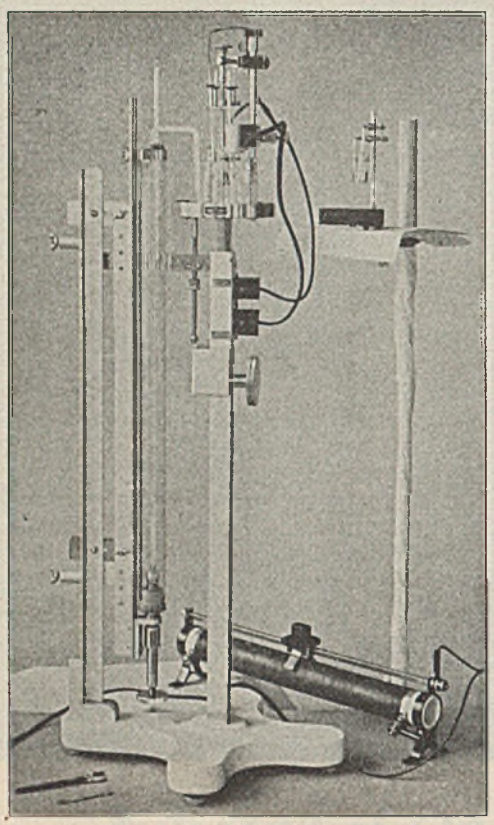
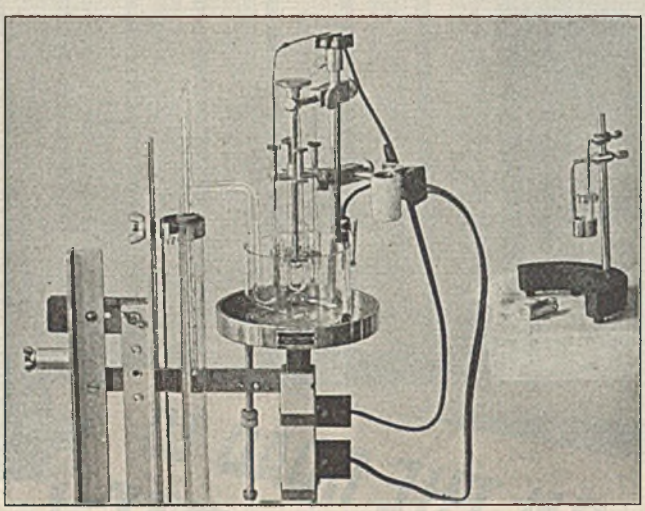
For the micro determination of the constituents of 25 to 100 cu mm samples of combustible and inert gaseous mixtures with a precision which compares favorably with that obtained in macro procedures



5937-A. Fig. 1



5937-A. Fig. 2
Showing details of Reaction Platform

MICRO GAS ANALYSIS APPARATUS, BLACET-LEIGHTON, A. H. T. Co. Specification. For the quantitative determination of the constituents of small samples of combustible and inert gaseous mixtures containing acetylene, ammonia, benzene, carbon dioxide, carbon monoxide, ethane, ethylene, unsaturated hydrocarbons, hydrogen, hydrogen chloride, methane, nitrogen, nitrous oxide, oxygen, water, etc. Determinations can be made on 25 to 100 cu mm samples with a precision which compares favorably with that obtained in macro procedures.

See Francis E. Blacet, George D. MacDonald and Philip A. Leighton, *Industrial and Engineering Chemistry, Anal. Ed., Vol. 5, No. 4 (July 15, 1933), pp. 272–274;* and R. Nelson Smith and Philip A. Leighton, *Ibid., Vol. 14, No. 9, (September 15, 1942), pp. 758–759; etc., etc.*

Analyses are made in open end gas thimbles, using mercury as the sealing fluid. Constituents of samples are absorbed by means of selected solid or liquid reagents inserted through the mercury seal. The amount of absorption is measured by transfer of the gases to the micro burette before and after the reaction is completed in the gas thimble. Solid absorbents, such as yellow phosphorus, potassium hydroxide, cupric oxide, etc., are prepared in the form of fused beads supported in loops or on straight wires made of platinum; liquid absorbents, such as fuming sulfuric acid, are applied in holders containing a porous glass bead.

The apparatus consists of a water jacketed micro burette, graduated from 0 to 100 cu mm in 0.2 cu mm divisions, with ₹ interchangeable ground glass joint to metal needle valve for delicate and positive control of mercury column, mounted on an adjustable metal holder which can be removed easily and supported in inverted position for convenient cleaning and filling of the burette; and a rotating assembly for serially testing four gas samples in thimbles mounted on a reaction platform with improved rack and pinion movement; both mounted on a stable corrosion-resistant stand with Coors porcelain base. It also includes improved electrical equipment for heating and treating gas samples with heater and combustion coils; gas thimble holders for storage of gas samples; and a portable stand with pneumatic trough for transferring gas samples.

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ANALYTICAL EDITION

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Synthetic Rubber

A Spectroscopic Method for Analysis and Control

R. BOWLING BARNES, URNER LIDDEL, AND V. Z. WILLIAMS

Stamford Research Laboratories, American Cyanamid Company, Stamford, Conn.

INFRARED spectroscopy offers methods for the identification, analysis, and control of hydrocarbon mixtures, which have decided advantages over physicochemical methods now in use.

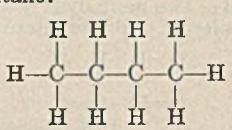
Ordinary chemical methods for the analysis of mixtures of hydrocarbons are long and tedious, and the trade has long resorted to the method of physical separation of the components by fractional distillation, with identification of the components by their boiling points. This is indeed an accurate method, but is time consuming. Even though the analysis can be accomplished in a matter of hours, many hundreds of gallons of product would be produced in a plant before an error in production could be caught in the laboratory. Therefore a method of analysis which would require at most a matter of minutes, and, in control, a matter of seconds, is highly desirable.

Another point for consideration is the fact that distillation requires samples in the liquid state. For the several hydrocarbons which are gases at normal temperatures, this requires condensation of the gases in order to obtain samples, and the usual difficulties of low-temperature distillations. The spectroscopic method, on the other hand, works equally well with gases or liquids, and requires only a very small fraction of the amount of material needed for distillation. Furthermore, this method is readily adaptable to use in the plant, since the spectrometer can be so placed as to allow the absorption cell to be connected into the production line. A portion of the product can be by-passed through the cell, thus obviating the necessity for transfer of samples to a separate laboratory. Lastly, the addition of a relay device permits automatic process control—a mechanical, continuous "watchman".

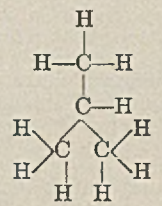
It has been pointed out many times in the literature (*1*, *7*, *8*) that the infrared spectrum of an organic compound is a unique property of that compound, and that, except in special instances, it retains that property on admixture with other compounds. These special instances are predictable, and are of no interest in discussing simple hydrocarbons. Thus, in general, in any particular hydrocarbon mixture, the concentration of a given component can be determined by infrared measurements, if at least one absorption band of this component can be found at a wave length for which the remainder of the mixture has negligible absorption. A determination of the per cent transmission at this wave length enables one to measure the amount of this component which is present.

That the problem of spectrochemical analysis by infrared is essentially simple may be readily seen by considering the various molecules of the components as mechanical systems. The origin of infrared spectra lies in the mechanical motions of the atoms of the molecules. (Actually, of course, the origin of infrared spectra lies in the periodic variation of the dipole moment of the molecules. However, this is a complicated function and a discussion is out of place here. It can be found in any standard text on spectroscopy.) Therefore, in discussing the mechanical analogy, we are approximating the actual spectroscopic situation, since each absorption frequency is related to a particular mechanical frequency. If we have several bodies tied together by suitable springs, and allow these masses to move freely, restrained only by the springs, they will perform certain motions with particular or characteristic frequencies. [The correlation between motions of gross bodies bound by steel springs and motions of atoms in molecules has been shown by Kettering, Shutts, and Andrews (*4*).] These frequencies can be made to vary by changing any of three factors—the masses involved, the strength of the springs, or the orientation of the masses.

Choosing a specific chemical compound as an example, let us take the molecule butane:



These carbon and hydrogen atoms will vibrate with certain frequencies which will give rise to a characteristic spectrum. If we simply rearrange this same number of atoms into another structure, we have isobutane:



From the mechanical analogy, it may be seen that this reorientation of the masses will give rise to characteristic frequencies different from those of *n*-butane.

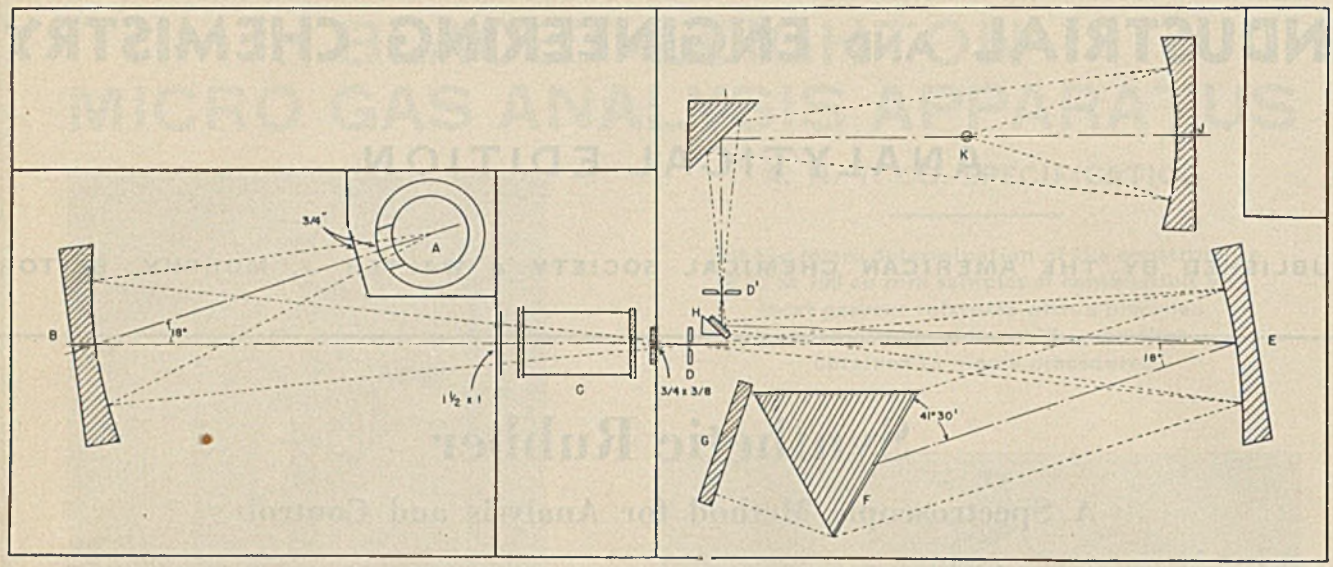


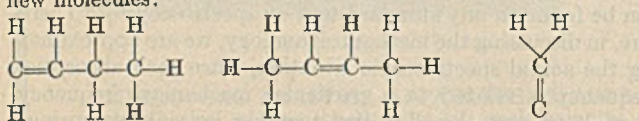
FIGURE 1. SCHEMATIC DESIGN OF A COMPACT, OPTICALLY POWERFUL INSTRUMENT FOR MAKING SPOT ANALYSES

Base, 10-inch channel iron

		Diameter, cm.	Focal length, cm.	Diopter
A.	Source, Nernst glower			
B.	Spherical mirror	9	10	10
E.	Spherical mirror	9	25	4
J.	Spherical mirror	9	7.14	14

C. Sample cell
D, D'. Slits
F. 60° salt prism, 6 cm. high, 7.5-cm. base
G. Plane mirror, 7 × 6 cm.
H. Plane mirror, 1 × 1.5 cm.
I. Prism, B. & L. 1.25-inch glass
K. Thermocouple

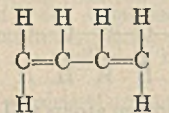
Now, if we remove two hydrogen atoms, we can form three new molecules:



Butene-1 Butene-2 Isobutylene

The decrease in mass caused by the removal of the hydrogen atoms can be neglected, as it is only 3 per cent of the total mass. Of importance, however, is the fact that we have double the strength of one of the "springs" holding the atoms together, and hence have a faster frequency of motion between two carbon atoms than occurs in either of the butanes. Furthermore, each of these new molecules is a mechanical system different from the other two.

Finally, by removal of two more hydrogen atoms from butene-1, we arrive at the molecule of prime interest, butadiene:



At first glance, it might be assumed that the frequency of the >C=C< spring here might be the same as in the previous molecules. Actually, however, another physical phenomenon appears, because there is a strong interaction between the motion of the carbon atoms, in this case roughly analogous to the interaction between two coupled pendulums. The organic chemist calls such molecules "conjugated" hydrocarbons or conjugated dienes, and knows that their chemical properties differ markedly from the monoolefins.

The foregoing molecules which have been chosen for purposes of illustration constitute the so-called C_4 fraction of petroleum distillations. Since they have different frequencies of motion, they give rise to characteristic infrared spectra, and so may be detected and measured quantitatively in hydrocarbon mixtures. In view of the fact, however, that the correlation between infrared absorption bands and mechanical motion holds in general, the application of this method of spectrochemical analysis is in no way limited to the cases herein cited. This method may be used in the cases of many rather subtle organic analyses, such as the differentiation of isomers, rates of oxidation, polymerization, etc.

It is the purpose of this paper to present the infrared spectra of certain compounds of particular interest in the manufacture of synthetic rubber, and to describe a method for analysis and for production control. The spectra of several synthetic rubbers are also shown.

Experimental

The apparatus used in this work has been described (*2, 3, 5*), together with the spectra of a large number of organic compounds and the analytical and identification techniques used.

The spectra shown in this article were obtained from an automatic recording, rock salt prism, Littrow spectrograph. The usual working range is from 3750 cm.$^{-1}$ (2.7μ) to 750 cm.$^{-1}$ (13μ). The average spectral slit width employed decreases from 15 cm.$^{-1}$ at 3000 cm.$^{-1}$ to 5 cm.$^{-1}$ at 800 cm.$^{-1}$.

Within experimental error, all measurements made on this instrument follow Beer's law, $I/I_0 = e^{-kcx}$. Here I/I_0 is the per cent transmission (or $1 - I/I_0$, the per cent absorption) at a particular frequency, k is an absorption constant of a compound at that frequency, c is the concentration of the compound, and x is the path length used. I/I_0 is the direct experimental result measured with a spectrograph and the accuracy with which it can be measured is determined by the characteristics of the instrument being used. Bearing this in mind, there are two pertinent considerations concerning the exponents kcx. First, except for a few special cases, at a given frequency I/I_0 remains constant so long as the product cx remains constant. Hence the concentration—(i. e., pressure in case of gas work)—or the cell length can

be chosen arbitrarily to suit experimental conditions so long as the other factor can be changed to give a suitable cx value. Second, if k and x are held constant, a consideration of the exponential graph for Beer's law shows that a small error in the measurement of I/I_0 represents different absolute errors in the measurement of the concentration, depending upon whether the concentration is high or low. Roughly speaking, the absolute concentration of a component can be measured more accurately when the concentration is low than when it is high. These points are elaborated below.

The spectra shown have been plotted between 2000 cm.$^{-1}$ and 750 cm.$^{-1}$, because this is generally the region from which the most information can be obtained. Results obtained outside of the region shown are discussed wherever they are of importance. These spectra are obtained from automatic recordings of the transmission through an empty and a sample-filled cell. These records are measured at given frequency intervals and the quotient of each pair of measurements is plotted as a graph of frequency in cm.$^{-1}$ *vs.* per cent transmission. Since there may be a change in experimental conditions between these two records and since there is no attempt to take account of scattered light, the intensity scale shown is of relative but not absolute importance. However, the frequency position of the bands is accurate within the experimental error of the instrument.

Automatic recording of cell-empty, cell-filled transmissions at a given frequency for "spot" analyses is chiefly of value in measuring a long series of similar samples. Moreover, the size of the authors' recording drum limits the amount of the cell-empty transmission energy which can be used. Hence all quantitative results given in this paper were obtained by visual reading of the galvanometer spot from an auxiliary lamp and scale system.

A description of the methods of infrared spectrochemical analysis is best achieved by taking a simple example in which it is desired to obtain the concentration of one component in a mixture of known materials. The spectra of all the pure compounds present in the mixture are compared and an absorption band is chosen which is unique to the particular component of interest. Transmission measurements at the frequency of the chosen band are made on a series of known standards in which the concentration of the one component is varied. A working calibration sheet is prepared by making a plot of concentration against these measured transmissions. Once the calibration data are obtained, any unknown may be analyzed by filling the absorption cell, measuring the per cent transmission on the instrument, and reading the answer off the work sheet—an operation requiring a very few minutes. By choosing a band unique to another component of the mixture and preparing a calibration work sheet at that frequency, two components may be measured successively. This process can be continued as long as these unique bands or combinations of such bands can be found. Working calibration sheets such as those described above have been obtained for various mixtures and are illustrated below.

There may be some objection that this method of analysis does not make allowance for the absorption of the empty cell or for the scattered light present in the instrument. It is true that corrections for these factors could be made and the working data could be plotted as a straight-line graph of log I/I_0 against concentration. Such corrections, however, are a function of the particular instrument used and would be of no value for analyses made on another instrument. Moreover, a working sheet on this basis would require additional calculations before it could be used. In actuality, the accuracy of any analysis depends ultimately upon the accuracy with which a per cent transmission can be measured, and the computational step from this measurement to concentration analysis should be as short as possible.

Because of the persistent requests for suggestions concerning infrared apparatus the authors have designed in this laboratory a small, very simple spectrometer with sufficient resolution and optical power to satisfy the demands of the problems at hand. This instrument was built by the Porocel Corporation and is now in very satisfactory service for hydrocarbon analyses at their plant.

A schematic diagram of the spectrometer is given in Figure 1. The light path through the instrument is shown in dotted line. The instrument has an aperture of f. 3.5 and the slits are adjustable to give the combination of energy and resolution desired for a given analysis. The design is a typical 60° rock salt prism Littrow mounting. In its present use, the absorption cell is introduced into the light path on a cell way and hand readings are made from a galvanometer and scale. If the instrument is to be used as a control relay in the plant, the absorption cell could be fixed in the light path and gas from a by-pass in the production line would be blown continuously through it. The introduction of a suitable device in the amplifying system would make possible the opening and closing of valves, variation in heat input in the process control, or the sounding of alarms. The instrument may be used for the analysis of liquid or solution samples merely by introducing a suitable absorption cell in the light path.

A photograph of the completed spectrometer is shown in Figure 2. The apparatus is shown from the operator's viewpoint with the galvanometer lamp and scale used in making analyses. The two glass leads to the absorption cell can be seen projecting above the instrument. Figure 3 shows the completed spectrometer with the covers removed and a foot rule laid on the base for size comparisons. This view is directly comparable with the schematic diagram of Figure 1, so that the component parts may be determined by corresponding positions on the base. The performance of this small spectrometer with respect to optical power, resolution, and reproducibility far exceeded expectations at the time it was designed.

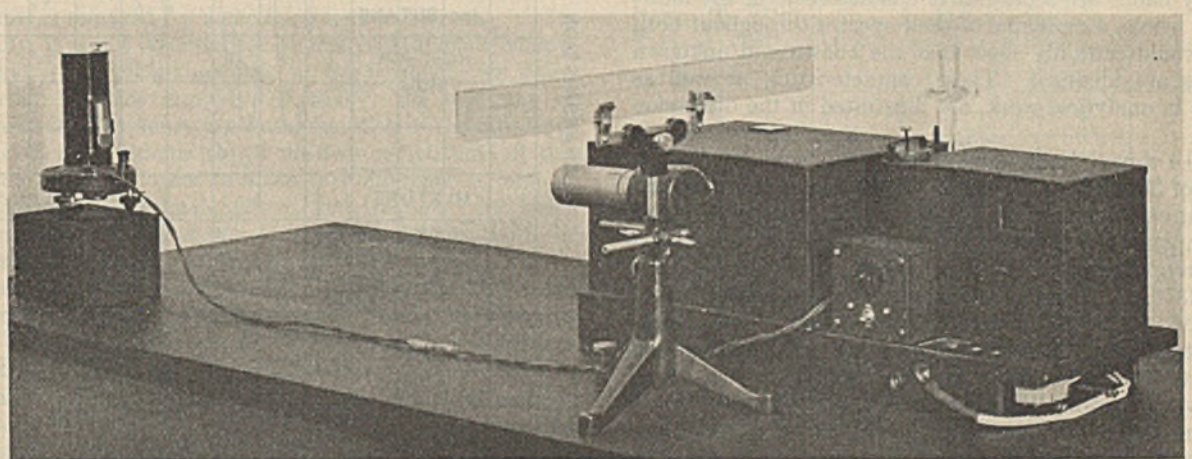


FIGURE 2. SMALL INFRARED SPECTROMETER FOR SPOT ANALYSES

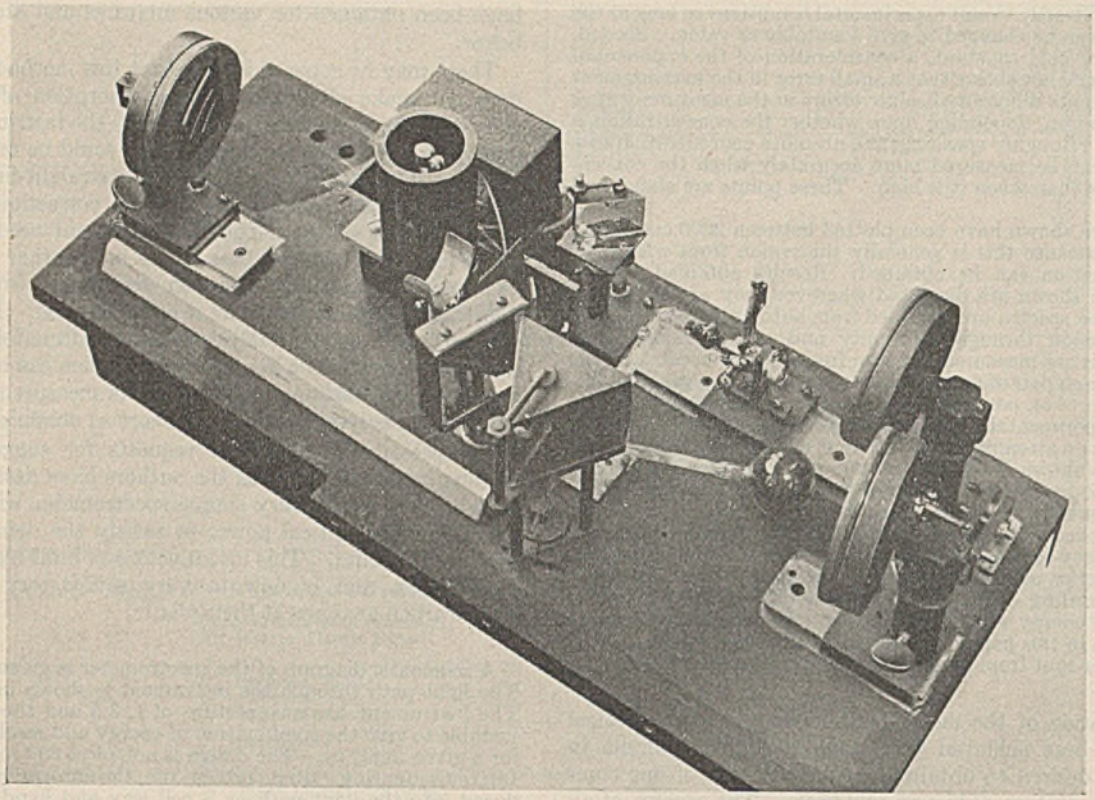


FIGURE 3. SMALL INFRARED SPECTROMETER WITH COVERS REMOVED

Discussion

An infrared spectrum may be divided roughly into two parts, a high-frequency and a low-frequency region, the division line being somewhere in the neighborhood of 1300 cm.$^{-1}$. In the high region, the observed absorption bands are likely to be caused essentially by a vibration of a specific pair or group of atoms within a molecule, such as O—H, C—H, N—H, C=O, C=C, aromatic rings, etc. In the low region, on the other hand, bands arise from vibrations in which all the atoms of the molecule take part. Upon comparing the spectra of an unknown and a known molecule, complete coincidence of bands both in position and intensity in the high region indicates that the same atomic groups are present, but does not necessarily permit the conclusion that the molecules are identical. On the other hand, since the lower frequency bands are more nearly characteristic of the molecule as a whole, a matching of their spectra throughout both regions would certainly show that the known and unknown molecules are identical. These characteristics, as well as their use in analytical work, are illustrated in the discussion below.

Figure 4 shows the spectra of *n*-butane, isobutane, and a mixture of 36 per cent *n*-butane, 60 per cent isobutane, and 4 per cent other gaseous hydrocarbons. Both spectra of the pure compounds have strong bands at 1450 cm.$^{-1}$, a general C—H group bending vibration. Again, both have a band around 1375 cm.$^{-1}$ which is characteristic of a methyl group absorption. This band in isobutane is double—one component at 1375 cm.$^{-1}$, the other at 1360 cm.$^{-1}$. This doubling is characteristic of a terminal isopropyl group.

However, spectral differences and not similarities are of prime importance in analytical work. It is immediately apparent that *n*-butane has a strong band at 975 cm.$^{-1}$, a region in which there is negligible absorption in isobutane. The reverse situation is true at 1180 cm.$^{-1}$ where the iso form has a band and the normal has not. Both these bands are seen to appear in the mixture. Therefore, an analysis for one component in the presence of the other is a simple

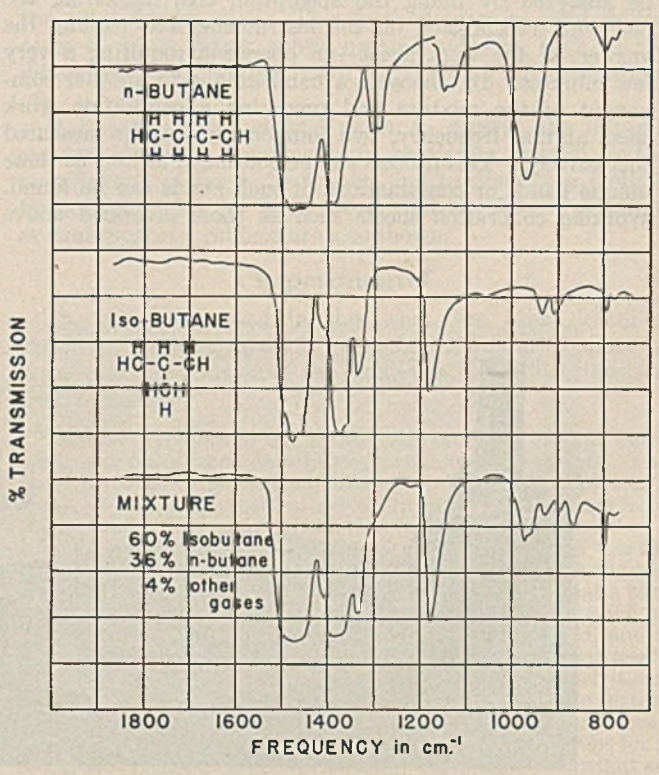


FIGURE 4. INFRARED SPECTRA OF PURE AND MIXED C_4 SATURATED HYDROCARBONS

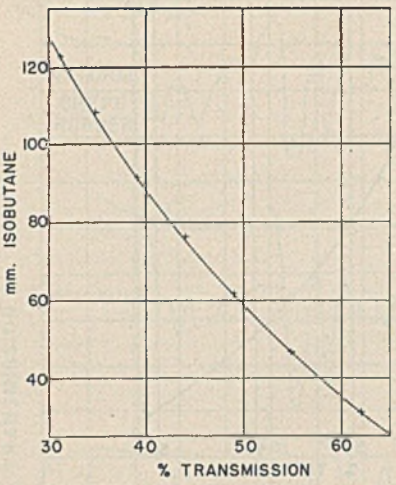


FIGURE 5. WORKING CALIBRATION CURVE AT 1180 CM.$^{-1}$

Per cent transmission of various pressures of isobutane in a mixture of approximately 150 mm. of butane + isobutane. Scattering of points is less than 1 mm. in 150, or an accuracy of better than ±1 % of total composition.

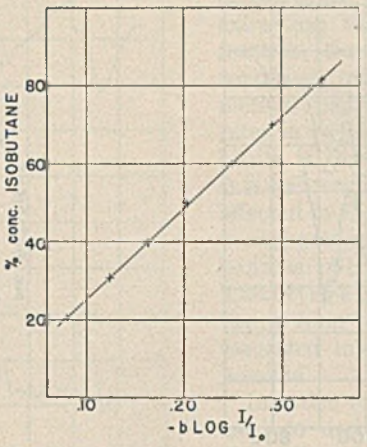


FIGURE 6. GRAPH OF FIGURE 5 REDUCED TO A STRAIGHT-LINE PLOT OF LOG PER CENT TRANSMISSION *vs.* ISOBUTANE CONCENTRATION

Values of Figure 6 have been corrected for scattered light and variation in total pressure. Constant *b* includes cell length, total pressure, and absorption coefficient *K*.

TABLE I. ISOBUTANE CONTENT

Chemical	Spectroscopic
%	%
38.3	38.3 (standard)
20.8	21.3
0.0	0.26
43.9	42.7
48.8	48.7

matter according to the technique outlined above. The working calibration sheet shown in Figure 5 was obtained by setting the instrument for the isobutane band at 1180 cm.$^{-1}$, filling an absorption cell with mercury manometric measures of isobutane, making up the mixtures with *n*-butane to about 150-ml. total pressure, and determining the per cent transmission of the prepared samples. A smooth curve drawn through these points shows a scattering of less than ±1 mm. of isobutane or an accuracy of better than ±1 per cent of the total mixture, in spite of the rough method of filling the cell. The time to obtain this curve was 3 hours. After this working sheet is once obtained, the time to analyze any unknown after the cell is filled would be no more than 5 minutes. If the *n*-butane content is also desired, another 5 minutes is required for a measurement at 975 cm.$^{-1}$. Incidentally, it is not essential for an analysis that one component have a negligible absorption at the frequency for which the second absorbs strongly. Actually, the accuracy of analysis at any frequency is a linear function of the difference in absorption coefficients (k) of the two components at this frequency. It is in order to make this difference as large as possible that analyses are made at that frequency for which one component has a strong absorption while that of the other is negligible.

In order to illustrate the method of working with a log per cent transmission plot, the values of Figure 5 were corrected by a constant factor for scattered light, cell window absorption, and variation in total pressure; and the straight-line graph of Figure 6 was plotted. This method of treating the data is theoretically much more rigorous. The resultant accuracy (or point scattering) is the same in Figure 6 as in Figure 5, although a greater time is required to prepare the calibration sheet. However, the method of log I/I_0 plotting is useful for extrapolation to a range of concentration for which calibration standards might be difficult to procure.

With respect to this particular mixture, the authors measured some samples for their isobutane content, in order to compare results with those obtained by fractional distillation. Here one sample was taken as a standard and the method of Figure 6 was used for measuring the others. Table I gives the results for each method of analysis. This comparison offers a further proof of the accuracy of spectroscopic methods.

Figure 7 shows the spectra of the unsaturated C_4 hydrocarbons. It was pointed out above that a double bond would be a much stronger "spring" than a single bond. This stronger spring is apparent in butene-1, butene-2, and isobutylene, where a C=C vibration occurs at 1650 cm.$^{-1}$. In butadiene, however, the presence of two strong springs connected by a weaker one causes two strong bands, one at 1825 cm.$^{-1}$, the other at 1600 cm.$^{-1}$. The butene-1 was rated at 95 per cent purity. Because of the presence of the weak bands at 1825 cm.$^{-1}$ and 1600 cm.$^{-1}$, it is likely that butadiene is a major impurity in the butene-1.

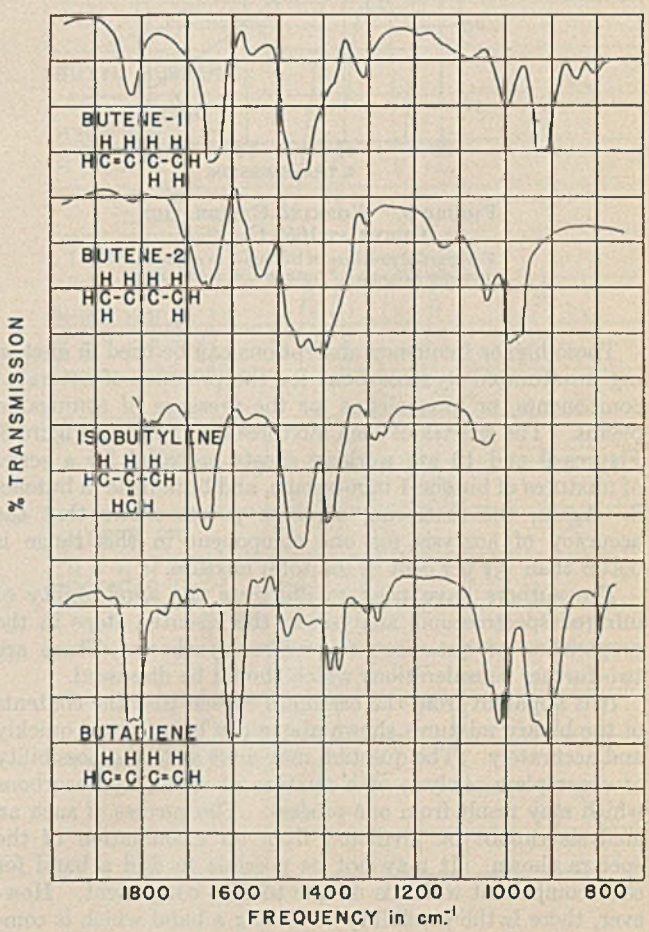


FIGURE 7. INFRARED SPECTRA OF C_4 UNSATURATED HYDROCARBONS

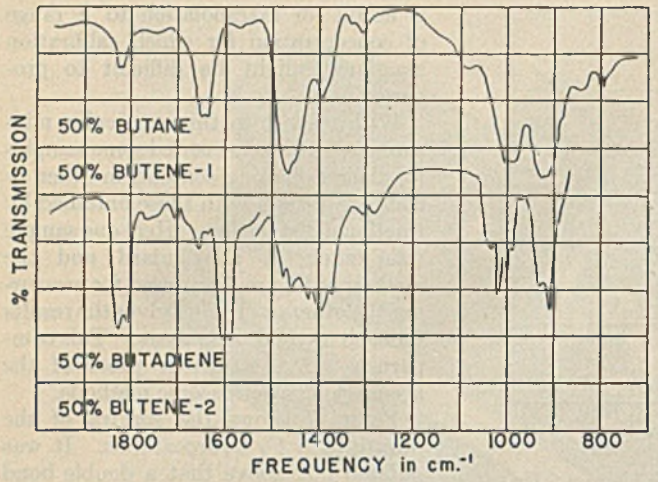


FIGURE 8. INFRARED SPECTRA OF MIXED UNSATURATED C_4 HYDROCARBONS

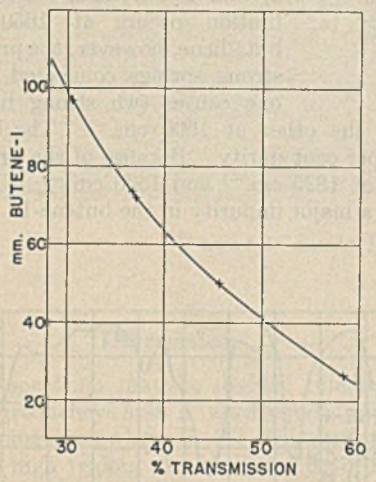


FIGURE 9. WORKING CALIBRATION CURVE AT 1650 CM.$^{-1}$

For partial pressure of butene-1 in approximately 120 mm. of butene-1 + *n*-butane

These higher frequency absorptions can be used in analyzing unsaturated hydrocarbons for the presence of saturated components, or monoolefins for the presence of conjugated olefins. The spectra of such mixtures are shown in Figure 8. Figures 9 and 10 are working sheets prepared for a series of mixtures of butene-1 in *n*-butane, and butadiene in butene-2. Again, the scattering of these points shows that the accuracy of analysis for one component in this range is better than ±1 per cent of the total mixture.

The authors have tried to illustrate the applicability of infrared spectroscopic analysis to the essential steps in the preparation of butadiene for rubber synthesis. There are two further considerations which should be discussed.

It is apparent from the examples chosen that the contents of the binary mixtures shown above can be analyzed quickly and accurately. The question may arise as to the possibility of a complete analysis of a mixture of all C_4 hydrocarbons which may result from one process. The success of such an analysis cannot be predicted from an examination of the spectra shown. It may not be possible to find a band for each component which is unique to that component. However, there is the possibility of finding a band which is common to two components and a band unique to one of these two. The second component can then be found by a difference method. An extension of this method may lead to the necessity of allowing for three or four components at one frequency. Such a situation may tend to lower the over-all accuracy of the analysis to a slight extent. However, this method can be used without appreciable loss of time. In

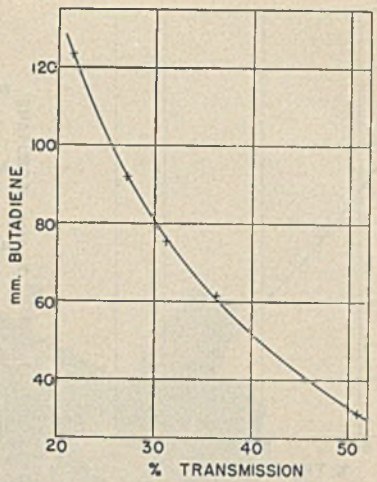


FIGURE 10. WORKING CALIBRATION CURVE AT 1825 CM.$^{-1}$

For partial pressure of butadiene in approximately 150 mm. of butadiene + butene-2

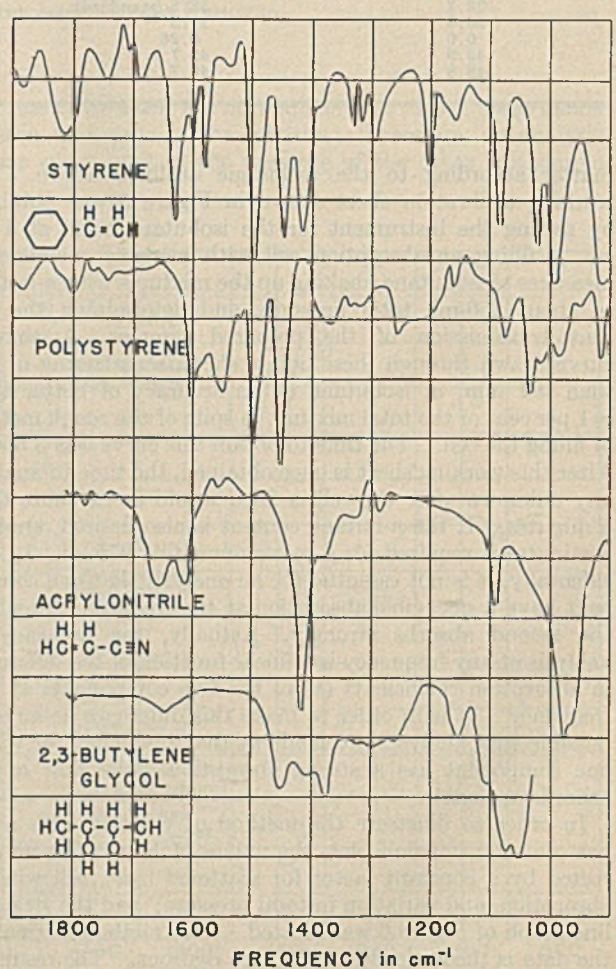


FIGURE 11. INFRARED SPECTRA OF SOME INTERMEDIATES IN PRODUCTION OF SYNTHETIC RUBBER

Samples studied as liquid or solid films

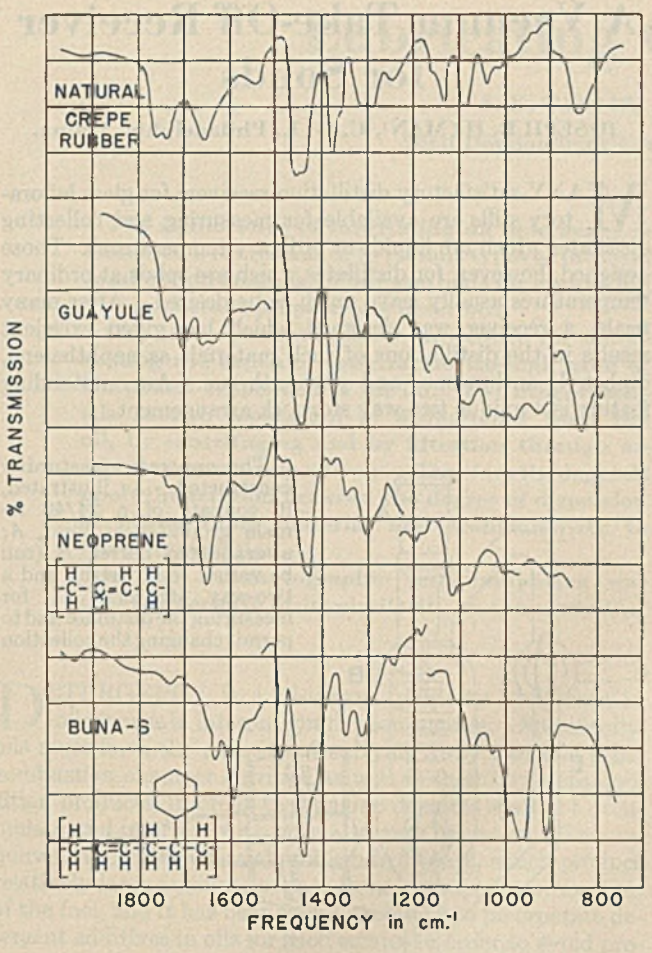


FIGURE 12. INFRARED SPECTRA OF NATURAL AND SYNTHETIC RUBBERS

fact, a routine analysis performed in this laboratory measures the presence of six impurities in a mixture with an accuracy of ±1 per cent of the total mixture.

The second point concerns the fact that the working sheets shown measure the presence of one component between 20 and 80 per cent concentration. It can be seen from the steepening slope above 80 per cent concentration or from a consideration of the exponential nature of Beer's law, that the accuracy of direct analysis for one component falls off as the concentration of that component approaches 100 per cent. In fact, for such high concentrations it is more accurate to analyze for the lesser components and obtain the major component by difference. Below 20 per cent, the accuracy of analysis for a component increases markedly. Consider an analysis at 1180 cm.$^{-1}$ for a 1 per cent concentration of isobutane in *n*-butane. Since the absorption of the *n*-butane is practically negligible, the absorption cell can be filled to atmospheric pressure. Isobutane will be present to 7.6-mm. pressure and can be measured with an accuracy better than ±1 mm. Hence the accuracy for this concentration of isobutane is better than 1 part in 760 or approximately ±0.1 per cent of the total mixture.

The infrared spectra of a few other intermediates in the production of synthetic rubber are shown in Figure 11. The spectrum of polystyrene has been included to illustrate the spectral changes which occur upon polymerization. The monomer styrene bands at 1625 cm.$^{-1}$, the C=C vibration, and at 1410 cm.$^{-1}$, the bending vibration of the vinyl C=H group, have completely disappeared in the polymer. These unique bands are of great value in measuring the amount of monomer present in a monomer-polymer mixture. By extracting time interval samples from a polymerization reaction, the rate of reaction under various experimental conditions can be measured quickly and accurately. The method suggests itself as a possibility for measuring reaction rates in rubber synthesis. A characteristic band of acrylonitrile is the C≡N absorption at 2240 cm.$^{-1}$ (this region is not shown in Figure 12). Since this chemical group is not affected in the synthesis of Perbunan and Hycar, it is found to reappear in the spectra of these rubbers. Again a unique band is offered for quantitative work. The spectrum of 2,3-butylene glycol shows little absorption in the unsaturated region from 1900 cm.$^{-1}$ to 1600 cm.$^{-1}$. An analysis for unsaturated materials in this compound is therefore readily possible.

In order to show that infrared spectroscopy can also be used to derive information concerning some natural and synthetic rubbers themselves, several spectra are shown in Figures 12 and 13. (The formulas for the unit structure of these compounds were taken from Powers, *6*.)

These spectra exhibit marked differences, so that an unknown rubber can be identified by comparison of its spectrum with that of a known sample. Moreover, the spectra offer information concerning the structure of the molecules themselves.

These samples of rubber were the purest ones available to the authors at the time. Their history is not well known, and some oxidation may have occurred which would lead to the presence of spurious bands in the spectrum. The general spectrum, however, must be close to that of the

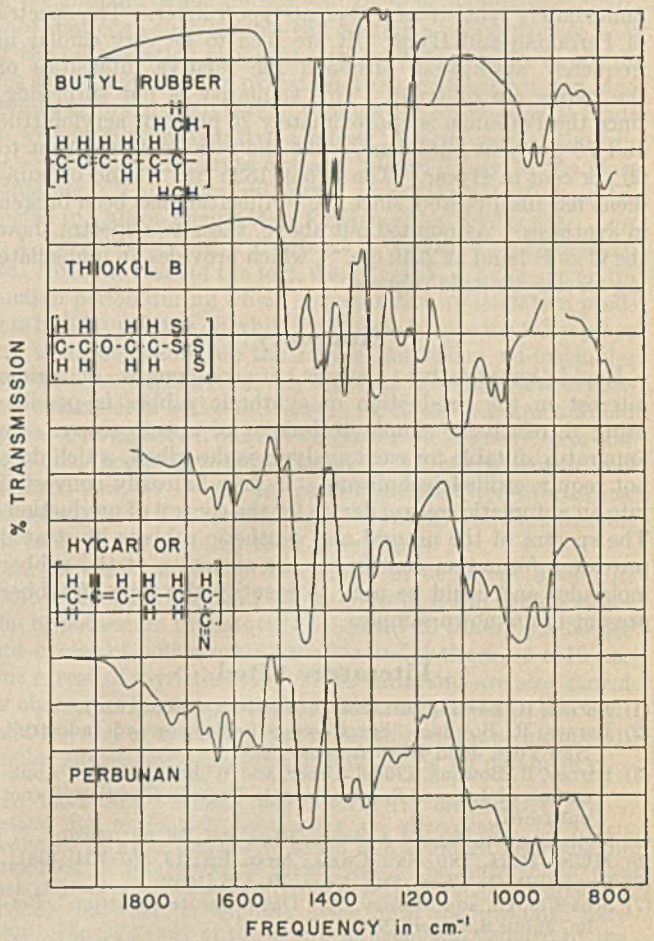


FIGURE 13. INFRARED SPECTRA OF SYNTHETIC RUBBERS

pure substance. The method of preparing samples for study was to form a "gluey" state of the rubber by mixture with a suitable material as carbon tetrachloride or acetylene tetrachloride. A film of this glue was spread on a rock salt plate and the solvent was evaporated.

Strong bands are observed in the spectrum of pure natural crepe rubber at 1750 cm.$^{-1}$, 1610 cm.$^{-1}$, and 1375 cm.$^{-1}$. It was known that this sample was 6 years old. This fact is evident in the spectrum, for the C=O band at 1750 cm.$^{-1}$ shows that considerable oxidation has taken place. The strength of this band could be used to make a quantitative analysis of the amount of oxidation in a sample for correlation with elasticity or abrasion tests. The 1610 cm.$^{-1}$ band is probably caused by a C=C bond while the 1375 cm.$^{-1}$ absorption points to the presence of methyl linkages. The small shoulder at 1360 cm.$^{-1}$ suggests that some of these may be terminal isopropyl groups. Somewhat similar bands are seen in the spectrum of guayule, although the greater number of absorptions in the region from 1600 cm.$^{-1}$ to 1800 cm.$^{-1}$ points to a greater complexity of unsaturated material in the guayule than in the crepe rubber.

The bands at 1650 cm.$^{-1}$ in Neoprene GN and at 1635 cm.$^{-1}$ in Buna S are caused by the presence of the C=C linkage. Neither compound has indication of methyl groups at 1375 cm.$^{-1}$. Butyl rubber and Thiokol B show no absorption in the 1650 cm.$^{-1}$ region, suggesting that there is far less unsaturation present in these samples than in the previous two. Butyl rubber has a peculiar double band at 1375 cm.$^{-1}$. This band splitting is different from that exhibited by an isopropyl group and may be characteristic of a tertiary butyl group. The bands at 1595 and 1495 cm.$^{-1}$ observed in Buna S are characteristic of an aromatic ring. The presence of these bands in an unknown rubber sample would immediately label it as a styrene-type rubber. The spectra of Perbunan and Hycar OR are seen to be very similar in frequency absorption, although the relative intensities of the bands are different. This similarity is not surprising, since the Perbunan is approximately 25 per cent acrylonitrile and 75 per cent butadiene, while the ratio is 40 per cent to 60 per cent in Hycar. The strong 1825 cm.$^{-1}$ band of butadiene has disappeared, since the conjugation has been broken in synthesis. As pointed out above, these two spectra show the C $\equiv$ N band at 2240 cm.$^{-1}$, which provides an immediate tag for acrylonitrile rubbers.

Summary

Rapid quantitative analysis of compounds of prime interest in the production of synthetic rubber is possible using a relatively simple technique of spectroscopy. An apparatus suitable for such analyses is described, which does not require skilled technicians. It may be easily converted into an automatic control device for the control of production. The spectra of the natural and synthetic rubbers illustrated provide information concerning the structure of the rubber molecules and could be used to identify the type of rubber present in unknown samples.

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A Vacuum Take-Off Receiver for Solids

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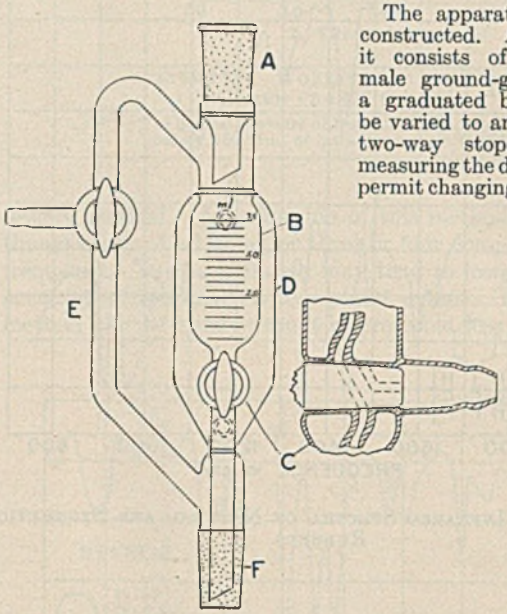
MANY satisfactory distillation receivers for glass laboratory stills are available for measuring and collecting distillates which are liquids at ordinary temperatures. Those designed, however, for distillates which are solids at ordinary temperatures usually leave much to be desired. After many trials, a receiver was designed which has given excellent results in the distillations of such materials as naphthalene, diphenyl, anthracene, and phenanthrene. An outstanding feature is a unique two-way stopcock arrangement.



The apparatus is sturdily constructed. As illustrated, it consists of a 24/40 female ground-glass joint, *A;* a graduated barrel, *B* (can be varied to any size), and a two-way stopcock, *C*, for measuring the distillate and to permit changing the collection flask; a jacket, *D*, enclosing both the barrel and the stopcock, to permit circulation of a suitable liquid at any desired temperature; an equalizing line with a two-way stopcock, *E;* and a 24/40 male joint, *F*, for connecting a collection flask.

Stopcock *C*, as shown in the diagram, has a tapered glass tail for a hose connection blown directly onto the end of the barrel casing. Stopcocks of this type usually have an extended barrel to which the hose is attached or have a small extension out of the top of the barrel casing. The first type is unsatisfactory, as turning the cock causes the hose to twist and often results in breaking the stopcock seal. The second type does not permit the stopcock to be jacketed. By the arrangement illustrated in *C*, the cock turns freely and there is no danger of loss of vacuum.

In actual distillation, when a fraction is to be taken, stopcock *E* is turned so that the lower portion of the equalizing line is isolated from the still; then stopcock *C* is turned to permit release of the vacuum in the collection flask; after a new receiver is put in place, the hose connected to *C* is attached to a vacuum line and the pressure is reduced; the stopcocks then are returned to their proper positions.

The apparatus is not limited to the collecting of solids or to distillations made at reduced pressures. It works equally as well at atmospheric pressure and when the proper cooling medium is circulated it is very satisfactory for use with low-boiling distillates.

Acknowledgment

The author wishes to acknowledge the helpful construction suggestions made by the Ace Glass Company, Vineland, N. J., which made the apparatus to the author's specifications.

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Lubricating Oil Detergency

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The significance of lubricating oil detergency is discussed with reference to various types of internal combustion engines. Four methods for evaluating this important property are described.

One is a chromatographic procedure for determining the depth of penetration through sand of lampblack suspended in the oil. Two other methods involve separation of asphaltenes from the oil, by centrifuging and by filtration through an asbestos mat, respectively. The fourth depends upon the relation between the degree of dispersion of oil-insoluble material and transmissivity to infrared radiation.

In the chromatographic test, lampblack was selected because of its similarity to the sooty fuel deposits in Diesel engines, and the laboratory results are in good agreement with experience on the Caterpillar Diesel. On the other hand, the procedures employing asphaltenes measure the peptization of oil oxidation products and correlate more nearly with ratings of the Lauson and certain other engine tests.

It is concluded that the laboratory tests are of interest in elucidating the nature of deposit formation in engines and, within limits, also provide a useful means for evaluating the detergent characteristics of lubricating oil additives. However, specific application of the tests requires consideration of the type of material deposited in a given engine as well as the operating temperature.

DETERGENCY is a necessary property of lubricating oils for use in internal combustion engines. Specifically, oils must have the capacity of carrying away soot and other combustion chamber detritus, as well as their own decomposition products, in order that engine deposits shall not accumulate and interfere with normal engine operation. This requirement creates a special problem in Diesels, which produce relatively large amounts of soot from incomplete combustion of the fuel, and it has been found necessary to incorporate detergent additives in oils for such service in order to avoid premature engine failure from ring sticking. High-output engines, which operate at high piston and cylinder wall temperatures, also create special detergency problems in regard to lacquering and ring sticking. For low-output engines, operating on clean burning fuels, the inherent detergency of plain mineral oil is usually sufficient to keep the engine clean.

While acceptable detergent additives have been on the market for a number of years, the development of newer and better additives has been hampered by the lack of simple laboratory methods of testing oils specifically for detergent properties. Engines have had to be used for this testing, even in the preliminary stages of testing experimental additives, although their use has simultaneously involved other variables, the least desirable of which has been a wide variation in quantity of material to be dispersed in supposedly comparable tests. Other reasons for desiring small-scale laboratory tests for detergency are small sample requirements, speed of testing, reproducibility, and at least a partial separation of variables. This paper describes four methods that have been found useful for investigating detergency on an empirical basis in this laboratory and the field of usefulness of each.

Detergency is complex and a single test cannot be expected to provide an absolute evaluation that will fit the diversity of conditions that prevail in an engine. From a physicochemical point of view it is a generic term and includes a variety of actions, such as solubilization, emulsification, and base exchange (*2*). The experiments indicate, however, that, in an engine, lubricating oil detergency acts in general to prevent deposition on solid surfaces rather than to remove deposits already formed. The success of a test depends upon a judicious compromise of a number of effects and its usefulness upon an understanding of the limitations of each method. The greatest difference found in the detergency requirements of lubricating oils lies in the fact that the materials to be dispersed vary from soot (derived from fuel) to asphaltenes (derived from oxidized lubricants).

The difference in action of low viscosity index oils (in the range 30 to 60) and high viscosity index oils may be mentioned as an example of the complex nature of lubricating oil detergency. There is generally no difficulty in securing representative samples during the course of oxidation of high V. I. oils in the Indiana oxidation test and there is considerable assurance that each such sample will contain a proportionate part of the oil oxidation products. In contrast, low V. I. oils do not so readily maintain oxidation products in suspension, and serious errors in analysis can result when an attempt is made to withdraw representative samples; a large part of the oxidation products separate out and adhere firmly to the glass container. This same difference in detergent action is reflected in Lauson engine tests for lacquering tendencies. Low V. I. oils generally begin to deposit lacquer on the piston skirt, etc., from the start of the test, while high V. I. oils have an induction period during which concentration of oxidation products builds up in the oil while little or no lacquering takes place and then, depending on the engine conditions, oil-insolubles may even remain at a relatively constant value after the deposition of lacquer is well established. From this picture it would appear that high V. I. oils have a greater detergency than low V. I. oils; however, it is for precisely the reverse reason that undoped low V. I. oils are considered best for lubricating Diesel engines. This anomaly may eventually be explained on the basis of factors other than detergency.

As an example of the complexity of detergent action in another field, McBain and others show that the detergent action of potassium myristate is increased 20 times by a 50 per cent excess of potassium hydroxide and 4 times by a 10 per cent excess of myristic acid. Wide variations are also shown for change in the concentration of the neutral soap, the temperature, and even the method of making up the solutions (*3*). Similar effects probably exist in lubricating oil detergency.

In 1938, Bray, Moore, and Merrill (*1*) described a simple method for evaluating lubricating oil detergency, in which specimens of white cloth were soiled in a standard dispersion of lampblack in the lubricant under test. The soiled cloth was washed in an equal-volume mixture of oil and naphtha (Stoddard solvent) and then in naphtha alone until the washings were clear. The efficiency of the detergent agent was denoted by the grayness of the washed cloth in comparison with a blank test in which no additive was present. No study was made of this test

TABLE I. EFFECT OF HEAT ON OXIDIZED OIL SOLUBILITY

(Centrifugal detergency test)

Procedure	Treatment before Centrifuging: Mixing Hours	Mixing ° C.	Storage[a] Hours	Storage[a] ° C.	Oil-Insolubles, in Reference Oxidized Oil[b] % by weight
A	2	30	..	..	0.81, 0.82, 0.83
B	10 min.	30	1	110	0.66
C	1	110	1	110	0.65, 0.66
D	1	150	2	30	0.62
E	1	150	1	150	0.58, 0.62
F	1	250	1	250	0.86, 0.82

[a] In centrifuge tube just prior to centrifuging, no stirring.
[b] SAE 30 Western oil, oxidized in Indiana oxidation apparatus and composited for these experiments.

in the present investigation, because the dilution with naphtha introduced an undesirable factor of uncertainty.

A simple setting test for detergency was examined in some detail. In this test, oils were shaken up with 0.2 per cent by weight of lampblack and allowed to stand at room temperature, and observations were made of the settling times to give a clear meniscus, a cloudy oil, and a clear oil. The test was applied to thirty oils of comparable viscosity, but of different base stocks and with different additives. There was no correlation of the rating given the oils in this test and the rating obtained with general engine experience. In fact, some very effective detergents were rated poor and a few plain oils without detergent additives were rated excellent.

A static peptization test was also tried without success. In this test, about 0.3 gram of powdered asphaltenes was placed in a small vial and 10 ml. of oil to be tested were gently added. Once each day the vial was gently inverted and inspected to see if any detergent action had taken place. There was no other stirring in the test. A series of oils was tested simultaneously on one rack and, while there was a difference in detergent action, the relative rating had no apparent significance.

The four tests discussed below were found to give useful information.

Centrifugal Detergency Test

The centrifugal detergency test is based upon the use of a high-speed centrifuge to determine the weight percentages of insolubles in oxidized oils without dilution. In order to test for detergency, a reference oxidized oil is run with and without the additive under test. The reduction, if any, of oil-insoluble material that results from the additive is taken as a measure of its detergency value. Since different oxidized oils are used from time to time, the results have relative significance only. The merit of this method of testing for detergency is that it measures one specific aspect only, the ability of the additive to increase the degree of dispersion of the oil-insoluble material beyond an arbitrary but presumably reproducible limit. Only indirectly does it measure the ability of an additive to displace oil-insolubles on a hot metal surface or to clean them off after they have been deposited.

Oil-insolubles are determined without dilution as follows:

The oil sample is thoroughly shaken, and 20 to 25 grams are weighed into a clean, dry centrifuge tube to 0.01 gram and centrifuged for 45 minutes in a de Laval 100-ml. precision centrifuge (10,000 × gravity). This time is satisfactory for almost all oxidized SAE 30 and lighter-viscosity oils; longer times are required for the more viscous oils and for oils that do not settle readily. The centrifuged oil is poured out of the tube in such a manner as to disturb the precipitate as little as possible. The tube is clamped in an inverted position and heated gently with a Bunsen burner, and as much oil as possible is drained from the tube. After cooling to room temperature, 50 ml. of isopentane are added and the precipitate is broken up with a rod or wire and thoroughly dispersed in the wash liquid. The tube is centrifuged for 15 minutes to repack the precipitate. All but about 0.5 ml. of the isopentane is removed with a pipet and the washing procedure is repeated with a second 50-ml. portion of isopentane. The washed precipitate is allowed to stand at room temperature until all visible isopentane has evaporated. The precipitate is then dried at 100° C. for 1 hour, brought to room temperature, and weighed in the centrifuge tube to 0.1 mg. The results are reported as percentage of oil-insolubles on the basis of the oil charged into the centrifuge tube.

The results of heating an oxidized oil to different temperatures before centrifuging for oil-insolubles are given in Table I.

Mild heating to 110° or 150° C. increases the degree of dispersion of oil-insolubles; but higher temperatures, in the range of 250° C., apparently accelerate coagulation, and the quantity of centrifugally separable oil-insolubles is increased. Stirring or storage for 1 hour at room temperature does not alter the increased dispersion brought about by mild heating.

Table II shows the effect of diluting an oxidized oil with various fresh oils on the dispersion of oil-insolubles. Dilution of the oxidized Western oil with an equal quantity of fresh oil of the same kind does not alter the degree of dispersion of the oil-insoluble material at any temperature. In other words, fresh and oxidized Western oils are compatible as regards the precipitation of oil-insoluble materials. In contrast, when fresh Mid-Continent and Pennsylvania oils are added to the oxidized Western oil, the dispersion of the oxidation products is decreased and precipitation of these materials could occur. However, this ill effect is reduced when the diluted oils are heated to 100° C. or above. A similar effect is indicated for the more viscous Mid-Continent oil, but the reproducibility of the test is poor and the result is somewhat uncertain.

As shown in Table III, detergent additives increase the dispersion of oil-insolubles. Except in the case of the low-tem-

TABLE II. EFFECT OF DILUTION WITH FRESH OIL ON SOLUBILITY

(Centrifugal detergency test)

Procedure	Treatment before Centrifuging: Mixing Hours	Mixing ° C.	Storage[a] Hour	Storage[a] ° C.	Oil Insolubles[b]: Reference oxidized oil[b]	Reference Oxidized Oil Diluted with Equal Volume of Fresh[c]: Western 30	Mid-Continent 30	Penn 30	Mid-Continent 60
					Per cent by weight				
A	2	30	.	...	0.81, 0.82, 0.83	0.86, 0.91	0.94, 1.01	1.01, 1.02	
C	1	110	1	110	0.65, 0.66	0.74, 0.78	0.90, 0.93	0.63, 0.89, 0.92	0.77, 0.89
E	1	150	1	150	0.58, 0.62	0.62, 0.63	0.80	0.76, 0.74	0.82

[a] In centrifuge tube just prior to centrifuging, no stirring.
[b] All oil-insolubles given on basis of undiluted oxidized oil.
[c] SAE Western oil, oxidized in Indiana oxidation apparatus and composited for these experiments.

TABLE III. EFFECT OF ADDITIVES ON OIL SOLUBILITY

(Centrifugal detergency test)

Procedure	Treatment before Centrifuging: Mixing Hours	Mixing ° C.	Storage[a] Hour	Storage[a] ° C.	Oil-Insolubles for Reference Oxidized Oil[b] Containing: Nothing	1% low temperature detergent	1% reference detergent	1% high temperature detergent[c]	1% benzene
					Per cent by weight				
A	2	30	...	...	0.81, 0.82, 0.83	0.70	0.84, 0.85	0.86	..
C	1	110	1	110	0.65, 0.66	0.48	0.50, 0.52	0.56	0.66
E	1	150	1	150	0.58, 0.62	0.46, 0.46	0.45, 0.47	0.49	0.64
F	1	250	1	250	0.86, 0.82	1.14, 1.03	0.44, 0.35	0.49	..
G	20 min.	300	...	...	.....	1.16	0.58	0.47	..

[a] In centrifuge tube just prior to centrifuging, no stirring.
[b] SAE 30 Western oil, oxidized in Indiana oxidation apparatus and composited for these experiments.
[c] Added in benzene solution.

perature detergent, mild heating is required to achieve the best results. Some detergents have a distinct temperature limitation and their use may actually increase the quantity of oil-insolubles that separate if they are heated to too high a temperature. Both the reference detergent, which has been found satisfactory for commercial use, and the high-temperature detergent show a useful effect even when heated to 300° C. for a short time. The effect of benzene is included in the table because some additives are blended into lubricating oil in the laboratory in this solvent. As can be seen, its effect is negligible.

The three detergent additives have been tested in several Diesel engines and an effectiveness corresponding to the indications of the solubility data in the table has been noted at high engine temperatures. For example, the ring sticking time in the Fairbanks-Morse Diesel engine operated under severe conditions was increased two times with the reference detergent and ten times with the high-temperature detergent as compared with the base oil; the low-temperature detergent was not tried in this engine.

Table IV. Infrared Detergency Test

	Fresh Oil	Oxidized Oil A	Oxidized Oil B	Oxidized Oil C
Oil-insolubles, % by weight, centrifuge				
Plain	...	.....	0.41	0.79
With 1% high-temperature detergent	...	.....	0.19	0.60
Infrared transmission, % 9700 Å.[a]				
Plain	100	9.4, 7.8	14.5	5.0
With 1% high-temperature detergent	97	31.0, 41.0	29.7	5.6

[a] Not centrifuged.

Infrared Detergency Test

Since the degree of dispersion of a solid suspended in a liquid is associated with the ability to transmit light, the light-absorption curves of a few fresh and oxidized oils were examined in a Coleman electric photometer. They all showed a pronounced transmission band at 9700 Å. and this wave length was arbitrarily selected for examining the effect of additives on the dispersion of oil-insolubles.

In Table IV are given results obtained with one additive on three laboratory oxidized oils, chosen at random, and the original fresh oil. In all cases, the infrared light transmission of the oxidized oil was increased by simply adding a detergent to the oil sample after oxidation, whereas the transmission of the fresh oil was slightly reduced. The effect of this same additive in the centrifugal detergency test on two of these oils is included for comparison. It is concluded that transmission at this wave length is associated with an increased degree of dispersion of the insolubles involved and it would appear that the ultimate particle size achieved by the dispersive action of the detergent is very small. This follows from the fact that transmission of light is a function of scattering as well as true absorption. Assuming that absorption is negligible because of the selection of the wave length and that the scattering of light depends on particle size only, it follows that the particle size must have been reduced to a value commensurate with the wave length of light used in this test. A few other additives were examined by the infrared detergency test and all showed similar increases in light transmission when added to oxidized oil.

While the infrared detergency test has some interesting possibilities and should prove useful for securing information that cannot be obtained in other ways, it was abandoned in favor of the centrifugal detergency test because the results of the latter could be interpreted with less uncertainty. Both tests presumably measure similar actions of detergents.

Wood River Detergency Test

A detergency test was investigated and developed by F. L. Johnston, J. B. Harkness, and G. A. Siegelman of the Wood River Research Laboratories of the Shell Oil Company, Incorporated. In it, the percentage of added asphaltenes that a given oil (and additive) will carry through an asbestos filter is taken as a measure of the detergency of the oil.

Experimental Procedure. To a 20-gram sample of the oil in a test tube (15 × 150 mm.) are added 2 ml. of benzene solution containing 20 mg. of asphaltenes. The sample is shaken vigorously, placed in an oil bath, and aged at 150° C. for 16 hours. The sample is removed, shaken again, allowed to cool to room temperature, and filtered through a dried and tared Gooch crucible with an asbestos pad. The precipitate is then washed exhaustively with 60–80 naphtha to remove the oil, and the crucible is dried for 1 hour at 110° C. (230° F.), allowed to come to room temperature, and again weighed. The percentage peptization is computed from the difference between the observed gain in weight and that which would occur if there were no peptization, using the following equation:

$$\% \text{ detergency} = 100\ 1 - \left(\frac{\text{weight of asphaltenes in crucible}}{\text{weight of asphaltenes added}}\right)$$

The asphaltenes are prepared by diluting an oxidized oil with naphtha, filtering and washing the precipitate, and removing the asphaltene fraction by benzene extraction. For special investigations it may be desirable to vary the procedure and add solid asphaltenes free of a solvent. Great care must be taken in removing the benzene because large variations in asphaltene solubility can result from even mild overheating. A practical procedure is to weigh out about 20 mg. of dry asphaltenes to 0.1 mg. and add 1000 times this weight of the fresh oil to be tested.

Since the quality of asphaltenes will vary greatly from one preparation to another, the results can have relative significance only. If metal salts are present in the asphaltene preparation, certain anomalies may be encountered in testing some additives and, for that reason, oil samples oxidized in the laboratory in the absence of metals are recommended as a source of asphaltenes for this work.

Table V. Effect of Naphthenate Additions

(Wood river detergency test)

Oil	Detergency Rating, %
Plain mineral oil, Mid-Continent SAE 10	22
Oil + 0.5% by weight sodium naphthenate	49
Oil + 0.5% by weight barium naphthenate	78

Table VI. Comparison of Laboratory Rating and Engine Lacquer Rating

(Wood River detergency test)

Detergent	Concentration % by weight	Detergency %	Lacquer Reduction in Lauson Engine %
A	1.0	64	89
B	2.0	58	50
C	2.0	40	38
D	1.4	14	13

Additional agitation during the aging period has been found to produce no significant effect. The substitution of a nitrogen atmosphere over the sample during the aging likewise has no effect, although a loss of detergency, probably through the destruction of the additive as well as an increase in asphaltenes, may result if oxygen is bubbled through the sample during the 16-hour aging period. In general, the effectiveness of a detergent increases up to 0.5 or 1 per cent concentration, after which the detergent action is constant for a given additive and is then mainly dependent upon the solubility distribution of the asphaltenes used.

In Table V is shown the increase in detergency secured by the addition of a sodium naphthenate and a barium naphthenate to a SAE 10 oil. Table VI shows the improvement obtained in the detergency rating in this test by the addition of several detergents to one base oil and the corresponding improvement in the Lauson engine lacquer rating.

Chromatographic Detergency Test

The chromatographic detergency test was developed here following a verbal report that General Motors were using a filtration test to measure the ability of an oil to hold soot and other engine deposits in suspension. In their test, a dirty used oil that remained dirty after filtering through a Gooch crucible filled with asbestos was given a "one star" rating. In the same way by passing the dirty filtrates through additional clean crucibles, the oil was given up to a maximum of a "three star" rating.

In order to provide a more discriminating test for development research and to reduce the maximum of three filtrations to one, a glass tube was substituted for the Gooch crucible in the General Motors test. A number of filtering media were studied, using lampblack suspensions in oils known to have widely different detergent activities. Lampblack was chosen for the early experiments because of its similarity to the soot of Diesel engines and was retained because it gave useful correlations.

The experiments showed that the depth of penetration of the lampblack could be readily observed and that the penetration was greatest for those oils rated most detergent by general Diesel engine experience. For a given oil, the depth of penetration varied greatly from one filtering medium to another but appeared to be largely dependent upon the coarseness of the packing. The relative ratings of the representative oils tested were roughly the same in different filtering media.

The actual limit of lampblack penetration in a homogeneous filter bed is not always clear, especially in the longer penetrations. It does not terminate abruptly, but tapers off in a poorly defined zone of diminishing intensity.

Table VII. Relative Rating of Mineral Oils

(Chromatographic detergency test)

Oil	Number of Darkened Rings SAE 10	SAE 30	SAE 70
Mid-Continent	1	3, 4	6, 6
Western	2	3, 2, 3	2, 3

In order to give a clear index of penetration, disks of filter paper are placed in the filtering tube at regular intervals. The edges of these papers are usually either distinctly blackened or not visibly altered by the lampblack, and the number of filter paper disks that have been darkened at the end of a test is taken as a measure of detergency. When tests are to be run at 150° C. or higher, layers of calcium carbonate powder are substituted for the paper, since paper chars when heated. The calcium carbonate-layered tubes are more difficult to prepare and unless the calcium carbonate is very carefully washed free of fines, undiluted oils cannot be filtered at room temperature.

The chromatographic test for detergency is carried out in Pyrex tubes, 38 cm. long by 7 to 8 mm. in inside diameter and constricted slightly at the bottom end. These are fitted with a cotton plug, on which are packed 10 or 12 alternate layers of sand and filter paper disks. Common sea sand is washed, ground, and separated to a particle size range of 15 to 150-micron average diameter; 0.4 ml. is used in each layer. Whatman's No. 4 filter paper is cut with a cork borer into disks approximately 8.4 mm. in diameter. The packed tubes are adjusted for moisture content for 2 hours just prior to making a test, by drawing through them air that has first been passed through 22 per cent potassium hydroxide solution and an adequate spray trap. To 10 grams of oil to be tested are added 20 mg. of Monsanto Germantown Bear Brand lampblack. The mixture is stirred vigorously for 5 minutes at room temperature and 1 ml. is measured and transferred into the filtering tube by means of a hypodermic syringe. The connection between the potassium hydroxide wash bottle and filtering tube is broken only long enough to add the sample to the latter. A vacuum of 60 to 67.5 cm. (24 to 27 inches) is maintained in the filtering tube for the duration of the test, 16 to 20 hours. The number of darkened filter paper edges is recorded at the end of the test.

Table VIII. Relative Rating of Detergent Additives

(Chromatographic detergency test)

Additive	Test Rating, No. of Darkened Rings	Caterpillar Engine Rating
None	2, 3	Poor
I	9, 11	O. K.
II	9	O. K.
III	7, 9	O. K.
IV	8, 8, 8	O. K.
V	7	O. K.
VI	6, 6	Insufficient detergency
VII	5, 4, 6	Insufficient detergency
VIII	4, 3	Poor
IX	6	Poor

Naphtha washing after oil filtration and before observing lampblack penetration is optional, but results between washed tubes cannot be compared with those from tubes that have not been washed with naphtha. Naphtha washing is usually desirable for the more viscous oils, SAE 40 and higher.

The calcium carbonate powder for high-temperature tests should be freed of fines by washing with naphtha and decanting repeatedly until all material that does not settle rapidly has been removed. Layered tubes of this sort are most readily filled by washing the solids into the tube in naphtha suspension and settling each layer with suction before adding the next. It is preferable to maintain a layer of naphtha above the packed solids in the tube until the packing is complete, after which the tubes are sucked dry and used without water conditioning. The tube with filter paper disks is packed dry with thorough tamping.

Of the variables that influence the penetration of carbon black in the filtering tube, water content has been found to be the most important for filtration at room temperature. While other factors, such as particle size of the filtering medium, relative quantities of materials used, degree of vacuum, etc., regulate the extent of penetration, the relative rating of a series of oils and additives can be altered by varying the moisture content of the tube packing. Water added to the suspension of lampblack in the oil also has a pronounced and sometimes erratic effect. Precautions for maintaining the moisture content of the filtering tubes constant are included in the described procedure and care should be exercised that water is not mixed in the oil. The observation that water affects the results of this test is important, because it is believed that traces of water have a similar important effect on lubricating oil detergency in engines.

Violent agitation, such as is produced by ultrasonic vibration of the lampblack suspension, increased the penetration of one sample from 7 to 11 rings. The maximum effect of an additive is generally reached at 0.5 to 1.0 per cent by weight concentration. With additive concentration at usual values, reduced penetrations may occur when the lampblack concentration is raised above 0.2 to 0.5 per cent by weight, although some additives can tolerate up to 2.0 per cent by weight lampblack with no pronounced reduction of effectiveness. Some additives begin to lose their effectiveness at 170° C. while others are fully effective, as measured by this test, up to 250° C.

Figure 1 illustrates the appearance of the filtration tubes in the chromatographic detergency test. This series of tubes was used to examine different batches of a particular additive. Batch 3 showed a definite superiority over the first two.

In Table VII are given the relative ratings of different grades of Western and Mid-Continent oils in this test. The higher rating given the more viscous Mid-Continent oils is not reflected in Diesel engine performance of these oils. The low rating given all three Western oils indicates that the relative

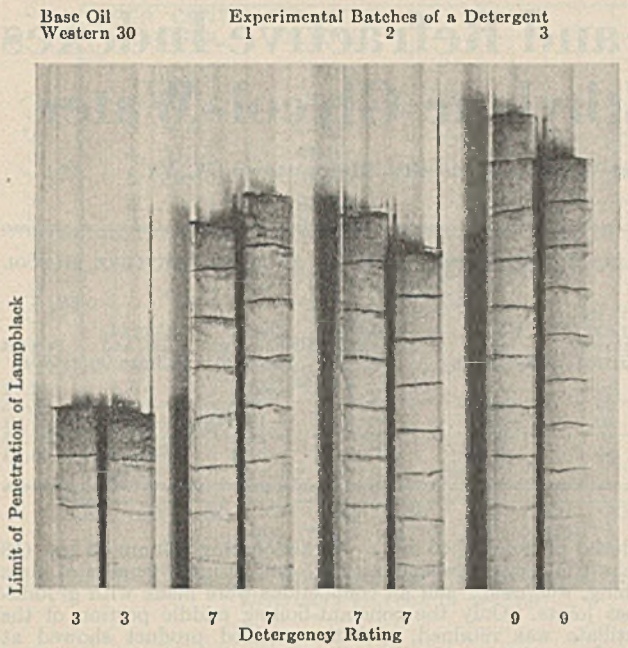

FIGURE 1. APPEARANCE OF CHROMATOGRAPHIC DETERGENCY TEST

Unequal height of sand layers in different tubes is caused by variations in tube diameters.

rating is not dependent upon viscosity differences as such. While the results of the chromatographic detergency test may be accepted as revealing a real difference in detergent properties of the oils examined, the results indicate that a high rating does not necessarily guarantee satisfactory Diesel engine performance. However, when a high rating is secured by an additive in a base oil of low rating, considerable reliance can be placed on improved engine performance as regards detergency, and the test is proposed only for this purpose. The data in Table VIII illustrate this use of the test and give an indication of the engine rating of the oils tested.

TABLE IX. COMPARISON OF CHROMATOGRAPHIC AND WOOD RIVER DETERGENCY TESTS

Additive	Chromatographic Rating, Darkened Rings		Wood River Rating	
	Eastern oil SAE 10	Western oil SAE 30	Eastern oil SAE 10	Western oil SAE 30
			%	%
None	1	1	51, 47	79, 83
1.4% detergent A	4, 2	6, 6, 5	−277	−100
1.0% low-temperature detergent	...	7, 6	...	−260, −290
2% detergent B	5, 6	7, 7	−14	2
1% detergent C	8, 7	7, 6, 8	81	75
10% voltol oil	...	6, 9	..	84
2.5% reference detergent	...	8, 8	..	76

Comparison of Wood River and Chromatographic Detergency Tests

The relative ratings of oils and additives are not the same in the chromatographic and Wood River detergency tests. Table IX gives a comparison of these two tests for several additives in two base oils. Only the last three additives are rated good in both tests, while the other three are rated fair to good in the chromatographic test and are given negative ratings in the Wood River test. The negative rating is taken to mean that additives as well as asphaltenes were removed on the asbestos filter in the latter test. Incidentally, additives that are effective as detergents in critical parts of an engine and yet aid in accumulating insolubles on a filter would be advantageous in engines, provided that the additive is not too quickly removed from the oil by the latter activity.

The lack of agreement in the ratings given by the two tests focuses attention on the necessity of considering two distinct types of detergency requirements. Indications are that the chromatographic detergency test correlates more nearly with the detergency requirements of the Caterpillar Diesel, while the Wood River test is more closely related to the lacquer rating of the Lauson engine. This difference appears more understandable when the predominant types of insolubles produced in the two engines are compared with the dispersed materials in the two tests.

Interchange of dispersed materials in the two tests provided no useful results. When lampblack is substituted for asphaltenes in the Wood River test, all detergency ratings are zero or negative. When asphaltenes are substituted for lampblack in the chromatographic test, the depth of penetration is poorly defined because the asphaltenes develop only mildly discolored rings at best. The complex character of the asphaltenes probably also contributes to the indifferent penetrations because of the range of specific penetrations involved.

While the laboratory detergency tests indicate a difference of detergent requirements for different classes of engine deposits, further studies must be made under conditions corresponding more closely to those parts of an engine that suffer most from a lack of detergency, in order that the questions raised may be answered more directly and reliably.

Conclusion

Four methods of evaluating the detergency of lubricating oils measure the ability of a lubricant to disperse or peptize oil-insoluble materials such as oxidized lubricants or soot. It is possible to use these tests profitably to predict the cleanliness-maintaining action of an oil in engines, providing consideration is made of the type of material most likely to be deposited as well as the temperature conditions under which the engine operates. In addition to their direct application to engines, which requires further correlation, they are useful tools in oil research and give quantitative data on many speculations made in the past on the detergent aspects of lubricating oils.

Acknowledgment

Grateful acknowledgment is made to H. C. Kennedy for his careful execution of the laboratory work described in this paper.

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CORRECTION. In the article entitled "Preparation of Diphenylthiocarbazide and Diphenylthiocarbazone" [IND. ENG. CHEM., ANAL. ED., 14, 953 (1942)], the last sentence in the first paragraph on page 954 should read as follows: "The yield is about 15 grams, 50 per cent of the theoretical based on the phenylhydrazine taken."

OLIVER GRUMMITT

Freezing Points, Densities, and Refractive Indexes of the System Glycerol–Ethylene Glycol–Water

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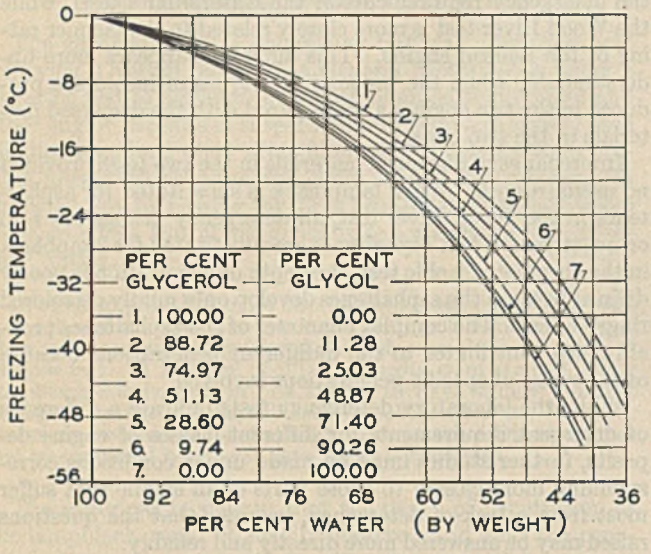

FIGURE 1. FREEZING TEMPERATURES FOR BLENDS OF GLYCEROL–ETHYLENE GLYCOL IN WATER

SINCE glycerol and ethylene glycol are both used extensively in the automobile antifreeze industry, the freezing points of aqueous mixtures of these compounds should be of interest. The following material gives the freezing points of mixtures of these three compounds, and an easy and exact method of analysis of any mixture of them.

Preparation of Solutions

The ethylene glycol was furnished by the Carbide and Carbon Chemicals Corporation and was purified by distillation at a reduced pressure of 40 mm. A fractionating column 45 cm. (18 inches) in length, packed with 7.5-cm. (3-inch) lengths of glass tubing, was used; and all connections were made with ground-glass joints. Only the constant-boiling middle portion of the distillate was retained, and the purified product showed at 25° C. an absolute density of 1.1101 and a refractive index of 1.4300.

The glycerol used was Baker's analyzed, C. P. material. It was purified (from water) by double distillation at a reduced pressure of 3 mm. Only 20-cm. (8-inch) fractionating column was used in order to prevent decomposition by superheating. Bumping was effectively eliminated in both these distillations by filling the distilling flask to the level of the liquid with coarse-fiber Pyrex brand glass wool, and using a large distilling flask (3 liters) heated uniformly by an oil bath. The purified glycerol showed at 25° C. an absolute density of 1.2580 and a refractive index of 1.4720. Comparison with the density tables given in the International Critical Tables (*6*) and the Bosart and Snoddy tables (*1*) showed this glycerol to be 100 per cent pure. The glycerol and ethylene glycol were kept in 2-liter Erlenmeyer flasks, equipped with soda-lime tubes and siphon tubes. The liquid was forced out through the siphon tube by means of dried air and was thus exposed to undried air for only a very short time.

Ethylene glycol–glycerol mixtures were prepared by weighing to the closest milligram varying amounts of glycerol (10 to 125 grams) from

TABLE I. FREEZING POINTS OF AQUEOUS ETHYLENE GLYCOL SOLUTIONS (° C.)

% by Weight of Ethylene Glycol	Curme and Young (*3*)	Bureau of Standards (*5*)	Olsen, Brunjes, and Olsen (*10*)	Conrad, Hill, and Ballman (*2*)	Present Work
10	−2.5	−2.9	−3.3	−3.6	−3.71
20	−7.7	−9.7	−7.8	−8.3	−8.45
30	−13.9	−17.6	−13.5	−14.4	−14.91
40	−23.3	−26.0	−22.1	−22.6	−24.05
50	−33.9	−37.0	−35.4	−34.6	−36.11

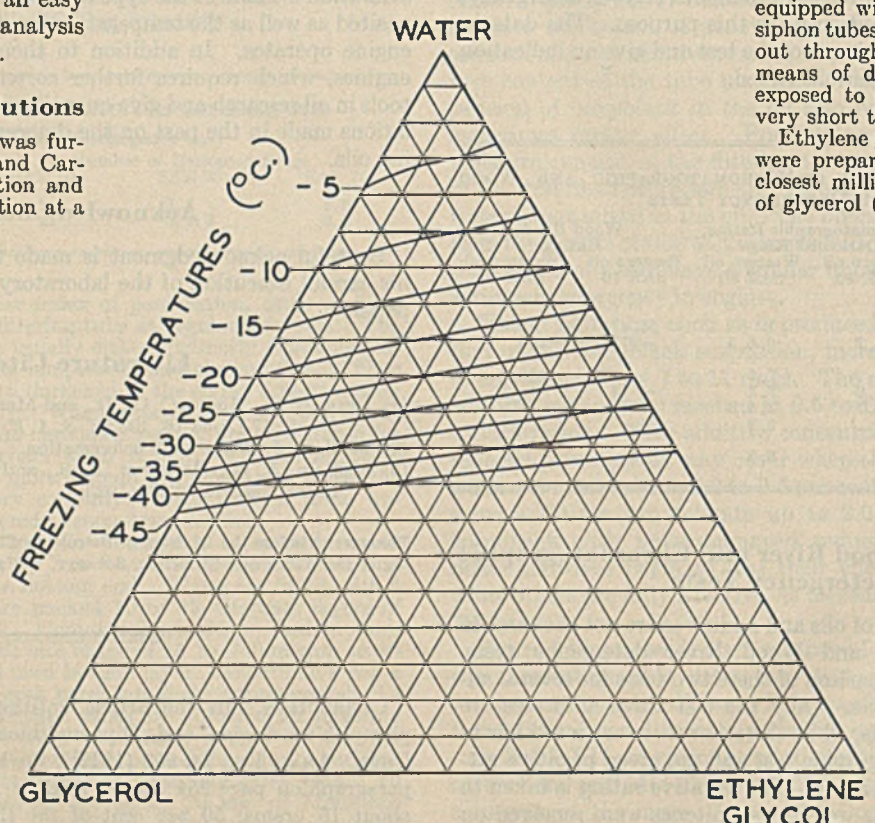

FIGURE 2. FREEZING POINT ISOTHERMS

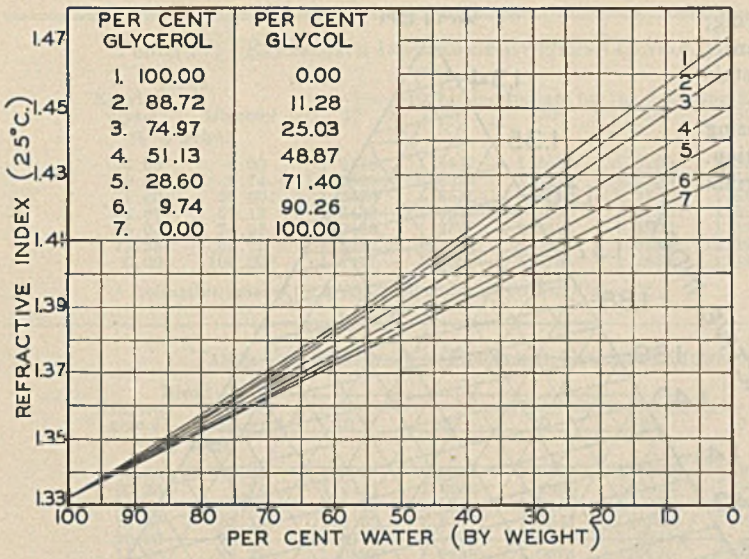

FIGURE 3. REFRACTIVE INDEXES AT 25° C. FOR BLENDS OF GLYCEROL–ETHYLENE GLYCOL IN WATER

TABLE II. FREEZING POINTS OF AQUEOUS GLYCEROL SOLUTIONS (° C.)

% by Weight of Glycerol	Lane (8)	Bureau of Standards (6)	Olsen, Brunjes, and Olsen (10)	Feldman and Dahlstrom (4)	Present Work
10	−1.6	−1.7	−2.3	−1.9	−1.99
20	−4.8	−4.8	−5.5	−5.4	−5.21
30	−9.5	−9.4	−9.8	−9.7	−9.92
35	−12.2	−12.3	−12.4	..	−12.65
40	−15.4	−15.6	−15.7	−15.6	−15.93
45	−18.8	−19.4	−18.6	..	−19.90
50	−23.0	−25.8	−23.8	−23.6	−24.55
55	−28.2	..	..	..	−30.40
60	−34.7	..	−37.2	−35.5	−37.90

a weighing buret. Ethylene glycol was then added in a similar manner to give solutions having approximate ratios of glycol to glycerol of 9 to 1, 7.5 to 2.5, 5 to 5, 2.5 to 7.5, and 1 to 9. Quantities of these blends were then weighed into ground-glass stoppered Erlenmeyer flasks and definite weights of water added from a standard, calibrated buret to make solutions containing approximately 10, 30, 50, 70, and 90 per cent of each blend. For the freezing point curves, it was necessary to weigh out two more samples for each blend at approximately 60 per cent blend in order to establish the eutectic point.

Freezing Point Determination

The freezing point apparatus used in this work consisted essentially of a 200-junction, copper-constantan thermocouple (one hundred junctions at each end), one end immersed in an ice bath and the other end in the solution, the freezing point of which was being determined. Provision for attaining equilibrium at each end was made by appropriate means of stirring, and the voltage developed was measured by means of a Leeds & Northrup Type K potentiometer and a Leeds & Northrup Type C galvanometer. The use of a thermocouple containing this number of junctions permitted an accuracy well within the limits of errors of the calibration values, using equipment generally available in most chemical laboratories. The thermocouple was calibrated by means of the following highly purified substances: water (0° C.), aniline (−5.98° + C.), carbon tetrachloride (−22.85° C.), chlorobenzene (−45.20° C.), and chloroform (−63.45° C.). The accuracy of these values vary from ±0.04° at 0° to ±0.06° at −65.0° C. The liquids being studied were cooled by the use of a bath of solid carbon dioxide and ethyl alcohol, separated from the freezing point tube containing the liquid by means of a Dewar flask. For the lower freezing points this Dewar flask was replaced by an ordinary freezing point tube, thus furnishing an air jacket.

Both cooling and heating curves were run, and three check determinations were made on both the freezing point and the melting point. The exact freezing points and melting points were obtained by extrapolation in the manner explained by Mair, Glasgow, and Rossini (9). The freezing point values differed from the melting point values by 0.02° to 0.05°, the latter figure applying to the lower freezing points.

Freezing Point Data

Tables I and II compare the values obtained in the present work with the freezing points obtained by other investigators for aqueous solutions of ethylene glycol and aqueous solutions of glycerol.

The values obtained in the present work are in good agreement with the average of the values obtained by the other investigators. The values for the lower temperatures should be much more accurate than the values obtained by the other investigators, because visual observation does not enter into the determination of the freezing point with the apparatus used in the present work. The increased accuracy due to this independence from visual observation is due to the fact that mixtures of ethylene glycol and glycerol with water get extremely viscous at low tem-

TABLE III. FREEZING POINTS OF ETHYLENE GLYCOL–GLYCEROL BLENDS IN AQUEOUS SOLUTIONS

Blend		Freezing Points for the Following % by Weight of Total Blend in Aqueous Solution					
Ethylene glycol	Glycerol	10	20	30	40	50	60
% by weight		° C.	° C.	° C.	° C.	° C.	° C.
100.00	0.00	−3.71	−8.45	−14.91	−24.05	−36.11	...
90.26	9.74	−3.54	−8.26	−14.57	−23.46	−35.35	...
71.40	28.60	−3.24	−7.71	−13.83	−22.21	−33.59	−49.84
48.87	51.13	−2.99	−7.10	−12.80	−20.50	−30.71	−46.12
25.03	74.97	−2.81	−6.64	−11.72	−18.59	−28.05	−43.29
11.28	88.72	−2.57	−6.18	−10.88	−17.20	−26.09	−40.45
0.00	100.00	−1.99	−5.21	−9.92	−15.93	−24.55	−37.90

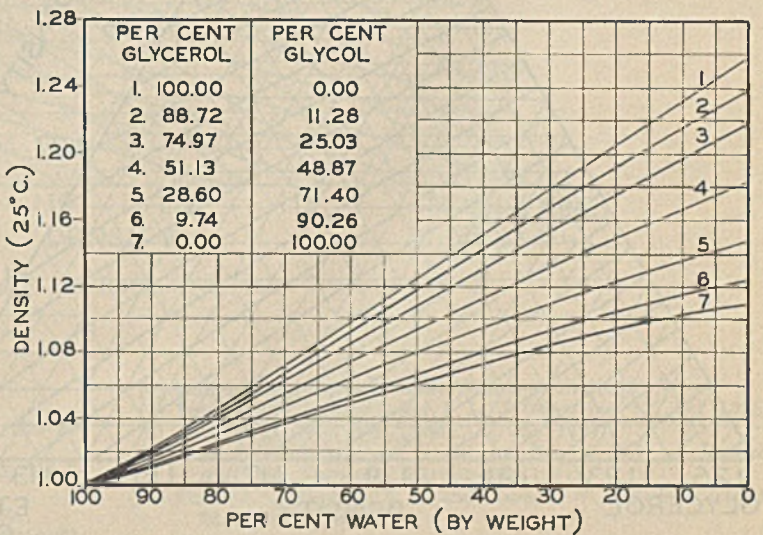

FIGURE 4. DENSITIES AT 25° C. FOR BLENDS OF GLYCEROL–ETHYLENE GLYCOL IN WATER

peratures and tend to trap air bubbles during stirring; this causes the solution to take on a milky appearance, making it very difficult to determine whether any crystals are present or not.

Figure 1 shows the curves obtained by plotting the freezing point against the per cent of total blend in aqueous solution. From these curves, values were taken for plotting isothermal curves on a triangular coordinate for every 5° as shown in Figure 2.

Table III lists the freezing points for compositions covering practically the whole range investigated. These data were obtained from the freezing point–composition curves.

Density and Refractive Index Data

The refractive indexes and absolute densities (all weighings reduced to vacuum and water of maximum density taken as unity) were determined at 25° C. using an Abbe refractometer and Leach pycnometers. Figures 3 and 4 show the curves obtained by plotting, respectively, the refractive index and density against the per cent of total blend in aqueous solution. From these curves, values were taken for plotting the constant refractive index and the constant density curves on the triangular coordinates shown in Figures 5 and 6.

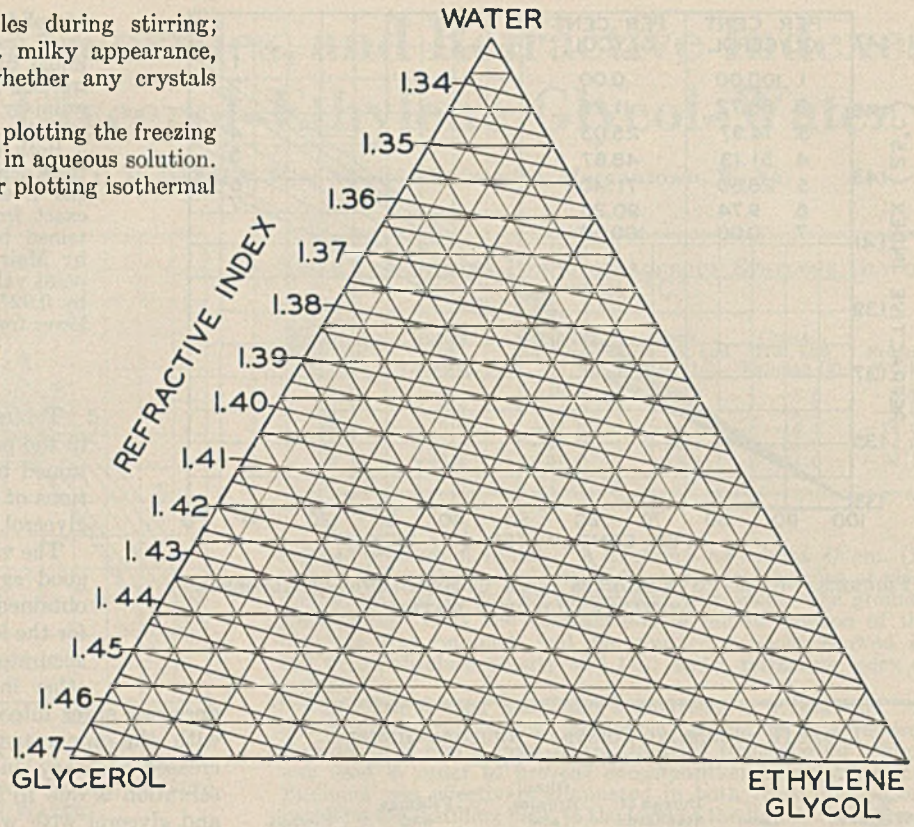

FIGURE 5. CONSTANT REFRACTIVE INDEX CURVES AT 25° C.

Tables IV and V list the refractive indexes and densities for compositions covering the whole range of this system. These data were obtained from the refractive index–composition and the density-composition curves.

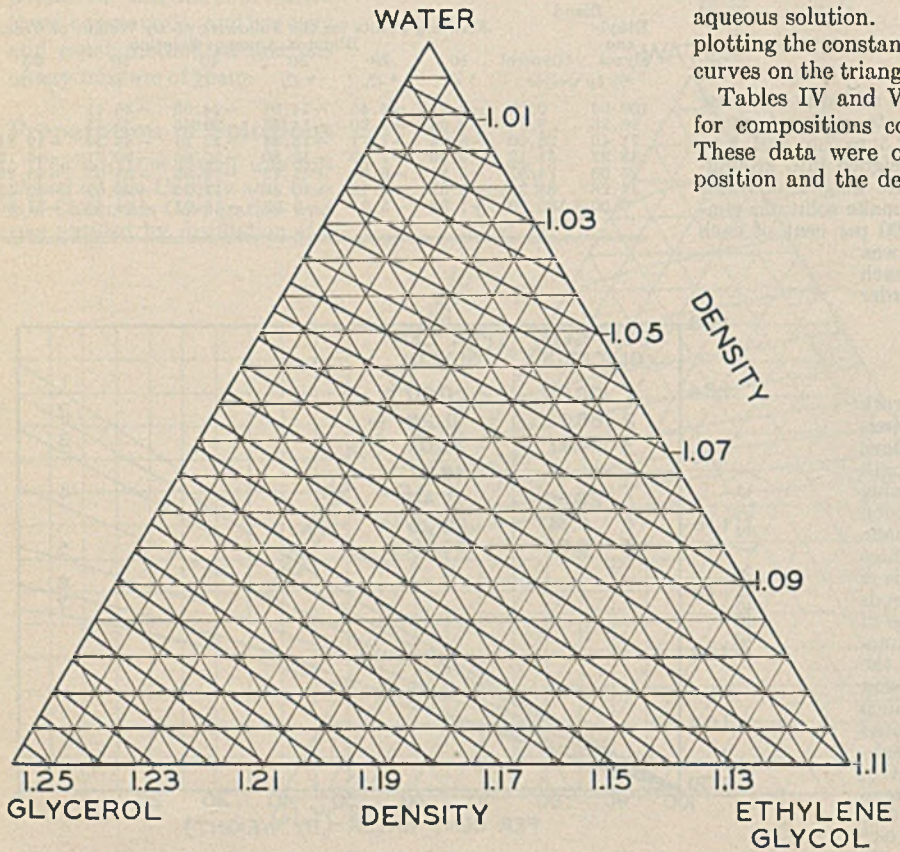

FIGURE 6. CONSTANT DENSITY CURVES AT 25° C.

Figure 7 shows both the constant density curves and the constant refractive index curves. These curves intersect each other at an angle of approximately 30°. This makes it possible to determine accurately the amount of glycerol and ethylene glycol in an aqueous solution by merely obtaining the density and refractive index for that solution. The point of intersection of the density and refractive index curves for that solution gives the composition immediately. This method of analysis is more convenient, easy, and exact than any chemical means hitherto developed, and should be of great value in determining the ethylene glycol content (as an impurity) in glycerol, for the hygroscopic nature of both of these substances makes it a ternary system.

TABLE IV. REFRACTIVE INDEXES OF ETHYLENE GLYCOL–GLYCEROL BLENDS IN AQUEOUS SOLUTIONS AT 25° C.

Blend: Ethylene glycol, % by weight	Blend: Glycerol, % by weight	Refractive Indexes for the Following % by Weight of Total Blend in Aqueous Solution: 10	20	30	40	50	60	70	80	90	100
100.00	0.00	1.3416	1.3517	1.3621	1.3723	1.3828	1.3927	1.4025	1.4118	1.4210	1.4300
90.26	9.74	1.3420	1.3522	1.3628	1.3736	1.3843	1.3946	1.4045	1.4148	1.4245	1.4343
71.40	28.60	1.3425	1.3533	1.3643	1.3757	1.3871	1.3981	1.4092	1.4202	1.4311	1.4420
48.87	51.13	1.3432	1.3547	1.3666	1.3786	1.3904	1.4026	1.4147	1.4269	1.4393	1.4516
25.03	74.97	1.3438	1.3558	1.3683	1.3809	1.3939	1.4073	1.4209	1.4343	1.4482	1.4624
11.28	88.72	1.3442	1.3563	1.3693	1.3826	1.3960	1.4100	1.4243	1.4388	1.4529	1.4670
0.00	100.00[a]	1.3447	1.3569	1.3701	1.3838	1.3977	1.4123	1.4273	1.4427	1.4568	1.4722

[a] Refractive indexes for glycerol taken from Iyer and Usher (7).

TABLE V. DENSITIES OF ETHYLENE GLYCOL–GLYCEROL BLENDS IN AQUEOUS SOLUTIONS AT 25° C.

Blend: Ethylene glycol, % by weight	Blend: Glycerol, % by weight	Densities for the Following % by Weight of Total Blend in Aqueous Solution: 10	20	30	40	50	60	70	80	90	100
100.00	0.00	1.0097	1.0231	1.0367	1.0495	1.0619	1.0733	1.0848	1.0946	1.1032	1.1101
90.26	9.74	1.0111	1.0259	1.0405	1.0544	1.0682	1.0813	1.0937	1.1051	1.1157	1.1250
71.40	28.60	1.0128	1.0300	1.0469	1.0631	1.0791	1.0952	1.1100	1.1239	1.1379	1.1521
48.87	51.13	1.0150	1.0349	1.0541	1.0738	1.0929	1.1110	1.1289	1.1478	1.1670	1.1832
25.03	74.97	1.0172	1.0406	1.0629	1.0857	1.1078	1.1302	1.1527	1.1755	1.1976	1.2187
11.28	88.72	1.0193	1.0438	1.0674	1.0921	1.1165	1.1411	1.1666	1.1918	1.2161	1.2399
0.00	100.00[a]	1.0207	1.0453	1.0707	1.0971	1.1238	1.1511	1.1784	1.2055	1.2320	1.2580

[a] Densities for glycerol taken from Bosart and Snoddy tables (1).

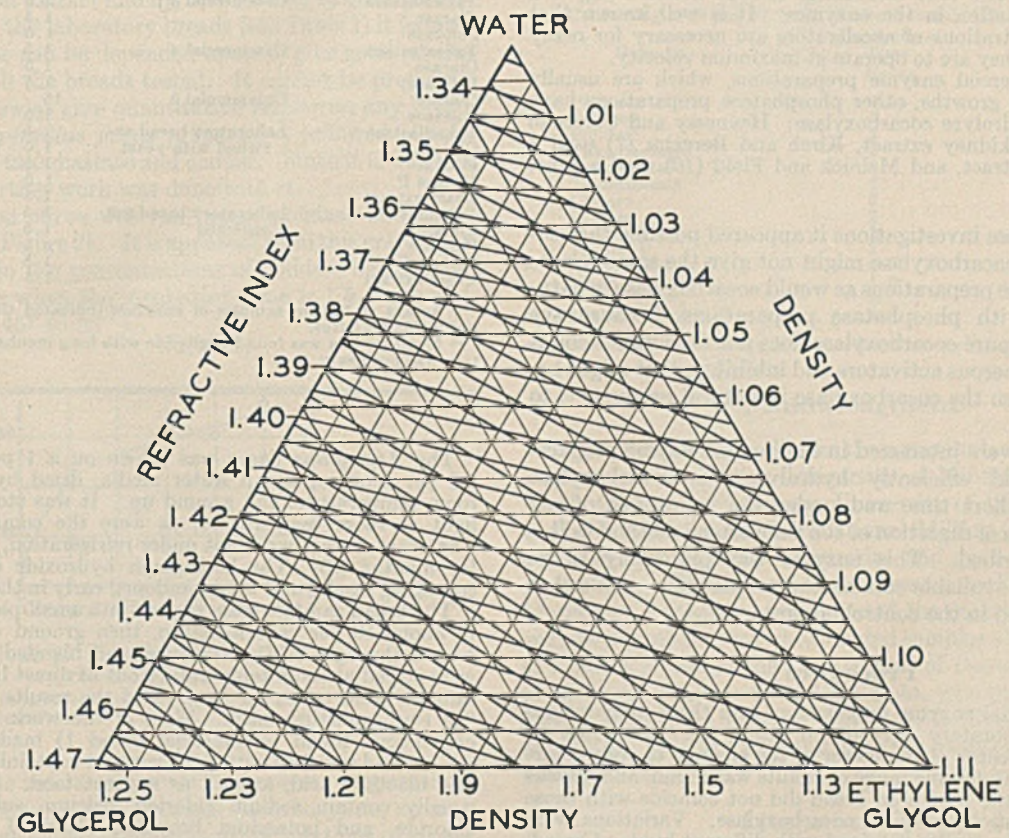

FIGURE 7. CONSTANT DENSITY AND REFRACTIVE INDEX CURVES AT 25° C.

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Determination of Thiamine in Bread by the Thiochrome Method

A Comparison of Phosphatase-Containing Enzyme Preparations

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ANDREWS and Nordgren (*1*) have suggested that a great deal of the difficulty experienced in assaying bread for thiamine by the thiochrome method may be due to the presence of cocarboxylase formed from the free thiamine by the action of yeast.

These authors raised the incubation temperature and lengthened the incubation time in order to accomplish complete hydrolysis of cocarboxylase. Lipton and Elvehjem (*8*) have shown that thiamine can be coupled with phosphate by yeast to give cocarboxylase, and Livshits (*9*) found that yeast may be capable of synthesizing thiamine.

Conner and Straub (*3*) and Hennessy, Tarshis, and Perlman (*5*) have shown that several commercial enzyme preparations will quantitatively hydrolyze aqueous solutions of synthetic cocarboxylase. The latter authors also found aluminum, mercuric, and ferric ions inhibiting, ferrous accelerating, and considerable variation in the enzymes. It is well known that optimum concentrations of accelerators are necessary for many phosphatases if they are to operate at maximum velocity.

Besides commercial enzyme preparations, which are usually made from mold growths, other phosphatase preparations have been used to hydrolyze cocarboxylase: Hennessy and Cerecedo (*4*) used a beef kidney extract, Kirch and Bergeim (*7*) used a yeast-glycerol extract, and Melnick and Field (*10*) used a yeast powder.

In view of these investigations it appeared possible that solutions of pure cocarboxylase might not give the same results with phosphatase preparations as would cocarboxylase in natural products with phosphatase preparations, because the work done with pure cocarboxylase does not take into account the effect of numerous activators and inhibitors that might become active when the cocarboxylase in natural products is to be hydrolyzed.

The authors were interested in finding an enzyme preparation that would efficiently hydrolyze the cocarboxylase in bread in a short time and under the conditions of pH and temperature of digestion of the thiochrome method as it is generally prescribed. This enzyme was preferably to be either a readily available commercial preparation or one that is easily prepared in the control laboratory.

Procedure

Four commercial enzyme preparations and three preparations made in this laboratory were tested, using the thiochrome reaction as an indicator of the power of the enzyme to split cocarboxylase. A considerable range of results was found, and, as was to be expected, the results on bread did not coincide with those on aqueous solutions of pure cocarboxylase. Variations were also noticed in the results obtained with different kinds of bread. The assay procedure was that of Conner and Straub (*3*) with minor modifications. The instrument used for the final readings was a Coleman Model 12 electronic photofluorometer. The enzyme preparations were as follows:

Enzyme	Source
Takadiastase	Parke, Davis & Co.
Clarase	Takamine Laboratory, Inc.
Mylase P	Wallerstein Laboratories
Polidase	Schwarz Laboratories, Inc.
Aspergillus flavus	Grown in this laboratory
Yeast-glycerol extract	Prepared in this laboratory after Kirch and Bergeim (*7*)
$La(OH)_3$ solution	Used in water solution. Suggested by the work of Bamann and Meisenheimer (*2*), who found that $La(OH)_3$ cleaves sodium glyceryl-β-phosphate

TABLE I. ASSAYS OF BREADS

Enzyme[a]	Bread	Incubation Time[b]	Thiamine
		Hours	*Micrograms/g.*
Takadiastase	Commercial 1	16.5	3.11
Takadiastase	(sponge process)	1.5	2.55
Clarase		16.5	3.09
Clarase		1.5	2.52
Clarase		20	3.15
Clarase		18	3.08
Polidase		20	3.10
Polidase		1.5	3.28
Polidase		18	3.01
No enzyme (blank determination)		1.5	1.62
Takadiastase	Commercial 2	1.5	3.51
Clarase		1.5	3.03
Polidase		1.5	4.35
No enzyme (blank determination)		1.5	1.41
Takadiastase	Commercial 3	1.5	3.96
Clarase		1.5	3.42
Polidase		1.5	5.04
Takadiastase	Commercial 4	1.5	1.32
Clarase		1.5	1.32
Polidase		1.5	1.71
Clarase	Commercial 5	16	3.12
Polidase		1.5	3.33
Takadiastase	Laboratory bread enriched with yeast	1.5	1.02
Clarase		1.5	1.35
Polidase		1.5	2.40
Mylase P		1.5	2.13
Yeast extract		1.5	1.14
Takadiastase	Laboratory bread not enriched	1.5	0.98
Clarase		1.5	0.78
Polidase		1.5	1.20
Mylase P		1.5	0.99
Yeast extract		1.5	0.51

[a] Assays of 1-gram samples of enzymes indicated that they were essentially thiamine-free.
[b] Moisture loss was found negligible with long incubation, and no correction was necessary.

The *Aspergillus flavus* was grown on a 1 per cent peptone–4.5 per cent sugar–tap water media, dried by an air blast at room temperature, and ground up. It was stored out of direct light at room temperature, as were the commercial enzymes. The yeast extract was kept under refrigeration, where it is stable for months (*6*). The lanthanum hydroxide did not give encouraging results and was abandoned early in the investigation.

The bread samples were broken into small pieces and air-dried to about 10 per cent moisture, then ground until 99 per cent passed through a 36 grits gauze, and blended carefully. They were stored at room temperature out of direct light. Four-gram samples were taken for assay, and the results are given on the "as run" moisture basis. Most of the work was done with a commercial bread (commercial bread 1) made by the sponge process and enriched with a concentrate containing iron, thiamine, and nicotinic acid, as well as a yeast food. Such yeast foods usually contain sodium chloride, calcium sulfate, ammonium chloride, and potassium bromate. Table I summarizes the results of 68 assays on this bread and 21 assays on six other assorted breads. The figures given represent averages of results, all of which agree within 5 per cent.

It is apparent here that the polidase gives the best results, whether the time is 1.5 hours or overnight, with mylase P giving second best. For commercial bread 1 mylase P and the yeast preparation did quite as well as polidase, as will be seen in Figure 1. In all cases takadiastase and clarase gave quantitative results only after incubation periods of many hours.

Using the sponge process bread (commercial bread 1) time curves were run on all the enzyme preparations. The results

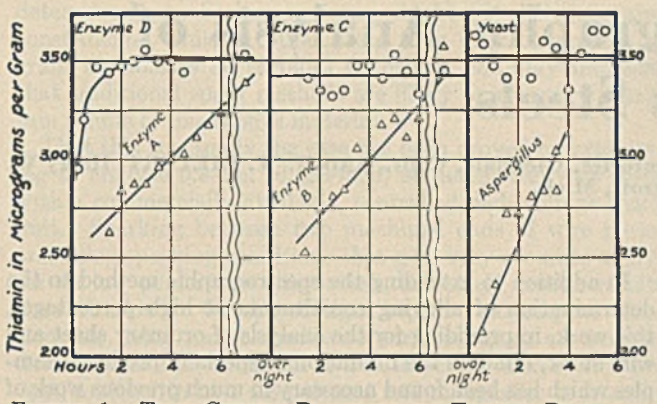

FIGURE 1. TIME CURVES PRODUCED BY ENZYME PREPARATIONS ON SPONGE PROCESS COMMERCIAL BREAD 1

All preparations were 3% suspensions in the buffer, except the mold, which was 1.1%. Temperature, 45°–50° C. *A*, takadiastase; *B*, clarase; *C*, mylase P; *D*, polidase

are shown in the series of small graphs in Figure 1. The curves were continued until further hydrolysis gave no more thiamine, with one exception.

It is evident from these graphs that only polidase, mylase P, and the yeast extract gave complete hydrolysis in 1.5 hours, and since the yeast extract and mylase P failed to give quantitative results on the laboratory breads (see Table I) it follows that only polidase can be depended upon to give good results in 1.5 hours on all the breads tested. It cannot be predicted that it would likewise give quantitative results on any bread tested. The *Aspergillus flavus* preparation followed closely the curves set by takadiastase and clarase. Since it is difficult to prepare, no further work was done with it.

A concentration curve was run, using polidase and commercial bread 1 (Figure 2). It is apparent from this graph that it is inadvisable to use concentrations of polidase below 3 per cent in the buffer when the incubation time is 1.5 hours and the temperature 45° to 50° C.

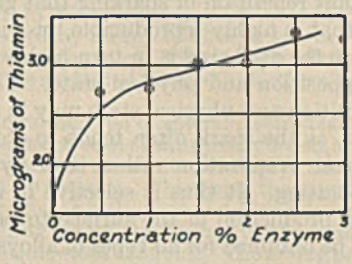

FIGURE 2. CONCENTRATION CURVE WITH POLIDASE

Incubation time 1.5 hours, temperature 45° to 50° C.

To compare the action of the various enzyme preparations on aqueous solutions of pure synthetic cocarboxylase, a series of experiments was undertaken using water solutions of cocarboxylase made 0.04 *N* with sulfuric acid. The buffer solution of enzyme was added as usual. Some 125 assays revealed such variations that no attempt was made to draw any definite conclusions as to the relative merits of the enzymes. Some typical results are shown in Table II. Only polidase gave quantitative recovery consistently. The causes for this, while unknown are assumed to be inhibitors and accelerators that appear in varying amounts as contaminants in the apparatus.

It seems reasonable to conclude from these experiments that variable results with enzymes used to hydrolyze cocarboxylase in bread—for example, the variation in the results obtained with mylase P and the yeast extract—may be attributed to the differences existing between various kinds of enzymes, and perhaps also to the presence of varying amounts of activators and inhibitors in different breads. It also seems reasonable to assume that experiments with aqueous solutions of pure cocarboxylase may not give the same results as with cocarboxylase in bread. It is recommended that, in using any enzyme preparation, the enzyme be allowed to incubate for at least 18 hours in order to ascertain the shortest incubation time possible with that particular enzyme and bread.

Summary

An enzyme was found that gave complete hydrolysis in 1.5 hours on all breads tested when they were assayed for thiamine content by the thiochrome method. Other enzymes gave results that varied considerably with the kind of bread used. When used with solutions of pure cocarboxylase, all but one of the enzymes gave results inconsistent with those obtained when they were used on bread. These variations are assumed to be due to inherent differences between various kinds of enzymes and to the presence of varying amounts of activators and inhibitors in different breads.

TABLE II. HYDROLYSIS OF AQUEOUS SOLUTIONS OF COCARBOXYLASE

(Containing 10.0 and 12.5 μg. of cocarboxylase per 50-ml. aliquot)

Enzyme	Incubation Time	Recovery
	Hours	%
Polidase	0.25	100.0
Polidase	2	100.0
Polidase	3	100.0
Takadiastase	2	2.5
Takadiastase	2	50.0
Takadiastase	3	10.0
Clarase	2	100.0
Clarase	2	10.0
Mylase P	2	100.0
Mylase P	2	60.0
Yeast extract	0.50	100.0
Yeast extract	1.5	78.0
Yeast extract	2	10.0
Blank (no enzyme)	1.5	0.0

Acknowledgment

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Quantitative Spectrographic Analysis of Stainless Steels

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A spectrographic method of analysis is described that allows rapid and accurate quantitative analyses of all metallic constituents in stainless steel stock. A direct current arc and a specially shaped carbon electrode allow chromium and nickel analyses accurate to 2 per cent of the quantity being measured. At the same time, satisfactory silicon, manganese, columbium, molybdenum, and titanium analyses can also be made. Complete analytical details are furnished, including a method of calculating results that takes into consideration the wide variation in iron content likely to be experienced in this type of material.

IN THE last five years the spectrograph has been applied successfully to the analysis of cast iron and low alloy steels (*1, 3*). In these fields the great economic advantage of a method so rapid and accurate that it can give analysis in the foundry before the metal is cast, and so convenient that one analyst can do the work of five, was bound to be appreciated. With the advent of the war, such advantages are even more valuable. However, the great need at the moment is to extend these methods to more and more alloys, so that testing methods can be made to keep step with the tremendous increase of metal production that is being experienced at this time.

The authors' laboratories have attempted to contribute to that need by developing a method of stainless steel analysis that is sufficiently accurate and rapid to be used for the routine analysis of chromium, nickel, manganese, silicon, columbium, molybdenum, titanium, and any other significant element by a single spectrographic procedure. The method represents a distinct step forward, as it allows the analysis of chromium up to 28 per cent and of nickel up to 20 per cent with results precise to ±2 per cent of the quantity being measured. This produces analyses, such as chromium at 28 ± 0.56 per cent, at 18 ± 0.36 per cent, and at 12 ± 0.24 per cent; and nickel at 20 ± 0.4 per cent, at 12 ± 0.24 per cent, and at 8 ± 0.16 per cent. At first glance, this accuracy may not appear as acceptable as chemical methods, but a careful comparison, using repeat chemical analyses by different laboratories and spectrographic analyses by different operators, indicated that the latter suffer much less from random accidental errors than the former, and hence should be just as acceptable, particularly for routine testing. When the determinations of minor constituents are compared, the spectrographic method proves more accurate than the chemical method. Thus the combination of acceptable consistent analyses at the higher percentages, and very precise analyses at the lower percentages for a number of the metallic elements in stainless steel, makes this method of distinct value.

In addition to extending the spectrographic method to the determination of alloying constituents at high percentages, this work, in providing for the analysis of ordinary sheet and wire stock, removes the limitation of specially produced samples which has been found necessary in much previous work of comparable accuracy.

Sampling of Alloys

In connection with the latter point, it will be of advantage to discuss the sampling problem of alloys with special reference to spectrographic work. This problem is related to the particular light source used to produce the spectrum of the sample, depending upon whether it is an electric spark or an arc. The spark is characterized by its repetitiousness and very small sample consumption, the direct current arc by its continuousness and relatively large and complete sample consumption. Numerous authors have characterized the spark as being highly reproducible and hence most suitable for accurate quantitative analysis, and the direct current arc as being nonreproducible and hence suited only for qualitative or semiquantitative work. That this last conclusion is not necessarily true and that the sampling problem can be solved to a considerable extent by employing an arc is the basic premise of the method to be presented.

In the case of the spark, either 60 or 120 electrical strokes per second are passed between electrodes of the material to be analyzed. Exposures from 10 to 60 seconds give thousands of strokes which play about the top of the sample exposed and thus thoroughly sample the surface presented for analysis. It is this constant repetition of sparking that gives an aggregate exposure that is highly reproducible, providing the surface presented to the discharge is in turn highly reproducible, both as to composition and physical state. This reproducibility of composition and physical state may in some cases be very important, as the spark often tends to etch the surface exposed by direct evaporation rather than by first melting and then evaporating. It thus is selective in its action and only by careful production of the surface to be sparked can assured results be obtained for all types of alloys.

To make certain that at least the composition of the surface exposed to the spark is representative of the sample, numerous methods of sampling have been worked out. The most convenient one is to take drillings or filings from various points on the sample and briquet them into an electrode. This furnishes a composite surface for sparking, which, as a whole, represents the average analysis of the sample—is average in composition. However, this procedure does not ensure a reproducible physical state on the sparking surface, irrespective of the sample's metallurgical history. That it has been highly successful in many instances indicates that for certain types of materials, such as the low percentage alloys, for instance, the physical state is well reproduced by ordinary metallurgical practice.

However, for high percentage alloys, such as stainless steel, produced under a variety of metallurgical conditions, no method of sampling, short of dissolving the metal completely or melting it under controlled conditions, would be expected to produce a sample representative in composition and uniform in physical form. The fact that certain of the elements

determined may be in some cases combined with the major constituents, while in other cases they may be present in grain boundaries as carbides or oxides, certainly indicates that traditional spark methods are likely to give very uncertain results of this type of material.

That this is actually the case has been proved by attempting to analyze 0.3-cm. (0.125-inch) stainless steel wire stock with a commercially available, controlled high-voltage spark unit. Sparking between two machined ends of wire pieces and using sparking conditions that give very accurate analyses on low percentage alloy steels, extremely erratic results were obtained. Groups of eight repeat analyses, on three samples, using remachined wire surfaces, gave an average deviation for the chromium to iron intensity ratio of 10.1 per cent, a nickel to iron 4.8 per cent and manganese to iron 5.4 per cent. Silicon, at approximately 0.5 per cent, could not be measured at all under these discharge conditions. This is good evidence that the composition of these wires is not sufficiently uniform for direct spark analysis. If briquetting were to be used, the average results obtained from the eight runs of each sample would be obtained with less random error. However, when these averaged results were plotted against percentage composition, no consistent relationship appeared, proving that the physical form of the alloys was not sufficiently consistent to enable direct spark analysis of either rod or briquetted samples.

Method of Sampling and Arcing

It was with these facts clearly in mind that a method of sampling and arcing was developed that allows accurate analysis of material, irrespective of its metallurgical history.

The basic idea is very simple. If a fine file is used, filings averaging 0.04 mm. in diameter can be easily obtained from sheet or wire stock. These can be taken from a number of portions of the piece to be tested and thoroughly mixed to produce a sample of good average composition. A 3-mg. portion of these filings contains about 1500 particles, which should be an adequate number to provide a good average composition. This conclusion is borne out by the reproducibility obtained upon duplicate analysis. This 3-mg. portion is placed upon a special carbon electrode and cemented in place with a drop of sugar solution which has been preceded by a drop of alcohol to wet the carbon. The surface tension of the sugar solution spreads the stainless steel particles over the surface of the electrode where they are deposited upon drying.

Figure 1 shows the type of special electrodes used for both the top and bottom electrodes of the direct current arc. Upon arcing, the discharge starts at the center post above the sample and holds to it for about 30 seconds. This heats the sample sufficiently by conduction, so that it melts first and then evaporates up into the arc. The melting erases the history of the solid sample and allows a reproducible state of affairs. The arcing proceeds smoothly as the molten metal stays spread out in a thin layer covered with many small bumps. There is no tendency to form a single large bead of metal, which usually causes arc wandering and unstability of the discharge. Rather, the center post burns away smoothly, vaporizing much of the metal as it does. This is followed by vaporization of the conical platform that carries the sample. When nothing remains but the stub, one is assured that every bit of the sample has been evaporated. This arcing to completion assures accurate results for the determination of refractory substances used as stabilizers, such as compounds of titanium and columbium, as well as for the more volatile metals.

With the sampling problem solved, the next question is, will such an arc give reproducible results; will the intensities of certain chromium, nickel, silicon, manganese, etc., lines in the spectrum bear a certain reproducible relationship to certain iron lines in the spectrum? To answer this question, it is necessary to determine first which spectrum lines are most suited to the purpose. The usual procedure is to vary such things as arc current, distance between electrodes, etc., and determine which line ratios of intensity remain the most constant for these changes. However, in this case, since these quantities could be controlled very accurately, it appeared best to study the variation in line intensity ratios as a function of the total amount of sample on the electrode. This was done, not because this quantity cannot be closely controlled, but rather because variations in this discharge will result mainly from differential vaporization effects, and change of discharge characteristics with time through the arcing cycle. By deliberately changing the basis for such effects, line pairs can be chosen that minimize the effect of possible variations.

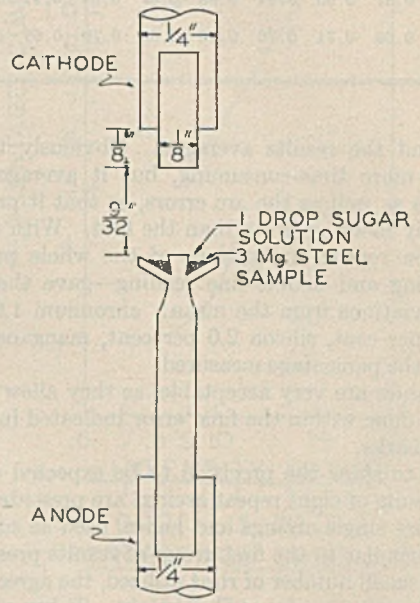

Figure 1. Special Carbon Electrodes Turned from Rod Stock

After making the final choice of line pairs, the whole problem of reproducibility was reviewed by running a number of arcings on a wide variety of samples to determine what the average variation in selected line intensity ratios would be. This was preferred to the customary procedure of repeating the process over and over again on the same sample, as in that case the properties of the particular sample used determine the results obtained to a large extent. If many samples are used, an average reproducibility can be determined that is significant for the entire practical problem on hand. The average deviations from the means obtained from 80 determinations of each element were as follows: chromium 2.5 per cent, nickel 2.6 per cent, silicon 2.8 per cent, manganese 3.2 per cent, all of the percentage measured.

This reproducibility is not quite so good as some that have been reported on specially prepared samples (*2*), but it is very good when the wide variety of samples used is considered. However, it still is not sufficient for the accurate determination of the high percentages desired. In general, there is a very simple way to improve analytical accuracy—that is, repeat the procedure and average the results. The gain in precision is equal to the square root of the number of determinations averaged to produce a single final determination. By averaging two arcings, the precision is thus increased 1.4 times. Two ways of handling this duplication are possible. One is to use two arcings to produce a single spectrogram, the other is to use each arcing to produce a single spectrogram. In the first case, one set of readings on the various line blacknesses with the densitometer gives the average result automatically, while in the second case two sets of readings are

TABLE I. REPEAT ARCINGS

Sample analysis: chromium 18.7%; nickel 9.9%; silicon 0.46%; manganese 0.50%. Exposure: single without averaging two. Percentage deviation of analysis obtained by applying average percentage deviation to working curves of Figures 3 and 4. Deviations for two arcings obtained by averaging all possible pairs of results, calculating average deviations from these averages, and then applying these to working curves.

Intensity Ratios	1	2	3	4	5	6	7	8	Average	Average % Deviation	% Deviation of Analysis 1 arcing	% Deviation of Analysis 2 arcings
$\frac{I_{Cr}}{I_{Fe}}$	1.27	1.28	1.26	1.24	1.27	1.31	1.30	1.32	1.281	1.6	2.2	1.4
$\frac{I_{Ni}}{I_{Fe}}$	1.29	1.29	1.31	1.33	1.26	1.31	1.31	1.26	1.295	1.5	2.5	1.6
$\frac{I_{Si}}{I_{Fe}}$	0.87	0.90	0.94	0.88	0.87	0.94	0.91	0.89	0.900	2.3	2.8	1.8
$\frac{I_{Mn}}{I_{Fe}}$	0.69	0.71	0.70	0.66	0.65	0.70	0.69	0.65	0.681	3.1	3.5	2.1

required and the results averaged. Obviously the second method is more time-consuming, but it averages over all other errors as well as the arc errors, so that it produces results slightly more accurate than the first. With it, average deviation on repeat applications of the whole procedure—double arcing and double line reading—gave the following average deviations from the mean: chromium 1.8 per cent, nickel 1.8 per cent, silicon 2.0 per cent, manganese 2.3 per cent, all of the percentage measured.

These values are very acceptable, as they allow consistent work to be done within the final error indicated in the introductory remarks.

In order to show the precision to be expected on a short run, the results of eight repeat arcings are presented in Table I. These are single arcings and hence, used as such, should give errors similar to the first group of results presented. In view of the small number of runs utilized, the agreement with the over-all average appears about right. Taken as pairs and averaged, the results should correspond to the second group of results. Again the agreement appears proper, illustrating the gain in accuracy obtained by this procedure.

With a reproducible method of handling complex metallurgical samples devised, what other problems arise in high percentage analysis that are not present at low percentages? The main one is the internal standard problem. In low alloy steels, the intensity of each element's lines is compared to iron, and since iron varies only from 90 to 100 per cent, even in extreme cases, this comparison can be made without regard to the iron content of the sample. At first consideration, this may appear as poor practice if it is desired to measure the various alloying elements very accurately. Actually, the intensities of most iron lines change very little for a substantial percentage change in the region 90 to 100 per cent iron. Only because of this can the comparison be made without regard to the iron content. However, in stainless steel work, the iron content may vary from as low as 55 per cent to as high as 90 per cent. In this case, the percentage change of iron will cause an appreciable change in iron line intensities, which certainly cannot be ignored.

At first it appeared that the only way to solve this problem was to introduce another element which was not present in steels as an internal standard. Some work was done along this line, using gold. Although the results were promising, it was found possible to go back to iron as the internal standard if corrections for variations in the iron content of the samples were made.

With the two basic problems solved, means for preparing a reproducible record easily, and means of interpreting that record, the rest of the work proceeded along conventional lines. However, in order to make that work available to other workers in the field, the method and results obtained with it are described in detail.

Apparatus and Method

SAMPLE PREPARATION. Use a No. 2 American-Swiss spark-plug file and obtain a total of approximately 100 mg. of filings from a number of different points on the sample. Sawed edges make good surfaces for filing, as then the body of the metal can be sampled. Collect these filings on a piece of paper, transfer them to a glass vial, and mix them thoroughly by stirring. Prepare a small scoop by drilling a conical hole on the side of a 0.31 × 0.16 cm. ($^1/_8$ × $^1/_{16}$ inch) rectangular rod, close to one end. This hole is about 0.175 cm. (0.070 inch) in diameter and conical. It is produced with an ordinary machinist's drill. Adjust the depth of the hole so that 3 mg. of sample will just fill the hole when it is scooped out of the vial with the rod, tapped, and leveled with a spatula. This can be done with a few trials. With a little experience, an operator can fill such a scoop with 3 ± 0.15 mg. every time. This represents a possible error of 5 per cent in weighing. However, since the intensities of all lines of the elements to be measured are compared to iron line intensities, this does not introduce an appreciable error in the result. This is particularly true for the lines chosen, as these varied little in intensity ratio with a large change in the total amount of sample.

ELECTRODE PREPARATION. All lower electrodes should be prearced 6 seconds after the current has been adjusted to 10 amperes on short circuit of the electrodes. This vaporizes the impurities common to graphite electrodes not of superpure grade that may interfere with certain of the determinations to be made, such as silicon and titanium. Besides this, the prearcing puts the electrode in a more porous form, so that the molten metal can more readily obtain intimate contact with it. A decided drop in reproducibility is noted if electrodes are not prearced, so that this represents an important point that should be given careful attention. Prearcing can be done for a group of lower electrodes just prior to their final preparation.

With the scoop, load 3 mg. of sample into each of two electrodes. This provides the duplicate electrodes necessary for very high precision. Tap the base of the electrode to distribute the sample around the center post. Place a drop of alcohol on the electrode to wet it, followed by a drop of sugar solution containing 4 mg. of sugar per drop. Gently heat the electrode over a flame until the sugar solution dries. Do not overheat, as carbonizing the sugar produces a variation in the starting condition of the electrodes.

ARCING CONDITIONS. The electrodes are centered accurately on the optical axis by means of a projection system. Vertically they are arranged 0.352 cm. ($^9/_{61}$ inch) above and below the optical axis, giving a total electrode spacing of 0.703 cm. ($^9/_{32}$ inch) between electrodes. All measurements are made to the platform rather than the tip of the lower electrode. The arc is struck by moving the upper electrode down onto the lower, and then rapidly raising it until it reaches its original position, determined by a stop.

The arc current supplied by a commercial spectrographic rectifier unit is adjusted to 10 amperes with the electrodes short-circuited. Upon striking the arc, the current is about 7.5 amperes. It gradually decreases as the arc gap widens. No adjustment of the electrode positions or of the current is made while the arc is on.

The arc is allowed to vaporize sample and electrode until the entire platform is consumed and only the stub remains. This requires about 80 seconds.

The entire arcing is photographed to produce a single spectrogram.

SPECTROGRAPH EMPLOYED. The spectra of iron, chromium, and nickel are all very complex. Thus, it is essential to have a spectrograph of adequate resolution and dispersion for stainless steel analysis. The instrument utilized was a commercial grating spectrograph (A. R. L.-Dietert) having a high resolving power, 48,000, and adequate dispersion, 7 A. per mm.

The instrument utilizes 35-mm. motion picture film, which provides the most uniform emulsion obtainable.

ILLUMINATION OF SPECTROGRAPH SLIT. The arc is placed 28 cm. beyond the height-limiting aperture of the astigmatic grating spectrograph, which is, in turn, 18 cm. beyond the

TABLE II. SPECTRUM LINES

Elements	Wave Length of Line	Transmission Readings (Typical Example)	Intensities Deduced from Figure 2
Chromium	2879.3	18.3	1.38
Nickel	2821.3	19.0	1.34
[a]	3101.6		
Manganese	2933.1	39.1	0.79
Silicon	2506.9	44.1	0.72
Columbium	2950.9	...	...
Molybdenum	2816.2	...	...

[a] For low percentage nickel alloys.

TABLE III. SPECTRUM LINES OF IRON

Wave Length	Known Relative Intensity Values	Transmission (Typical Example)
2950.2	1.50	16.2
2872.3	1.00	28.3
2869.3	2.13	10.1
2840.4	1.08	25.9
2838.1	2.30	9.2
2828.8	0.76	41.2
2804.5	2.59	8.0

primary slit. No condensing lens is used. This setup of the astigmatic spectrograph gives spectra with sharply defined edges, such as are obtained with a stigmatic instrument.

FILM PROCESSING. To obtain the best results, the uniformity of film sensitivity must be matched with uniformity of development. This is accomplished with commercial apparatus made specifically for handling film under reproducible conditions of developer agitation and temperature. High-speed washing and drying allow complete film processing in 4 to 5 minutes.

FILM MEASUREMENT. A projection comparator-densitometer provides a very simple method for finding the lines required and measuring their degree of blackness. The identification of lines is made by a linear direct-reading scale on a master plate which allows them to be located by their wave lengths. Their transmission values are then read with a high-precision photoelectric densitometer.

SPECTRUM LINES EMPLOYED. Table II gives the spectrum lines of the various elements used in making analyses. Table III gives the various spectrum lines of iron used, both as internal standards and for film calibration. These iron lines have been carefully chosen, so that they remain constant as to their relative values over large changes in iron content.

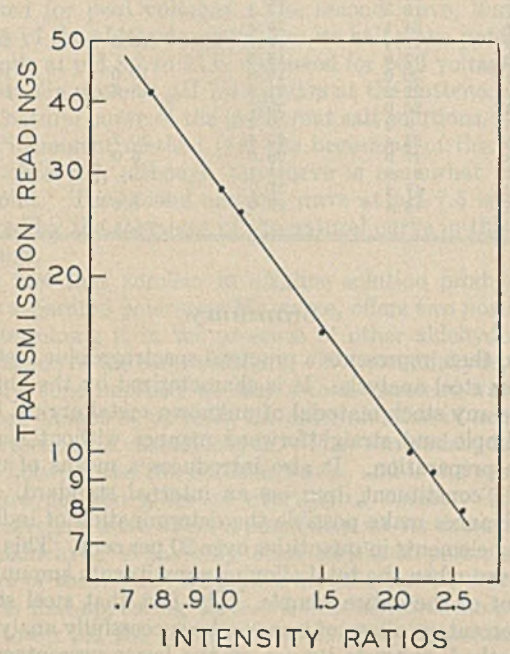

FIGURE 2. TYPICAL FILM BLACKENING CALIBRATION CURVE

Determined from lines of known relative intensity

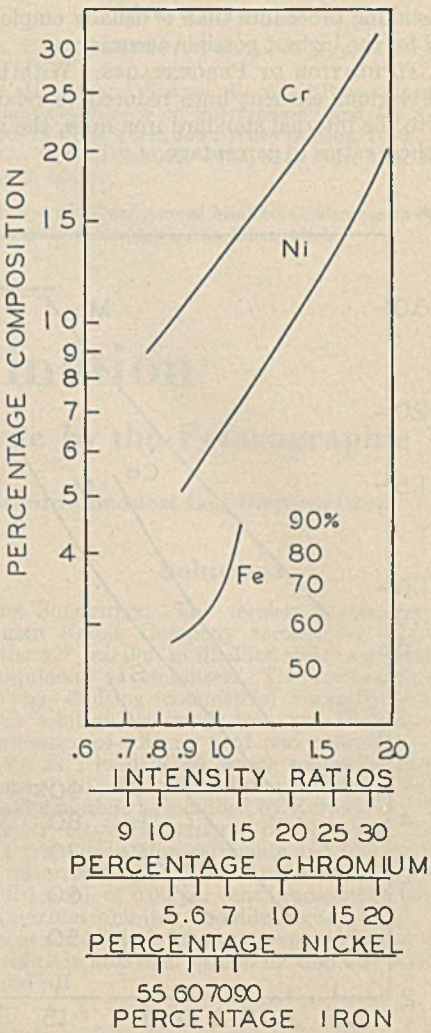

FIGURE 3. WORKING CURVES AND SCALES FOR CHROMIUM AND NICKEL DETERMINATIONS

With unity point correction curve for iron content of sample

The "known values of intensity" have been determined by standard stepped-sector methods. They should be rechecked with the spectrographic equipment to be used, as variations in the exact positioning of the arc may cause differences.

Calculations

CALCULATION OF RELATIVE INTENSITY VALUES. This group of iron lines is read in each spectrogram and plotted as shown in Figure 2—log transmission as ordinate, log intensity as abscissa. This gives the usual film-blackening calibration curve inverted, by which differences in developing conditions, film contrast, etc., are eliminated from the results. Using this curve, the transmission readings of the lines of the elements to be determined are converted to relative intensity values, relative to the iron 2872 Å. line being considered as of intensity unity. These are given for the typical example shown.

This procedure is repeated for each spectrogram, so that each relative intensity is deduced from the best average curve through all the iron line values for that exposure. This is a

more painstaking procedure than is usually employed, but it is essential for the highest possible accuracy.

Final Calculation of Percentages. With the intensities of the various element lines reduced to relative values compared to the internal standard iron lines, the next step is to reduce these ratios to percentage.

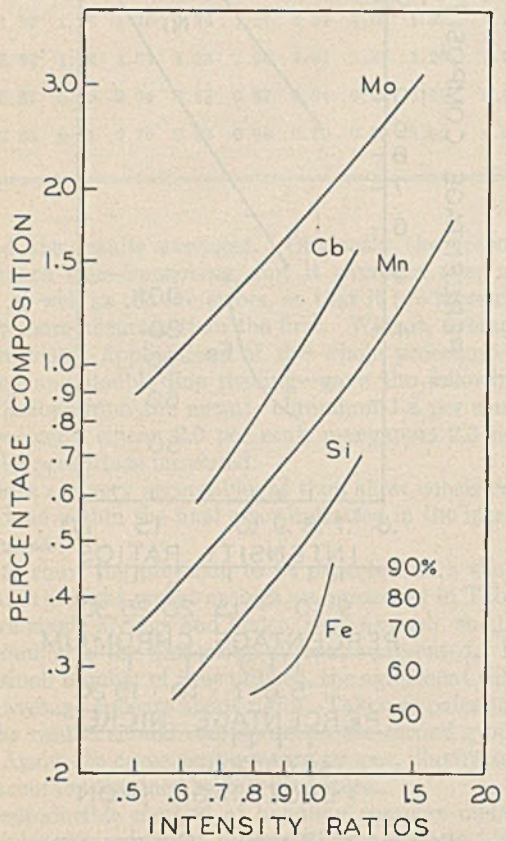

Figure 4. Working Curves for Molybdenum, Columbium, Manganese, and Silicon

With unity point correction curve for iron content of sample

The way to do this, so that a final working scale can be abstracted from the results, is to plot percentage as ordinates, and I_l/I_{Fe} (relative intensity to iron) as abscissa using log scales. This gives curves such as are shown in Figures 3 and 4. By replacing relative intensity values along the abscissa by the corresponding percentages, a rule for the direct reading of percentages is made available, to replace the log intensity scale of Figure 2. It should, of course, be adjustable horizontally. An arrangement like this is the basis for the conventional calculating machine.

So far this follows the usual procedure for the reduction of data to the final percentage. However, an innovation is introduced at this point to allow taking into consideration the variable iron content. Since the intensities of the iron lines will increase with the increased concentration of iron, a correction is made by moving the unity point of the relative intensities (Figures 3 and 4) to the right for an increase in iron. Thus, by introducing a curve for iron relating the percentage of iron to the intensity of the basic iron line, 2872 Å., and using this curve to locate the unity point of the system, a multicomponent system of analysis is made possible, instead of the essentially single-component system usually employed.

At first glance this appears involved, but actually it is simple. In most work, the approximate iron content is known. Thus, starting with this value, the unity point of the system is determined. The various elements are determined with respect to this iron value, and are then totaled, including the iron content. If they add up to 100 ± 2 per cent, the correct iron percentage was utilized. If the sum departs from 100 too much, the iron content must be evaluated in the following manner:

For the sum equal to a value over 100, the original iron estimate was too high. As a first trial, reduce the iron percentage by half the amount of the total exceeding 100 per cent. Recalculate the other elements and recheck the total. Readjust the iron value again if necessary, and recalculate.

In actual practice, the results are insensitive to variations in the iron percentage, especially at the higher iron percentages. Here an error in iron content of 5 per cent causes only a 2 per cent error in the element determinations. Thus, the iron content is usually known with sufficient accuracy just from the type of alloy being tested, so that a single calculation suffices for the determination of each of the other elements.

Comparison between Chemical and Spectrographic Determinations

Table IV indicates the degree of comparison to be expected between chemical and spectrographic results.

A number of chemical determinations were checked by two laboratories. The variations experienced were, in some cases, as great as those of the spectrographic method. Thus, in interpreting differences between the chemical results and the spectrographic results, a certain amount of the error must be attributed to the chemical method as well as the spectrographic. If the difference is shared between the two methods, the errors of the spectrographic method appear small enough for much routine work.

In the case of manganese, silicon, molybdenum, and columbium, the general agreement between the two methods was satisfactory. However, repeat chemical determinations on these elements, carried out by different laboratories, varied much more than repeat spectrographic results for these elements, so that no detailed comparison is given.

Table IV. Comparison of Methods

Sample No.	Chromium		Nickel	
	Chemical	Spectrographic	Chemical	Spectrographic
2001	16.5	16.7	10.2	9.9
2002	17.8	18.1	9.2	9.1
2003	18.6	18.2	9.2	9.0
2004	18.8	18.0	9.2	9.3
2005	19.1	19.5	10.6	10.5
2006	21.5	21.1	11.9	11.8
2007	13.3	13.2	...	...
2008	24.1	23.9	...	...
2009	17.4	17.7	12.5	12.4
2010	17.9	18.5	...	...
2011	20.0	19.8	10.0	10.5
2013	22.2	21.8	13.7	13.4
2014	26.0	25.7	...	...
2015	9.2	9.2	...	...
2016	23.4	23.4	20.2	20.2
2018	27.9	28.0	5.0	5.2
2021	11.9	11.4	...	...
2022	17.4	17.7	...	...
2023	18.4	19.0	8.6	8.2

Summary

This, then, represents a practical spectrographic method of stainless steel analysis. It is characterized by the ability to analyze any stock material of unknown metallurgical history in a simple and straightforward manner without elaborate sample preparation. It also introduces a means of using a variable constituent, iron, as an internal standard. These two advances make possible the determination of individual alloying elements in quantities over 20 per cent. This can be done even when the total alloying constituents amount to 45 per cent of the entire sample. The fact that steel stock of high percentage alloys of iron can be successfully analyzed by this method, suggests its use on the lower percentage alloy steels in cases where its sampling advantages are important.

Though the method is not so precise for the higher percentage alloying elements as chemical analyses carried out by ex-

perienced analysts working under carefully controlled conditions, it offers the advantages of a time-saving routine procedure for the analysis of many alloys produced by the steel industry.

Acknowledgment

The authors wish to express their appreciation to John Disario and A. P. Johnston, of Los Angeles, for their cooperation in certain phases of this problem.

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Acrolein Determination

In the Presence of Formaldehyde and Acetaldehyde by the Polarographic Method

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ACROLEIN can be identified by certain color tests (*2*, *6*, *7*, *12*) and by the preparation of derivatives (*1*, *8*, *13*). The literature on its quantitative determination is meager.

Schiff's reagent has quantitative application (*9*). Neuberg (*10*) made use of the insolubility of the 2,4-dinitrophenylhydrazone for a quantitative precipitation method. Zappi and Labriola (*15*) studied the application of several aldehyde methods to the quantitative determination of acrolein, and found the Ripper bisulfite method satisfactory, as did Ivanov (*3*). Another method uses the reaction with iodine to give iodoform, with back-titration of the excess iodine (*5*).

The above methods are inaccurate when other aldehydes are present with acrolein. This paper describes the application of a reliable polarographic method to the quantitative determination of acrolein in the presence of formaldehyde and acetaldehyde.

Acrolein shows two reduction-potential waves (Figure 1). The first wave, presumably due to reduction of the double bond, has its half-wave potential at −0.83 volt at pH 4.8; −0.98 volt at pH 5.8; and −1.04 volts at pH 7.0 to 11.0, corrected for pool voltages. The second wave, due to reduction of the aldehyde group, has its half-wave potential at 1.44 volts at pH 8.7 to 11.0, corrected for pool voltage. The first acrolein wave at pH 7.5 appears at the flattened portion of the natural curve of the indifferent salt solutions. By use of the increment method (*11*) the beginning of this wave is easily observed, although the curve is somewhat steep at this point. The second acrolein wave at pH 7.5 is entirely obscured by the steepness of the natural curve in this potential range.

The fact that acrolein in alkaline solution produces two widely separated polarographic waves, offers two possibilities for determining it in the presence of other aldehydes often encountered in the same solution. The acetaldehyde wave is partially superimposed on the second acrolein wave, and therefore cannot be used for the determination of acrolein in the presence of acetaldehyde. The formaldehyde wave occurs between the two acrolein waves, and if the concentrations are not too great no overlapping occurs. Therefore either acrolein wave offers a possibility for its determination if formaldehyde is the only other aldehyde present. In a mixture of these three aldehydes acrolein can be determined from its first-appearing wave. At an elevated temperature and a lower pH the acrolein wave heights are greater. Reproducibility is better at a lower pH. A lithium phosphate–buffered solution at approximately pH 7 containing 0.01 molar lithium chloride proved to be the best indifferent salt solution for determining acrolein from its first wave.

Solutions

Aldehyde Solutions. The acrolein solution was prepared from Eastman Kodak Company acrolein by distilling it and collecting the 52° fraction in distilled water containing 0.01 per cent hydroquinone as stabilizer. The formaldehyde solution was made by diluting commercial formalin solution. The acetaldehyde solution was made from paraldehyde by distilling it in the presence of sulfuric acid and collecting the fraction, boiling point 22°, in distilled water containing 0.01 per cent hydroquinone. All aldehyde solutions were analyzed by the Ripper bisulfite method as modified by Kolthoff and Furman (*4*).

Indifferent Salt Solutions, pH 11, were prepared by dissolving 1 gram of lithium carbonate and 0.01 mole of lithium chloride in water, adding 1 ml. of 0.2 per cent methyl red alcoholic solution and 1.5 ml. of 0.02 per cent bromocresol green alcoholic solution as maxima inhibitor, and diluting to 1 liter.

Solutions of pH 8.7 and 9.6 were prepared in the same way as the pH 11 solution and then carbon dioxide was added to adjust to the desired pH.

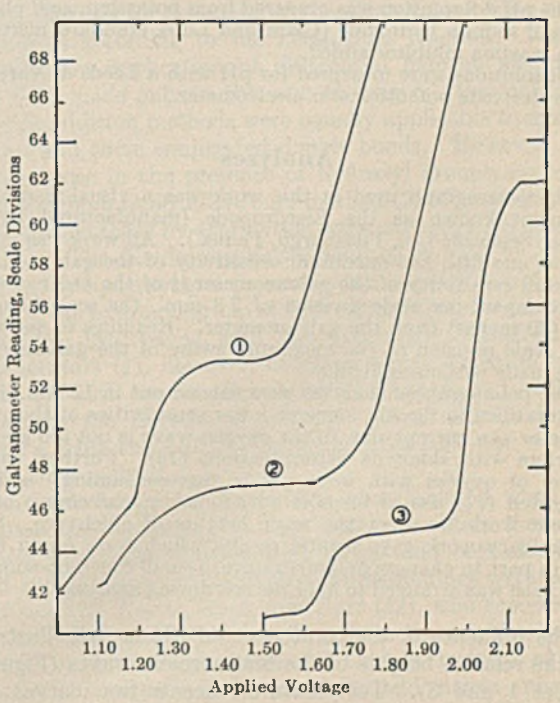

Figure 1. Current Voltage Curves for Acrolein

Sensitivity, 1/5. Volume, 110 ml.
1. 2.71 mg. of acrolein, pH 7.5, 28.8°
2. Residual current, pH 7.5, 28.8°
3. 2.71 mg. of acrolein, pH 11.0, 24.8°

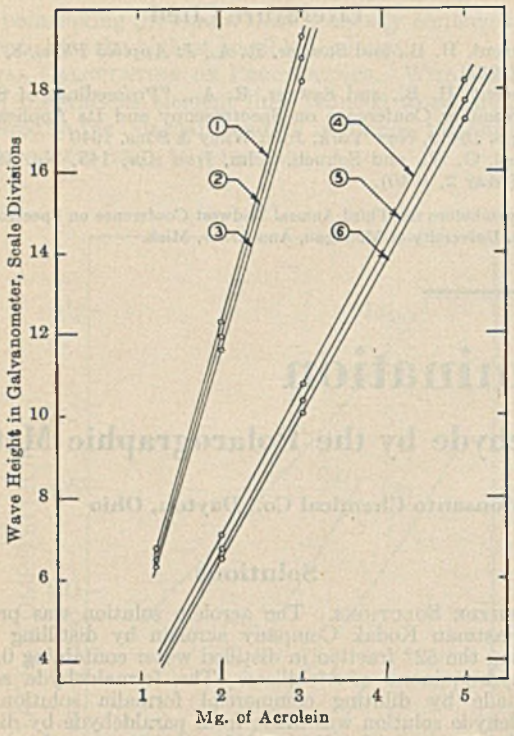

FIGURE 2. EFFECT OF TEMPERATURE ON WAVE HEIGHT

Sensitivity, 1/5. Volume, 110 ml.
Upper curves, acrolein second wave, pH 11
1. 28.8°. 2. 26.8°. 3. 24.8°
Lower curves, acrolein first wave, pH 7.5
4. 28.8°. 5. 26.8°. 6. 24.8°

The pH 7.5 solution was prepared by neutralizing 0.02 mole of phosphoric acid with lithium hydroxide, adding the maxima inhibitor, and diluting to 1 liter.

The pH 5.8 solution was prepared from potassium dihydrogen phosphate and sodium hydroxide (Clark and Lubs' standard mixtures) with maxima inhibitor added.

The pH 4.8 solution was prepared from potassium acid phthalate and sodium hydroxide (Clark and Lubs' standard mixtures) with maxima inhibitor added.

All solutions were measured for pH with a Leeds & Northrup glass electrode potentiometer electrometer.

Analyses

The polarograph used in this work was a visual-reading instrument known as the Elecdropode (manufactured by the Fisher Scientific Co., Pittsburgh, Penna.). All work was carried out at one fifth the maximum sensitivity of the galvanometer. The full sensitivity of the galvanometer is of the order of 0.006 microampere per scale division of 2.3 mm., the scale being 75 cm. (30 inches) from the galvanometer. Readings to one tenth of a scale division of the maximum swing of the galvanometer were made without difficulty.

The polarographic analyses were carried out in 125-ml. lipless beakers open to the air, since at lower sensitivities of the galvanometer the current due to the oxygen wave is not too great to interfere with aldehyde determinations (*14*). Further, the removal of oxygen with inert gas is time-consuming; and the suggested (*14*) use of bisulfite was found in the course of the present work to affect the wave heights of aldehydes. Since preliminary work gave erratic results which were found to be due in part to changes in temperature, a small constant-temperature bath was arranged to hold the cell during analysis.

The influence of temperatures and pH is well illustrated by the relative heights of the first acrolein waves (Figure 1, curves 1 and 3). Temperature-concentration curves were made (Figure 2). In the case of both acrolein waves, temperature control is necessary for accurate results since 1° of temperature change causes an 0.8 per cent error. The effect of pH on the accuracy of acrolein determinations may be seen in Figure 3, which shows curves for concentration-wave heights at pH 4.8 to 11 of the first acrolein wave. Curves for pH 4.8 and 11 are included, although the values are not reproducible, since when the analysis is repeated immediately on the same solution the wave height has become smaller. Figure 3 also shows the effect of pH on the second acrolein wave.

TABLE I. DETERMINATION OF ACROLEIN

Acrolein Present	Formaldehyde Present	Acetaldehyde Present	Wave Height[a] Found	Wave Height[a] Average	Acrolein Found[b]	Error	
Mg.	*Mg.*	*Mg.*			*Mg.*	*Mg.*	%
0.952	0.0	0.0	3.9 3.8	3.85	0.98	+0.028	+2.9
0.943[c]	17.7	6.14	3.5 3.6	3.55	0.93	−0.013	−1.3
1.904	0.0	0.0	6.0 6.1	6.05	1.83	−0.074	−3.9
1.904	10.6	6.14	6.2 6.1	6.15	1.87	−0.034	−1.8
2.856	0.0	0.0	8.9 8.6	8.75	2.90	+0.044	+1.5
2.856	0.0	6.14	8.9 8.7	8.80	2.91	+0.054	+1.9
3.808	0.0	0.0	11.0 11.0	11.0	3.78	−0.028	−0.7
3.808	17.7	0.0	10.9 11.1	11.0	3.78	−0.028	−0.7

[a] Galvanometer current increase in scale divisions as determined by increment method.
[b] See curve for pH 7.5 in Figure 3.
[c] Correction for concentration.

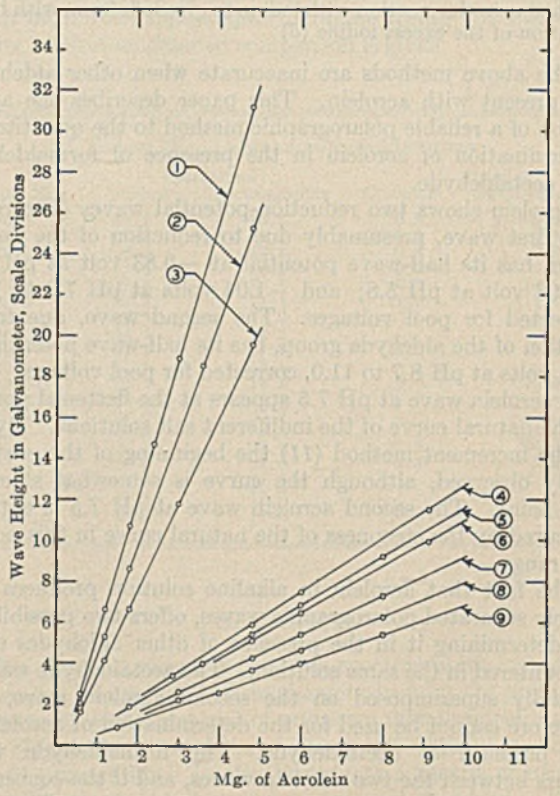

FIGURE 3. EFFECT OF pH ON WAVE HEIGHT

Sensitivity, 1/5. Temperature, 28.8°. Volume, 110 ml.

Acrolein second wave
1. pH 11.0
2. pH 9.6
3. pH 8.7

Acrolein first wave
4. pH 5.8
5. pH 7.5
6. pH 4.8
7. pH 8.7
8. pH 9.6
9. pH 11.0

Results

A buffered solution at pH 7.5 was selected as best for (1) reproducibility, (2) ease of observance of the beginning and end of the first acrolein waves by the increment method without the necessity of first plotting the polarographic curve, (3) maximum wave height, and (4) noninterference of formaldehyde and acetaldehyde. At the half-wave potential of −1.0 volt the drop weight was 0.0045 gram, and the drop rate was 4.02 seconds.

Table I contains representative data obtained at pH 7.5, 28.8° C., and a galvanometer sensitivity of one fifth.

Summary and Conclusions

The quantitative polarographic determination of acrolein in the presence of formaldehyde and acetaldehyde is most accurately made in a lithium chloride solution buffered at pH 7.0 to 8.0 with lithium phosphate. During the determination, the temperature must be held constant within ±0.05°.

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Woburn Iodine Absorption Method

Use in Combination with Partial Iodine Values for the Determination of Diene Numbers

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IN THE preceding paper (*22*) it was shown that iodine bromide solutions of 1.6 to 2 times the concentration of Hanus' solution can be used for determining the total unsaturation of oils and fatty acids containing conjugated double bonds. It was indicated that the "total iodine value" thus obtained could be used, in combination with partial iodine values, for determining the diene value of certain oils and fatty acids.

Previous Methods

The use of total and partial iodine absorption values for determining conjugation is not new.

Kaufmann (*14*) measured the iodine values of eleostearic acid for saturation of one, two, and three double bonds, using bromine with and without irradiation and thiocyanogen, respectively, in an attempt to calculate the composition of tung oil. Qualitative methods for showing the presence of conjugated double bond systems based on their characteristic rapid primary absorption of Wijs' reagent which is followed by a slow completion of the addition, were used by Boeseken (*3*, *4*), Gelber (*10*), and later by Forbes and Neville (*9*), while Kass *et al.* demonstrated that the conjugation could be qualitatively detected by the use of a rapid Hanus method in conjunction with the Rosenmund-Kuhnhenn and other modified Wijs procedures (*11*). Scheiber (*27*) and Boettcher (*5*) used Hanus' solution for the same purpose, while Smit (*28*) made use of a similar behavior toward oxidation with peracetic acid.

Toms (*30*) calculated the percentage of eleostearic acid in tung oil from the difference between the bromine-vapor iodine value (*26*) and the Wijs iodine value, and his method was later improved by Bolton and Williams (*6*), who replaced the ordinary Wijs value by the "instantaneous" iodine value, found by extrapolating two Wijs iodine numbers for 0.5 and 3 hours, respectively.

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The need for such differential halogen absorption methods was temporarily placed in doubt when in 1936 Kaufmann and Baltes (*15*) and subsequently Ellis and Jones (*8*) completed direct methods for determining the diene value, using maleic anhydride according to the Diels-Alder reaction. While a quantitative application of differential halogen absorption had been made only in the case of tung oil, the maleic anhydride addition methods were equally applicable to systems of two and three conjugated double bonds. However, difficulties arose in the presence of hydroxyl groups, especially with the more rapid Ellis-Jones method (*7*, *25*) and attempts to overcome these by acetylation were only partially successful (*1*). The Kaufmann-Baltes method is said to be unaffected by hydroxyl groups, and tests made in this laboratory showed the hydroxyl group of hydrogenated castor oil (*25*) to cause no disturbance with this procedure. Bickford and collaborators (*1*), however, obtained diene values on castor oil of 3.2 with the Kaufmann-Baltes method, and of 9.9 to 10.2 with the Ellis-Jones method. Bruce and Denley (*7*) found a diene value of 19.7 for commercial castor oil with the Ellis-Jones method.

An imperfection in dienometry was disclosed when McKinney and Jamieson (*18*) were unable to obtain theoretical maleic anhydride addition with alpha- and beta-eleostearic acid and the alpha-triglyceride. These findings were largely confirmed by Norris, Kass, and Burr (*24*), who reported the addition of eleostearic acid to be only in the neighborhood of 85 per cent of the theoretical. Certain isomers of eleostearic acid are said to be unable to add maleic anhydride although they contain the conjugated structure (*13*).

Such observations have not diminished the usefulness of the maleic anhydride addition methods, which are frequently

employed in industrial analysis. It is evident, however, notwithstanding the increasing use which is being made of spectroscopic methods for determining conjugation, that an independent method based on differential halogen absorption is desirable.

Table I. Partial Iodine Values of 9,11- and 10,12-Octadecadienoic Acids

Sample Weight *Gram*	Wijs Solution *Ml.*	$CHCl_3$ *Ml.*	Time *Min.*	Excess %	I. V. Found	Partial I. V., Theoretical	Material
0.1218	12	10	2	163	92.2	90.5	9,11-acid
0.1887	20	10	2	173	95.2	90.5	9,11-acid
0.3232	32	10	2	159	93.8	90.5	9,11-acid
0.1832	24	10	0.5	239	94.7	90.5	9,11-acid
0.2994	20	20	2	80	91.5	90.5	9,11-acid
0.2856	16	16	2	52	90.8	90.5	9,11-acid
0.1396	8	8	2	60	89.9	90.5	10,12-acid
0.2023	11	11	2	52	89.8	90.5	10,12-acid

Quantitative Relationship

When Toms (*30*) in 1928 calculated the percentage of alpha-eleostearic triglyceride in tung oil from the formula $(q - p)\frac{100}{87.7}$, where q and p are the total and partial iodine values, he unwittingly introduced the concept of diene value, although this term was suggested only 8 years later by Kaufmann and Baltes (*15*). The number 87.7, which he calculated as the difference between the iodine values for the addition of 18 and 12 atoms of iodine, respectively, to the triglyceride is actually the diene value according to the later definition, which was based on the consideration, that for each molecule of maleic anhydride added one double bond less than originally present remains. The relationship, total iodine value minus partial iodine value equals diene value, is a general one, if partial iodine value is understood to represent the value obtained when all nonconjugated double bonds, one half of all conjugated double bonds occurring in pairs, and two-thirds of all conjugated double bonds occurring in triplets, have added iodine. That this is necessarily so may be shown as follows:

Let a mixture contain x per cent of nonconjugated substances with an iodine value of X, y per cent of substances having a pair of conjugated double bonds with a true or total iodine value of Y, and z per cent of substances having three conjugated double bonds with a total iodine value of Z. Each of the three portions may in itself be a mixture of various constituents, in which case X, Y, and Z represent the average iodine values of each portion. The total iodine value of the mixture is then

$$I.\ V._T = \frac{1}{100}(xX + yY + zZ)$$

its partial iodine value by the above definition:

$$I.\ V._P = \frac{1}{100}(xX + {}^1/_2yY + {}^2/_3zZ)$$

and its diene value:

$$D.\ V. = \frac{1}{100}({}^1/_2yY + {}^1/_3zZ)$$

The difference, total minus partial iodine value, is then:

$$\frac{1}{100}(xX - xX + yY - {}^1/_2yY + zZ - {}^2/_3zZ) = \frac{1}{100}({}^1/_2yY + {}^1/_3zZ)$$

which equals the expression for the diene value.

Application to Dienometry

In order to use this relationship for the determination of the diene value of a substance it is necessary to select adequate methods for the determination of the two iodine values, total and partial.

Total Iodine Value. There exists, to the authors' knowledge, no halogen absorption method that can claim the accuracy and universality of properly conducted quantitative hydrogenation procedures. However, these require time-consuming preparations and a complicated apparatus which is not ordinarily available in the average industrial laboratory. The Woburn iodine absorption method (*22*), on the other hand, has all the required simplicity and speed at only a slight sacrifice in accuracy. It fulfills the necessary prerequisite of measuring the total unsaturation for both conjugated and nonconjugated substances, which is not possible with the conventional absorption methods.

The difficulty in its application to substances of mixed unsaturation lies in the fact that threefold conjugation, as in eleostearic acid, is best measured with the 0.40 *N* solution at 0° C., while 9,11-linoleic acid gives somewhat high results under these conditions and is better characterized by 0.32 *N* iodine bromide solution under otherwise similar conditions. If the 0.32 *N* solution was used for 1 hour at 20° C., however (method A), fair agreement with theoretical values was obtained in either case, and this modification, therefore, shows promise for total iodine value determination when systems with both two and three conjugated double bonds are present. If either double or triple conjugated systems are known to predominate, the accuracy may be improved by modifying the conditions slightly, according to the findings of the previous investigation.

Partial Iodine Value. This value in the past, has received attention only in the case of tung oil (*12*). The ordinary Wijs iodine value was considered to represent this stage adequately, where two out of three double bonds in eleostearic acid had been saturated with halogen, until Bolton and Williams (*6*) extrapolated a time series of Wijs numbers of tung oil and obtained its true partial or instantaneous iodine value. A simpler procedure, requiring one 2-minute determination in an ice bath instead of two exposures of 0.5 and 3 hours, respectively, was suggested by one of the authors (*21*) and shown to give the theoretical two-thirds addition for alpha-eleostearic acid. If this method is to be used in the determination of the diene value it must be adapted to systems of two conjugated double bonds as well.

The best-known example of a fatty acid containing a system of two conjugated double bonds is Mangold's 9,11-linoleic acid, formed by the dry distillation of ricin–elaidic acid (*19*). After repeated recrystallizations from petroleum ether, samples of this acid (m. p. 53. 5–54° C.) were subjected to the modified Wijs method in ice, as previously suggested for tung oil (*21*). The values obtained (Table I) are close to the theoretical partial iodine value, but from 2 to 5 points higher. It is evident that the addition of halogen to the second double bond proceeds easier than the corresponding addition to the third double bond in eleostearic acid.

In order to provide for somewhat milder conditions, a smaller excess of Wijs solution was employed, and instead of using 10 ml. of chloroform as solvent for the sample with 25 ml. of Wijs solution, an equal volume of chloroform and Wijs solution was employed. The resulting values agreed with theory within one unit (Table I, lines 5–6).

A new solid conjugated isomer of linoleic acid was found in the fatty acids resulting when dehydrated castor oil (Isoline, unbodied) was isomerized, using a commercial process for shifting isolated double bonds into the conjugated position (*31*). Disruptive oxidation showed that this new acid, melting at 57° C., which is not identical with Mangold's acid, is 10,12-octadecadienoic acid (*20*).

TABLE II. IODINE NUMBERS

Fatty Acid	Wijs Iodine No. (0.5 Hr.)	Woburn Iodine No. Method A (0.32 *N*)	Woburn Iodine No. Method D (0.4 *N*)	Partial Iodine No.	% Excess Short Wijs Iodine No.	Woburn Diene No.
Linseed, split	189.0	185.7	189.5[a]	190.6	268	−4.9 or −1.1
Sunflower, distilled	132.0	133.5	....	132.0	237	1.5
Soybean, split	146.5	143.7	....	145.7	229	−2.0

[a] Method D was used on linseed fatty acids because lower iodine values were found with method A.

After repeated recrystallizations from petroleum ether, ethyl ether, and ethyl alcohol, the new acid, which was handled in an atmosphere of carbon dioxide to prevent oxidation, gave almost theoretical partial iodine values when analyzed according to the procedure found most suitable for Mangold's acid (Table I, lines 7–8).

In analyzing mixtures of fatty acids containing both types of conjugation—i. e., double and triple—it must be borne in mind that the conditions giving the most accurate results are not identical in the two cases. That it is possible, however, to obtain almost theoretical partial iodine values with blends containing both types of conjugation is seen by comparing Tables III and IV. In determining the partial iodine value of such blends it is preferable to keep the excess of reagent between 200 and 250 per cent and follow the procedure used with tung oil. This is necessary in the presence of linolenic acid, which otherwise is not completely saturated, owing to the somewhat retarded addition of halogen to this nonconjugated acid.

Previously, it had been thought that the partial iodine number gave low results with nonconjugated oils and fatty acids, particularly those containing linolenic acid, because not enough time elapsed for the halogen to add completely to the last isolated double bond. However, the iodine values given in Table II for some fatty acids indicate that the partial iodine method may be used to determine the total unsaturation of nonconjugated fatty acids, including linseed fatty acids, if the excess of the reagent is 200 to 250 per cent. It may also serve as a method of control in isomerizing fatty acids to their conjugated form.

The determination of total iodine values was as follows:

Method A, 0.32 *N* IBr, 1 hour at 20° C. Method B, 0.40 *N* IBr, 3 hours at 0° C. Method C, 0.32 *N* IBr, 3 hours at 0° C. Method D, 0.40 *N* IBr, 1 hour at 20° C. The partial iodine values were determined in thin-walled Erlenmeyer flasks in the ice bath as previously described (*21*), following the details originally suggested for tung oil—i. e., 200 to 250 per cent excess of Wijs solution (25 ml.), 10 ml. of chloroform, and 2 minutes, unless otherwise stated.

DIENE VALUES. These were determined by subtracting partial iodine values from the total (Woburn) values and compared to theoretical figures and, in some cases, to conventional (maleic anhydride) diene determinations (Table III).

The blends analyzed (Tables III and IV) were made up as follows:

Blend A. 50.0% 9,11-octadecadienoic acid
50% soybean fatty acids (I. V. = 138.8)

Blend B. 5.505% beta-eleostearic acid
31.40% 10,12-octadecadienoic acid
63.095% sunflower fatty acids (I. V. = 132.0)

Blend C. 35.71% beta-eleostearic acid
34.25% 10,12-octadecadienoic acid
30.04% linseed fatty acids (I. V. = 179.3)

The diene values obtained for beta-eleostearic acid were 89.7 and 90.8, depending on details followed in the determination of the total unsaturation. The latter value, which is based on method B, agrees well with the calculated value. This method, which had previously been recommended for tung and oiticica oils (*22*, page 788) was employed for calculating the diene value of two of the tung oil samples previously described (*22*, page 786). The resulting values agree well with the corresponding Ellis-Jones maleic anhydride diene values. The iodine value of 224.2 for sample 4, which coincides with the break in the curve for varying excess (*22*, page 787), gives a diene value somewhat lower than those by either of the conventional methods.

TABLE III. CALCULATED VALUES OF BLENDS

Blend	Total Iodine Value	Partial Iodine Value	Diene Value
A	160.0	114.7	45.3
B	155.2	121.7	33.4
C	213.5	150.0	63.6

With oiticica oil the use of the ice bath (method B) is especially necessary because of the known influence of the keto group in licanic acid on iodine value determinations at ordinary temperatures. While conventional iodine absorption procedures fail entirely with this oil (*11*), both the total and partial iodine values determined with the methods here described gave reproducible results and the differential diene value lay between the Ellis-Jones and the Kaufmann-Baltes values.

While 9,11-linoleic acid gave satisfactory results, the differential diene value of the new 10,12-linoleic acid was several points too high. The methyl ester of this acid, however, gave practically theoretical diene values. Blend B, which contained the new acid as the principal conjugated constituent, also gave a somewhat high differential diene

TABLE IV. COMPARISON OF DIFFERENTIAL IODINE ABSORPTION AND DIENE VALUES

Substance	Woburn Iodine Value	Partial Iodine Value	Diene Value by Difference	Maleic Anhydride Diene Value	Theoretical Diene Value
Beta-eleostearic acid	272.7 (A) 273.8 (B)	183.0	89.7 90.8	.....	91.2
Tung oil 4[a]	226.6 (B) 224.2[b]	160.5	66.1 63.7	75.0 (KB) 66.2 (EJ)	.. ..
Tung oil 5[a]	227.1 (B)	159.5	67.6	73.5 (KB) 69.1 (EJ)	..
Oiticica oil, sample 1	203.9 (B)	139.7	64.2	71.4 (KB) 59.5 (EJ)	..
Oiticica oil, sample 2	201.6 (B)	138.3	63.3	60.3 (EJ)	..
9,11-Linoleic acid	183.3 (A)	91.2[c]	92.1	.....	90.5
10,12 - Linoleic acid	186.7 (A)	89.9[c]	96.8	.....	90.5
10,12-Methyl linoleate	173.4 (A)	86.4[c]	87.0	.....	86.2
Blend A	160.6 (A)	113.3[c]	47.3	.....	45.3
Blend B	161.8 (A) 157.3[d]	124.1 ..	37.7 33.2	....	33.4 ..
Blend C	213.0 (A)	149.1	63.9	.....	63.6
Castor fatty acid	89.3 (A)	87.0	2.3	.....	..
Walnut oil	157.4 (A)	156.1	1.3	.....	..

[a] Samples described in (*22*).
[b] From excess *vs.* iodine value curve in previous paper (*22*).
[c] 50 to 80% excess reagent; volumes of Wijs solution and chloroform equal.
[d] 390% excess of reagent.

TABLE V. COMPARISON OF DIENE VALUES FOR COMMERCIAL PRODUCTS AND SAMPLES

Substance	Woburn Iodine Value[a]	Partial Iodine Value	Diene Value by Difference	Maleic Anhydride Diene Value
Conjulin F. A.[b], sample 1	190.4 (D)	117.5	72.9	18.0 (EJ)
Conjulin F. A., sample 2	178.7 (D)	116.2	62.5	24.7 (EJ)
Conjulin F. A., sample 3, distilled	186.0 (D)	131.9	54.1	.....
Conjusoy F. A.[b]	136.7	94.4	42.3	14.1 (EJ)
Conjusoy F. A., distilled	137.1	95.4	41.7	8.8 (KB) 10.5 (EJ)
Dehydrated castor oil, sample 1	155.1	122.2	32.9	18.4 (KB)
Dehydrated castor oil, sample 2	155.3	118.2	37.1	13.5 (KB)
Dehydrated castor fatty acid, distilled	163.9	130.0	33.9	22.8 (EJ)
Isomerized isoline[c] fatty acids	165.4	101.2	64.2	39.2 (EJ)
Isomerized walnut fatty acids	159.6	96.5	63.1	.....
Heat-treated oiticica oil[d]	182.4 (B)	121.9	60.5	42.1 (EJ)

[a] Method A, except when otherwise indicated.
[b] Conjulin F. A. and Conjusoy F. A. are trade names for isomerized linseed and soybean fatty acids, respectively.
[c] Isoline is the trade name for dehydrated castor oil as manufactured by Woburn Degreasing Company of N. J.
[d] This sample, known in the trade as Cicoil, was obtained through the courtesy of the Brazil Oiticica Company. Its viscosity was 21 poises.

value, except when a smaller excess of reagent than usual was employed in the Woburn iodine value determination.

Summing up the data presented in Table IV, in most cases the observed values are in good agreement with theory. The diene value determination by differential halogen absorption, in its present form, may therefore be accepted as a simple and rapid method for obtaining a good quantitative estimate of conjugation in fatty acids and esters. Certain limitations in the scope of applicability are discussed below.

Limitations

There are certain compounds, other than those containing conjugated double bonds, which behave abnormally with halogenating reagents. They, too, add variable amounts of halogen, depending on the length of contact, excess of reagents, kind of reagent used, and other secondary factors.

Among such compounds van Loon (*17*) lists hydroxy acids, oils with much unsaponifiable matter, polymerized oils or acids, and 6,7-stearolic acid containing an acetylenic or triple bond. With polymerized oils and esters, this behavior was also observed by Steger and van Loon (*29*) and by Kino (*16*) who traced the increased iodine value of these products upon prolonged time of contact to substitution. Morrell (*23*) found that the iodine values of the maleic anhydride–licanic acid addition products were too high after long treatment with Wijs reagent and ascribed it to keto-enolization. Rossmann (*26*) tested undecylenic and cinnamic acids among compounds with sterically hindered or inactive bonds requiring larger excess of bromine vapor for complete saturation.

It might be thought that any of these compounds would give a higher iodine value with the Woburn method than with the modified Wijs procedure and, therefore, show an apparent diene value. However, it was shown in the case of castor fatty acids (Table IV) that the difference between the two iodine values was only 2.3 points. Even if it is assumed that this was caused entirely by the hydroxyl group of ricinoleic acid and that no conjugated acid was present, the error caused by the hydroxyl group is small. The resulting differential diene value is smaller than those observed on castor oil with either of the methods using maleic anhydride (*1*, *7*). The values for oiticica oil (Table IV) similarly show that the keto group does not affect the differential halogen absorption values adversely if the Woburn iodine value is determined at 0° C. (method B).

On the other hand, the authors' observations indicate that polymerized oils definitely have differential iodine absorption values, regardless of the presence of conjugated double bonds. Their method will, therefore, not be applicable to bodied oils. This may be due not only to substitution, as observed by Kino (*16*), but to sterically hindered double bonds remaining in cyclic polymers as well. Other types of inactive bonds might also result in an apparent diene value and this factor must be considered whenever substances of unknown or doubtful composition are tested by the new method.

Listed in Table V are the constants of a number of substances for which the Woburn diene value differs markedly from the diene values obtained by other methods, the difference being definitely larger than any experimental errors previously observed. The isomerized fatty acids listed in this table have been made semicommercially by treatment with aqueous alkali (patents pending), designed to cause the shifting of double bonds from the isolated to conjugated position (*31*). The two dehydrated castor oils were unbodied samples having viscosities of F-G (Isoline, raw) and G-H in the order listed.

In all these oils or fatty acids the diene value by difference is considerably larger than the conventional maleic anhydride values. The question naturally arises whether this disagreement is due to the presence of some of the above discussed inactive double bonds in the isomerized products, to a failure of maleic anhydride to react with all conjugated double bonds present, or to both these causes. To decide this, tests were made to determine whether the extent of maleic anhydride addition could be increased.

TABLE VI. DIENE VALUES WITH MALEIC ANHYDRIDE IN ORTHODICHLOROBENZENE

Substance	Period of Refluxing	Diene Values
	Hours	
Tung oil, sample 5[a]	3	69.1, 69.7
Conjulin F. A.[b]	3	50.4, 50.8
Conjusoy F. A.[b]	1	26.8
	2	35.4
	3	40.6, 41.9
	18	44.7, 46.0
Soybean fatty acid distilled	1	3.8
	2	14.7
	3	11.9, 16.9
	18	47.6

[a] Sample described in (*22*).
[b] Trade names.

Maleic Anhydride at Higher Temperatures

The use of higher boiling solvents in the refluxing operation of the Ellis-Jones method suggests itself as a means of promoting the addition. Of several solvents which were tried for this purpose, *o*-dichlorobenzene (technical), boiling at 175° C., appeared most promising. The details of these determinations were similar to those of the Ellis-Jones method, except that enough ether was added before hydrolyzing to obtain a mixture floating on water, thus facilitating the extraction of residual maleic anhydride in the separatory funnel. Blank runs were made as usual under identical conditions. They showed that no appreciable reaction took

place between maleic anhydride and the solvent during the refluxing period.

Some diene values thus obtained are listed in Table VI. The values for tung oil are seen to be identical with those obtained in toluene solution in the Ellis-Jones procedure (compare Table IV). All values obtained on conjugated soybean and linseed fatty acids, on the other hand, were much higher than the figures obtained with the Ellis-Jones or the Kaufmann-Baltes methods (compare Table V). With Conjusoy fatty acids the 3-hour values equaled the differential iodine absorption values, but with Conjulin fatty acids they were somewhat lower.

Quantitative conclusions which might be drawn from these maleic anhydride diene values at higher temperature are made difficult, however, by the fact that ordinary distilled soybean fatty acid, when similarly treated in *o*-dichlorobenzene solution, also led to substantial diene values. The values obtained, with both conjugated and nonconjugated fatty acids, depended on the length of refluxing, suggesting that a rearrangement of the double bonds into the conjugated position takes place during the determination. [The reaction of maleic anhydride with nonconjugated fatty acid esters at 200° to 250° C. has been studied thoroughly by Bickford *et al.* (*2*).]

For short refluxing periods the addition values of the isomerized fatty acids are much larger than those of the unisomerized, distilled fatty acids, and the difference between the two corresponding values is larger than the conventional diene values for the conjugated fatty acids. It follows, therefore, that the amount of conjugation originally present is larger than would be indicated by the conventional diene value. The 1-hour values listed show, for example, that the true diene value of the Conjusoy sample is certainly not below 23—i. e., 26.8 − 3.8—although it may be higher. The value of 14, obtained with the Ellis-Jones method on the same sample (Table V) was, therefore, due to incomplete addition. Similar conclusions must be drawn concerning the Kaufmann-Baltes method, since both methods gave comparable results with the conjugated products.

Further evidence that the conventional methods yield incomplete diene values with these products is seen in a comparison with spectroscopic data. (The spectroscopic determinations are taken from a private communication by Bradley and Richardson, whose courtesy in permitting its use is gratefully acknowledged.) The spectroscopically calculated diene values of the samples of Conjulin and Conjusoy fatty acids, listed in Table V, were 36.9 and 29.8, respectively. Assuming these values to be correct, the conventional diene values would indicate only about one half of the conjugated double bonds present in these products. The Woburn diene values, on the other hand, would be too high.

Summary

The quantitative relationship, total iodine value − partial iodine value = diene value, can form the basis for determining the diene value of fatty acids and oils.

The Woburn iodine method, which makes use of iodine bromide solutions 1.6 to 2 times the concentration of Hanus' solution, may serve to determine total iodine values.

The partial iodine values may be determined with Wijs solution, by limiting the time of contact to 2 minutes at ice-bath temperature. This procedure is almost similar to one previously evolved for tung oil. Theoretical partial iodine values are obtained for Mangold acid and for a new solid conjugated isomer of linoleic acid—i. e., 10,12-octadecadienoic acid—showing that this method is applicable to conjugated dienes. The modified Wijs procedure can be used to determine total unsaturation in nonconjugated fatty acids, by using the correct excess of reagent.

The diene values obtained by differential iodine absorption for tung oil and oiticica oil compare well with maleic anhydride diene values. The total and partial iodine values of oiticica oil are reproducible in spite of the inapplicability of other iodine absorption procedures to this oil. With pure conjugated fatty acids and esters, the differential diene values, in general, are in good agreement with calculated values.

The new method is not applicable in the presence of polymerized oil and certain types of unsaturation, which may cause differential iodine absorption due to steric hindrance.

Certain commercial fatty acids and oils of the conjugated type have differential diene values much higher than their maleic anhydride addition values, determined by standard methods.

Maleic anhydride does not react completely with the conjugated double bonds present in these manufactured products at the temperature used in the standard diene methods.

A slow reaction takes place between maleic anhydride and nonconjugated fatty acid in *o*-dichlorobenzene and the amount of anhydride added after 18 hours equals that added to the corresponding conjugated fatty acid.

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Analytical Data for the Systems Carbon Tetrachloride–Acetic Acid–Benzene and Carbon Tetrachloride–Tetrachloroethylene

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IN THE course of investigations on the ternary system benzene–carbon tetrachloride–acetic acid and the binary system carbon tetrachloride–tetrachloroethylene, it was found necessary to have methods of analysis for each system. Two methods were developed for the ternary system and the analytical data obtained for the binary system.

The carbon tetrachloride, benzene, and tetrachloroethylene were of the best grade obtainable from the Eastman Kodak Company. The acetic acid used was Baker's Analyzed material. As a check on purity, the carbon tetrachloride and tetrachloroethylene were redistilled. The boiling range of the carbon tetrachloride was found to be 0.3° C. and that of the tetrachloroethylene, 2° C. The middle portion of the latter compound was used. The refractive indexes of the solvents are given in Table I.

TABLE I. REFRACTIVE INDEXES

	Experimental	Literature (2)
Benzene	1.4985 at 25° C.	1.5017 at 20° C.
Carbon tetrachloride	1.4549 at 25° C.	1.4630 at 20° C.
Acetic acid	1.3706 at 25° C.	1.3715 at 22.9° C.
Tetrachloroethylene	1.4993 at 25° C.	1.5055 at 20° C.

Procedure and Results

In the first method of analysis for the ternary system, the sample was weighed, and the acid determined volumetrically with standardized sodium hydroxide solution. Thymol blue was used as the indicator. Phenolphthalein as ordinarily prepared in alcoholic solution could not be used because the alcohol changes the refractive index of the benzene–carbon tetrachloride layer. However, thymol blue made up in a basic aqueous solution (*1*) proved satisfactory. The layer or phase containing benzene and carbon tetrachloride appears as the titration is carried out. The other layer consists of water, sodium acetate, thymol blue, and excess sodium hydroxide. The two layers were separated in a centrifuge, and the refractive index of the benzene–carbon tetrachloride layer was determined in an Abbe refractometer, which was held at 25.0° ± 0.1° C. The index of refraction was then compared with a standard curve (Figure 1) that represented the refractive indexes of synthetic mixtures of benzene and carbon tetrachloride plotted against the per cent by weight. These synthetic mixtures were made up by volume at the 10 per cent divisions. Corrections were made for the densities of the liquids at room temperature. The values for the refractive indexes at 25° are given in Table II.

That the method of analysis is capable of sufficient precision to be useful was demonstrated by the following experiments.

Five mixtures of carbon tetrachloride and benzene covering the whole range of the solution (refractive indexes 1.4642, 1.4750, 1.4816, 1.4918, and 1.4963) were made up and (*a*) the refractive indexes of the solutions were determined. Then equal quantities of the solutions were mixed with (*b*) water, (*c*) water and sodium acetate solution, (*d*) water and sodium hydroxide, and finally (*e*) indicator solution. The last four mixtures, in each case, were shaken thoroughly and then allowed to stand for 0.5 hour. Finally they were centrifuged for about 2 minutes and the refractive index of the carbon tetrachloride–benzene layer was redetermined. It was found that the values had not changed by more than ±0.0005 in any sample.

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TABLE II. BENZENE AND CARBON TETRACHLORIDE

Benzene, Weight %	Refractive Index at 25° C.
0	1.4549
10	1.4626
20	1.4693
30	1.4744
40	1.4793
50	1.4837
60	1.4873
70	1.4906
80	1.4936
90	1.4961
100	1.4985

The results from the sample having refractive index 1.4750 are given below as representative of the group:

Sample	Refractive Index
(*a*)	1.4750
(*b*)	1.4749
(*c*)	1.4749
(*d*)	1.4751
(*e*)	1.4750

It may be concluded that solubility effects are not great enough to change seriously the refractive index of mixtures of benzene and carbon tetrachloride.

A preliminary run on the analysis of the mixture was performed. Three milliliters of acetic acid in water required 77.42 ml. of sodium hydroxide solution (27 grams of sodium hydroxide per liter) for neutralization; 1 ml. of acetic acid in 6 ml. of the sample of carbon tetrachloride and benzene whose refractive index is given above, required 26.00 ml. of sodium hydroxide solution for neutralization. This is a check within 1 per cent. The layer of carbon tetrachloride and benzene was separated and found to have a refractive index of 1.4754, which is in good agreement with the previous values. Three solutions containing benzene, carbon tetrachloride, and acetic acid were then made up

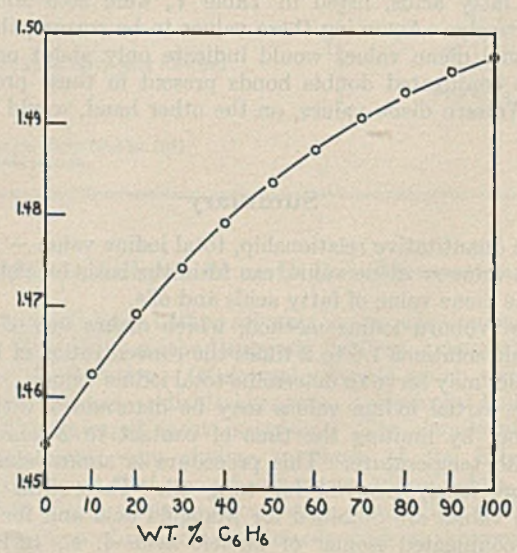

FIGURE 1. REFRACTIVE INDEX OF SYSTEM BENZENE–CARBON TETRACHLORIDE AT 25° C.

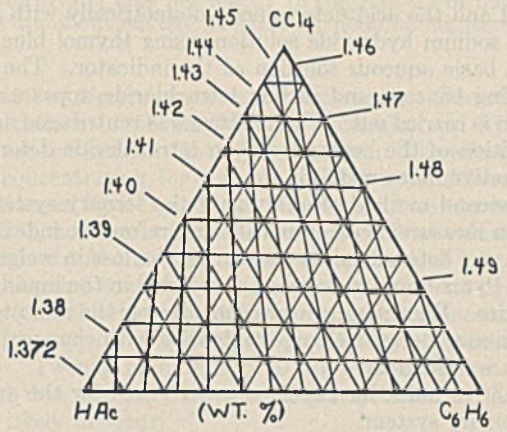

FIGURE 2. LINES OF CONSTANT REFRACTIVE INDEX FOR TERNARY SYSTEM AT 25° C.

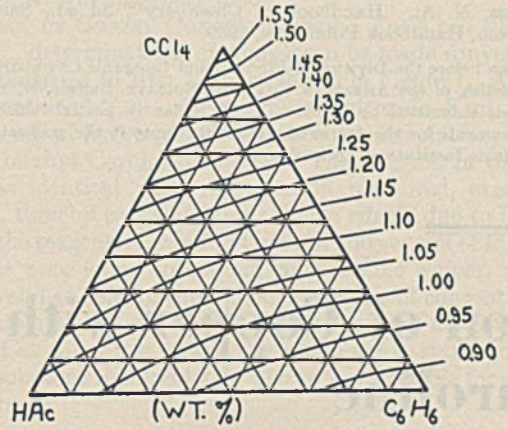

FIGURE 3. LINES OF CONSTANT DENSITY FOR TERNARY SYSTEM

accurately at approximately the following concentrations, expressed in per cent by weight:

Benzene	Carbon Tetrachloride	Acetic Acid
10	10	80
80	10	10
10	80	10

The corresponding percentages, as determined by the method of analysis outlined above, were in all cases within 1 per cent.

A more rigorous experiment was then performed. Twelve solutions were made up, each with 5 ml. of water, 1 ml. of acetic acid, and 3 drops of thymol blue solution. Solutions 3, 4, 11, and 12 each had 1 ml. of carbon tetrachloride. Solutions 5 and 6 each had 5 ml. of carbon tetrachloride. Solutions 7, 8, 11, and 12 each had 1 ml. of benzene. Solutions 9 and 10 each had 5 ml. of benzene. Titrations were carried out for the acid with standardized sodium hydroxide solution:

Solution	Titer
1	29.75
2	29.88
3	29.78
4	29.85
5	29.77
6	29.87
7	29.70
8	29.65
9	29.91
10	29.92
11	29.72
12	29.85
	Av. 29.80

It is felt that these experiments demonstrate satisfactorily that the method of analysis is practicable and that mixtures containing other water-soluble acids might be analyzed in a similar way.

In later work the analysis of the ternary system was based on the measurement of the density and refractive index.

Representative mixtures were made up covering the three-component diagram and their refractive indexes were determined at 25.0° ± 0.1° C. The densities were determined by the "loss of weight principle". A drop of Pyrex brand glass was suspended in the liquids by means of a fine tungsten wire. Rigid temperature control over the mixtures was not necessary because the change of density with change of composition was so great. The densities were measured at 27° ± 0.5° C. Sixteen and fifteen samples were used to construct the ternary density and refractive index diagrams, respectively. In transposing the refractive index data to the ternary diagram, six graphs were made, one along each 0 per cent line and one along each 25 per cent line (total of 6), plotting refractive index against per cent by weight. It was then decided what intervals would be useful to plot. For refractive index, the 0.01 intervals were marked on the six graphs—that is, 1.40, 1.41, 1.42, etc. These values were then marked on the ternary diagram at the appropriate compositions. A line was drawn through all points representing the same value of refractive index (Figure 2). The same procedure was followed in transposing the experimental density data to the ternary graph (Figure 3).

The experimental refractive index and density data are presented in Tables III and IV, respectively.

The analysis of the binary mixture, carbon tetrachloride–tetrachloroethylene, was most conveniently done by refractive index. Solutions were made up accurately by weight at

TABLE III. REFRACTIVE INDEXES OF TERNARY MIXTURES

Acetic Acid, Weight %	Benzene, Weight % 0	25	50	75	100
100	1.3706	....	....	....	....
75	1.3851	1.4011	....	....	....
50	1.3991	1.4184	1.4330	....	....
25	1.4229	1.4425	1.4555	1.4657	....
0	1.4549	1.4724	1.4837	1.4923	1.4985

TABLE IV. DENSITIES OF TERNARY MIXTURES

Acetic Acid, Weight %	Benzene, Weight % 0	20	25	50	70	75	80	100
100	1.0304	....	....	....	....	....	....	....
75	1.1287	....	0.9846	....	....	....	....	....
50	1.2410	....	1.0603	0.9314	....	....	....	....
25	1.3825	....	1.1675	1.0102	....	0.8966	....	....
0	1.5866	1.3533	....	1.1234	1.0091	....	0.9507	0.8734

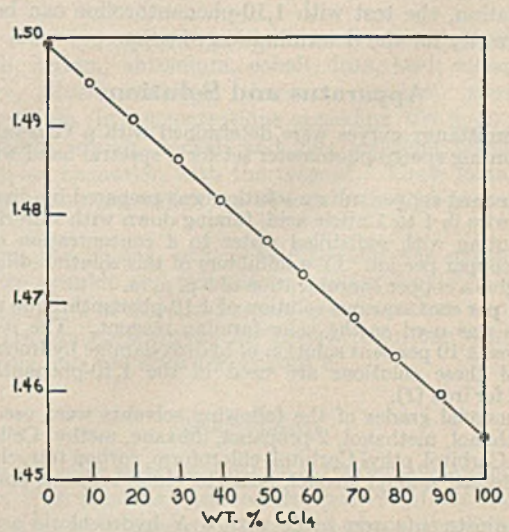

FIGURE 4. REFRACTIVE INDEX OF SYSTEM CARBON TETRACHLORIDE–TETRACHLOROETHYLENE

TABLE V. CARBON TETRACHLORIDE AND TETRACHLOROETHYLENE

Carbon Tetrachloride, Weight %	Refractive Index at 25° C.
0	1.4993
9.85	1.4949
19.16	1.4907
29.65	1.4862
39.54	1.4818
49.75	1.4771
58.29	1.4733
69.94	1.4684
79.59	1.4640
89.94	1.4596
100.00	1.4549

the approximate 10 per cent divisions. When the results were plotted as refractive index against per cent by weight, it was found that a straight line fitted the data most accurately (Table V).

Summary

Two methods have been developed for the analysis of the ternary system, benzene–carbon tetrachloride–acetic acid, and one method for the binary system, carbon tetrachloride–tetrachloroethylene.

In the first method for the ternary system, the sample was weighed and the acid determined volumetrically with standardized sodium hydroxide solution, using thymol blue made up in a basic aqueous solution as the indicator. The phase containing benzene and carbon tetrachloride appears as the titration is carried out. The mixture was centrifuged and the composition of the benzene–carbon tetrachloride determined by refractive index methods.

The second method of analysis of the ternary system was based on measurements of density and refractive index. The density was determined by measuring the loss in weight of a drop of Pyrex brand glass when suspended in the liquids from a fine wire. Rigid temperature control over the mixtures was not necessary because change of density with change of composition was so great.

Refractive index measurements were used for the analysis of the binary system.

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Colorimetric Determination of Copper with 1,10-Phenanthroline

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TARTARINI (*5*) in a discussion of new color reactions involving cuprous salts, reported that 2,2′-bipyridyl and 1,10-phenanthroline form copper complexes which can be reduced with hydroxylamine to give highly colored cuprous compounds. The reaction is brought about by treating an ammoniacal solution of a cupric salt with the reagent and reducing. All cuprous salts containing ammonia or strong organic bases are either colorless or very pale. With a slight modification, the test with 1,10-phenanthroline can be used satisfactorily for the determination of copper.

Apparatus and Solutions

Transmittancy curves were determined with a General Electric recording spectrophotometer set for a spectral band width of 10 mμ.

A standard copper sulfate solution was prepared by dissolving copper wire in 1 to 1 nitric acid, fuming down with sulfuric acid, and diluting with redistilled water to a concentration of 0.05 mg. of copper per ml. Five milliliters of this solution diluted to 50 ml. give a copper concentration of 5 p. p. m.

A 0.1 per cent aqueous solution of 1,10-phenanthroline monohydrate was used as the color-forming reagent. The reducing agent was a 10 per cent solution of hydroxylamine hydrochloride. Both of these solutions are used in the 1,10-phenanthroline method for iron (*1*).

Commercial grades of the following solvents were used: acetone, ethanol, methanol, 2-propanol, dioxane, methyl Cellosolve, methyl Carbitol, ethyl Carbitol, chloroform, carbon tetrachloride, ethyl acetate, *n*-amyl alcohol, benzene, chlorobenzene, and ethyl ether.

pH adjustments were made with 6 *N* hydrochloric acid and 6 *N* ammonium hydroxide.

Standard solutions of the anions studied were prepared from the alkali metal salts. Nitrates, chlorides, and sulfates were used for the cations. Each solution contained 10 mg. of the ion in question per ml.

The Color Reaction

The order in which the reagents are added is of primary importance, the two following conditions being necessary: copper must be in the form of the ammonia complex and the reagent must be added before the hydroxylamine. Ammonia concentration is a critical factor but is easily controlled by neutralizing the solution to litmus and adding a measured quantity of 6 *N* ammonium hydroxide.

Formation of a complex cuprous salt such as $Cu(C_{12}H_8N_2)X$ is responsible for the orange color (*5*). The time required for precipitation to occur in aqueous solutions varies with the copper concentration. Precipitation is prevented entirely by the presence of certain alcohols or other solvents miscible with water. Other undesirable properties of aqueous solutions of the complex which can be avoided by addition of a solvent are instability of the color, nonconformity to Beer's law, and change of hue with copper concentration.

Forty per cent methyl Carbitol is most satisfactory of the solvents tried, although ethyl Carbitol can also be used. Fading occurs within 2 days with dioxane, methyl Cellosolve, or the alcohols. With methyl Carbitol, the slight change in 2 days is not measurable by visual means. Acetone reacts with the hydroxylamine.

Extraction of the color with any of the following solvents is not feasible: *n*-amyl alcohol, benzene, chlorobenzene, chloroform, carbon tetrachloride, ether, and ethyl acetate.

The 5-chloro, 5-bromo, 5-methyl, nitro, and 5-nitro-6-methyl derivatives of 1,10-phenanthroline have no advantage over the parent compound from the standpoint of sensitivity or stability of the colored complex. 2,2′-Bipyridyl is less sensitive and

2,2′,2″-terpyridyl does not react. In contrast, all of these compounds are about equally effective for determining ferrous iron (*1*, *4*).

Effect of Concentration of Reagents. The effects of concentrations of phenanthroline, ammonia, and hydroxylamine are not independent of one another. The effect of ammonia concentration, for example, is different for different concentrations of hydroxylamine. These variables can be easily controlled, however. For solutions with a final volume of 50 ml., 2 ml. of 6 *N* ammonium hydroxide, 10 ml. of 0.1 per cent phenanthroline solution, and 5 ml. of 10 per cent hydroxylamine hydrochloride are sufficient for copper concentrations up to 10 p. p. m. Concentration of methyl Carbitol is not critical. Twenty milliliters in 50 ml. of solution are recommended, but less may be used if precipitation of dissolved salts is likely to occur.

Buffering with ammonium acetate offers no advantage because the required amount of free ammonia must be present before reducing the copper, in which case proper adjustment of pH will have been made.

Effect of Copper Concentration. For a cell thickness of 1 cm., determinations of copper can be made conveniently in the range 0.1 to 10 p. p. m. as shown in Figure 1. All solutions contained 2 ml. of ammonium hydroxide, 8 ml. of reagent, 5 ml. of hydroxylamine hydrochloride solution, and 20 ml. of methyl Carbitol in 50 ml. The solution in the back cell was identical with the solution measured, except for copper, thereby canceling any adverse effects due to impurities in the reagents. Methyl Carbitol and several of the other solvents were found to contain considerable copper. Beer's law is valid at 435 mμ throughout the range of concentrations susceptible to measurement. The molecular extinction coefficient at 435 mμ is 7030. For visual work, standards such as the solutions represented in Figure 1 are reliable for at least 24 hours.

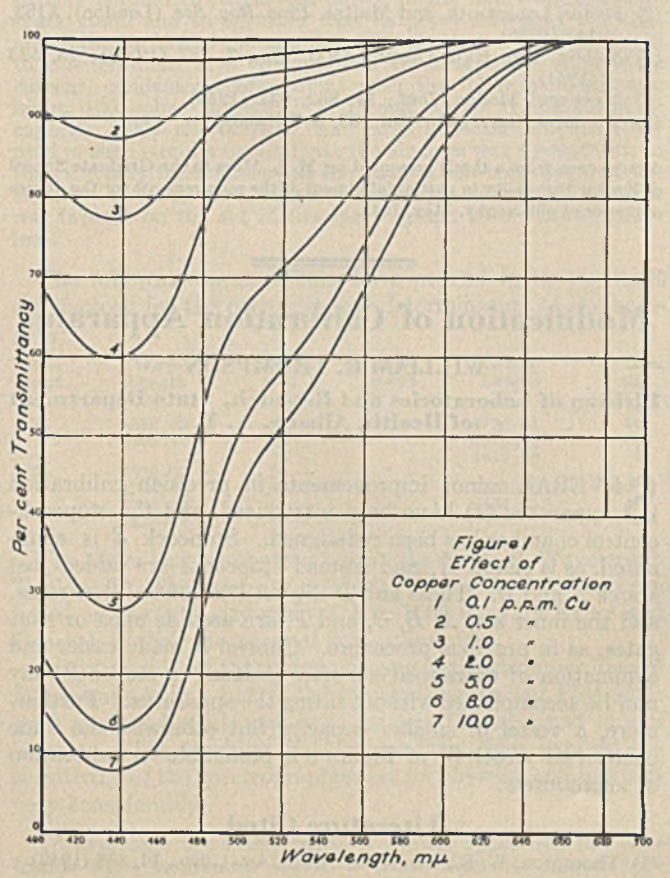


Table I. Effect of Diverse Ions

Ion	Added as	Present P. p. m.	Error %	Amount Permissible P. p. m.
Ba^{++}	$Ba(NO_3)_2$	500	3	300
Be^{++}	$Be(NO_3)_2$	100	0	100
Cd^{++}	$Cd(NO_3)_2$	10	2	10
Co^{++}	$Co(NO_3)_2$	10	6	5
Ni^{++}	$Ni(NO_3)_2$	5	2	5
Sr^{++}	$Sr(NO_3)_2$	500	10	100
Zn^{++}	$Zn(NO_3)_2$	10	2	10
CO_3^{--}	Na_2CO_3	500	3	300
CHO_2^-	HCO_2H	500	3	300
$C_2O_4^{--}$	$(NH_4)_2C_2O_4$	500	3	300
$C_3H_5O_3^-$	$CH_3CHOHCO_2H$	500	3	300
$C_4H_4O_6^{--}$	$(NH_4)_2C_4H_4O_6$	500	3	300
$C_6H_5O_7^{---}$	$(HO)C(CO_2H)(CH_2CO_2H)_2$	500	3	300
$C_7H_5O_2^-$	$C_6H_5CO_2Na$	500	3	300
$C_7H_5O_3^-$	$C_6H_4(OH)CO_2Na$	500	3	300
CN^-	KCN	2	14	0
$Cr_2O_7^{--}$ (Cr)	$K_2Cr_2O_7$	25	2	25
$P_2O_7^{--}$	$Na_4P_2O_7$	500	4	300
SCN^-	KSCN	500	6	250
$S_2O_3^{--}$	$Na_2S_2O_3$	30	18	5
VO_3^-	KVO_3	500	14	100

Effect of Diverse Ions. The phenanthroline method for copper is comparatively free from interference by the anions. Few cations interfere aside from those which precipitate in the presence of ammonia.

In determining the extent of interference for the various ions, the following procedure was adopted: A measured volume of solution containing the ion in question was added to 5 ml. of the copper sulfate solution containing 0.05 mg. of copper per ml. After neutralizing to litmus with 6 *N* ammonium hydroxide or hydrochloric acid, the following were added in order: 2 ml. of 6 *N* ammonium hydroxide, 10 ml. of reagent solution, 1 ml. of hydroxylamine solution, and 20 ml. of methyl Carbitol. The solution was then diluted to 50 ml. with redistilled water and the transmission curve run. Ammonium acetate was added in a number of cases without any apparent advantage. If interference was pronounced, smaller quantities of the ion were used successively until an estimate of the limiting permissible concentration could be made, 2 per cent error being set arbitrarily as a reasonable tolerance. These values are summarized in Table I.

For solutions containing 5 p. p. m. of copper, the following ions may be present in concentrations of at least 500 p. p. m. without causing an error greater than 2 per cent: ammonium, calcium, lithium, magnesium, potassium, sodium, acetate, arsenate, arsenite, bromide, chlorate, chloride, fluoride, iodate, iodide, molybdate, nitrate, nitrite, orthophosphate, perchlorate, periodate, silicate, sulfate, sulfite, tetraborate, and tungstate.

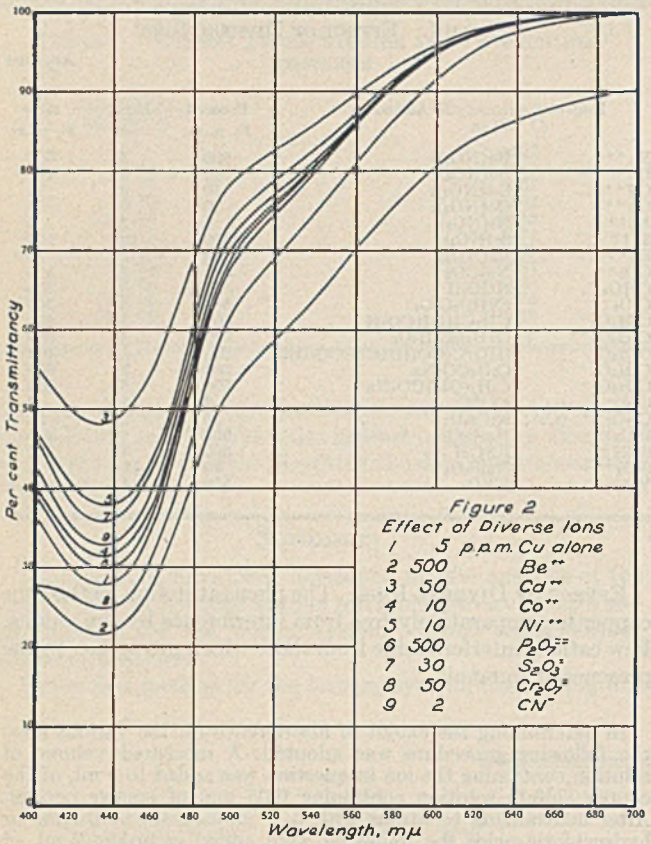
The following cations precipitate: aluminum, antimony, bismuth, cerium, chromium, cobalt, iron, lead, manganese, mercury, silver, titanium, thorium, uranium, zirconium, and beryllium (in concentrations exceeding 100 p. p. m.).

Cadmium, cobalt, nickel, and zinc interfere presumably by complex formation with the reagent. These metals also interfere in the determination of iron with 1,10-phenanthroline (*1*).

Among the anions, serious interference was encountered only with cyanide and thiosulfate. These should be absent. Large amounts of dichromate and metavanadate also interfere. Transmission curves for several solutions containing interfering ions are shown in Figure 2.

Discussion

Since the color reaction must be carried out in ammoniacal solution, iron must be removed irrespective of the fact that it, also, gives an intense color with 1,10-phenanthroline. This separation can be made by careful precipitation with ammonia as directed by Mehlig (*3*). When the filtration has



been completed, copper is present as the ammonia complex and, if the concentration is high enough to give a measurable color, no advantage over the ammonia method (*3*) can be gained by using 1,10-phenanthroline. If the concentration is too low, however, the proposed method may be used with good results for concentrations greater than 0.1 p. p. m.

In using the method for copper ores, results were calculated by the following procedure.

Instead of comparing the transmittancy of the unknown solution with a standard, the change in transmittancy produced by adding a known amount of copper to the unknown was determined. The unknown concentration, x, was then calculated according to Beer's law expressed as follows:

$$T_x = T_{a+x}^{\frac{x}{a+x}}$$

$$x = \frac{a \log T_x}{\log \frac{T_{a+x}}{T_x}}$$

in which T_x is the transmittancy of an aliquot of the sample and T_{a+x} the transmittancy of a similar aliquot containing a small additional quantity of copper, a. Uncertainties arising from the presence of other constituents, turbidity, extraneous color, or other sources, except presence of copper in the reagents, are thus compensated. Foster, Langstroth, and McRae have applied this principle successfully to the spectrographic determination of lead (*2*).

1,10-Phenanthroline is somewhat less sensitive as a copper reagent than is sodium diethyldithiocarbamate. The latter reagent forms an insoluble copper salt requiring use of gum arabic or extraction with a suitable solvent. Methyl Carbitol is not satisfactory for preventing precipitation with the diethyldithiocarbamate method nor can gum arabic be used with the phenanthroline method.

Recommended Procedure

Treatment of Sample. Prepare a solution of the sample by appropriate means to contain 0.01 to 0.5 mg. of copper (see *3* for copper ores and mattes).

If necessary to remove any metals precipitated by ammonia, make the solution just basic to litmus by adding 15 *N* ammonium hydroxide, filter into a conical flask, and wash the precipitate with 3 *N* ammonium hydroxide until the washings emerge colorless. Remove the flask, wash the precipitate into the original container, and dissolve the material with a minimum amount of concentrated sulfuric acid. Then repeat the precipitation and washing with ammonium hydroxide as before, and catch the filtrate in the same conical flask. Evaporate the solution to 15 ml.

Measurement of Desired Constituent. Neutralize the solution to litmus with 6 *N* hydrochloric acid or ammonium hydroxide and add the following in order: 2 ml. of 6 *N* ammonium hydroxide, 10 ml. of 0.1 per cent 1,10-phenanthroline solution, 1 ml. of 10 per cent hydroxylamine hydrochloride solution, and 20 ml. of methyl Carbitol.

Dilute to 50 ml. with water, mix well, and measure or compare the color by any of the usual means. A blue filter such as Corning No. 556 is recommended for filter photometers.

Summary

The intense, brown color of cuprous-phenanthroline complex may be used as the basis of a colorimetric method for the determination of copper. Beer's law is valid for concentrations from 0.5 to 10 p. p. m. of copper, at least, this being the range most suitable for measurements with a 1-cm. transmission cell. The colored system is stable for at least 24 hours.

Of the metals whose compounds are soluble under the conditions used, only cadmium, cobalt, nickel, and zinc interfere seriously. Metals which precipitate with ammonia are removed during the course of the procedure. Among the anions studied, only cyanide, dichromate, and thiosulfate interfere appreciably.

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Abstracted from a thesis presented by M. L. Moss to the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of doctor of philosophy, May, 1942.

Modification of Calibration Apparatus

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SEVERAL minor improvements in precision calibration apparatus (*1*) have been introduced, and the stopcock-control chamber has been redesigned. Stopcock *E* is eliminated, as is clamp *H*, and instead stopcocks are added just above *A* and *B*. These and *D* are used to control flow rates, and the inner set, *A*, *B*, *C*, and *F*, are used as open or shut gates, as in previous procedure. Control is made easier and elimination of entrapped air after greasing a stopcock may now be accomplished without tilting the apparatus. Furthermore, a vessel of smaller capacity but otherwise the same pattern as vessel *W* of Figure 3 is preferable for calibration of microburets.

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Spectrographic Detection and Determination of the Halogens

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New England Spectrochemical Laboratories, West Medway, Mass.

THE spectrochemical analysis of the halogens has been confined, with one exception, to the detection and determination of fluorine as calcium fluoride (*1*, *5*, *7*) or silicon fluoride (*6*). Pfeilsticker (*8*) has analyzed salts for bromine, chlorine, and iodine at reduced pressures by the use of a special, very sparklike spark (2000-microfarad condenser, 0.05–0.1 millihenry inductance, at 220 volts). Nevertheless, the intensity of the spark lines of the halogens tabulated by Exner and Haschek (*2*) suggested strongly that it would be possible to detect all the halogens spectrographically in the usual spark source, if it were not for the presence of air lines, principally of nitrogen. The air lines could not be quenched by the usual expedient of introducing self-inductance into the spark circuit because the sparklike qualities of the source would thereby be degraded toward the arc, and the spark lines of the halogens would become exceedingly faint. Despite this difficulty, it seemed feasible to determine the halogens spectrographically without the use of special auxiliary equipment and this possibility was investigated.

All spectrograms were taken in the first order of a 3-meter grating spectrograph, dispersion 5.6 Å. per mm. Eastman 33 plates were used for determining chlorine and bromine; Eastman spectroscopic I-F plates for fluorine and iodine. Overlapping higher order spectra were eliminated by suitable Corning glass filters placed before the spectrograph slit. The finely divided samples were introduced into the cored crater of 0.6-cm. (0.25-inch) graphite electrodes (National Carbon Co., Acheson grade) and flattened off level with the rim of the crater. The loaded electrode was placed in the lower clamp of a spark stand. A sharply pointed electrode was placed in the upper clamp of the spark stand and so adjusted that its point was exactly 2 mm. above the surface of the sample. The electrodes were connected to the terminals of a conventional spectrographic, alternating current, condensed-spark circuit with the following constants: input, 130 volts; output, 25,000 volts, 17 to 21 milliamperes; capacity 0.004 microfarad. The large inductance customarily used in such circuits to eliminate the air lines was shorted out, so that the only inductance was the natural self-inductance of the circuit. All leads were made as short as practicable. The spark was focused on the slit of the spectrograph by a simple quartz lens.

The following spectral lines have proved to be the most satisfactory for the detection and determination of the halogens:

Element	Wave Length Å.	Intensity	Element	Wave Length Å.	Intensity
F	6856.02	60	Cl	4794.54	20
	6902.46	40		4810.06	10
				4819.46	8
Br	4678.69	10			
	4704.86	30	I	5161.19	50
	4785.50	15		5464.61	30
	4816.71	10			

The wave lengths are taken from the M. I. T. Wavelength Tables (*4*). The intensity estimates are the authors' and are based on the intensity scale of 1 to 1000 used by Exner and Haschek in their measures of the air lines (*2*).

Air lines did not interfere with any of the strongest lines of fluorine and iodine, but all the chlorine lines except 4794.54 Å. were masked by nitrogen lines, as was the strongest line of bromine. The masking of these sensitive lines reduced the sensitivity of the spectrographic test for chlorine and bromine very considerably.

To overcome the interference of the air spectrum, the electrodes were introduced inside a 5-cm. diameter Pyrex tube through the center of two rubber stoppers (the interelectrode gap was still maintained at 2 mm.). Off-center holes in each stopper held 3-mm. diameter glass tubes. Carbon dioxide was introduced through the lower tube and escaped through the upper tube. A few ounces of carbon dioxide pressure sufficed to sweep the tube clear of nitrogen in less than a minute. It was, of course, necessary to use pure carbon dioxide. Carbon dioxide generated from dry ice was found to be contaminated with chlorine, and ordinary commercial carbon dioxide contained traces of nitrogen and chlorine. Through the courtesy of E. N. Ellis of the S. S. White Dental Mfg. Co. of Philadelphia, the authors obtained U. S. P. carbon dioxide customarily used in anesthesia. No nitrogen or chlorine could be detected in this gas by spectrographic methods.

The spectrographic sensitivity of the halogens varied with the matrix in which they were found and the cations to which they were attached. In general, amounts of the following orders of magnitude could be detected with certainty:

Element	Limit of Identification *Micrograms*	Sensitivity %
Br in CO_2	500	0.3
Cl in CO_2	900	0.5
F in air	200	0.1
I in air	100	0.07

where the limit of identification refers to the quantity sensitivity, and the limit of sensitivity refers to the concentration sensitivity, in accordance with the definitions given by Feigl (*3*). One half these limiting amounts could be detected in many materials, one fourth these amounts in some materials.

Satisfactory quantitative determinations of chlorine were carried out in the carbon dioxide atmosphere in the range from 1 to 15 per cent of chlorine when 150 mg. of sample, introduced in five separate loads, were sparked. The accuracy of the determinations varied from ±10 per cent of the amount of chlorine found when the chlorine content was less than 5 per cent to ±5 per cent when the chlorine content was 15 per cent. The precision of the determinations was uniformly of the order of ±5 per cent.

Summary

Materials containing the halogens were sparked without prior chemical treatment in the usual high-voltage spectrographic spark. By this method the halogens could be determined with an accuracy comparable to that obtained in the spectrochemical analysis of metals. In the case of chlorine and bromine the most sensitive lines were masked by air lines and observations were carried out in a carbon dioxide atmosphere in a simple cylindrical tube. The limits of detection were 0.5 per cent for chlorine and 0.3 per cent for bromine, in a carbon dioxide atmosphere; 0.1 per cent for fluorine and 0.07 per cent for iodine, in air.

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Cerate and Periodate Oxidimetry

Perchlorato-Cerate and Periodate Ions as Oxidants in the Determination of Organic Compounds

G. FREDERICK SMITH AND F. R. DUKE[1], University of Illinois, Urbana, Ill.

THE most satisfactory general procedures for the quantitative determination of organic compounds are those of Stamm (*5*) and Malaprade (*2*). The Stamm procedure involves oxidation by the permanganate ion in alkaline solution. The practical difficulties in its application make its use unusually troublesome. The Malaprade procedure, in which periodic acid is used as the oxidant, is not so generally applicable but is much to be preferred over the Stamm procedure.

The perchlorato-cerate ion, $Ce(ClO_4)_6^{--}$, in perchloric acid solution, has been employed by Smith and Duke (*4*) in the determination of glycerol, and the same type of procedure for the determination of an extensive series of organic compounds is described in the present work. The applications described are limited to oxidations in acid solution, and, in general, to aliphatic organic compounds. The results are stoichiometric rather than empirical, the reactions are rapidly complete at ordinary temperatures, and oxidation-reduction indicators are applicable throughout.

In this procedure, as well as in the Stamm and Malaprade procedures, the determination is not specific or selective in its action without supporting analyses of the reaction products obtained with variations of reaction conditions.

A bibliography of previous work on the general subject of the quantitative oxidation of organic compounds has been given by one of the present authors (*3*).

Oxidation of Organic Compounds Using Periodic Acid

A typical oxidation of an organic compound using periodic acid may be illustrated by citing the determination of glycerol as described by Fleury and Fatome (*1*). The reactions are as follows:

$$CH_2OH.CHOH.CH_2OH + 2Na_3H_2IO_6 + 2H_2SO_4 \rightarrow 2HCHO + HCOOH + 2NaIO_3 + 2Na_2SO_4 + 5H_2O$$

$$Na_3H_2IO_6 + Na_3AsO_3 + 2NaHCO_3 \rightarrow Na_3AsO_4 + NaIO_3 + 2Na_2CO_3 + 2H_2O$$

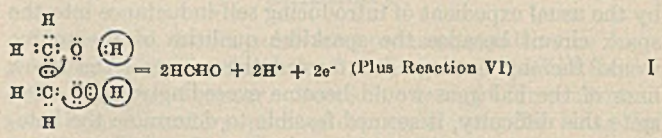
The oxidation of glycerol in dilute sulfuric acid solution is complete in 5 minutes at ordinary temperatures with an excess of periodate. An excess of sodium bicarbonate is added and the excess of periodate reduced, using excess of standard arsenite solution. The excess arsenite is then determined using standard iodine solution with starch as indicator. The presence of iodate from the reduction of periodate does not interfere.

The principles involved in the oxidation of various types of organic compounds which may be determined by a series of reactions similar to those of the determination of glycerol using periodate, will be apparent from the following discussion of the Malaprade procedure.

Two electrons and two protons are removed from organic compounds containing hydroxyl groups in the alpha position to each other (the ethylene glycol type of structure). The electronic structure of the remainder of the molecule is unstable and must be capable of rearrangement to a stable configuration.

In the case of organic compounds having two aldehyde groups, an aldehyde and alcoholic group, or an aldehyde and keto group in the alpha position to each other, each pair of groups hydrates and is thus converted into the ethylene glycol type of configuration and oxidized in a similar manner.

Formaldehyde, acetaldehyde, propionaldehyde, etc., are oxidized by periodic acid only after an extended period of time. Illustrative reactions are those of the oxidation of ethylene glycol, glyoxal, glycollic aldehyde, and pyruvic aldehyde.



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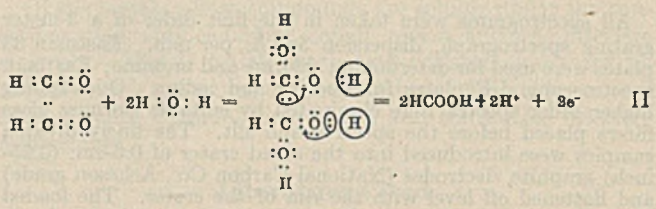

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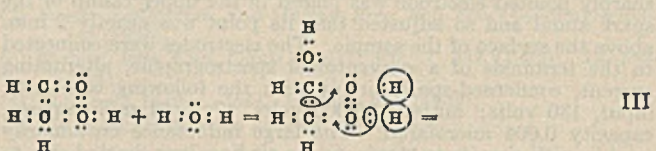

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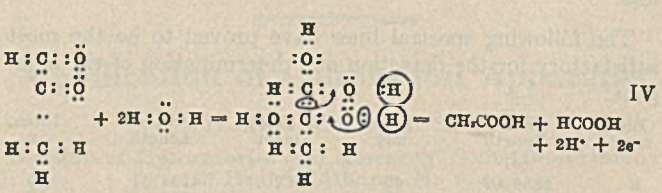


IV

Polyhydroxy compounds in general are oxidized using excess of periodate—for example, ethylene glycol, erythritol, arabitol, and mannitol. These reactions are summarized in the following general formulation:

$$CH_2OH.(CHOH)_n.CH_2OH + (n+1)HIO_4 = (n+1)\,HIO_3 + n(HCOOH) + 2HCHO + H_2O$$

In the case of polyhydric dicarboxylic acids in which n is greater than 1, such as tartaric acid, trihydroxyglutaric acid, and saccharic acid, the general formulation is

$$COOH.(CHOH)_n.COOH + (n-1)HIO_4 = (n-2)HCOOH + 2CHO.COOH + (n-1)HIO_3$$

[1] Present address, Frick Chemical Laboratory, Princeton University, Princeton, N. J.

TABLE I. COMPOSITE RESULTS IN DETERMINATION OF ORGANIC COMPOUNDS BY THE PERCHLORATO-CERATE–OXALATE PROCEDURE

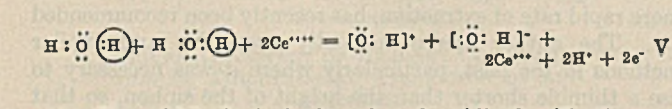
Sample	Sample Wegnt	$Ce(ClO_4)_4^{--}$		$C_2O_4^{--}$		No. of Determinations	Temp.	Time	Results Found	Accuracy	Equivalents Required
	Mg.	*Ml.*	*N*	*Ml.*	*N*		*° C.*	*Min.*	*Mg.*	*Av. %*	
Glycerol	23.90	25.00	0.1036	4.952	0.1032	4	45	15	23.925	+0.10	8
Glucose	27.54	25.00	0.1040	6.940	0.1036	5	26	45	27.580	+0.15	12
Sucrose	59.88	50.00	0.1040	6.302	0.1038	5	24	45	59.920	+0.07	26
Cellulose	29.72	50.00	0.1035	32.272	0.1035	5	27	120	29.774	+0.18	12
Biacetyl	171.4	75.00	0.0927	28.00	0.1050	1	24	5	171.8	+0.4	2
	83.6	25.00	0.0927	3.75	0.1050	1	24	5	82.7	−0.6	2
	49.1	25.00	0.0927	11.12	0.1050	1	24	5	49.1	0.0	2
	504.9	150.00	0.0927	20.54	0.1050	1	24	5	504.9	0.0	2
Acetyl acetone	100.83	100.00	0.1043	41.578	0.1056	4	25	10	100.92	+0.09	6
Tartaric acid	88.15	50.00	0.1032	22.775	0.1036	4	26	10	88.225	+0.08	6
Malonic acid	104.32	100.00	0.1032	41.605	0.1036	4	26	10	104.375	+0.05	6
Citric acid	44.925	50.00	0.1032	18.173	0.1036	4	10	30	44.825	0.00	14
Malic acid	107.0	100.00	0.1032	38.205	0.1036	4	25	15	107.075	+0.02	8

These two reactions indicate that tartaric acid requires 6 equivalents for oxidation and mannitol 10 equivalents. All the periodic acid oxidations require an even number of equivalents and are thus not empirical in any instance.

Oxidation of Organic Compounds by Perchlorato-Ceric Acid in the Presence of Perchloric Acid

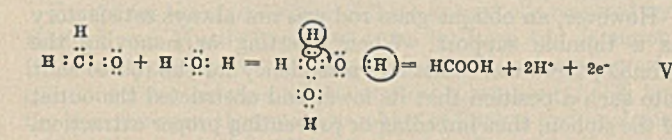
The principles governing the oxidation of aliphatic organic compounds by use of perchlorato-ceric acid in the presence of 4 molar perchloric acid are as follows:

1. In each case an even number of equivalents of oxidant is required. End products are fatty acids, ketones, aldehydes (other than formaldehyde), and carbon dioxide. In all cases the oxygen demand is stoichiometric rather than empirical. All reactions are carried out at room temperature or at slightly elevated temperatures only.
2. The simplest type of oxidation follows the direct liberation of two protons and two electrons (reaction I + II is an illustration). Only those compounds, the electronic configuration of which is capable of rearrangement to a stable form after the removal of two protons and two electrons, are oxidizable. Only compounds in which the protons are removed from oxygen are readily oxidized. This demands a 1,2 oxygen-containing compound.
3. The carbonyl group before oxidation must hydrate to the glycol form (reaction III is an illustration). Compounds containing an active methylene are oxidizable. The double bond of the enol, formed by such active methylene group, is saturated by hydroxyl groups resulting from reaction V.



V

4. Formaldehyde is hydrated and oxidized with the consumption of two oxygen equivalents in accordance with reaction VI.

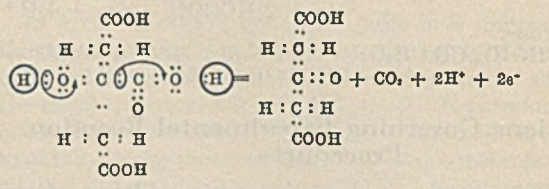


VI

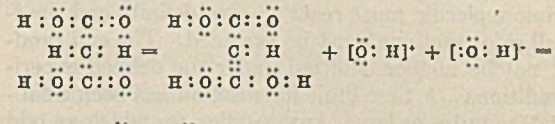
In this case one proton is detached from direct union to carbon, a specific property of cerate oxidimetry as distinct from periodate oxidimetry. The reaction is rapid. In case aldehydes other than formaldehyde are involved or the ketone group is present unsubstituted by oxygen in the alpha position, either no reaction results under the condition of the experiment or empirical results are obtained.

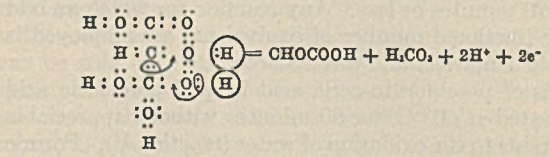
5. Oxalic acid is instantly oxidized at ordinary temperatures. This reaction is used in the determination of excess perchlorato-cerate ion. Nitro-ferroin is used as internal indicator for this reaction. The standardization of the perchlorato-cerate solution in this case should be carried out by titration with standard oxalate rather than by the reverse procedure.

The above rules are all illustrated in the oxidation of citric acid developed in reactions VII, VIII, IX, and X.

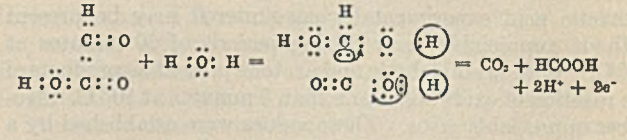


VII

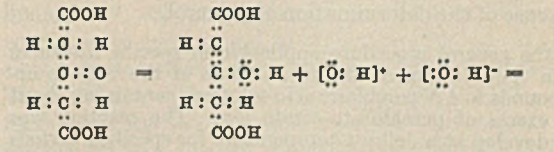




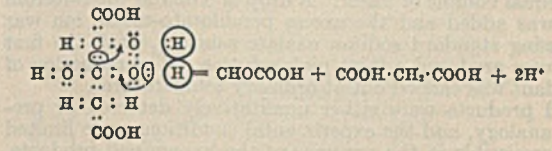
VIII



IX



X



The citric acid is first oxidized to acetone dicarboxylic acid (reaction VII) with the consumption of 2 equivalents. The enolized form of the acetone dicarboxylic acid then has the double bond broken with the consumption of two more equivalents, as illustrated by reactions V and VIII, forming 1,3-dicarboxy-2,2,3-trihydroxypropane, which is oxidized by 2 more equivalents to form one mole of malonic acid and one mole of glyoxylic acid. The glyoxylic acid is then hydrated and oxidized to formic acid with the consumption of 2 more equivalents, according to reaction IX. Lastly, the mole of malonic acid enolizes and the double bond is broken by the reaction of the positive and negative hydroxyl, accounting for the use of 2 additional equivalents of oxidant and forming another molecule of glyoxylic acid (reaction X). Lastly, two more equivalents of oxygen are required to oxidize this glyoxylic acid in accordance with reaction IX. The

total number of oxygen equivalents demanded is 14. The total reaction is shown as follows:

$$C_6H_8O_7 + 14Ce(ClO_4)_6^{--} + 5H_2O = 4CO_2 + 2HCOOH + 84ClO_4^- + 14H^+ + 14Ce^{+++}$$

The formulas for the group oxidation of various type reactions are given in the following summary:

XI. Polyhydric Alcohols

$$CH_2OH.(CHOH)_n.CH_2OH - (2n + 6)e^- + (n + 2)H_2O = (n + 2)HCOOH + (2n + 6)H^+$$

XII. Polyhydric Alcohol Monocarboxylic Acids

$$CH_2OH.(CHOH)_n.COOH - (2n + 4)e^- + (n + 1)H_2O = (n + 1)HCOOH + (2n + 4)H^+ + CO_2$$

XIII. Polyhydric Dicarboxylic Acids

$$COOH.(CHOH)_n.COOH - (2n + 4)e^- + nH_2O = nHCOOH + 2CO_2 + (2n + 4)H^+$$

XIV. Aldoses

$$CH_2OH.(CHOH)_n.CHO - (2n + 4)e^- + (n + 2)H_2O = (n + 2)HCOOH + (2n + 4)H^+$$

XV. Ketoses

$$CH_2OH.(CHOH)_n.CO.CH_2OH - (2n + 8)e^- + (n + 3)H_2O = (n + 2)HCOOH + (2n + 8)H^+ + CO_2$$

Conditions Governing Experimental Reaction Procedures

For the quantitative and stoichiometric oxidation of organic compounds several requirements must be fulfilled. Each organic molecule must react to give definite end products and all side reactions must be excluded. The end products must not be further oxidized under the defined experimental conditions. A time limit for reaction has been established of 120 minutes or less. Any reaction for which an odd number or fractional number of equivalents are employed is known to be empirical and unsatisfactory.

Solutions of perchlorato-ceric acid in 4 *N* perchloric acid may be heated at 60° C. for 60 minutes without appreciable loss in titer due to the oxidation of water (reaction V). Formic acid is not appreciably oxidized by the same cerate solution, at the same temperature, in the same period of time. Amounts of acetic acid experimentally encountered may be present without appreciable error during periods of 60 minutes at 50° C. With acetaldehyde and acetone present as products of the reaction of oxidation, more than 5 minutes at 10° C. introduces appreciable error. These values were established by a procedure duplicating that described by the present authors (*4*) for the case of the determination of glycerol.

Testing the general procedure applicable in specific instances consisted in the addition of known amounts of the various organic compounds to 4 *N* perchloric acid solutions containing about a twofold excess of perchlorato-cerate ion. The reaction was allowed to develop at a definite temperature for specified periods of time and at the end the reacting solution was diluted by the use of an equal volume of water. A drop of 0.025 *M* nitro-ferroin indicator was added and the excess perchlorato-cerate ion was titrated, using standard sodium oxalate solution, until the first drop of excess produced a faint pink solution. The reduction of excess oxidant was carried out at ordinary temperatures.

The end products were either qualitatively detected or predicted by analogy, and the experimental conditions were limited to those permissible in the presence of the known end products. If the oxygen equivalent calculated did not agree with that experimentally found, side reactions were indicated demanding altered conditions or elimination of the procedure from practical application.

The results of the analyses of a series of pure organic compounds are given in Table I.

Summary

The mechanism of quantitative oxidation of organic compounds using periodic acid as oxidant has been described. The same mechanism has been applied to the oxidation of organic compounds using the perchlorato-cerate ion, $Ce(ClO_4)_6^{--}$, in the presence of 4 *M* perchloric acid. The latter procedure has been studied for a considerable number of individual cases and the type reactions involved make possible its application to a more extensive series of compounds.

The use of the perchlorato-cerate ion and periodate ion in these procedures follows the same general scheme. The organic compound is oxidized by excess of oxidant in acid solution for a specified time at a given temperature, after which the excess oxidant is evaluated using a standard reducing agent. The use of periodic acid is more complicated than that of the perchlorato-cerate ion because the excess periodate ion must be determined in the presence of the iodate ion, necessitating the use of an added standard solution. The perchlorato-cerate ion is more extensive in its applicability, the number of oxidation equivalents required is larger, and the speed of the reactions in general greater than in the case of the periodate ion.

All reactions by both procedures are stoichiometric rather than empirical, and an exceptional degree of accuracy is obtainable in all cases. The methods apply to aliphatic compounds and are not specific in their action but require special treatment to isolate the substance to be determined if interfering substances are present.

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Thimble Supports for Faster Soxhlet Extraction

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THE insertion of a piece of glass rod at an angle into the extractor (midpiece) of a Soxhlet apparatus to raise the thimble off the bottom, thus securing better drainage and a more rapid rate of extraction, has recently been recommended (*1*). The author has occasionally used this and similar methods in the past, particularly where it was necessary to use a thimble shorter than the height of the siphon, so that undissolved matter might have been displaced from the thimble mechanically if the liquid had been allowed to rise above the top of the thimble.

However, an oblique glass rod was not always satisfactory as a thimble support. When inserting or removing the thimble, there was sometimes a tendency for the rod to shift into such a position that its lower end obstructed the outlet of the siphon, thus impeding or preventing proper extraction.

If this trouble is encountered, a ground-glass stopper with a flat or mushroom-type top may be inverted in the bottom of the extractor to support the thimble without obstructing the siphon. To avoid breakage, it should be carefully inserted or removed with crucible tongs or forceps. By somewhat diminishing the volume of liquid which can collect at each filling, the glass stopper promotes more frequent siphoning and faster extraction. A weighing bottle, or large homeopathic vial, of appropriate height and diameter, inverted in the extractor, also serves as an effective thimble support in some cases.

Literature Cited

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Thiocyanogen Absorption of Linseed Oils

Thiocyanogen Absorption of Linoleic and Linolenic Acids and Composition of Linseed Oils

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IN CONNECTION with studies of fat metabolism in flaxseed and of the fat acid distribution responsible for the wide variations in the degree of unsaturation of linseed oils, it became necessary to determine the composition of a large number of oils. Before undertaking this work it seemed desirable to apply to linseed oil some of the refinements of technique recently shown to be necessary for the analysis of mixtures of oleic, linoleic, and linolenic acids.

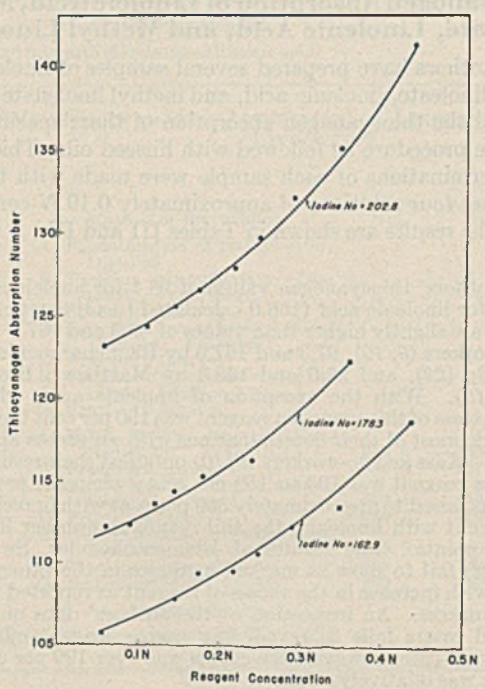

FIGURE 1. EFFECT OF REAGENT CONCENTRATION ON THIOCYANOGEN ABSORPTION OF LINSEED OIL

Approximately same amount of reagent used for all points of each curve.

Except for the composition of three linseed oils reported by Hilditch and Murti (*7*) and of seven by Rose and Jamieson (*25*), nearly all analyses of linseed oils have been based on calculations using Kaufmann's theoretical constants (*11*, *12*) for the thiocyanogen absorption numbers of linoleic and linolenic acids. Kimura (*16*) in 1929 presented evidence which placed Kaufmann's theoretical constants in doubt. Nevertheless, they were widely used until recently when several laboratories investigated the validity of Kaufmann's values. As a result of their work (*7*, *9*, *10*, *19*, *22*, *23*, *26*) the thiocyanogen values have been modified, so that in using the method originally proposed by Kaufmann one can now obtain results which are much more accurate than previously possible.

Thiocyanogen absorption numbers of oleic, linoleic, and linolenic acids and esters determined by several groups of investigators have been summarized by Riemenschneider, Swift, and Sando (*22*). Matthews, Brode, and Brown (*19*) have since reported values for their carefully purified specimens of these acids. The results obtained by the several groups are in fair agreement, but it is clear that the absorption of thiocyanogen depends upon the conditions under which the determination is made. The concentration of reagent (*9*, *10*, *22*), the excess of reagent over that added to the ethylenic bonds (*9*, *10*, *19*, *22*, *23*), the length of time permitted for addition of the reagent (*9*, *10*, *19*, *22*), the temperature of the reaction (*19*, *22*), and the solvent (*19*) are factors which alter the thiocyanogen number of linoleic and linolenic acids. Oleic acid, on the other hand, gives nearly theoretical thiocyanogen absorption numbers by most procedures.

In order to obtain accurate results it is imperative that thiocyanogen should add to the double bonds of the fat acids of the oil in the same ratio that it adds to the fat acids when the empirical constants are determined. It therefore seemed necessary to determine those factors which must be controlled to obtain precise thiocyanogen absorption constants of linseed oil, and the empirical constants for the thiocyanogen absorption of linoleic and linolenic acids by following the procedure finally adopted for linseed oil.

Thiocyanogen Absorption of Linseed Oil

To obtain the data shown in Figure 1, equal volumes of the most concentrated solution used with each oil were diluted with acetic acid reagent to the weaker concentrations. It can be seen that the concentration of the thiocyanogen reagent must be held within narrow limits if reproducible results are to be obtained. The thiocyanogen number is approximately 3 points higher with the 0.2 *N* solution recommended by the American Oil Chemists' Society (*1*) than with the 0.1 *N* solution recommended by the A. O. A. C. (*2*). The higher iodine number oil showed a greater increase in the thiocyanogen number with increase in reagent concentration than the lower iodine number oil. This is readily explained by the larger amount of linolenic acid in the former (Table VI).

The rate of addition of thiocyanogen to linseed oil (Figure 2) follows closely the rate predicted from studies of oleic (*23*), linoleic (*17*, *23*), and linolenic (*9*, *19*) acids. Thiocyanogen continues to add beyond 24 hours, but the rate of addition is slow enough so that this appears to be a suitable reaction time. These results differ markedly from those of Gay (*5*), who found that the thiocyanogen number of linseed oil increased rapidly up to about 48 hours, and from those of Kaufmann

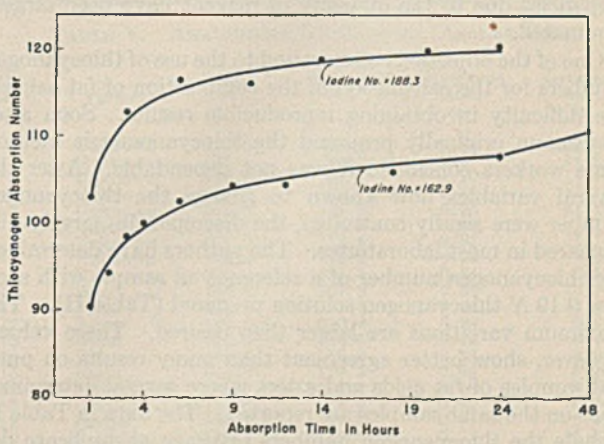

FIGURE 2. RATE OF ADDITION OF THIOCYANOGEN TO LINSEED OILS

TABLE I. EFFECT OF EXCESS OF THIOCYANOGEN REAGENT

Oil 11		Oil 18		Oil 19		Oil 21		Oil 22	
Thiocyanogen No.	Excess thiocyanogen %	Thiocyanogen No.	Excess thiocyanogen %	Thiocyanogen No.	Excess thiocyanogen %	Thiocyanogen No.	Excess thiocyanogen %	Thiocyanogen No.	Excess thiocyanogen %
106.6	51	125.1	55	114.5	63	117.2	53	112.1	129
106.0	63	125.4	95	115.5	96	119.0	123	113.5	204
106.7	107	127.2	172	114.6	115	120.9	192	114.7	209
107.4	130	126.4	183	115.6	204	120.9	201	114.2	377
107.0	214	126.6	293	114.7	306	121.7	259	...	...
107.8	234	...	...	...	...	...	...	...	...
108.4	271	...	...	...	...	...	...	...	...
107.8	292	...	...	...	...	...	...	...	...

Iodine No.: oil 11, 162.9; oil 18, 202.8; oil 19, 178.2; oil 21, 188.3; oil 22, 176.6.

and Keller (*13*) and Griffiths and Hilditch (*6*), who found no increase after 18 hours.

In Table I, thiocyanogen numbers of several linseed oils obtained by varying the excess of thiocyanogen reagent are shown. The original concentration of reagent was approximately the same in each case. The excess reagent is expressed as $\frac{\text{amount unused}}{\text{amount added to double bonds}} \times 100$. These data indicate that the thiocyanogen numbers increase slightly with a greater excess of reagent, but the increment of increase is small. Over much of the range studied the differences are about the same as expected from the precision of the thiocyanogen determination (see Table II). We can expect somewhat better precision for the results in Table I because, with the exception of a few repeat determinations, the thiocyanogen values for each oil were determined with the same solution.

The extent of thiocyanogen addition to double bonds should be determined by the concentration of thiocyanogen and not by total unused reagent. In actual practice sufficient time is not allowed for the reaction to reach equilibrium, and the initial rate of addition may also be modified. Nevertheless, the authors believe the "effect of excess reagent" is primarily due to the concentration at the time the thiocyanogen not added to the double bonds is titrated. Relatively large differences in the per cent of excess reagent cause relatively small changes in thiocyanogen concentration. With a solution of original 0.2 *N* concentration and percentages of excess of 50, 100, 200, 300, and 400, the corresponding thiocyanogen concentrations are 0.067 *N*, 0.10 *N*, 0.133 *N*, 0.15 *N*, and 0.16 *N*. By increasing the excess reagent above 200 per cent the increased absorption would probably be small, if not negligible.

Nearly all of the authors' determinations on linseed oil which have been used to calculate composition have had 180 to 230 per cent excess reagent. The average has been 206 per cent. Within this range and with this great an excess, discrepancies due to the quantity of reagent have been largely eliminated.

One of the objections often raised to the use of thiocyanogen numbers for the calculation of the composition of fat acids is the difficulty in obtaining reproducible results. Soon after Kaufmann originally proposed the thiocyanometric method some workers concluded it was not dependable. After the several variables now known to change the thiocyanogen number were rigidly controlled, the discrepancies largely disappeared in most laboratories. The authors have determined the thiocyanogen number of a reference oil sample with each new 0.19 *N* thiocyanogen solution prepared (Table II). The maximum variations are larger than desired. These values, however, show better agreement than many results on purified samples of fat acids and esters where several determinations on the same samples are reported. The data in Table II include the thiocyanogen numbers (average of duplicate determinations) of each solution and not a single determination on the reference samples was excluded. In practice the precision of the method is better than the data in Table II indicate. When the iodine number of linseed oil is plotted against the thiocyanogen number, most of the points fall close to a straight line. Whenever a determination gives a point several units from this line, it is repeated. Almost without exception the repeat value falls very close to the line. Recently the authors have routinely determined the thiocyanogen number of each oil sample with two or more solutions. Numerous repeats have been necessary and occasionally one of duplicate determinations is out of line. Nevertheless, they believe very few of their values deviate more than 1 point from the "true" thiocyanogen number.

Thiocyanogen Absorption of Linoleic Acid, Methyl Linoleate, Linolenic Acid, and Methyl Linolenate

The authors have prepared several samples of linoleic acid, methyl linoleate, linolenic acid, and methyl linolenate and determined the thiocyanogen absorption of these specimens by the same procedure as followed with linseed oil. Thiocyanogen determinations of each sample were made with two and sometimes four solutions of approximately 0.19 *N* concentration. The results are shown in Tables III and IV.

The authors' thiocyanogen values of 98.1 for linoleic acid and of 167.4 for linolenic acid (168.0 calculated to a theoretical iodine number) are slightly higher than values of 96.3 and 167.3 by Kass and co-workers (*9, 10*), 97.3 and 167.6 by Riemenschneider, Swift, and Sando (*22*), and 96.6 and 166.3 by Matthews, Brode, and Brown (*19*). With the exception of linolenic acid, where the average excess of thiocyanogen reagent was 190 per cent the authors have made most of their determinations with an excess above 200 per cent. Kass and co-workers (*9, 10*) obtained their results when the excess reagent was 100 to 150 per cent; when the per cent of excess was raised to approximately 350 per cent with linoleic and to 250 per cent with linolenic, the thiocyanogen number increased about 3 points. The results of Riemenschneider, Swift, and Sando (*22*) fail to show as marked increases in the thiocyanogen number with increase in the excess of reagent as reported by Kass and co-workers. An inspection of the authors' data on the fat acids and esters fails to reveal any excess reagent effect. In nearly every case, however, the excess was over 190 per cent and the range was relatively small.

Matthews, Brode, and Brown (*19*) show, as predicted, that the thiocyanogen number of linolenic acid increases more with increase in amount of reagent than does that of linoleic acid. The

TABLE II. REPRODUCIBILITY OF THIOCYANOGEN ABSORPTION NUMBER OF LINSEED OILS

Oil 21		Oil 22		Oil 23	
Thiocyanogen solution No.	Thiocyanogen No.	Thiocyanogen solution No.	Thiocyanogen No.	Thiocyanogen solution No.	Thiocyanogen No.
15	121.1	23	114.5	37	117.0
16	119.0	24	115.2	39	115.9
17	122.1	24	115.7	42	114.9
17	120.8	25	113.7	44	116.6
18	121.4	26	114.0	45	117.4
19	121.1	29	113.5	46	116.4
19	121.2	30	115.6	47	115.4
..	...	31	113.6	..	...
..	...	32	114.9	..	...
..	...	33	116.4	..	...
..	...	34	115.3	..	...
..	...	35	113.2	..	...
..	...	36	114.1	..	...
..	...	37	114.8	..	...
..	...	39	116.1	..	...
Av.	121.0		114.7		116.2
Av. deviation from mean	±0.59		±0.85		±0.71
Maximum deviation from mean	2.0		1.7		1.3

Iodine No.: oil 21, 188.1; oil 22, 176.6; oil 23, 178.8.

TABLE III. THIOCYANOGEN ABSORPTION OF LINOLEIC ACID AND METHYL LINOLEATE

Sample		Method of Preparation	Iodine No.	Thiocyanogen No.	Linoleic Acid Iodine No.	Linoleic Acid Thiocyanogen No.
Linoleic acid	A	Saponification of methyl ester. Distilled. Fraction 1	...	...	179.2	98.0
	B	Fraction 2 (above)	...	...	179.5	96.0
	C	Pyridine debromination. Not distilled	...	...	181.4	99.4
	D	Distilled (above) Fraction 1	...	...	181.2	98.0
	E	Distilled (above) Fraction 2	...	...	181.4	97.8
	F	Pyridine debromination. Distilled	...	...	181.2	98.6
	G	Saponification of methyl ester. Distilled	...	...	181.7	98.3
	H	Redistilled acid fractions	...	...	179.7	97.4
Methyl linoleate	A	H_2SO_4–MeOH debromination. Not distilled	172.0	93.3	180.6[a]	98.0[a]
	B	Distilled (above)	172.5	93.6	181.1	98.3
	C	H_2SO_4–MeOH debromination. Distilled. Fraction 1	172.6	94.1	181.2	98.8
	D	Fraction 2 (above)	172.2	93.6	180.8	98.3
	E	H_2SO_4–MeOH debromination. Distilled	172.4	94.1	181.0	98.8
	F	Redistilled ester fractions	171.4	93.5	180.0	98.2
		Av. thiocyanogen No.				98.1

[a] Methyl linoleate calculated as linoleic acid.

TABLE IV. THIOCYANOGEN ABSORPTION OF LINOLENIC ACID AND METHYL LINOLENATE

Sample		Method of Preparation	Iodine No.	Thiocyanogen No.	Linolenic Acid Iodine No.	Linolenic Acid Thiocyanogen No.
Linolenic acid	A	Saponification of methyl ester. Distilled	...	...	271.2	167.7
	B	Pyridine debromination. Distilled. Fraction 1	...	...	271.6	167.0
	C	Fraction 2 (above)	...	...	272.3	167.6
	D	Saponification of methyl ester. Distilled. Fraction 1	...	...	271.7	168.1
	E	Fraction 2 (above)	...	...	272.5	168.3
	F	Pyridine debromination. Distilled. Fraction 1	...	...	272.5	168.8
	G	Fraction 2 (above)	...	...	272.5	168.5
Methyl linolenate	A	H_2SO_4–MeOH debromination. Redistilled. Fraction 1	259.9	158.0	273.0[a]	166.0[a]
	B	Fraction 2 (above)	258.8	157.3	271.8	165.2
	C	H_2SO_4–MeOH debromination. Distilled. Fraction 1	259.4	159.1	272.5	167.1
	D	Fraction 2 (above)	259.7	159.2	272.8	167.2
	E	H_2SO_4–MeOH debromination. Distilled. Fraction 1	259.9	159.4	273.0	167.4
	F	Fraction 2 (above)	259.3	159.8	272.4	167.8
	G	Redistilled ester fractions	259.1	159.1	272.2	167.1
		Av. thiocyanogen No.				167.4

[a] Methyl linolenate calculated as linolenic acid.

lower thiocyanogen values on the carefully purified samples prepared in Brown's laboratory (*19*) may be due to a lower reaction temperature (16° C.), and it is possible, though not likely, that the 10 per cent carbon tetrachloride lowered the absorption. Low thiocyanogen absorption numbers of linolenic acid samples stored for some time have been observed (*19*). The authors' thiocyanogen determinations were started the same day the linolenic samples were distilled. The iodine numbers were determined within a few hours after distillation. Most of the determinations on the linoleic samples were also made soon after preparation, but the iodine numbers of the linoleic samples, unlike the linolenic samples, dropped only slightly when stored several days in the refrigerator.

With few exceptions, the thiocyanogen numbers of the fat acids and esters (Tables III and IV) show excellent agreement in each group. The average thiocyanogen number of linolenic acid samples, 168.0, and of methyl linolenate samples, 166.8, a difference of 1.2 points, is difficult to explain. An average of these values must be chosen but it leaves some doubt as to the best choice of the empirical constant.

Norris, Kass, and Burr (*21*) favor the use of alcohol–hydrochloric acid (Rollett, *24*) for debromination and esterification in the preparation of esters of linolenic acid, but the authors have found Kimura's methyl alcohol–sulfuric acid reagent more satisfactory. Preparations of methyl linolenate by Rollett's method (*24*) had an iodine number 2 to 3 points lower than those shown in Table IV which were prepared by modifying Kimura's method. The thiocyanogen numbers were also lower, but when calculated to a theoretical iodine number, they were essentially the same as those shown in Table IV. After the hexabromostearic acid and zinc dust were ground together in a mortar, debromination in methyl alcohol proceeded smoothly. For the preparation of linolenic and linoleic acids, the authors find debromination in pyridine (*14*) much less tedious than saponification of the esters.

Composition of Linseed Oil

For several years samples of flaxseed grown at various locations throughout the United States and Canada have been sent to this laboratory. Samples with a range in iodine numbers from 127.8 to 202.8 have been collected. A linseed oil with an iodine number under 140 may seem surprising. Adverse climatic conditions, high temperature, and insufficient moisture while the seed ripens sometimes produce an oil in flaxseed with an iodine number this low. These conditions were not uncommon in 1936. Occasionally low iodine number oils have been produced in the Northwest Great Plains in other years.

The analytical results necessary for the calculation of the fat acid glycerides are shown in Table V.

The saturated acids were determined by the Bertram method (*3*), which gives higher values than the older lead-salt method. These are minimum values because the iodine number of the saturated acid specimens is essentially zero. No attempt was made to determine the different saturated acids. Small amounts of C_{20} and C_{24} acids (*25*) have been reported in linseed oil, but most of the saturated acids are stearic and palmitic. In order to calculate the fat acid composition as glycerides, the neutralization equivalent of several samples of saturated acids was determined. The average molecular weight of the saturated fat acids was 275.7. The neutralization equivalent of the saturated acids from several oils varied somewhat more than expected.

When substituting constants in simultaneous equations to secure expressions which give the percentages of the fat acids as glycerides, it is sometimes necessary to make corrections for the presence of the unsaponifiable matter and free fat acids in the oil. With linseed oil, however, corrections for these components do not appear to be necessary. The unsaponifiable portion has an iodine and thiocyanogen number not greatly different from that of the oil. Iodine values of 159.0, 164.5, 167.8, and 162.4 and thiocyanogen values of 106.2 and 116.5 have been obtained on individual samples. The combined unsaponifiable fraction of approximately 75 determinations, which accumulated over several months, gave an iodine number of 145.5 and a thiocyanogen

TABLE V. ANALYTICAL RESULTS ON LINSEED OILS

Oil No.	Variety	Iodine No.	Thiocyanogen No.	Unsaponifiable %	Saturated Acids %
1	Unknown	127.8	89.1	1.34	15.37
2	Unknown	135.4	95.8	1.27	14.84
3	Unknown	146.2	99.7	1.10	12.00
4	Unknown	154.6	103.7	0.97	11.12
5	Unknown	164.8	107.2	0.94	11.19
6	Unknown	166.1	106.8	0.84	11.71
7	Unknown	177.0	113.5	0.82	11.12
8	Unknown	193.6	122.2	0.79	9.76
9	Unknown	200.0	124.4	0.84	8.52
10	Bison	162.8	107.8	0.88	10.70
11	Bison	162.9	107.8	0.91	13.12
12	Bison	164.5	110.8	0.98	9.92
13	Bison	176.8	114.6	0.85	8.46
14	Bison	181.1	115.6	0.83	8.96
15	Bison	185.6	119.5	0.86	8.96
16	Linota	176.9	115.1	1.10	8.33
17	Linota	199.2	125.0	0.80	6.94
18	Linota	202.8	127.2	0.91	6.93
19	Redwing	178.2	115.5	0.98	9.01
20	Redwing	195.1	122.9	0.95	7.96

TABLE VI. COMPOSITION OF LINSEED OILS

(Expressed in per cent of glyceride fraction)

Oil No.	Variety	Saturated Glycerides %	Oleic Glycerides %	Linoleic Glycerides %	Linolenic Glycerides %
1	Unknown	16.3	40.5	22.7	20.5
2	Unknown	15.7	42.5	11.8	30.0
3	Unknown	12.7	37.1	19.3	30.9
4	Unknown	11.7	33.6	19.5	35.2
5	Unknown	11.8	26.4	22.2	39.6
6	Unknown	12.3	23.6	24.6	39.5
7	Unknown	11.7	21.4	18.5	48.4
8	Unknown	10.3	15.6	15.4	58.7
9	Unknown	9.0	11.9	19.4	59.7
10	Bison	11.3	30.3	18.1	40.3
11	Bison	13.8	28.6	13.9	43.7
12	Bison	10.5	33.8	11.7	44.0
13	Bison	8.9	25.3	19.2	46.5
14	Bison	9.4	21.1	21.2	48.3
15	Bison	9.4	22.0	14.4	54.2
16	Linota	8.8	26.1	17.9	47.2
17	Linota	7.3	15.1	19.0	58.6
18	Linota	7.3	14.2	16.7	61.8
19	Redwing	9.5	24.7	17.2	48.6
20	Redwing	8.4	16.1	18.4	57.1

of 90.4. Since these values do not differ greatly from those of the total oils (the IN/TN ratio is close to that of the oil), and the unsaponifiable fraction makes up approximately 1 per cent of the oils, it is unlikely that corrections for this fraction would amount to more than 0.2 point.

It follows that the equations recommended by Kaufmann and Baltes (*12*), which include an expression for the unsaponifiable matter, cannot be applied to linseed oil unless the iodine and thiocyanogen values of the unsaponifiable are determined and included in the expressions. If, on the other hand, the unsaponifiable portion is deleted in the calculations, the error due to its presence in the oil will be small, if not negligible.

The free fat acids, rarely higher than 1.5 per cent of the total, would if esterified reduce the iodine and thiocyanogen numbers slightly. Only in cases where the free fat acids were unusually high would an error of more than 0.1 per cent result by considering the free fat acids as glycerides in the calculations.

There is some question as to whether or not oleic acid adds thiocyanogen quantitatively. Most results (*22*) indicate a thiocyanogen number 0.4 to 0.5 point lower than the theoretical, but oleic acid is difficult to obtain completely free of saturated and linoleic acids. The presence of these would result in low thiocyanogen values. Matthews, Brode, and Brown (*19*) state that their pure samples of oleic acid absorb thiocyanogen quantitatively, and Wheeler, Riemenschneider, and Sando (*26*) obtained nearly quantitative results on triolein. The authors, therefore, favor the theoretical value of 89.9.

When simultaneous equations employing thiocyanogen absorption numbers of 89.9 for oleic acid, 98.1 for linoleic acid (Table III), and 168.0 for linolenic acid (Table IV), and theoretical iodine numbers of 89.9, 181.0, and 273.5, are solved, the following expressions are obtained:

$$\begin{aligned} \%\ \text{oleic acid} &= 1.649\,TN - 1.246\,IN - 0.638\,S + 63.8 \\ \%\ \text{linoleic acid} &= -3.269\,TN + 1.391\,IN - 1.689\,S + 168.9 \\ \%\ \text{linolenic acid} &= 1.624\,TN - 0.146\,IN + 1.329\,S - 132.9 \end{aligned}$$

where TN = thiocyanogen absorption number, IN = iodine absorption number, and S = per cent saturated acids.

By a similar process, with the above thiocyanogen and iodine numbers converted to values corresponding to glycerides, the expressions become:

$$\begin{aligned} \%\ \text{oleic glyceride} &= 1.724\,TN - 1.303\,IN - 0.638\,S + 63.8 \\ \%\ \text{linoleic glyceride} &= -3.426\,TN + 1.457\,IN - 1.693\,S + 169.3 \\ \%\ \text{linolenic glyceride} &= 1.701\,TN - 0.154\,IN + 1.330\,S - 133.0 \end{aligned}$$

The composition of the linseed oils listed in Table V, expressed as glycerides, is shown in Table VI.

With the fat acid composition calculated as per cent of glycerides of the total glyceride fraction, the results should be directly comparable to results obtained by analysis of the fat acids obtained by saponification. A comparison of the percentages of fat acids calculated from analysis of the fat acids with those calculated from analysis of the oil should indicate the precision of the authors' methods of analysis. A few results on fat acids obtained by saponification of some of the linseed oils are shown in Table VII.

For the most part, the results on the fat acids agree well with those on the oils. When the numerous sources of errors in the procedures for fat acid analysis are considered, these results seem satisfactory. Analysis of synthetic mixtures of methyl esters of oleic, linoleic, and linolenic acids by Riemenschneider, Swift, and Sando (*22*) and of the acids by Matthews Brode, and Brown (*19*) gave results similar to those in Table VII.

Analyses of fat acids obtained by saponification have certain advantages, but the authors prefer to make determinations on the oil itself when it is as unsaturated as linseed oil. Many fat acid samples prepared by methods which require prolonged heating in strongly alkaline solutions (*4*) gave percentages of fat acids much different from the oils. The iodine numbers were invariably lower than should have resulted from the parent oils. Without exception the linolenic acid content of these fat acids was lower than that of the oils. Linseed oil is fairly stable, but the manipulations necessary for the preparation of fat acids may produce changes. A shift of ethylenic bonds, polymerization, and oxidation may occur. When the fat acid samples were prepared by a quick saponification the fat acid composition was reasonably close to that of the oils (Table VII).

The composition of linseed oil, when calculated from empirical constants, shows quite a different picture from the composition calculated from the older theoretical constants. The chief differences are that the oleic and linolenic acid content is considerably higher, and the linoleic acid content considerably lower, than formerly believed. Linoleic acid values higher than 40 per cent have often been reported (*8*). When the composition of the linseed oils shown in Table V is recalculated by employing theoretical constants proposed by Kaufmann and Baltes (*12*) the values are much different from those shown in Table VI. One sample gave an oleic acid content of less than 4 per cent.

The composition of the oils in Table VI agrees well with that published by Rose and Jamieson (*25*), who, although they did not determine constants for the thiocyanogen absorptions, made their calculations using constants reported by others for 0.1 N thiocyanogen solutions. Hilditch and Murti (*7*) reported on the composition of three linseed oils by recalculation of previously published results using their thiocyanogen numbers. Unless the conditions under which the former determinations were carried out are known, and the thiocyanogen constants determined under similar conditions, this is a bold procedure. Unfortunately, the data in most cases are insufficient to permit a recalculation of the results already published. When any of the known factors which affect the thiocyanogen absorption are changed, other predetermined empirical values are necessary. The authors agree with Rose and Jamieson (*25*) that "in the majority of cases where thiocyanogen values are given in the literature for linseed and other oils of the same type containing notable quantities of linolenic acid, there is apparently no way of knowing the empirical values which should be used for recalculating the percentages of the individual unsaturated acids in these oils."

Experimental

DETERMINATION OF IODINE NUMBER. Wijs 1-hour absorption, 100 to 150 per cent excess.

DETERMINATION OF THIOCYANOGEN NUMBER. Fifty grams of the lead thiocyanate (*1*) were suspended in 500 cc. of the acetic acid reagent (glacial acetic refluxed 3 hours with 10 per cent acetic anhydride) and approximately 450 cc. of the acid

containing 17 grams of bromine were added in small portions. After the lead bromide had been filtered out the clear thiocyanogen solution was titrated and the concentration adjusted to 0.18 to 0.19 *N* (recently 0.185 to 0.190 *N*) by addition of acetic acid reagent.

Determinations were carried out in 250-cc. glass-stoppered flasks held at 20 ± 1° C. for 24 hours. Titration has been described (*1, 9*).

Determination of Saturated Fat Acids and Unsaponifiable Matter. The unsaponifiable matter was determined by the modified Kerr-Sorber method (*8*) and the saturated fat acids by the modified Bertram (*3*) method as given by Jamieson (*8*) with the following changes. (*a*) The sulfuric acid and bisulfite were added alternately and in small portions to destroy the excess permanganate and manganese oxides, and to reduce frothing. A few drops of capryl alcohol were added to aid in reducing the frothing. (*b*) The extraction of the fat acids, before and after the precipitation of the magnesium soaps, was made with smaller quantities of petroleum ether, first with 150 cc., then decreasing to about 75 cc. for the third and fourth extractions.

Linseed Oil Samples. The oil samples were pressed from finely ground flaxseed warmed to about 60° C.

Table VII. Comparison of Results by Analyses of Oils and Fat Acids

Oil No.	Saturated		Oleic		Linoleic		Linolenic	
	Oil[a]	Fat acids	Oil[a]	Fat acids	Oil[a]	Fat acids	Oil[a]	Fat acids
	%	%	%	%	%	%	%	%
7	11.7	11.9	21.4	23.8	18.5	14.7	48.4	49.7
8	10.3	10.0	15.6	17.0	15.4	13.8	58.7	59.1
10	11.3	11.1	30.3	30.3	18.1	19.2	40.3	39.4
11	13.8	14.2	28.6	27.8	13.9	13.1	43.7	44.8
12	10.5	10.8	33.8	34.7	11.7	10.2	44.0	44.2
13	8.9	9.4	25.3	25.7	19.2	17.2	46.5	47.6
15	9.4	9.6	22.0	23.5	14.4	15.0	54.2	51.8
16	8.8	8.4	26.1	27.7	17.9	17.6	47.2	46.3
19	9.5	9.7	24.7	26.0	17.2	13.0	48.6	51.2
20	8.4	8.7	16.1	17.9	18.4	15.6	57.1	57.8

[a] Results on oils expressed as glycerides.

Quick Saponification and Separation of Fat Acids. Fat acids shown in Table VII were prepared by the following method: To 25 grams of linseed oil in a 2-liter Erlenmeyer flask, approximately 25 ml. of 95 per cent ethyl alcohol were added. The flask was then placed in a boiling water bath and when the temperature of the oil solution reached that of the boiling water bath, 5 ml. of 50 per cent sodium hydroxide (specific gravity 1.53) were added slowly from a pipet. Saponification was complete in 3 to 5 minutes. The soap solution was then removed from the water bath and diluted with approximately 800 ml. of water, and the unsaponifiable material was extracted by ether. Dilute sulfuric acid (1 + 4) was added to liberate the fat acids. After the fat acids were washed with several portions of water, they were dissolved in petroleum ether and dried with anhydrous sodium sulfate and the solvent was removed on the steam bath at reduced pressure. During all the steps following saponification a stream of nitrogen was passed through the solutions.

Preparation of Bromides of Linoleic and Linolenic Acids. Fat acids of linseed oil and sunflower oils (*4*) dissolved in dry ethyl ether and in petroleum ether were cooled to approximately −20.56° C. (−5° F.) by placing outside on a cold day, and brominated.

The linolenic hexabromide was filtered out, washed copiously with ether, dissolved in the minimum amount of hot (about 80° C.) 1-4,dioxane (*18*), filtered by the aid of a steam-jacketed funnel, and then allowed to cool. When crystallization began, an equal volume of petroleum ether was added. The crystalline hexabromide was filtered after standing at 0° C. overnight and the product washed well with petroleum ether. The hexabromide was recrystallized two more times in this manner and once treated with decolorizing carbon before filtration of the solution in hot dioxane. After a final crystallization from boiling xylene, the melting point was 182.5° C. (melting point tube inserted at 175° C.).

The linoleic tetrabromide was first washed with petroleum ether, then crystallized four times by the addition of 3 volumes of petroleum ether to a saturated solution of tetrabromide. Before the third recrystallization the ethyl ether solution was treated with decolorizing carbon, warmed to a boil, and filtered. The product was less soluble in ethyl ether than reported by McCutcheon (*17*). Melting point = 115–115.5° C.

Preparation of Linoleic Acid, Methyl Linoleate, Linolenic Acid, and Methyl Linolenate. All debrominations, solvent removals, and distillations were carried out in all-glass apparatus with ground-glass connections and in an atmosphere of nitrogen. The methyl alcohol used was distilled from magnesium methoxide. The final products were taken up in petroleum ether (Skellysolve F, boiling point 30° to 60° C.). After the solutions had been dried with anhydrous sodium sulfate the petroleum ether was removed by the use of a water pump and the products were distilled. A vacuum receiver of the type described by Noonan (*20*) permitted a separation into fractions without disturbing the distillation. The usual practice was to collect a sample in a receiving flask until the boiling point became constant (fraction 1), then turn the stopcock of the receiver to separate the constant-boiling fraction (fraction 2).

Methyl Linolenate. Forty grams of hexabromostearic acid and 40 grams of zinc dust were ground together in a mortar and suspended in 90 ml. of methyl alcohol. After refluxing 1 hour, a solution of 15 ml. of concentrated sulfuric acid in 100 ml. of methyl alcohol (*15*) was slowly added. The solution was refluxed 1 hour and the ester separated (*22*). The petroleum ether solution was washed with dilute sodium carbonate. One product distilled at 109° C. at 0.018 mm., another at 114° C. at 0.030 mm.

Linolenic Acid. Samples A, D, and E were prepared by saponification of methyl linolenate in the cold in a completely filled flask as suggested by Kass, Loeb, Norris, and Burr (*9*). After most of the alcohol was removed at reduced pressure, the solution was diluted with water and extracted with petroleum ether to remove any unsaponified ester before liberation of the acid.

Samples B, C, F, and G were prepared by pyridine debromination (*14*). To 40 grams of hexabromostearic acid and 40 grams of zinc ground together in a mortar, 120 ml. of freshly distilled pyridine were added. As the reaction was rather violent, the flask was cooled with ice water. After the initial exothermic reaction subsided, the flask was immersed in a boiling water bath for 15 minutes. While still warm the semisolid mass was transferred to a separatory funnel with the aid of approximately 100 ml. of concentrated hydrochloric acid in 400 ml. of water, and petroleum ether. The lumps of zinc salt were broken up with a glass rod and shaken up with petroleum ether. The pyridine was extracted with water. One product distilled at 126° C. at 0.05 mm., another at 137° C. at 0.07 mm.

Methyl Linoleate was prepared by same method as methyl linolenate except that 40 grams of tetrabromide, 30 grams of zinc, and 110 ml. of methyl alcohol were used to debrominate, and a solution of 8 ml. of concentrated sulfuric acid in 70 ml. of methyl alcohol was used to complete esterification. Products distilled at 121° C. at 0.04 mm. and at 147° C. at 0.32 mm.

Linoleic Acid. Methyl linoleate was saponified in the same manner as methyl linolenate. Tetrabromostearic acid was debrominated with zinc in pyridine as described for hexabromostearic acid. Fractions were collected at 129° C. at 0.018 mm. and at 133° C. at 0.070 mm.

After preparation, the acid and ester fractions were placed in vials, most of the air was displaced by nitrogen, and the stoppered vials were stored in the refrigerator.

Summary

The thiocyanogen absorption of linseed oil has been studied to show the importance of controlling the concentration of reagent, the absorption time, and the excess of reagent. Samples of linoleic acid, methyl linoleate, linolenic acid, and methyl linolenate were prepared to determine the empirical thiocyanogen absorption numbers by the authors' method. The average thiocyanogen absorption values for linoleic, 98.1, and for linolenic, 168.0, were then used in equations to obtain expressions to calculate the composition of linseed oils. Linseed oils with iodine numbers from 127.8 to 202.8 were analyzed. A comparison of the results calculated from analyses of the fat acids, obtained by saponification, with those calculated from analyses of the original oils, showed fair agreement. The range of the saturated glycerides was from 7.3 to 16.3 per cent, of the oleic glycerides from 11.9 to 42.5 per cent, of the linoleic glycerides from 11.7 to 24.5 per cent, and of the linolenic glycerides from 20.5 to 61.8 per cent. Linseed oil contains more linolenic acid, more oleic acid, and less linoleic acid than formerly supposed.

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Cryoscopic Analysis of Styrene, Indene, and Dicyclopentadiene

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SMALL quantities of certain impurities in monomeric styrene, indene, and dicyclopentadiene markedly affect the properties of polymers produced therefrom. Limitations of analytical methods for the detection of small quantities of impurities based on bromine absorption or on the accurate measurement of several physical properties are well known to investigators in this field.

A method for determining the amount of impurity in hydrocarbons from freezing and melting curves is described by Mair, Glasgow, and Rossini (*1*) but is not designed for control work where speed is essential.

It was believed that an analytical procedure based on the depression of the freezing point of the hydrocarbon would provide a precise and sensitive method, dependent on the relative number of molecules of hydrocarbon impurities present and practically independent of the type of impurities.

The development of the procedure entailed: (1) the preparation of "100 per cent" styrene, indene, and dicyclopentadiene, respectively, and (2) the design of a suitable apparatus for measuring freezing points, and the determination of the molal dispersions, K_f, of the compounds stated in (1).

Purification of Light Oil Hydrocarbons

The light oil hydrocarbons were purified by successive recrystallizations. The liquid phase was separated from the solid by means of a centrifuge. The centrifuge cakes were not washed. Advantage of equilibrium melting was taken during centrifuging. The criterion of purity consisted of identity of freezing points of the solid and liquid phases of the final crystallization. The hydrocarbons thus processed were assumed to be pure. The purified compounds had the following physical properties.

Compound	Freezing Point, ° C. (Cor.)	Refractive Index, n_D^{20}
Styrene	−30.60	1.5469
Indene	− 1.50	1.5764
Dicyclopentadiene	33.6	....

Measurement of Freezing Points and Molal Depressions

Styrene. The cell in which the freezing points were determined was a glass tube 38 × 1.6 cm. (inside diameter) surrounded by a tube 39 × 2.5 cm. (inside diameter) which provided an air bath. A specially constructed mercury thermometer, 37.5 cm. long, graduated directly to 0.01° C. in the range of −30° to −38° C. was used to measure temperatures. The thermometer was calibrated by the National Bureau of Standards.

A 15-cc. sample, which immersed the thermometer to the −37° mark, was found to be sufficiently large. The freezing cell and air bath were immersed to the −35° mark on the thermometer scale in a carbon dioxide–acetone bath contained in an unsilvered quart Dewar vessel. The bath was maintained at a temperature approximately 5° C. lower than the freezing point of the solution under examination. The solution, the freezing point of which was being determined, was stirred mechanically with a 12-gage Nichrome wire, provided with two loops surrounding the thermometer below the solution level. The highest temperature observed after crystallization began was taken as the freezing points.

Solutions of varying composition were prepared from purified styrene and purified *m*-xylene. The freezing points of the solutions were determined and the molal depression constant was calculated for each ΔT between consecutive solutions. Two observers independently checked each freezing point with the deviation shown, along with the complete data, in Table I.

Indene and Dicyclopentadiene. Purified indene and purified dicyclopentadiene, respectively, were diluted with *p*-

Table I. Freezing Data of Styrene-Xylene Solutions

Styrene *Mole %*	Freezing Point °C. (cor.)	Super-cooled °C.	Maximum Deviation between Observers °C.	K_f
100	−30.60	0.5	0.00	..
99.77	−30.69	0.6	0.00	4.9
99.58	−30.78	0.5	0.00	5.3
99.22	−30.95	0.4	0.00	4.9
99.08	−31.01	0.6	0.00	4.9
98.15	−31.44	0.4	0.00	5.1
96.61	−32.15	0.6	0.01	4.9
95.30	−32.76	0.7	0.01	5.0
			Av.	4.95

Table II. Freezing Data

Indene Mole %	Freezing Point °C. (cor.)	Supercooled °C.	Kf
	Indene–p-Xylene Solutions		
100	−1.50	0.3	..
99.34	−1.91	0.4	6.86
98.85	−2.20	0.3	6.90
98.47	−2.46	..	6.88
97.93	−2.78	0.4	6.88
		Av.	6.89
Dicyclopentadiene Mole %	**Freezing Point °C.**		
	Dicyclopentadiene–p-Xylene Solutions		
100	33.6	0.1	..
99.63	32.25	0.1	55.1
99.25	31.1	0.1	48.2
98.88	29.5	0.1	51.0
98.38	27.1	0.1	50.0
96.79	21.4	0.1	49.3
		Av.	50.7

xylene (freezing point 13.3° C.) and the freezing points were measured by means of a thermometer, graduated directly to 0.01° C., calibrated by the National Bureau of Standards. The freezing point technique was in general identical with that described under styrene. Results are summarized in Table II.

Discussion

The data presented indicate a precise analytical method for the detection of small quantities of impurities in the hydrocarbon examined. Compounds higher in molecular weight than xylene produce a proportionally lower freezing point depression per unit weight, and impurities of lower molecular weight produce a depression proportionally higher. High molecular weight polymers in small amounts would not be detected by this method. However, impurities of boiling points diverging appreciably from the boiling point of the light oil hydrocarbons in question can be separated by fractional distillation. Chemically reactive compounds, such as organic acids which produce an abnormally high K_f, should be determined and removed by chemical means. The cryoscopic procedure is used in research and control work only between concentrations of 92 to 100 per cent.

The true freezing points of the solutions of purified compounds and xylene are slightly higher than those herein reported, for reasons discussed by Mair, Glasgow, and Rossini (*1*).

The high K_f and convenient freezing point of dicyclopentadiene suggest a possible use in the determination of molecular weights as a substitute for camphor. The reactivity of the compound, especially with respect to oxidation, would necessitate some precautions not required in the case of camphor.

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Determination of Sulfur Dioxide in Beer

A Modification of Monier-Williams Method

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DURING the determination of sulfur dioxide in beer by the Monier-Williams method (*1*), it was noticed that a precipitate of barium sulfate was formed in the oxidizing medium (3 per cent hydrogen peroxide). This was found to be due to an excess of barium hydroxide added in removing the sulfate ion from the peroxide by the regular procedure. The presence of this precipitate indicated that any sulfur dioxide determinations run using this hydrogen peroxide were in error by the amount of sulfate precipitated, as sulfate in this form is not titratable.

An attempt was made to improve the accuracy of the procedure as well as increase the rapidity with which the hydrogen peroxide could be prepared by eliminating the effect of the sulfate by another method not involving the use of barium hydroxide, as it is difficult to avoid adding an excess of this reagent. This was accomplished by neutralizing the acid in the peroxide with 0.1 *N* sodium hydroxide to pH 4.0, using a glass electrode (the neutralization could be made to the bromophenol blue end point). pH 4.0 is the neutral point in the titration of the sulfuric acid by this method.

The sulfuric acid in the peroxide is not removed as it is when barium hydroxide is used, but the effect of any acid present in the peroxide is eliminated by neutralization, thus avoiding any error in the volumetric result due to sulfuric acid in the peroxide.

Should it be necessary to check the volumetric result gravimetrically, a blank on the hydrogen peroxide must be determined by precipitating the sulfate present with barium chloride and this blank deducted from the total barium sulfate.

Experimental

Excess Barium Ion in Hydrogen Peroxide Neutralized with Barium Hydroxide. Two separate portions of 3 per cent hydrogen peroxide were prepared in regular manner by diluting Superoxol (Merck 30 per cent) and precipitating the sulfate present with 10 per cent barium hydroxide solution to the bromophenol blue end point. The precipitate of barium sulfate was allowed to settle for 5 days at 5° C., when the solution was filtered, standardized with 0.1 *N* potassium permanganate, and diluted to exactly 3 per cent.

Gravimetric blanks (Table I) were determined on the peroxide solutions to estimate the excess barium ion and the error due to its presence. This was done by adding 1 ml. of concentrated sulfuric acid to 20 ml. of the peroxide, and calculating the weight of

Table I. Determination of Blanks

(20-ml. sample)

Sample	SO_2 Equivalent to Excess Barium Ion P. p. m.
1	0.84
2	1.02
Av.	0.93

barium sulfate thus precipitated to the equivalent amount of sulfur dioxide. The presence of excess barium ion results in a negative error and the blank must be added to any determinations of sulfur dioxide run when using peroxide prepared as directed above, as the first portion of sulfuric acid formed by the oxidation of sulfur dioxide will be precipitated and thus cannot be titrated, giving a low result.

Preparation of Hydrogen Peroxide (Barium-Free). Twelve milliliters of Superoxol (Merck 30 per cent) were diluted to 100 ml. with distilled water, and then neutralized with 0.1 *N* sodium hydroxide to pH 4.0, using a glass electrode. About 4 ml. of the base were required. The strength was then determined by permanganate titration and the neutral solution diluted to exactly 3 per cent. Experience has shown, however, that this standardization is necessary only when the Superoxol is of old stock and considerably too weak, as the peroxide solution required need only be approximately 3 per cent in strength.

Titration blanks were determined on this solution, using a 20-ml. sample, by running a regular sulfur dioxide determination, replacing the usual beer sample with 300 ml. of distilled water. The peroxide was then washed into a 100-ml. beaker and the pH determined. In all cases the pH was 4.0. Had the pH been either higher or lower than 4.0, the solution would have been titrated to that pH using either 0.01 *N* sodium hydroxide or 0.01 *N* sulfuric acid, and this titration subtracted or added to the titration for each regular sulfur dioxide determination, depending on the standard solution used.

Sulfur Dioxide Determinations (Table II). Volumetric determinations were run by the Monier-Williams method (*1*) using both peroxide neutralized with barium hydroxide to the bromophenol blue end point and peroxide neutralized to pH 4.0 (glass electrode) with 0.1 *N* sodium hydroxide. The titrations with 0.01 *N* sodium hydroxide to pH 4.0 were made using a glass electrode.

The procedure as outlined in the Monier-Williams method (*1*) was used in gravimetric determinations, with the exception that the barium sulfate precipitate was digested by keeping the solution at incipient boiling for one hour, then holding it on the steam bath for one hour, and allowing it to stand overnight.

Table II. Gravimetric-Volumetric Checks

(Hydrogen peroxide neutralized with barium hydroxide)

Sample No.	SO_2 Volumetric Uncorrected	SO_2 Volumetric Corrected[a]	SO_2 Gravimetric	SO_2 Difference Uncorrected	SO_2 Difference Corrected
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
1	11.2	12.0	12.35	1.15	0.35
1	10.9	11.7	12.10	1.20	0.40
Av.	11.1	11.9	12.20	1.10	0.30
2	13.4	14.2	14.35	0.95	0.15
2	13.8	14.6	14.50	0.70	−0.10
Av.	13.6	14.4	14.43	0.83	0.03

[a] Blank compensating for excess barium in peroxide added.

Discussion of Results

For convenience, the blanks on the peroxide solutions (Table I) were calculated to parts per million of sulfur dioxide in determinations using 300 ml. of beer, rather than to milligrams of sulfur dioxide.

Correcting the results of volumetric determinations of sulfur dioxide (Table II) in beer for the error due to excess barium ion in the peroxide brings them into closer agreement with the results determined gravimetrically.

In determinations of sulfur dioxide in beer, the use of peroxide neutralized with sodium hydroxide gives results approximately 1 part per million higher than those in which the peroxide was neutralized with barium hydroxide (Table III).

It can be seen from Tables II and III that several separate determinations of sulfur dioxide in the same beer sample can be checked within 0.5 p. p. m.

Summary

The proposed method of preparing hydrogen peroxide by neutralizing with 0.1 *N* sodium hydroxide to a pH of 4.0 is very rapid, thus making the determination available at any time; while preparation by the regular method requires a 5-day wait and a filtration before the peroxide can be used.

The presence of excess barium ion in the hydrogen peroxide prepared by neutralization with barium hydroxide to the bromophenol blue end point, as done in the regular procedure, causes an error approximately equivalent to 1 part per million of sulfur dioxide when a 300-ml. sample of the beer is used.

Hydrogen peroxide prepared by neutralizing with 0.1 *N* sodium hydrogen hydroxide to a pH of 4.0 eliminates the above error without otherwise impairing the accuracy of the procedure.

Gravimetric checks of the procedure may still be made if desired, providing a blank is run to determine the quantity of sulfuric acid present in the peroxide solution used.

The bromophenol blue indicator gives a satisfactory end point when 0.1 *N* sodium hydroxide is used. However, the end point is not sharp when 0.01 *N* sodium hydroxide is used, and the use of the potentiometric method of titration eliminates the uncertainty of the final end point with the lower concentration acid.

The above procedure is not restricted to beer alone, but may be used for determination of sulfur dioxide in many other materials such as malt, barley, corn sirup, wine, etc.

Table III. Reproducibility and Comparative Results

[Hydrogen (1) neutralized with sodium hydroxide, (2) neutralized with barium hydroxide]

Sample No.	H_2O_2 Neutralization	SO_2	Average	Average Diff.
		P. p. m.	*P. p. m.*	*P. p. m.*
3	1	5.6	5.6	0.50
3	2	4.8, 5.5	5.1	
4	1	15.8, 16.0	15.9	1.3
4	2	14.6	14.6	
5	1	8.8, 8.7	...	...

Acknowledgment

Grateful acknowledgment is made to Karl B. Goetsch and John B. Thompson for their many helpful suggestions in the preparation of this paper.

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Perchloric Acid Method for Determination of Silicon in Ferrosilicon

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IN THE tentative A. S. T. M. method for the determination of silicon in ferrosilicon (*1*), a large excess of sodium peroxide is used to reduce the violence of the reaction. The large quantity of sodium chloride subsequently formed in the solution increases the solubility of silica and produces low results (*5*). Two dehydrations with hydrochloric acid are needed to separate the silica quantitatively. This requires a great deal of time, particularly because of slow evaporations. Perchloric acid may be used as a dehydrating agent for silicon-rich ferrosilicon, if the samples are first decomposed (*3*, *7*).

A rapid empirical procedure has been evolved from these methods for the analysis of simple ferrosilicon samples containing approximately 25 to 75 per cent of silicon. Precise results may be obtained in about 5 hours with a minimum amount of manipulation.

TABLE I. DETERMINATION OF CORRECTION FACTOR

Sample[a]	Si Present	Si Found[b]	Error	Ratio of Si Present: Found
	%	%	%	
1	26.83	26.36	0.47	1.018
2	50.0	49.28	0.72	1.015
3	63.16	62.15	1.01	1.016
4	75.60	74.39	1.21	1.016
5	96.80	96.00[c]	0.80	1.008[d]
		Average correction factor		1.016

[a] Analyzed samples 1 and 3 were obtained from the Union Carbide and Carbon Research Laboratories, Niagara Falls, N. Y.; samples 2, 4, and 5 (refined silicon) are Bureau of Standards samples 59, 58, and 57, respectively.
[b] Each value represents average of duplicate determinations.
[c] One-fourth factor weight of sample was used.
[d] Omitted from average.

Procedure

A fusion mixture is prepared by grinding 10 grams of anhydrous sodium perchlorate (*8*), 20 grams of anhydrous sodium carbonate, and 70 grams of sodium peroxide in a mortar heated to about 100° C. The mixture is dried for 1 hour at 110° C. and kept in a desiccator.

About 1 gram of the fusion mixture is melted in a 16-ml. nickel crucible, which is slowly rotated as the melt sets to produce a uniform lining about 0.47 cm. ($^3/_{16}$ inch) in height. A 0.2336-gram sample (one-half factor weight) of ferrosilicon, ground to 200-mesh and dried for 1 hour at 110° C., is carefully mixed with 3 grams of the fusion mixture in the lined crucible with an iron rod. Particles adhering to the rod and to the sides of the crucible are brushed down, and the mixture is covered with about 1 gram of the fusion mixture.

The mixture is fused by revolving the crucible around the outer edge of a low burner flame. At the beginning of the reaction, which is detected by a characteristic sound, the crucible is set aside for about 3 minutes, after which it is heated at 700° C. for 15 minutes. The crucible when slightly cooled is inverted over the opening of a Parr bomb plate which is placed over a 200-ml. platinum dish. The melt may be removed easily by tapping the bottom of the crucible, although it is sometimes necessary to heat the crucible for a moment with a burner.

The platinum dish is partly covered with a watch glass and 60 to 75 ml. of water, followed by 12.5 ml. of 12 *N* hydrochloric acid, are added to the melt. The crucible is rinsed with about 10 ml. of water, filled with 3 *N* hydrochloric acid which is left until the residue has completely dissolved, and again rinsed with water. All the washings are to be added to the contents of the dish. The analysis is rejected if any particles of unreacted ferrosilicon are observed.

The acidic solution is transferred to a 400-ml. Pyrex beaker, and 50 ml. of 70 per cent perchloric acid are added. The mixture is concentrated to heavy white fumes over a hot plate set at 85° to 135° C.; the evaporation may be greatly hastened by the use of a drying lamp. The beaker, covered with a Pyrex watch glass, is then heated above 200° C. for 20 minutes, during which the acid should boil freely and reflux along the sides of the beaker.

The mixture is cooled to about 100° C., diluted with 200 ml. of boiling water, and digested over a steam bath for 15 minutes. The precipitate is washed three times with hot 1 per cent hydrochloric acid, using a total of 125 ml., transferred to a 41-H Whatman filter paper (*2*), and washed six times with hot water.

The precipitate is ignited in a platinum crucible at 1050° C. for 30 minutes, after the paper has been charred and burned over a low flame. The crucible is cooled in a desiccator and weighed. The silica is moistened with concentrated sulfuric acid, and dissolved in 15 to 20 ml. of pure 48 per cent hydrofluoric acid. The solution is evaporated to dryness, and the crucible weighed again.

The per cent of silicon in the sample is found by multiplying the weight of silica (loss by hydrofluoric acid treatment) by 203.2, which is the product of 200 and a correction factor of 1.016 (Table I).

Discussion

Practically all the sources of error in the procedure are negative, as Hawley (*4*) and others (*6*) have pointed out in connection with similar determinations. The most significant error results from the loss of a definite amount of silicic acid which escapes dehydration, but conditions may be controlled in such a way that the total loss of silica is nearly proportional to the per cent of silica in the sample. A correction factor may therefore be introduced to avoid the necessity of a second dehydration.

It is evident from the calculations summarized in Table I that highly precise results may be obtained for ferrosilicons containing approximately 25 to 75 per cent of silicon by applying the correction factor of 1.016. The factor should be checked occasionally by the analysis of a ferrosilicon of known silicon content, particularly if the procedure is changed significantly. For instance, the method may be applied to refined silicon by reducing the size of the sample to one-fourth factor weight if a correction factor of 1.008 is used.

Summary

In a simplified method for the determination of silicon in a comparatively wide range of ferrosilicon samples, the sample is fused with a mixture of sodium peroxide, sodium carbonate, and sodium perchlorate. The melt is then decomposed with water and hydrochloric acid, and the resulting silicic acid is dehydrated with perchloric acid. A correction factor is introduced to eliminate the necessity for a second dehydration.

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CONDENSATION of the dissertation submitted by Sigurd O. Rue to the Graduate College of the State University of Iowa in partial fulfillment of the requirements for the Ph.D. degree.

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Photoelectric Fluorimeter

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THE alternating current–operated fluorimeter shown in Figure 1 attains its sensitivity by the use of vacuum photocells whose current is amplified by an electronically stabilized feedback amplifier of conventional design, similar to that described by Krebs, Perkins, Tytell, and Kersten (*1*).

An ultraviolet lamp housed in a cylinder open at both ends (*C*, Figure 1) provides the light which passes through four 0.47 × 2.5 cm. ($^3/_{16}$ × 1 inch) vertical slits and emerges in the direction of the white arrow. The cells for holding the liquid whose fluorescence is to be determined are supported on two platforms of an "elevator". One cell is shown in position at *G* and the platform for the other is at *E*. Part of the fluorescence from the liquid enters two photocells contained in housings (one shown at *F* and the other opposite it) after it passes through filters held in position at *D*. One of the two windows through which the fluorescent light passes is shown at *H* and the other is opposite it. The filters may be selected to transmit a part of the fluorescent light without transmitting too much of the light diverted toward the photocells from the ultraviolet beam by the liquid. A liquid which does not fluoresce will then have a reading of almost zero on the meter.

After the fluorimeter is wired, *R*5 is adjusted until the voltage across the filament leads of *T*9 is 12.6. This is the only permanent adjustment which need be made. To measure fluorescence with the instrument, *S*2 is closed; after about a minute *S*1 is closed, the ultraviolet lamp is turned on, and the whole apparatus is allowed to warm up for 15 minutes. The liquid is then poured into cell *G* and the solvent into a similar cell which is placed on the platform at *E*. The door is closed and the meter brought to zero by turning rheostats *R*12 and *R*9 (one is for coarse and the other for fine adjustment). Next, cell *G* is raised to a position formerly occupied by the one on platform *E* by means of rod *A* which slides without rotating in tube *B*. The reading of the meter will then indicate a number proportional to the fluorescent light from the sample. The cells are raised and lowered several times to assure the operator that the circuit and the light have remained in a stable state during the several seconds needed to take the readings. One may also use only one cell, in which case the meter is set to zero when this cell is in position *G*.

The sensitivity of the instrument depends on the size of the resistor, *R*10. Figure 3 shows a calibration curve made when *R*10 had a value of 1000 megohms. The ultraviolet lamp was found to be exceedingly stable against voltage fluctuations, probably because its gaseous discharge acted as a negative, while its transformer acted as a positive resistance, resulting in a combination which produced a fixed light output independent of small line voltage variations.

The following parts are needed: *R*1, 5000 ohms, 0.5 watt; *R*2, 7500 ohms, 10 watts; *R*3, 100,000 ohms, 2 watts; *R*4, 5000 ohms, 10 watts; *R*5, 100,000-ohm potentiometer; *R*6, 100,000 ohms, 2 watts; *R*7, 1000 ohms, 25 watts, adjusted so that 0.15 ampere flows through it; *R*8, 2500 ohms, 2 watts; *R*9, 1000-ohm potentiometer; *R*10, 1000 megohms; *R*11, 150 ohms, 0.5 watt; *R*12, 1000-ohm potentiometer; *R*13, 50,000 ohms, 2 watts; *R*14, 3 megohms, 2 watts; *R*15, 120,000 ohms, 2 watts; *R*16, 20,000 ohms, 0.5 watt; *C*1 16-mfd., 450-volt, electrolytic condenser; *B*1, 22.5-volt B-battery (this will need to be changed about once per year); M, 0-200 microammeter; *S*1, double-pole single-throw toggle switch; *S*2, double-pole single-throw toggle switch; *TR*, Thordarson T13R09 transformer; *CH*1, Thordarson

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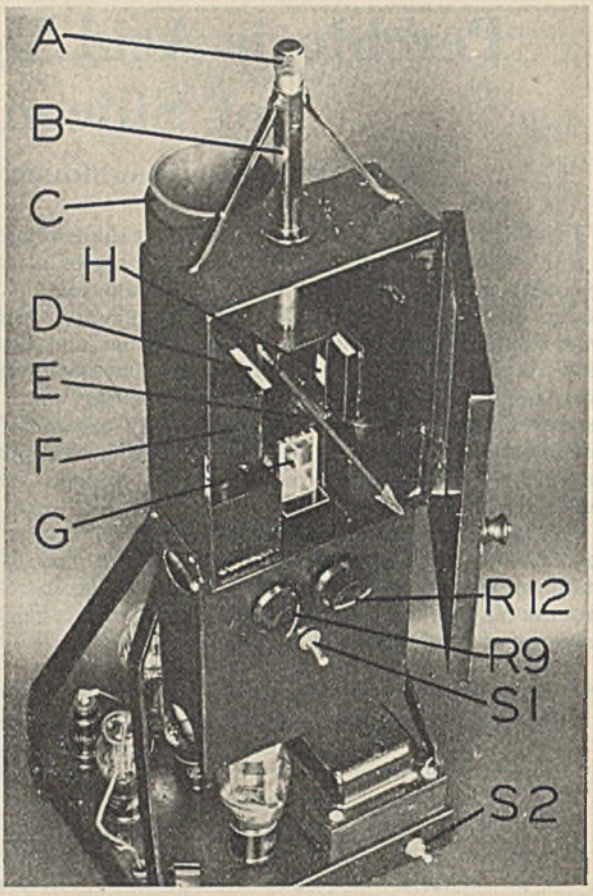

FIGURE 1. FLUORIMETER

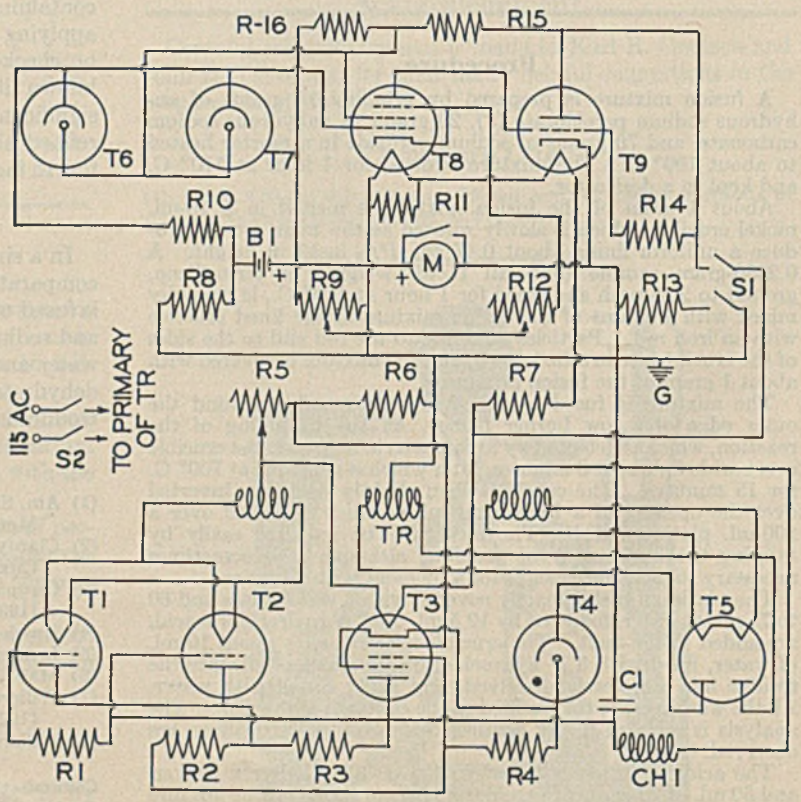

FIGURE 2. DIAGRAM OF CONNECTIONS

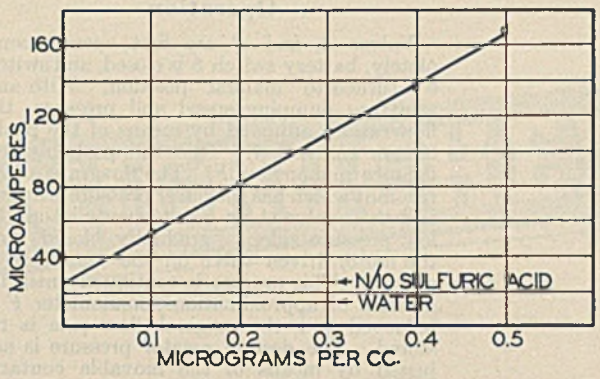

FIGURE 3. GRAPH OBTAINED WITH FLUORIMETER USING QUININE SULFATE IN 0.1 *N* SULFURIC ACID

T74C29 choke; *T*1, 2A3; *T*2, 2A3; *T*3, 12SJ7; *T*4, VR-105-30; *T*5, 5Z3; *T*6, R. C. A. 929; *T*7, R. C. A. 929; *T*8, 12J7-GT; *T*9, 12SF5. See Figure 2 for diagram of connections.

The ultraviolet lamp is a Westinghouse BH-4, 100-watt with a natural red-purple bulb. A special transformer and socket for the lamp are also needed. The filters are Corning 5 cm. (2 inches) square, polished; those used for the curve shown in Figure 3 were No. 4308, 3.14 mm. thick, and No. 3389, 1.51 mm. thick. The cell used for Figure 3 had a capacity of 25 ml. and was made of glass with one window of ultraviolet transmitting glass to permit a larger amount of the ultraviolet light to enter the liquid. Many liquids will fluoresce enough to give satisfactory readings when ordinary glass cells are used.

Acknowledgment

The writers wish to acknowledge the assistance of members of the Department of Biological Chemistry of the University of Cincinnati, who carefully prepared all the solutions used in testing the fluorimeter.

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A Constant Pressure and Flow Ratio Regulator for Continuously Mixing Two Gases

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A FLOW regulator was required which would introduce two components of a gas mixture into a reaction chamber at a constant predetermined ratio of flows and a constant reaction pressure, regardless of variations in reaction chamber pumping speed, rate of chemical reaction, initial pressures, and differential consumption rates of reactants.

The following system meets these requirements, and while it was designed for flows of each gas in the range 0.40 to 20.0 cc. per second at 50 to 200 mm. of mercury, it should be equally applicable to other flow and pressure ranges. The design is such that sparking regulator contacts are not exposed to the mixed gases. Thus even with a pair of gases which form an inflammable mixture, there is no danger of spark ignition.

A constant flow ratio is maintained on the principle that the ratio of flows through two resistances will remain constant regardless of changes in absolute values of pressure or flow, providing the ratio of the mean pressures remains constant. For a reactor feed system, where the reactor pressure is the common final pressure of the two gas inlet systems, this condition can be readily maintained by establishing and maintaining an equality of pressures between the inlet lines at some other point, for the ratio of mean pressures in the lines between this point and the reactor thus remains constant at 1. This can be accomplished by a control U-tube manometer.

Description

A schematic diagram of the flow system and the electrical circuits is shown in Figure 1. The control manometer, *F*, has two fixed electrical contacts on one limb. Valves *A* and *B* between the limbs of manometer *F* and the flowmeters, *M* and *Q*, provide the desired adjustment of ratio of resistances in the lines between *F* and the reactor. *E* is an open manometer, for control of pressure; it has a movable double contact arm in the open side. The contacts are slightly displaced vertically in *E* and *F*. Both manometers are sufficiently long to allow evacuation of any portion of the system, and both have a third contact sealed into the bottom.

All manometer tubes should be at least 10 mm. in inside diameter to prevent excessive sticking of the mercury meniscus. Manometer *F* is filled with mercury to a position midway between the contact points when both sides are balanced.

The motor-driven valves *C* and *D*, details of which are shown in Figure 2, are a modified form of the valve described by Fowler (*1*), and consist of a thin-walled brass tube, flattened and bent into a U. The valve is opened or closed by expanding or contracting the U by means of a rotating lead screw.

For other flow ranges, different sizes of tubing from that shown will be necessary. An ordinary needle valve is satisfactory for the higher flow speeds.

The lead screw of the U-type valve, or the stem of the needle valve, is turned by a 4 r. p. m. Telechron motor, Type C2M. By reversing one of the pole pieces in each of the Telechron motors, the motors can be made to turn in either direction.

The choice of valve operating speeds will be governed mainly by the resistances of the connecting gas lines and by the volume of the reactor. These lines should be as short as possible for the most rapid compensation without hunting. In the present system, it was necessary to place the reactor about 360 cm. (12 feet) from the control apparatus and to make the lines of 0.6-cm. (0.25-inch) inside diameter tubing; thus, a rather slow valve operating speed was required. The reactor had a volume of about 500 ml. These figures may serve as a starting point for the selection of speeds for other conditions.

Parts 1, 2, 3, and 4, in Figure 1, are battery-operated relays which require not more than 20 milliamperes current to energize, and have load capacities of 0.2 ampere. Double-pole double-throw relays are used for 1, 2, and 4, and a single-pole double-throw relay for 3. Part 6 is a double-pole double-throw switch with a neutral, or "off", position, for changing from manual to automatic control of the valves. Group 7 consists of four double-pole single-throw push-button switches for manual control of both motors in either direction.

Testing the Circuit

The circuit may be tested by shorting manometer contacts, as indicated in Table I, and observing the response of the valves.

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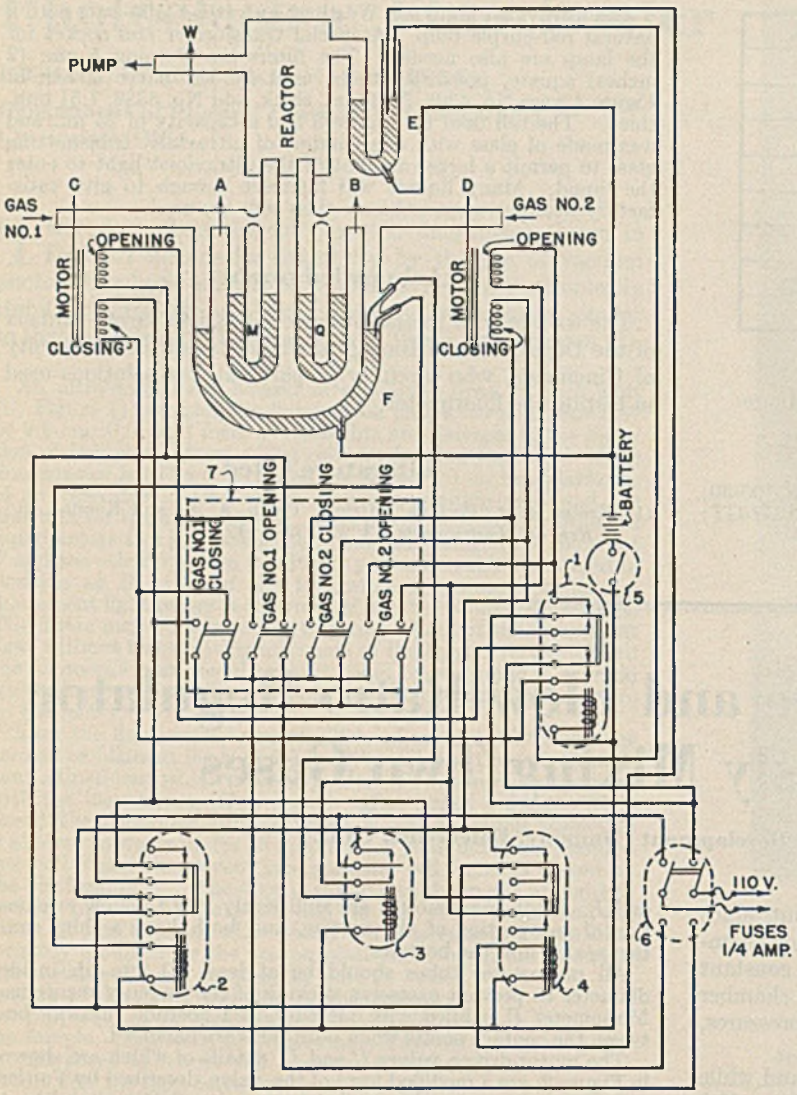

FIGURE 1. DIAGRAM OF CONSTANT PRESSURE AND FLOW RATIO REGULATOR

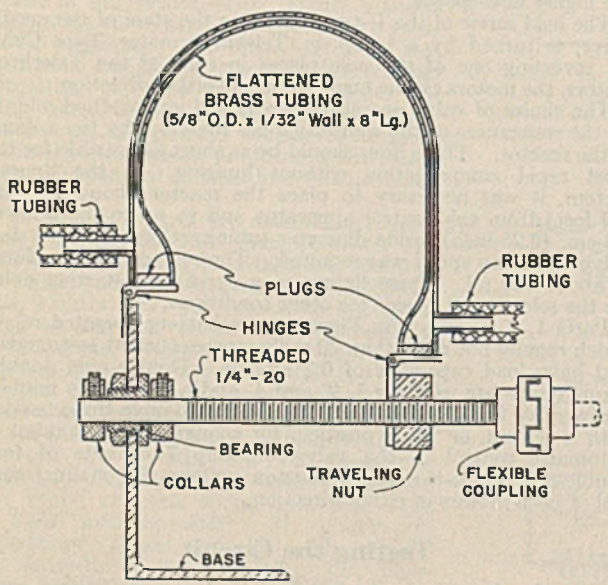

FIGURE 2. MOTOR-OPERATED VALVE FOR SMALL FLOW RATES

Operation

Valves *A* and *B* are first opened completely, battery switch 5 is closed, and switch 6 is turned to "manual" position. With any arbitrary pumping speed and pressure, the flow ratio is adjusted by means of the push-button controls, 7. This will probably unbalance manometer *F*. The flow rate of the reactant which has the lower pressure is noted, and then valve *A* or *B*, whichever is on the low pressure side, is gradually closed, and the motor-driven valve on the same side is opened. This process is continued until, by a series of approximations, manometer *F* is balanced and the original flow rate is restored. The desired reactor pressure is adjusted by means of the movable contacts in *E*. Switch 5 can now be changed from "manual" to "automatic", and the system will begin regulating. If the total flow speed requires correction, this can be accomplished by adjustment of valve *W*. This will not interfere with the established values of flow ratio or pressure. In subsequent runs, the original conditions will be automatically restored, providing valves *A*, *B*, and *W* are not changed.

Application

The unit has been used to control the input of two reactants into a chamber in which rapid reaction can be initiated; such reaction results in a sudden pressure change of some 30 per cent. The regulator satisfactorily restores the pressure to its original value by changing the flow speed of the component gases, without changing the component ratio. Even with the long lines which the author used to connect reactor and regulator, the time to compensate for such large pressure changes was only on the order of a minute, after which compensation for minor fluctuations was much more rapid and was generally made to a constancy, in both pressure and flow speed, of about 1 per cent. At the lower end of the range of flow speeds hunting set in to some extent, but this could be readily eliminated, if desired, by reducing the speed of the valve motors, by using interchangeable lead screws of lower pitch, or by reducing the length of the flow lines between regulator and reactor.

TABLE I. MANOMETER CONTACTS

Conditions		Contacts[b] Manometer *F*		Manometer *E*		Motor Valves	
Flow ratio[a]	Pressure	Upper	Lower	Upper	Lower	Gas 1	Gas 2
Gas 1 high	High	In	In	In	In	Closes	..
Gas 2 high	High	Out	Out	In	In	..	Closes
Balanced	High	Out	In	In	In	Closes	Closes
Gas 1 high	Balanced	In	In	Out	In	..	Opens
Gas 2 high	Balanced	Out	Out	Out	In	Opens	..
Balanced	Balanced	Out	In	Out	In	..	..
Gas 1 low	Low	Out	Out	Out	Out	Opens	..
Gas 2 low	Low	In	In	Out	Out	..	Opens
Balanced	Low	Out	In	Out	Out	Opens	Opens

[a] "High" and "low" refer to required flow.
[b] "In" and "out" indicate that particular contacts are in or out of mercury, and therefore corresponding circuits should be closed, or opened, respectively.

Acknowledgment

The author wishes to thank George C. Eltenton for valuable suggestions.

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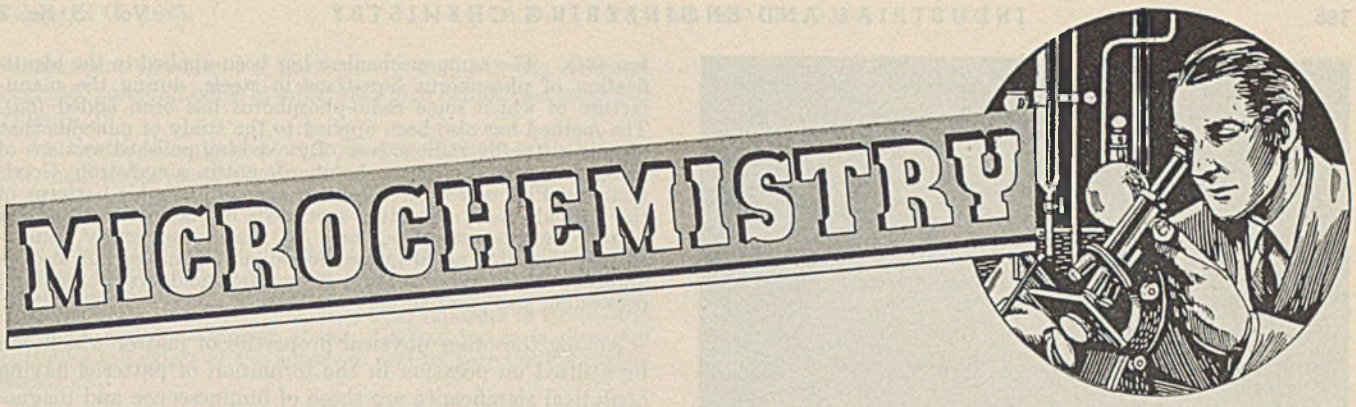

Analytical Patterns in the Study of Mineral and Biological Materials

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DURING the past half century numerous investigators have devised printing methods whose primary object was to ascertain the distribution of a particular component in the samples studied by them. These isolated individual methods include autoradiography employed by mineralogists in the study of radioactive inclusions in rocks (*36*, *50*), the methods of sulfide (*6*), phosphide (*37*), and iron oxide (*57*) printing employed in metallurgical laboratories, and the more generally applicable electrographic method devised by Glazunov for the study of liquations in alloys (*14–16*, *18–26*).

When an effort is made to correlate these diverse techniques, one can visualize the development of a system of two-dimensional analytical chemistry in which a selected plane of the massive sample plays a role similar to that of the representative sample of powdered material in classical analysis. The primary object of such a system of analysis would be to reveal the mode of distribution of the chemical components as an adjunct to the established methods of qualitative and quantitative analysis. This analytical approach frequently facilitates the detection of minor constituents where these have crystallized as distinct units and permits a fairly complete qualitative analysis of the major components without causing appreciable injury to the prepared surface. Each component is characterized by an "analytical pattern" which may be described as an imprint of the surface on an external medium that can be interpreted in terms of its chemical composition as a result of the controlled mechanism of its formation.

Table I. Classification of Analytical Patterns

A.	Produced by physical agencies
	1. Autoradiograph
	2. Autoluminograph
	3. Magnetograph
B.	Produced by chemical reactions
	1. Contact print
	2. Electrographic print

Classification of Patterns

The analytical patterns can be classified in two distinct groups as represented in Table I. In the first classification are those patterns made possible by some intrinsic physical property of one of the components, such as radioactivity, characteristic luminescent effects, or the presence of marked paramagnetism. These patterns are characterized by the absence of any loss of sample whatsoever during their execution and all enjoy the simplicity of the readily available photographic emulsion as their recording medium. Such simple physical mechanisms are available for only relatively few of the elementary constituents. In general it is necessary to strip a film from the surface by some appropriate solvent or electrolyte and then to make its components evident through the use of selective chemical reactions.

When the stripping is effected by means of solvents the processed pattern is commonly known as a contact print. In the case of electrically conducting materials the surface film can be transposed electrolytically and the finished print is termed an electrograph (*11*, *18*). These chemical methods of stripping do not involve the removal of a complete plane, such as would be secured by cutting the specimen with a microtome. Such uniform sampling could be effected only in the case of perfectly homogeneous metals. In general, when a solvent acts on a complex surface a preferential solubility usually exists for one or more of the components. This factor is of considerable importance, as by the judicious selection of a stripping agent it is often possible to improve the specificity of a given analytical pattern.

Of the patterns produced by physical agencies, the autoradiograph is by far the most important. The alpha-rays emitted by radioactive elements activate a photographic emulsion the same way as ordinary light rays. This actinic action was applied by Mügge (*55*) as a means of studying the distribution of thorium and uranium compounds in minerals by contacting the specimen with an emulsion for a suitable period of time and noting the blackening produced on subsequent development. A thin sheet of black paper or aluminum foil is usually placed between the plate and the specimen to avoid direct chemical action or to prevent luminescent light from activating the emulsion and thereby interfering with the specificity of the autoradiographic pattern.

The period of exposure varies with the activity of the specimen and the speed of the film. The writer has found the small 3 × 4 cm. x-ray film, in common use by dentists, convenient for test purposes. Although it is possible to secure an image by simply placing the specimen on the film ensemble, better definition results if the specimen is exposed in a light-tight can directly against the film removed from its protective jacket of paper and

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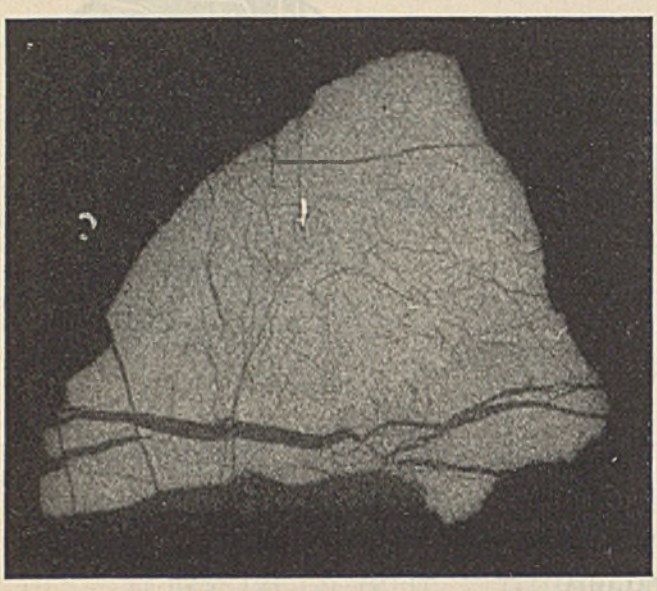

FIGURE 1. AUTORADIOGRAPH OF PITCHBLENDE FROM JOHANNGEORGENSTADT, BOHEMIA

Area enlarged 50-fold, exposure 24 hours. Pattern reveals presence of very fine veins of nonradioactive material.

lead foil. After overnight exposure the film is developed with a solution consisting of one part of Eastman print developer D-72 and two parts of water. The film records considerable fine detail of the radioactive surface, which is best observed on an enlargement print (Figure 1).

This mechanism was employed by Lacassagne (*51*) as a means of studying the distribution of polonium salts injected into the blood stream of animals. After autopsy the organs were fixed and thin sections were contacted with photographic plates. The intensity of the developed images served as a measure of the distribution of the polonium and the prints frequently exhibited the cellular detail characteristic of the tissue. The localization of lead in animal organs has likewise been established by Lomholt (*53*) and independently by Behrens and Bauman (*7*), using radio-active isotopes of lead as tracers. The autoradiographic techniques has also proved fruitful in the study of occlusion within large crystals grown in solutions containing traces of radioactive ions (*33*), and in investigations of the behavior of radioactive elements in alloys during melting, rolling, and wire-drawing processes (*63*, *64*).

The field of autoradiography has been extended with the discovery of methods for synthesizing radioactive isotopes of the lighter elements. These radioactive isotopes serve as indicators for tracing the diffusion of the normal elements found in living structures and the technique can be employed wherever a radioactive isotope of suitable life can be synthesized for the element in question.

The assimilation of phosphorus in plants and the distribution of iodine in thyroid tissue have thereby been recorded by Hamilton (*34*). The same mechanism has been applied in the identification of phosphorus liquations in steels, during the manufacture of which some radio-phosphorus has been added (*66*). The method has also been applied to the study of minerals that are not naturally radioactive. By exposing polished sections of manganese ores to electron bombardment in a cyclotron, Goodman and Picton (*28*) have secured autoradiographic patterns of the normally nonradioactive specimens owing to the formation of radio-manganese. Goodman (*27*) has subsequently demonstrated that this novel autoradiographic technique can also be applied in the study of the ores of tungsten, gold, arsenic, sodium, potassium, and phosphorus.

Among the other physical properties of matter which can be utilized on occasion in the formation of patterns having analytical significance are those of luminescence and magnetism. The latter property is sufficiently pronounced only in the case of metallic iron and some of its mineral occurrences, such as magnetite and pyrrhotite, and can be demonstrated by sprinkling iron filings on a photographic printing paper placed over the magnetized specimen. This property is of little practical importance, as an iron pattern is more readily made by chemical methods. The writer has found luminescent properties serviceable in studying the homogeneous character of mineral specimens. After exposure to ultraviolet light, many substances continue to luminesce for a considerable period after their removal from the source of initial excitation. Although this afterglow is usually feeble and can be observed only in perfect darkness, it is of sufficient intensity to activate a photographic plate when it is brought in direct contact with it. A relatively brief exposure of 2 or 3 minutes suffices, so that there is very little danger of confusing the resultant picture with the pattern produced by any radioactive constituents that might be present. This simple mechanism furnishes a permanent record of the evanescent

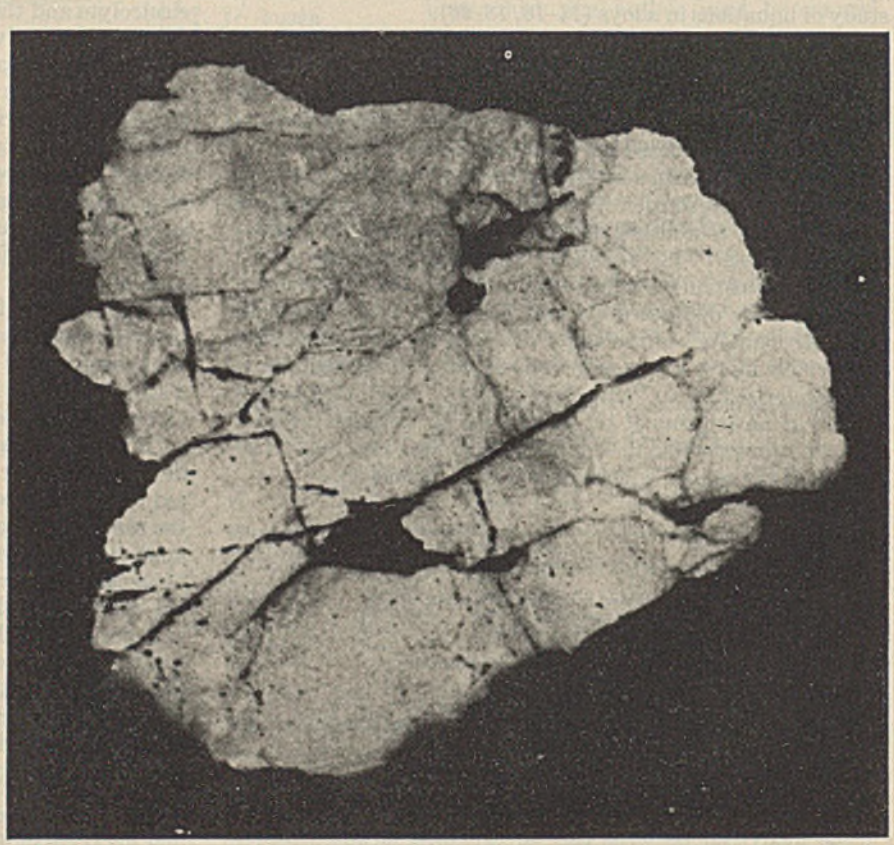

FIGURE 2. AUTOLUMINOGRAPH OF ANTOZONITE, A VARIETY OF FLUORITE FROM ONTARIO, CANADA

Area enlarged 16-fold, exposure 2 minutes. White luminescent areas are produced by calcium fluoride; black nonluminous veins reveal presence of intrusions, which on analysis proved to be limestone and silica.

luminescent effect and is particularly useful in the study of occluded foreign bodies in minerals which exhibit the property (Figure 2).

An electrical method of reproducing surfaces with low relief, such as leaves of plants and coins, has been described by Prat and Schlemmer (*59*).

In this method, termed electrography, the specimen is placed in direct contact with a dry photographic emulsion and the unit is sandwiched between two iron plates connected to the secondary of a high-voltage spark coil. After a brief exposure to the electric discharge and development of the plate, an image is produced outlining the ridges of the specimen and its surrounding corona discharge.

This process does not yield an analytical pattern, since the print furnishes no information about the chemical composition of the specimen. It is of value, however, as a means of recording the path of a high-voltage pulsating current through the material under investigation, and suggests an extension of the electrographic method to samples composed of conducting particles embedded in a nonconducting matrix.

The sulfur print method for exhibiting the distribution of sulfides in iron alloys is one of the earliest examples of an analytical pattern produced by chemical reaction (*6*).

This imprint is secured by pressing the polished section against a sheet of photographic paper that has been moistened with 2 per cent sulfuric acid and maintaining the contact for about 2 minutes. The acid causes the evolution of hydrogen sulfide, which reacts with the silver halides to form dark brown silver sulfide. A permanent print is secured by rinsing off the acid, fixing in a hypo bath, and washing in running water. The contact printing method has since been applied to the identification of phosphate minerals in rocks by Leitmeier (*52*), to copper, nickel, cobalt, and iron oxide liquations by Niessner (*58*), and in the investigation of mineral sections by Gutzeit (*31*).

The electrographic method of printing, devised by Glazunov (*17*) and independently by Fritz (*11*), is essentially a reversal of the principle employed in electroplating.

In this method an absorbent paper moistened with a suitable electrolyte is inserted between a cathode of inert material such as aluminum and the polished face of the alloy which serves as the anode. This unit is connected to a battery of dry cells and a current is allowed to flow for several seconds. During this electrical exposure ions leave the surface of the specimen and migrate into the permeable medium, where their presence can be made manifest by reaction with selective reagents. The simplicity and elegance of this technique soon attracted many devotees, who applied the method to the solution of problems in metallurgy (*1*, *35*, *45*, *46*), mineralogy (*31*, *47*), and biology (*67*).

The electrographic method is of course applicable only to materials that are conductors of the electric current. While the number of conducting minerals constitutes only a minor fraction of all the known species, many of the conducting minerals are fairly common and are often ores of economic importance, such as galena, pyrites, and sulfide minerals in general. In the biological field the method is applicable to the localization of those constituents which are normally present within the tissue in an ionic state.

The Media

The ideal medium for the execution of patterns by chemical agencies is one that is structureless, is preferably transparent, conducts the electric current, is resistant to the action of the etching and developing reagents, and finally does not become distorted while drying. While no one of the media listed in Table II meets all the requirements, a good approximation is usually available to meet the demands of individual patterns.

Absorbent smooth-textured papers are satisfactory as printing media only when an image of the macrostructure of the specimen suffices for the study. Any attempt at magnifying the pattern brings out the fibrous structure of the medium and prevents observation of detail. The outstanding advantage of this medium is its extreme resistance to the action of concentrated volatile acids, and if a prehardened paper is selected such as C. S. & S. 507, it will exhibit little shrinkage after washing.

Table II. Media Suitable for Analytical Patterns

1. Hardened absorbent papers
2. Gelatin-coated paper
3. Gelatin plates and films
4. Thin plaster castings
5. Transparent water-permeable plastics

Dense paper coated with a film of pure hardened gelatin makes a satisfactory medium for contact and electrographic work which permits the resolution of fine detail on the developed pattern. Such paper, free from silver halides, can be obtained from manufacturers of photographic paper. The dense backing of the gelatin-coated paper is rendered electrically conducting by allowing the sheets to soak in a bath of a suitable electrolyte for at least 10 minutes. The gelatin coating tends to hydrolyze on prolonged action of strong mineral acids; nevertheless the medium can be employed for contact printing, provided the acids are cold, and are diluted with equal volumes of water and the paper is washed shortly after the development process. The opaque backing necessitates the observation of the print by reflected light and requires a microscope provided with a vertical illuminator if photomicrographs of the pattern are to be made.

Transparent nonconducting gelatin-coated films suitable for contact printing can be made by extracting the silver halides from photographic film and hardening the gelatin coating. This is accomplished by placing the film in a chrome alum hypo bath for 20 minutes, washing in running tap water for about 1 hour, agitating in distilled water for about 15 minutes, and finally drying in a dust-free atmosphere. The chief advantage of this medium over gelatin paper in contact printing resides in its transparency. This permits the projection of the print onto a screen and its microscopic examination by means of transmitted light.

In an effort to develop a transparent medium that also conducts the electric current, it was discovered that certain varieties of commercial cellophane, that have not been waterproofed, serve

Figure 3. Microphotographic Detail from Lead Pattern of Section of Bronze, ×20

Bronze contacted for 15 seconds against plaster casting moistened with 1 per cent acetic acid. Lead entering solution is immediately fixed on medium as lead sulfate, which is rendered visible by conversion to black lead sulfide on exposure to hydrogen sulfide vapors. Microscopic examination of pattern demonstrates that lead is segregated in minute spherules within bronze matrix.

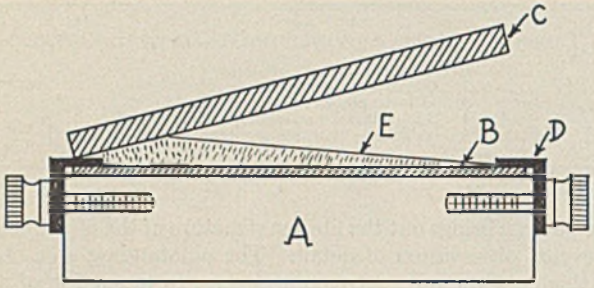

FIGURE 4. CROSS-SECTIONAL VIEW OF MOLD FOR CASTING THIN SHEETS OF PLASTER OF PARIS

A. Hardwood block
B. Glass backing plate
C. Glass surfacing plate
D. Angle railing machined from metal or plastic
E. Slurry of plaster of Paris

as conductors of the second class when moistened with an electrolyte. The thin film is subject to shrinkage while drying so that it is serviceable only in the examination of macrostructures. The distortion of the image can be minimized by preshrinking the cellophane in a water bath and drying prior to its being moistened with an electrolyte. This medium is particularly adapted to the study of the iron pattern in conjunction with potassium ferrocyanide as the developing agent, as the blue image exhibits no bleeding during the washing process, in marked contrast to the behavior on the surface of most other media.

The thin castings of plaster of Paris developed by the writer for producing sodium and potassium patterns of vital tissue (*67*) have unique properties which merit attention. When the plaster casting is moistened with water it furnishes its own electrolyte in the form of a dilute solution of calcium sulfate. By virtue of the fixed source of sulfate ion, the medium serves as a reagent for the fixation of lead sulfate. These inherent properties offer a very simple approach to the study of lead patterns (Figure 3). Water-insoluble reagents such as zinc sulfide, barium carbonate, aluminum oxide, etc., are readily incorporated into the plaster sheet during the casting process. This makes the medium of value not only in electrographic work but also in many other analytical techniques where a fine-grained capillary matrix sensitized with such reagents is of importance, as in spot tests, in capillary analysis, and in the execution of microchromatographic separations of complex organic materials.

The following process is employed by the writer for the preparation of thin plaster sheets that are substantially free from minute air bubbles:

The glass backing plate (Figure 4, *B*) is cleaned with alcohol, placed in the mold, and rubbed dry with a soft lintless paper. To fill a cast having a total volume of 20 ml., 30 grams of plaster are added to a 50-ml. beaker containing 20 ml. of distilled water and the mixture is rubbed into a uniform paste with the aid of a rubber-tipped rod. The thin paste is immediately transferred to the center of the backing plate and the entire mold is bounced against a sponge rubber mat with sufficient force to cause the mix to spread over the greater area of the plate. The surface is then aerated momentarily with ethyl alcohol vapor, and the mold is again briefly agitated. The vibration brings the air bubbles to the surface, where they can be destroyed by the lowering of the surface tension caused by the alcohol vapor. This process is continued until the ascension of the occluded air ceases, but should not be prolonged for more than 2 minutes.

The unit is now removed from the rubber mat and the mold is tilted sufficiently for the mix to flow towards the lower rail. The heavy surfacing plate, *C*, is then placed on the lower rail and rotated downwards with a slow firm motion until it is brought in contact with the opposite support. The excess plaster is wiped off, and about 15 minutes later, when the plaster has set, the upper plate is removed by wedging a thin blade under its corners. The rails are removed and the bottom plate with its adhering sheet of plaster is set aside to dry at a temperature not exceeding 80° C. It is good practice to make several castings late in the afternoon and to permit the sheets to dry slowly at room temperature overnight. When thoroughly dry, the casting separates readily from its backing plate. The sheets are then cut into squares of suitable size by making scratch lines with a razor blade and bending the plaster along the lines of demarcation.

The thickness of the plaster castings can be varied from about 0.4 mm. and up by selecting rails of suitable gage and grinding them down to the desired height. It is well to standardize on sheets 1 mm. thick, as these possess adequate mechanical strength and are sufficiently translucent to permit the observation of colored compounds by transmitted light. In the preparation of specially sensitized castings with water-insoluble reagents, the water employed in the casting process is replaced by an equal volume of a thin suspension of freshly precipitated and washed reagent. In making plaster sheets for chromatographic analysis, about 20 per cent of powdered alumina or dry precipitated chalk is added to the plaster powder and mixed with the water as in the standard casting procedure. The plaster medium itself frequently serves as a chromatographic adsorbent.

Preparation of the Specimen

Minerals sectioned by any one of the standard procedures employed in petrographic laboratories for microscopic examination of their structure are also suited for the preparation of their analytical patterns. The equipment necessary for mounting specimens in plastics may not always be available and the following simple and inexpensive procedure will be found adequate.

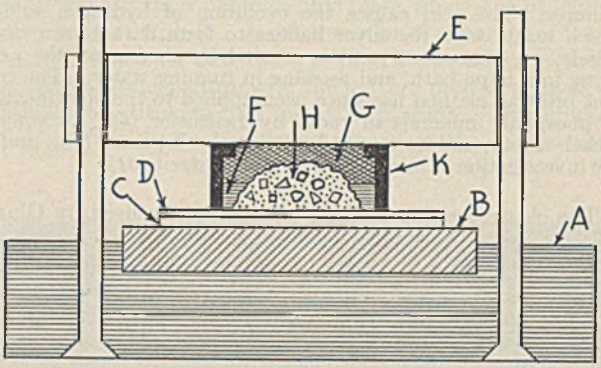

FIGURE 5. PRESS FOR EXECUTION OF ANALYTICAL PATTERNS

A. Wooden block with groove lined with lead foil
B. Electrode sliding on *A*
C. Paper pad similar to C. S. & S. No. 470
D. Medium for recording pattern
E. Lead casting
F. Sealing wax
G. Fusible metal
H. Embedded specimen
K. Plastic screw cap

The sample is first trimmed to fit into a plastic screw cap about 3 to 4 cm. in diameter, the greater part of whose top has been drilled away, so that substantially only a threaded wall remains (Figure 5, *K*). An approximately plane surface is then prepared by grinding the specimen on a glass plate covered with a slurry of 100-mesh Carborundum powder and water until the desired surface is exposed. The mineral is washed free from grit, dried, and placed in an oven at 110° C. on a metal plate covered with a sheet of sized paper. After about 5 minutes the plate is removed from the oven, the warm specimen is covered with the screw cap, and molten sealing wax is poured through the opening until the cap is completely filled. Commercial sealing wax may be used, but it is best to prepare one free from inorganic fillers by heating a mixture of 60 grams of flaked shellac, 45 grams of rosin, and 23 ml. of turpentine until vigorous bubbling sets in (*61*).

When the unit cools to room temperature the mounting is separated from the paper and the grinding is continued until the surface of the mineral is exposed. The section is then polished on fine emery paper until the scratches from the grinding process are removed. In mounting minerals that conduct the electric current, only the lower half of the specimen is embedded in wax. After solidification, the upper half is scraped free from any ad-

hering wax and tarnish and the screw cap is filled with a low-melting alloy. One suitable for this purpose can be prepared by melting together 50 parts of bismuth, 25 parts of lead, and 12.5 parts each of tin and cadmium.

Biological materials require no special mounting prior to their electrographic analysis when the constituents exist as ions in the tissue. A parallel-faced slice about 4 mm. thick is cut from a fresh organ and is drained on lintless filter paper and then exposed to the sensitized emulsion. The orientation of non-ionic inorganic constituents can be established by destroying the organic matter in a thin section and making contact prints of the residual ash. The slow combustion of a thin section of tissue mounted on a glass slide results in an ash skeleton in which the inorganic constituents are deposited exactly as in the original tissue. This pattern, analogous to the total ash determination in classical analysis, is known as a spodogram (*60*). The ash resulting from the microincineration of the tissue is in many respects akin to a polished mineral section and its constituents can be studied by the methods of contact printing.

Preparation of the Pattern

The apparatus employed in the execution of the patterns (Figure 5) is essentially a press for maintaining uniform contact of the specimen with the printing medium.

A metallic foil, *A*, serves to make electrical contact with a sliding slab, *B*. This electrode may be of any inert metal such as aluminum, platinum-clad steel, or heavily silver-plated brass. Hiller (*38*, *41*) and Gutzeit (*29*) employ as electrode material a layer of sponge rubber or felt covered with an envelope of aluminum foil. The alkali hydroxides formed during the electrolysis tend to corrode the cathode, particularly if it is aluminum. Any visible corrosion should be removed by burnishing the surface with fine steel wool; otherwise it will constitute an added irregular resistance and prevent the formation of a perfect image. The paper pad, *C*, between the cathode and the medium, *D*, equalizes the pressure on the face of the specimen and facilitates uniform contact. The pad is rendered conducting with an appropriate electrolyte and is drained of excess fluid before being placed on the electrode. The medium is likewise moistened with electrolyte, and buffering and developing agents, and thoroughly blotted between absorbent paper. The specimen is then brought in contact with the medium, by lowering it at an angle, so as to avoid the inclusion of air bubbles. The electrode with its assembly is transferred to the groove in the base of the press, so that the specimen is centered with reference to the lead casting, *E*, which when lowered subjects the specimen to a reproducible pressure. This casting should weigh about 500 grams, and when necessary, the pressure can be augmented by placing added weights. In making autoradiographic prints a small C-clamp suffices for contact of the specimen against the film.

When the surface is to be stripped electrolytically the electrodes are connected to the appropriate terminals of a 22.5-volt B battery in series with a variable rheostat of about 500 ohms' resistance and a meter having a range of about 100 milliamperes. Although the electrode potentials of most elements is below 3 volts, it is necessary to apply a much higher voltage in order to overcome the resistance of poorly conducting minerals and the internal resistance of the medium. The duration of the current seldom exceeds 30 seconds, the optimum exposure being determined by trial on sucessive prints. The intensity of the current, and its duration can be approximated in the case of fairly homogeneous specimens with the aid of Faraday's second law

$$ti = 96{,}500\ Adn/W$$

In this expression i is the current in amperes, t is its duration in seconds, A is the area of the polished surface in sq. cm., W and n are the atomic weight and valence of the particular ion whose pattern is to be rendered, and d is the weight of the element in grams per sq. cm. that must be electrolyzed in order to produce the desired intensity of color on development.

Experience with quantitative drop reactions on paper shows that in general 50 micrograms of most of the metals produce brilliantly colored products when the reaction is confined to an area of 1 sq. cm. (*70*). Using this density factor the equation reveals that when copper is electrolyzed an exposure of 15 milliamperes for 10 seconds per sq. cm. of surface will result in a print of good color intensity. This is in fair agreement with actual working exposures resulting in satisfactory patterns of copper alloys and ores. Since this equation does not take into consideration the electrical energy expended in decomposing the electrolyte, the calculated exposures must be considered as first approximations useful in gaging the initial trial exposure.

In exposing a specimen composed of several different mineral species, the current passing through the individual crystals will vary with their resistivity and cross-sectional area. Under these circumstances the sample comprises a series of resistances connected in multiple, and the current may be carried almost entirely by the component of highest conductivity, with the result that the pattern will be underexposed with respect to the poorly conducting constituents. Stripping of a more uniform character is achieved by moistening the medium with a mineral acid instead of a neutral electrolyte (*41*).

After exposure, the image is rendered visible by treating the medium with a reagent solution that produces a characteristic insoluble colored compound with the ion in question. Since the number of specific reactions are rather limited, interfering ions brought into solution during the stripping process must be removed prior to development. To prevent diffusion of the element whose pattern is being processed, it is good practice to fix the ion in the medium as a sparingly soluble compound. This principle is illustrated in the method for the formation of chloride patterns (*67*).

The medium consists of gelatin-coated paper impregnated with silver chromate. On electrical exposure the chloride ion is fixed as insoluble silver chloride, and the print can be washed with dilute nitric acid in order to leach out excess reagent and other compounds that enter the emulsion during electrophoresis of the tissue. After washing in water, the colorless image is rendered visible by reducing the silver chloride to metallic silver with the aid of a photographic developing solution. A typical example of the resultant chloride pattern is shown in Figure 6.

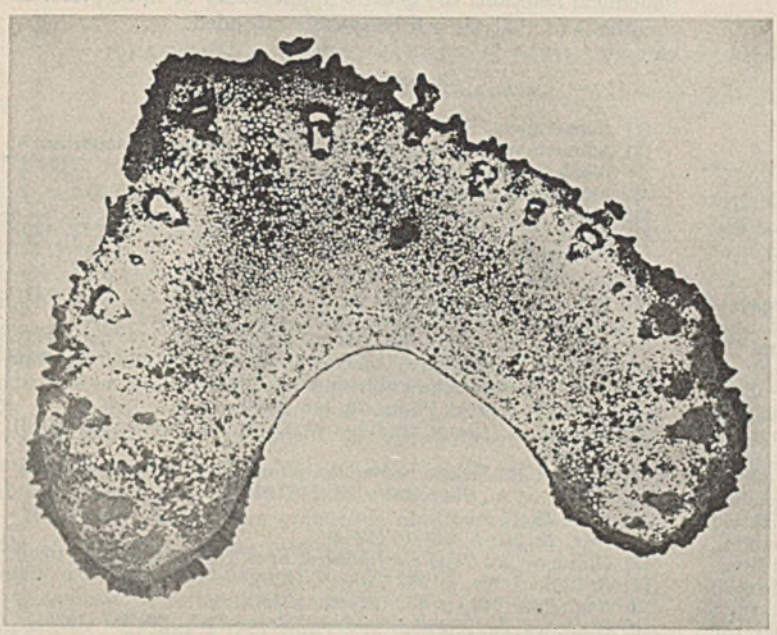

FIGURE 6. CHLORIDE PATTERN OF CROSS SECTION OF CELERY

Area enlarged 20-fold, exposure 5 ma. for 15 seconds. Pattern exhibits physical structure of cell walls and demonstrates concentration of chloride ion in epidermal wall, vascular bundles, and central collenchyma tissue.

In contact printing, solution of the surface film is effected by the action of dilute mineral or organic acids, ammonia, and alkalies. Refractory ores can often be attacked by moistening the medium with a mixture consisting of 1 part of nitric acid, 1 part of hydrochloric acid, and 2 parts of water. In specific cases a selective stripping for one or more components can be achieved through the action of potassium cyanide (silver), ammonia and hydrogen peroxide (arsenic), tartaric and phosphoric acids (antimony), bromine water (gold), sulfurous acid (manganese), etc. The optimum method of attack for any particular pattern depends on the nature of the mineral and is best established by preliminary tests.

As an example of the applications of the contact printing technique, consider a mineral section containing copper, nickel, cobalt, iron, and sulfur as principal components. A joint pattern of the copper, nickel, and cobalt can be rendered by bringing the polished surface into contact with gelatin paper moistened with equal volumes of ammonia and water. These elements form soluble ammines and their presence is made manifest by developing the medium with a saturated alcoholic solution of dithiooxamide (*10*) which forms a dark green derivative with copper, and blue and brown compounds with nickel and cobalt, respectively. Individual patterns can be secured by developing successive prints with alpha-benzoinoxime (copper, bright green), dimethylglyoxime (nickel, red), and alpha-nitroso-beta-naphthol (cobalt, brown). The iron pattern is made by stripping the surface with 20 per cent hydrochloric acid and washing the medium with 5 per cent ammonium hydroxide. The iron remains fixed as ferric hydroxide, and is separated from the bulk of the accompanying copper, nickel, and cobalt chlorides. The iron pattern is developed by immersing the print in a 2 per cent solution of potassium ferrocyanide containing about 0.1 per cent of hydrochloric acid which converts the ferric hydroxide into Prussian blue.

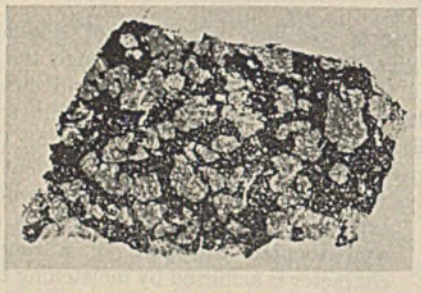

FIGURE 7. SULFIDE PATTERN OF A COPPER ORE

Specimen of chalcocite intergrown with bornite, from Butte, Montana. Area of pattern, full size. Exposure, 40 ma. for 10 seconds.

Since most sulfides are good conductors of the electric current, the electrographic technique is advantageously applied in the execution of the sulfide pattern of minerals.

The specimen is placed on a sheet of matte photographic printing paper (Eastman, Azo Grade A, No. 2), which has been moistened with a 1 per cent solution of sodium chloride and the mineral is connected to the negative pole of the battery. A current of 5 milliamperes per sq. cm. is allowed to flow for about 10 to 15 seconds, causing the migration of the negatively charged sulfide ion to the aluminum anode and its fixation as brown silver sulfide on the medium. Excess silver halides are then extracted by means of hypo and the print is finally washed in water (Figure 7). When the current is passed through the moist photographic paper the area acquires a pink tint, which is probably caused by the formation of reduction products of the silver halides. These subhalides dissolve in the hypo solution and do not cause any interference with the sulfide pattern. In applying the method to the localization of traces of sulfides, the exposure should not be prolonged beyond 1 minute, to avoid possible reduction of the emulsion to metallic silver. The brown colored pattern recorded on the paper is specific for sulfides in the absence of selenides and tellurides, which also react with the emulsion with the formation of black silver derivatives. The more commonly occurring arsenides and antimonides do not interfere and the method can be applied to the detection and localization of traces of sulfide segregations in minerals where arsenic and antimony predominate.

TABLE III. CLASSIFIED GUIDE TO THE LITERATURE ON ANALYTICAL PATTERNS

C, contact print; E, electrographic print; R, autoradiographic print

Constituent	Mineral Sections	Metals and Alloys	Tissues	Plated Surfaces
Aluminum	..	..	..	E (*20*)
Antimony	C (*13*); E (*32, 41*)	E (*15*)	..	C (*62*)
Arsenic	C (*13*); E (*32, 41*); R (*27*)	..	..	..
Bismuth	C (*13*); E (*41*)	E (*15, 42*)	..	..
Cadmium	..	E (*15*)	..	E (*35*)
Chlorides	..	..	E (*67*)	E (*68*)
Chromium	..	E (*4, 23, 24, 35*)	..	E (*8, 69*)
Cobalt	C (*13, 30*); E (*39*)	C (*53*); E (*15, 44, 48*)	..	..
Copper	C (*13*); E (*39*)	C (*53*); E (*17, 44*)	..	E (*69*)
Gold	R (*27*)	..	..	E (*20, 69*)
Iodides	..	..	R (*34*)	..
Iron	C (*13, 30*); E (*41*)	E (*1, 17, 46*)	..	C (*54*)
Iron oxides	..	C (*56, 58*)	..	..
Lead	C (*13*); E (*41*)	E (*22, 35*)	R (*7, 53*)	..
Manganese	E (*29, 41*); R (*28*)	E (*15*)	..	..
Molybdenum	E (*41*)	..	..	..
Nickel	C (*13, 30*); E (*47, 49*)	E (*19, 25, 48*)	..	E (*23, 69*)
Nitrites	..	..	..	C (*43*)
Palladium	E (*29*)	E (*35*)	..	..
Phosphates	C (*52*); R (*27*)	..	R (*34*)	..
Phosphides	..	C (*37, 57, 58*); R (*66*)	..	..
Polonium	..	R (*9, 64*)	R (*51*)	R (*9*)
Potassium	R (*27*)	..	E (*67*)	..
Silver	E (*40*); C (*65*)	E (*17, 35*)	..	E (*69*)
Sodium	R (*27*)	..	E (*67*)	..
Sulfides	C (*12, 13, 30*)	C (*2, 5, 6, 37, 57*)	..	..
Thorium	R (*36*)	R (*63*)	..	..
Tin	..	E (*15*)	..	E (*20*)
Titanium	E (*3, 41*)	..	..	..
Tungsten	R (*27*)	..	..	..
Uranium	R (*36, 50*)	..	..	..
Zinc	E (*41*)	E (*15, 35*)	..	E (*35, 69*)

Further details of these techniques can be secured by consulting the original papers classified in Table III, which lists the methods applicable to the execution of analytical patterns of minerals, vital tissues, alloys, and electroplated surfaces. Most of these methods were developed during the past 10 years by a comparatively small number of workers. It is to be expected that with further developments in the synthesis of radioactive isotopes and with the discovery of new selective chemical reactions the general applicability of these pictorial methods of analysis will be greatly extended.

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Yeast Microbiological Methods for Determination of Vitamins

PYRIDOXINE

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THE discovery that pyridoxine belongs to the group of substances known as bios factors or yeast growth factors (*3*, *4*) was followed by attempts to use the effect in assay methods (*4*, *9*). Since the initial use of a yeast growth method for the assay of pyridoxine (*4*), certain improvements in the method have been made. The technique and apparatus have been simplified and the medium has been improved, but the principal change is the choice of a yeast strain especially selected for its specific response to pyridoxine. In a pyridoxine-free medium this yeast (culture 4228) grows very slightly, but if pyridoxine be added to the medium an extensive growth is observed, comparable to that observed in the presence of yeast water or malt extract.

Wherever possible the apparatus and materials required for the conduct of the assay have been chosen from the least expensive standard items available. The technique has recently been described in detail (*1*). Yeast suspensions, in selected 18-mm. Pyrex test tubes, are shaken in a Fisher-Kahn shaking apparatus at 30° C. Some workers (*9*) do not shake the yeast suspensions during the growth period but in the authors' hands this method has yielded irregular results. The extent of yeast growth after 16 and 18 hours is estimated by densitometric measurements, made directly on the tubes with the aid of a Lumetron No. 400 photoelectric colorimeter. A Mazda lamp provides the light, which is reduced in intensity by a gray glass-wire screen combination in place of a filter.

Solutions

Because the methods used in the determination of pyridoxine may also be employed for the determination of other vitamins—e. g., pantothenic acid, biotin, and inositol—it is convenient to prepare individual solutions of the various components of the basal medium and to combine them in the different ways required by the several methods.

SUGAR AND SALTS SOLUTION. One liter contains 200 grams of C. P. dextrose (anhydrous), 2.2 grams of monopotassium phosphate, 1.7 grams of potassium chloride, 0.5 gram of calcium chloride ($CaCl_2.2H_2O$), 0.5 gram of magnesium sulfate, 0.01 gram of ferric chloride, and 0.01 gram of manganese sulfate.

POTASSIUM CITRATE BUFFER. One liter contains 100 grams of potassium citrate ($K_3C_6H_5O_7.H_2O$) and 20 grams of citric acid ($14H_3C_6H_5O_7.H_2O$).

Table I. Effect of Volume and Acid Concentration of Extraction Medium on Extraction of Pyridoxine from Dry Yeast

(100 mg. of 200 B dry yeast suspended in volume noted, heated at 20 pounds for 1 hour)

Volume	Concentration of Sulfuric Acid	Pyridoxine Determined
Ml.	*N*	*γ/g.*
10	0.055	31.0
50	0.055	37.0
100	0.055	37.0
180	0.055	39.3
180	0.0	27.2
180	0.028	38.4
180	0.111	37.4
180	0.222	35.7

Casein Hydrolyzate Solution, 80 ml. of S. M. A. Corp. "vitamin free" casein hydrolyzate (10 per cent solution) neutralized to pH 4.0 to 6.0 (alkacid paper), and diluted to 100-ml. volume.

Thiamine Solution, 10 micrograms per ml.

Inositol Solution, 1 mg. per ml.

Biotin Solution, S. M. A. Corp. biotin concentrate No. 5000, diluted so that it contains approximately 0.8 microgram per ml.

Calcium Pantothenate Solution, 200 micrograms per ml.

Yeast Inoculum

Culture 4228, a strain of *Saccharomyces carlsbergensis*, is carried on Difco malt agar slants. A slant is incubated for 24 hours at 30° and is then stored in the refrigerator for not more than 2 weeks. To prepare an inoculum for the assay a fresh slant is prepared 24 hours earlier and is also incubated at 30°. A quantity of fresh growth is removed by a sterile wire loop and suspended in 10 ml. of sterile 0.9 per cent saline in a colorimeter tube. The concentration of the yeast is estimated with the densitometer and is adjusted to a concentration of 1 mg. of moist yeast per ml. with additional sterile saline. The densitometer is conveniently calibrated with a suspension of moist compressed baker's yeast instead of culture 4228. With the authors' instrument a suspension of moist baker's yeast, 1 mg. per ml., shows a light absorption of 20 per cent. Five milliliters of the adjusted suspension are then added to 45 ml. of saline in an Erlenmeyer flask. The final suspension thus contains 0.1 mg. of moist yeast per ml. and is ready for use.

Preparation of Samples for Assay

Although pyridoxine is relatively soluble, it is extracted from most plant and animal tissues with difficulty. The Texas workers (*9*) have observed that autolysis increases the pyridoxine content of animal tissue extracts, but this technique is inapplicable to most foods and tissues. The authors have found that acid extraction increases the pyridoxine content of extracts as measured by the yeast method. The efficiency of the extraction depends upon the volume of the extraction medium as well as the presence of acid. This is demonstrated by the data of Table I. For most substances extraction with 180 ml. of 0.055 *N* sulfuric acid yields maximal values and this treatment has been used throughout this communication unless otherwise specified.

Insoluble materials should be powdered if dry, or macerated with water in a Waring blendor, or its equivalent, if infrangible. A portion containing between 2 and 4 micrograms of pyridoxine is suspended in 180 ml. of 0.055 *N* sulfuric acid—i. e., 1 ml. of 10 *N* acid and 179 ml. of water. The suspension or solution is heated in an autoclave at 9-kg. (20 pounds) pressure for 1 hour, then cooled, neutralized to pH 5.2, and diluted to 200-ml. volume. If the solution is turbid it is centrifuged and the clear supernatant liquid is used for the assay. In exceptional cases—e. g., white flour—the turbidity remains but can be removed if the extract is treated with a knife point of clarase at 37.5° for 30 minutes, followed by centrifugation.

Wheat and wheat products do not yield maximal pyridoxine values when treated as above, but require more acid. It has been found that 180 ml. of 0.44 *N* sulfuric acid—i. e., 8 ml. of 10 *N* sulfuric acid and 172 ml. of water—yield nearer maximal assay values with these substances.

The efficiency of the acid extraction of pyridoxine has been checked by comparison with enzymic digestion. Although the digestion with clarase does not always yield results as high as the acid extraction, the tendency is clearly toward the same values. Table II contains several instances where the two methods of extraction have been compared. Either clarase or polidase may be used, but the authors prefer clarase because it has less color and less pyridoxine. Their sample contained 1 microgram per gram. The enzymic digestion is performed as follows:

A portion of the sample containing between 2 and 4 micrograms of pyridoxine is suspended in a small quantity of water in a graduated test tube, 0.5 ml. of potassium citrate buffer (pH 5.2) is added, and the volume is made to 10 ml. The tube is steamed for 30 minutes, cooled, and a quantity of clarase equal to the weight of the sample, but not less than 100 mg., is added. A few drops of benzene are added, and the tube is tightly corked and incubated for 3 days at 37.5° or 2 days at 45°. At the end of the incubation the tube is steamed for 20 minutes and the contents are diluted to 200 ml.

The problem of obtaining pyridoxine extracts suitable for assay is not simply one of preparing a soluble form of pyridoxine; some soluble vitamin concentrates were found to contain a bound form of pyridoxine which was not active in the test until acid autoclaved or enzymically digested.

Method

Five milliliters of basal pyridoxine-free medium plus a solution of the unknown or of pure pyridoxine are placed in a series of test tubes together with water to make the total volume in each tube 9 ml. The tubes are plugged and steamed for 10 minutes,

Table II. Pyridoxine Content of Miscellaneous Substances

Description	Pyridoxine Determined	Pyridoxine Literature Values
	γ/ml.	*γ/g.*
Citrus		
Lemon juice (whole juice)	0.35	
Orange juice (whole juice) A	0.52	
Orange juice (whole juice) B	0.60	
	γ/g.	
Meat		
Pork liver (fresh)	5.9	3.3 (*8*), 1.7 (*9*)
Beef liver (fresh)	7.1	7.3 (*8*), 0.4 (*9*)
Pork muscle (fresh)	6.8	6.1 (*8*), 1.23 (*9*)
Beef muscle (fresh)	2.3	4.0 (*8*), 0.81 (*9*)
Liver concentrate powder, 1 to 20	41.8	45.0 (*2*)
	γ/ml.	*γ/ml.*
Milk		
Pasteurized (whole)		
A	0.56	1.3 (*8*), 1.7 (*6*)
B	0.51	
C	0.50	
D	0.60	
D[a]	0.50	
Evaporated (whole, not diluted)	0.62	
	γ/g.	*γ/g.*
Dry (skim milk)	5.5	
Wheat and wheat products		
Whole wheat[b]	4.8	4.6 (*7*), 7.6 (*6*)
Whole wheat[a]	4.7	
White flour (patent)[b]	1.2	2.2 (*7*)
Whole wheat bread (air dry)[b]	4.2	
White bread (air-dry)[b]	1.0	
	γ/day	
Urine		
Normal 24-hour excretion		
Subject A	143	
Subject B	128	
Subject C	127	
24-hour excretion following ingestion of 5.0 mg. of pyridoxine		
Subject A	241	
Subject B	284	
	γ/g.	
Yeast		
Baker's (fresh) A	6.9	
Baker's (fresh) B	4.9	
Baker's (fresh) C	9.1	
Brewer's (dry)	39.3	55 (*2*), 54 (*6*)
200 B-(dry)	40.0	
200 B-(dry)[a]	39.6	
Yeast extract (dry) A	120.0	
B	62.0	

[a] Clarase digestion, 3 days at 37.5° C. at pH 5.2.

[b] Extracted at higher acid concentration—i. e., 180 ml. of 0.44 *N* sulfuric acid, 1 hour at 20 pounds.

TABLE III. TYPICAL PROTOCOL

[To each tube are added 5 ml. of basal pyridoxine-free medium plus ingredients noted below. After sterilization 1 ml. of yeast suspension (0.1 mg. of moist yeast) is added to each. The tubes are then shaken at 30° for 16 hours, and the absorption measured, then returned to incubator for 2 hours and measured again.]

No.	H_2O	Added	16 Hours: Absorption	16 Hours: B_6 $m\gamma$ per tube	16 Hours: B_6 γ/g. or γ/ml.	18 Hours: Absorption	18 Hours: B_6 $m\gamma$ per tube	18 Hours: B_6 γ/g. or γ/ml.	Av.
	Ml.	Ml.	%			%			γ/ml.
1	4	0	17.5	..	.....	21	..	.....	.....
2	3.5	0.5 ≙ 5 $m\gamma$ of B_6[a]	30	..	.....	35	..	.....	.....
3	3	1.0 ≙ 10 $m\gamma$ of B_6	38.5	..	.....	44	..	.....	.....
4	2.5	1.5 ≙ 15 $m\gamma$ of B_6	45	..	.....	50.5	..	.....	.....
5	2	2.0 ≙ 20 $m\gamma$ of B_6	49	..	.....	57	..	.....	.....
6	1	3.0 ≙ 30 $m\gamma$ of B_6	56.5	..	.....	64.5	..	.....	.....
7	0	4.0 ≙ 40 $m\gamma$ of B_6	62.5	..	.....	68.5	..	.....	.....
8	3	1.0 ≙ 0.01 ml. of whole orange	29	4.5	0.45	35.5	5.5	0.55	
9	2	2.0 juice[b]	38.5	10.0	0.50	45	10.7	0.54	0.53
10	1	3.0	45	15.7	0.52	52.5	16	0.53	
11	0	4.0	51	22.3	0.55	58	21	0.53	
12	3	1.0 ≙ 0.01 ml. of approved	29.5	4.7	0.47	36	5.7	0.57	
13	2	2.0 pasteurized milk[c]	37.5	9.5	0.48	45	10.7	0.54	0.50
14	1	3.0	43.5	14.5	0.48	51	15	0.50	
15	0	4.0	48.5	19.5	0.49	56	19	0.48	
16	3	1.0 ≙ 0.1 ml. of urine, normal	34	7.2	0.072	40	8	0.08	
17	2	2.0 male[d]	45	15.7	0.078	51.5	15.3	0.077	0.075
18	1	3.0	51	22.2	0.074	59.5	22.5	0.075	
19	0	4.0	56	29	0.073	63	28	0.07	
									γ/g.
20	3.5	0.5 ≙ 0.05 mg. of yeast extract	32	6	120	37	6.3	126	
21	3	1.0 dry[e]	42	13	130	47	12	120	122
22	2	2.0	54	26	130	59	22	110	
23	1	3.0	61.5	39	130	66.5	34	113	
24	3.75	0.25 ≙ 0.125 mg. of liver pow-	30.5	5.2	41.6	35.5	5.5	44	
25	3.5	0.5 der 1 to 20[f]	38.5	10.2	40.8	44	10	40	41.8
26	3	1.0	51	23.2	44.4	56.5	20	40	
27	3.5	0.5 ≙ 0.25 mg. of dry 200 B	38.5	10.2	40.8	45	10.7	42.8	
28	3	1.0 yeast[g]	50.5	21.7	43.4	54.5	18	36	40.5
29	2	2.0	62.5	40	40	68	40	40	

[a] Pyridoxine hydrochloride. One millimicrogram (1 $m\gamma$) = 0.001 microgram. A solution containing 10 $m\gamma$ per ml. may be preserved for at least a month without deterioration if acidified and protected from light. It may be protected from bacterial attack by benzene or by sterilization.

[b] 2 ml. of freshly prepared orange juice added to 177 ml. of water plus 1 ml. of 10 N H_2SO_4. Heated at 20 pounds for 1 hour, cooled, neutralized to pH 5.2, and diluted to 200-ml. volume.

[c] 2 ml. of whole milk treated as in [b].

[d] 20 ml. of urine (normal subject); 24-hour excretion diluted to 2000 ml. volume, treated as in [b] except 159 ml. of water added.

[e] 100 mg. of dry soluble yeast extract dissolved in 179 ml. of water and treated as in [b] except final dilution to 1000-ml. volume.

[f] 100 mg. of liver concentrate powder 1 to 20 suspended in 179 ml. of water and treated as in [b].

[g] 100 mg. of dry 200 B yeast suspended in 179 ml. of water and treated as in [b].

cooled, and inoculated with 1 ml. each of the yeast inoculum. The tubes are then shaken at 30° for 16 to 18 hours and the yeast growth is estimated in the densitometer. A reference curve, which consists of a series of tubes containing 0, 5, 10, 15, 20, 30, and 40 millimicrograms of pyridoxine, is included in each assay run.

The basal medium contains the following ingredients for each 5 ml.: sugar and salts solution, 2.5 ml.; potassium citrate buffer, 0.5 ml.; casein hydrolyzate, 0.5 ml.; thiamine solution, 0.25 ml.; inositol solution, 0.25 ml.; biotin solution, 0.10 ml.; calcium pantothenate solution, 0.125 ml. For a set of 40 tubes, for example, forty times the above amounts are measured into a mixing cylinder and diluted to 200-ml. volume.

A typical assay run is given in Table III, which is the actual protocol of an assay in which representative materials were assayed. The results of the reference series (Figure 1) in per cent absorption are plotted on ordinary graph paper against millimicrograms of pyridoxine and the values for the unknowns obtained by interpolation. It is desirable to make readings at both 16 and 18 hours and to average all results except obvious slips, to obtain the estimated values. All results are reported on the basis of pyridoxine hydrochloride (Merck) employed as a reference standard.

TABLE IV. RECOVERY OF PYRIDOXINE

Substance		Pyridoxine Content (by Assay)	Pyridoxine Added	Total Pyridoxine (by Assay)	Recovery
	Ml.	γ/l.	γ	γ/l.	%
Urine					
A	1000	60	100	153	93
B	1000	60	100	150	90
C	1000	159	100	283	124
D	1000	187	100	284	97
	Gram	γ/g.		γ/g.	
Yeast extract					
A	1	132	100	233	101
B	1	113	100	203	90
C	1	48	100	165	117
Dry yeast					
A	1	42	50	94	104
B	1	40	50	101	122
				Av.	104

Results

The number of pyridoxine assays by other methods which have been published is inadequate to provide a basis for critical comparison with the results of the yeast microbiological assay method. Table II contains the estimations made on a series of representative substances, compared with estimations which have been reported in the literature, obtained principally by the animal growth method of Conger and Elvehjem (*2*). The chemical method of Swaminathan (*6*) has provided a few results, but the chemical method of Scudi *et al.* (*5*) is apparently not sensitive enough for use with samples of ordinary potency.

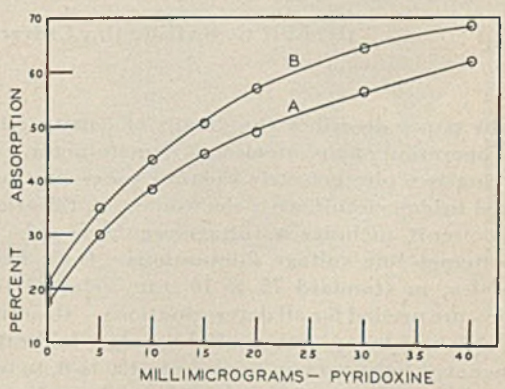

FIGURE 1. REFERENCE CURVE

A, 16 hours; *B*, 18 hours

The values obtained on fresh meat samples agree with the results of Waisman and Elvehjem (*3*). The Texas workers (*9*) report very low values for meats. In a more recent publication (*10*) the Texas group report assays on a number of foods, but apparently they have not changed their method of assay and the results are still very low.

The authors' values for fresh milk, about 0.5 microgram per gram, are significantly lower than the two values which have so far been reported. Stronger acid digestion and

clarase digestion both failed to give higher results. The analysis of dry skim milk is consistent with the fresh milk assays—i. e., about 11 times higher. The wheat assays are satisfactory except for the patent flour, which is lower than reported.

The assay of urine gave 135 micrograms per day as the average 24-hour excretion for 10 days by three normal male adult subjects. Ingestion of 5.0 mg. of pyridoxine was followed by an excess output of about 0.1 mg. during the next 24 hours. This is a rather low figure, but it may be estimated with reasonable precision and its smallness alone does not detract from its clinical significance.

The success of recovery experiments does not of itself establish the validity of an assay method, but it is a favorable sign. Table IV gives the results obtained when pyridoxine hydrochloride was added to a series of urines, yeast extracts, and dry yeasts. Each mixture was carried through the complete procedure. The recovery was calculated on the basis of the pyridoxine added and the average of 9 determinations was 104 per cent.

With regard to the specificity of the yeast microbiological method for pyridoxine determination, the following arguments can be adduced in favor of a specific response: The estimated values for the various extracts which have been tested show no perceptible change at different testing levels —i. e., no drift; no change is observed if the test is extended from 16 to 18 hours; acid digestion and enzymic digestion produce essentially the same extraction of the active principle; with a few exceptions the yeast method gives results which agree reasonably well with results obtained by the rat growth method; and added pyridoxine may be recovered from urine, yeast, and yeast extracts without appreciable loss or gain.

Summary

A microbiological method for the determination of pyridoxine employs a yeast strain (No. 4228) characterized by a specific response to pyridoxine. The yeast is grown in test tubes which are shaken at 30° for 16 to 18 hours. Yeast growth is estimated with the aid of a photoelectric colorimeter.

Recovery experiments are described and the pyridoxine content of a series of representative foods and other substances is reported.

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A Null-Reading Photoelectric Microdensitometer

For Use in Turbidimetry and Abridged Spectrophotometry

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The paper describes the details of construction and operation of a photoelectric densitometer, employing two photoelectric vacuum tubes in a balanced bridge circuit with electronic amplification. The circuit includes a voltage-regulator tube to counteract line voltage fluctuations. Only 1-ml. samples, in standard 75 × 10 mm. selected test tubes, are needed for all determinations. Readings are obtained by adjusting a light wedge, calibrated in per cent light transmission from 100 to 0, to balance the amount of light absorbed by the unknown over the control. The electronic circuit, operating on the null-reading principle, is used only to indicate the balance point. Once a calibration curve is obtained for any substance it can always be used for estimating the unknown sample, since the light wedge cannot change its calibration. That the data follow the Bouguer-Beer law can be seen from the linearity of the graphs obtained from a series of dilutions of India ink and copper sulfate, using the proper color filters. The instrument will be used in the measurement of the degree of turbidity resulting from mixtures of antigen and antibody in studies of precipitins.

IN ALL branches of biological research there is an urgent need for better methods of measurement, methods of greater speed, greater sensitivity, and greater reliability. To that end this paper is submitted, giving the details of construction, operation, and performance data of a new instrument for the measurement of colored and turbid solutions—viz., a photodensitometer, employing two photoelectric vacuum tubes in a balanced bridge circuit, with electronic amplification, in a stabilized circuit known to have great sensitivity and reliability.

This instrument is the culmination of a year's design, building, and testing by the writer in an attempt to find a better and more rapid method of measuring the turbidity of solutions. The writer (*2*) has been interested in a phase of serology, on the study of animal relationships, where the technique employed (*1*, *4*) involved measuring the amount of precipitate formed in the precipitin reaction between the interactions of antisera with homologous and heterologous antigens. The precipitate so obtained in the reaction has previously been measured by centrifugation at a known rate of speed for a fixed time in calibrated capillary tubes. While the results obtained have been statistically reliable, the time involved in their measurement has been a severe drawback. To this end it was determined to attempt to measure the

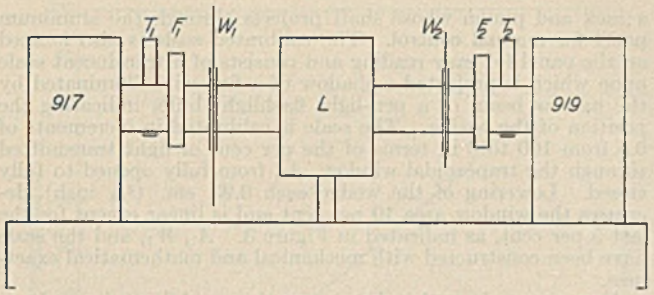

FIGURE 1. BASIC MECHANICAL ARRANGEMENT OF INSTRUMENT

T_1, T_2. Holders for test tubes containing samples to be compared. F_1, F_2. Filter compartments. W_1. Calibrated light wedge. W_2. Compensating light wedge for initial null setting. *L*. Ventilated light compartment for illuminating photocells found in compartments 917 and 919

amount of precipitate in suspension by means of photometry on the basis of the Bouguer-Beer law, applied to turbid systems (*8*). While the instrument was designed for this one specific purpose, it can be applied to many related problems as well as to the measurement of the density of colored solutions by the use of the proper color filters.

Instrument Types

Since the first report (*17*) of the application of the photoelectric principle to nephelometry and densitometry, many papers describing a number of instruments have appeared. These instruments fall into five general classes.

1. Many instruments (*6*, *7*, *10*, *18*, *19*) employ a sensitive galvanometer to measure directly the current output from a single self-generating barrier layer (photovoltaic) cell under variable conditions of light transmission. The difficulties are numerous. (*A*) Very sensitive and hence very delicate suspension galvanometers must be employed in the better instruments to measure the minute currents of the photovoltaic cell. (*B*) Such small currents can be amplified only with difficulty so that it is not practical to use an amplifier circuit in order to permit use of more rugged, less sensitive, and hence less delicate galvanometers. (*C*) The light source must be very constant; the ordinary 110-volt alternating current line supply is hardly adequate and the better instruments must therefore employ storage batteries. (The alternating current line voltage in the author's laboratory fluctuates steadily between 117 and 122 volts with frequent excursions to 115 and 125 volts. Even the best voltage-compensating transformers cannot maintain the necessary constant voltage required for illumination of the single photoelectric cell instruments, *16*.) This makes the instrument nonportable as well as requiring attention, frequent charging, and eventual replacement of the battery. (*D*) Individual photoelectric cells, while stated to be similar, will vary sufficiently to require new calibration curves in the event of their replacement. (*E*) Deterioration of photoelectric cells will require that calibration curves be constantly checked for reliability of the data.

2. A few instruments (*17*, *21*) have been constructed which use a single photoelectric vacuum tube and a direct reading galvanometer. The difficulties listed above still apply to these vacuum tube instruments.

3. Some instruments (*5*, *13*, *15*, *20*) employ a single photoelectric vacuum tube with an electronic amplifier system where essentially the photoelectric cell electronic emission is amplified so that sensitivity can be increased and more rugged meters (usually a milliammeter) employed to measure the current output. However, objections *C*, *D*, and *E* still apply to these vacuum tube instruments.

4. Some instruments (*3*, *9*, *23*) employ two photovoltaic cells in a bridge circuit using a galvanometer to determine the null point and a calibrated potentiometer for obtaining readings in a linear scale of the percentage of light transmitted through the solution on a logarithmic scale for a direct reading of density. While these instruments compensate for line voltage fluctuation and photocell deterioration, they are limited in their sensitivity by the circuit employed and by the galvanometer sensitivity.

5. Other instruments (*11*, *22*) use photoelectric vacuum tubes in a bridge circuit with methods of reading the transmission of light or the density as in the previous type. These instruments can use an electronic amplifier system (*12*, *14*) to increase the sensitivity to a degree limited only by the amplifier system employed. The instrument described in the present paper is of this type, employing a stabilized amplifier system.

The instruments described in this brief review by no means cover the entire field, but are mentioned only to describe the essential types used by various workers to study many problems: analytical chemistry, routine clinical analyses, spectrophotometry, etc. A very complete list of contributors to this field is found in the literature (*8*, *14*).

Basic Design of the Instrument[1]

The instrument described in the present paper attempts to improve further upon the use of photoelectric cells in turbidimetry and abridged spectrophotometry.

It employs two RCA photoelectric vacuum tubes in a "null" circuit with one stage of vacuum tube amplification. Since both photocells are illuminated by the same light source, changes in its intensity will have the same effect on both photoelectric cells, but no effect on the null reading. The amplifier circuit, as well as the photoelectric cells, is voltage-regulated by means of a VR 105–30 voltage regulator tube, so that changes in line voltage have a negligible effect upon the circuit. All vacuum tubes are working at minimum potential for long life and stability. Inherent in the design of the instrument is the principle that a very small change in the light intensity falling on one photoelectric cell over that falling upon the other cell, after they had been previously adjusted for the null point, causes a change in the electronic emission of one photoelectric cell over that of the other, so that the grid of the electronic amplifier

[1] Since this paper was accepted for publication improvements in design include (1) an interchangeable condensing lens system to increase sensitivity when using certain filter combinations, (2) the addition of RCA 926 photoelectric vacuum tubes, with greater sensitivity in the blue region of the visible spectrum, and (3) adaptations for use of square test tubes of precision manufacture (6 × 6 mm. in inside diameter and 75 mm. in length).

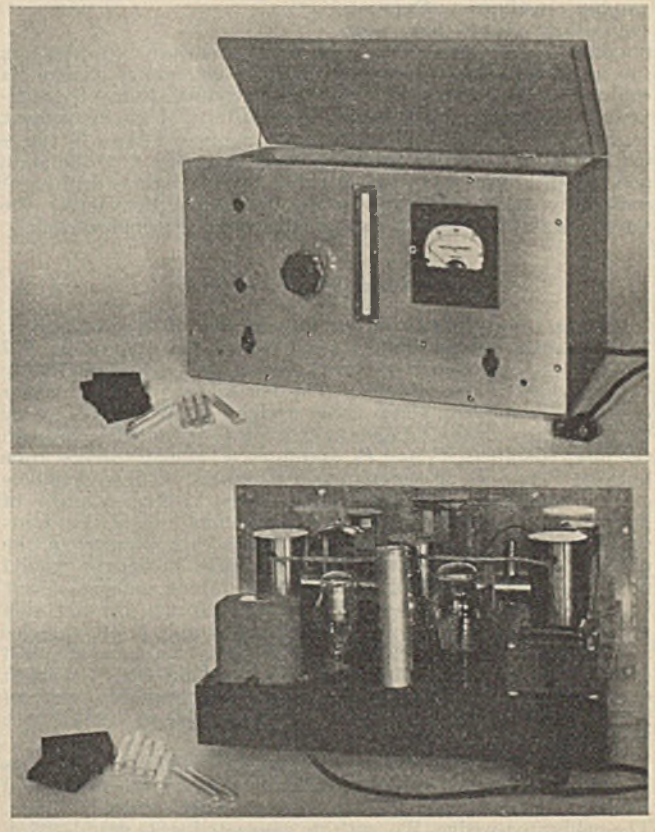

FIGURE 2. PHOTOGRAPH OF DENSITOMETER

Above. Cover open ready for use
Below. Instrument as seen from back with cabinet removed

becomes either more or less negative than it was when at the null point. This in turn causes the microammeter in the plate circuit to register either zero or full-scale deflection. A light wedge is then adjusted to cause the meter to register the null reading again, bringing the photoelectric cells back to the same degrees of illumination and electron emission as originally. The amount of movement of the wedge required to bring the instrument back to the null point is a measure of the amount of light absorbed by the unknown sample. The instrument is thus adjusted to an arbitrary null point, for each reading, by regulating the light which falls upon the two photoelectric cells.

Two similar sample tubes when placed successively in the path of light going to one photoelectric cell will not cause a change in the null point, while if one of the tubes is replaced by an unknown tube of differing density, less light will pass through to fall on the one photoelectric tube and the calibrated light wedge will have to be adjusted to return the instrument to the null reading. Graphs are plotted, on semilogarithmic paper, of the wedge readings obtained from solutions of known concentration, and the unknown sample concentrations are obtained by reference to the known graphic readings. Once a calibration curve has been obtained for any substance, it can always be used for estimating the unknown sample, since the light wedge cannot change its calibration, as is possible using direct readings from photocell-meter instruments. The degree of sensitivity of the instrument to minute differences in light intensity can be regulated by varying the voltages applied to the photoelectric cells, or to the plate and screen of the electronic amplifier tube. Various degrees of sensitivity are incorporated in the instrument by varying the screen voltage with preset resistor clips and a multipole switch.

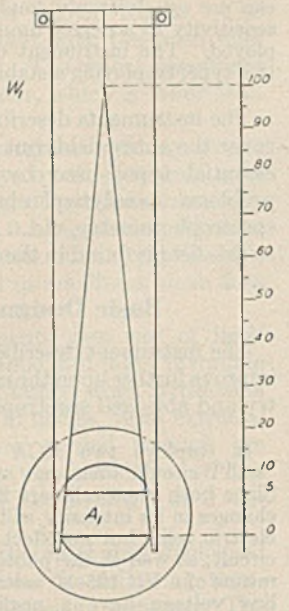

FIGURE 3. DIAGRAMMATIC VIEW OF LIGHT-DECREASING WEDGE AND ASSOCIATED CALIBRATED SCALE

A_1. Trapezoidal window area admitting light to filter, sample, and then to photocell 917. Area of trapezoid = $\frac{1}{2}(a + b)\,h$ where base, a, = 21 x, top, b, = 19 x and altitude, h, = 10 x. 10 x = $\frac{3}{8}$ inch. Lowering of wedge from scale reading of 100 to 90 decreases light window area 20 square units, or 10 per cent of total, etc. Window, wedge, and scale are constructed with precision.

Mechanical Design

Figures 1 and 2 illustrate the basic design of the instrument. The housings for the lamp, L, and for the two photocells, 917 and 919, are constructed of brass sheeting and tubing and rigidly supported on a steel chassis. The horizontal tubes of smaller diameter, connecting these three upright compartments, carry the light through the tapered light wedges, W_1 and W_2, through the filter chambers, F_1 and F_2, and in turn through the test tube holders, T_1 and T_2, to the photocell chambers. These chambers are all blackened inside and light-tight. Diffusion disks of flashed opal glass are used for even illumination of the light-adjusting wedges and so that the entire cathode area of the photocells will be utilized. The test tube holders of round brass tubing are of suitable size for 10 × 75 mm. serological test tubes and have milled vertical slots 3 by 10 mm. on each side for the transmission of light through the sample (1-ml. samples are sufficient for all analyses). The filter chambers are 0.93 cm. ($\frac{3}{8}$ inch) in width to allow for several 3.1-cm. ($1\frac{1}{4}$-inch) filter circles in combination for limiting the frequency range of light transmission. The filters are assembled in hard-rubber holders for easy replacement.

W_2 is the light-compensating wedge for balancing differences in light transmission of different filters and for the initial null setting. It is adjusted by means of a vertical screw and cam. The calibrated wedge, W_1, with its associated scale graduated in per cent of light transmission is illustrated diagrammatically from an end view in Figure 3. It is similar to W_2 except for its precision manufacture. This wedge is raised and lowered with a rack and pinion whose shaft projects through the aluminum panel for manual control. The calibrated scale is also located on the panel for easy reading and consists of a translucent scale upon which is projected a shadow of a fine wire illuminated by the narrow beam of a pen-light flashlight bulb, indicating the position of the wedge. The scale is calibrated in increments of 0.5 from 100 to 0 in terms of the per cent of light transmitted through the trapezoidal window, A_1, from fully opened to fully closed. Lowering of the wedge each 0.93 cm. ($\frac{3}{8}$ inch) decreases the window area 10 per cent and is linear except for the last 5 per cent, as indicated in Figure 3. A_1, W_1, and the scale have been constructed with mechanical and mathematical exactness.

Also mounted on the chassis are the amplifier tube, voltage regulator tube, rectifier tube, filter condenser, choke coil, and transformers. Under the chassis are the resistors, with all the wiring thus concealed from view. All critical circuits of the photoelectric cells and amplifier tube are shielded to eliminate any stray capacitance effect by the operator.

Photoelectric Vacuum Tube and Amplifier Circuit

The circuit used in employing the two photoelectric vacuum tubes with their amplifier is shown in Figure 4. The rectifier and filter circuit supplies the high voltage to the voltage divider, R_1 R_2 R_3. The voltage across the resistor, R_2 R_3, is maintained at 105 volts by the voltage regulator tube, VR 105-30. The suppressor grid of the 38 amplifier tube and the cathode are grounded. The resistor, R_3, is below ground potential, so that the cathode of photoelectric cell 917 is adjusted for 22 volts below ground potential, while the anode of photoelectric cell 919 is operated almost an equal potential above ground. The 919 cathode and the 917 anode lead to the 38 grid and are essentially at ground potential when the photocells are equally illuminated or at zero illumination. The plate of the 38 amplifier is set at a low potential (25 volts) for stability and constancy of operation of the amplifier. The screen is set at a sufficient positive potential for the desired sensitivity and is variable in several steps (5, 10, and 15 volts).

A resistor in series with the meter and plate will prevent excessive plate current flow for the protection of the microammeter. Operating the circuit in this fashion with low plate, screen, and photoelectric cell potentials ensures stability and constancy of vacuum tube characteristics, even though extreme sensitivity is forsaken. However, the sensitivity which the instrument exhibits is much more than even the best of mechanical construction and experimental error warrant. If still greater sensitivity were to be desired and could be used, all voltages could be proportionately increased. The writer has

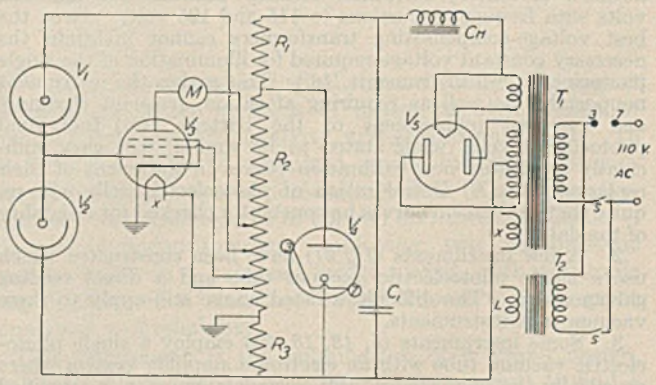

FIGURE 4. BALANCED CIRCUIT WITH HIGH DIFFERENTIAL SENSITIVITY AND STABILIZED HIGH-VOLTAGE SOURCE

V_1 = RCA 919; V_2 = RCA 917; V_3 = 38; V_4 = VR 105-30; V_5 = 80.

R_1 = 7000 ohms, 25 watts; R_2 = 7500 ohms, 75 watts; R_3 = 2000 ohms, 25 watts.

M = 0–200 microammeter, Weston, Model 301; C_1 = 16-mfd. electrolytic condenser (negative terminal insulated from ground); T_1 = power transformer (Thordarson T13R11).

Filament winding to 38 amplifier reduced from 6.3 to 4.0 volts by series resistor; T_2 = 115-5V step-down transformer (U. T. C. S-54) for photocell illuminating lamp; S = switches inserted in primaries of both transformers T_1 and T_2.

Contact pins 3 and 7 of VR 105-30 are inserted in A. C. power input lead as a safety measure; Ch = choke (Thordarson T68Co8).

Power supply indicating pilot light and dial illuminating lamp with appropriate resistors are not shown. Microammeter is protected by 150,000-ohm series resistor to limit current flow to slightly less than full scale. Heater of 38 amplifier is tied back approximately 9 volts above ground.

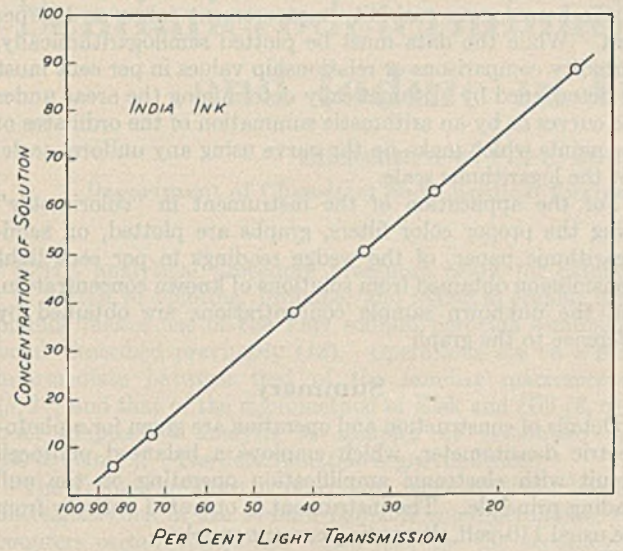

FIGURE 5. INDIA INK CALIBRATION CURVE

Concentrations of samples tested are in terms of per cent concentration of arbitrary India ink standard dilution (sample 2) taken as 100 per cent.

experimented with possible variations and has found that the instrument has much more sensitivity to differences in light intensity upon the two photoelectric cells than is needed.

The electrical circuit functions on the principle that if more light falls on one photoelectric cell (917) than on the other (919), after the circuit has previously been balanced for an arbitrary null point, the microammeter will deflect to zero, indicating that the grid of the 38 amplifier tube has become temporarily more negative as a result of greater electron flow from the cathode to the anode of the 917 photocell. To return the circuit to balance at the null point, wedge W_1 must be adjusted to reduce the amount of light falling upon photocell 917. The amount of movement of wedge W_1 indicates by its calibrated scale the area of the window which was removed to cause the proper decrease in light transmission to the 917 to compensate for the amount of light absorbed by the unknown solution, over the control, when placed in the path of light going to photoelectric cell 919.

Operation of the Instrument

The light from L (Figure 1) is allowed to fall upon both photoelectric cells (917 and 919). In the light path to each of these photoelectric cells is placed one of the two identical control samples, T_1 and T_2 (usually distilled water). With the calibrated wedge, W_1, set at 100 for full light transmission, wedge W_2 is adjusted so as to cause the microammeter to register the arbitrary null reading (100 microamperes). Then sample T_2 is removed and a solution of some substance of unknown concentration is inserted in its place. If more light is absorbed by this unknown tube (less transmitted) than was absorbed in sample tube T_2, W_1 is moved down so as to decrease the amount of light passing through T_1 until the same arbitrary null reading is again obtained.

The reading from the calibrated scale of W_1 is taken as showing the amount of light transmitted by the unknown tube compared with the control sample. Its concentration is obtained by referring to the graph obtained previously by plotting the light transmission of a series of tubes of increasing but known concentrations of the same substance prepared in the same way. Readings are made only on the basis of the amount of movement of the wedge required to compensate for the amount of light absorbed by the unknown sample tube over the amount absorbed by the standard comparison tube, which is a fixed or constant amount for all densitometric studies. The two photoelectric tubes in the null circuit are used only to determine to what position W_1 must be moved to again obtain a balance in the circuit.

Since the mechanical setup of the apparatus is stable under all conditions, the instrument does not depend upon the photoelectric cells for anything but an indication of the light balance point for the null reading. In fact, even though the photoelectric vacuum tubes are replaced by tubes of different sensitivities, the instrument can be adjusted for the same null reading by mechanically adjusting the null setting wedge, W_2. Replacement of the amplifier, voltage regulator, and rectifier tubes causes but minute and insignificant deflections in the null point of the instrument, which can easily be reset.

TABLE I. RELIABILITY OF INSTRUMENT

[In being able to duplicate data using a dilute India ink solution (sample 1) as a standard of 100% concentration]

Concn. of Sample	Light Transmission						
	7-1-42	7-1-42	7-1-42	7-1-42	7-1-42	7-2-42	7-7-42
% of standard	%	%	%	%	%	%	%
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	96.0	96.1	96.3	96.1	96.2	96.0	96.0
2	91.7	91.7	92.0	91.7	91.7	91.6	91.4
5	80.7	80.4	80.4	80.5	80.5	79.6	80.4
10	65.0	65.3	65.3	65.3	65.0	64.2	65.1
25	38.2	38.5	38.4	38.6	38.4	38.0	38.5
50	17.0	17.3	17.3	17.3	17.2	17.4	17.2
75	7.3	7.5	7.6	7.5	7.5	7.0	7.4
100	3.1	3.5	3.4	3.5	3.4	3.2	3.4

Performance Data

For testing the reliability of the instrument in turbidity studies, readings were made using various dilutions of an arbitrary India ink standard (sample 1). The data obtained without the use of filters are illustrated in Table I and show the reliability of the instrument in being able to duplicate the readings over a period of days. The results are very consistent, even though from day to day differences are to be expected in line voltage fluctuations and in duration of use of the instrument. Figure 5 illustrates the data obtained in a similar study from a different India ink standard (sample 2) but using essentially monochromatic light by means of Corning No. 396 and Wratten No. 74 filters. That the readings follow the Bouguer-Beer law is shown by the linearity of the data as plotted.

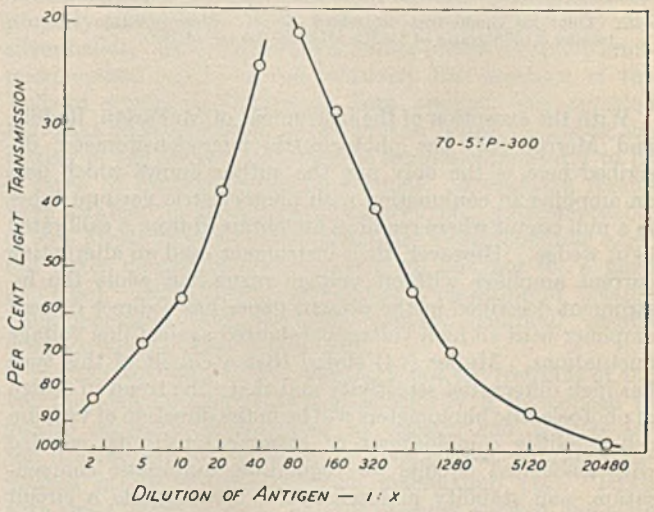

FIGURE 6. DEGREE OF TURBIDITY

Expressed in per cent light transmission resulting from 0.5 ml. of anti-pig rabbit serum reacted against 0.5 ml. of increasing dilutions (decreasing concentrations) of its homologous pig serum antigen.

Figure 6 shows the results of a serological study using a constant dilution of anti-pig rabbit serum (70-5) reacted against serial dilutions of its homologous pig serum antigen (P-300). The graphical data illustrated here are typical and correspond with the volumetric studies of Baier (*1*). They also illustrate the application of the instrument to studies of serological relationship (*2*).

Since the photoelectric cells employed in the instrument are very sensitive in the red and infrared (greatest sensitivity at 800 millimicrons), readings were obtained against dilutions

of a standard solution of copper sulfate, which contained approximately 6 per cent (6.1336 grams of cupric sulfate pentahydrate per 100 ml. of water) of copper sulfate. Wratten No. 70 filters were used in obtaining the data illustrated in Figure 7.

Discussion

The need of a reliable and rapid method for the determination of turbidity in 1-ml. samples led to the development of the present instrument. The various instruments available were unsatisfactory, chiefly because they required larger samples for analysis. At the same time it was obvious that storage and B-batteries are bulky and troublesome, so that there was a need for a circuit incorporating voltage regulation in order to use the usual 110-volt alternating current source. Furthermore, individual photoelectric cells vary in sensitivity and linearity, so that a bridge circuit with a mechanical mechanism to measure per cent light transmission would minimize these possible sources of error.

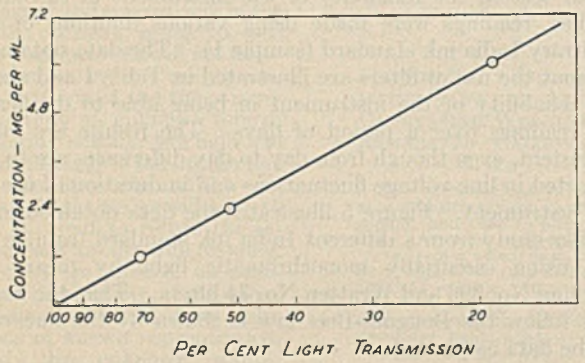

FIGURE 7. CALIBRATION CURVE FOR COPPER SULFATE

Dilutions made from standard copper sulfate solution containing 6.1336 grams of $CuSO_4.5H_2O$ in 100 ml. of H_2O.

With the exception of the instrument of McFarlan, Reddie, and Merrill (*12*) the photoelectric microdensitometer described here is the only one the author knows which uses an amplifier in conjunction with photoelectric vacuum tubes in a null circuit where readings are obtained from a calibrated light wedge. However, their instrument used an alternating current amplifier without voltage regulation while the instrument described in the present paper has a direct current amplifier with all high voltages stabilized against line voltage fluctuations. Müller (*14*) stated that a circuit of this type has high differential sensitivity and that "the trend in design of photoelectric photometers will be in the direction of vacuum cell–amplifier combinations of extreme sensitivity coupled with associated circuits for regulation, automatic compensation, and stability maintenance." He suggests a circuit having such possibilities, very much like the one utilized in the present instrument.

From the graph and table presented for India ink it can be seen that the data essentially illustrate the author's contention that the instrument shows great sensitivity and reliability. Within the limits studied in the dilutions the graphs are straight lines, following closely the theoretical aspects of the Bouguer-Beer law, with an inverse logarithmic relationship between light transmission and concentration.

Since in turbidity studies this linearity is shown between concentration and per cent light transmission, the instrument will be used in determining the degree of turbidity resulting from the reaction of an antiserum against varying dilutions of homologous and heterologous antigens. Heterologous turbidity measurements will be expressed in terms of per cent of the homologous turbidity measurement taken as 100 per cent. While the data must be plotted semilogarithmically, turbidity comparisons or relationship values in per cent must be determined by arithmetically determining the areas under the curves or by an arithmetic summation of the ordinates of the points which make up the curve using any uniform scale, not the logarithmic scale.

For the application of the instrument in "colorimetry" using the proper color filters, graphs are plotted, on semilogarithmic paper, of the wedge readings in per cent light transmission obtained from solutions of known concentration, and the unknown sample concentrations are obtained by reference to the graph.

Summary

Details of construction and operation are given for a photoelectric densitometer, which employs a balanced photocell circuit with electronic amplification operating on the null reading principle. The instrument is operated entirely from the usual 110-volt alternating current supply.

The apparatus includes a regulated high-voltage source unaffected by variation in the usual line voltage.

Readings in terms of per cent transmission of light are obtained by means of a calibrated wedge decreasing the size of a light-transmitting window.

The photocells are used only to indicate balance in the circuit, not to indicate per cent light transmission. Only 1 ml. of a sample is needed and selected 10 × 75 mm. serological test tubes are used.

Data are given to show its use as a turbidimeter and abridged spectrophotometer.

Acknowledgment

The writer wishes to acknowledge with appreciation the invaluable aid in the circuit design and the many helpful suggestions freely given by Hans U. Hjermstad, vice president of the Federal Electric Company of Chicago. He is also indebted to the staff of the Physics Department of the University of Wisconsin in Milwaukee for their many suggestions and for the use of the machine shop facilities.

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Semimicrodetermination of Chlorine, Bromine, and Iodine in Organic Compounds

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THE analytical procedure presented below for determination of chlorine, bromine, and iodine in organic compounds makes use of the Parr sodium peroxide semimicrobomb described previously (*12*). Operations are on a scale intermediate between that of the familiar macromethod (*8, 11*) and that of the micromethod of Elek and Hill (*2, 6*)—i. e., adapted to analysis of samples of semimicro size (20 to 50 mg.). The determination is gravimetric.

The Volhard volumetric procedure, when applied following decompositions in the sodium peroxide semimicrobomb, encounters certain difficulties which decrease the accuracy of this usually excellent method. The conditions of volume and salt concentration make necessary a solution of thiocyanate not weaker than 0.02 *N* if end points are to be satisfactory. With thiocyanate solution of this strength, and with semimicrosamples, the effective titrations are relatively small (especially with bromine and iodine), and the reproducibility of results is thus impaired. The alternative of increasing the samples considerably, so as to obtain larger effective titrations, was judged to be undesirable, as it would involve some danger of approaching or exceeding the capacity of the oxidizing charge, and would increase the consumption of sample material in some cases to that of the macromethod.

Direct titration of halide ion by silver nitrate with the aid of an adsorption indicator—e. g., dichlorofluorescein (*7, 10*)—was not submitted to trial, as the relatively high concentration of electrolytes unavoidably present constitutes a possible interference (*7*; cf. *1*).

Following decompositions of samples in the sodium peroxide bomb bromine is present partly as bromate [Beamish (*2*) reported bromate to be formed in a steel bomb but not in a nickel bomb], and iodine is present almost wholly as iodate. When a volumetric determination is intended it is ordinarily satisfactory to add an excess of standard silver nitrate solution to the alkaline extract, then to acidify, add hydrazine sulfate to reduce bromate or iodate, and finally to titrate excess of silver ion (*8*). When a gravimetric determination of halogen is intended it is necessary to filter the liquid before addition of silver nitrate, as the alkaline extract of the bomb contents contains undissolved particles. It is permissible to filter the alkaline extract through asbestos in a Gooch crucible, provided any dark residue on the filler is extracted with dilute nitric acid. Such residue may consist of, or contain, some metallic compound contributed by the bomb itself, and in one series of trials (see results for *o*-iodobenzoic acid in Table I) indications were clear that the residue contained part of the halogen.

In the gravimetric determination of chlorine a preliminary filtration of the alkaline extract is unnecessary, as the absence of chlorate makes it possible to acidify the solution, filter, and then add silver nitrate. In the gravimetric determination of bromine or iodine it is essential to add either the silver nitrate or the reducing agent before the solution is acidified. The addition of silver nitrate to the filtered alkaline solution, as in the volumetric procedure (*8*) led to irregularity in the determination of bromine, though it was satisfactory in the determination of iodine. Results for bromine were too high, and the precipitate was discolored after acidification of the liquid, suggesting contamination of the precipitate; the use of nitric acid in excess only partially eliminated this effect. In the determination of iodine no similar interference was observed, but in this case the initial precipitate of silver iodide was small, most of the iodine (present as iodate) being precipitated later from acid solution, so that contamination of the precipitate in the alkaline solution was slight. In order to have available if possible a single procedure useful for the gravimetric determination of both bromine and iodine, the feasibility of reducing bromate and iodate in the alkaline liquid was investigated. It was found that the small amount of bromate usually present is readily reduced by hydrazine in the hot alkaline solution, and that the relatively larger amount of iodate is completely reduced if the heating period is somewhat extended. To complete the analysis the solution is acidified and filtered, and the halide is precipitated by silver nitrate.

The use of glass ampoules for liquid samples introduces difficulties which are not readily overcome, and which are absent if gelatin capsules are used. In the sodium peroxide bomb a glass ampoule undergoes a silica fusion of its thinner parts (*13*), and later acidification of the liquid may cause precipitation of gelatinous silicic acid which presents an obstruction to the convenient gravimetric determination of halide. An earlier study of this difficulty (*17*), as it affects the macroprocedure, showed that satisfactory volumetric determination of halogen is possible by the Volhard titration properly executed (*9, 15*) in presence of the silicic acid and silver halide. Attempts to use a similar procedure in the semimicromethod confirmed the relatively low precision of the Volhard semimicrotitration as here applied, and revealed also that glass ampoules, if not broken before or during the mixing of the charge, may protect samples sufficiently to be a cause of incomplete decompositions. Acceptable results were obtained for chlorine in substances readily decomposable or not highly volatile (*o*-chloroaniline, *o*-chlorobenzoic acid; the latter decomposed in presence of, but not in, a glass ampoule). With substances relatively resistant or volatile (chlorobenzene, bromobenzene, *n*-butyl chloride) results were variable and not satisfactory.

It was found that liquid samples, if not too resistant or too volatile, can be decomposed satisfactorily if introduced in gelatin capsules, as suggested by Pflaum and Wenzke for the decomposition of fuming or deliquescent fluorine compounds in the sodium peroxide macrobomb (*14*). Trials with small (5 × 15 mm.) gelatin capsules showed complete destruction of the capsules, no increase in the blanks, and satisfactory gravimetric results. The use of gelatin capsules apparently introduces no complication; the analysis may be completed as for solid substances. Results by this procedure, which is the one recommended for liquid substances, are given in Table I.

The applicability of the sodium peroxide bomb method, on any scale, to the analysis of liquids appears to be limited. A considerable experience with the macro- and semimicromethods in this laboratory does not encourage great confidence in the certainty of complete decompositions of liquids which are resistant to decomposition or even moderately volatile, if samples are introduced enclosed in vessels (ampoules or capsules) which may afford a certain amount of

initial protection. Volatile liquids may be handled with reduced danger of loss, and may be decomposable with increased certainty, if a little anhydrous alcohol (or other solvent which is halogen-free and not too volatile or resistant) is introduced into the gelatin capsule before the sample is added and weighed (*16*). Light or readily electrified solids may not be properly decomposable in the sodium peroxide bomb. Specimens of halogenated fluoresceins, which may be very susceptible to frictional electrification, could not be satisfactorily analyzed by the macroprocedure. Even when an overlying layer of sodium peroxide was added to the previously well mixed charge visible quantities of undecomposed sample were present on the internal surface of the cover after decomposition, the alkaline extract showed fluorescence, and the results were variable and too low.

Procedure for Gravimetric Semimicrodetermination of Chlorine, Bromine, and Iodine

The Parr semimicrobomb required was described in a previous paper (*12*). Gooch crucibles, of 10-ml. capacity, should be prepared for use in a manner analogous to that described in the procedure for determination of sulfur (*12*), and should be brought to "constant" weight (within 0.03 mg.). Gelatin capsules, required for liquid samples, can be secured from drug supply houses. Those used were approximately 5 mm. in diameter and 15 mm. in length, and weighed about 0.04 gram.

Solid Substances. Transfer to the bomb cup 0.2 gram of powdered potassium nitrate (cf. *3*). Introduce the finely powdered (cf. *3*) sample, which should be of size to yield about 20 to 50 mg. of silver halide, and should be weighed with an accuracy of 0.02 mg. Add powdered sucrose (starch, lactose, benzoic acid) in such amount that the total weight of sample and other carbonaceous material is 0.2 gram. Weigh rapidly 4 grams (±0.1 gram) of granular sodium peroxide (low in chlorine) and transfer it to the cup. A satisfactory procedure, especially in humid weather, is to support the bomb cup on the left-hand pan of a trip scale, place a small beaker on the right-hand pan, and introduce water to counterpoise the bomb cup, into which the sodium peroxide may then be weighed directly and rapidly. A measuring cup of suitable capacity would be convenient and sufficiently accurate. Make certain that the gasket is in good condition, and then adjust the bomb cover in place and secure it firmly by tightening the screw collars. To mix the charge, shake the apparatus vigorously, and finally tap the bottom of the cup against the table top to settle the charge.

Liquid Substances. Transfer to the bomb cup 0.2 gram of powdered potassium nitrate, 0.16 gram of sucrose, and 4.0 grams of granular sodium peroxide. Fasten the cover in place, and thoroughly mix the charge by shaking. Weigh the sample (to yield 20 to 50 mg. of silver halide) in a gelatin capsule. Remove the cover of the sodium peroxide bomb, and thrust the capsule and the contained sample into the center of the previously mixed charge, so that the capsule is completely covered. Fasten the cover in place, but do not attempt further mixing of the bomb contents.

Decomposition. Support the apparatus in a vertical position by means of a clamp and ring stand, and at such height that the cup can be heated by the flame of a blast lamp seated on the table top. For the present, and until the explosion hazard of this apparently safe apparatus is better known, it is advisable to wear goggles and to place a safety glass screen, or an iron plate, in front of the bomb while the charge is ignited. Adjust the blast flame so that it is 7.5 cm. in length and 3 to 4 mm. in diameter. First bring the tip of the flame to a point about 1 cm. below the bomb for about 15 seconds, then move the flame so that the tip touches the bottom of the cup, and continue heating thus for 30 seconds more. A longer or more severe heating may melt the gasket.

Analysis. Cool the bomb, finally in tap water, and remove the cover. Wash the lower surface of the cover with a fine stream of hot water, collecting the washings in a 125-ml. beaker. Wash the cup externally (discard these washings) and then, with the aid of a thick glass rod, place the cup on its side in the beaker. Add water to a volume of 25 ml., cover the beaker, and warm gently to dissolve the fused mass. Using the glass rod, lift the bomb cup above the liquid and wash the cup externally with a fine stream from the wash bottle. Then grasp the cup between the fingers and wash the interior, collecting the washings with the main extract. Cover the beaker and heat the liquid until gas evolution practically ceases, indicating decomposition of

Table I. Determination of Chlorine, Bromine, and Iodine in Organic Compounds

(Following decomposition in the sodium peroxide semimicrobomb gravimetric procedure)

Compound	Sample	Silver Halide	Halogen Found	Halogen Calculated
	Mg.[a]	*Mg.*[a,b]	%	%
p-Aminophenol hydrochloride	23.78	23.36	24.30	
	24.69	24.26	24.31	
	34.38	33.46	24.08	
	38.85	38.36	24.43	
			Av. 24.28	24.36
p-Nitrochlorobenzene	30.35	27.40	22.33	
	45.59	41.18	22.39	
			Av. 22.36	22.51
o-Chlorobenzoic acid	39.94	36.31	22.49	
	33.65	30.63	22.52	
	39.96	36.03	22.30	
	24.67	22.49	22.55	
	24.65	22.41	22.49	
	23.40	21.24	22.45	
	19.57	18.09	22.87	
			Av. 22.52	22.65
Methylene-*p*-chloroaniline $(ClC_6H_4N.CH_2)_3$	16.28	16.72	25.41	
	22.04	22.31	25.04	
	23.37	23.64	25.02	
	21.64	22.00	25.15	
	28.71	29.22	25.18	
			Av. 25.16	25.40
3-*p*-Chlorophenyl-6-chloro-3, 4-dihydroquinazoline	21.42	22.11	25.54	
	24.70	25.15	25.19	
			Av. 25.32	25.59
o-Chloroaniline samples in gelatin capsules	33.78	38.00	27.83	
	35.91	40.08	27.61	
			Av. 27.72	27.79
p-Bromoaniline	48.50	53.25	46.72	
	46.66	50.81	46.34	
	37.22	40.84	46.69	
			Av. 46.58	46.47
p-Dibromobenzene	16.73	26.70	67.91	
	22.40	35.42	67.29	
	34.40	54.64	67.59	
			Av. 67.60	67.75
Methylene-bis-*p*-bromoaniline (1) Alkaline solution filtered, $AgNO_3$ added, acidified, N_2H_4 added	12.96	14.53	47.71[c]	44.89
	21.68	23.37	45.87	
	18.68	20.25	46.13	
	14.91	16.38	46.75	
(2) As in (1), but large excess of HNO_3	24.62	26.00	44.94[d]	
	27.19	28.93	45.28	...
(3) Hydrazine added to alkaline solution; recommended procedure	12.98	13.62	44.66	
	28.96	30.60	44.93	...
			Av. 44.80	...
N-(2-benzalamino-5-bromobenzyl)-4-chloroaniline	24.60	20.81	36.00	
	28.88	24.60	36.28	
	30.54	25.71	35.83	
			Av. 36.04	35.99
3-*p*-Bromophenyl-6-bromo-3,4-dihydroquinazoline	26.47	27.08	43.54	
	26.45	27.05	43.52	
			Av. 43.53	43.66
n-Butyl iodide, b. p. 130.4–131.3° corrected. Samples in gelatin capsules	21.68	27.59	68.79	
	26.48	33.69	68.77	
	25.95	33.06	68.87	
			Av. 68.81	68.98
o-Iodobenzoic acid (1) Alkaline solution filtered and black residue washed with water	28.93	23.27	43.48[e]	51.17
	43.58	36.12	44.82	
	22.56	13.44	32.20	
(2) Alkaline solution filtered and black residue dissolved in dilute HNO_3	42.10	39.75	51.04[e]	
	20.76	19.59	51.01	
			Av. 51.03	
(3) Filtered alkaline solution treated with $AgNO_3$, then acidified and N_2H_4 added	20.87	19.72	51.08	
	52.06	49.07	50.95	
			Av. 51.02[f]	
(4) Reduction by hydrazine in alkaline solution; liquid filtered, acidified, $AgNO_3$ added. Recommended procedure	24.58	23.30	51.24	
	28.12	26.73	51.38	
	23.63	22.31	51.14	
	25.99	24.43	50.81	
			Av. 51.14	

[a] Weighings made on semimicrobalance.
[b] Blanks deducted.
[c] Results by this procedure are all much too high, owing to contamination of AgBr by precipitation in alkaline solution.
[d] Excess nitric acid decreased but did not eliminate contamination of AgBr precipitated in alkaline solution.
[e] Black residue removed by filtration of alkaline extract contained iodine, which was recovered by washing with dilute nitric acid.
[f] Iodine was satisfactorily determined by this procedure, in which only the small precipitate of AgI formed in alkaline liquid is subject to contamination, most of iodine (initially present as iodate) being precipitated later after reduction by hydrazine in acid solution.

excess sodium peroxide, an operation which is shortened if some granular Alundum (grain size 16) is introduced (*5*).

If only chlorine is to be determined acidify the cooled solution by cautious addition of 10 ml. of concentrated nitric acid, which is run in slowly along a rod extending obliquely into the covered beaker. If bromine or iodine is to be determined, introduce into the hot (not boiling) alkaline liquid 1 gram of pure hydrazine sulfate, stir to dissolve the salt, and heat the solution just below boiling until the slow effervescence ceases (about an hour for reduction of iodate and a shorter time for bromate). Cool the solution, and acidify by adding 10 ml. of concentrated nitric acid in the manner outlined above. Approach the neutral point carefully, with the liquid not more than moderately warm. If a yellow or brown color appears when the solution becomes acid the reduction of bromate or iodate was probably incomplete. In this case the prompt addition of a little hydrazine sulfate may save the analysis.

Filter the acidified solution through paper, collecting the filtrate in a 250-ml. beaker. Wash the paper with hot water, collecting the washings with the main liquid. Add water if needed to bring the volume to 125 ml. Introduce slowly and with stirring 5 ml. of 1.7 per cent silver nitrate solution. Boil the liquid for some minutes to coagulate the precipitate, and allow to stand for several hours or overnight away from strong light. The analyses reported below were allowed to stand overnight.

Decant the liquid through a 10-ml. Gooch crucible, and wash the precipitate in the beaker several times with 1 to 100 nitric acid by decantation. Transfer the precipitate completely to the filter, cleaning the beaker by use of a small policeman and a fine stream of 1 to 100 nitric acid, and wash the precipitate on the filter twice with 1 to 100 nitric acid. Using very light suction, wash filter and precipitate with alcohol and then with ether. Dry the crucible and precipitate at 130–140° for an hour, transfer to a desiccator, and when the crucible has cooled to room temperature set it in the balance case. After 10 to 15 minutes weigh the crucible to 0.02 mg. Repeat the drying, etc., until it is certain that the weight is constant within 0.03 mg.

Conduct several blank analyses in an identical manner, using no sample but 0.2 gram of sucrose. The precipitate of silver chloride may be barely visible, and it must be allowed to stand overnight before filtration, or it may be incompletely retained on the filter. In the work described the best obtainable grade of sodium peroxide was used, and the blanks for two lots were as follows: (1) 0.52, 0.46, 0.53 mg., average 0.50 mg.; (2) 0.32, 0.35, 0.40 mg., average 0.36 mg. If hydrazine sulfate is used in the analysis, a like amount must be included in the blank unless the salt is entirely free of chloride.

Results obtained by the procedure described are presented in Table I, which includes also the results of experimental trials made to test several points mentioned in the introduction.

Determination of Halogen in Liquid Substances Weighed and Decomposed in Glass Ampoules

The uncertainties introduced when the sample is contained in a glass ampoule include possible protection of the sample from complete decomposition if the ampoule is not broken before or during the mixing of the charge, and the interference by the silicic acid which may separate when the alkaline extract is acidified. The presence of silicic acid excludes any convenient procedure for the accurate gravimetric determination of halogen, and it interferes with a volumetric procedure which requires filtration to remove silver halide, for the complete washing of a precipitate which contains gelatinous silicic acid may not be feasible. Trials were therefore made using the Volhard volumetric method with the filtration omitted, the back-titrations being made in the presence of the precipitate of silver halide and silicic acid and of nitrobenzene to protect the silver halide (*4*). Blanks were determined by the same procedure. The silver nitrate and thiocyanate solutions were 0.04 *N* and 0.02 *N*, respectively.

Results by this procedure were satisfactory in the cases of *o*-chlorobenzoic acid (decomposed in presence of, but not in, a glass ampoule) and *o*-chloroaniline introduced in a glass ampoule. For *o*-chlorobenzoic acid results were 22.74 and 22.64 per cent chlorine; calculated 22.65 per cent. For *o*-chloroaniline results were 27.73, 28.08, and 27.90 per cent chlorine; calculated 27.79 per cent. Analyses of *n*-butyl chloride, chlorobenzene, and bromobenzene, all introduced in glass ampoules, gave results many of which were too low. In some cases this could be attributed to incomplete decompositions. In the determination of bromine accuracy was unavoidably decreased, owing to the analytical disadvantage inherent in the small volumes of the effective titrations. It appears from the results of these trials that a procedure which involves decomposition of the sample introduced in a glass ampoule, and determination of halogen by the Volhard titration without filtration—i. e., in presence of the precipitate of silver halide and of silicic acid—is practicable, provided complete decomposition of the sample is assured, though results for bromine and iodine may be expected to be less accurate and concordant than is desirable. The use of gelatin capsules as containers for liquid, volatile, or hygroscopic substances, and the gravimetric determination of the halogens are recommended as more satisfactory procedures.

Work is now in progress on the extension of the use of the semimicrobomb for the determination of phosphorus and of arsenic in organic compounds.

Summary

A gravimetric procedure is described for the semimicrodetermination of chlorine, bromine, and iodine in organic compounds following decomposition in the Parr sodium peroxide semimicrobomb. The volumetric semimicrodetermination of halogens by the Volhard titration is not recommended, especially for bromine and iodine, as the effective titrations, using solutions of such strengths that satisfactory end points are obtained, are too small to ensure results of acceptable accuracy and consistency.

The determination of halogen in liquid samples weighed and introduced in glass ampoules may be obstructed by the protective effect of the containers, which may be the cause of incomplete decompositions, and by the precipitation of silicic acid when the alkaline liquid from the decomposition is acidified. These disadvantages are avoided by use of small gelatin capsules for liquid samples.

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Spectrographic Limit of Identification of Potassium

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IT IS well known (*6*) that spectrographic tests are of high sensitivity for elements in the first two columns of the periodic table and of low sensitivity for elements in the last few columns of the periodic table, and (*2*, *4*, *7*, *9*) that the intensities of the emission lines of any element are a function of the spectrographic and photographic techniques used, the matrix in which the element appears, the anions with which it is associated (*3*), and the intrinsic brightness of its lines.

There is general agreement among spectroscopists in this country that the direct current arc is the most satisfactory source to use in qualitative spectrographic analysis, and that when dealing with very small amounts of materials the lower electrode, which holds the sample, should be the cathode (*7*, *9*). In the case of potassium, Steadman, Hodge and Horn, (*8*) have reported that they could detect from 1 to 5 micrograms in the direct current arc, using the potassium lines 4044.1 and 4047.2 Å. Using these lines, however, the authors found that the spectrographic sensitivity of potassium varied by factors of several hundredfold and that, in general, the sensitivity was not so great as it was reported to be. They therefore investigated the spectrographic limit of detection of potassium in the direct current arc for both the sensitive (*6*) doublets:

λ	Int.		λ	Int.
4044.14	800	and	7664.91	9000
4047.20	400		7698.98	5000

the strongest lines of the element, arising from the lowest energy values. These lines will hereafter be referred to simply as the doublets at 4000 Å. and at 7600 Å. These latter lines are seldom used because specially sensitized plates are required to photograph them, and the dispersion of prism spectrographs is low in that wave-length region.

Procedure

The spectrograms were taken in the first order of a 3-meter grating spectrograph (*1*), dispersion 5.6 Å. per mm. The 4000 Å. doublet was photographed on Eastman 33 plates, the 7600 Å. doublet on Eastman Spectroscopic I-N plates. Both types of plates were processed in accordance with the manufacturer's directions. When photographing the 4000 Å. doublet the overlapping second order spectrum was absorbed by a Corning No. 738 filter placed before the slit; in the case of the 7600 Å. lines by a Wratten Cine-Red filter. An enlarged image of the arc was formed on the slit of the spectrograph with a quartz lens and was so adjusted that the image of the cathode fell just off the edge of the Hartmann slide delimiting the slit length.

The direct current arc was operated at 15 amperes (250-volt input). The voltage across the electrodes varied from 40 to 50 volts, depending on the sample being arced. The interelectrode gap was maintained manually at 3 mm. as the electrodes burned away, with the aid of an enlarged image formed on a target by an auxiliary lens. The electrodes were 0.6-cm. (0.25-inch) diameter spectrographic graphite electrodes manufactured by the National Carbon Co. In the lower (cathode) electrode a crater 5 mm. wide and 4 mm. deep was drilled by a special fixture. The upper electrode was pointed in a pencil sharpener reserved exclusively for this purpose.

For each sample a fresh pair of electrodes was introduced in the arc stand, and preburned for 40 seconds to volatilize any traces of metal that might have been introduced in preparing the electrodes. Then a 40-second spectrogram was taken to record any impurities intrinsically present in the electrodes, and the cathode was removed and cooled rapidly in a metal block. Samples containing potassium as an impurity were weighed directly into the cathode. Potassium salts were dissolved in distilled water (which had been found potassium-free) and 0.1 ml. of solution of the appropriate concentration was placed in the cathode, which was then dried at 110° C. The loaded electrode was replaced in the arc stand, the spectrograph shutter opened, the arc started, and the spectrograph plate-holder racked down every 40 seconds until the sample was completely consumed. The exposure time used was the optimum exposure dictated for the authors' spectrograph by the twin opposing factors of maximum exposure for faint lines and minimum exposure for the background to permit maximum visibility for faint lines. In general, the bulk of the potassium was volatilized during the first exposure.

TABLE I. LIMIT OF DETECTION OF POTASSIUM

K Introduced as	Limit of Detection 4044–4047	7664–7698	Column 2 / Column 3
	Micrograms		
KCl	120	0.6	200
KOH	180	0.08	2250
K_3PO_4	95	0.08	1200
$KMnO_4$	320	0.2	1600
$K_2Cr_2O_7$	240	0.8	300
$KC_2H_5SO_3$	200	0.2	1000
Na_2CO_3	10	0.04	250
$Na_2C_2O_4$	16	0.04	400
V_2O_5	40	0.04	1000
Glass[a]	20	0.06	330

[a] Bureau of Standards borosilicate glass, standard 93

Results

The limit of detection of potassium was studied for six of its salts and for four compounds containing it as a minor impurity. The observed limits of detection are listed in Table I. The first column tabulates the form in which the potassium was introduced in the arc, the second column lists the limit of detection for the 4000 Å. doublet, the third column lists the limit of detection for the 7600 Å. doublet, and the last column gives the ratio of the two limits of detection given in the preceding columns.

The figures represent the smallest amount of potassium (introduced in the form listed in column 1) which can always be detected with certainty. One half of these amounts may or may not be detected. One quarter of these amounts will usually, but not invariably, not be detected. Whenever one line of a doublet was present, the second line invariably was present.

The striking variation in the spectrographic sensitivity of potassium demonstrated in Table I can be attributed primarily to uncontrollable changes in the conditions of excitation in the arc produced by variations in the constituents being volatilized. This phenomenon is most prominently shown by the two samples whose major constituent is sodium. While the volatile sodium is coming off in the arc, very little carbon is volatilized from the electrodes and the cyanogen bands are correspondingly faint, thus allowing faint potassium lines to be seen. In general it may be said that the limit of detection of potassium at the 4000 Å. doublet is set primarily by the intensity of the heavy cyanogen bands (*8*) rather than by the faintness of the potassium lines. This point is further demonstrated by the fact that where the theoretical ratio of intensities of the 7600 Å. doublet to the 4000 Å. doublet is roughly 10 to 1, the observed ratio of the limits of detection at 4000 Å.

to the limits of detection at 7600 Å. (column 4, Table I) varies from 200 to 2200.

Summary

The spectrographic limits of detection of potassium in the direct current arc for the doublets 4044–4047 and 7664–7698 have been studied for six potassium salts and four compounds containing potassium as minor impurities. The limits of detection vary widely, depending on the form in which the sample is introduced. The limit of detection for the 7600 Å. doublet is several hundred times lower than for the 4000 Å. band, largely because the latter are masked by heavy cyanogen bands. The limit of detection of the 4000 Å. doublet varies from 10 to 300 micrograms for the materials investigated, and for the 7600 Å. doublet it ranges from 0.04 to 0.8 microgram.

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Relation of Shape to the Passage of Grains through Sieves

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NOTWITHSTANDING the widespread use of sieves for sizing sands and other particulate materials, many problems relating to the meaning and interpretation of sieving data remain unsolved or partially solved. One such problem concerns the actual diameters of grains passed or retained by sieves. Although many factors are known to influence the size of grain passed or retained, very few data have been published, either on the net effect of these factors or on their relative importance.

Recently microprojection measurements on a natural stream sand showed that from 30 to 50 per cent of the grains retained on sieves of a $\sqrt{2}$ series had intermediate diameters larger than the rated openings of the next largest sieve (*4*). Thus the effective size of the sieve openings, as measured by the intermediate grain diameters, was considerably larger than the rated size of the openings. The passage of these oversize grains through the sieves was thought to be due mainly to two factors—(1) the relative importance of the few oversize holes in the sieves, and (2) the passage of flattened grains diagonally through the sieve openings. This article presents some data on the quantitative importance of these two factors and outlines several types of investigations in which shape-calibrated sieves can be used advantageously.

Laboratory Technique

A 60-gram sample of channel sand from the Rio Grande near Los Lunas, N. M., was placed in a nest of standard wire-mesh sieves, in which the size of openings increased from fine to coarse approximately as the $\sqrt{2}$. After 14 minutes of shaking in a Rotap mechanical shaker, the sand retained on each sieve was removed and placed in a separatory funnel partially filled with acetylene tetrabromide (specific gravity 2.92). The "heavy" minerals that sank and the "light" minerals that floated were removed to separate filter papers, washed with alcohol, and dried. After removal of magnetite, the heavy mineral separates consisted dominantly of ilmenite, pyroxene, and hornblende, and smaller amounts of garnet, apatite, mica, and other minerals. The light mineral separates were composed of quartz, feldspar, calcite, and altered grains.

Parts of the light and heavy mineral separates were split out with an Otto microsplit (*2*), mounted in a three-dimensional measuring wedge (*3*), and placed on the stage of a petrographic microscope equipped with a mechanical stage and micrometer ocular. Each grain was identified and the long diameter, a, the short diameter, c, and the intermediate diameter, b_i, that controls passage of grains through sieve openings were measured. The ratio of breadth to length, b_i/a, and the flatness ratio, c/b_i, were computed for each grain. These ratios are dimensionless numbers that have a possible range from one to zero.

All grains measured in each size grade were classified according to flatness ratio (0.999–0.900, 0.899–0.800, etc.) and the average intermediate diameter was computed for each flatness class. The averages for the smaller flatness ratios were based mainly on measurements of heavy mineral grains.

These data are presented in Table I and Figure 1. For comparison, the arithmetic mean diameter of each size grade,

TABLE I. AVERAGE INTERMEDIATE DIAMETERS FOR DIFFERENT FLATNESS RATIOS

Flatness Ratio	Size Grade 0.351–0.246 mm. Grains measured	0.351–0.246 mm. Average intermediate diameter	0.246–0.175 mm. Grains measured	0.246–0.175 mm. Average intermediate diameter	0.175–0.124 mm. Grains measured	0.175–0.124 mm. Average intermediate diameter	0.124–0.088 mm. Grains measured	0.124–0.088 mm. Average intermediate diameter
	No.	*Mm.*	*No.*	*Mm.*	*No.*	*Mm.*	*No.*	*Mm.*
0.999–0.900	48	0.314	45	0.228	57	0.166	70	0.115
0.899–0.800	67	0.326	61	0.243	71	0.175	83	0.128
0.799–0.700	99	0.341	82	0.252	102	0.184	100	0.135
0.699–0.600	83	0.358	83	0.264	108	0.193	93	0.138
0.599–0.500	57	0.367	48	0.277	73	0.200	42	0.143
0.499–0.400	26	0.385	30	0.267	28	0.210	42	0.151
0.399–0.300	8	0.408	6	0.296	11	0.227	11	0.153
0.299–0.200	15	0.419	2	0.290	4	0.211	...	...
0.199–0.100	9	0.425	..	...	6	0.258	...	...

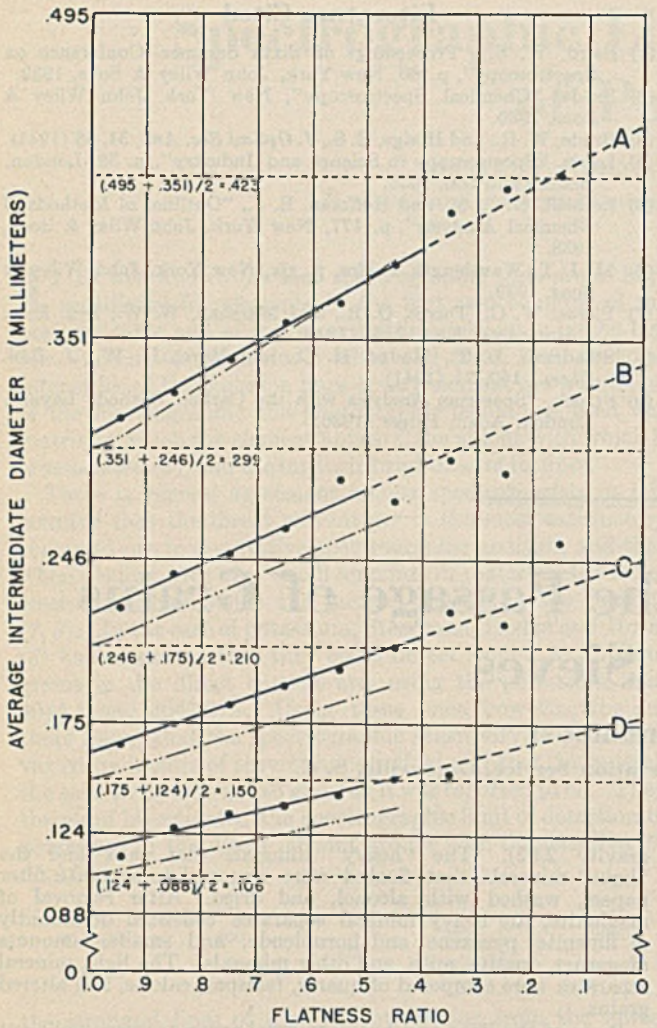

FIGURE 1. RELATION OF FLATNESS RATIO, MEASURED INTERMEDIATE DIAMETER, AND ARITHMETIC MEAN INTERMEDIATE DIAMETER

A, *B*, *C*, and *D* are for 0.351- to 0.246-mm., 0.246- to 0.175-mm., 0.175- to 0.124-mm., and 0.124- to 0.088-mm. size grades, respectively.

computed by averaging the rated openings of the two limiting sieves, is shown in Figure 1 by dashed horizontal lines.

Discussion

It is readily apparent from Figure 1 that (1) the average intermediate diameter, b_a, of particles in each sieve fraction is inversely proportional to the flatness ratios; (2) the relationship is linear when plotted arithmetically; (3) at 1.00 flatness the measured intermediate diameter is slightly larger than the arithmetic mean intermediate diameter; and (4) the flatness ratio at which the measured intermediate diameter is the same as the arithmetic mean intermediate diameter of the next larger size grade increases with decreasing grain size.

Apparently there are two ways in which shape is related to the size of grain that will pass or be retained by a particular sieve. First, if all the openings in a sieve were square and were exactly the same size, flattened grains would pass diagonally through the openings. Secondly, in the manufacture of sieves, some variation in the size and shape of openings is unavoidable. Consequently, the size and shape of grains passed or retained will be related to the number, size, and shape of the offsize openings.

The extent to which diagonal passage will occur depends, not only on the flatness ratio, but also on the cross section of the particle in the b-c plane. If the particles were rectangular in cross section, only those with flatness ratios less than 0.40 would be passed diagonally through holes too small to pass them in the normal way. In the case of grains of elliptical or rhombic cross section, however, grains of higher flatness ratios would be passed diagonally. The maximum effect would be for particles of rhombic cross section. For such grains, the relation between intermediate diameter and flatness has been determined graphically, and is indicated for each size grade by a dash-dot curve in Figure 1. In these determinations, the grains were assumed to lie in their most stable position on a plane surface, the same assumption made in measuring the actual particles. For particles with flatness ratios approximating zero, the limiting intermediate diameter would be $\sqrt{2}$ times the rated openings.

The difference in average intermediate diameter between the theoretical rhombic-section curves and the experimental curves of Figure 1 is the minimum that cannot be explained by diagonal passage of particles through square holes. Because most grains are not rhombic in cross section, the actual differences due to offsize openings are probably much greater, particularly in the flatness range from 0.9 to 0.6. Cross-sectional measurements were not made during the present investigation, and consequently the effect of diagonal passage of grains cannot be evaluated more precisely at this time. Whatever the actual relations between flatness ratio, intermediate diameter, and arithmetic mean intermediate diameters may be for a particular substance passing square holes, that relation would be the same for all size grades, except as the probability of grains passing the sieves is affected by the relative area of wire and mesh (*1*).

Of the offsize openings in sieves, those that are oversize will be more important than those that are undersize in determining the size and shape of grains passed or retained. Thus, if all offsize holes were square and were present in the same relative proportions in the different sieves, the measured average intermediate diameter would increase, but the new relation between flatness ratio, intermediate diameter, and arithmetic mean intermediate diameter would be the same for all size grades. But actually there will be many more offsize rectangular openings than offsize square holes, and more variation will occur in sieves of fine mesh than in sieves of coarse mesh. In consequence, oversize grains of low flatness ratio will be passed in greater numbers, and this effect will be more pronounced as the sieve mesh becomes finer. This is shown in Figure 1, where the flatness ratio at which the average intermediate diameter equals the arithmetic mean intermediate diameter of the next larger size grade increases from about 0.17 for the 0.351 to 0.246-mm. size grade to about 0.45 for the 0.124 to 0.088 mm. size grade.

The grain measurements also were analyzed to determine the relation, if any, between the breadth/length ratio and the size of grains passed or retained. No definite relation was found. Few of the grains had breadth/length ratios less than 0.50, however, and consequently the data did not provide satisfactory information regarding extremely elongated particles.

From the data presented, there seems little doubt that sets of sieves may be calibrated for both size and shape from three-dimensional measurements of particulate materials. For much routine work, the time required for calibration by methods described above will be prohibitive. Since much of the difference between measured and arithmetic mean intermediate diameters is due to the oversized openings in the sieves, it may be possible to simplify and shorten shape calibration by establishing a direct relationship between experimental curves of the type shown in Figure 1 and the standard

deviations of the offsize openings as determined by the method of Weber and Moran (*5*). Unfortunately, such a relationship could not be established by the present investigation, which was a by-product of a larger problem. The shape data were obtained several years after the sample was sized, and the sieves, though still available, had been used so much during the intervening period that any relationships based on measurements of their openings at the later data would be of questionable validity. There is also the possibility that standard samples can be used effectively in shape calibration of sieves.

For some research problems, however, shape-calibrated sieves may yield information that will amply justify the time required for calibration. One such problem is the correlation of textural data obtained by sieving, elutriation or sedimentation, and microprojection. Preliminary studies by the writer, for example, have shown that about 80 per cent of sand grains in a size grade will settle through water at the same rate as would spheres with diameters between the rated sieve openings. In the same samples, 30 to 50 per cent of the grains have intermediate diameters larger than the rated sieve openings.

Shape-calibrated sieves also can be used to provide data on the relation of grain shape to such mass properties as permeability, porosity, and strength, and to give a sounder basis for determining average grain volume, weight, or surface, and specific surface. After sieving, the long and intermediate diameters in each size grade would be measured by microprojection methods. Then, using the average intermediate diameter and the calibration curves, an average flatness ratio and an average short diameter would be obtained. Thus two-dimensional measurements, requiring no more time than other microprojection methods that are now in use, would yield three-dimensional shape data.

The curves of Figure 1 were obtained on a particular set of sieves which have certain average openings and certain dispersion of offsize openings. Sets of sieves having different average openings or different dispersion of offsize openings will require separate calibration.

Acknowledgment

The writer is very grateful to Matthew Weber, Jr., for his valuable suggestions and criticisms, particularly for his analysis of the diagonal passage of grains of rectangular cross section through square holes.

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Photometric Routine Estimation of Traces of Lead by Dithizone

Application to Materials Used in the Manufacture of Cosmetics

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THE desirability of a simple method for routine analysis of raw materials for traces of lead is obvious, particularly in analytical laboratories of the food, pharmaceutical, and cosmetic industries. It is further desirable that the method involve the use of only stable, readily prepared reagents and be capable of yielding reliable results within reasonable limits of error with as few special precautions as possible.

The method of Bambach (*4*) is rapid and useful if used as recommended—i. e., as a limit test. In fact, a very similar test has been incorporated in the United States Pharmacopœia (*10*) for use on reduced iron. However, much of its advantage is lost in attempting to use it for actual routine analysis because of the necessity of daily preparation of color standards which are unstable. It would appear that recourse to one of the well-known photometric procedures (*3*, *7*, *8*) is the logical solution if a dithizone method is to be used. Hence, the method here described is an adaptation of these procedures with provision for elimination of interferences not ordinarily encountered in biological materials. The only special instrument used is a comparatively inexpensive photoelectric colorimeter, the Lumetron model 400 made by the Photovolt Corporation, which uses standard 18 × 150 mm. test tubes as sample holders. The advantage of numerous sample holders is readily apparent when several samples are run simultaneously, as is frequently the case in a control laboratory. This instrument, with one of the filters with which it is regularly equipped (transmission maximum at 530 mμ), gives very satisfactory results.

The authors have had extensive experience with the A. O. A. C. electrolytic method (*2*) and have found it very satisfactory on materials which contain at least 50 p. p. m. of lead. Materials containing less lead may, of course, be run by this method, but much time is lost in preparing the necessarily large samples. Even in the case of such substances as zinc oxide, which are most readily analyzed by the electrolytic method, the dithizone procedure here described has been found more rapid.

All the reagents used, with the exception of the dithizone solution, will keep indefinitely in glass-stoppered Pyrex bottles. If the dithizone solution is properly prepared (see reagents) and stored in the dark at 40° F., it should remain in usable condition for at least several months (*6*). Few of the reagents are specially purified, and comparatively small quan-

TABLE I. ACID-EXTRACTABLE LEAD

Substance	Sample	Lead Found	Av.	Blank	Av.	Net	Net
	Gram	*Micrograms*					*P. p. m*
TiO_2	0.5	9.5, 9.2	9.35	1.7, 1.4	1.55	7.8	16
Talc	1.0	1.6, 1.4	1.5	0.6, 0.6	0.6	0.9	0.9
Kaolin	1.0	2.3, 2.3	2.3	1.6, 1.4	1.5	0.8	0.8
$BaSO_4$	1.0	16.4, 17.4	16.9	0.7, 0.4	0.6	16.3	16

TABLE II. RECOVERY OF ADDED LEAD, ACID-EXTRACTABLE

Substance	Sample	Lead Av., Table I	Lead Added	Lead Found	Av.
	Gram	*Micrograms*		*Micrograms*	
TiO_2	0.5	7.8	4	11.8, 11.9	11.85
Talc	1.0	0.9	2	2.8, 3.0	2.9
Kaolin	1.0	0.8	2	2.6, 2.9	2.75
$BaSO_4$	1.0	16.3	5	18.3, 18.4	18.35

tities of these are used, so that preparation of reagents is not frequently necessary. While more accurate results may be obtained by the use of lead-free reagents, the authors have obtained acceptable results within the desired magnitude of error with ordinary reagent grade chemicals. It is important, however, to run blanks carefully in all determinations.

The method as set forth below is specific for lead, thallium, and bismuth. Bismuth, if present, may be readily removed by extraction with dithizone at pH 2 (*11*). No sample of any of the materials described in this article has been found to contain bismuth and the authors have accordingly not felt justified in including a separation step in the procedure. Instead, it is recommended that only such samples as appear to be unusually high in lead be examined for bismuth which, if present, may then be removed as suggested. The presence of thallium, which is determined with the lead except when hydrogen sulfide is used in preparation of the sample, is extremely unlikely. Thallium may, of course, be eliminated by a preliminary hydrogen sulfide isolation of the lead. However, the authors have not yet encountered a single sample which contained thallium.

The accuracy and reproducibility of results obtained by this procedure are definitely within 2 micrograms and very probably, certainly in the case of acid-extractable lead and for acid-soluble substances, within 1 microgram.

Typical analyses, in duplicate, are shown in Tables I and III. Known amounts of lead were also added to these same samples as a check on the results (Tables II and IV). In the case of barium sulfate, poor recovery of added lead is obtained (Table II, acid-extractable), probably because of adsorption, since good recovery is obtained in the determination of total lead.

It is felt that the distinction between acid-extractable and total lead is very important and has not been given sufficient consideration in the past. The U. S. Food and Drug Administration, for example, sets a limit of 20 parts per million on lead in cosmetic materials but fails in most cases to specify how this is to be determined. It is common practice, in cases of acid-insoluble substances, to determine only acid-extractable lead. This seems justified on the assumption that lead which is not extractable by hot acid will probably produce no dermatological effects. It is, therefore, recommended that for ordinary control work only acid-extractable lead be determined, although procedures for total lead are included here.

Reagents

Water. Distilled water is redistilled in Pyrex. The use of rubber stoppers or corks is avoided.

Ammonium citrate. Ammonium citrate is prepared for use according to the directions of Bambach (*3*).

Potassium cyanide. A 10 per cent solution is prepared according to the directions of Bambach (*3*) with the following variation: The saturated, deleaded solution is boiled briefly before dilution. This serves to eliminate chloroform and prevents the solution from darkening on standing.

Hydroxylamine hydrochloride. A 20 per cent solution is prepared by the method of Bambach (*3*).

Ammonia-cyanide solution, 75 cc. of concentrated ammonium hydroxide and 100 cc. of 10 per cent lead-free potassium cyanide, made up to 500 cc. with redistilled water (*7*).

Chloroform. Chloroform is reclaimed for use by the method of Biddle (*5*). The alcohol used as preservative is freshly distilled over potassium hydroxide and is placed in the receivers before distillation of the chloroform (*6*).

Dithizone. Dithizone is purified by the conventional method (*1*). A purified product is now available (Eastman Kodak Co.) and may be used directly.

Dithizone in chloroform, 12.0 mg. per 1000 ml. by direct weight, is stored in a refrigerator.

Nitric acid, concentrated, 1 to 1, and 1 per cent (10 ml. of concentrated nitric acid plus water to make 1000 ml.). The reagent grade acid is not purified.

Sulfuric acid, concentrated and 1 to 1. The reagent grade acid is not purified.

Ammonium hydroxide, hydrochloric acid, sodium sulfate, and potassium bisulfate. Reagent grade chemicals are used directly.

Copper sulfate solution, 0.16 gram of C. P. copper sulfate per 100 cc.

Hydrofluoric acid, reagent grade.

Standard lead solution (for calibration). Recrystallized lead nitrate is dissolved in 1 per cent nitric acid and diluted to 1 or 2 micrograms per ml. with 1 per cent nitric acid.

TABLE III. TOTAL LEAD

Substance	Sample	Lead Found	Av.	Blank	Av.	Net	Net
	Grams	*Micrograms*					*P. p. m.*
$MgCO_3$	2.0	7.8, 8.0	7.9	2.2, 2.5	2.3	5.6	2.8
ZnO	0.1	2.9, 2.9	2.9	1.4, 1.2	1.3	1.6	16
Zinc stearate	0.5	9.2, 8.4	8.8	2.3, 2.0	2.2	6.6	13
Ocher[a]	0.1	4.2, 3.6	3.9	0.2, 0.2	0.2	3.7	37
$CaCO_3$	5.0	5.4, 5.0	5.2	5.0, 4.6	4.8	0.4	0.1
TiO_2	0.2	14.6, 14.0	14.3	3.6, 3.4	3.5	10.8	54
Talc	2.0	18, 16.3	17.15	14.8, 14.1	14.45	2.7	1.4
Kaolin	0.5	14.8, 14.0	14.4	6.0, 5.1	5.5	8.9	18
$BaSO_4$	1.0	27.0, 27.4	27.2	3.0, 2.6	2.8	24.4	24

[a] An iron oxide pigment.

Procedure for Preparation of Sample

Reasonable precautions to avoid contamination should be taken and, in any event, blanks should be treated exactly like the samples. Only Pyrex, Vycor, or platinum containers should be used. All containers should be washed with hot 1 to 1 nitric acid and thoroughly rinsed before use.

ACID-EXTRACTABLE LEAD. The sample is digested with nitric acid and the lead determined on the extract.

TOTAL LEAD. The entire sample is brought into solution according to the methods described below. In the case of acid-soluble substances, the results are for total lead.

Magnesium Carbonate. Dissolve 2.00 grams of the sample in 10 ml. of 1 to 1 nitric acid. Boil to eliminate carbon dioxide, cool, and dilute to about 75 cc. in a separatory funnel. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red with ammonium hydroxide. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution.

Zinc Oxide. Dissolve 0.100 gram of the sample in 1 ml. of 1 to 1 nitric acid and wash the solution into a small separatory funnel with several portions of water. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red with ammonium hydroxide. Add 0.5 ml. of hydroxylamine hydrochloride solution and 10 ml. of potassium cyanide solution.

Zinc Stearate. Ash 0.500 gram of the material in a silica or Vycor crucible in a muffle furnace at 475° to 500° C. for 15 to 20 minutes. Cool and wet the residue with 3 drops of concentrated nitric acid. Evaporate to dryness over a low flame and return to the muffle for 20 to 30 minutes. A clean white ash should result. The nitric acid treatment may be repeated if

necessary. Dissolve in 1 ml. of 1 to 1 nitric acid and wash into a small separatory funnel with several portions of water. Add 3 ml. of ammonium citrate solution and 0.5 ml. of hydroxylamine hydrochloride solution and make alkaline to phenol red with ammonium hydroxide. Then add 10 ml. of potassium cyanide solution.

Cosmetic Ochers (ferric oxides). Boil 0.100 gram with 2 ml. of concentrated hydrochloride until dissolved. Add 3 drops of nitric acid and boil to eliminate chlorine and oxides of nitrogen, adding a little water if necessary. Do not allow the solution to go to dryness. Transfer to a separatory funnel and add 5 ml. of ammonium citrate solution and 5 ml. of hydroxylamine hydrochloride solution. Make alkaline to phenol red with ammonium hydroxide and add a 2-ml. excess. Cool thoroughly and add 6 ml. of potassium cyanide solution.

Calcium Carbonate. Dissolve 5.00 grams of the sample in 15 ml. of 1 to 1 nitric acid, boil, and cool the solution. Add 5 ml. of ammonium citrate solution and 5 ml. of copper sulfate solution and adjust the pH to 3.0 to 3.4 (bromophenol blue) with ammonium hydroxide. Saturate with hydrogen sulfide, filter, and wash with 3 portions of 3 per cent sodium sulfate solution. (The pH of a 3 per cent solution of sodium sulfate solution is adjusted to 3.0 to 3.4 and the resulting liquid saturated with hydrogen sulfide before using it as a wash.) Dissolve with 4 ml. of hot 1 to 1 nitric acid, catching the filtrate in the same flask in which the precipitation was carried out. Wash with six small portions of hot water, adding the washings to the filtrate. Boil to eliminate sulfide and transfer to a small separatory funnel. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red, adding a 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution. [It is possible to hold calcium in solution, at least temporarily, at this pH in the presence of ammonium citrate. However, in the case of calcium carbonate, where a rather large (5.0-gram) sample is advisable because of low lead content, a sulfide separation is preferable to the use of the very large amounts of ammonium citrate and large volumes of solution which otherwise become necessary.]

Table IV. Recovery of Added Lead, Total Lead

Substance	Sample	Lead Av., Table III	Lead Added	Lead Found		Av.
	Grams	*Micrograms*				
$MgCO_3$	2.0	5.6	5	10.9,	9.1	10.0
ZnO	0.1	1.6	2	3.5,	3.9	3.7
Zinc stearate	0.5	6.6	5	11.4,	10.5	11.0
Ocher	0.1	3.7	5	8.8,	8.8	8.8
$CaCO_3$	5.0	0.4	5	5.7,	5.3	5.5
TiO_2	0.2	10.8	4	13.9,	13.3	13.6
Talc	1.0	1.4[a]	5	6.6,	6.2	6.4
Kaolin	0.5	8.9	4	14.1,	11.9	13.0
$BaSO_4$	0.5	12.2[a]	5	16.0,	17.1	16.6

[a] Calculated.

Titanium Dioxide. Acid-Extractable Lead. Add 0.500 gram to 10 ml. of 1 to 1 nitric acid in a 15-ml. graduated centrifuge tube, digest 1 hour in boiling water bath with frequent agitation, centrifuge, and pour off the liquid into a small separatory funnel. Add 5 ml. of 1 to 1 nitric acid and 5 ml. of water to the centrifuge tube, break up the packed mass with a glass rod, and digest for 15 minutes on the water bath. Centrifuge and add the liquid to the original extract. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red with ammonium hydroxide, adding a 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution.

Total Lead. Fuse 0.200 gram of the titanium dioxide with 2.00 grams of potassium bisulfate in a covered Vycor crucible. Use only a Bunsen flame and heat only until a clear melt is obtained. Rotate the crucible as the melt solidifies, so as to spread it in a thin layer over the sides. After the melt has cooled, add 4 ml. of ammonium citrate solution and 5 to 6 ml. of water. Heat carefully until a clear solution is obtained. Transfer to an Erlenmeyer flask, washing well with a few portions of water, cool, and adjust the pH to 3.0 to 3.4 (bromophenol blue) using dilute sulfuric acid. Add 5 ml. of copper sulfate solution.

Saturate with hydrogen sulfide, filter, and wash thoroughly (ten times) with 3 per cent sodium sulfate solution. (The pH of a 3 per cent solution of sodium sulfate solution is adjusted to 3.0 to 3.4 and the resulting liquid saturated with hydrogen sulfide before using it as a wash.) Dissolve the precipitate with 4 ml. of hot 1 to 1 nitric acid, catching the filtrate in the flask in which the precipitation was effected. Wash the paper with six small portions of hot water, adding the washings to the filtrate. Boil to eliminate sulfide and transfer to a small separatory funnel. Add 3 ml. of ammonium citrate solution, and make alkaline to phenol red with ammonium hydroxide, adding a 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution. [Titanium is not precipitated at the pH of extraction in the presence of citrate, but even small amounts completely prevent the extraction of lead by dithizone (see Table V[1]). Hence a preliminary isolation of the lead as sulfide was resorted to. The precipitate of lead sulfide and copper sulfide must be washed very thoroughly to remove all trace of Ti^{++++}. Since the amount of Ti^{++++} necessary to interfere with the lead extraction is small, it was thought that double precipitation of the sulfides would be advisable. However, experiments showed this to be unnecessary, providing the sulfides are well washed.]

Talc. Acid-Extractable Lead. Follow the procedure for titanium dioxide exactly, using a 1.00-gram sample.

Total Lead. To a platinum crucible containing 3 grams of hydrofluoric acid and 6 ml. of 1 to 1 sulfuric acid, add 1.00 gram of the talc. Place in a sand bath heated to 200° to 250° C. and take to fumes of sulfur trioxide. Cool, add 1 gram of hydrofluoric acid, and again heat to sulfur trioxide fumes. Repeat once more with 0.5 gram of hydrofluoric acid. [The sample of talc must be fumed strongly for some minutes to drive off excess fluoride. If much fluoride ion remains it is difficult to obtain a clear solution.] Cool, dilute with a little water, wash into a separatory funnel, add 3 ml. of ammonium citrate solution, and make alkaline to phenol red with ammonium hydroxide, adding 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution.

Kaolin. Acid-Extractable Lead. Follow the procedure for titanium dioxide but use 5 per cent nitric acid for both extractions in place of the more concentrated acid and use a 1.00-gram sample. [When the acid concentration is too high (1 to 1), considerable aluminum is extracted. This leads to poor results.]

Total Lead. To a platinum crucible containing 1.5 grams of hydrofluoric acid and 5 ml. of 1 to 1 sulfuric acid, add 0.500 gram of the kaolin. Place in a sand bath at 200° to 250° C. and heat to fumes of sulfur trioxide. Cool, add 5 drops of nitric acid to destroy organic matter, and again heat to sulfur trioxide fumes. Transfer to an Erlenmeyer flask with about 25 ml. of water, add 5 ml. of ammonium citrate solution, and boil for a few minutes until a clear solution is obtained. Cool and adjust the pH to 3.0 to 3.4 (bromophenol blue). Add 5 ml. of copper sulfate solution.

Saturate with hydrogen sulfide, filter, and wash 5 or 6 times with 3 per cent sodium sulfate solution. (The pH of a 3 per cent sodium sulfate solution is adjusted to 3.0 to 3.4 and the resulting liquid saturated with hydrogen sulfide before using it as a wash.) Dissolve with 5 ml. of boiling 1 to 1 nitric acid, catching the filtrate in the flask in which the precipitation was effected. Wash with six portions of hot water, adding the wash to the filtrate. Boil to eliminate hydrogen sulfide, cool, and transfer to a small separatory funnel. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red with ammonium hydroxide, adding a 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution. [The extraction of lead in the presence of much aluminum is very unsatisfactory (see Table VI). A preliminary isolation of the lead as sulfide eliminates this difficulty completely.]

Barium Sulfate. Acid-Extractable Lead. Follow the procedure for titanium dioxide exactly. Use a 1.00-gram sample.

Total Lead. Mix 1.00 gram in a platinum crucible with 2 grams of sodium carbonate and 3 grams of potassium carbonate. Fuse over a Bunsen burner to a clear, mobile liquid. Cool, add a small amount of water, and heat cautiously over a flame. This will loosen the melt and enable its transfer to a beaker. Boil with 20 to 25 ml. of water until the mass is thoroughly disintegrated, filter the hot solution, and wash 6 to 8 times with 4-ml.

[1] The results shown in Tables I to V include a reagent blank of approximately 1.4 micrograms.

Table V. Effect of Titanium on Extraction of Lead by Dithizone

Lead Added	Titanium	Lead Found
Micrograms	*Micrograms*	*Micrograms*
10	0	11.4
10	10	11.4
10	500	11.4
10	5,000	11.1
10	50,000	3.4
10	100,000	0.2

portions of hot 5 per cent sodium carbonate solution until the wash liquid is free of sulfate. Finally wash twice with small portions of water, discarding this wash. To the combined filtrate and washings add 5 ml. of ammonium citrate solution, 0.5 ml. of hydroxylamine hydrochloride solution, and 1.0 ml. of potassium cyanide solution (Solution 1).

Dissolve the residue on the filter in 2 ml. of hydrochloric acid diluted with about 5 ml. of water. Wash with several small portions of water, adding the washings to the acid solution. Boil for a few minutes to eliminate carbon dioxide and transfer to a separatory funnel. Add 3 ml. of ammonium citrate solution and make alkaline to phenol red with ammonium hydroxide, adding 0.5-ml. excess. Add 0.5 ml. of hydroxylamine hydrochloride solution and 1 ml. of potassium cyanide solution. The solution at this point should have a volume of about 75 ml. and be perfectly clear (Solution 2).

Table VI. Effect of Aluminum on Extraction of Lead by Dithizone

Lead Alone		In Presence of 1.5 Grams of $Al_2(SO_4)_3.18H_2O$	
Extract No.[a]	Lead	Extract No.[a]	Lead
	Micrograms		*Micrograms*
1	10.6	1	5.3
2	1.0	2	3.8
3	0.5	3	2.0
4	0.0	4	0.7
		5	1.1
		6	0.6

[a] Successive 5-ml. portions of dithizone.

Extraction of Lead

The prepared sample is extracted with three successive 5-ml. portions of dithizone which are combined in a small (60-ml.) separatory funnel. In the case of barium sulfate (total lead), Solutions 1 and 2 are extracted separately and all extracts are combined in a single separatory funnel.

The combined extracts are then shaken with 20 ml. of 1 per cent nitric acid and the dithizone solution is discarded.

Determination of Lead

Four milliliters of the ammonia-cyanide solution and 2 drops of the hydroxylamine hydrochloride solution are added to the 20 ml. of acid containing the lead, 10.0 ml. of the dithizone are added from a buret, and the mixture is shaken for 30 seconds. The chloroform layer is then filtered through a 7-cm. acid-washed filter paper folded in eights and fitted directly into the mouth of one of a series of matched 18 × 150 mm. test tubes.

A "zero" standard is prepared by treating 10.0 ml. of the dithizone with 20 ml. of 1 per cent nitric acid containing 4 ml. of the ammonia-cyanide solution and 2 drops of hydroxylamine hydrochloride solution. This is used to set the photometer to 100 per cent transmission.

A reading is then taken on the prepared sample and on a blank which is prepared in exactly the same way as the sample, omitting only the material undergoing analysis, using the yellow-green filter (transmission maximum 5300 Å.). From these readings, the amount of lead is read from a calibration curve constructed by analysis of varying amounts of the standard lead solution.

Discussion

This procedure is not intended to replace the more accurate techniques employing dithizone (*3*, *7*, *8*, *11*), but rather to provide a comparatively simple method for use by the average control laboratory where a precision of 1 or 2 micrograms is adequate. In such a laboratory, speed and simplicity are important factors and the use of only simple, rugged instruments is desirable.

It is felt that too little consideration is given to the possible interference of various cations in the extraction of lead by dithizone, although at least one article in the literature (*9*) deals with this question. The authors have found (see Table V and VI) that the presence of aluminum definitely interferes with the extraction of lead and the presence of titanium prevents it completely.

It has been observed in this laboratory that the presence of only 5 to 10 mg. of titanium seriously interferes with the extraction of lead by dithizone and that 0.1 to 0.2 gram prevents it completely. Thus, in analyzing a sample containing 0.2 gram of titanium dioxide, if it is attempted to extract the lead directly (without recourse to preliminary separation by hydrogen sulfide), the blank will show more lead than the sample. This effect is not due to a simple shift in the pH of optimum extraction, since experiments have shown the same results at various pH from 7.0 to 11.0.

The procedure of adding known amounts of lead to samples for analysis was preferred to spectrographic analysis as a means of checking results. Three well-known spectrographic laboratories, working with a master sample of titanium dioxide, obtained values of 24, 45, and 66 p. p. m., respectively. The authors obtained 54 p. p. m. (see Table III), using the above dithizone procedure.

As an additional check, samples of zinc oxide, talc, and titanium dioxide were analyzed by the A. O. A. C. electrolytic method (*2*). Good agreement with the authors' dithizone procedure was obtained in each case.

Summary

A simple photometric procedure for the routine determination of lead by dithizone is described. The procedure is fairly rapid, requires very little special equipment, and is sufficiently accurate for most control work (within 1 or 2 micrograms).

The method has been in use for some time on a number of common materials used in the cosmetic industry. The results have been very satisfactory and the procedure could be readily adapted to virtually any material. Detailed methods for the preparation of samples of such materials as are commonly used in the manufacture of cosmetics have been worked out. These methods of preparation could be followed by analysis according to any of the known dithizone procedures.

Aluminum and titanium have been found to interfere with the extraction of lead by dithizone.

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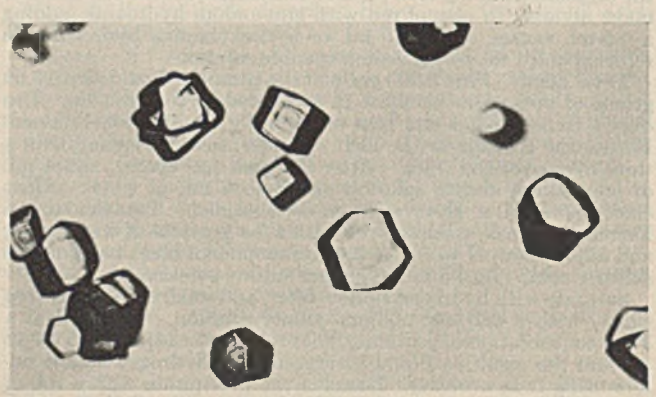

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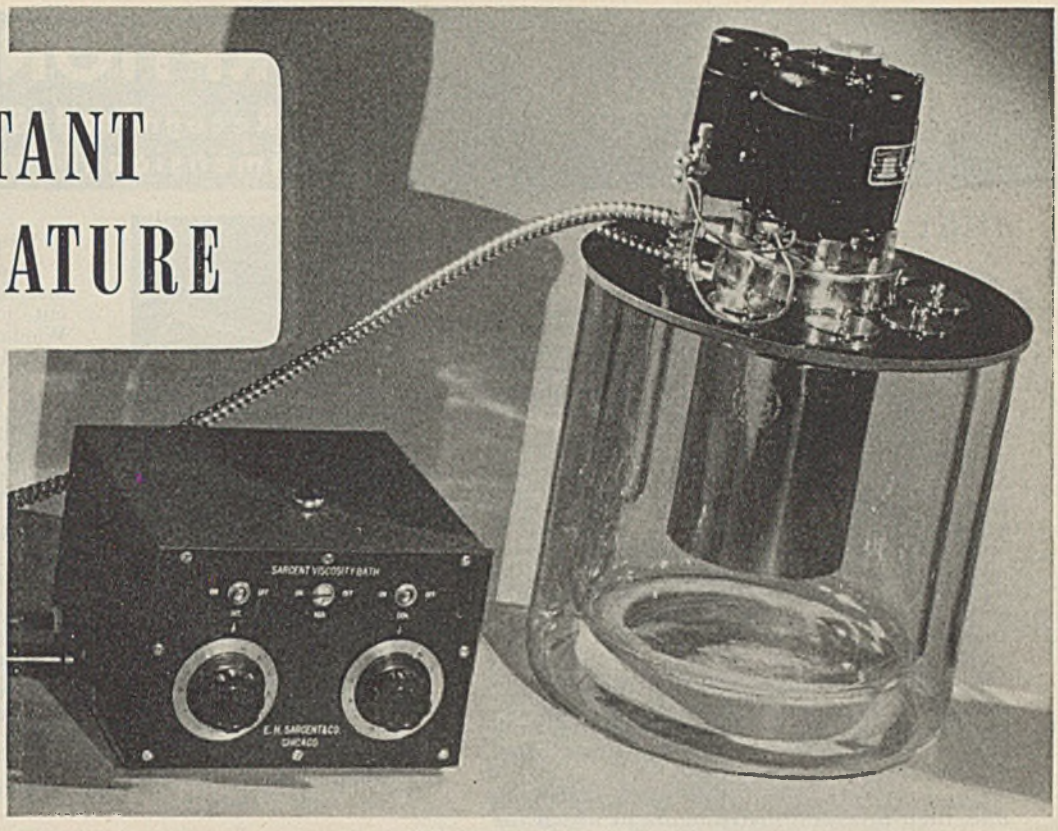

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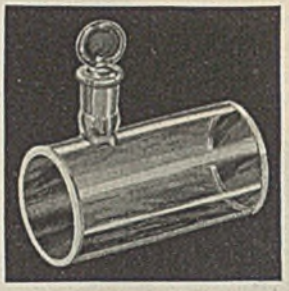

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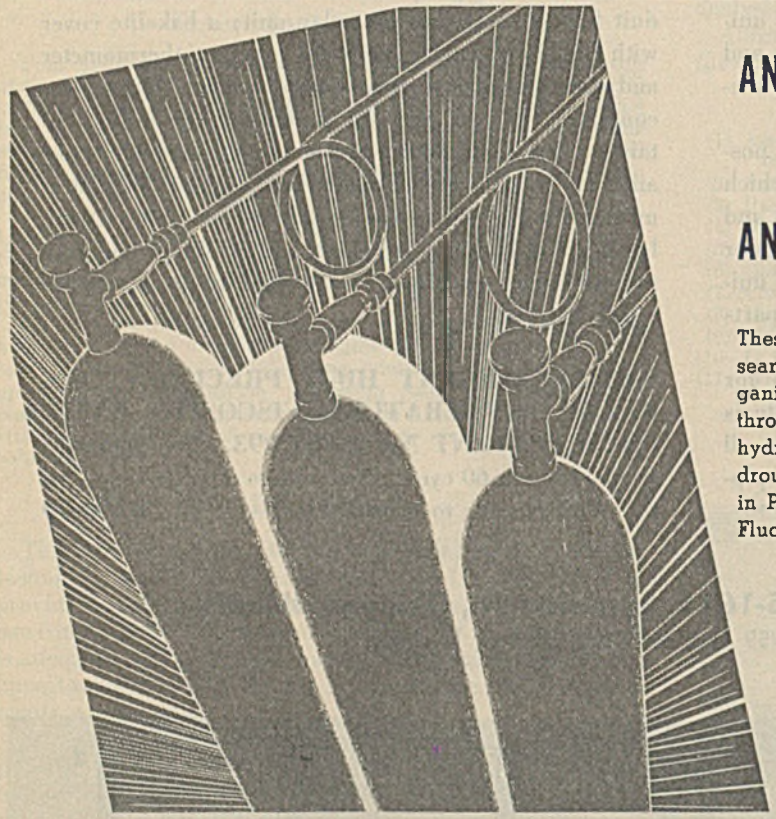

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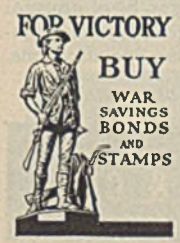

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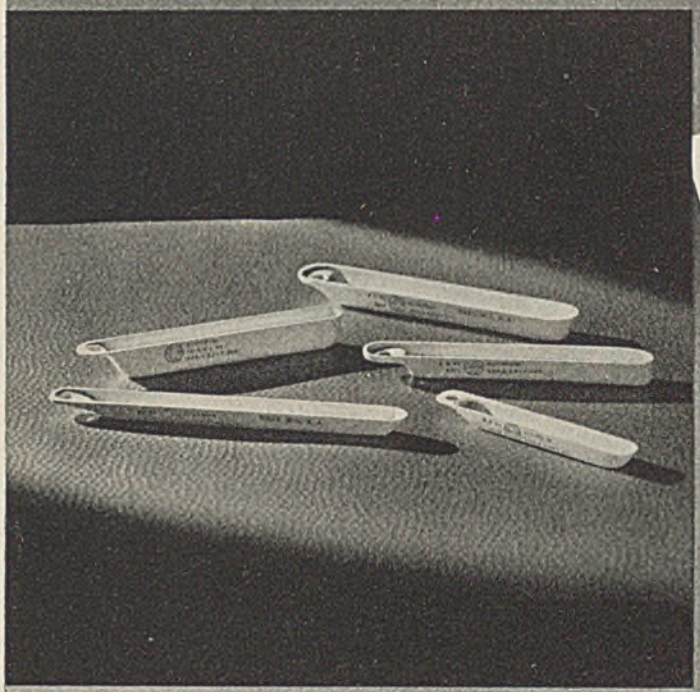

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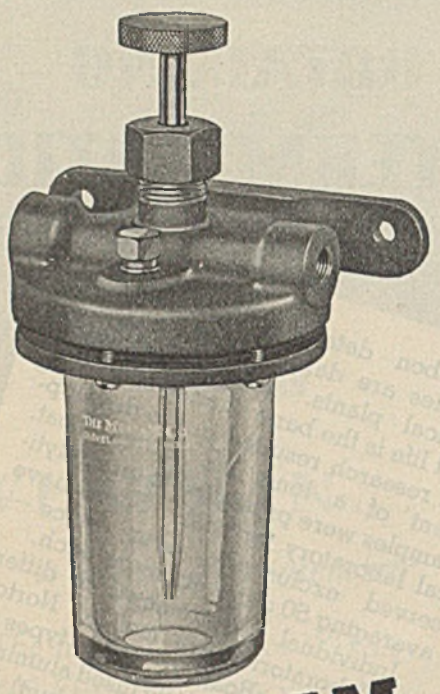

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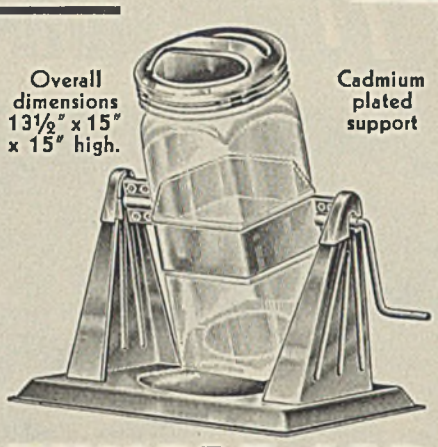

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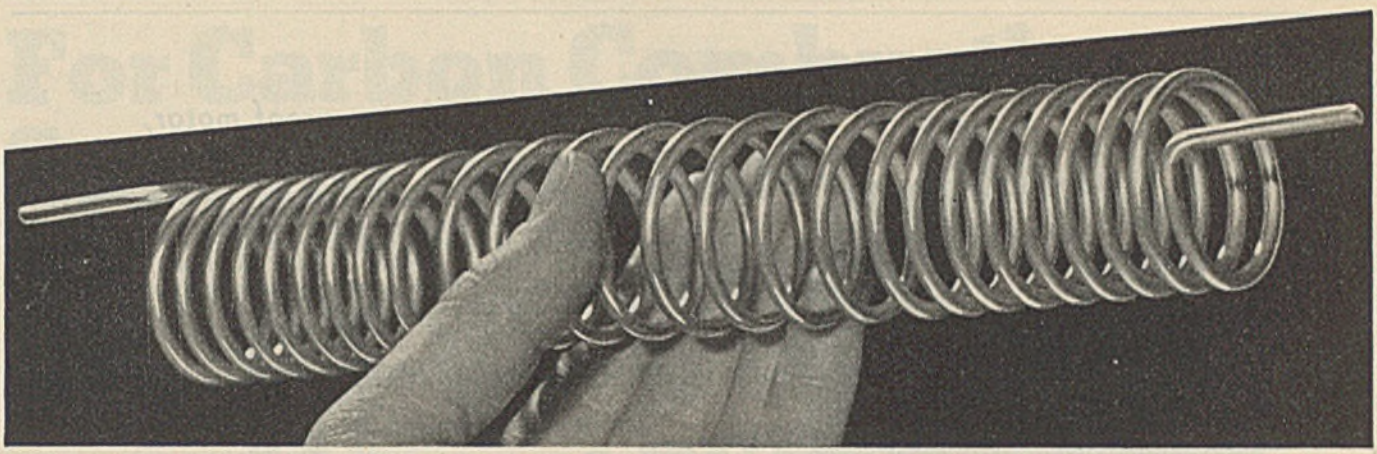

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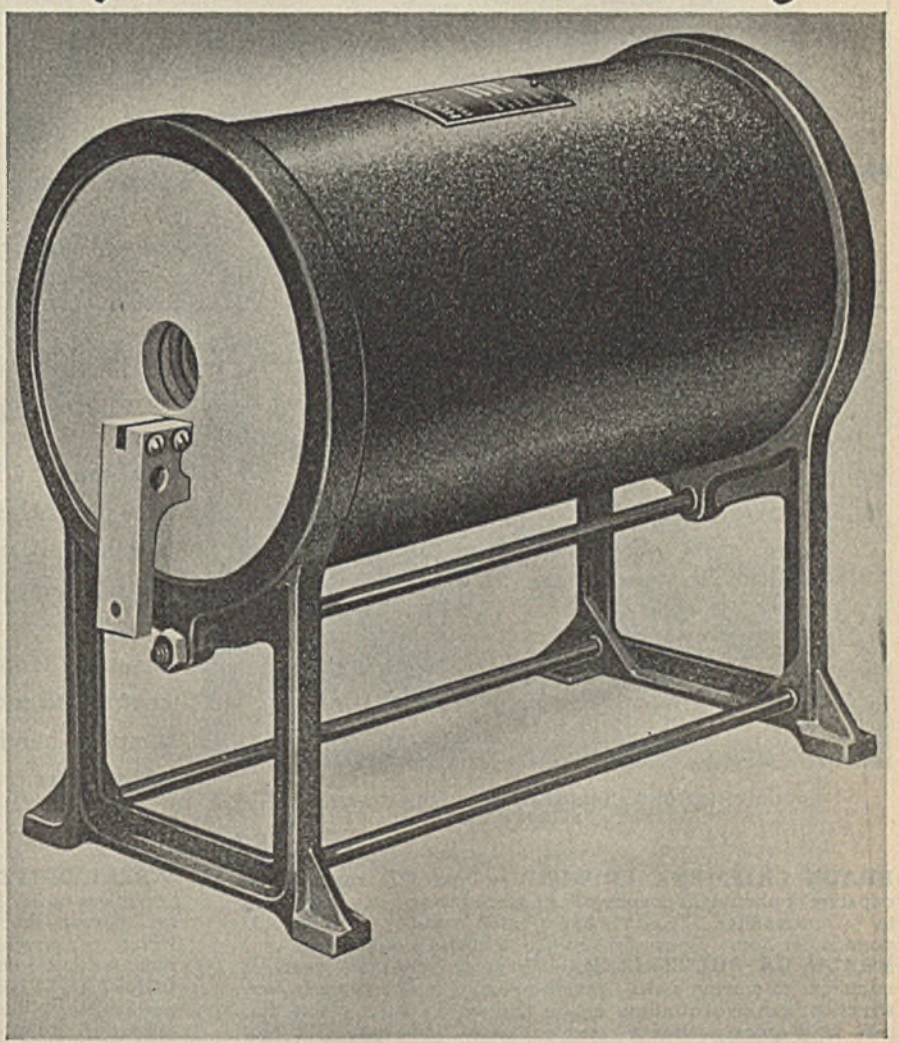

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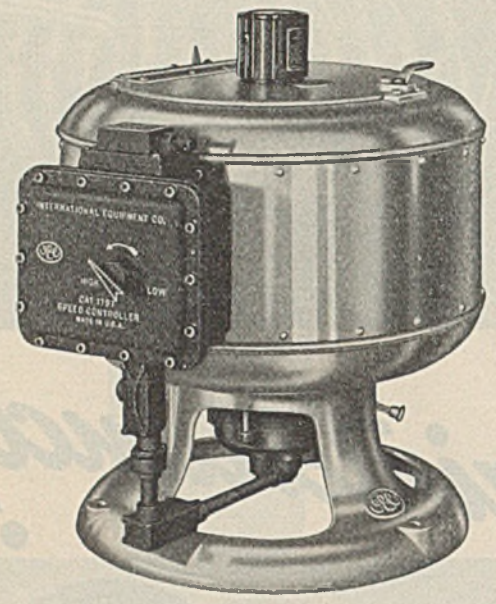

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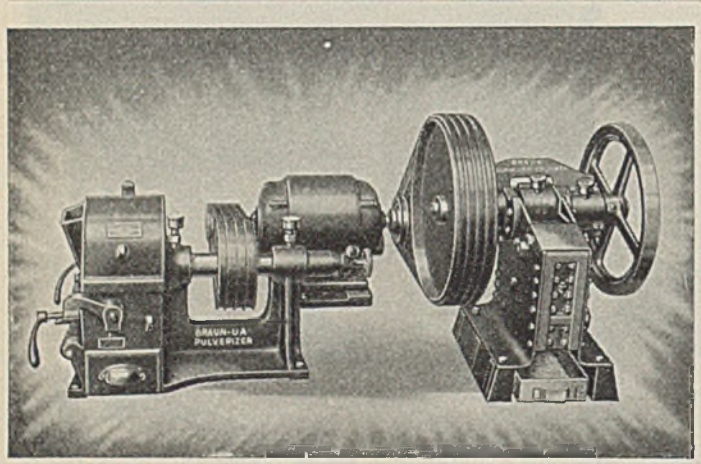

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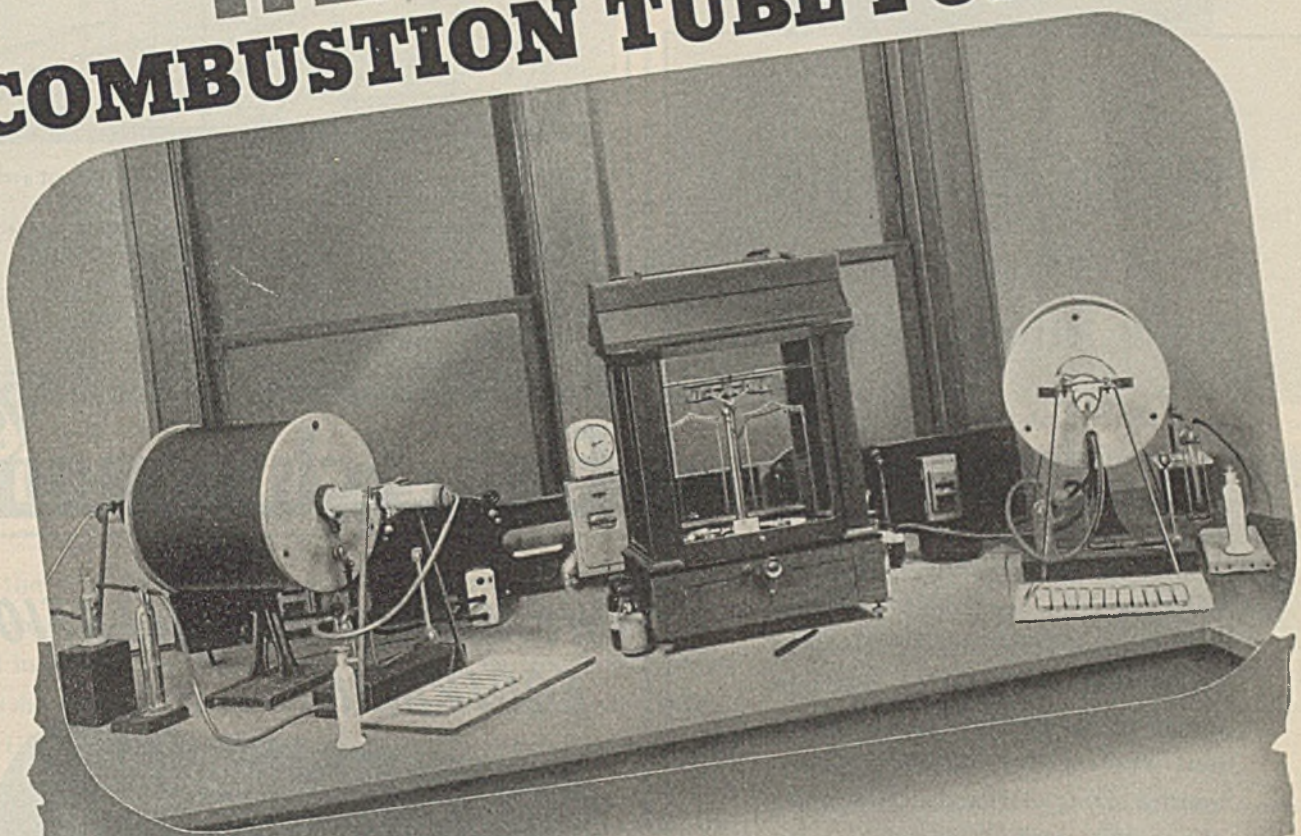

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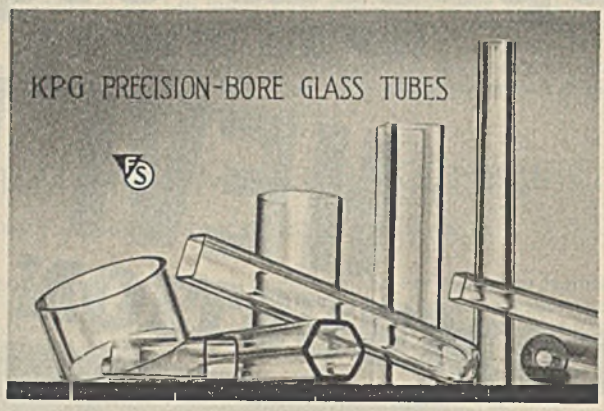

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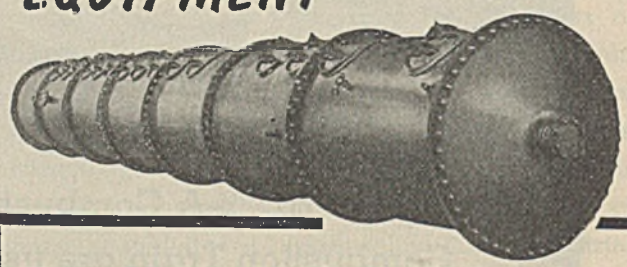

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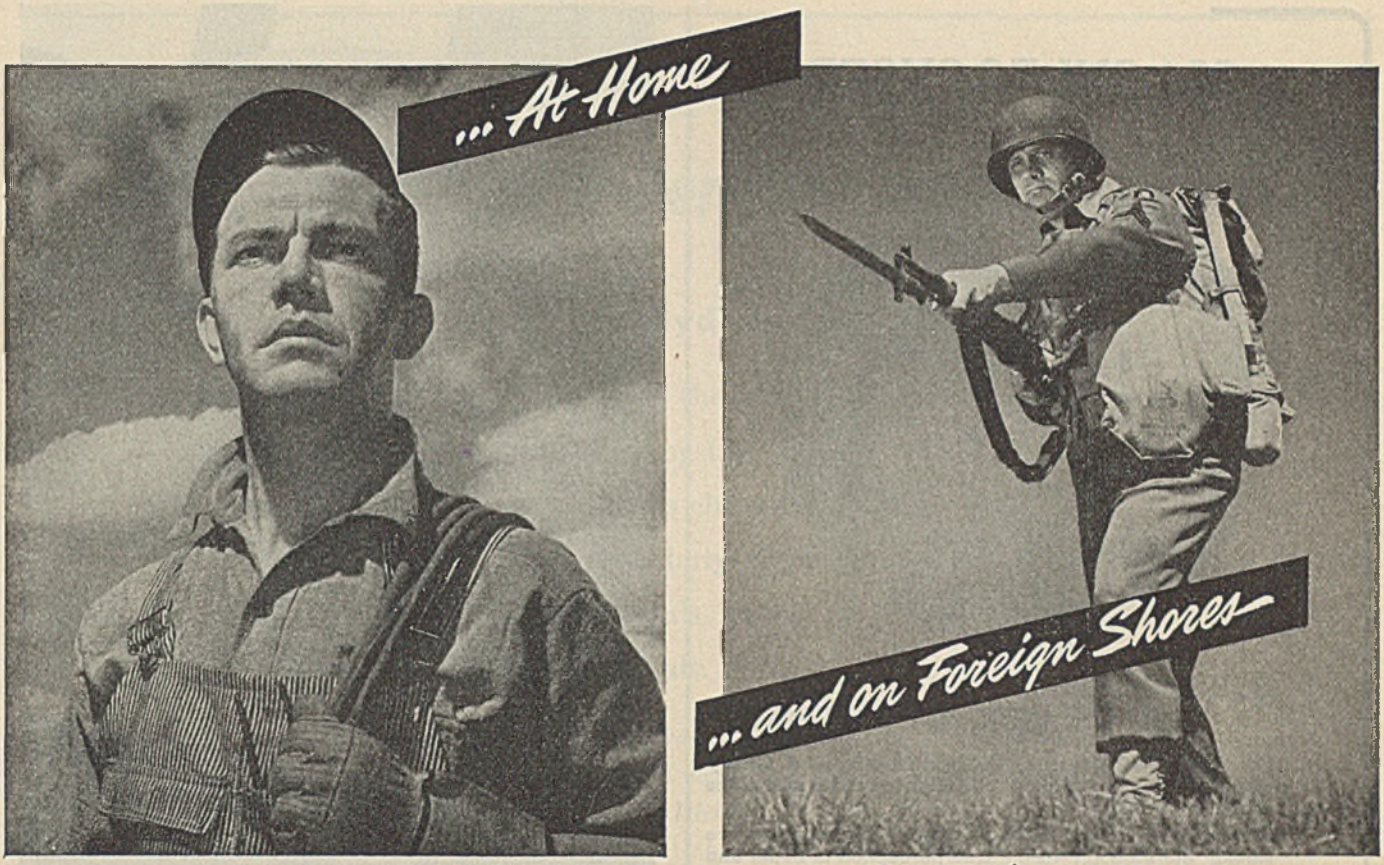

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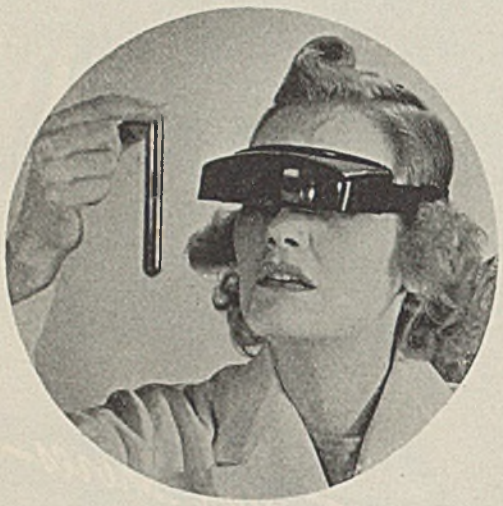

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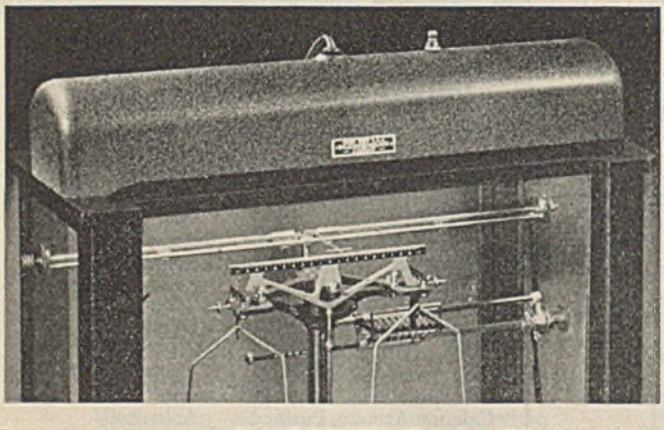

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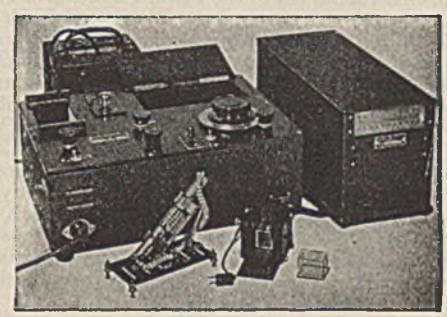

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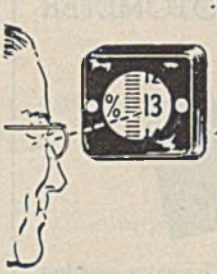

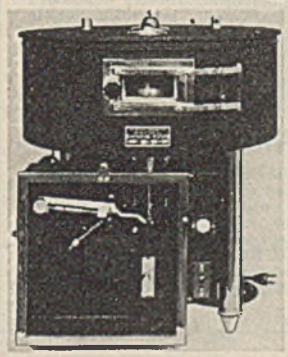

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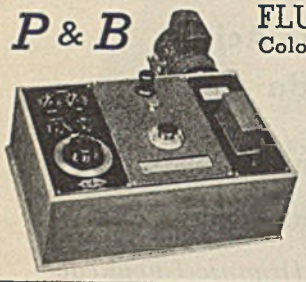

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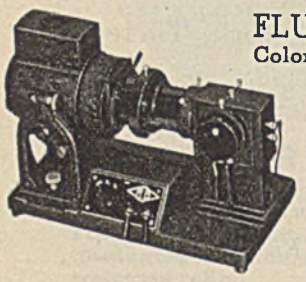

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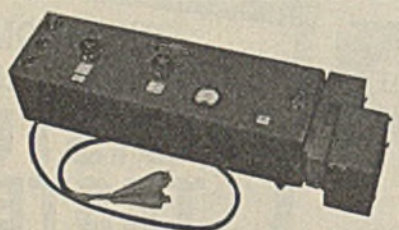

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