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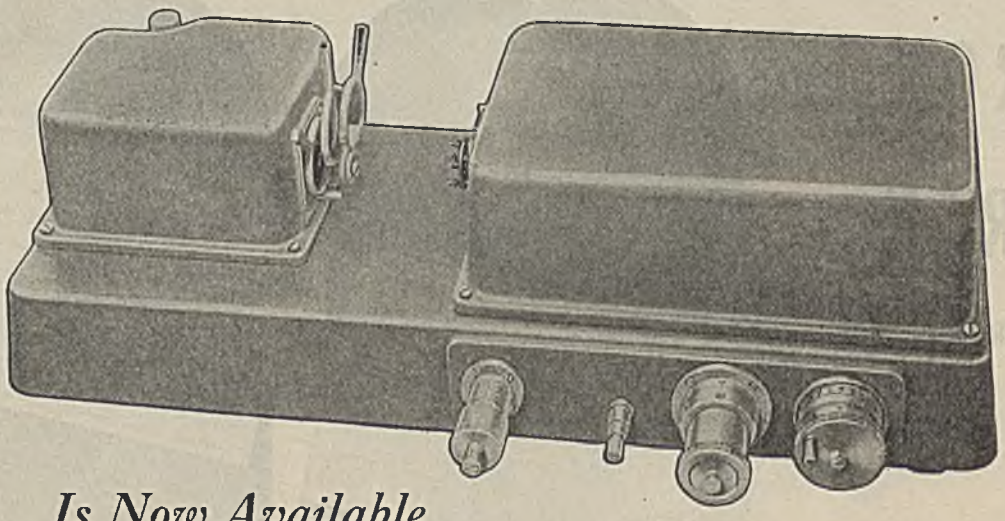

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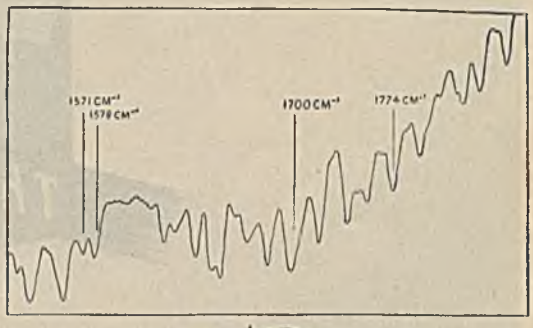

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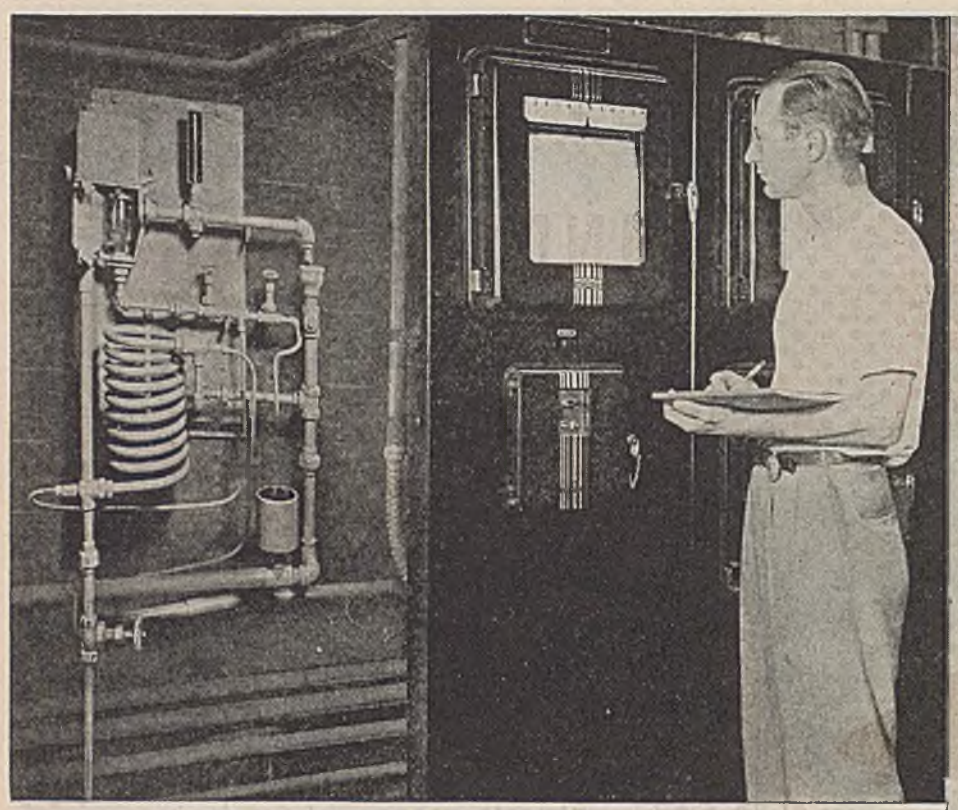

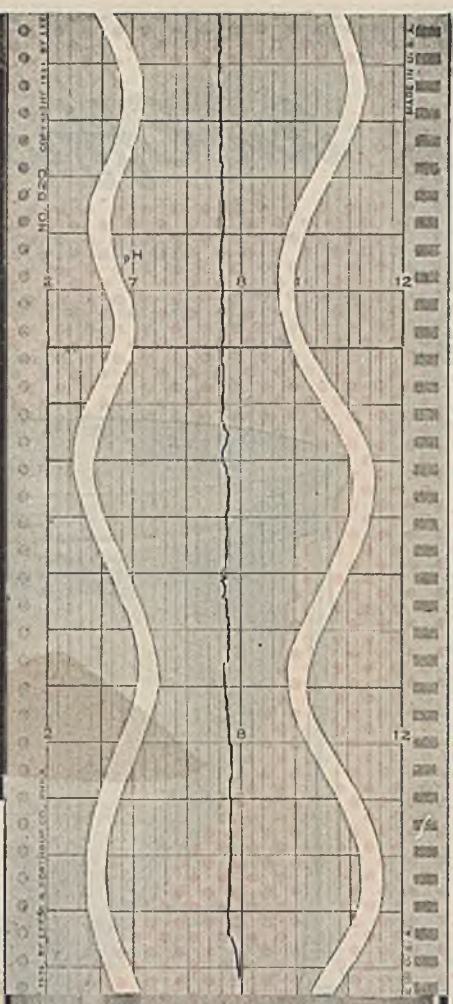

Micromax pH Control, feedwater plant, Wyandotte Chemicals, Inc., Wyandotte, Mich. Right, typical pH chart of finished water in a holding tank.

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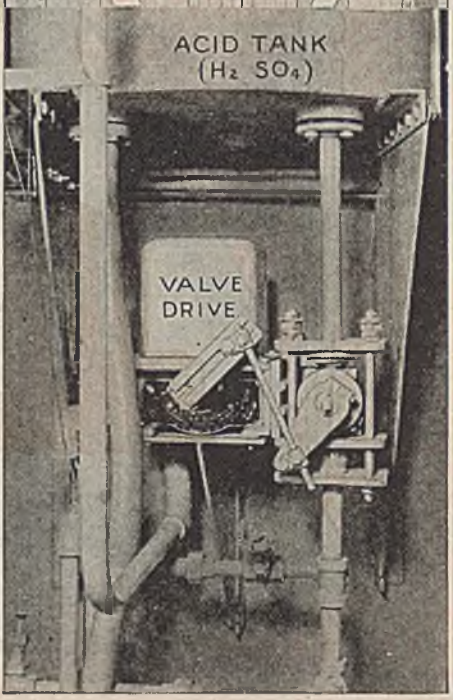

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Applicability of Newer Physical Methods for Hydrocarbon Analyses

W. J. SWEENEY, Esso Laboratories, Elizabeth, N. J.

Some of the newer physical methods are finding wide application in hydrocarbon analyses. Many highly complex problems are greatly simplified by a judicious use of these methods. The author summarizes briefly the advantages resulting therefrom in the analysis of petroleum products.

OVER the past several years great progress has been made in applying the principles of physics to methods of analysis for hydrocarbons. For security reasons, very little of this work has yet been published. The purpose of the present article is merely to indicate the applicability of this published information to the needs of research in the petroleum industry. A short bibliography is appended for more detailed references on the subject.

Experience has shown that it is profitable to take analytical work out of the routine class and to consider it as a vital part of petroleum research, development, and manufacturing. The physicists and the manufacturers of optical and electrical instruments have opened up a new field in this class of analytical work. This development is still in its infancy; but it has made such strides as to warrant confidence of its future indispensability in serving the petroleum industry in laboratory research as much as other physical methods have helped in oil field production.

This new analytical technique will not replace older methods entirely. It has already been amply demonstrated by experience

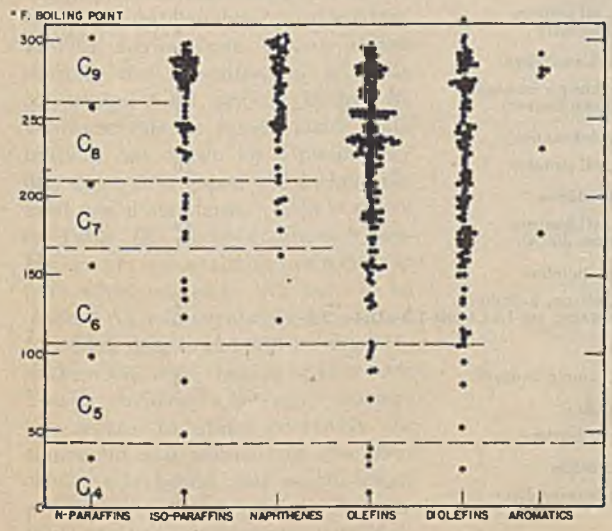

Figure 1. Distribution of Hydrocarbon Groups by Boiling Point

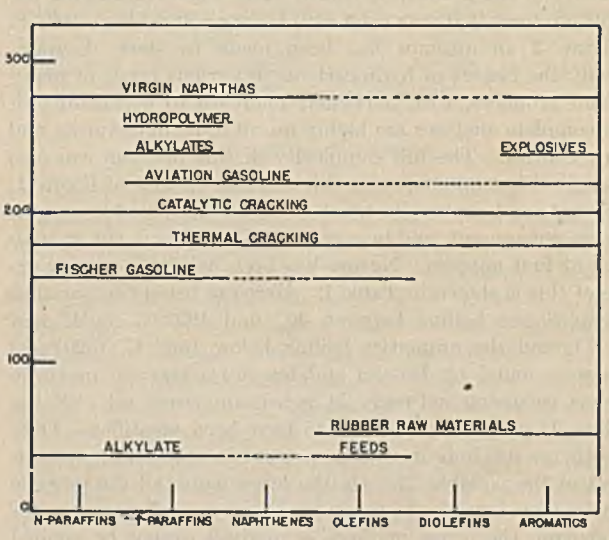

Figure 2. Classes of Hydrocarbons

that all analytical methods, new and old, are less competitive and more complementary when they are most efficiently applied. Moreover, in the present state of the art, it is not always possible to decide beyond question which instrument is best for any purpose. As an example of the variety of equipment for which a modern petroleum research laboratory can have full-time use, a partial list of the physical instruments currently used by the Esso Laboratories-Research Division of the Standard Oil Development Company is as follows:

20 Fractional distillation columns (Fenske packing)
6 Podbielniak Hyd-Robot columns
2 Podbielniak manually operated columns
1 Gaertner infrared spectrograph
1 Beckman infrared spectrophotometer
1 Perkin-Elmer infrared spectrograph
2 Beckman ultraviolet spectrophotometers
1 ARL-Dietert emission spectrograph
1 Westinghouse mass spectrograph
1 Raman spectrograph (accessible but not owned)

This list does not, of course, include all the conventional chemical equipment that was used prior to the introduction of the newer techniques and is still being used concurrently with them.

PROBLEMS OF PETROLEUM ANALYSIS

A satisfactory way to become oriented as to the interdependence of these methods is to consider the complexity of petroleum and of the problems of its analysis. To illustrate this point, in Figure 1 the isomers of normal paraffins, isoparaffins, naphthenes,

Table I. Hydrocarbons Found in Typical Crudes

(A.P.I. Project 6)

Paraffins, 40–102° C.	Naphthenes, 40–102° C.	Aromatics, 80–160° C.
2,2-Dimethylbutane	Cyclopentane	Benzene
2,3-Dimethylbutane	Methylcyclopentane	Toluene
2-Methylpentane	Cyclohexane	Ethylbenzene
3-Methylpentane	1,1-Dimethylcyclopentane	*p*-Xylene
n-Hexane	1,3-Dimethylcyclopentane (T)	*m*-Xylene
2,2-Dimethylpentane	1,2-Dimethylcyclopentane (T)	*o*-Xylene
2,4-Dimethylpentane	Methylcyclohexane	Isopropylbenzene
2,3-Dimethylpentane		*n*-propylbenzene
2-Methylhexane		
3-Methylhexane		
n-Heptane		
Total Possible Compounds		
15	52	8

olefins, diolefins, and aromatics are grouped according to boiling point, up to and including hydrocarbons of 9 carbon atoms. The complexity of the problem is readily seen, when considering the possible mixtures that may exist with boiling points above 100° F. In Figure 2 an attempt has been made to show diagrammatically the classes of hydrocarbons in various types of materials and products, with particular reference to petroleum, for which complete analyses are highly useful in manufacturing and refining control. The full complexity of this problem can also be visualized by superimposing this diagram on that of Figure 1.

Fortunately, however, in most cases all the possible components are not present, and in practice the problem is not so complex as at first appears. Nature has been helpful. A good example of this is shown in Table I. Here are listed the paraffins and naphthenes boiling between 40° and 102° C. (104° and 216° F.), and the aromatics boiling below 160° C. (320° F.) which were found by Rossini and his co-workers (*4*) in virgin naphthas occurring naturally in petroleum crude oil. Of the paraffins, 11 out of the possible 15 have been identified. Only 7 naphthenes are indicated to be present in appreciable quantities out of the possible 52. On the other hand, all the possible aromatics were found to be present.

In general, the newer methods of analysis cannot be applied directly to mixtures containing a large number of components. For this reason, it is necessary to "divide to conquer". To obtain accurate and reliable results, the number of components should be decreased to about 8, and preferably fewer, by employing all available means of separation, such as fractional distillation.

As an illustration of what can be done along these lines, Figure 3 shows a combination method for the analysis of a complete C_4-hydrocarbon cut. The butadiene content can be determined directly by ultraviolet absorption, but the remaining compounds are analyzed by infrared absorption, only after making appropriate separations to reduce the complexity, and that entails the removal of the butadiene. The amount of *n*-butane and isobutane is determined on a part of the sample from which the olefins have been removed with bromine. Infrared measurements are then made on another part of the total sample; these measurements are corrected for the amount of *n*-butane and isobutane previously found; and the amount of each of the C_4-olefins is then calculated.

By using this method, the required calculations are greatly reduced as compared to those required when the analysis is made by infrared alone on the total sample. A preliminary fractional distillation as in the Podbielniak Hyd-Robot (*5*) also contributes to the accuracy of this type of analysis by removing C_3 and C_5 hydrocarbons, which definitely interfere with the infrared analysis. It is not necessarily true that the method here outlined is the best for analyzing the C_4 mixture; but it is true that the best method will involve an analogous system of simplifying short cuts based on good judgment. Just as the expert golfer uses a variety of golf clubs, an expert in hydrocarbon analysis will use the combination of instruments best suited to the particular job.

While these newer methods have resulted both in shortening the time required for certain analyses and in permitting other analyses to be done which were not previously possible, it is not therefore to be inferred that earlier workers were wholly in the dark. The "old-fashioned" chemical and physical methods have been and are being used to good effect in obtaining analyses, both as to type and as to specific hydrocarbons, of many materials important to the war effort—for example, the components of aviation gasoline and of synthetic rubber. There will always be a need for the older methods. Such techniques as distillation, fractional or azeotropic, crystallization, solvent extraction, bromination, or other chemical methods of separation and identification, will be useful and necessary parts of the analytical stock in trade. In particular, they will be used for type analyses on complex mixtures, as a guide to the choice of optical methods and of the proper individual hydrocarbons to be prepared in utmost purity for background standards in spectral analysis. It is obviously impossible to stock a "bank" with standard samples of all conceivable hydrocarbons. A laboratory working in a

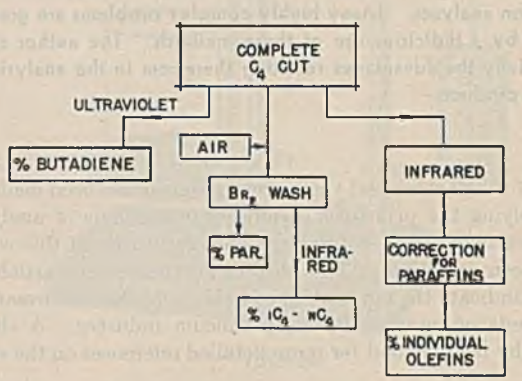

Figure 3. Combination of Methods for Analysis of C_4 Cut

Table II. Hydrocarbons Required for Spectrometer Calibrations

(A.P.I. Project 48)

Paraffins		
C_1-C_4, all isomers	5	
C_5, all isomers	3	
C_6, all isomers	5	
C_7, all isomers	9	
C_8, liquid isomers	17	
C_9, lower isomers	5	
C_9-C_{10}, normal compounds	2	
		46
Aromatics		
C_6-C_8, all isomers	6	
C_9, all isomers	8	
		14
Alkyl cyclopentanes		
C_6-C_7, known isomers	7	
C_8, known isomers	11	
		18
Alkyl cyclohexanes		
C_8-C_9, all isomers	10	
		10
Aliphatic olefins		
C_2-C_5, all isomers	12	
C_6, below 50° C.	1	
		13
Aliphatic diolefins		
C_3-C_4, allene, butadiene	2	
C_5, isoprene; *cis*-1,3-, *trans*-1,3-; 1,4-; 2,3-	5	
		7
Acetylenes		
C_2-C_4, simple isomers	4	
		4
Cyclic olefins		
C_5, cyclopentene	1	
		1
Cyclic diolefins		
C_5, cyclopentadiene	1	
		1
		114

given field can make type analyses of its more important raw materials, intermediates, and products, thereby determining what hydrocarbons are of greatest immediate importance in that field. Then the standard samples can be prepared.

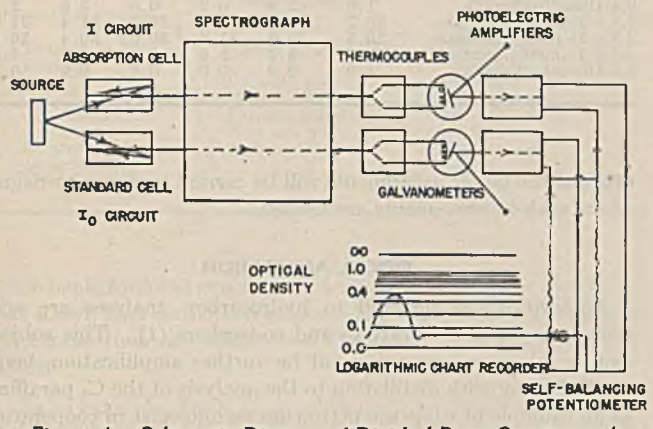

Figure 4. Schematic Diagram of Divided Beam Spectrograph

Regarding the choice and availability of hydrocarbon standards, a number of organizations have contributed as part of the war effort an important fund of information on the hydrocarbons present in aviation gasoline and in materials for synthetic rubber. This includes not only hydrocarbons isolated by analysis as at the National Bureau of Standards and the Pennsylvania State College, but also those prepared by synthesis under the auspices of the American Petroleum Institute—e.g., Ohio State University—and in the laboratories of the National Advisory Committee for Aeronautics, the General Motors Corporation, and several petroleum laboratories. Although some of these hydrocarbons may not be present in commercial streams, it is desirable to have them available as standards to facilitate research on manufacturing methods. Generally it is just as important to have standards representing the undesirable hydrocarbons. For example, in research on aviation gasoline, it is as important to have data on the methylheptanes which have poor antiknock quality as on the desirable trimethylpentanes, in order to obtain accurate analyses on the latter.

With advice from various expert sources, the committee of a newly constituted A.P.I. project (Project 46, Hydrocarbons for Spectrometer Calibration) has drawn up a preliminary list, which it is hoped will include the most useful standards. This is shown in Table II. Some of these hydrocarbons are now available in a relatively pure state, but some will have to be made. All will eventually be purified to a high degree and will be available to those who need them at or near cost. This is obviously a necessary cooperative service to utilize effectively the equipment and methods of spectrometry. It is hoped that contributions will come to that committee (refer to R. P. Anderson, American Petroleum Institute, 50 West 50th St., New York, N. Y.), both in hydrocarbons which might be available and in suggestions as to important hydrocarbons which should be added to the list.

PERSONNEL

If it is admitted that the various analytical techniques, new and old, must be used interdependently for greatest effectiveness, it can be seen that an investigator working alone needs to have mastered both fundamental chemistry and fundamental physics and must have developed some degree of skill in instrumentation. When working in a group, the chemist and the physicist can spend more effort in their respective fields; but they must work as an integrated team, so that the procedures worked out will be compatible with the ends in view and with the facilities available. In other words, the analytical procedure should be only sufficiently accurate for the job at hand, should be built around the type of equipment and operators available, and should be changed as circumstances and availability of new facilities dictate. In order to satisfy these requirements it is therefore necessary that close liaison be effected between the analytical staffs and the people who use the analytical results—namely, the manufacturing, development, and research departments.

INSTRUMENTATION

Among the most important factors contributing to the success of the newer methods have been the skill and the ingenuity of the instrument manufacturers. Without this contribution all the skill displayed by the analyst in the development of techniques would amount to little. As an example, there may be mentioned one recent development which was made by analysts at the Esso Laboratories, but which was made possible by the contributions of the instrument people. This development is the modification of a Gaertner infrared monochrometer to permit the recording of infrared spectra directly in terms of optical density. This modification has been of the greatest value in permitting the obtaining of "working" spectra of pure hydrocarbons directly from the instrument with a large saving in time. The modifica-

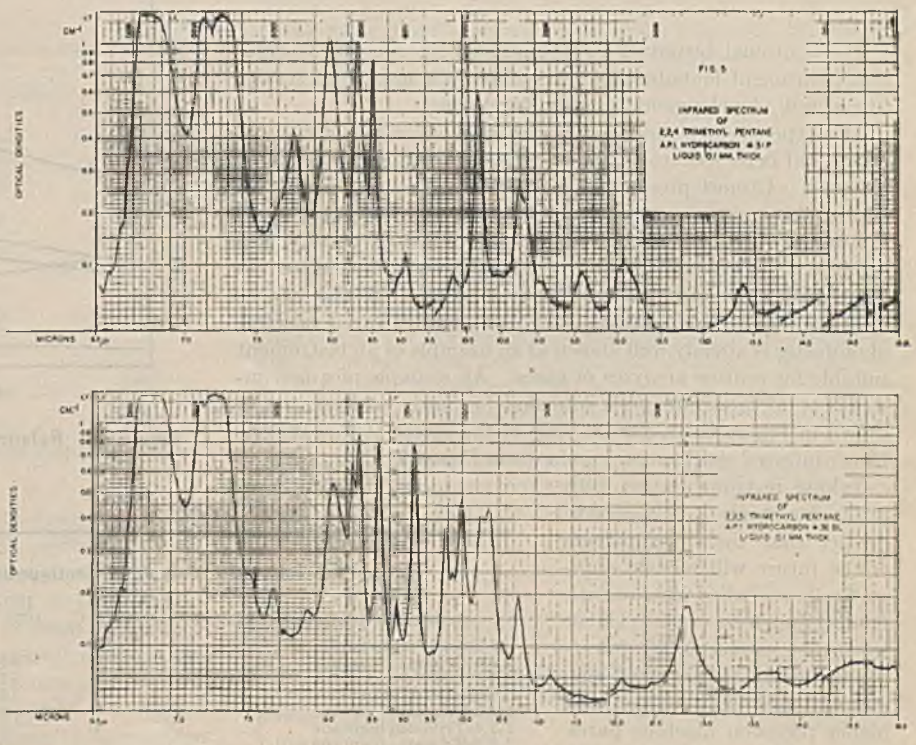

Figure 5. Infrared Spectra

Above. 2,2,4-Trimethylpentane. A.P.I. hydrocarbon 31 P. Liquid 0.1 mm. thick.
Below. 2,2,3-trimethylpentane. A.P.I. hydrocarbon 36 BL

tions are shown in Figure 4 where the light from the source is split into two beams, one passing through the cell containing the sample and the other through an empty, similar cell, after which both beams enter the monochrometer. The amount of energy from each of these beams is picked up by separate thermocouples, amplified, ratioed with a self-balancing bridge, and the result recorded directly in optical density. Stability and other characteristics essential for good quantitative analysis have been found entirely satisfactory. Figure 5 shows the spectra of 2,2,4-trimethylpentane and of 2,2,3-trimethylpentane as they were taken directly from the recorder.

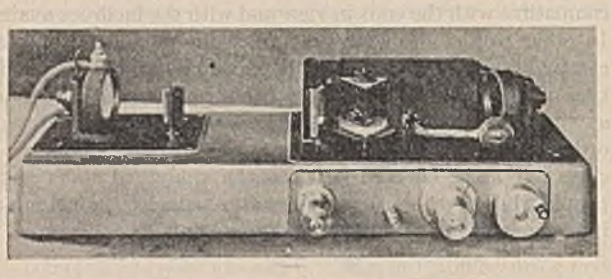

Figure 6. Perkin-Elmer Infrared Spectrometer

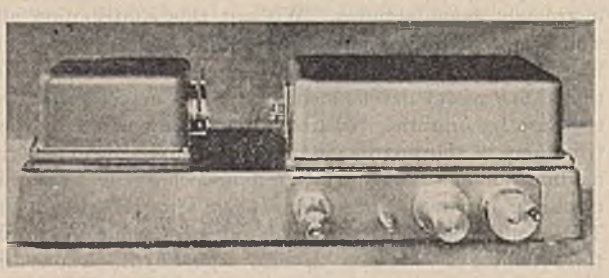

Figure 7. Optics of Perkin-Elmer Infrared Spectrometer

The continual introduction of improved instruments is proof that instrument manufacturers recognize their important service to research, development, and manufacturing.

The type of instruments under discussion—for example, infrared and mass spectrographs (*6*)—requires highly skilled workmanship. Utmost precision is needed in the optical, electrical, and mechanical trains of the apparatus, some of which are custom-built. The price range at the present time usually falls between the limits of $2000 and $20,000, depending upon the complexity of construction and the number of units that can be built to standardize design. The Beckman infrared spectrophotometer is already well known as an example of an instrument suitable for routine analyses of gases. An example of a new instrument of moderate price intended for fairly wide service is shown in Figure 6. A partial view of the optics of this Perkin-Elmer infrared spectrometer is shown in Figure 7.

Taking instrumentation with infrared as typical, it is easy to predict that the developments of the future will include automatically recording instruments, monochrometers with higher resolution, more sensitive detecting systems, better amplifiers, more intense light sources, and higher precision machine parts. One would also predict that the improvements to come in the other of the newer instruments will be carried to a similar degree where such improvements are needed.

Table III. Analyses of Synthetic Octane Blend

Constituent	Synthetic Composition	Analysis No. 1	Analysis No. 2	Analysis No. 3	Analysis No. 4	Analysis No. 5
2,2,4-Trimethylpentane	27.8	27.0	28.0	28.2	27.4	27.9
2,5-Dimethylhexane	1.7	0.0	2.3	1.8	1.4	2.3
2,4-Dimethylhexane	1.6	2.9	0.2	0.0	2.6	2.1
2,2,3-Trimethylpentane	30.7	32.2	32.1	32.4	31.4	31.0
2,3,4-Trimethylpentane	29.5	30.6	31.4	30.6	30.4	30.1
2,3,3-Trimethylpentane	6.8	6.4	6.0	7.0	5.9	6.6
2,3-Dimethylhexane	1.9	0.9	0.0	0.0	0.9	0.0

TYPICAL APPLICATION

Applications of infrared to hydrocarbon analyses are adequately covered by Brattain and co-workers (*1*). This subject can be taken as a case in point for further amplification, being applied along with distillation to the analysis of the C_8 paraffins, as an example of what the petroleum technologist in cooperation with the instrument designer can do. One important application of infrared spectrometry, when it is combined with suitable distillation, is the analysis for the individual paraffins in a mixture such as a commercial "isooctane" fraction. Distillation is an integral part of this program, to obtain cuts having not more than 8 components and to make separations where spectral differences of the hydrocarbons are not so pronounced as might be wished In Table III there are presented the infrared analyses of one synthetic mixture containing 7 of the isomeric octanes, to show that both accuracy and reproducibility are well within the limits required for most applications.

Previously, analyses of such mixtures as paraffinic alkylates have been done in an empirical way on the basis of boiling points in an "analytical distillation". In general, this has required a

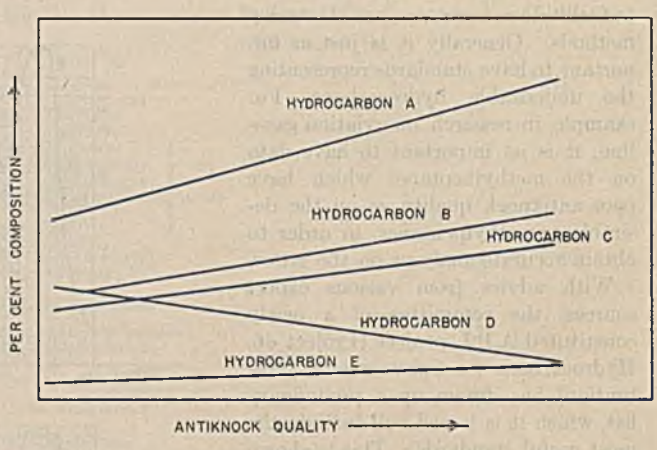

Figure 8. Relationship between Composition and Antiknock Quality

Table IV. Infrared Analysis of Contiguous Cuts Distilled from Experimental Fuel

Boiling Range	94–99° C.	99° C.	99–100° C.	100–109° C.	109–112° C.	113–114° C.		114° C.	
2,3-Dimethylpentane	17	..	..	..	..	..	..	..	..
2,2,4-Trimethylpentane	83	98	91	35	4	0	0	0	0
2,5-Dimethylhexane	..	1	6	35	30	14	8	4	0
2,4-Dimethylhexane	..	1	2	15	16	14	7	0	1
2,2,3-Trimethylpentane	..	0	1	9	12	8	6	5	4
2,3,4-Trimethylpentane	..	0	0	5	30	45	51	54	51
2,3,3-Trimethylpentane	..	..	..	0	8	19	26	33	40
2,3-Dimethylhexane	..	..	..	1	0	0	2	4	4

Table V. Analyses

Ultraviolet Absorption	Routine Infrared	Research Infrared
Benzene	Isobutane–*n*-butane	Individual C_6 paraffins
Toluene	Isopentane–*n*-pentane	Individual C_7 paraffins
C_8 aromatics	C_3-C_4-C_5 paraffins	Individual C_8 paraffins
C_9 aromatics	Isobutylene purity	Cuts of alkylate
Butadiene	Isobutylene	Cuts of hydrocodimer
	Methyl chloride	Cyclopentane
Ultraviolet Emission	Isobutane	C_4 saturates
		Amylenes
Potassium	Mass Spectrograph	Isohexenes
Barium		Benzene
Lead	Exhaust gas	Toluene
	C_4 cut	C_8 aromatics
Raman	Paraffin gases	C_9 aromatics
	Flue gas	
C_8 aromatics		
C_9 aromatics		
Simple paraffins		

large background of experience on similar products, for experience has shown that distillation plateaus in petroleum distillates represent more than one component when complex mixtures are distilled.

As an example, Table IV gives the infrared analyses of blended cuts prepared by Fenske (2) from the distillation of an experimental fuel in a still of 1-barrel capacity, with a fractionating column having 100 equivalent plates. Considering 2,2,3-trimethylpentane (b.p. 109.8° C.) it will be noted that this compound begins to show in the cut taken off at 99–100° C., increases to a maximum in the cut taken off at 109–112° C., and is still present to an appreciable extent in the cut taken off at 114° C. At the time the distillation was made the infrared technique was not available; with the aid of refractive indices and other physical constants, it was possible to translate the distillation data to accurate analyses in terms of the major components. However, this interpretation required much more experience and time than were subsequently needed when the infrared technique was applied.

A large number of aviation fuel-blending agents have been analyzed by infrared and the data have been found useful in a practical way for guiding research and process control. Figure 8 shows empirically the effect of change in composition of a blending agent on antiknock quality, the change in composition being due to changes in operating conditions.

Table VI. Savings by Optical Methods

	Method	Time *Hours*	Time by Other Methods *Hours*
Benzene and toluene	Ultraviolet	0.75	4
C_8 aromatics	Ultraviolet	1	(50)
Butadiene	Ultraviolet	0.25	1
Potassium	Ultraviolet	1	8
Barium	Ultraviolet	0.75	2.5
Sodium	Ultraviolet	1	8
Lead	Ultraviolet	1	2.5
Total C_4 cut	Infrared	1	3
Iso- and *n*-butane	Infrared	0.25	0.5
Iso- and *n*-pentane	Infrared	0.167	5
C_3, C_4, and C_5 paraffins	Infrared	0.75	5
C_8 paraffins	Infrared	6	(200)
C_7 paraffins	Infrared	6	(200)
C_4 paraffins	Infrared	2	(200)
C_4 olefins	Infrared	3	16

Regarding more general applications of spectrometry, Table V lists a number of analyses found possible to perform by spectrographic means. This list by no means exhausts all the possibilities. In particular, much wider application of the emission spectrograph should prove a valuable asset in analytical work.

As to the savings which are possible by optical methods, an attempt has been made to present a rough estimate in Table VI. In almost all the cases shown there is an appreciable saving in time over conventional methods. In some cases, the advantage for the spectrographic method is more than 200 hours. In practice, these older time-consuming methods would rarely, if ever, be used. The estimates given in parentheses are not so firm as the others, but they are reasonable for methods based upon fractional distillation at very high reflux ratios.

SUMMARY

Much progress has been made in applying the newer techniques of physics to hydrocarbon analysis, and greater advances are to be expected. However, no one method is self-sufficient, and its usefulness is dependent largely on judicious combination with other methods. It might be expected that the marked decrease in time required to complete an analysis should result in a decrease in operating costs of an analytical laboratory. It may happen, however, that when the utility of such analyses becomes apparent, much more analytical work is undertaken. In such a case the total cost may go up; but this may be fully justified by a marked gain in speed of research, control of operations, and improvement of product quality.

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Standards for Analytical Filter Papers

Methods have been developed at the National Bureau of Standards, Washington, D. C., for testing filter papers and applying the methods to the establishment of standards of quality. These include rate of flow of water, retention of fine precipitates, and bursting strength of wet paper, and an improved method for determination of ash content. Other tests considered by the bureau as desirable for a thorough evaluation of filter papers are thickness, weight per unit area, alpha-cellulose, copper number, and pH.

The bureau has completed testing the various types and grades of analytical filter papers made in England, Sweden, and the United States, and found them all to be of good and in most respects of equal quality.

CORRECTION. In the article on "Sulfuric Acid Extraction in Hydrocarbon Type Analysis" [IND. ENG. CHEM., ANAL. ED., 16, 558 (1944)], two errors appeared. The estimated accuracy of the volume per cent of saturates should be ±2% instead of ±0.2% as printed. In Table III, column 2, the last blend number should be 7 instead of 6 as printed.

CLYVE C. ALLEN

Spectrochemical Analysis with the Air-Acetylene Flame

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IN THE spectrochemical analysis of biological and related materials certain elements may be encountered occasionally at levels of concentration that do not require methods of maximum sensitivity. In such cases the prime requisites are reproducibility and accuracy of results and convenience of procedure, qualities which are more easily met by the air-acetylene flame method than by any other form of excitation. In spite of this and other advantages, spectrographers in this country have made little use of the flame method, and therefore the authors' experience in the adaptation and application of technical procedures is given in this article. Much of the material is not original, but the information is widely scattered in the literature and since many scientific journals are not readily available at the present time, its presentation should be useful.

PRINCIPLE

With the air-acetylene flame it is possible to detect 34 elements (*5*), among which are those of greatest interest from a biological standpoint. The elements that can be detected are indicated within the blocked-off portions of Table I. The approximate molar sensitivities of detection are indicated in footnotes. The flame is used in the analysis of solutions which are carried as fine mists in the air supplied to the acetylene. The resulting light is then analyzed (photographed) by means of an appropriate quartz spectrograph. The photographed lines are measured with a suitable densitometer, the densities of the lines obtained under rigidly controlled conditions of exposure and photography being proportional to the amount of element which produced them.

EQUIPMENT

Figure 1 illustrates the atomizer and burner, while Figure 2 shows the equipment in place for use. In details of construction this equipment follows Lundegårdh's (*6*, *7*) latest design as described by McClelland and Whalley (*8*). Although the general construction is evident from the illustration, a number of measurements and details have been omitted to make the drawing simpler, and these are given below. Ready-built atomizers and burners may be imported at an approximate cost of $900, but the equipment illustrated was built for about $200, the most costly item being the fabrication of the platinum-iridium components. The cost may be further reduced by using less expensive and less resistant alloys for the metal parts, but these may have to be replaced frequently.

Air at 2.8 kg. per sq. cm. (40 pounds per square inch) is taken from a compressor and passes through the brass tube, *A*. The air in passing through the small hole in *C* (platinum-iridium jet 0.4 cm. in outside diameter, 0.2 cm. in inside diameter except 0.04-cm. opening at top) and the platinum-iridium disk, *F* (0.1-cm. hole widening to a 45° angle) sucks up the fluid which has entered the ebonite lower portion of nozzle *D* (1.2 cm. in diameter) and carries it as a spray through the opening (0.3 cm.) in the adjustable ebonite upper portion of nozzle *E*. (The brass tube, *A*, to which *C* and *D* are fixed, is held in place in the glass 19/38 $ male joint by means of the lock nut, *B*, and a rubber washer.) The larger droplets of the spray issuing from *E* are returned to the main portion of the solution when they strike the walls of the glass vessel, while the air carrying the fine mist is led to the burner through *G*. Opening *G* must be large enough, so that liquid flowing down the walls is not forced through it; if this should occur, the liquid would fill the narrower opening in which this outlet ends, thus producing a pulsating air stream through the burner. The opening in *D* should be made low enough so that the atomizer can handle 2 cc. of sample if required to do so. The atomizer illustrated, when set in a vertical position, can handle 5 to 20 ml. of solution, but when set at an angle it works well with only 2 ml.

The air compressor is operated at pressures between 6.7 and 8.1 kg. (95 and 115 pounds) by means of a Cutler-Hammer, Style D, regulator switch and is provided with an outlet carrying a 3.5-kg. (50-pound) maximum adjustable pressure regulator.

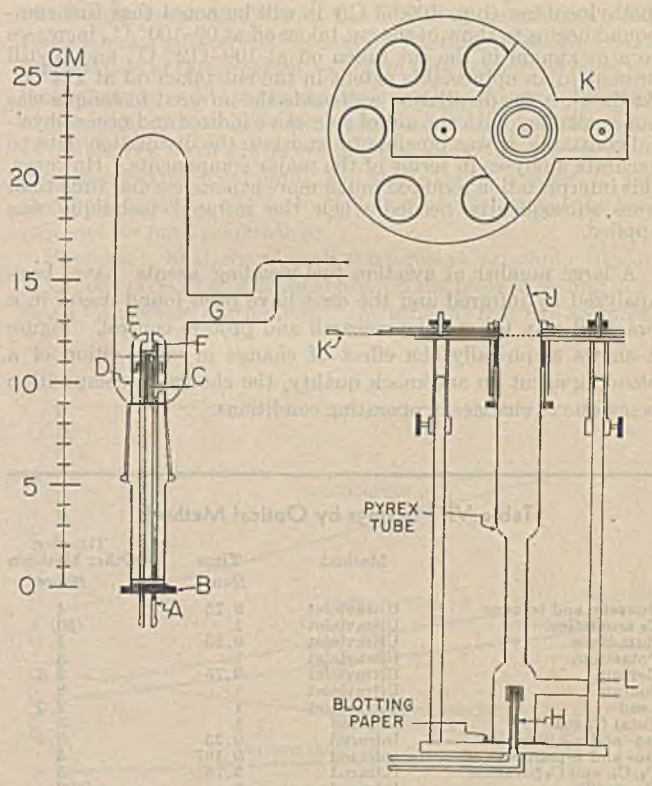

Figure 1. Details of Construction of Lundegårdh Atomizer and Burner

Table I. Elements Susceptible of Flame Analysis

Shell	I	II	III	IV	V	VI	VII		VIII		0
1	1 H	.....	.....	....	...	....	.....	....	....	....	2 He
2	3 Li[a]	4 Be	5 B	6 C	7 N	8 O	9 F	....	....	....	10 Ne
3	11 Na[a]	12 Mg[b]	13 Al	14 Si	15 P	16 S	17 Cl	....	....	....	18 A
4	19 K[b]	20 Ca[a]	21 Sc[d]	22 Ti	23 V	24 Cr[b]	25 Mn[a]	26 Fe[b]	27 Co[b]	28 Ni[b]	36 Kr
	29 Cu[a]	20 Zn[c]	31 Ga[d]	32 Ce	33 As	34 Se	35 Br	....	....	....	...
5	37 Rb[a]	38 Sr[a]	39 Y[d]	40 Zr	41 Cb	42 Mo	43...	44 Ru[a]	45 Rh[d]	46 Pd[b]	...
	47 Ag[a]	48 Cd[c]	49 In[d]	50 Sn	51 Sb	52 Te	53 I	....	....	....	54 Xe
6	55 Cs[b]	56 Ba[b]	57–71 Rare earths[e]	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	...
	79 Au[a]	80 Hg[c]	81 Tl[a]	82 Pb[b]	83 Bi	84 Po	85...	....	....	....	86 Rn
7	87...	88 Ra	89 Ac	90 Th	91 Pa	92 U	93...	94...	....	....	...

[a] 0.0001 molar. [b] 0.001–0.0001 molar. [c] 0.001 molar. [d] Undetermined. [e] Rare earths, Dy, Gd, La, Nd, and Pr, all undetermined.

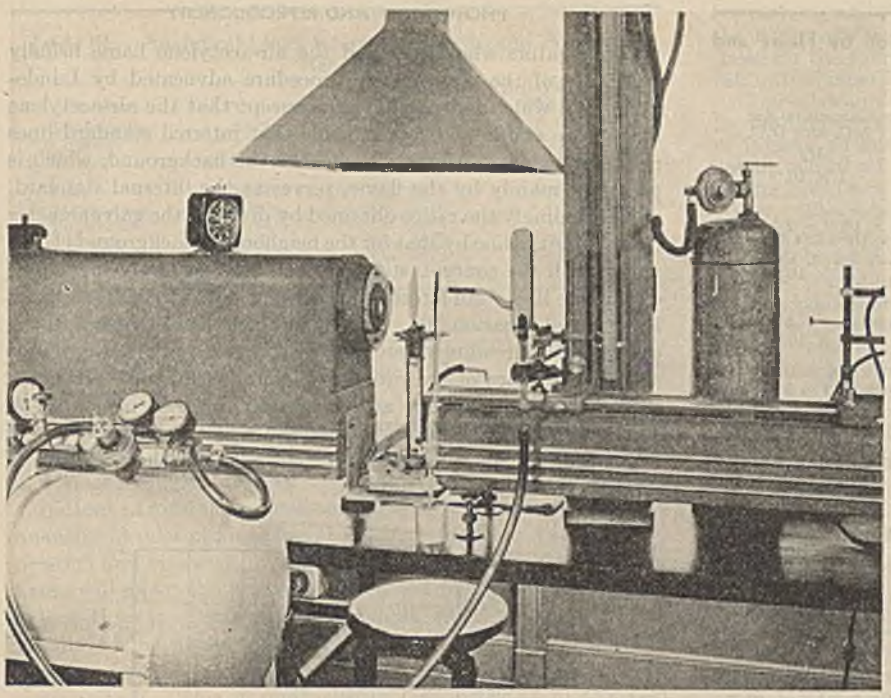

Figure 2. Burner Equipment Set Up for Use

In the moderate range of pressures at which the compressor operates, the regulator delivers air at 2.8 ± 0.035 kg. per sq. cm. (40 ± 0.5 pounds per square inch) pressure.

The mist issuing from *G* is led to the burner through *L* and is mixed with acetylene in the narrow portion of the Pyrex tube. Acetylene at 25-cm. water gage enters the burner through *H* (platinum-iridium jet 0.4 cm. in outside diameter, 0.2 cm. in inside diameter, with screw cap of same material holding in place a platinum-iridium disk 0.3 cm. in diameter with a centered hole 0.05 cm. in diameter). The air-acetylene mixture issues through a platinum wire screen (45-gage, 0.002-cm., 0.008-inch, wire) carried in the rotatable disk, *K*, and finally passes through the platinum-iridium cone, *J*.

Acetylene is obtained from a Prest-O-lite Type B (40 cubic feet) cylinder and is maintained at 25 ± 0.2 cm. water gage by means of a Type OO (10-pound) Prest-O-lite regulator and a second needle valve between the regulator and the water manometer. Before entering the burner the acetylene is passed through a water bubbler which keeps the gas moist and prevents clogging of the orifice in *H* by material which otherwise would deposit, because of drying of the mist (*8*). The bubbler also serves to remove minute particles of cylinder-packing material which would clog the small orifice. It is good practice, before inserting a full Prest-O-lite cylinder, to blow off (in the open air) a sufficient amount of acetylene to make certain that acetone spray is not carried along with the acetylene.

The burner is so placed that the cone of the flame is in the optical axis and in the case of a Bausch & Lomb medium quartz spectrograph, it is set 5.5 to 6.0 cm. from the slit. Its height is so adjusted that the tip of the blue inner cone of the flame is about 10 mm. below the opening of the slit of the spectrograph. In this position maximum intensity is obtained (*5*) and the amount of light entering the slit is further increased by reflection from a plane chrome-plated mirror set immediately behind the flame (not shown in Figure 1). A smaller spectrograph than the one indicated can be employed, but its dispersion must be sufficient to separate cleanly the manganese triplet at 4031 Å. from the potassium doublet at 4044/7 Å. In the particular work described the standard period of exposure was 2 minutes. Eastman No. 33 plates were used for the determination of all lines from Mg 2852 Å. to Sr 4607.3 Å., and Panchromatic plates for lines at longer wave lengths, particularly for Na 5890/5.9 Å. and Li 6707.9 Å.

SENSITIVITIES

In practice the sensitivity and the reproducibility obtainable depend on a strict standardization of the details of operation. Each atomizer and burner operates most efficiently and yields maximum sensitivity at air and acetylene pressures which are unique, dependent upon the size of the orifices of the spray and acetylene jet. Lundegårdh worked at air pressures varying from 30 to 180 pounds per square inch and 35-cm. water gage acetylene (*5*); McClelland and Whalley employed air at 100 pounds per square inch, 40-cm. acetylene (*8*); Ells (*2*) air at 30 pounds per square inch and acetylene at 22 cm.; while Griggs and co-workers (*4*) used air at 37.5 pounds per square inch and acetylene at 26 cm. The authors used air at 40 pounds per square inch and acetylene at 25 cm. These conditions were chosen as the most suitable for the simultaneous analysis of certain concentrations of magnesium, copper, sodium, iron, manganese, potassium, calcium, and strontium when all of these cations or various combinations of them were present in the samples. Whenever ultimate sensitivity is required, however, each element presents a problem in itself and the best conditions for its detection must be determined beforehand.

The variations in the line intensities of certain elements, due to varying air pressures at a fixed acetylene pressure of 25 cm., are graphically illustrated in Figure 3. These relative intensities were obtained by means of an intensity calibration standard placed on the plate with a step sector. The density-intensity plot of the plate calibration was made as described by Pierce and Nachtrieb (*12*) for use in correcting for background. Figure 3 demonstrates that with an acetylene pressure of 25 cm. the air pressures which are satisfactory for the maximum sensitivity of detection of sodium and manganese, among others, are not suit-

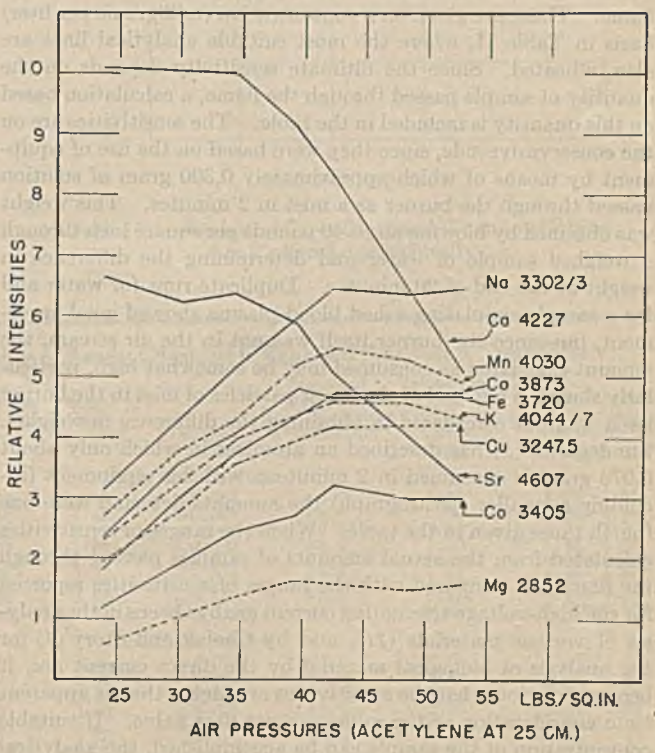

Figure 3. Variations in Line Intensities Obtained with Variations in Air Pressure

Table II. Comparison of Sensitivities of Detection by Flame and Arc Excitation

Element	Line	Sensitivity	Element Passing through Burner in 2-Min. Exposure	Element on Arc A.C. and D.C.
	Å.	Mg./l.	Mg.	Mg.
Ag	3280.7	5	1.5×10^{-3}	1×10^{-5}
Au	2676	20	6.0×10^{-3}	.....
Ba	5535.5	100	3×10^{-2}	.....
Ca	4226.7	0.4	1.2×10^{-4}	$1 \times 10^{-4} - 2 \times 10^{-5}$
Cd	3261.1	200	6×10^{-2}	$2 \times 10^{-4} - 1.7 \times 10^{-5}$
Co	3526.9	1	3×10^{-4}	1.1×10^{-4}
Cr	3578.7	0.5	1.5×10^{-4}	1×10^{-5}
Cs	4553.3	50	1.5×10^{-2}	.....
Cu	3247.5	0.5	1.5×10^{-4}	$1.7 \times 10^{-4} - 5 \times 10^{-6}$
Fe	3849.9[a]	5.0	1.5×10^{-3}	$6 \times 10^{-5} - 5 \times 10^{-6}$
Hg	2536.5	200	6×10^{-2}	2×10^{-4}
K	4044.2/7.2	8	2.4×10^{-3}	.....
Li	6707.9	0.1	3×10^{-5}	1.4×10^{-6}
Mg	2852.1	5.0	1.5×10^{-3}	$6 \times 10^{-5} - 1 \times 10^{-5}$
Mn	4030.8[b]	0.3	9×10^{-5}	$1.3 \times 10^{-3} - 1 \times 10^{-6}$
Na	5890/5.9	0.2	6×10^{-5}	.....
	3302.3/2.9	11.5	3.5×10^{-3}	2×10^{-3}
Ni	3414.8	10	3×10^{-3}	$2 \times 10^{-4} - 1.5 \times 10^{-5}$
Pb	4057.8	100	3×10^{-2}	$1 \times 10^{-4} - 8 \times 10^{-7}$
Pd	3634.7	20	6×10^{-3}	.....
Rb	4201.8	10	3×10^{-3}	.....
Ru	3725.9/8.0	10	3×10^{-3}	.....
Sr	4607.3	0.2	6×10^{-5}	$6 \times 10^{-5} - 5 \times 10^{-6}$
Tl	3775.7	0.4	1.2×10^{-4}	2×10^{-5}
Zn	3072.1	3000	9	$2 \times 10^{-3} - 3 \times 10^{-4}$

[a] 3719.9 is somewhat more sensitive.
[b] This is really a triplet.

able for the most sensitive detection of calcium and strontium. In the first case maximum sensitivity is obtained at 42.5+ pounds' air pressure, and in the second case, between 25 and 30 pounds' air pressure. Therefore when calcium and strontium must be determined simultaneously with other elements, the conditions chosen must be the most favorable for the detection of the cation present in the smallest amount. When the above test is repeated at a lower acetylene pressure (22 cm.) the shapes of the intensity variation curves are essentially the same, but occur at lower levels. Some increase in the intensity levels is obtained at higher acetylene pressures, but the gain for all practical purposes is not sufficiently great to warrant the increased use of acetylene.

Lundegårdh (*5*) has determined the ultimate sensitivities of detection of most of the elements detectable by the air-acetylene flame. These are given on a concentration (milligrams per liter) basis in Table II, where the most suitable analytical lines are also indicated. Since the ultimate sensitivity depends on the quantity of sample passed through the flame, a calculation based on this quantity is included in the table. The sensitivities are on the conservative side, since they were based on the use of equipment by means of which approximately 0.300 gram of solution passed through the burner as a mist in 2 minutes. This weight was obtained by blowing air at 40 pounds per square inch through a weighed sample of water and determining the difference in weight at the end of 20 minutes. Duplicate runs for water and for a sample simulating ashed blood plasma showed good agreement, but since the burner itself was not in the air stream, the amount calculated as consumed may be somewhat high, particularly since the removal of the larger particles of mist in the burner itself was not considered in obtaining the difference in weights. Lundegårdh (*5*) has described an atomizer in which only about 0.075 gram is consumed in 2 minutes; with his equipment (including a smaller spectrograph) the amounts detected were one fourth those given in the table. When the ranges of sensitivities calculated from the actual amounts of samples passing through the flame are compared with the ranges of sensitivities reported for the high-voltage alternating current arc by Owens in the analysis of various materials (*11*), and by Cholak and Story (*1*) for the analysis of biological material by the direct current arc, it becomes obvious that the sensitivities are higher than is apparent from consideration of the milligram per liter value. If suitable concentration of the sample can be accomplished, the analytical range for many elements is only slightly inferior to that obtained with the stronger excitation methods.

PHOTOMETRY AND REPRODUCIBILITY

Investigators who have used the air-acetylene flame usually make use of the photometric procedure advocated by Lundegårdh (*5*), which is based on the concept that the air-acetylene flame is so stable and reproducible that internal standard lines need not be used. As a consequence the background, which is produced mainly by the flame, serves as the internal standard, and accordingly the ratios obtained by dividing the galvanometer reading for the line by that for the neighboring background (L/H) varies with the concentration, in the flame, of the element producing the line. An alternative method, called the L-H method, is also used occasionally. In this method the differences in the galvanometer readings between the lines and the backgrounds are used in place of the ratios. The standardization curves resulting from these data are illustrated for iron in Figure 4. Both curves show appreciable curvature at the higher levels of concentration, and consequently a decrease in the accuracy of analysis in this region is implied.

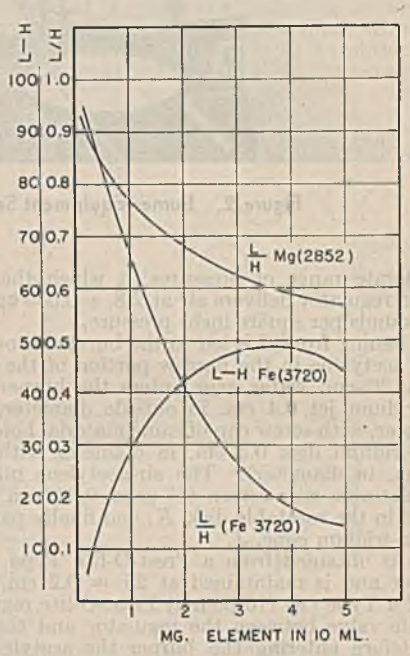

Figure 4. Typical Analysis Curves Obtained with L/H or L-H Method of Photometry

In order to correct for variations in the sensitivity of individual plates, as well as for slight variations in the excitation conditions, Lundegårdh derives a master curve by averaging the results for the standard solutions taken on numerous plates. Samples to be analyzed are next placed on the same plate with a series of standard solutions. The L/H ratios of the samples are then corrected by applying a factor representing the mean ratios between the L/H values of the standard solutions on the particular plate and the corresponding L/H values of the master curve.

This procedure has been used successfully, but it appears to have a number of disadvantages. A more convenient calibration curve, particularly one with a linear response over the entire analytical range, would increase the analytical accuracy at higher concentrations and would reduce considerably the frequency with which an analysis must be repeated after dilution of a sample to bring it into the most favorable portion of the L/H curve (*5*). This apparently can be accomplished merely by altering the manner in which the data are plotted. If the L/H ratios are plotted on a logarithmic scale, a more linear calibration results, as can be seen from Figure 5. In the particular case

Table III. Analytical Lines, Internal Standards, and Analytical Ranges

Element	Wave Length	Internal Standard	Analytical Range, Mg./10 Ml.
Magnesium	2852	Co 3405	0.05–5.0
Copper	3247.5	Co 3405	0.04–2.5
Sodium	3302/3	Co 3405	0.30–25.0
Iron	3719.9	Co 3873	0.05–5.0
Manganese	4030	Co 3873	0.005–0.30
Potassium	4044/7	Co 3873	0.06–4.0
Calcium	4227	Co 3873	0.005–0.20
Strontium	4607	Co 3873	0.004–0.30

illustrated, the 5-mg. value is slightly high, causing a slight deviation from linearity for values above 3 mg., for the reason given below.

The method of correcting for variations in individual plate sensitivity in the L/H method is inconvenient, time-consuming, and uneconomical. Moreover, it does not correct truly for variations in excitation conditions arising from differences in the quantity of mist produced in atomizing samples of varying composition and viscosity. Variation in the quantity of mist in the flame will affect the intensity of the test line to a much greater extent than it affects the background, since the latter is due mainly to the flame. Neither method (L/H or L-H) corrects properly for the effect of deep background which arises from the sample itself. This is most clearly demonstrated in the L-H curve of Figure 4, where the background subtractions are too high, since the L-H value for 5 mg. of iron is lower than that obtained with 2.5 mg. of iron. When using the L/H method under the same conditions, the L/H values will be high and they will tend to flatten the curves at the higher concentrations, as in Figure 4, or cause them to deviate from the linear relationship, as indicated in Figure 5.

Reproducible results therefore can be obtained with the L/H or L-H method only by the strict standardization of all the possible variants. In spite of the fact that in the flame as compared to the arc there is little interionic action to affect line intensities, the presence of moderate quantities of certain cations (magnesium, sodium, potassium, iron, and aluminum, among others) will produce marked changes in the background. As a result it is not possible with the L/H or L-H method to derive a single analytical curve which can be used with equal accuracy for the analysis of materials showing large differences in matrix composition.

All the above-mentioned disadvantages, however, disappear if internal standard lines are used in place of background and if the plate calibration and background correction of modern photometry are employed.

The method used by the authors is the conventional one, in which the intensity ratio (*I* line/*I* standard) is obtained from the plate intensity calibration pattern made for each batch of plates, the intensity of each line being corrected for the background in which it lies by the method of Pierce and Nachtrieb (*12*, *13*). Plate intensity calibration marks are obtained with a rotating step sector (6 steps, factor of 2) and a low amperage arc (2.5 amperes) across 7-mm. diameter brass rods. Eastman No. 33 plates are used for the analysis of all lines from 2852 to 4607 Å. and because of the variation of gamma with wave length separate plate calibrations are obtained for the 2850 to 3600 A. and for the 3600 to 4607 Å. regions. The slopes of the H and D curves taken at a number of wave lengths in each region show such slight differences that no appreciable error is introduced by the use of a single standard line for each region. The slopes of the group of H and D curves for the 3600 to 4600 Å. region, however, are somewhat greater than those for the shorter wave-length region and therefore a separate calibration must be obtained for each region.

Concentration calibrations were obtained for eight elements by diluting serially a stock solution containing all the elements of interest in addition to 10% hydrochloric acid and 5 mg. of cobalt per 10 ml. of solution. The diluent consisted of a 10% hydrochloric acid solution of cobalt chloride, 5 mg. of cobalt per 10 ml. The cobalt line at 3405 Å. was used as the standard for all lines between 2850 and 3600 Å., while the cobalt line at 3873 Å. was employed for analysis in the region between 3600 and 4607 Å. Two-minute exposures, air pressures of 40 ± 0.5 pounds per square inch, and acetylene pressures of 25 cm. were employed. These conditions were chosen for the reasons given under "Sensitivities" and also because at the adopted conditions the intensity ratios showed practically no variation due to small changes in the excitation conditions.

The elements for which analytical curves have been derived, the internal standard lines used, and the analytical ranges covered (expressed as milligrams per 10 ml. of solution) are given in Table III. The lower concentration values in many cases can be reached only by doubling or tripling the exposure period. Tests on standard solutions have shown that the intensity ratios given by the increased exposures fit the values obtained by extrapolating calibration curves derived at higher concentrations and with 2-minute exposures. The characteristic analytical curve obtained when log intensity ratio is plotted against log concentration is illustrated in Figure 6, the data being taken

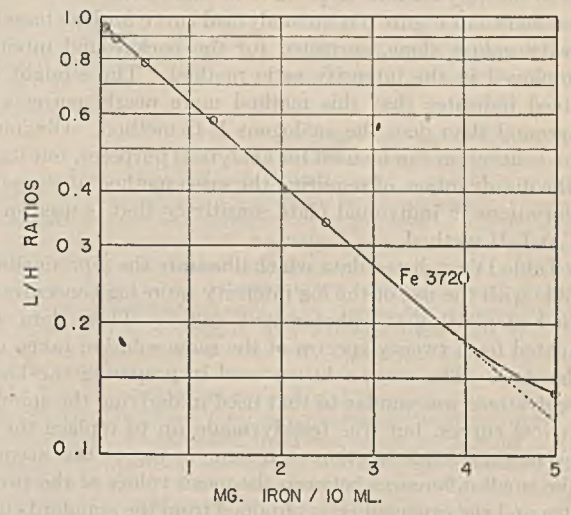

Figure 5. Analytical Curve Obtained When L/H Ratios Are Plotted Logarithmically

Table IV. Reproducibility of Results Obtained in Repeated Analyses of Same Sample

Element	Amounts Added *Mg.*	Expected Ratio from Standard Curve	Mean Ratio Obtained	Std. Dev.	Standard Deviation % or Coefficient of Variability	Values from Calibration Curve *Mg.*	Spread Calculated as Due to Standard Deviation % *Mg.*
			Individual Spectra				
Magnesium	1.50	0.62	0.61	±0.053	±8.7	1.49	1.35 –1.62
Copper	0.60	1.40	1.46	±0.067	±4.6	0.65	0.62 –0.68
Sodium	7.50	2.25	2.30	±0.068	±2.95	7.60	7.4 –7.8
Iron	1.50	1.07	1.07	±0.033	±3.1	1.50	1.46 –1.55
Manganese	0.09	1.23	1.21	±0.041	±3.4	0.089	0.086–0.092
Potassium	1.2	0.98	1.04	±0.033	±3.2	1.25	1.21 –1.29
Calcium	0.12	2.20	2.08	±0.11	±5.3	0.112	0.106–0.118
Strontium	0.09	1.35	1.35	±0.042	±3.1	0.09	0.088–0.092
			Duplicate Spectra				
Magnesium	1.50	0.62	0.61	±0.046	±7.6	1.49	1.38 –1.60
Copper	0.60	1.40	1.45	±0.057	±3.9	0.64	0.61 –0.66
Sodium	7.50	2.25	2.30	±0.041	±1.8	7.60	7.45 –7.75
Iron	1.50	1.07	1.07	±0.025	±2.3	1.50	1.47 –1.53
Manganese	0.09	1.23	1.21	±0.025	±2.1	0.089	0.087–0.090
Potassium	1.2	0.98	1.03	±0.016	±1.55	1.25	1.23 –1.27
Calcium	0.12	2.20	2.08	±0.075	±3.6	0.112	0.108–0.116
Strontium	0.09	1.35	1.34	±0.024	±1.8	0.09	0.088–0.091

Table V. Effect of Size of Sample on Analytical Reproducibility

Ml. of Original Sample in Atomizer	Mg. of Element per Liter of Tap Water Ca	Mg	Na	K	Fe	Sr
1	31.0	..	..	...	..	...
5	29.0	..	..	...	..	...
100	..	5.0	..	1.6	..	0.13
200	..	5.25	9.5	1.5	..	0.135
500	..	4.9	10.0	1.6	0.11	0.126

Table VI. Effect of Method of Sample Preparation on Cation Recovery

Technique	Grams per Liter of Urine Mg	Na	K	Ca
Dry ashing	0.10	3.55	2.0	0.029
Sulfuric acid–nitric acid digestion	0.115	4.00	2.20	0.037
Nitric acid–hydrogen peroxide digestion	0.110	3.90	2.20	0.037
Untreated	0.10	3.90	2.10	0.035

from the same plate used for Figures 4 and 5. The slopes of all the concentration calibration lines except that for magnesium show only slight variation from that of the iron analytical line, the differences being due to the reversibility shown by some of the lines (*12*). The greater steepness of the magnesium-analytical line cannot be attributed solely to the factor of reversibility, but regardless of the real reason for this phenomenon it is evident that the magnesium plot in Figure 6 is superior for analytical purposes to the very shallow L/H curve of Figure 4.

Also shown in Figure 6 is an analytical curve for iron based on intensity values alone, corrected for the background intensity as employed in the intensity ratio method. The straight line obtained indicates that this method more nearly corrects for background than does the analogous L-H method. Obviously, such a calibration can be used for analytical purposes, but its use has the disadvantage of requiring the same method of correcting for variations in individual plate sensitivity that is used in the L/H or L-H method.

In Table IV are listed data which illustrate the reproducibility possible with the use of the log intensity ratio-log concentration method of plotting the photometric values. These data were calculated from twenty spectra of the same solution taken on a single plate. The stock solution used in preparing the known concentrations was similar to that used in deriving the standard analytical curves, but was freshly made up to replace the depleted original stock solution. In some instances this accounts for the small differences between the mean values of the twenty spectra and the expected ratio obtained from the standard curve. The statistical analysis was made on the ratio values obtained, but corresponding concentration values are also given for these data. By way of comparison, it is interesting to note Mitchell's study of 15 spectra of a 0.000125 *M* manganese chloride solution (*9*), in which he obtained a standard deviation of ±6.41% in the L/H ratios of the single spectra and ±4.5% when duplicates were used. Lundegårdh (*5*) reports that in general the error of determination never exceeds 5 to 7%, and in dealing with the elements calcium and manganese, the errors are not more than 1 to 2%.

The errors of analysis indicated in Table IV may have been increased by the use of a single calibration pattern for plates from the same production batch. In all probability the portion of the error due to the photometric procedure can be reduced by placing an intensity calibration pattern on each plate.

Table V is included in order to demonstrate further the reproducibility of certain analyses as obtained by the use of different aliquots of the same prepared sample of tap water. Excellent agreement in the results for the elements listed is shown. Indeed, the reproducibilities of results for the ranges of concentration listed in Tables IV and V compare favorably with those obtained by the most sensitive chemical methods, and in some cases they are far superior.

APPLICATIONS

Since the flame gives rise to the low-temperature lines typical of atoms or molecules in the lowest states of excitation, the spectra produced are very simple, being characterized by a minimum of masking of important analytical lines. This is particularly favorable in connection with the choice of a spectrograph, since it permits the use of a smaller, less expensive instrument possessing a dispersion sufficient merely to separate the manganese 4031 Å. lines from the potassium doublet at 4044/7 Å. In addition to the saving effected in the purchase of suitable equipment, further economies occur in the operation of the method itself due to the low cost of acetylene and the elimination of electrodes which are very expensive when spectroscopically pure.

The air-acetylene flame is characterized by the ease and accuracy with which it is possible to reproduce the conditions of excitation and by the comparative freedom from reactions between the various ions carried by the spray into the flame (*5*). The latter point is of distinct advantage in accurate spectrochemical analysis, since in most applications it eliminates the need for the use of buffer salts to ensure conformity in composition of samples and the standards used to derive the analytical curves. Therefore a single curve derived from water solutions of salts of the elements of interest can be applied to the analysis of many materials which vary widely in inorganic salt composition. This is true, however, only if the method of photometry accurately corrects for the background intensity. Such accurate corrections of background are obtained by the method of photometry employing internal standard lines and intensity calibration standards, when used in the manner described above.

The absence of reactions between the cations and anions of a sample in the flame simplifies the chemical procedures needed to prepare samples for analysis. Inorganic material and biological ash may be placed in solution by the use of dilute solutions of hydrochloric, nitric, or sulfuric acid, or by water alone (*5*). However, in order to protect the platinum components of the equipment, mixtures of hydrochloric and nitric acids must be avoided. In the case of fluid biological material, it is frequently possible to analyze the material without any preparatory chemical treatment. This is particularly true of samples of urine in which only 1 ml. of fresh urine, provided with the internal stand-

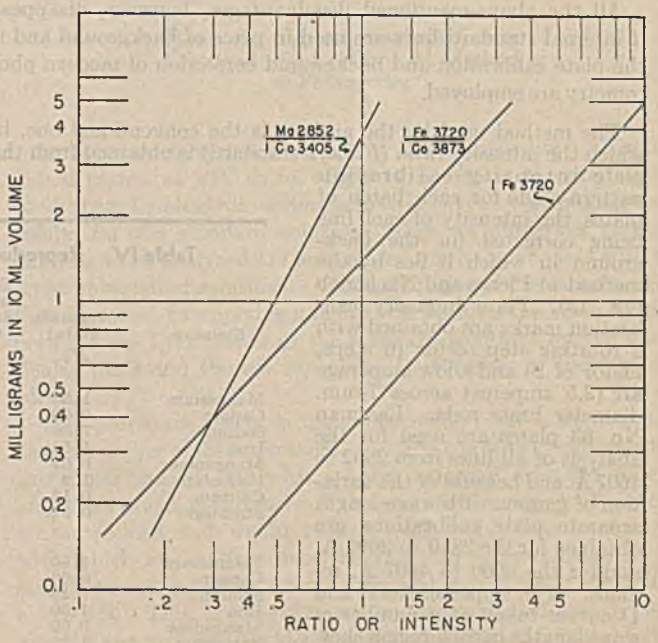

Figure 6. Typical Analytical Curves Obtained by Use of Internal Standards and Plate Calibration Procedures

Table VII. Applicability, Methods of Preparation, and Dilutions Required for Flame Analysis

Material	Amount of Sample	Preparation	Final Volume and Dilutions	Exposure	Cation Determined
			Ml.	*Min.*	
Tap water	500 ml.	Evaporate to dryness, dissolve in HCl and H_2O	10	2	Mg, Na, K, Sr, Fe, Cu, Mn
			× 100	2	Ca
Inorganic salts	0.1–1.0 g.	H_2O or H_2O + acid solution, evaporate to volume	10	2	34 elements in proper concentration
Whole blood	2 ml.	H_2SO_4-HNO_3-$HClO_4$ digestion	10	4	Mg, Na, Fe
			× 1	2	Na, Fe, K, Ca
Blood serum or plasma	2 ml.	H_2SO_4-HNO_3-$HClO_4$ digestion	10	4	Mg, K, Na
			× 1	2	Ca, K
Tissue samples	5 g.	H_2SO_4-HNO_3-$HClO_4$ digestion	10	2	Mg, Fe, Cu, Ca, Na
			× 1	2	K
Fruit and vegetable juices	50 g. or ml.	H_2SO_4-HNO_3-$HClO_4$ or HNO_3-H_2O_2 digestion	10	2	Mg, Cu, Fe, Mn, Sr, Na
			× 50	2	Na, K, Ca
Individual food	20–50 g.	H_2SO_4-HNO_3-$HClO_4$ or HNO_3-H_2O_2 digestion	10	2	Mg, Cu, Mn, Fe, Na, K, Ca
			× 20	2	Ca, Na, K, Mg
Soils (exchangeable base)	5 g.	Leach with H_2O or N NH_4Ac, evaporate to dryness, H_2O_2 to destroy organic matter	25 cc.	2	Mg, Mn, Sr, K, Na, Fe, etc.
			× 50	2	Ca
Alloys	0.1–1.0 g.	H_2O + acid, evaporate to volume	10	2	Any of 34 in proper concentration
			× ?	2	More abundant element

Table VIII. Typical Analyses with the Air-Acetylene Flame

Material	Mg	Cu	Na	Fe	Mn	K	Ca	Sr
	Grams per 1000 grams or ml.							
Tapwater	0.0049	....	0.010	0.00011	.....	0.0016	0.030	0.00013
Urine (human)	0.1000	....	3.90	.....	.....	2.200	0.035	.....
Orange juice (canned)	0.052	....	0.017	0.0024	0.00028	2.450	0.090	0.00022
Grape-fruit juice (canned)	0.054	....	0.0042	0.0010	0.00044	1.300	0.053	0.00050
Tomato juice (canned)	0.086	0.0004	3.075	0.0022	0.00094	3.050	0.00056	.....
String beans (canned)	0.155	....	1.600	0.0120	0.00155	1.400	0.210	<0.0001
	Grams per 100 grams							
Whole blood, rabbit 1	0.002	....	0.150	0.030	.....	0.150	0.0090	.....
Whole blood, rabbit 2	0.006	....	0.165	0.045	.....	0.200	0.0073	.....
Whole blood, rabbit 3	0.0043	....	0.175	0.0475	.....	0.200	0.0068	.....
Blood plasma, rabbit 1	0.0018	....	0.400	.....	.....	0.016	0.019	.....
Liver (rabbit)	0.0134	....	0.140	0.0185	0.00018	0.260	0.016	.....
Liver (human)	0.0166	....	0.120	0.0096	0.0002	0.250	0.0073	.....
Brain (human)	0.0180	....	0.120	0.0052	.....	0.380	0.0073	.....
Kidney (rabbit)	0.0134	....	0.150	0.0088	0.00011	0.340	0.0050	.....

ard and diluted to 10 ml., suffices for the analysis of the alkalies. The accuracy of analysis in this case is illustrated in Table VI, where the results obtained by the use of the untreated sample are compared with the results obtained by the analysis following ashing of the organic materials by three distinctly different procedures. Direct analysis has also been applied to milk samples (*5*), and it is probable that it can be adapted to laked blood, blood serum, and blood plasma.

When the organic matrix of biological material must be destroyed, dry-ashing is satisfactory except in the case of sodium, potassium, and calcium where, as can be seen from Table VI, the results are lower than those obtained following ashing by other procedures. Although the wet-ashing technique is decidedly superior, a number of difficulties occur which force the analyst to vary the technique to fit the different conditions encountered. In the case of biological material other than bone, organic matter can be destroyed by sulfuric and nitric acids, supplemented by the use of perchloric acid. In these cases all the salts will remain in solution when the digest is diluted to the desired volume or can be made to go into solution by addition of a little hydrochloric acid and application of heat. When material contains much calcium, organic matter is destroyed by the use of nitric acid and Superoxyl. This is a more tedious procedure than the technique employing sulfuric acid, but it has the advantage of producing residues which in most cases are readily soluble in water or in dilute acids. For the detection of minor elements, it may also be necessary further to concentrate the prepared samples by evaporation, but this is limited to the point at which crystallization of salts occurs. The removal of these salts or other insoluble matter (silica) by filtration is not a safe procedure, since many elements may be lost through occlusion or adsorption. Precipitates are not particularly inimical to accurate analysis except when they are so heavy that they tend to clog the orifices of the equipment (*5*), or when they cannot be dispersed uniformly in the mist. Apparently it is not necessary for all the organic matter to be destroyed (Table VI), and therefore analyses can be made on biological material in which the ashing process has been continued only until the sample can be placed in solution or dispersed finely and uniformly in the desired volume.

Since the means of preparing samples may vary widely, specific details are not given and the analyst must choose modifications which best suit his purpose, on the basis of his own experience. However, the general methods found satisfactory for various materials are indicated in Table VII, which also illustrates the size of sample employed, the dilution required for the determination of the elements, and the wide applicability of the flame method. In general, when acids are used, an attempt is made to keep their concentration at about 10% of the total volume of sample, but this may vary somewhat without affecting the results. The authors have applied the method mainly to the analysis of biological material.

Data on its applicability to other materials may be obtained from papers by Mitchell and Robertson (*10*) and Ells and Marshall (*3*), who applied the technique to the determination of exchangeable base in soil samples. The purpose of the ammonium acetate leach indicated in Table VII is to prevent the removal of large amounts of aluminum (*3*), high concentrations of which are said to interfere in the determination of calcium and strontium (*10*). The interference is said to be due to the depression of the intensities of the strontium and calcium lines and this interionic action appears to be the only one noted in the literature. Ells (*2*) states that the interference occurs when aluminum is present in a concentration exceeding 1 mg. per liter. Examples of the application of the method to the analysis of inorganic salts and alloys may be obtained from the paper by McClelland and Whalley (*8*). These investigators routinely determined calcium and sodium in magnesium and aluminum powders and alloys, as well as copper, manganese, and magnesium in aluminum. They also used the method to determine calcium, potassium, and sodium in blast-furnace and lime-kiln dusts, as well as in various inorganic salts.

For the purpose of easy computation of results, analyses are always made on a 5- or 10-ml. aliquot of each sample. This volume should include the proper amount of cobalt (5 mg. per 10 ml.) which furnishes the internal standard lines. Exposure periods may vary from 2 to 10 minutes. In the internal standard line method of photometry, as used in this work, the intensity ratios do not show significant variations with the duration of the exposure, and therefore the concentration values may be read directly from the calibration curve. By increasing the period of exposure it is also possible to detect minor elements present in amounts below the lower limit of the calibration curve merely by extrapolating the line to lower limits.

Concentrated samples are flamed first in order to detect the minor elements, and then diluted with a water solution of cobalt chloride (5 mg. of cobalt per 10 ml.) as indicated in Table VII for the determination of the more abundant elements. Between samples, the atomizer and burner are cleaned by spraying distilled water through the system for 1 to 2 minutes. Two and 4-minute exposures are employed, without the preliminary stabilizing period which is customary with the L/H method of photometry. Air pressures of 40 pounds per square inch and acetylene pressures of 25 cm. are not the most suitable conditions for the detection of minute traces of calcium and strontium, but high sensitivity is not required for the former, which is normally present in abundance, while the latter can be detected in very

small amounts by proper concentration of the sample (Table VII). The sodium doublet at 3302/3 Å. is not so sensitive as the 5890/5.9 Å. doublet, but it is satisfactory for the analysis of most biological materials and is not affected appreciably by small contaminations. The yellow sodium doublet at 5890/5.9 Å. is so sensitive that its use requires special precautions in order to prevent contamination by sodium from glassware.

In Table VIII are listed typical results obtained with various materials. In a number of instances minor elements known to be present have not been detected. This can be remedied by the use of larger samples or by employing longer periods of exposure than were used in the examples cited. Owing to the scarcity of suitable analytical lines in the region from 4607 to 6700 Å., internal standard methods in this region have not yet been developed. Of the prominent lines in this region, the barium line at 5535.5 Å. was not considered because it is too diffuse, while the strong gadolinium line at 5696 Å. (*5*) could not be tested because of lack of a suitable salt of this rare element. Analysis in this region (lithium particularly) at present is carried out by employing the L/H method in which the L/H values are read from a curve plotted from the ratios obtained for standard solutions taken on the same plate as the samples.

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Determination of Ethyl Acetate in the Presence of Acetaldehyde

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In the determination of ethyl acetate in the presence of a large amount of acetaldehyde erratic results are due to the tendency of acetaldehyde to consume alkali in a varying and irregular manner. The method described here involves the quantitative oxidation of acetaldehyde to acetic acid carried out simultaneously with the saponification of the ester. A separate bisulfite determination of the acetaldehyde is made to correct the saponification value for the acetic acid produced from the aldehyde.

IN THE course of a research investigation, it was necessary to determine the amounts of ethyl acetate and acetaldehyde present in a mixture containing a high concentration of acetaldehyde. Since the material frequently contained polymerized aldehyde in addition to acetaldehyde and ethyl acetate, it was impossible to determine one component in the sample and estimate the other by difference.

Methods for estimating esters and aldehydes in such materials as distilled liquors are well known (*1*). The esters are determined by saponification, while the aldehydes are determined colorimetrically with sulfite fuchsin solution or volumetrically with sodium bisulfite (*2*). The determination of acetaldehyde in the samples under consideration presented no difficulty; the Kolthoff and Furman modification (*7*) of the Ripper (*9*) bisulfite addition method gave accurate results.

While the determination of ethyl acetate in the presence of acetaldehyde by saponification is satisfactory in cases of low concentrations of acetaldehyde (*3*), erratic results were frequently noted in the present study where the concentrations of the two components were much greater and where the ratio of aldehyde to ester was high.

Since the source of the error was traced to the tendency of acetaldehyde to consume alkali in a varying and irregular manner the successful use of the saponification reaction depended upon quantitative removal of the acetaldehyde from the solution, or quantitative conversion of the aldehyde to a form consuming alkali in a regular and reproducible manner. Because removal of the acetaldehyde by precipitation or other means immediately available was considered to be too time-consuming, this method was not investigated. The quantitative oxidation of formaldehyde to formic acid with hydrogen peroxide in the presence of alkali has been described (*6*). Various references indicated that the analogous reaction for acetaldehyde also occurred (*4, 5, 8*). This suggested the possibility of oxidizing the acetaldehyde to acetic acid during the saponification reaction, thus allowing a simultaneous determination of the acetaldehyde and ethyl acetate. In the following procedure the sum of the two constituents is determined, followed by a correction for the acetaldehyde present, determined from a sulfite precipitation value.

PROCEDURE

Determine the density of the sample by means of a pycnometer, observing the usual precautions necessary when measuring the density of volatile liquids. Pipet 5 ml. of the mixture into a 100-ml. volumetric flask, make up to the mark with distilled water, and mix thoroughly.

ACETALDEHYDE DETERMINATION. Pipet 5 ml. of the diluted sample into 50 cc. of 0.1 *N* sodium bisulfite solution contained in a 250-ml. glass-stoppered iodine flask. (This solution should contain 5 to 10% of ethyl alcohol and should be standardized daily.) Shake the sample intermittently for about 30 minutes, then wash the neck of the flask with distilled water and add an amount of standard iodine solution exactly equivalent to the sodium bisulfite. Titrate the excess iodine with standard sodium thiosulfate solution, using starch indicator near the end point.

Table I. Oxidation of Acetaldehyde to Acetic Acid

Sample No.	Acetaldehyde Present	Acetic Acid Produced[a]	Error
	Milliequivalents	*Milliequivalents*	%
1	0.43	0.43	0.0
2	0.86	0.84	2.3
3	1.29	1.27	1.6
4	1.72	1.74	1.2
			Av. 1.3

[a] Corrected for free acetic acid in acetaldehyde.

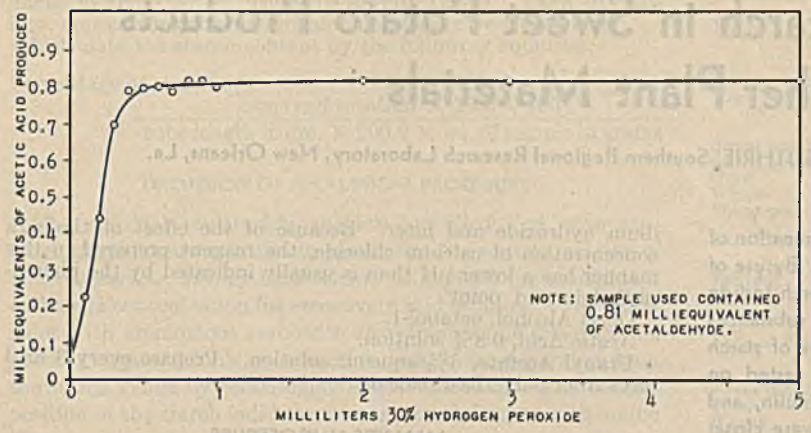

Figure 1. Effect of Excess Hydrogen Peroxide in Oxidation of Acetaldehyde to Acetic Acid

Table II. Determination of Ethyl Acetate and Acetaldehyde

Sample No.	Acetaldehyde Added	Ethyl Acetate Added	Acetaldehyde in Mixture	Ethyl Acetate in Mixture	Sum of Two Components	Sum Found by Analysis	Ethyl Acetate Found	Error in Ethyl Acetate Determination
	Milliequivalents		%	%	*Milliequivalents*			%
1	0.52	3.83	12	88	4.35	4.31	3.79	−1.04
2	1.03	3.83	21	79	4.86	4.90	3.87	+1.04
3	1.03	3.08	25	75	4.11	4.13	3.10	+0.65
4	1.03	2.31	31	69	3.34	3.39	2.36	+2.16
5	1.03	1.54	40	60	2.57	2.55	1.52	−1.30
6	2.06	1.54	57	43	3.60	3.55	1.49	−3.24
							Av.	1.57

The number of milliequivalents of acetaldehyde is calculated as follows:

$$\text{Milliequivalents of acetaldehyde} = \text{ml. of } Na_2S_2O_3 \times N\ Na_2S_2O_3$$

Ethyl Acetate Determination. Pipet 5 ml. of the diluted sample into an iodine flask containing approximately 100 ml. of water, 25 ml. of 0.5 *N* sodium hydroxide, and 5 ml. of 30% hydrogen peroxide. Secure the stopper of the flask, place the flask on a steam hot plate, and heat 15 minutes. (Samples reach a temperature of approximately 80° C. and a maximum pressure of about 360 mm. of mercury.) Remove the flask and allow it to stand for one hour. At the end of this time carefully open the flask, and wash the neck with distilled water. Titrate the excess alkali with 0.5 *N* hydrochloric acid, using phenolphthalein as indicator. Run a blank determination concurrently with the samples.

$$\%\ \text{ethyl acetate} = \frac{\text{milliequivalents of alkali consumed} - \text{milliequivalents of acetaldehyde present} \times 0.088 \times 100}{0.25 \times \text{density of sample}}$$

DISCUSSION

Oxidation of Acetaldehyde to Acetic Acid. In order to study the effect of varying amounts of hydrogen peroxide on the oxidation of acetaldehyde, the following experiments were undertaken:

One-milliliter samples of a standard solution of acetaldehyde (0.81 milliequivalent per ml.) were added to flasks containing approximately 100 ml. of water, 25 ml. of 0.5 *N* sodium hydroxide, and from 0 to 5 ml. of 30% hydrogen peroxide. After heating as described in the procedure, the remaining alkali was titrated with 0.5 *N* hydrochloric acid; thus the amount of acetic acid produced was found.

Results are shown in Figure 1. Even when no hydrogen peroxide is present, a slight amount of acid is produced. This means that the acetaldehyde is oxidized by air under the conditions of testing, since a correction was made for the small amount of acetic acid initially present in the acetaldehyde. Apparently no deleterious effect is produced by a large excess of hydrogen peroxide, and, since Figure 1 shows that more than a stoichiometric relationship is necessary, it is advisable to maintain an excess. This excess is ensured by the amount recommended in the procedure.

After determination of the quantity of hydrogen peroxide required for complete oxidation of acetaldehyde, samples containing varying amounts of aldehyde were prepared and the amount of acetic acid produced was determined. Table I shows the quantitative nature of the oxidation.

Experiments were also carried out to determine whether there would be any oxidation of the ethyl alcohol produced in the saponification. No appreciable amount of acid was produced when alkaline solutions of ethyl alcohol were treated with varying amounts of hydrogen peroxide, and it was concluded that this component would cause no errors in the method.

RESULTS

To test the application of the method to various mixtures of ethyl acetate and acetaldehyde, solutions were prepared as discussed below and analyzed according to the procedure previously described. The accuracy of the method may be judged by data in Table II.

Acetaldehyde Solution. A standard acetaldehyde solution was prepared by diluting 12 to 15 ml. of chilled redistilled acetaldehyde to 250 ml. with chilled carbonate-free distilled water. This solution was standardized against the bisulfite solution by the method described above.

Ethyl Acetate Solution. A standard ethyl acetate solution was prepared by diluting 18 to 20 ml. of redistilled ethyl acetate to 250 ml. with carbonate-free distilled water. The amount of ester in terms of milliequivalents per ml. was determined.

SUMMARY

The reaction of acetaldehyde with alkali during the saponification of ethyl acetate has been noted in mixtures of these two compounds. A method eliminating the erratic results so produced involves oxidation of the aldehyde with hydrogen peroxide in alkaline solution. Saponification data are corrected for the effect of the acetic acid produced by the oxidation reaction, by a separate bisulfite determination of the acetaldehyde. Average accuracy within 2% is reported.

ACKNOWLEDGMENT

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Determination of Starch in Sweet Potato Products and Other Plant Materials

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A polarimetric method has been developed for the determination of starch in sweet potato products and other plant materials. By use of the specific and quantitative precipitation of starch as starch iodide and of uranyl acetate as a protein precipitant, the effect of substances which interfere with most methods for the determination of starch has been largely eliminated. When the method was tested on samples containing large amounts of protein, pectin, inulin, and other interfering substances, the starch values obtained were closer to the true starch content than were found by either the malt-diastase or Hopkins method. The addition of a number of different substances commonly found in biological materials did not significantly alter the starch values obtained with the proposed method. The procedure described should be applicable to materials containing 10% or more starch on the moisture-free basis. The specific rotation for the conversion of polarimetric readings to starch was found to be 200.9 for the proposed method.

MANY of the methods currently available for the determination of starch are limited in application because they fail to give true starch values when the sample contains pectin, nonstarch polysaccharides, proteins, and other interfering substances. With sweet potato material it is especially important to eliminate the interference of pectin. In developing the proposed method two principles brought out by the work of Denny (*4*) have been followed. (1) A starch method to be generally applicable must give correct values when pectin, nonstarch polysaccharides, and proteins are present in the sample. (2) The method should give zero or very low values on samples containing little or no starch by qualitative tests, but containing high amounts of substances which might possibly interfere. Parts of the methods of Hopkins (*7*), Denny (*5*), Pucher and Vickery (*9*), and Sullivan (*10*) have been used in the proposed method. Only the procedure finally adopted and data to establish its validity are given here.

The proposed method consists of treatment of the sample at boiling temperature with dilute ammonium carbonate solution, precipitation of the starch with iodine, decomposition of the starch iodide, reprecipitation with iodine, decomposition of the starch iodide, precipitation of the starch with alcohol, dispersion in calcium chloride, precipitation of any remaining protein with uranyl acetate, and determination of the optical rotation. The method is recommended for samples containing 10% or more starch on the moisture-free basis.

REAGENTS

Celite, an analytical filter aid.

Ammonium Carbonate Solution. Dissolve 3.0 grams of reagent quality ammonium carbonate in water and dilute to 1 liter.

Sodium Chloride Solution. Dissolve 200 grams of reagent quality sodium chloride in water and dilute to 1 liter.

Iodine–Potassium Iodide Solution. Dissolve 30 grams of iodine and 50 grams of potassium iodide in water and dilute to 250 ml.

Sodium Thiosulfate Solution. Dissolve 125 grams of sodium thiosulfate pentahydrate in water and dilute to 1 liter.

Hydrochloric Acid, approximately *N*.

Ethyl Alcohol, 95% by volume.

Ethyl Alcohol, 70% by volume.

Calcium Chloride Solution, 2 parts of crystalline calcium chloride hexahydrate plus one part of water. Adjust to a density of 1.30, make very faintly pink to phenolphthalein with 0.1 *N* sodium hydroxide and filter. Because of the effect of the high concentration of calcium chloride, the reagent prepared in this manner has a lower pH than is usually indicated by the phenolphthalein end point.

Octyl Alcohol, octanol-1.

Acetic Acid, 0.8% solution.

Uranyl Acetate, 5% aqueous solution. Prepare every 3 or 4 days from pulverized reagent.

ANALYTICAL PROCEDURE

Weigh accurately 0.9 to 1.0 gram of the finely ground sample, 80-mesh or finer, into a 100-ml. heavy Pyrex centrifuge tube (30 by 170 mm., without pourout) and add 2.0 grams of Celite. Add 50 ml. of ammonium carbonate solution and stir with a strong glass rod until the material is completely wetted. Place in an oil bath maintained at 117–120° C. and boil for 30 minutes, adding a drop or two of octyl alcohol to prevent excessive foaming, if necessary. Stir the suspension frequently during the 30 minutes' boiling. If excessive foaming persists after the addition of octyl alcohol, it may be controlled by raising the tube so that it does not extend so deeply into the bath. After the initial foaming has subsided, the tube should be lowered so that vigorous boiling is resumed.

Cool to room temperature, rinse off the stirring rod, and place it aside for subsequent use with the same sample. Add 20 ml. of sodium chloride solution and 2.5 ml. of the iodine–potassium iodide solution. Almost fill the tube with water and stopper with a tightly fitting rubber stopper. Invert and shake gently, so that all the starch reacts with the iodine. Precipitation usually occurs in about 5 minutes. Let the solution stand for at least 5 minutes after precipitation starts. Remove stopper and wash any adhering starch iodide into the tube with small quantities of water from a wash bottle. Centrifuge at about 2000 r.p.m. for 10 minutes.

Pour off and discard the supernatant liquid. Add 20 ml. of the 20% sodium chloride solution to the precipitate. Suspend the starch iodide with the glass rod, previously set aside, and add sodium thiosulfate solution until the starch iodide is decomposed. A long, thin, flexible spatula is more convenient for suspending the starch iodide than the glass rod. The glass rod should be saved, however, for the final dispersion of the starch. About 3.5 ml. of the thiosulfate solution will be required on relatively pure starches and excess should be avoided.

Add 10 ml. of *N* hydrochloric acid, 2.5 ml. of iodine–potassium iodide solution, and enough water to fill the tube. Restopper and shake gently. After the starch iodide has precipitated, centrifuge as before and discard the supernatant liquid. Add approximately 50 ml. of 95% ethyl alcohol and thoroughly suspend the starch iodide. Add sodium thiosulfate solution until the blue color is discharged, avoiding excess, since too much may cause cloudiness in the final dispersion. Add water to make approximately 70% alcohol, stopper, and shake thoroughly. Allow to stand about 15 minutes and centrifuge 10 minutes. Wash the precipitated starch once with 50 ml. of 70% alcohol by volume. Add a total of 60 ml. of concentrated calcium chloride solution, a small amount at a time, and stir with the rod, previously set aside, until the material is suspended and free from lumps. Add 3 ml. of 0.8% acetic acid, place in the oil bath, and boil for 18 minutes. A shorter period of boiling may lead to cloudiness in the final solution, while a longer period may give values which are slightly low. Raise the tube and while still hot add 5 ml. of 5% uranyl acetate solution and stir well.

Transfer the contents of the tube with water to a 100-ml. volumetric flask, cool to room temperature, make to volume, add 1 ml. of water to correct for volume occupied by Celite, and shake thoroughly. A small correction for the volume occupied by tissue residue could also be made here. Transfer to a dry centrifuge tube and centrifuge for 10 minutes at 2000 r.p.m. Protect the solutions against evaporation by use of rubber caps. Occasionally some floating material is present in the supernatant liquid and can be removed readily by filtering through Whatman No. 4 paper or an equivalent fast paper.

Place the supernatant or filtrate in either a 2- or a 4-dm. polarimeter tube, depending on clarity, and read in a polar-

imeter at about 25° C. using the sodium D line. Take 10 readings, approaching the match point alternately from each side.

Calculate the starch content by the following equation:

$$\text{Percentage of starch} = \frac{\text{observed rotation} \times 100 \times 100}{\text{tube length in dm.} \times 200.9 \times \text{wt. of sample in grams}}$$

DISCUSSION OF ANALYTICAL PROCEDURE

The material undergoing analysis is boiled first with ammonium carbonate solution to neutralize the natural acidity of plant material. It may be necessary to increase the ammonium carbonate concentration for excessively acid samples. The treatment with ammonium carbonate also disperses or dissolves the pectins, sugars, soluble proteins, etc., which are separated from the starch iodide by centrifuging and decantation. The decomposition of the starch iodide and second precipitation with iodine liberate impurities which may have been carried down with the first starch iodide precipitate. After the last addition of thiosulfate, 70% alcohol is used to remove excess sodium thiosulfate and materials not soluble in water but soluble in 70% alcohol. This step promotes clarity of the final solution. Calcium chloride is used in preference to other solubilizing agents because of its ability to disperse starch readily and its use in other methods (*3*, *5–10*). Starch dispersed in calcium chloride solution does not degrade appreciably even on long standing. Solutions that had stood 24 to 48 hours showed no significant changes in rotations. Starch solutions which had been stored in glass-stoppered containers for 30 to 90 days showed an increased dextrorotatory power due to evaporation. The acidity during solubilization with calcium chloride is somewhat greater than that recommended by Hopkins (*7*) and Mannich and Lenz (*8*). This increased acidity appears to have no adverse effect on the starch and promotes greater clarity. Uranyl acetate is added at the end to remove any proteins not removed by previous treatment. Other protein precipitants were tried but proved unsatisfactory. The use of uranyl acetate as a protein precipitant will probably prove useful in the Hopkins method and in other polarimetric methods for starch.

In determining the starch content in cornstarch, wheat starch, and waxy maize starch, the final solution was usually too cloudy to read in a 4-dm. tube. Shaking the solution vigorously with about 10 ml. of carbon tetrachloride, centrifuging, and decanting the starch dispersion gave clear solutions. Chloroform may be used in place of carbon tetrachloride, but in this case correction must be made for the solubility of chloroform in the starch dispersion.

The method can probably be shortened when used with materials known to be relatively free of pectin and protein by making only one precipitation with iodine.

DETERMINATION OF FACTOR FOR CONVERSION OF POLARIMETRIC READINGS TO STARCH

Since pure starch or starch of known purity has probably never been prepared, it is not possible to determine accurately the specific rotation, $[\alpha]_D$, of starch by dissolving a weighed sample and reading it in a polarimeter. It is, however, possible to estimate the specific rotation of starch by determining the amounts of the major impurities, either before or after purification of the starch and assuming that the rest of the sample is starch. It is also possible to estimate the factor by assuming some other starch method to be correct and using the value so obtained for calculating the specific rotation. Mannich and Lenz (*8*) and Hopkins (*7*) recommended the use of +200 for the specific rotation of wheat starch. However, since the value varies somewhat with the method, depending on the degree of degradation of the starch, it was necessary to determine the proper factor to use with the proposed method.

The first procedure used to arrive at the factor for the method was to determine the nonstarch material in the starch samples under investigation. Ash was determined by ignition at 550° C. for 3 hours. Nitrogen was determined by the Kjeldahl procedure, followed by nesslerization of the distillate. Protein was calculated by use of the appropriate factor. Extractives were obtained by a 16-hour extraction with 85% methanol followed by a 5-hour extraction with ethyl ether. Moisture values were calculated from the loss in weight of samples dried approximately 16 hours at 96° to 100° C. in a vacuum oven equipped with a drying train. The results are shown in Table I.

Table I. Estimation of Factor by Correction for Major Impurities

Kind of Starch	Nonstarch					Starch (by Difference)	$[\alpha]_D$ (Calculated)
	Moisture	Ash	Protein	Extractives	Total		
	%	%	%	%	%	%	
Sweet potato A	12.52	0.17	0.08	0.21	12.98	87.02	199.5
Sweet potato B	12.01	0.36	0.05	0.26	12.68	87.32	198.9
Corn	12.44	0.07	0.21	0.45	13.17	86.83	198.5
Wheat	12.45	0.11	0.27	0.41	13.24	86.76	196.4
Waxy maize	11.99	0.10	0.25	0.19	12.53	87.47	197.7

Table II. Estimation of Factor by Use of Starch Put through Proposed Method

Kind of Starch	Ash	Moisture	Starch (by Difference)	$[\alpha]_D$ (Calculated)
	%	%	%	
Sweet potato B	0.52	13.63	85.85	200.1
Corn	2.94	12.10	84.96	201.0
Wheat	1.53	10.44	88.03	201.9
Waxy maize	0.32	11.48	88.20	200.6

The value for the specific rotations of sweet potato, corn, and waxy maize starches put through the proposed method is approximately 199 when estimated by this procedure. Wheat starch gave a lower value, which may be due to the presence of some impurities which were not accounted for.

The second procedure for arriving at the factor was to assume that the malt-diastase method gives correct values on reasonably pure starches when the 0.93 factor is used to convert glucose to starch. On this basis, the specific rotations found with the proposed method were 202.0, 200.6, 200.3, 198.4, and 199.9, respectively, for starches in the order listed in Table I.

The third procedure for obtaining the factor for the method was to use the starch obtained from the final calcium chloride solution.

Eight 2.0-gram samples of each starch were put through the method and the combined final solution was dialyzed, in Visking sausage casing, against running distilled water for 2 days to remove the calcium chloride and other salts. After concentration of the solution by pervaporation the starch was precipitated with an alcohol-ether mixture and washed three times with ethyl alcohol and twice with ethyl ether. The precipitated starch was dried in vacuo over sulfuric acid. After air-equilibration, moisture and ash were determined. Specific rotation was then found by dissolving a sample in calcium chloride solution, using just sufficient heat to ensure complete dispersion. The materials dispersed readily and the preparation from waxy maize dispersed in the cold. The addition of dilute acetic acid was unnecessary. The results are given in Table II.

The calculated values for the specific rotation are now in closer agreement and wheat starch shows less divergence from the other starches. The average specific rotation found for the starches by this procedure is 200.9. Since this procedure gives slightly higher and more consistent values than the first procedure, the value 200.9 has been selected for use with the proposed method. The average value of 200.2 found by assuming the correctness of the malt-diastase method, with 0.93 factor, is supporting evidence. The value 200.9 is probably a close approximation for the value of the specific rotation of the starch as it exists at the time of polarization in the proposed method.

COMPARISON WITH MALT-DIASTASE AND HOPKINS PROCEDURES

The proposed method was compared with the official A.O.A.C. malt-diastase method for starch (*1*, p. 359), the factor 0.93 (*2*)

Table III. Comparison of Methods for Determination of Starch

Sample No.	Kind of Material	Per Cent Starch on Moisture-Free Basis: Malt-diastase	Hopkins, 200 factor	Hopkins, 204.8 factor	Proposed method
1	Sweet potato starch A	98.2	100.9	98.5	98.8
2	Sweet potato starch B	98.4	100.5	98.1	98.0
3	Waxy maize starch	98.3	100.4	98.0	97.9
4	Cornstarch	98.2	100.1	97.8	97.8
5	Wheat starch	98.1	99.1	96.7	96.9
6	Ground corn	70.5	74.2	72.5	71.6
7	Bread	67.4	68.2	66.6	64.6
8	Sweet potato residual pulp, water process, dried	55.5	56.4	55.1	54.8
9	Sweet potato by-product pulp, lime-water process, dried	62.1	50.4	49.2	61.4
10	Sweet potatoes, dehydrated, food type	60.5	45.9	44.8	41.9
11	Sweet potatoes, dehydrated, food type, extracted	70.8	76.1	74.3	70.3
12	Sweet potatoes, dehydrated, stock-feed type	59.1	61.8	60.4	59.3
13	Cottonseed meal	9.8	−4.4	−4.3	−0.6
14	Orange rind	12.8	22.6	22.1	−0.2
15	Jerusalem artichokes	27.4	−18.0	−17.6	−0.3
16	Peanut meal	9.1	3.5	3.4	6.7
17	Gladiolus leaves	1.3	0.3	0.3	0.4

being used instead of the official 0.90 factor to convert glucose to starch, and with the tentative Hopkins method (*7*), the final solution being clarified by centrifuging with Celite. The samples chosen ranged from relatively pure starches to samples containing little or no starch, but large amounts of other substances that might possibly interfere. Since stable, homogeneous samples were required for the comparison of methods, dry, finely ground materials were used. However, analysis of fresh plant materials by the proposed method should present no difficulties other than those of sampling, sample preservation, and preparation. The conventional preparation of fresh sample materials by dropping into hot alcohol (*1*, p. 125) is suggested. A description of the samples follows:

1. Sweet potato starch A. A.O.A.C.-1942 sample for collaborative studies on starch methods.
2. Sweet potato starch B. A laboratory-prepared starch, extracted and purified without use of lime water by the Sweetpotato Products Division of this laboratory.
3. Waxy maize starch, furnished by the American Maize-Products Company, Roby, Ind.
4. Cornstarch. A.O.A.C.-1943 sample for collaborative studies on starch methods.
5. Wheat Starch. A.O.A.C.-1943 sample.
6. Ground Corn. A.O.A.C.-1943 sample.
7. Bread. A.O.A.C.-1943 sample.
8. Sweet potato pulp. A residual pulp from water-extraction of starch without use of lime water, dried at 60° C. in a mechanical convection oven. Furnished by the Sweetpotato Products Division of this laboratory.
9. Sweet potato pulp. A dried residual pulp, typical of lime-water process by-product, from the Laurel Starch Plant, Laurel, Miss.
10. Sweet potato (dehydrated). Peeled, blanched, and dehydrated sweet potatoes, prepared for food use by the Sweetpotato Products Division of this laboratory.
11. Sweet potato (dehydrated). Same as 10 except Soxhlet-extracted with 80% alcohol before analysis.
12. Sweet potato (dehydrated). Whole sweet potato cossettes, dried for the production of stock feed, from the Sweetpotato Products Division of this laboratory.
13. Cottonseed meal. Composite of cottonseed kernels, thoroughly extracted with ethyl ether before analysis.
14. Orange rind. Composite of many kinds of orange peel that had been dropped into boiling alcohol, ground in food chopper, Soxhlet extracted with 80% alcohol, dried, and ground in ball mill.
15. Jerusalem artichokes. From T. A. Kiesselbach of the University of Nebraska. Prepared for analysis in same manner as orange rind.
16. Peanut meal. Peanut kernels successively extracted with petroleum ether, ethyl ether, 95% alcohol, dried, and ground finely.
17. Gladiolus leaves, local garden variety. Dropped into boiling alcohol, dried, and ground finely.

Comparative results by the official A.O.A.C. malt-diastase, Hopkins, and the proposed methods are shown in Table III.

On the relatively pure starches the malt-diastase method and the proposed method are in good agreement. The Hopkins method values (factor, 200) are higher on the starches than the values by the other two methods. This is clearly due to the use of the factor 200 for the specific rotation of starch in the Hopkins method. The factors calculated for the Hopkins method, after correcting for impurities and moisture, are 202.8, 202.6, 202.0 200.0, and 201.8, respectively, for the starches in the order listed in Table I. If we assume the values by the proposed method to be correct, the factors calculated for the Hopkins method are 204.3, 205.2, 204.9, 204.5, and 204.9 for these starches, or an average of 204.8. Recalculation of the Hopkins values with the assumed correct value of 204.8, in order to eliminate the question of factors from the comparison, brings the starch content of the starches into good agreement by all three methods, as is shown in Table III. Earle and Milner (*6*) have recommended 203.0 for the factor in their modification of the Hopkins method.

On the sweet potato materials the agreement between the proposed method and the malt-diastase method is good with the exception of the sample of dehydrated sweet potatoes, sample 10. In this case the high malt-diastase values are due to the presence of sugars and other nonstarch carbohydrates. After this sample was extracted with 80% alcohol in a Soxhlet extractor to remove a large amount of sugars, including those formed during the dehydration process, the agreement was good. With the exception of the limed sweet potato pulp, sample 9, which gave low Hopkins values due to interference of the lime with the dispersion of the starch, the Hopkins method gave higher values than the proposed method on sweet potato samples. This is true even if the factor 204.8 is used instead of 200 and may be due to interference of pectin in the Hopkins method. The high value in the case of bread with the malt-diastase procedure is probably due to the presence of sugars, dextrins, or degraded starch in the sample.

The results with the orange rind, Jerusalem artichokes, gladiolus leaves, cottonseed meal, and peanut meal are of special significance with regard to the validity of the three methods. These samples were chosen because they were low in starch and high in substances that might possibly interfere. The true starch content of the orange rind sample is probably close to 0.4%. This is based on colorimetric measurements of the blue color produced with iodine. The proposed method gives a negative value of 0.2% on this sample, which is much closer to the truth than 12.8% by the malt-diastase and 22.6% by the Hopkins method. This shows that the pectin present in orange rind does not interfere with the proposed method but does interfere with the other two methods. The Jerusalem artichoke sample was starch-free by qualitative test with iodine. The proposed method gave a negative value of 0.3% on this sample, which is much closer to zero than the values of 27.4 and −18.0 found with the malt-diastase and Hopkins methods, respectively. The gladiolus leaves, which were starch-free, gave about the same values by the proposed and Hopkins methods. The cottonseed meal was used as a sample high in proteins which show negative optical rotation. Its true starch content was close to 0.2% based on colorimetric measurements. The value of −0.6% is closer to the true starch value than −4.4% by the Hopkins method. This shows that the interference of protein is small in the proposed method. The high value for cottonseed meal with the malt-diastase method is probably due to the presence of raffinose in the sample. The peanut meal contained considerable starch, 6.7% by the proposed method. As expected, the malt-diastase method gave a high value with peanut meal, probably due to the presence of nonstarch carbohydrates in the sample, and the Hopkins method gave low values due to the negative optical rotation of proteins.

It will be seen from the above comparison of methods, that in practically every case of disagreement the proposed method gave a value closer to the true value than the other two methods.

DETERMINATIONS MADE IN PRESENCE OF VARIOUS SUBSTANCES

The method was tested in the presence of sugars, amino acids, proteins, pentosans, pectin, and other substances often found in biological materials. In each case 200 mg. of the substance were added to an accurately weighed starch sample and the mixture was analyzed by the proposed method. The results are given in Table IV.

Table IV. Determination of Starch by the Proposed Method in the Presence of Added Substances

Substance Added[a]	Starch Found, Air-Dry Basis: Control	Control plus substance
	%	%
Raffinose (commercial)	85.9	86.0
Sucrose (commercial)	85.9	86.3
Gum arabic (commercial)	86.5	86.5
Egg albumin (commercial)	86.5	86.6
Inulin (commercial)	86.3	86.5
Gluten (laboratory purified)	86.4	86.6
l-Cystine (commercial)	86.1	86.5
l-Proline (commercial)	86.1	86.5
White potato dextrin (commercial)	86.1	87.3
Levulose (commercial)	86.6	86.7
Maltose (commercial)	86.6	86.8
Malic acid (commercial)	86.4	86.8
Sweet potato pectin (laboratory preparation)	86.0	86.2

[a] 200 mg. of each substance added to 1-gram samples of sweet potato starch B.

Table V. Recovery of Added Starch[a]

	A	B	C	D
	Mg.	*Mg.*	*Mg.*	*Mg.*
From 1.0 gram of cottonseed meal				
Starch added	28.1	68.7	112.1	156.5
Starch recovered	29.4	69.6	112.9	155.8
From 1.0 gram of orange rind				
Starch added	24.9	69.2	115.5	159.0
Starch recovered	26.0	70.6	118.1	158.8

[a] Calculated as empirically pure starch.

The only substance listed that showed evidence of interference was white potato dextrin. This material gave a red color with iodine and interfered slightly. Higher polymer dextrins, which give a violet or violet-blue color with iodine and which may be found in slightly degraded starch, do not precipitate completely when the starch is precipitated with iodine in the method and if present may be readily detected in the supernatant liquid. They are partially carried down with the starch iodide and consequently behave in an anomalous manner in the method. Consequently the results on samples containing higher dextrins are of doubtful validity. Glycogen acts in much the same way. When it is analyzed alone by the method, it is almost completely removed, but in the presence of starch it is carried down with the starch iodide and included as starch.

RECOVERY OF ADDED STARCH

Sweet potato starch was added in various quantities to 1.0-gram samples of cottonseed meal and orange rind and the mixture analyzed by the proposed method. Controls without starch were also run and the polarimetric readings subtracted from the polarimetric readings of the samples to which starch had been added. The results given in Table V show that the method works well in the presence of large amounts of pectin and protein.

PRECISION AND ACCURACY

Duplicates on 1-gram samples of relatively pure starches usually agree within 0.4%, or 4 mg. of starch. The agreement is dependent to a great extent on the precision obtainable from a polarimeter. In most instruments this precision is ±0.01 angular degree. The deviation between duplicates, then, due to the instrument alone, for a 1-gram sample in a 4-dm. tube may be expected to be as great as 0.25%, even if the highest precision of the instrument is attained. This variation becomes increasingly important as the starch content decreases. Based on experiments with 1-gram samples containing large amounts of pectin and protein, it may be concluded that the method gives values that are within 10 mg. of the true starch content even on samples containing large quantities of the substances which interfere in most starch methods. The latter statement assumes that the factor 200.9 for the specific rotation of starch in the final solution is essentially correct. As an illustration of the reproducibility of the method, duplicate determinations on sample 12 gave 59.3 and 59.3% starch on the dry basis, while duplicate determinations made 5 months later gave 59.8 and 59.8%. An analyst, who had not used the method previously, obtained 59.3 and 59.5% on the same sample.

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Colorimetric Assay of Quaternary Ammonium Salts

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RECENTLY a method was described for the determination of germicidal quaternary ammonium salts in dilute solution (*1*). Since publication of this paper, comments from various correspondents have made it clear that some workers find it difficult to clarify—i.e., dry—the ethylene dichloride dye solution without affecting its color intensity. Re-examination of this point led to the decision to use benzene instead of ethylene dichloride. Benzene is not so good a solvent for the colored salt, but, being lighter than water, it can conveniently be clarified by centrifugation, thus avoiding all danger of contamination. At the same time, to compensate for the loss of solvent power, two other changes were made: (1) The amount of sample was reduced to one fourth—50 micrograms. (2) The light filter was changed from one transmitting at about 540 mμ to one transmitting at about 600 mμ. The method, as now used, is as follows:

In a 125-ml. Squibb separatory funnel, take 50 ml. of water containing 50 to 75 micrograms of the quaternary compound. Ordinary stopcock grease should be avoided. Starch-glycerol lubricant is satisfactory (*2*). Add 5 ml. of 10% sodium carbonate solution, 1 ml. of aqueous 0.04% bromophenol blue indicator solution, and exactly 10 ml. of benzene. (The indicator solution should be prepared on the day it is to be used. Dissolve 40 mg. of bromophenol blue powder in 100 ml. of water containing 1 ml. of 0.1 *N* sodium hydroxide.) Shake steadily for 2.5 to 3 minutes, let the layers separate roughly (20 to 30 seconds), and then swirl the funnel contents. Let stand several minutes or until well separated. Rinse a 15-ml. centrifuge tube with a portion of the lower aqueous layer, discard this layer entirely, and then run the colored benzene layer into the tube. Stopper the tube with a clean rubber diaphragm stopper and centrifuge for a few minutes at about 1000 r.p.m., if necessary to clarify. Transfer to a dry Klett-Summerson colorimeter tube, and read, using filter No. 60.

Changes in technique involve no change in rationale of method. Limit of error is about ±2%, with occasional errors ±5%.

LITERATURE CITED

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Spectrophotometric Study of the Oxidation of Quenching Oils

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With the wartime development of large-scale production of aluminum alloy castings, especially for airplane motors, there has been little information available on the choice and performance of quenching oils for these alloys. In one large foundry it was found that the strain residual in quenched castings increased markedly with continued use or aging of a well-known quenching oil in the 5000-gallon tank. A spectrophotometric study has been undertaken to evaluate the changes which have occurred in this oil with an extension to a study of the rate of oxidation of several other commercial oils recommended for quenching, at high temperatures by a laboratory procedure, with and without addition agents and in the presence and absence of the aluminum alloy as catalyst. Spectrophotometric data are believed related to the light scattered by colloidal particles or precipitables as indicated by absorption curves and electron micrographs. Oxidation stability and absence of precipitable polymer particles are correlated with the quality of quenched castings as measured in terms of residual strain.

RECENTLY aluminum alloy motor castings quenched in a particular oil in a 5000-gallon tank have exhibited an increasing amount of internal strain with continued use or aging of the quenching oil. This residual strain is indicated both by x-ray diffraction patterns and by mechanical measurement of bow when strain is relieved by sawing the casting nearly through vertically. These castings of aluminum alloy were quenched rapidly from a temperature of 480° C. (900° F.) in this quenching oil maintained at approximately 45° C. This hot quenching of the metal very evidently oxidized the oil, producing a by-product which in some manner impaired the heat-conduction properties of the oil. The aluminum alloy castings seemed particularly sensitive to this change in the heat-conduction properties. It was the authors' problem to find some reliable method of studying and evaluating the deterioration of this quenching oil.

The method chosen was based on spectrophotometric values of the oil at different stages of use. It was then applied to a variety of other commercial quenching oils oxidized under laboratory conditions in order to gain some idea as to whether the optical data might indicate relative stabilities, as, of course, would be shown by a number of more familiar and accepted tests in petroleum laboratories.

TYPE OF OILS

The results of ten commercial quenching oils are presented here. These samples were chosen from among a large number of commercial quenching oils and represent oils supposedly of the best oxidation stability available. They were essentially paraffin-base oils of light color, A.P.I. gravity near 30, and boiling largely between 300° and 400° C. Some of the quenching oil samples contained commercial polar and nonpolar additives designed to increase the stability of the oil and impart desirable heat-conduction properties. All the oils contained varying amounts of aromatic compounds of lower stability than the bulk of the oil. Table I lists the properties, as far as known, of these ten commercial quenching oils.

PRODUCTS OF OXIDATION

Fenske (*2*, *3*) in heating lubricating oils at 140° to 170° C. accounted for most of the oxygen absorbed. His results indicate that about half of the oxygen absorbed goes into the formation of water, while the remainder may be accounted as carbon dioxide, carbon monoxide, volatile acids, fixed acids, and isopentane-insolubles (polymers and lacquers). The isopentane-insolubles or precipitables have been classified according to solubility, color, and melting point, but there appears to be no clear line of demarcation. They probably are polymers of low molecular weight (less than 2000). Their solubility is low in isopentane and moderate in chloroform, ether, and benzene (*6*).

APPARATUS AND TECHNIQUE

Throughout the history of petroleum analysis, color has been used in a qualitative sense in testing for purity. The older methods of visual comparison with colored disks or standards is gradually giving way to more reliable photometric methods utilizing standard color filters (*1*). Further refinements of these color tests lie in the use of prism or grating instruments capable of measuring light transmission through oils at any wave length near the visible region.

In this work, a Cenco-Sheard Spectrophotelometer was used. The sensitivity below 400 mμ was low with darker colored oils. In this range with dark-colored oils an error of 5% in the transmission readings was possible. In the rest of the range an error of 2% limited the accuracy. The large slit width required in testing dark-colored oils in the ultraviolet range led to a spectral width of the transmission readings as much as 10 millimicrons. The error due to overlapping orders was small but appreciable as determined by the use of filters. In order to obtain a satisfactory

Table I. Properties of Quenching Oils

Oil	Properties
1	Commercial paraffin-base oil containing nonpolar additives
2	Commercial paraffin-base oil containing nonpolar additives
3	Highly refined, paraffin-base, colorless oil, containing no additives
4	Experimental straight-run quenching oil without any additives
5	Paraffin-base oil for commercial quenching oils
6	Oil 5 containing 0.85% of lard oil and 0.3% of polar additives
7	Experimental paraffin-base quenching oil containing additives
8	Similar to oil 7
9	Pure, colorless, light paraffin oil
10	Commercial lubricating oil containing special polar addition agents

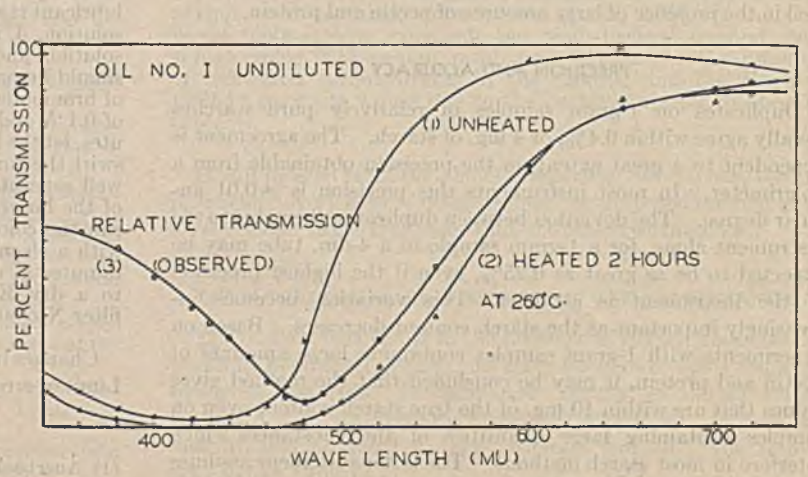

Figure 1. Transmission *vs.* Wave Length

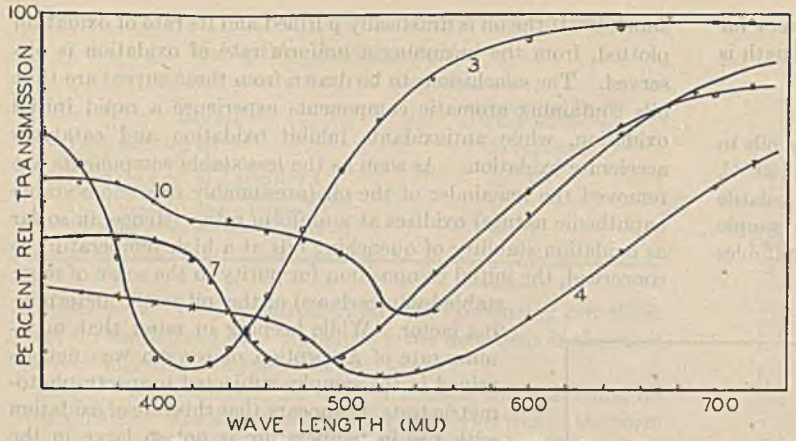

Figure 2. Relative Transmission vs. Wave Length
Commercial quenching oils heated 2 hours at 260° C. (undiluted)

Table II. Transmission vs. Wave Length
(Oil 1 heated at 260° C. for 2 hours, undiluted)

Wave Length	T_1 Unheated	T_2 Heated	T_1/T_2 (Calculated)	T_1/T_2 (Observed)
340	15	10	66	52
360	9.0	4.5	50	52
380	4.5	2.5	55	48
400	3.0	1.3	43	40
420	3.0	1.0	33	32
430	3.0	1.0	33	28
440	3.0	0.8	27	24
450	3.7	0.8	22	19
460	6.0	0.8	13	12
470	...	..	...	8
480	28	1.6	5.7	7
490	...	...	...	9
500	49	6.5	13	13
520	69	17	25	24
550	85	30	35	43
600	98	69	70	70
650	102	88	86	85
700	98	87	89	91
720	97	90	93	92

accuracy with dark-colored oils it was necessary to dilute the samples. Petroleum ether was chosen as a cheap, easily handled solvent.

EXPERIMENTAL CURVES AND NUMERICAL RATING OF OILS

If the transmission *vs.* wave-length values for a typical quenching oil are plotted, curve 1 in Figure 1 is obtained. Curve 2 represents the spectral curve for the same oil heated 2 hours at 260° C. If the ratio quotient of these two curves is plotted with the transmission value of the unheated oil at 100 we obtain a curve similar to function 3 on the graph. Table II lists the calculated and observed values for these relative transmissions. The value of the relative transmission curves lies in their evaluation of the oxidation products formed in the oil. The exact nature of the oxidation products producing these curves is unknown, though thought to be tied up with the formation of the polymer "precipitables".

These relative transmission curves are observed to vary widely from oil to oil in the position and character of the absorption peak (minimum transmission), as can be seen in Figure 2 in which several commercial quenching oils are graphically compared. In general, the more highly refined oils show absorption peaks further toward the violet than do oils containing aromatic compounds. This shift in absorption peaks is misleading in oils which are evaluated on the basis of visible color only. An oxidized oil of light color may actually contain more oxidation and polymer products than a dark-colored oil similarly treated. Under some conditions, usually oils heated at relatively low temperatures (150° C.), abnormal curves may be obtained which show several relative absorption peaks occurring after heating for various lengths of time.

DILUTION EFFECTS

When working with dark-colored oils or light-colored oils having a large absorption in the near ultraviolet region, it is necessary to dilute the samples with a neutral solvent to obtain a maximum sensitivity. A high-boiling petroleum ether was chosen. Dilution with ethyl ether and chloroform resulted in curves similar to those with petroleum ether; only a slight difference was noted in the lower relative transmission values of the oils diluted in chloroform. This lowering of the transmission values is believed to be associated with the solubility of the "insolubles" in chloroform over that of the petroleum ether. The use of a low-boiling petroleum ether (isopentane) is not practical, since it rapidly precipitates the polymers from the more highly oxidized oils. Dilutions were accomplished with the aid of calibrated pipets.

The most obvious result of dilution is the large shift in the absorption peak. This amounts to as much as 110 mμ in oils diluted up to 5% with petroleum ether. All oils investigated exhibited approximately the same displacement of the absorption peak with dilution. Figure 3 illustrates this effect. The shift in absorption is not dependent on purity, sample 3 being nearly a pure paraffin oil.

It is obvious that Beer's law will not be rigorously followed by a solution showing such a large shift of the absorption peak as is exhibited with heated quenching oils. Figure 4 shows that there is a uniform change upon dilution in the general nature of the curves. The region of the curve on the ultraviolet side of the peak exhibits a steeper slope than the curve on the red side. These properties must be considered before investigating Beer's law.

To study the extent to which Beer's law is followed, the per cent composition (by volume) is plotted against the extinction (log $1/T$) at the peak absorption and at a constant wave length (500 and 550 mμ). The graph in Figure 5 shows a curved line for extinctions at peak absorption in the case of the three oils plotted. At 550 mμ the curve, though of lower slope, approaches a straight line. When a filter photelometer is used, the curve is a modification of those at 500 and 550 mμ for any given oil. From these

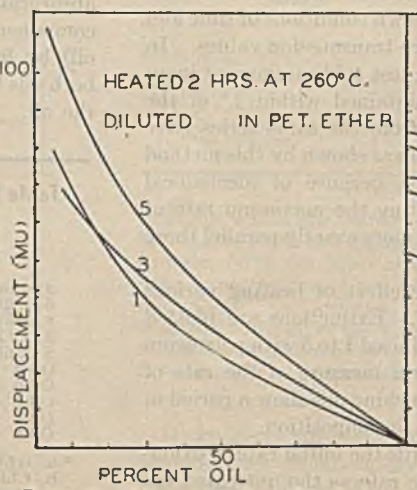

Figure 3. Absorption Peak Displacement vs. Per Cent Composition

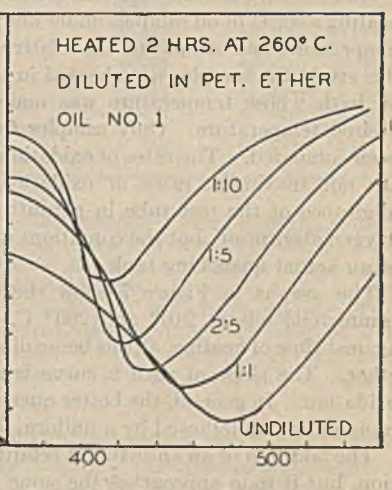

Figure 4. Relative Transmission vs. Wave Length

data it is apparent that Beer's law may be used to correct for dilution only to a rough approximation when the wave length is given.

EVALUATION OF OXIDATION STABILITY

Fenske (*2*, *3*) reports the rate of oxidation of lubricating oils to double with each 10° rise in temperature between 140° and 180° C. He has likewise found that the rate of production of volatile and fixed acids, lacquers, and precipitables follows a simple logarithmic function. Small discrepancies with precipitables have been assigned to their "solubility" in isopentane.

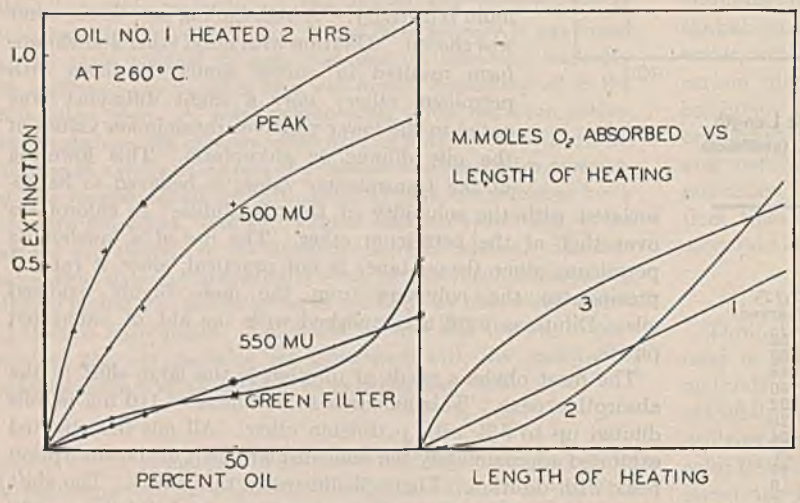

Figure 5. Extinction *vs.* Per Cent Composition

Figure 6. Rate of Oxidation

Three types of curves become evident when the length of heating time is graphed against the amount of oxygen absorbed.

The graph in Figure 6 illustrates this. Curve 1 is characteristic of relatively pure hydrocarbon oils and indicates a uniform rate of oxidation. Curve 2 shows a "period of induction" where the rate of oxidation is slight. After a short time, the oil usually oxidizes at a rate equal to or greater than that of a saturated hydrocarbon. This period of induction may be ascribed to the effect of some natural or added inhibitor. Curve 3 is characteristic of oils heated in the presence of a metallic catalyst, such as the metal of the casting to be quenched. In lubricating oils the metals usually catalyzing oxidation are lead, iron, and copper; in the quenching of aluminum alloys, small percentages of copper may be the predominant catalysts, although many of the authors' experiments indicate that aluminum is not wholly inert (see Table IV).

It is possible to study the relative rates of oxidation of oils by heating a series of oil samples under known conditions of time and temperature and comparing the relative transmission values. In this study the samples were heated in test tubes immersed in an oil bath whose temperature was maintained within 1° of the desired temperature. Only samples from the same series have been compared. The rates of oxidation as shown by this method are not maximum rates of oxidation because of mechanical hindrance of the test tube in permitting the maximum rate of oxygen absorption, but the conditions more exactly parallel those of an actual quenching tank.

The graphs in Figure 7 show the effect of heating various commercial oils at 210° and 260° C. Extinctions are plotted against time of heating, all oils being diluted 1 to 5 with petroleum ether. The slope of such a curve is a measure of the rate of oxidation. In general, the better quenching oils show a period of rapid oxidation followed by a uniform decomposition.

The addition of an antioxidant retards the initial rate of oxidation, but it soon approaches the same rate as the untreated oil. Heating in the presence of a metal catalyst (aluminum alloy turnings) further accelerates initial oxidation in most circumstances. If the oil is drastically purified and its rate of oxidation plotted, from the beginning a uniform rate of oxidation is observed. The conclusions to be drawn from these curves are that oils containing aromatic components experience a rapid initial oxidation, while antioxidants inhibit oxidation and catalysts accelerate oxidation. As soon as the less stable components are removed the remainder of the oil (presumably of a more stable naphthenic nature) oxidizes at a uniform rate. Hence, in so far as oxidation stability of quenching oils at a high temperature is concerned, the initial composition (or purity in the sense of more stable hydrocarbons) of the oil is the determining factor. While keeping in mind that maximum rate of absorption of oxygen was not attained in the samples subjected to spectrophotometric tests, it appears that this rate of oxidation with rise in temperature is not so large in the range 200° to 260° C. as it is at lower temperatures except during the short initial oxidation. In these tests the rate of oxidation has doubled in the 50° rise between 210° and 260° C.

STUDY OF ADDITIVE ACTION

Most additives used in lubricating oils contain the following essential ingredients: detergents, antioxidants, film strengtheners, and polar compounds designed to improve the lubrication at the surface of the metal. Additives used in quenching oils act primarily to improve oxidation stability and heat conduction.

The function of detergents is to minimize sludge formation and maintain a clean metal surface by inhibiting lacquer formation and buffer acids produced on oxidation (*7*). Most antioxidants contain hydroxyl or amino groups or compounds of phosphorus, sulfur, selenium, antimony, arsenic, and germanium. Many theories concerning their mechanism in oils may be found in the literature (*2*, *3*, *4*). One important function of some antioxidants lies in poisoning the metallic catalysts. Phosphites have recently been found to increase the proportion of water formed by absorbed oxygen (*2*, *3*), while forming phosphides with suspended metals.

With the aid of the spectrophotometer it is possible to study the antioxidant properties of additives. In general, additives containing antioxidants inhibit the oxidation of the less stable components of the oil. When the antioxidant is added to a highly purified oil the oxidation of the oil is accelerated. This effect may be ascribed to the oxidation of the antioxidant itself. In Table III are compiled the relative transmission values at peak absorption for quenching oil 6 with the additive and its separate components and for sample 6 drastically purified (filtered system oil) by long oxidation and filtration with activated clays. In both oils the halogenated ester has little effect on the oxidation of the oil. The detergent and sulfurized olefin show the greatest

Table III. Relative Transmission Values at Peak Absorption

(Undiluted, heated at 200° C.)

Sample		Heated 1.5 Hours	Heated 3.5 Hours
System oil filtered		32 (445 mμ)	7 (455 mμ)
S.O. filtered + a[a]	0.3%	27	8.5
S.O. filtered + b	0.06%	31	10
S.O. filtered + c	0.12%	27	8.0
S.O. filtered + d	0.12%	34	11
Oil 6		15 (465 mμ)	3.9 (480 mμ)
Oil 6 + a	0.3%	24	7.7
Oil 6 + b	0.06%	15	4.2
Oil 6 + c	0.12%	22	7.3
Oil 6 + d	0.12%	21	8.0

[a] a. GLC-I composed of b, c, and d.
b. Chlorinated methyl ester.
c. Detergent.
d. Sulfurized olefin.

Table IV. Additive Action

(Heated 3 hours at 210° C. Petroleum ether)

Sample	Transmission Values at Peak Absorption
Oil 6 and lard oil	28 (420 mμ)
Oil 6, lard oil, and Al	31.5
Oil 6, lard oil, and additive I	43
Oil 6, lard oil, additive I, and Al	39
Oil 6, lard oil, and additive II	34
Oil 6, lard oil, additive II, and Al	42

inhibitor action on the untreated oil while producing but slight effect on the filtered oil. Apparently the detergent is the most "active" ingredient in the additive.

Table IV demonstrates the effect of heating the quenching oil with a metal. The aluminum used in this series was in the form of turnings obtained from an aluminum alloy casting. Two types of additives were introduced into samples of the oil: GLC-I, the same as discussed above, and GLC-II containing a phosphite. In all cases the additive has a beneficial effect in inhibiting oxidation. The effect of the aluminum turnings is relatively slight. The increased stability produced by heating oil 5 with aluminum may be attributed to the formation of an aluminum soap with the acids produced by oxidation. The additive GLC-I seems to react with the metal in such a way as to limit its value in inhibiting oxidation, while the second additive is favored by the presence of the aluminum.

CHANGES IN OIL UPON QUENCHING

A series of quenching oil samples from the quenching tanks of a large aluminum foundry was obtained. To the base oil (No. 5) had been added 0.85% lard oil for wetting purposes. Subsequent heating tests indicate little further decomposition of the lard oil over that of the quenching oil when heated at 260° C. To this base oil containing lard oil was added 0.3% of additive GLC-I. If the transmission curves for this composite oil are determined with respect to the oil without the additive, a slightly increased absorption of light is observed which may be ascribed to the color of the additive (Figure 8). However, after the additive has been in the oil for several months the oil actually becomes lighter in color. The effect of decreased light absorption is even more marked in the case of additive GLC-II (freshly added). In this instance, the absorption peak occurs near the absorption peak of the heated oils.

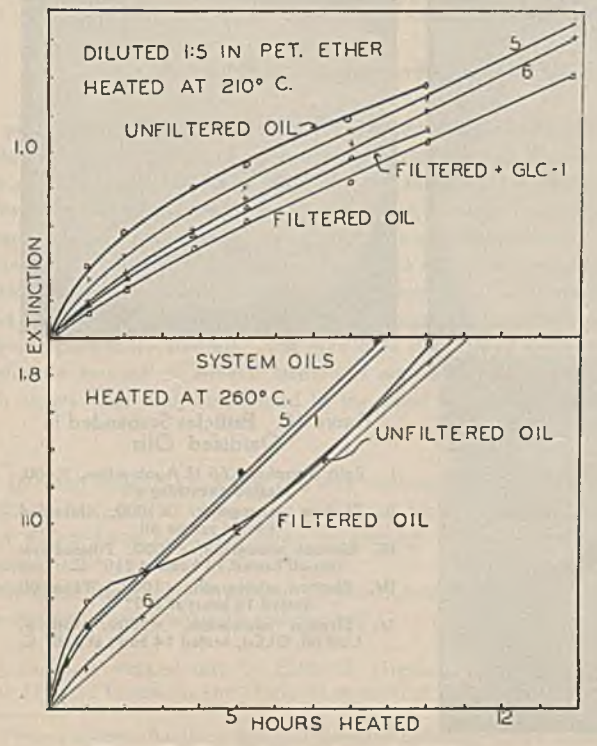

Figure 7. Extinction vs. Length of Heating

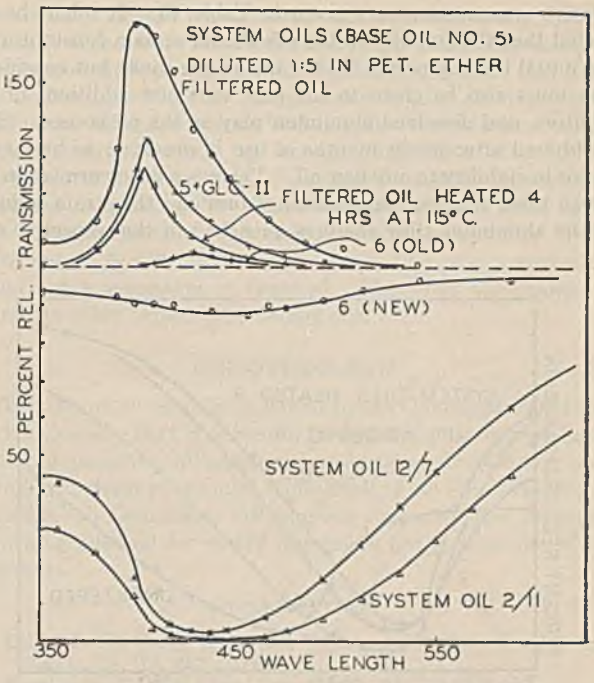

Figure 8. Relative Transmission vs. Wave Length

Table V. Transmission Values of System Oil Relative to Oil Number

(At different dates with continuous use in quenching tank)

Wave Length, Mμ	12/7	12/18	1/12	2/11 Unfiltered	2/11 Filtered
340	43	40	32	30	112
360	42	37	30	29	115
380	39	32	25	24	117
390	...	...	...	...	137
400	17	16	12	12	170
410	...	...	5	2.2	167
420	3.7	3.1	2.6	1.7	158
430	2.6	2.0	1.8	1.2	142
440	2.3	1.8	1.2	1.0	135
450	2.9	2.0	1.1	1.0	129
460	4.0	2.3	1.3	1.2	...
470	6.0	3.4	2.0	1.8	117
480	8.7	5.0	3.0	2.5	...
500	17	11	7.0	5.2	111
540	36	29	21	18	104
600	63	56	47	44	100

Table VI. Strain in Quenched Castings Compared with Stability of System Oils

(Relative transmission at maximum absorption. Heated at 200° C. Diluted 1 to 5 with petroleum ether)

Sample	Average Strain in Castings, Inches of Bow on Stress Relief	Heated 1 Hour	Heated 2 Hours
Oil 6	0.055	48 (420)	35 (420)
12/7	0.095	34 (465)	12 (460)
12/18 (first additive)	0.065	42 (470)	19 (475)
1/12 (new additive)	0.045	66 (480)	27 (480)
2/11 unfiltered	0.052	54 (475)	29 (485)
2/11 filtered	0.030	85 (410)	62 (410)
9/11 (6 months' use of oil without further filtering)	0.045	70 (410)	58 (410)

Table V lists the transmission values for samples of quenching oils taken from the quenching tanks at different dates. These samples have not been heated subsequent to removal from the quenching tanks and are relative to the base oil (5) with lard oil and GLC-I (all diluted 1 to 5 with petroleum ether). The transmission values show increasing oxidation over a period of 4 months when the oil was filtered with a clay adsorbent. The filtered oil has a peak transmission 170% greater than the original oil.

If these system oils (from the quenching tanks) are further heated under the laboratory conditions described and the relative transmission curves of the laboratory heated to unheated oils are determined, some information may be had as to the stability of the oil at any time during use in the tank. These values (for peak

relative transmission) are given in Table VI. It might be expected that the stability of the oils should remain constant after the initial heating period (in the quenching tank) but consideration must also be given to the part that new additions of oil, additive, and dissolved aluminum play as the oil is used. The oil filtered after many months of use is observed to be far superior in stability to any new oil. There is a good correlation between these relative transmission values and the strain induced in the aluminum alloy castings quenched in the respective oils.

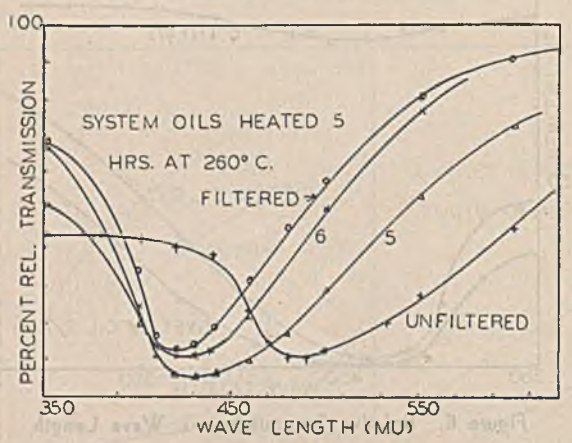

Figure 9. Per Cent Relative Transmission *vs.* Wave Length

The strain values in Table VI are the amount of bow in inches in the casting during stress relief when the casting is sawed nearly through with a series of parallel saw cuts by a standard procedure for the foundry. Experience in motor performance has shown that a bow less than 0.25 cm. (0.050 inch) has never resulted in failure, but that it is essential to maintain a value less than this maximum. The relation between relative transmission and bow is not linear in this instance, but this could hardly be expected with an oil which is continually being renewed with fresh oil, two additives of two additive blends, and other variables operating. Without exception, however, in quenching in this 18,925-liter (5000-gallon) tank and in 567-liter (150-gallon) tanks in which single castings are submersed, the lower the relative transmission index number, the higher the residual strain in the castings. It is a curious fact that the variations in strain follow the index numbers in Table VI (relative changes of the oil at any given stage on further oxidation) and not those of Table V (ratio of the transmission of the oil at any stage in the tank to that of the original unused oil).

A further effect is noted when the filtered system oil is heated for 4 hours at 115° C. Under these circumstances the heated oil shows a transmission 126% greater than the unheated filtered oil. Some inhibitor appears to be in this oil, perhaps a stable product from the original additive or from the clay adsorbent. The nature of the transmission *vs.* wave-length curves for a few of the system oils is given in Figure 9. It is interesting to note the correlation between the absorption curves for samples heated in the laboratory alone and the oils from the quenching tanks.

THEORY OF OPTICAL EVALUATION OF OIL DETERIORATION

The above data suggest a theory upon which the observed phenomenon may be explained. Two basic readings may be observed: the natural and relative transmissions which mean natural color and color induced in the oils upon heating. The natural transmission curves are broad and affected to a lesser extent by heating and diluting than the relative transmission curves. The natural transmission curves are the result of colored materials originally present in the oil in addition to any products of oxidation, while the relative transmission curves are characteristic of the heated products of the oil alone. There appear to be three fundamental processes at work in the oil to produce the relative transmission values: absorption, fluores-

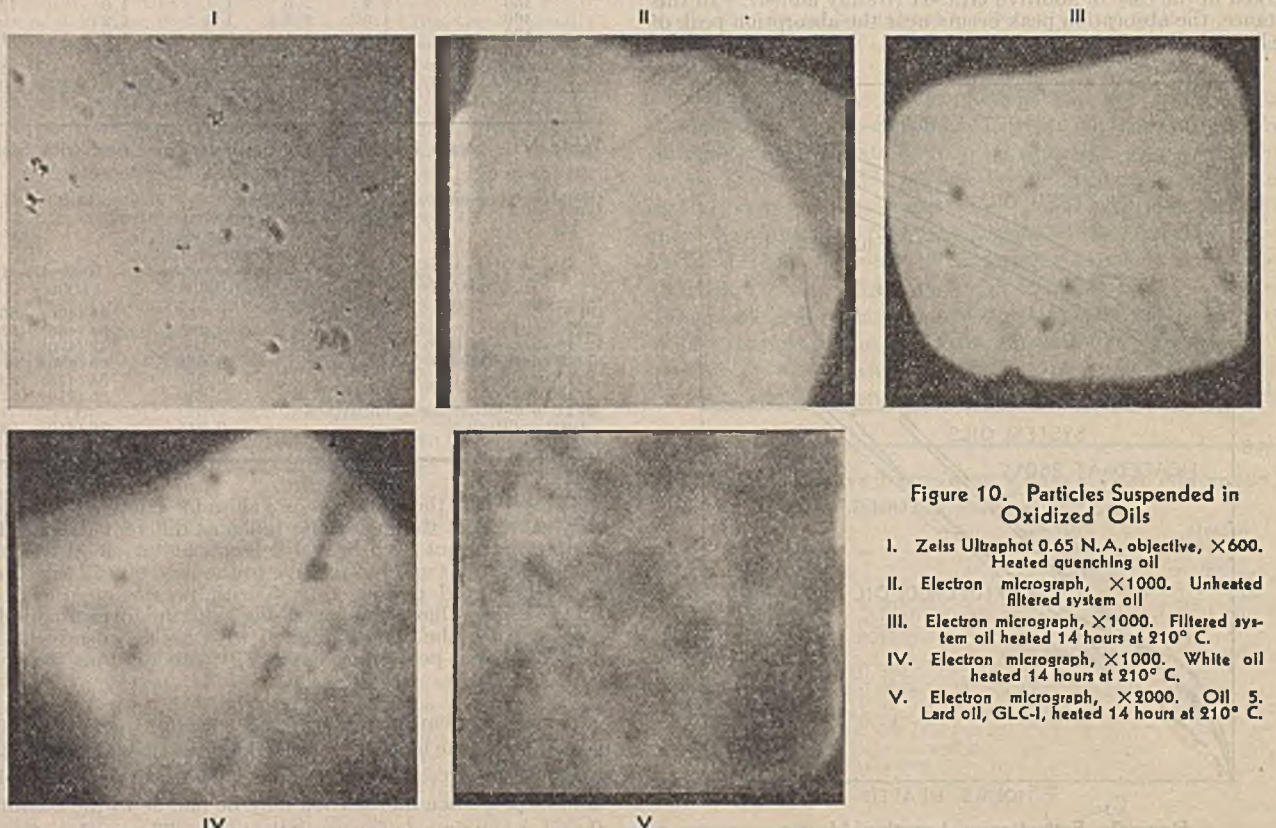

Figure 10. Particles Suspended in Oxidized Oils

I. Zeiss Ultraphot 0.65 N.A. objective, ×600. Heated quenching oil

II. Electron micrograph, ×1000. Unheated filtered system oil

III. Electron micrograph, ×1000. Filtered system oil heated 14 hours at 210° C.

IV. Electron micrograph, ×1000. White oil heated 14 hours at 210° C.

V. Electron micrograph, ×2000. Oil 5. Lard oil, GLC-I, heated 14 hours at 210° C.

cence, and scattering. To what extent each of these processes enters has not been determined, though scattering is believed to play a large part.

The large shift in the absorption peak on dilution with little variation with different solvents does not seem to find adequate explanation on the basis of absorption, while the nature of the curve does not seem to favor explanation on the basis of fluorescence. If absorption were due to fluorescence the transmission curve would be expected to change sharply on the long wave-length side rather than on the short wave-length side of the absorption peak.

The remaining explanation lies in scattering of the light. The presence of finely divided particles suspended in oxidized oils has been known for some time. The "precipitables" are probably a class of these particles. The word "class" is used, since the precipitables can be only the larger of the particles.

Figure 10 illustrates the type of particles to be found in the oil. Photograph I was taken of an undiluted heated oil sample at 600 magnification with a Zeiss 0.65 N.A. objective lens in a Zeiss Ultraphot. The particles appear spherical with a tendency to coalesce. Measurement of the size of these particles leads to approximately 0.5μ. II to V are electron micrographs at 1000 to 2000 diameters. The specimens were diluted approximately 1 to 20 with a benzene drop placed on a collodion film backed with a 200-mesh copper screen and the excess oil removed. It is of interest that any particles appear, since they might be expected to dissolve in the benzene if they are the precipitables.

If these relative transmission curves are actually a measurement of the amount of particles in the oil, the results may indicate the mechanism by which the conduction properties of a quenching oil change on use. The presence or growth of large quantities of particles may have a large influence on the heat conduction from a hot casting. This is indicated by the fact that when particles are completely removed by filtration as proved by electron micrographs, the residual strain in castings quenched in this oil as measured by the bow when the castings are sawed nearly through vertically is decidedly lower than that found in castings quenched in the same oil before filtration. Furthermore, after this filtration following months of deterioration of the oil, remarkable stability is maintained, since in 6 months of continuous use the average strain in castings has increased only from 0.45 to 0.1125 cm. (0.030 to 0.045 inch) and the relative transmission value has decreased from 85 to 70. There is clear evidence from small-scale experiments that with each filtration the stability of this particular oil is still further improved, so that only a very occasional batch operation is required. This may not apply, of course, to other types of quenching oils.

ACKNOWLEDGMENTS

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Determination of Wax in Cotton Fiber

A New Alcohol Extraction Method

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A new technique is described for the determination of the wax of cotton fiber. It consists of a two-step process in which the wax is first extracted with hot 95% ethyl alcohol and then transferred to chloroform through a phase-separation process, in order to eliminate sugars, mineral constituents, and other nonwaxy constituents removed at the same time by the alcohol. The wax is extracted from cotton fiber more rapidly by hot alcohol than by chloroform, the most rapid and adequate of a considerable number of common wax solvents previously studied. The wax thus determined contains a negligible amount of mineral impurities and is less contaminated with sugars than that determined in the usual Soxhlet extraction.

IT IS often desirable to know the wax content of different varieties and strains of cotton, as well as of various cotton products which have been subjected to kiering, scouring, or other treatments.

Methods previously available for the determination of wax in raw cotton are not entirely satisfactory.

A method worked out by Clifford, Higginbotham, and Fargher (*3*) and based on the study of a number of solvents recommends extraction of 100 grams of cotton with chloroform "in a hot Soxhlet apparatus" for a period of 6 hours; "where it is necessary or desirable, however", the quantity can be reduced to 20 grams and the extraction period to only 3 hours. The use of chloroform, especially "hot", is somewhat objectionable, and the quantities of cotton prescribed are large, often not available, and also beyond the capacities of most conventional Soxhlet extractors. These investigators also recommend that carbon tetrachloride be employed "to give an approximate estimate of wax in cotton, the conditions of time and quantity of material being those already outlined in the case of chloroform". Carbon tetrachloride is rather selective in its action and thus gives a less complete extraction of the wax. These investigators studied a number of other solvents, although not ethyl alcohol.

Ahmad and Sen (*1*) employed a sample of only 2.5 grams of cotton and extracted for 4 hours. They used hot benzene which, according to Clifford, Higginbotham, and Fargher, extracts more completely than carbon tetrachloride, but less completely than chloroform. It is inflammable, in contrast to carbon tetrachloride.

In addition to chloroform, benzene, and carbon tetrachloride in the cold, Clifford, Higginbotham, and Fargher studied the selectivity and solvent extraction rate of hot and cold petroleum ethers (boiling point 40° to 60° and 60° to 70° C.), hot and cold ethyl ether, hot benzene, and hot chloroform. In referring to ethyl alcohol, they stated that it is known to dissolve substances other than wax from cotton. Their results showed that hot extraction, in general, removed in 30 hours or longer more waxy substance than cold extraction and that, of the solvents used, chloroform, benzene, and carbon tetrachloride, in the order

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named, were decreasingly efficient. In all cases the extraction seemed to take place in two stages. During the first stage the extraction is rapid; during the second it is slow, proceeds at a diminishing rate for a long period of time, and appears to be concerned with a different kind of material, difficultly soluble in the solvent. The authors concluded, therefore, that routine measurements with different solvents merely permitted the extractive matter to be divided into classes of varying solubility.

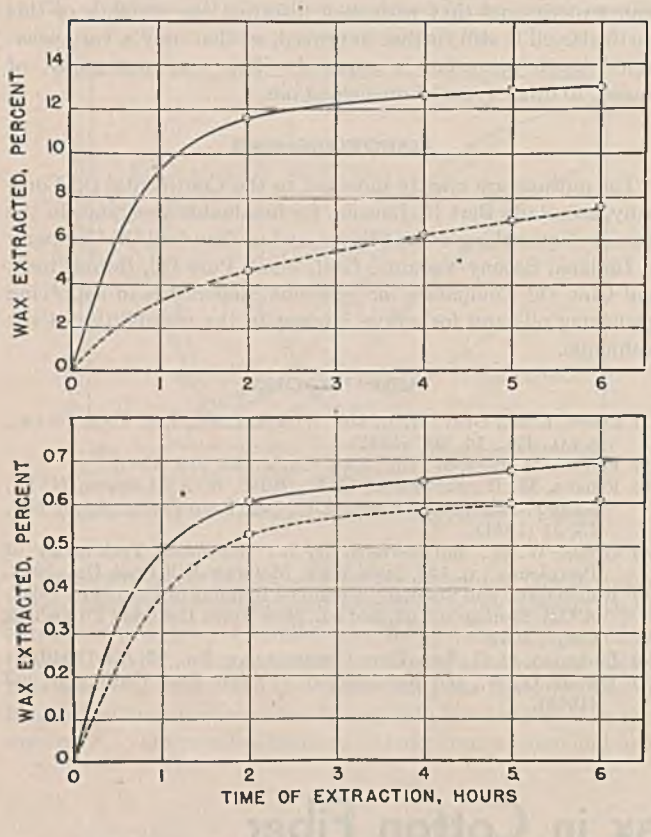

Figure 1. Comparative Amounts of Cotton Wax Extracted with 95% Ethyl Alcohol (Full Lines) and with Chloroform (Broken Lines)

Upper. Very high-wax (green lint) cotton, Sample 3120CX
Lower. Cotton of normal wax content, Sample 109CX

The heterogeneity of cotton wax from representative samples of American and Egyptian cottons was demonstrated by the rather extensive studies of Fargher and Probert (*7*), Clifford and Probert (*4*), and Fargher and Higginbotham (*6*). These workers found that the crude wax contains such widely diverse classes as long-chain aliphatic mono-, di-, and trihydroxy alcohols, aliphatic acids and esters, sterols, sterol glucosides, a mixture of hydrocarbons, resin acids, and resenes. It is therefore little wonder that the attempt to extract these substances simultaneously with a single "fat solvent" should be beset with considerable difficulty.

Hess (*8*, p. 205) points out that, in general, the maximum amounts of resins, fats, and waxy substances are extracted from cellulosic materials with acetone or hot alcohol. He credits Schunk (*8*, p. 206) with the statement that cotton wax is easily soluble in ether and hot alcohol and on cooling separates from the latter in the form of a white gel consisting of microscopically fine crystals. He gives Schwalbe credit (*8*, p. 249) for the statement that equal parts of alcohol and benzene are appropriate for the quantitative determination of resin, fats, and waxes in cellulosic materials. It will be noted that these authors prefer somewhat different solvents for the removal of waxes from these materials than those recommended by Clifford, Higginbotham, and Fargher (*3*) for their quantitative determination.

Maclean and Maclean (*9*, p. 67) point to the fact that alcohol often removes with ease fats and other lipids that are removed only in part or not at all by the usual fat solvents. Bloor (*2*, p. 264) considers that boiling alcohol gives the most complete extraction of lipids from tissues of any solvent.

A technique used by Thor and Smith (*11*), in which chloroform was employed to remove the fat and wax from the alcoholic extracts of pecan fruits before undertaking sugar analysis, first suggested a new technique employing alcoholic extraction, for the determination of wax in cotton fiber. In this the waxy materials are first extracted by the more rapid and inclusive solvent, 95% ethyl alcohol, and then separated with the aid of chloroform from the nonwaxy substances. The successful use of this method over a period of some four years on hundreds of samples of raw cotton has seemed to justify its publication.

COMPARATIVE EXTRACTIVE EFFICIENCIES OF CHLOROFORM AND ETHYL ALCOHOL

It is appropriate first to establish the advantage of ethyl alcohol over chloroform as a solvent for cotton wax.

Two cottons, one of low and the other of very high wax content, were chosen. For high wax content a 5-gram sample of green lint cotton, studied by Conrad and Neely (*5*) and having a wax content of about 13%, was employed. For the low-wax cotton a 10-gram sample of ordinary white cotton was used. In each case duplicate samples were weighed out and placed in the extractive compartments of "large" (50 × 250 mm.) Soxhlet extractors. To one set of extractors were added 250 ml. each of chloroform (U.S.P. XI, for anesthesia) while to the others was added an equal volume of 95% ethyl alcohol. Heat was applied to the flasks and the time was noted when the solvent began to condense and fall on the sample; the extractions were continued exactly 2 hours. The extractions were then interrupted, the flasks with extracts set aside, and new flasks with new solvent substituted. The extraction was again continued exactly 2 hours. Both sets of extractions were interrupted in the same way twice more at 1-hour intervals.

In the light of the work of Clifford, Higginbotham, and Fargher (*3*), the total material extracted by the chloroform may be considered to be wax. However, the waxy material in the alcoholic extract had to be separated from sugars and other nonwax constituents, removed at the same time, by mixing with chloroform and separating into two layers with water, in a manner described below. The accumulated percentages of wax, obtained at the end of the successive periods, are shown by curves in Figure 1.

By reference to Figure 1 it will be seen that not only did 95% ethyl alcohol extract the wax more rapidly than did chloroform, but at the end of 6 hours it had extracted a larger quantity. The total wax extracted in 6 hours with alcohol and separated with chloroform was 15% greater than that extracted with chloroform alone from Sample 109CX, and 75% greater than that extracted with chloroform alone from Sample 3120CX. After 2 hours alcohol had extracted as much as or more wax than was obtained with a 6-hour extraction with chloroform.

From Figure 1 it would appear that extraction is not entirely complete after 6 hours even with alcohol. In the case of routine work complete extraction is often not practicable.

For example, although Clifford, Higginbotham, and Fargher (*3*) actually recovered small but measurable quantities of wax after 35 hours, they recommended only 3 hours for the extraction of a 20-gram sample of cotton with hot chloroform. In the case of an Egyptian cotton extracted with hot chloroform they obtained 0.61% wax after 4 hours, but 0.72% at the end of 32 hours. On the other hand, using cold chloroform on the same cotton they obtained only 0.57% wax after 4 hours and 0.65% after 32 hours. On an American cotton cold chloroform gave them under the same conditions 0.60 and 0.63% wax, respectively. Ordinarily, an extraction period extending beyond about 6 hours is impracticable from a routine standpoint.

The sizes of samples used for the experimental results shown in Figure 1 were one half and one fourth as large, respectively, as the smallest recommended by Clifford, Higginbotham, and Fargher, and the extraction was continued for 6 hours instead of 3. For the low-wax cotton the percentage increase in total wax, obtained at the end of the 5th and 6th hours, as compared with that at the end of the preceding hour, was approximately the same for either alcohol or chloroform and less than 3.5%. With the high-wax cotton on the other hand, whereas alcohol extracted 2.14 and 1.58% additional wax during the 5th and 6th hours, respectively, chloroform extracted 10.5 and 8.1%, respectively. In view of the greater total quantity of wax removed, especially in the case

of the high-wax cotton, advantage of alcohol over chloroform—the most adequate solvent of those studied by Clifford, Higginbotham, and Fargher—is clearly demonstrated.

TRANSFER OF WAXES FROM ALCOHOL TO CHLOROFORM

The successful use of ethyl alcohol as a wax solvent requires a subsequent treatment to separate the wax from any sugars, amino acids, or other alcohol-soluble, nonwax substances.

Various preliminary experiments were carried out in which the alcohol was evaporated to a very small volume and the resulting mixture washed with water and with various wax solvents, including petroleum ether, ethyl ether, carbon tetrachloride, benzene, and chloroform. Invariably, emulsions were obtained which were unmanageable. Evaporation of the extracts to dryness before use of the wax solvents not only led to the same difficulties, but in addition, the waxy constituents were difficultly and incompletely redissolved.

The preliminary extraction of the cotton with water to remove the nonwax constituents, followed by extraction with alcohol to remove the waxes, proved unsatisfactory, since the water did not wet the cotton readily, often not completely even after 4 hours in the Soxhlet, and channeled through the sample. It was recognized also that hot water might melt some of the waxes and carry them over by entrainment, thus giving low wax results.

The successful procedure, finally adopted, based on the experiments of Thor and Smith (*11*) and additional suggestions by Thor (*10*), consisted in the combination of the hot alcoholic extract of the waxes with an equal volume of chloroform, giving a homogeneous solution, and the separation of this into two phases by the addition of water. The waxes are retained by the chloroform layer, whereas the nonwax substances go into the alcohol-water layer.

The completeness with which the wax was transferred to the chloroform layer was demonstrated for both high- and low-wax cottons by separating the first chloroform layer in a separatory funnel, adding new portions of chloroform, and determining the additional amounts of wax obtained. Thus, in a series of samples, using 100 ml. of chloroform for the original separation, and three successive 50-ml. quantities of chloroform to wash the alcohol-water layer, the distribution of wax in the successive portions of chloroform was 99.25, 0.55, 0.10, and 0.10% of the total, respectively. The alcohol-water layers, also, were evaporated to dryness and further examined, both for wax content and for nonwax alcoholic extractives. Any wax obtainable from these layers amounted to less than 0.02%, absolute, while the nonwax residue amounted to 0.75 to 1.30% of the sample, depending on the sample used. It is thus evident that the transfer of waxy substance from alcoholic solution to chloroform is very complete with the first 50-ml. chloroform wash.

AMOUNT OF ASH CARRIED TO CHLOROFORM WITH WAX

Experiments were conducted on both low- and high-wax cottons which showed that while the alcoholic extractions removed 37% of the total mineral constituents from the low-wax cotton and 25% from the high-wax cotton, the greatest weight of ash found in the wax was 0.7 mg., and the average was 0.3 mg. These figures are equivalent to a maximum error in the wax content of 0.44% or an average error of only 0.15%. It is thus evident that no appreciable error is introduced into the wax results through transfer of mineral constituents from the alcoholic to the chloroform solution.

AMOUNT OF SUGARS CARRIED TO CHLOROFORM WITH WAX

The question also arose as to whether any appreciable quantity of sugars (which are contained in cotton fibers in slight amounts and dissolve in 95% ethyl alcohol) could, because of slight solubility in chloroform, be transferred over into it and thus lead to greater apparent weight of wax than actually exists. Sugars would be about the only other nonmineral, nonwaxy constituents known to be present which are soluble in aqueous alcohol and might be soluble to a slight extent in chloroform. No exactly pertinent data could be found in the literature.

To decide this question duplicate 1-gram samples of dextrose, the principal sugar found in the fiber, as well as like samples of sucrose and levulose were dissolved in 100-ml. quantities of 95% ethyl alcohol, transferred to 100 ml. of chloroform, and washed with three additional successive 50-ml. portions of chloroform in the same way as was done above with the alcoholic extracts of the fibers. The chloroform extracts were evaporated to dryness, first on the steam bath and then in the vacuum oven at 80° C. The residues were weighed and the weights of the sugars found in this way are shown in Table I.

Table I. Sugars Transferred to 250 Ml. of Chloroform

(By distribution from 95% ethyl alcoholic solutions containing 1 gram of the sugar)

Sugar Taken	Residue from Chloroform	Sugar Recovered
	Mg.	*Mg.*
None	0.9	...
	0.9	...
Dextrose	3.4	2.5
	3.8	2.9
Sucrose	1.1	0.2
	1.2	0.3
Levulose	1.9	1.0
	1.8	0.9

By reference to Table I it will be seen that the chloroform itself contained a very slight residue. Very small quantities of sugar dissolved in the chloroform, the amount being somewhat the greatest in the case of dextrose. Assuming a 10-gram sample of cotton of about average wax content of 0.5% and dextrose as only sugar in the fiber, the error caused by a saturated solution of this sugar in 150 ml. of chloroform (two washes of 100 and 50 ml., respectively) is slightly over 3% of the wax. On the other hand, it is evident that in a conventional extraction where the chloroform would be used directly in the Soxhlet apparatus to remove the wax, and where through repeated siphoning the effective volume of the chloroform is many times that actually present, a much greater quantity of sugars could be dissolved if present. In fact, an actual experiment showed that under these conditions from 10 to 25 times as much sugar could be extracted in a 6-hour extraction period, and deposited in the extraction flask. The use of alcohol for the extraction, with subsequent transfer to chloroform, thus avoids a serious source of error which can be present if chloroform is used directly as the wax solvent.

PROPOSED METHOD FOR DETERMINATION OF TOTAL WAX IN COTTON FIBER

The following method is proposed for the determination of total wax in cotton fiber and similar materials.

Place 5 to 10 grams, depending on the wax content, of well cleaned fiber in a coarse thimble in a large Soxhlet (50 × 250 mm.) extractor assembled ready for operation. Add 250 ml. of 95% ethyl alcohol to the extraction flask and adjust the gas flame or other source of heat until the liquid siphons over at 3 to 4 minute intervals. Continue the extraction for 6 hours. Turn off the heat, lift condenser, and remove the thimble and sample from the extraction compartment. Replace condenser and continue heating until part of the alcohol has passed over to the extraction compartment of the Soxhlet and only 75 to 85 ml. of liquid remain in the extraction flask.

While still warm (above 60° C.), or after warming if the extract has been allowed to cool, transfer the alcoholic extract to a 500-ml. separatory funnel. Wash out the Soxhlet flask with several 5-ml. portions of hot 95% ethyl alcohol and add additional alcohol, so that the final volume is approximately 100 ml. Add 100 ml. of reagent or U.S.P. XI grade chloroform to the separatory funnel and mix thoroughly. This should give a completely homogeneous solution. Now add to this alcohol-chloroform solution 75 ml. of water and agitate somewhat to cause mixing and separation of two distinct layers, the chloroform layer being at the bottom. Do not agitate violently, as this is unnecessary and tends to cause permanent emulsions to form in some cases. Allow the two layers to stand until they become clear. This may take overnight. Draw off the chloroform layer and set aside in a 250- to 300-ml. Erlenmeyer flask. Add a fresh 50-ml. portion of chloroform to the separatory funnel,

agitate gently, and again allow the layers to separate. This time the separation should be complete in a couple of hours. The wax is now practically completely in the chloroform layer.

It is probably desirable to wash the chloroform solution of extracted wax at least once with water. Therefore drain the separatory funnel and discard the spent alcohol-water solution, which also contains the sugars and other alcohol-soluble, nonwaxy substances. Without washing the funnel, pour the chloroform solution of wax back; add about 100 ml. of distilled water, shake carefully, and allow the two layers to separate. When separation is complete, draw off the chloroform layer, receiving it into the same Erlenmeyer flask from whence it was last taken. Now add two 5-ml. portions of fresh chloroform successively to the separatory funnel, shake well, allow to separate, and draw off each in turn into the Erlenmeyer flask containing the main body of chloroform solution. This should complete the transfer of the wax back into this flask.

Remove the chloroform from the wax by evaporating in tared 100-ml. beakers on a steam bath. The beakers should not be filled more than half way at a time, because of the tendency for the chloroform to superheat and boil up slightly at times as well as to leave a deposit of wax above the solvent on the sides of the beaker. After the wax residue appears to be dry, cool and weigh the beakers, then heat them on the steam bath 30 minutes more, and again cool and weigh. If the weights are not constant, repeat reheating until two successive weighings agree within 0.1% of the residue weight.

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A Fractionating Column

For Continuous Production of Distilled Water of High Purity

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THE entire apparatus shown in Figures 1 and 2, the fractionating column of which is of primary interest here, was used to prepare redistilled water of high purity from a laboratory supply of rather poor quality. Long single tubes of small inside diameter and concentric glass tubes, in which the reflux is distributed on the walls of the tubes have been found to give an efficiency of up to the equivalent of more than 85 theoretical plates in a length of less than 150 cm. (5 feet) (*2*). The present apparatus may be regarded as two such columns in series, but arranged concentrically to prevent loss of heat and to reduce the over-all height.

Accessories shown serve to make the operation of the apparatus continuous and automatic. The constant-level device for controlling the input is a more sturdy modification of one previously reported (*1*). To maintain an easily controlled uniform flow of cooling water through the condenser, an obvious device was used (constant head, Figure 2).

Present circumstances do not justify a careful study of the heat exchange and other details of performance of the apparatus. Instead, certain seemingly valid assumptions were made, and the final effectiveness of the column was tested by a simple electrometric measurement of the conductivity of the distillate (water).

It is assumed that the greater part of any higher-boiling fractions are condensed in the outer space between the outer air-cooled shell and the middle concentric tube and are refluxed back into the boiling flask, and that, at least in the case of water, any heavier fractions which pass over the top in more than negligible traces are substances that will readily distill with steam and will be carried with the steam to the top of the condenser. The amount and composition of the first reflux (above) will, of course, depend on temperature and rate of flow of the vapors, temperature of the surrounding air, and other factors. The narrow space between the central and middle concentric tubes serves to conduct the remaining vapors to the bottom of the central space and to maintain or slightly increase their temperature toward the bottom of the space. These vapors then ascend through the central space to the condenser, which is maintained by slow flow of cooling water at near the temperature of condensation of water. As the condensate flows down the walls of the central tube, it is continually in contact with hot vapor flowing upward. Any lighter fractions which may have condensed and any gases redissolved in the water are assumed to be driven off again and returned to the top of the column. Gases and lower-boiling fractions are eventually driven out with a small portion of steam from the top of the condenser.

On several occasions the performance of the still was tested by measuring the conductivity of the distillate in a Barnstead purity meter in terms of an electrometrically equivalent concentration of sodium chloride in solution in pure water. The results are given in Table I. From these data it is evident that the first 150 to 200 ml. should be discarded, that the still is then clean and

Table I. Conductivity of Distillate (Water) Delivered at 3 to 4 Liters Per Day

Run	Sample, Portion of Run, Condition of Still, etc.	Temp. °F.	NaCl Equivalent P.p.m.
1	Water in reservoir	80	5.8
	First distillate taken, read when taken	130	0.3
	First distillate taken, after 30 min.	105	0.5
	Probable solution of electrolyte from glass		
2	Beginning of day, first 150 ml. discarded	...	...
	First sample, barely in zero range	95	< 0.1
	End of day, far into zero range	132	< 0.1
3	End of 2-liter run, last portion from reservoir in flask, far in zero range	132	< 0.1
4	Still idle 2 weeks; first 20 ml. discarded	...	...
	First fraction, 50 ml. after 20 ml. discarded	128	0.7
	40 ml. discarded, next 50 ml. taken / Second fraction, well in zero range	130	< 0.1

A considerable arc on the meter below 0.1 is marked "zero range".

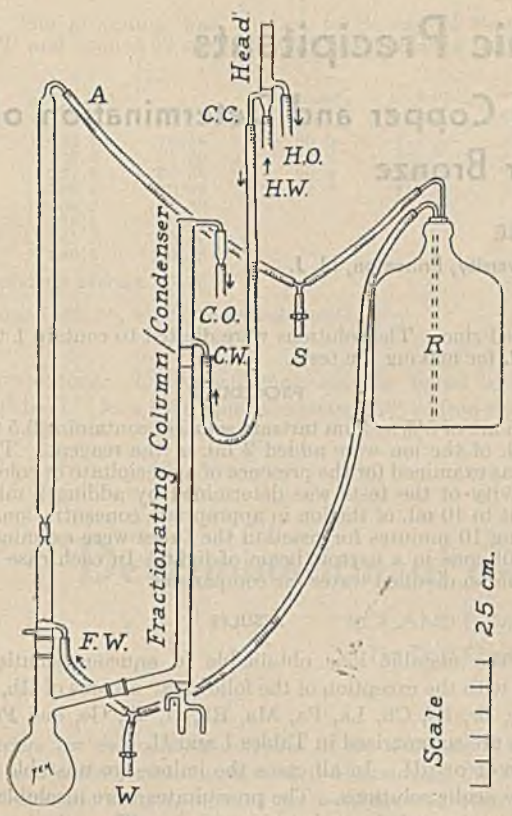

Figure 1. General Arrangement and Proportions of Column, Boiling Flask, and Accessories

A.	Air pressure communication, constant-level column to *R*
C.C.	Condenser cooling water control capillary
C.O.	Condenser cooling water overflow
C.W.	Condenser cooling water inlet
F.W.	Feed water (from *R* into boiling flask)
Head.	Constant head device
H.W.	Constant head device, inlet (tap water)
H.O.	Constant head device, overflow
R.	20-liter bottle, reservoir for feed water
S.	Tube with screwclamp for application of suction
W.	Tube for refilling *R* with water

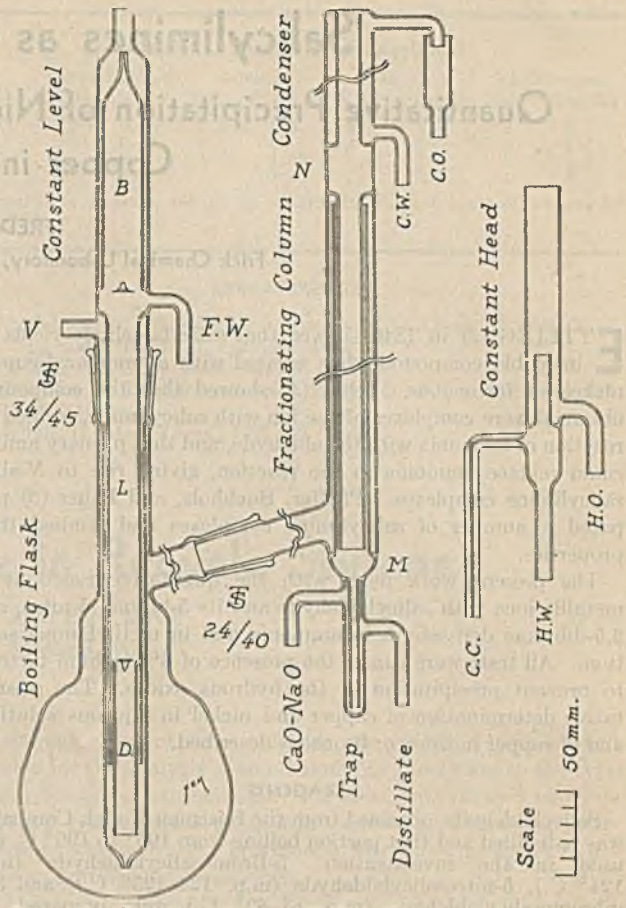

Figure 2. Details of Column, Boiling Flask, and Accessories

B.	Baffle
D.	Tube to check air flow and facilitate surge of water
L.	Constant-level tube
M.	Dead space below central tube
N.	Space separating condenser from top of fractionating column
V.	Vent
Other reference letters same as in Figure 1	
ⅎ	Standard-taper joints as indicated

delivering pure water, and that the production of a satisfactory distillate is maintained to the last portion of at least 20 liters from the reservoir.

Space *N*, Figure 2, separates the condenser from the fractionating column to prevent overcooling of the first reflux. Space *M*, which is undesirable and is made as small as possible, could be heated slightly to prevent condensation of lighter fractions, but for present purposes this has not been found necessary. The trap serves to prevent loss of vapor and to maintain sufficient back pressure to force the vapor through the column. If extreme precautions are to be taken, a soda-lime tube or other protective absorbent may be connected to the trap vent (CaO-NaO, Figure 2).

A uniform flow of cooling water is essential to proper operation of the still. This is adequately provided by a constant head or pressure of water acting against the resistance of the capillary (Figure 2, *C.C.*) and by the fixed level of the overflow from the condenser into an open cup (*C.O.* in both figures). The device marked "constant head" in Figure 2 is suitable. Its position above the column is indicated in Figure 1. By raising or lowering it, the rate of distillation and the portion of middle fraction rejected, hence also the quality of the distillate may be closely controlled.

The constant boiler level control device consists of two parts, built separately for convenience in fabrication and to decrease danger of breakage. Air entering the lower section through the vent, *V*, passes below the bottom edge of *L*, upward between the walls of *L* and *D*, through the V-shaped opening in the ring seal into *L* and then *B*, and through the capillary tip of *B* into the top section of this column. Water from *B* and *L* surges freely down *D* when displaced by air, quickly blocking further ingress of air, but it can flow only slowly past *B* through the V-shaped opening in the ring seal at the bottom of *B*. Air flows from the top of the column at *A* into *R*, permitting water to flow from *R* to *F.W.* If, in starting operation, the pressure in *R* is not sufficiently low to hold back excess water, it can be reduced further by applying suction slowly at *S*. The reservoir can be refilled through *W* by placing pinchclamps between *A* and *S* and between *F.W.* and *W* and applying suction at *S*. The earlier paper (*1*) gives a more satisfactory explanation of the constant-level control.

Table II. Dimensions of Still

Fractionating column, length 600 mm.		Condenser, length 300 mm.	
Outer tube (shell)	30 mm. O.D.	Outer tube	30 mm. O.D.
Middle tube	20 mm. O.D.		
Central tube	15 mm. O.D.	Central tube	15 mm. O.D.
Shell of lower section of constant-level control above standard taper joint, and entire upper section of column			32 mm. O.D.
Shell of lower section below standard taper joint			25 mm. O.D.
Tube *B* 24 mm. O.D.	Tube *L* 19 mm. O.D.	Tube *D*	14 mm. O.D.
Orifice at top of baffle, *B*, approx. 0.6 mm. I.D.			
Orifice at bottom of shell of lower section, approx. 1.5 mm. I.D.			
Bottom edge of *L* approx. 30 mm. above desired level in flask			
Trap at least 50 mm. long between lower end of inner tube and junction of distillate delivery tube, because of back pressure			
Control capillary of constant-head device approx. 1.0 mm. I.D. and 150 mm. long including bend			
Constant-head device large enough to avoid flooding and splashing			

Considerable latitude is probably permissible in the dimensions of various parts of the still. Those that may be informative or critical are listed in Table II.

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Salicylimines as Organic Precipitants

Quantitative Precipitation of Nickel and Copper and Determination of Copper in Brass or Bronze

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ETTLING (*2*) in 1840 showed that salicylaldehyde yields an insoluble compound when treated with ammoniacal cupric, nickel, or ferric ions. Schiff (*4*) showed that the compounds obtained were complexes of the ion with salicylimine, formed by reaction of ammonia with the aldehyde, and that primary amines could replace ammonia in the reaction, giving rise to *N*-alkyl salicylimine complexes. Pfeiffer, Buchholz, and Bauer (*3*) prepared a number of salicylimine complexes and studied their properties.

The present work deals with the qualitative reactions of metallic ions with salicylaldehyde and its 5-bromo, 5-nitro, and 3,5-dibrome derivatives in ammonia and in methylamine solution. All tests were run in the presence of 5% sodium tartrate to prevent precipitation of the hydrous oxides. The quantitative determination of copper and nickel in aqueous solution, and of copper in brass or bronze is described.

REAGENTS

Salicylaldehyde, obtained from the Eastman Kodak Company, was redistilled and that portion boiling from 195° to 196° C. was used in the investigation. 5-Bromosalicylaldehyde (m.p. 124° C.), 5-nitrosalicylaldehyde (m.p. 124–125° C.), and 3,5-dibromosalicylaldehyde (m.p. 81–82° C.) were prepared according to Beilstein (*1*).

IMINE SOLUTIONS. *Salicylimine.* Dissolve 1 gram of salicylaldehyde in 100 ml. of 10 to 90 ammonium hydroxide. *5-Nitrosalicylimine.* Dissolve 1 gram of 5-nitroaldehyde in 100 ml. of 10 to 90 ammonium hydroxide. *5-Bromosalicylimine.* Dissolve 0.25 gram of the 5-bromoaldehyde in 100 ml. of concentrated ammonium hydroxide. *3,5-Dibromosalicylimine.* Dissolve 0.2 gram of the 3,5-dibromoaldehyde in 100 ml. of concentrated ammonium hydroxide. The solutions darken and lose their usefulness after approximately 8 hours.

N-METHYLIMINE SOLUTIONS. Prepare in same way as imine solutions, using 25% aqueous methylamine in place of concentrated ammonium hydroxide. The solutions are stable for long periods of time.

SOLUTIONS OF INORGANIC IONS. Stock solutions of known approximate concentration were prepared from reagent grade salts, oxides, or metals, followed in some cases by reduction on amalgamated zinc. The solutions were diluted to contain 1 to 2 mg per ml. for making the tests.

PROCEDURE

To 5 ml. of 5% sodium tartrate solution containing 0.5 to 1 mg. per ml. of the ion were added 2 ml. of the reagent. The solution was examined for the presence of a precipitate or color. The sensitivity of the tests was determined by adding 2 ml. of the reagent to 10 ml. of the ion in appropriate concentration. After allowing 10 minutes for reaction the tubes were examined for a Tyndall cone in a narrow beam of light. In each case a blank was run on distilled water for comparison.

RESULTS

All the metallic ions obtainable in aqueous solution were tested with the exception of the following: all ions of Rb, Cs, Ra, Sc, Ac, Zr, Hf, Cb, La, Pa, Ma, Rh, Ir, Tl, Ge, and Po. The results are summarized in Tables I and II.

EFFECT OF pH. In all cases the imines are unstable in even weakly acidic solutions. The precipitates were insoluble in high concentrations of the amine or ammonia. Thus, the insolubility range in all cases is pH 7–8 to 11–12.

QUANTITATIVE APPLICATION OF SALICYLIMINE

Standard solutions of Cu^{++} and Ni^{++} were prepared by electrolyzing a known amount of the metal on platinum, removing the metal plate with nitric acid, and diluting to known volume. To pipetted samples of the standard solutions were added sodium tartrate to 5%, followed by an excess of ammonia and salicylimine solution prepared according to the above directions. The precipitates were filtered through sintered-glass crucibles and weighed after being dried to constant weight at 100° C. The factor for converting the copper precipitate to metal is 0.2092, and the factor for nickel is 0.1963.

Cu Taken, Mg.	Wt. of Ppt., Mg.	Cu Found, Mg.
9.85	46.9	9.81
9.85	47.1	9.85
24.87	118.6	24.81
24.87	118.6	24.81
49.58	236.9	49.56
49.58	236.6	49.50

Ni Taken, Mg.	Wt. of Ppt., Mg.	Ni Found, Mg.
8.91	44.9	8.81
8.91	45.1	8.85
22.57	115.4	22.65
22.57	115.2	22.61
44.78	227.6	44.68
44.78	228.0	44.76

DETERMINATION OF COPPER IN BRASS OR BRONZE. A 60- to 100-mg. accurately weighed sample of the alloy is dissolved in hydrochloric-nitric acid (2 ml. of each of the concentrated acids). The solution is diluted to approximately 25 ml. and sufficient sodium tartrate solution is added to make its over-all concentration 5%. After making distinctly ammoniacal with filtered ammonium hydroxide and cooling below 25° C., the salicylimine solution is added. After 5 minutes in the cold the precipitate is filtered through sintered-glass crucibles, dried for an hour at 100° C. (never above 105° C.), and weighed. Per cent copper is calculated as follows:

% Cu = weight of ppt. × 0.2092 × 100/sample

Table I. Reactions of Salicylaldehyde and Derivatives

Ion	Salicylaldehyde NH_3	Salicylaldehyde CH_3NH_2	5-Nitro NH_3	5-Nitro CH_3NH_2	5-Bromo NH_3	5-Bromo CH_3NH_2	3,5-Dibromo NH_3	3,5-Dibromo CH_3NH_2
Cu^{++}	Green ppt.	Green ppt.	Green ppt.	Green ppt.	Green ppt.	Green ppt.	Green ppt.	Green ppt.
Ni^{++}	Orange ppt.	Yellow ppt.	Orange ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.	Brown ppt.	Yellow ppt.
V^{++}	Red ppt.	Red ppt.	Orange ppt.	Red ppt.	Red ppt.	Red ppt.	Red ppt.	Red ppt.
Pd^{++}	Yellow ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.
Mn^{++}	....	....	....	....	....	....	....	Brown ppt.
Fe^{+++}	Red ppt.	Red ppt.	....	....	Red ppt.	....	....	....
Fe^{++}	Red color	Red ppt.	Red color	Blue ppt.	....	Lavender ppt.	....	Purple ppt.
Co^{++}	....	Brown ppt.	Brown ppt.	Brown ppt.	....	Brown ppt.	Brown ppt.	Brown ppt.
Hg^{++}	....	....	....	....	....	....	....	Yellow ppt.
Zn^{++}	....	....	....	....	....	....	....	Yellow ppt.
Cd^{++}	....	....	....	....	....	....	....	Yellow ppt.
ReO_4^-	....	....	....	....	....	....	....	Yellow ppt.

Qualitative reactions in 5% sodium tartrate. .... indicates no reaction.

weight. The procedure was applied to Bureau of Standards brass 37B and bronze 52 samples, with the following results:

Brass, Mg.	Wt. of Ppt., Mg.	% Cu	Bronze[a], Mg.	Wt. of Ppt., Mg.	% Cu
124.9	421.3	70.56	99.0	418.8	88.50
73.2	249.5	71.30	64.8	273.7	88.75
79.6	267.7	70.36	90.0	381.0	88.55
96.0	323.0	70.38	107.7	455.2	88.42
90.2	303.2	70.32			
67.0	225.9	70.53			
71.5	241.2	70.57			
89.0	300.3	70.58			
Bur. of Standards average		70.36			88.33

[a] Contains 0.13% Ni, which was not subtracted.

INTERFERENCES. The interferences may be found by reference to Table I. Less than approximately 0.3% of iron does not interfere.

Table II. Sensitivity of Reactions

Ion	Salicylaldehyde NH_3	Salicylaldehyde CH_3NH_2	5-Nitro NH_3	5-Nitro CH_3NH_2	5-Bromo NH_3	5-Bromo CH_3NH_2	3,5-Dibromo NH_3	3,5-Dibromo CH_3NH_2
Cu^{++}	2	0.75	3.5	1.5	4	1.5	4	2
Ni^{++}	2	0.75	3	1	3	2	3	1.5
CO^{++}	.	0.5	1	0.75	1.5	1	2	1.5
Fe^{++}	.	0.1	...	0.25	...	0.75	.	1

Sensitivity of tests, 10^6 ml. of solution in which 1 gram of ion can be detected.

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Determination of o-Xylene in Recycle Styrene

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A procedure for determining o-xylene in the presence of alkylbenzenes and olefinic compounds consists of removing the unsaturates with mercuric acetate, nitrating the o-xylene and other alkylbenzenes, and, using a modified Bost-Nicholson reaction, measuring the color produced by dinitro-o-xylene in a suitable photoelectric colorimeter. It is possible to detect less than 0.01% o-xylene in samples consisting largely of unsaturates.

IN THE production of Buna S rubber the recovery of unpolymerized styrene monomer is an important factor. Impurities such as 1,4-vinylcyclohexene, ethylbenzene, isopropylbenzene, *n*-propylbenzene, and *o*-xylene accumulate in varying amounts, thus making purification necessary to utilize the unpolymerized styrene. All these impurities except *o*-xylene, which boils at 144° C. (styrene boils at 145–146° C.), are easily removed by distillation. Therefore the analysis of styrene for *o*-xylene is often important.

Luszczak (*4*) developed a method for the determination of small amounts of xylene in xylene-toluene vapor mixtures in the air within buildings, in which 7 to 8 liters of air were shaken for half an hour in a flask with 50 to 100 ml. of alcohol. The xylenes, which give the same millimolar extinction constant, and toluene, whose extinction curve is not simultaneously influenced, were quantitatively determined from the ultraviolet spectrum of the alcoholic solution. Luszczak (*5*) also developed a similar method for the determination of benzene, toluene, and xylene in commercial benzene.

A method for analysis of *m*-xylene in xylene mixtures by nitration and fractional crystallization of 2,4,6-trinitro-*m*-xylene from acetone was described by Reichel (*7*).

Kester and Holmes (*3*) analyzed mixtures of paraffins, benzene, toluene, and xylene by means of fractionation followed by sulfonation of the cuts. Zaborowski (*12*) analyzed various mixtures of xylene, toluene, and gasoline by sulfonation with a known amount of sulfuric acid and titration of the excess acid.

An optical method was developed by Schildwächter (*8*) in which vapors of methanol, ethanol, diethyl ether, pentane, hexane, heptane, benzene, toluene, xylene, and various petroleum and coal-tar benzines were determined. The requisite percentages were calculated from data obtained by means of an interferometer.

Norris (*6*) devised a method for the determination of *o*-xylene in xylene mixtures by oxidation with potassium permanganate to the corresponding phthalic acids, which were separated and determined. However, this method is only about 90% quantitative.

Although some of these methods are accurate, none seemed suitable for the analysis of small amounts of *o*-xylene in the type of samples under examination. Furthermore, infrared methods (*11*) for *o*-xylene are not possible in this case because of the interference of ethylbenzene. Therefore it was found necessary to develop an entirely new procedure.

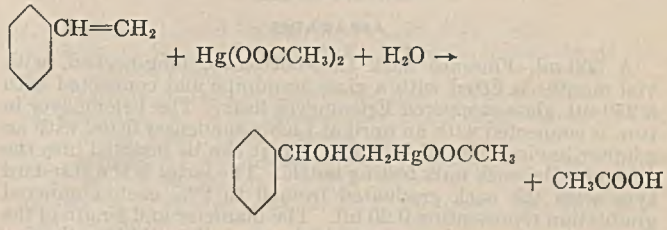
OUTLINE

I. The alkylbenzenes are separated from the styrene, 1,4-vinylcyclohexene, and any other olefinic compounds present by reacting the sample with aqueous mercuric acetate and subsequently steam-distilling the alkylbenzenes from the reaction mixture. Ethylbenzene is used as a carrier in the steam-distillation to aid in removing the *o*-xylene.

Whitmore (*9*) states that aqueous mercuric salts add the groups —HgX and —OH to the double bond of olefinic compounds. A general equation for this reaction may be shown as follows:

$$RCH{=}CH_2 + HgX_2 + H_2O \rightarrow RCHOHCH_2HgX + HX$$

the addition in general following Markownikoff's rule (*10*), mercury going to the carbon having the most hydrogen atoms. With styrene, the reaction with mercuric acetate would be:



Mercuric acetate reacts similarly with the unsaturated linkages of the 1,4-vinylcyclohexene.

In a few instances, particularly with the propenyl group, (—CH=CHCH$_3$), the mercuric salt does not add to the double bond, but instead oxidizes it to the glycol (*1*). Thus, if this reaction occurred with styrene, phenylglycol would be produced:

$$+ 2(CH_3COO)_2Hg + 2H_2O \rightarrow$$

$$+ 2CH_2COOHg + 2CH_3COOH$$

The formation of an oily liquid, sparingly soluble in water, indicates the presence of organo-mercury compounds, while the appearance of a precipitate of mercurous acetate after prolonged steam-distillation indicates the formation of glycols. It is thought that both reactions occur, although in what proportion has not been determined, since either reaction brings about the desired result—viz., the changing of all olefinic compounds to compounds that are not steam-distillable.

II. The alkylbenzenes, after removal of the olefinic compounds, are nitrated by means of a suitable nitrating acid and the dinitro compounds are treated with acetone and potassium hydroxide in accordance with the Bost-Nicholson color test (*2*). In this reaction the color first formed by dinitromonoalkylbenzene is a deep blue, while the color formed by dinitro-*o*-xylene is a deep green. The green color in the presence of the blue cannot be satisfactorily measured by a colorimeter. However, advantage is taken of the fact that the blue color gradually changes to a purplish-red under the influence of alkali, while the green color remains and is measured by the photoelectric colorimeter. To hasten the change of the blue color and make the red less intense, the reaction is modified by the addition of monoethanolamine.

REAGENTS

Propylene Glycol. This reagent should be of a good grade, free from volatile impurities other than water.

Mercuric Acetate Solution. Dissolve 320 grams of reagent grade mercuric acetate (anhydrous) in 800 ml. of distilled water (cold, to keep formation of mercuric oxide at a minimum). Filter into a 1000-ml. glass-stoppered bottle. On long standing a small amount of mercuric oxide will form, which may be disregarded.

Ethylbenzene, xylene-free.

Potassium Hydroxide, aqueous 50% solution. Dissolve 75.0 grams of reagent grade potassium hydroxide pellets in 75.0 ml. of distilled water.

o-Xylene. The xylene used for standards in this investigation was approximately 92% *o*-xylene, with 1 to 2% of *p*-xylene and approximately 6% of *m*-xylene.

Nitrating Acid. Mix equal parts, by volume, of concentrated C.P. nitric and sulfuric acids. Keep available sufficient of a single lot of this mixture to complete all determinations in a given series of analyses.

Nitric Acid, concentrated C.P.

Acetone, reagent grade. In this case also the accuracy of the colorimetric determination is enhanced if the reagent is kept in a single lot of consistent quality, and it should be used in connection with the same lot of nitrating acid.

Diethyl Ether, U.S.P.

Monoethanolamine. Use a technical grade, from a single lot of consistent quality and in connection with the same lot of nitrating acid.

APPARATUS

A 300-ml. Florence flask (flat-bottomed, long-necked, with vial mouth) is fitted with a glass standpipe and connected with a 250-ml. glass-stoppered Erlenmeyer flask. The Erlenmeyer in turn is connected with an upright Liebig condenser fitted with an adapter having a tip of such size that it can be inserted into the neck of a Babcock milk testing bottle. The latter is the standard type with the neck graduated from 0 to 8%, each numbered graduation representing 0.20 ml. The diameter and length of the glass tubing connections should be kept small to aid in collecting the organic distillate without distilling a large volume of water. Rubber stoppers are used to complete the connections, and replaced from time to time, since they slowly deteriorate.

A shaker on which 250-ml. Erlenmeyer flasks may be placed is needed. A second shaker is required, to which is attached a small box with a snug hinged cover and painted black inside. A sponge-rubber mat with openings to accommodate two 30-ml. (1-ounce) bottles is placed in this box. The bottles are the narrow-mouthed French square type, fitted with silver foil-lined screw caps. In this investigation the second shaker was a Fisher Scientific Co. model No. 6-213A, oscillating 275 to 285 times per minute.

Another small box with black interior is required. Any small cardboard box with cover will do. In this investigation the box was a cube measuring 12.5 cm. (5 inches) on the side.

A photoelectric colorimeter equipped with the proper filters is required. A Lumetron model 402-EM instrument, fitted with a B660 filter, was used in this investigation. Of those available, this filter was found to be most efficient in preventing interference from the red color of dinitroethylbenzene, etc., at the same time allowing full measurement of the green color of dinitro-*o*-xylene.

Other necessary apparatus includes capillary-tipped pipets made from medicine droppers, an interval timer or stopwatch, and a centrifuge for the Babcock bottles.

Table I. Sample Size

o-Xylene %	Sample *Ml.*	Alkylbenzene *Ml.*
Nil–0.10	5.0	0.15–0.20
0.10–0.5	5.0	0.20–0.25
0.50–1.0	3.0	0.2–0.3
1.0–3.0	3.0	0.4–0.6
3.0–6.0	3.0	0.6–0.8
6.0–20	2.0	0.5–0.7
20–50	1.0	0.4–0.9

SAMPLE SIZE

If the mixture is predominantly alkylbenzene, a 1-ml. sample is satisfactory.

With samples consisting largely of unsaturates, it is desirable to strike a balance between sample size and the volume of alkylbenzenes distilled over, depending on the percentage of *o*-xylene. When the *o*-xylene content is low, the smallest practicable amount of ethylbenzene is added to the sample to obtain a maximum concentration of *o*-xylene for the colorimetric determination. When the *o*-xylene content is high, more ethylbenzene should be added to increase the volume of alkylbenzenes, thus preventing too high a concentration of *o*-xylene. If sufficient or more than sufficient alkylbenzene is already present, no ethylbenzene is added. Table I serves as a guide for the proper sample size.

PROCEDURE

When a sample contains more than 99% styrene and less than 0.5% *o*-xylene, the procedure is as follows: Place 60 ml. of propylene glycol in a clean 250-ml. glass-stoppered Erlenmeyer flask and add, by means of a pipet, 5.0 ml. of the styrene sample. Determine the sample weight by weighing a similar amount. Add 0.20 ml. of xylene-free ethylbenzene to act as carrier for the *o*-xylene, then add 75 ml. of the mercuric acetate solution. Stopper the flask, securing the stopper firmly with friction tape, and shake vigorously on a shaking machine for 2 hours.

Connect the flask with the distillation apparatus and steam-distill the ethylbenzene and *o*-xylene into the Babcock bottle. Boil the water in the steam generator before heating the contents of the Erlenmeyer flask, in order to shorten the time the solution is hot while the alkylbenzenes are distilled. Continue the distillation until the bottle is about one-third full, or until just before the appearance of crystals of mercurous acetate, which rarely occurs before 35 to 40 ml. of distillate have been collected. Fill the Babcock bottle with distilled water and centrifuge at 1500 r.p.m. for 5 minutes. Measure and record the volume of the alkylbenzene layer. By means of a capillary-tipped dropping pipet transfer the alkylbenzene layer to a small vial, to be used as needed. The balance of the analysis should be completed within one working day, since the dinitro compounds decompose upon long standing.

Using a 0.1-ml. serological pipet graduated in 0.01 ml., transfer 0.050 ml. of the alkylbenzene mixture in the vial into the neck of a clean, dry 200-ml. volumetric flask, and wash immediately into the flask with 10.0 ml. of nitrating acid mixture. Shake the flask for a few seconds, then allow it to stand for 1 hour with occasional shaking, and finally allow it to cool in an ice bath.

Gradually add 25 to 30 ml. of distilled water, while shaking the flask, then add 10.0 ml. of concentrated nitric acid to dissolve the nitro compounds or to keep them in homogeneous suspension. Fill the flask to the mark with distilled water. Accurately pipet a 5.0-ml. aliquot of this solution into a small separatory funnel containing 10 ml. of distilled water. Make the solution in the funnel alkaline with 1.0 ml. of 50% potassium hydroxide; if the solution is made too basic the subsequent development of the colors may be lessened. Extract the alkaline solution with 10

Table II. Analyses of Known Solutions

o-Xylene Added	o-Xylene from Stock Solution	Total o-Xylene Present	o-Xylene Found
%	%	%	%
0.04	0.08	0.12	0.09, 0.13, 0.12
0.37	0.08	0.45	0.41, 0.42
3.69	0.08	3.77	3.82, 3.57, 3.73
10.8	0.07	10.9	11.0, 10.9
48.8	0.04	48.8	49.0, 48.2

% o-xylene in styrene for stock solution = 0.06, 0.07, 0.08, 0.08.
% o-xylene in stock solution = 0.08, 0.09.

ml. of diethyl ether, and again with 5 ml. of ether, collecting the extracts in a clean, dry 1-ounce narrow-mouthed French square bottle. Gently evaporate the ether on a steam bath, leaving the nitro compounds and the water which was dissolved in ether. Take care to evaporate just the ether; evaporation of all or part of the water will lessen the intensity of the colors.

Pipet 20.0 ml. of acetone and 1.0 ml. of monoethanolamine into the 1-ounce bottle and add 2.0 ml. of 50% potassium hydroxide from a buret. Close the bottle tightly with the silver foil–lined screw cap, place it in the box on the shaker, and, starting the timer from zero, shake the bottle for 15.0 minutes. Leaving the timer in operation, transfer the bottle to the black cardboard box, and allow it to stand so that the caustic solution settles out of the colored acetone solution. After 17.0 minutes from the start of the shaking, fill a 10-mm., 10-ml. absorption cell with the solution and immediately read the per cent transmittance on the colorimeter, using a B660 and neutral gray filter with the transmittance of water at 100%. Record the first reading. Read the per cent o-xylene from a curve prepared by running the same procedure on known solutions of ethylbenzene and o-xylene.

CALCULATION

Calculate the per cent o-xylene in the original sample as follows:

$$\frac{A(B + C)DE}{\text{sample weight}} = \text{per cent (by weight) of o-xylene}$$

where A = volume per cent of o-xylene in alkylbenzenes. This figure is obtained by the colorimetric procedure.

B = ml. of alkylbenzenes. This figure is obtained by reading the volume of the alkylbenzenes distilled into the Babcock bottle.

C = volume increment. If only the volume of alkylbenzenes actually measured in the Babcock bottle (B, above) is used in calculating the per cent of o-xylene, the results will be low, owing to mechanical loss and the solubility of the alkylbenzenes in water. The volume of this loss may be obtained by running several known synthetic samples containing up to 5 to 6% of o-xylene, using the same o-xylene that was used in making the curve, and adding empirically the volume increment necessary to obtain the correct answer. This volume increment is constant, and with the apparatus and Babcock bottles used in this procedure was found to be 0.05 ml.

D = correction factor. This factor is necessary when impure o-xylene is used in preparing the curve. In this investigation 92% o-xylene containing 6% m-xylene and 2% p-xylene was used, and the error introduced by the meta and para isomers in making up the curve was negligible, the correction factor being $\frac{100}{92} = 1.09$.

E = specific gravity of o-xylene. The figure 0.87 was used.

ANALYTICAL DATA

A stock solution was prepared by mixing together 175 ml. of styrene, 15 ml. of 1,4-vinylcyclohexene, and 5 ml. of ethylbenzene. Various solutions of known o-xylene content were prepared from this stock solution and o-xylene. Results of analyses of these known solutions are shown in Table II.

ERRORS INTRODUCED BY OTHER COMPOUNDS

Using pure chemicals, the colorimeter readings given by various compounds possibly occurring in the samples, as well as the calculated percentage error if 5.0% of the compound were present, are given in Table III. Since propylbenzene, isopropylbenzene, and diethylbenzene boil well above styrene and o-xylene, while benzene and toluene boil well below, styrene and o-xylene can be readily fractionated out.

DISCUSSION

In general, 15 ml. of mercuric acetate reagent are used for each milliliter of sample. This gives, roughly 1.5 moles of mercuric acetate per mole of sample, a sufficient excess. One exception is when the sample contains considerable 1,4-vinylcyclohexene; because this compound has two olefinic linkages, a greater proportion of mercuric acetate must be added. If the sample contains but a small amount of olefinic compounds, less reagent may be added if desired.

Usually the volume of propylene glycol added is the same as that of the mercuric acetate reagent. However, if a 5.0-ml. sample is required, 60 ml. of propylene glycol are added even though 75 ml. of mercuric acetate reagent are used. This cuts down the volume of liquid in the Erlenmeyer flask. Ethylene glycol may be used, but its solvent action is not so great.

The contents of the flask after shaking will be a clear, water-white solution on polymer-free samples. However, any sample that can be pipetted, even with great difficulty, does not contain enough polymer to affect greatly the accuracy of the determination if the o-xylene content is below 0.5%.

The precaution of boiling the water in the steam generator before heating the contents of the Erlenmeyer flask makes it possible to complete the steam-distillation before the occurrence of a secondary reaction in which mercurous acetate precipitates and a small amount of an oily liquid distills over, lessening the accuracy of the determination. A small amount of acetic acid distills over also, but it offers no interference.

In the colorimetric determination the first reading is taken because the colors produced slowly fade when subjected to light.

The smaller the percentage of o-xylene, the smaller is the absolute error introduced from reading the volume of alkylbenzenes and the greater is the sensitivity of the colorimetric curve; thus samples containing less than 0.2% of o-xylene and not more than 5% of alkylbenzenes can be determined with an accuracy of ±0.01%. When greater accuracy is desired or lower concentrations are to be determined, samples may be fractionated and the analyses made on selected cuts. Less than 0.01% o-xylene can be detected in a given sample. When large amounts of alkylbenzenes are present it is advisable to fractionate the material and determine the o-xylene on the proper fractions.

Table III. Errors Introduced

Compound	Reading for Pure Compound	% Error if 5.0% Present
Ethylbenzene	93.6	Nil
Isopropylbenzene	95.4	−0.04
Propylbenzene	94.4	−0.02
Toluene	82.5	+0.28
Benzene	89.7	+0.09
Diethylbenzene (mixture of isomers?)	97.3	−0.09
m-Xylene[a]	100.0	−0.16
p-Xylene	97.7	−0.10

[a] m-Xylene containing 10% o-xylene gave a reading of 79.8 which, when corrected by use of the curve, indicates a reading of 100 for pure m-xylene.

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Laboratory-Size Glass Circulating Evaporator

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THE evaporation of solutions for the purpose of reducing volume and concentrating solids is one of the more frequently encountered operations common to many academic and industrial laboratory research problems. Strangely enough, this unit operation has received very little critical attention and in most laboratories is carried out much as has been the custom of the last 30 years with a steam cone and round-bottomed flask as the basic apparatus. This paper describes a convenient and compact apparatus modeled along the lines of large industrial units commonly used for the evaporation of aqueous solutions to make possible recovery of dissolved solids.

The glass parts of the heat exchanger were constructed by sealing five 0.375-inch outside diameter (standard-wall 10-mm. Pyrex) pieces of tubing between two small bulbs. One tube was centered from bottom to bottom between the two bulbs and then four additional tubes were sealed in on the corners of an imaginary square with a diagonal equal to the diameter of the bulbs. The packing glands were the conventional gland, string packing, and follower type. The gland bodies were brazed to the end plates of the metal heat-exchanger jacket. Since only one end plate need be removed in order to assemble the heat exchanger, one end only was fixed to the metal jacket by means of six bolts on a 9-cm. (3.625-inch) bolt circle, this end of the heat exchanger jacket and the jacket end plate being fitted with companion flanges. The other end plate was brazed to the jacket.

Instead of the rubber sleeve connections shown in Figure 1, standard-taper ball and socket glass joints may be substituted. This aids somewhat in assembly and disassembly and removes danger of contamination from anything save glass.

The glass tubes shown entering the evaporator bowl and the centrifugal separator were bent at right angles to the line of entry in a plane parallel to the floor, in order that they might deliver vapor tangential to the wall and thus impart a rapid swirling motion to the vapor in these two parts of the apparatus.

In principle this evaporator functions as do any of the long-tube natural circulating evaporators found in the chemical industry. The liquor to be concentrated is fed into the evaporator below the level of the heat exchanger. The liquor in the heat exchanger tubes boils, and the vapor rising as froth forces slugs of liquor ahead of it at high velocity up the tubes and out into the disengaging space in the evaporator bowl. As the system repeats this process, the liquor is rapidly circulated from heat exchanger to bowl to return line to heat exchanger, etc. The distinctive advantage of this type of evaporator lies in the high coefficient of heat transfer realized, due to the great velocity of the liquid over the heating surface. The character of the flow of liquid and vapor through the tubes guarantees a thorough and rapidly repeated wetting of the whole heating surface.

To operate the apparatus, vacuum is applied to the receiver and liquid drawn up until the larger bowl is filled to within 2.5 or 5 cm. (1 or 2 inches) of the vapor inlet. The liquor feed line is then shut by means of a screw clamp on a section of rubber hose. The pressure is allowed to come to a minimum, or to some predetermined pressure if a vacuum controller is to be used. An intermittent leaks type of vacuum controller with an electrically actuated valve working from one of the conventional mercury-filled U-tube manostats is to be recommended, since the evaporator will operate much more smoothly at constant pressure. Steam is then turned on the jacket. The steam condensate line is conveniently placed below the surface of the water emerging from the surface condenser. No positive steam pressure is needed for aqueous solutions. The solution in the small tubes of the heat exchanger rapidly comes to boil and emerges from the top of the exchanger as a mixture of vapor and entrained liquor. The charge in the evaporator rapidly comes to its boiling point as the liquor circulates. The vapor and liquor emerging from the vapor outlet are given a circular motion by the bend in the end of the vapor outlet. The centrifugal force developed throws all drops of liquor or foam against the evaporator sides where they run down at once to the charge in the bowl. Any foam or entrainment not removed by the first centrifugal separator is taken out by the second.

ADVANTAGES OF APPARATUS

The unit will closely duplicate results obtained with tube evaporators of commercial size. Protein or other food product solutions, almost impossible to handle in a flask because of excessive foaming, may be evaporated with little or no difficulty.

Bumping is eliminated in most cases. When it does occur it results rather in a surging action in the heat exchanger tubes. This can be overcome if desired by allowing a small stream of air bubbles to enter the system by cracking the clamp on the liquor inlet line, which in this case must now open to the atmosphere.

Evaporation is rapid as compared with the usual setup and extremely so for glass surfaces exposed to steam.

Heat-sensitive materials receive a minimum of damage.

The recovery of solids from a solution is satisfactory. Aqueous solutions of organic or inorganic material on being evaporated to where solids separate in general give small crystal size with but little tendency to crust on the inner surfaces of the evaporator. Crusting is apparently prevented by the scouring action of the rapidly circulating liquor.

The unit may be used for continuous evaporation. In this event the liquor inlet is used for continuous feeding of fresh liquor and the finished liquor is drawn off continuously through a T in the return down-leg line.

The unit may be easily dumped, cleaned, and refilled. Dump-

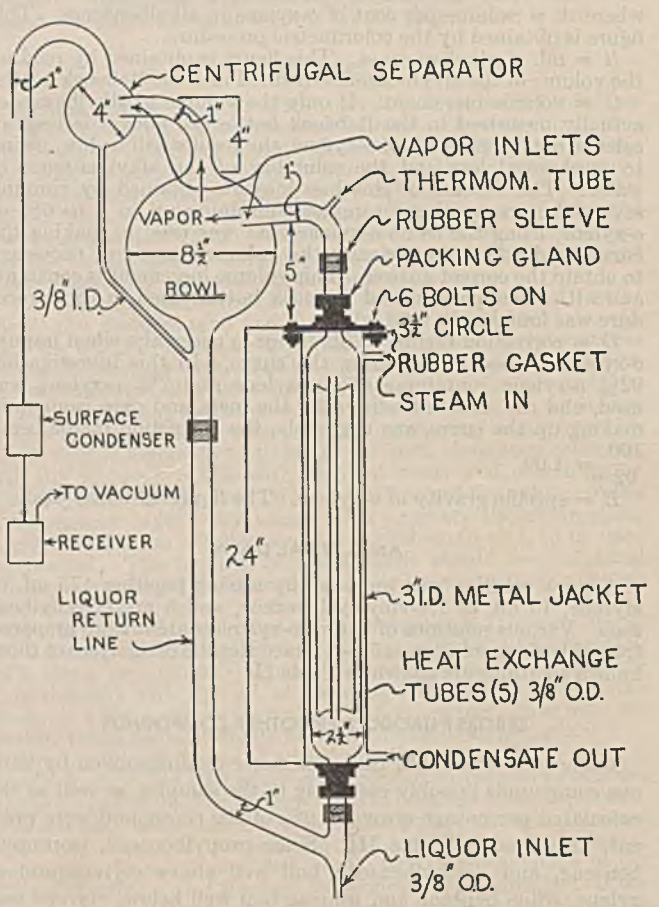

Figure 1. Diagram of Evaporator

Table I. Operation Characteristics

(Wall thickness of heat exchanger tubes, 1/12 inch)

Hg Pressure	Liquor Temp.	Steam Temp.	T	H_2O Evaporated	Glass Surface	H_2O Evaporated	B.t.u./Sq. Ft./Hour/° F.
Mm.	*° F.*	*° F.*		*Lb./hr.*	*Sq. ft.*	*Lb./hr./sq. ft.*	
103	126	212	86	11.40	0.955	11.95	142
85	119	212	93	12.15	0.955	12.73	140
65	109.5	212	102.5	13.15	0.955	13.78	138.5
		Operation of 12-liter flask on a steam cone					
97	123.8	212	88.2	10.35	1.88	5.5	66.2

ing merely requires the vacuum to be released, after which the charge will flow out under gravity. To recover the liquor or crystals adhering to the interior walls, it is only necessary to draw up about 50 to 100 cc. of water into the evaporator, allow the vacuum to build to a few hundred millimeters' pressure, and then suddenly open the inlet line. The rapid surge of air up through the heat exchanger tubes throws the small amount of wash liquor around violently, and on releasing the vacuum, it drains out and carries with it the portion of the original charge remaining on the walls of the apparatus.

With pure water this evaporator has the operation characteristics shown in Table I.

The authors have several such units in use in their laboratories, ranging from several times the size shown to small units suitable for handling as little as 100 cc. For the smaller units the heat exchanger is usually made up with only one glass tube through the steam jacket. The jacket is commonly made from scrap thin-walled boiler tubes, with rubber stoppers replacing the two packing glands on the jacket.

This type of evaporator may be fabricated from standard Pyrex tubes and flasks, or may be purchased complete from suppliers of special glass apparatus. (Ace Glass, Inc., has indicated a willingness to fabricate such an apparatus in any size desired.)

Identification of Some Important Unsulfonated Azo-2-naphthol Dyes

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A rapid and simple method has been developed for the identification of unsulfonated azo-2-naphthol dyes, by catalytic reduction of the azo bond, and the separation of the scission products with immiscible solvents, under controlled acid and alkaline conditions. Direct formation of the stable benzoyl derivatives of the reduction products precludes the necessity of isolating sensitive diamines and triamines.

AZO dyes prepared from diazotized primary aromatic amines and 2-naphthol constitute an important series of commercial colors, and their identification is important to the dyestuff chemist. Several analytical methods have been proposed, among which are hydrogenation of the azo bond (*2*, *3*, *10*, *11*, *12*), scission of the azo link with fuming nitric acid (*7*, *8*, *9*), and schematic identification by means of immiscible solvents (*4*, *5*, *6*).

Whitmore and Revukas (*10*, *11*) have shown the applicability of catalytic reduction to azo colors by the use of Raney nickel. Their procedure, however, involves moderately expensive equipment and consumes considerable time when nitrated dyes are hydrogenated, and they report that the isolation and identification of the reduction products are "laborious".

This paper proposes a rapid and simple method for the separation and characterization of the hydrogenation compounds which is based upon their differential solubility in water and ether under varying acid and alkaline conditions.

Cheronis and Koeck (*1*) have devised a simple semimicro hydrogenation apparatus, obtainable from the Wilkens-Anderson Company of Chicago, which the authors find very adaptable to the reduction of azo colors. Utilizing this outfit, it is possible to hydrogenate this series of dyes in peroxide-free dioxane (*11*), smoothly and quickly. Subsequent isolation of the reduction products, by the immiscible solvent procedure, and direct conversion into their benzoyl derivatives, eliminate the necessity of recovering the sensitive free amines.

Partial dehalogenation of chlorinated dyes, an inevitable accompaniment of all attempted reductions in neutral solvents, was overcome by acidification of the peroxide-free dioxane before hydrogenolysis of the color.

GENERAL PROCEDURE

Purification of Samples. The coloring matter is purified by crystallization from dioxane, and if or when necessary, water is added to this solvent to induce precipitation.

Preparation of Reduction Products. Freshly ground Adams-Voorhees platinum oxide (0.05 gram) is placed in the Cheronis hydrogenating unit, and the catalyst is suspended in 25 ml. of peroxide-free dioxane. Hydrogen gas, preferably from a tank, is bubbled through the suspension for 2 to 5 minutes, in order to convert the platinum oxide to colloidal platinum black.

Then 1 gram of dye and 2.5 ml. of concentrated hydrochloric acid are added to the platinum black suspension, the hydrogenating unit is heated by immersion in hot water, 80° to 90° C., and hydrogen gas is passed through the mixture at such a rate that continuous agitation is maintained. (Occasionally, the reduction products will clog the disperser, and it is convenient to have a clean one available for quick replacement. If a second unit is not on hand, it is necessary to remove the clogged disperser, and to clean it by immersion in a test tube containing hot dioxane. At or near the completion of the hydrogenolysis, it is advisable to wash down any dye particles adhering to the sides of the tube, with 5 to 10 ml. of dioxane.)

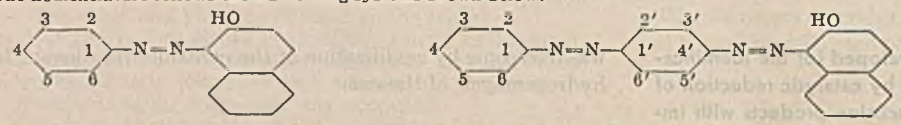
Separation of the Reduction Products. If a precipitate of the reduction products forms in the acidified dioxane solution, it is redissolved by the addition of 5 to 10 ml. of water and filtered into a 500-ml. Squibb separatory funnel. The filtrate is buffered with 250 ml. of a 5% sodium acetate solution, and the ether-soluble amino-2-naphthol, whose presence is indicated by a blue fluorescen e, and any primary monoamine, are extracted with two successive 100-ml. portions of ether.

The aqueous layer, containing any water-soluble primary polyamine, is separated from the ether and intimately mixed with 5 ml. of benzoyl chloride. The combined ether fractions are washed with two 50-ml. volumes of water, to remove residual polyamine, and the washings are run into the benzoylation mixture. Then 10 grams of solid sodium hydroxide are added, sufficient to make the aqueous solution alkaline, and after standing at room temperature for 30 minutes, with frequent stirring, the mixture is placed on the steam bath to expel dissolved ether. The benzoyl derivative of the diamine or triamine, which separates as a solid, is filtered off, washed thoroughly with water, and crystallized from a suitable solvent.

Table I. Identification of Unsulfonated Azo-2-Naphthol Dyes

	Dye	Melting Point Observed (uncorrected) ° C.	Melting Point Literature ° C.	Reduction Products of Diazo Component	Melting Point of Benzoyl Derivative of Reduction Product of Diazo Component Observed (uncorrected) ° C.	Melting Point of Benzoyl Derivative of Reduction Product of Diazo Component Literature ° C.	Reduction Time Min.
1.	Benzeneazo-2-naphthol[a, b]	131–2	131	Aniline	162–3[c]	163	15
2.	2-Methylbenzeneazo-2-naphthol	132–3	128	*o*-Toluidine	144–5[c]	145–6	20
3.	4-Ethoxybenzeneazo-2-naphthol	133–4	140	*p*-Phenetedine	172–3[c]	173	25
4.	4-Methylbenzeneazo-2-naphthol	133–4	130	*p*-Toluidine	157–8[c]	157	10
5.	4-Methoxybenzeneazo-2-naphthol	140–1	139	*p*-Anisidine	155–6[c]	156	30
6.	3-Methylbenzeneazo-2-naphthol	140–1	140	*m*-Toluidine	123–4[c]	125	15
7.	2-Ethoxybenzeneazo-2-naphthol	142–3	145	*o*-Phenetedine	Oil	...	20
8.	2,5-Dimethylbenzeneazo-2-naphthol	152–3	156	*p*-Xylidine	147–8[c]	140	15
9.	2,4,5-Trimethylbenzeneazo-2-naphthol	155–7	...	Pseudocumidine	169–70[c]	162	15
10.	2,4-Dimethylbenzeneazo-2-naphthol[d]	160–1	166	*m*-Xylidine	193–4[c]	192	15
11.	3-Chlorobenzeneazo-2-naphthol	160–1	...	*m*-Chloroaniline	120–21[c]	118–20	30
12.	4-Chlorobenzeneazo-2-naphthol[e]	161–2	160	*p*-Chloroaniline	191–2[c]	192–3	30
13.	2-Chlorobenzeneazo-2-naphthol	167–8	167	*o*-Chloroaniline	104–5[d]	99–101	30
14.	2-Methyl-4-chlorobenzeneazo-2-naphthol	170–1	172	2-Methyl-4-chloroaniline	142–3[e, d]	...	90
15.	2-Methyl-5-chlorobenzeneazo-2-naphthol	176–7	...	2-Methyl-5-chloroaniline	171–2[e, f]	...	30
16.	3-Methylbenzeneazo-3′-methylbenzeneazo-2-naphthol	175–7	...	*m*-Toluidine	123–4[c]	125	20
				2-Methyl-*p*-phenylenediamine	301–2[g, h]	...	
17.	2-Methoxybenzeneazo-2-naphthol	180–1	180	*o*-Anisidine	Oil	59.8	10
18.	2-Naphthaleneazo-2-naphthol	181–2	174	2-Naphthylamine	160–1[i]	161	20
19.	2,5-Dichlorobenzeneazo-2-naphthol[j]	183–4	...	2,5-Dichloroaniline	119–20[c]	120	30
20.	2-Methylbenzeneazo-2′-methylbenzeneazo-2-naphthol	188–9	...	*o*-Toluidine	144–5[c]	145–6	30
				2-Methyl-*p*-phenylenediamine	301–2[g, h]	...	
21.	3-Nitrobenzeneazo-2-naphthol	196–7	194	*m*-Phenylenediamine	241–2[g]	240	40
22.	Benzeneazobenzeneazo-2-naphthol	199–200	202	Aniline	162–3[c]	163	90
				p-Phenylenediamine	338–9[g]	Over 300	
23.	2-Nitro-4-methoxybenzeneazo-2-naphthol	206–7	...	4-Methoxy-*o*-phenylenediamine	251–2[g]	251–2	65
24.	5-Nitro-2-methylbenzeneazo-2-napnthol	210–11	206	1-Methyl-2,4-phenylenediamine	225–6[g]	224	45
25.	2-Nitrobenzeneazo-2-naphthol	213–4	212	*o*-Phenylenediamine	303–4[g]	301	30
26.	1-Naphthaleneazo-2-naphthol	232–3	224	1-Naphthylamine	158–9[i]	156, 161–2	20
27.	4-Nitro-2-methylbenzeneazo-2-naphthol	251–2	248	2-Methyl-1,4-phenylenediamine	301–2[g, h]	...	30
28.	4-Chloro-2-nitrobenzeneazo-2-naphthol[k]	255–6	252	4-Chloro-*o*-phenylenediamine	226–7[d]	230	55
29.	2,5-Dimethylbenzeneazo-2′,5′-dimethylbenzeneazo-2-naphthol	263–4	...	*p*-Xylidine	147–8[e]	148	120
				2,5-Dimethyl-*p*-phenylenediamine	311–12[g, l]	...	
30.	2-Nitro-4-methylbenzeneazo-2-naphthol	273–4	278	4-Methyl-*o*-phenylenediamine	263–4[g]	263–4	150
31.	2-Chloro-4-nitrobenzeneazo-2-naphthol[m]	289–90	282	2-Chloro-*p*-phenylenediamine	239–40[c]	228	50
32.	2,4-Dinitrobenzeneazo-2-naphthol[n]	312–13	302	1,2,4-Triaminobenzene	278–9[o, p]	260	50

[a] The nomenclature follows the numbering systems shown below:



[b] The benzoyl derivative of amino-2-naphthol, from all compounds investigated, was recrystallized from ethanol, M.P. observed 232–3° C. (uncorrected): M.P. literature, 226.5°, 235.5° C.

[c] Solvent, ethanol-water.

[d] To differentiate further between compounds 10 and 12, analyze for presence of halogen by any accepted procedure.

[e] Monobenzoyl derivative, $C_6H_3.CH_3.Cl.NHCOC_6H_5$. Chlorine calculated, 14.44%; found, 14.40%.

[f] Monobenzoyl derivative, $C_6H_3.CH_3.Cl.NHCOC_6H_5$. Chlorine calculated, 14.44%; found 14.47%.

[g] Solvent, acetic acid.

[h] Dibenzoyl derivative, $C_6H_3.CH_3.(NHCOC_6H_5)_2$. Nitrogen calculated 8.48%; found, 8.50%.

[i] Solvent, ethanol.

[j] 2,5-Dichloroaniline did not wash out of ether layer with *N* hydrochloric acid. It was isolated by evaporating ether and crystallizing residue from ethanol-water; M.P. 49° C. (uncorrected).

[k] Nearly all the diazo component was found as 4-chloro-2-nitraniline in ether layer, M.P. 117–18° C. (uncorrected). Its benzoyl derivative melted at 131–2° C. (uncorrected).

[l] Dibenzoyl derivative, $C_6H_2(CH_3)_2(NHCOC_6H_5)_2$. Nitrogen calculated, 8.14%; found, 8.18%.

[m] About one half of, diazo component was found in ether layer as 2-chloro-4-nitraniline, M.P. 107–8° C. (uncorrected). Its benzoyl derivative melted at 157–8° C. (uncorrected).

[n] About one half of diazo component was found unreduced to triamine form and was isolated from ether layer as 2,4-dinitraniline, M.P. 178–9° C. (uncorrected).

[o] Solvent, acetic acid–water.

[p] Benzoyl derivative of triamine (1,2,4-triaminobenzene) isolated from reduction of *p*-ethoxybenzeneazo-*m*-phenylenediamine, had same M.P.

Amphoteric amino-2-naphthol is separated from the primary monoamine, by removing the former with four successive 50-ml. portions of 2% sodium hydroxide solution, followed by two 50-ml. volumes of water, and is isolated as the benzoyl derivative by running the alkaline extracts into 5 ml. of benzoyl chloride, stirring vigorously after each addition. Dissolved ether is eliminated by heating on the steam bath, and after cooling to room temperature, the benzoyl derivative is filtered off, washed thoroughly with water, and crystallized from ethanol.

The ether solution, containing the primary monoamine, is shaken with four 50-ml. portions of *N* hydrochloric acid, followed by a 50-ml. volume of water. Dissolved ether is expelled by heating, and the acid solution is cooled. Ten grams of solid sodium hydroxide are added to liberate the free amine, and the mixture is treated with 5 ml. of benzoyl chloride. Upon vigorous agitation, the benzoyl derivative separates as a pasty mass, which is allowed to stand overnight. It is then filtered off, washed thoroughly with water, and crystallized from a suitable solvent.

If the melting point of the purified dye indicates the presence of 2,5-dichlorobenzeneazo-2-naphthol, 2,4-dinitrobenzeneazo-2-naphthol, 2-chloro-4-nitrobenzeneazo-2-naphthol, or 4-chloro-2-nitrobenzeneazo-2-naphthol, the residual ether is evaporated on a steam bath, and the residue is identified according to the directions given in the footnotes to Table I.

ACKNOWLEDGMENT

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Estimation of Vitamin C in Presence of Iron Salts

Stepwise Determination of Vitamin C and Ferrous Iron with Dichlorophenolindophenol

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REPORTS on the reductive interference of ferrous iron with the dichlorophenolindophenol reagent used in the estimation of vitamin C have appeared in the literature and procedures have been devised to minimize and remove this interference (*2–5*).

During the course of studies in this laboratory on isolated vitamin C–iron salt systems the authors have found that ferrous and ferric iron interferences can be avoided if a suitable medium is chosen for the dye titration. Thus vitamin C can be determined in the presence of ferrous iron if acetic acid is employed as the titration medium and in the presence of ferric iron if metaphosphoric acid is present in the medium. The observation that ferrous iron reduces dichlorophenolindophenol in the presence of metaphosphoric acid provides a basis for the stepwise determination of vitamin C and ferrous iron in the same aliquot.

Whether or not these observations may be applied in toto or in part to biological systems, pharmaceuticals, and food products where other interferences (*3*) in addition to iron may be present remains to be determined.

REAGENTS

Ferrous Iron Solution. Two grams of dried ferrous sulfate powder were dissolved in 160 ml. of distilled water containing 5 ml. of concentrated sulfuric acid by warming gently on the steam bath. The solution was treated with a rapid current of hydrogen sulfide for 0.5 hour, followed by a stream of nitrogen, and then made up to a volume of 1000 ml. with distilled water. An aliquot titrated with 0.01 *N* potassium permanganate contained 0.64 mg. of ferrous iron per ml.

8% Acetic Acid, 80 ml. of glacial acetic acid made up to liter with distilled water.

Table I. Influence of Medium on Reduction of Dichlorophenolindophenol by Ferrous Iron

(0.64 mg. of ferrous iron present)

Titration Medium[a]	Dichlorophenolindophenol Required for End Point, Ml.
8% acetic acid	0.05
3% metaphosphoric acid	9.1
6% metaphosphoric acid	9.2
Citrate reagent (Bessey)	Slowly fading end point
8% acetic acid adjusted to pH 3.5 with NaOH	0.05
8% acetic acid with 0.025% metaphosphoric acid present	3.2
8% acetic acid with 0.15% metaphosphoric acid present	Reduction very slow and not reproducible
8% acetic acid with 0.5% metaphosphoric acid present	5.0
8% acetic acid with 1.0% metaphosphoric acid present	7.3
8% acetic acid with 3% metaphosphoric acid present	9.0
	9.1

[a] Initial volume 25.0 ml.

Table II. Influence of Medium on Titration of Vitamin C with Dichlorophenolindophenol in Presence of Ferric Iron

(1.00 mg. of vitamin C and 0.75 mg. of ferric iron[a] present in all cases)

Titration Medium[b]	Dichlorophenolindophenol Required for End Point, Ml.
8% acetic acid	5.9[c]
8% acetic acid with 3% metaphosphoric acid present	9.2
3% metaphosphoric acid	9.3
6% metaphosphoric acid	9.2

[a] From ferric ammonium sulfate standardized by reduction and subsequent titration with $KMnO_4$.
[b] Initial volume 25.0 ml.
[c] Not reproducible.

6% Metaphosphoric Acid, 60 grams of crushed metaphosphoric acid sticks dissolved in distilled water and made up to liter. When not in use this solution was kept in the refrigerator.

Citrate and Metaphosphoric Acid Buffer, as described by Bessey (*1*)

Dichlorophenolindophenol Dye Solution, 50 mg. of ether-extracted dichlorophenolindophenol dissolved in 200 ml. of distilled water to which 42 mg. of sodium bicarbonate had been added. When not in use the dye was stored in the refrigerator.

Table III. Estimation of Vitamin C and Ferrous Iron

Sample	Total Dye[a]: 20 ml. of 8% acetic acid, Ml.	Total Dye[a]: Subsequent addition of 10 ml. of 6% metaphosphoric acid, Ml.	Found: Vitamin C, Mg.	Found: Ferrous iron, Mg.
0.64 mg. of ferrous iron	0.05	9.1	0.0	0.63
1.0 mg. of vitamin C	9.2	9.2	0.99	0.00
1.0 mg. of vitamin C and 0.64 mg. of ferrous iron	9.3	18.6	1.01	0.64
Vitamin C[b] capsule containing $FeSO_4$	12.8	21.6	34.7	15.1

[a] Dye standardized against U.S.P. vitamin C.
[b] Vitamin capsule containing 35 mg. of vitamin C and 15 mg. of iron, diluted to 25 ml., 1-ml. aliquot used for titration. Capsule found by oxidation and colorimetric estimation with thiocyanate to contain 15.0 mg. of iron

EXPERIMENTAL AND DISCUSSION

Table I shows that ferrous iron reduces dichlorophenolindophenol in the presence of metaphosphoric acid. This also happens when phosphoric acid is present and can be explained on the basis of the increase in reduction potential of the ferrous-ferric system when the effective ferric-ion concentration is reduced by complex formation with metaphosphate or phosphate ions.

The reverse of the above phenomena takes place in the presence of ferric iron. Here, as can be seen from Table II, the reduced dye is reoxidized in acetic acid medium but not in the presence of metaphosphoric acid. This reoxidation takes place at a much slower rate than the corresponding reduction by ferrous iron and at the present time is being investigated as the basis for a colorimetric estimation of iron.

From the above experiments it was apparent that vitamin C and ferrous iron could be determined stepwise on one aliquot by first titrating in acetic acid, then adding metaphosphoric acid and continuing the titration. Control analyses and the analysis of a vitamin capsule containing vitamin C and ferrous sulfate are given in Table III.

SUMMARY

Ferrous sulfate reduces dichlorophenolindophenol in the presence of metaphosphoric acid. Ferric iron oxidizes reduced dichlorophenolindophenol in acetic acid medium. Vitamin C and ferrous iron can be determined on one sample by stepwise titration with dichlorophenolindophenol.

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Determination of Small Amounts of Zinc

By Measurement of Fluorescent Turbidities

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A rapid and accurate method for the determination of zinc by measuring the fluorescence of a turbidity of zinc 8-hydroxyquinolinate is described. The range of the method is from 0.05 to 0.60 mg. and the accuracy is about 0.02 mg. The influence of variations in temperature, filters, method of procedure, amount of reactants added, and extraneous salts has been investigated. Other ions precipitated by 8-hydroxyquinoline in acetic acid–acetate solutions interfere. Zinc can be determined in the presence of magnesium by the method if calibration curves are constructed using approximately the amount of magnesium present in the unknowns.

ZINC forms a precipitate with 8-hydroxyquinoline which fluoresces brilliantly. It has been found possible to produce stable, reproducible turbidities of zinc 8-hydroxyquinolinate and to measure the turbidity accurately by means of its fluorescence. Most fluorescence methods developed previously have dealt with true solutions.

The method developed by White and Lowe (*14*) for aluminum using morin as the reagent is believed to be due to the formation of a colloidal solution of $Al(C_{15}H_9O_7)_3$ (*11*).

A number of workers (*4*, *5*) have used the fluorescence of the 8-hydroxyquinoline precipitates for qualitative detection of zinc and other metals. Lutz has described (*7*) a method for determination of zinc by measurement of the fluorescence of zinc salts with urobilin. Sandell (*10*) has published a method for the fluorometric determination of gallium as the 8-hydroxyquinoline complex dissolved in chloroform and suggests (*9*) that zinc could be determined similarly.

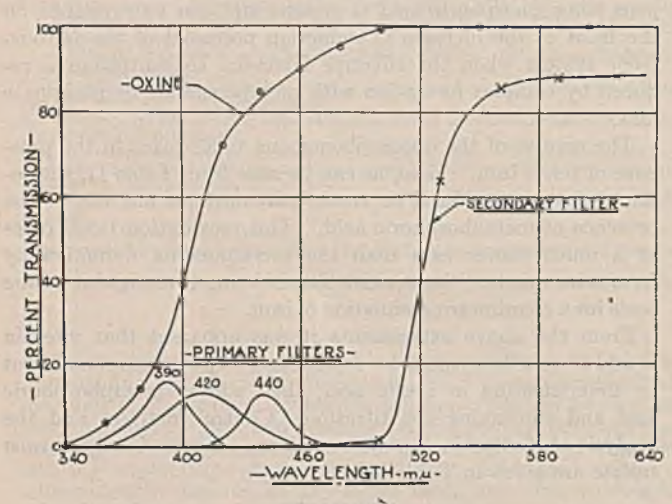

Figure 1. Transmission of Filters and Reagent

Zinc has been determined colorimetrically or nephelometrically by numerous methods. Among the most useful are the colorimetric methods employing dithizone (*9*); these are extremely sensitive and are employed for amounts of zinc ranging from a microgram up to about 1 mg. These methods require extraction with an organic solvent such as carbon tetrachloride. The nephelometric determination of zinc with ferrocyanide (*2*, *3*) covers the range of zinc from about 0.1 to 5 mg. but the average error is about ±0.05 mg. (*1*). Zinc can also be determined turbidimetrically (*15*) or nephelometrically (*8*) as the sulfide.

Teitelbaum (*13*) has described an indirect colorimetric method in which zinc is precipitated as the 8-hydroxyquinolinate complex salt, the precipitate is then dissolved in acid, and the liberated 8-hydroxyquinoline is determined by its reducing action on Folin's reagent. The method is applicable to about 0.008 to 0.09 mg. of zinc with an accuracy of 2 to 5%.

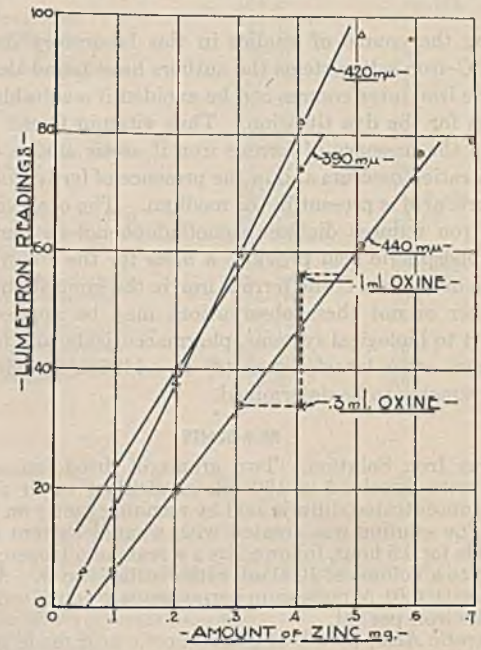

Figure 2. Influence of Filters and Amount of Reagent

The determination of zinc by measurement of the fluorescence of 8-hydroxyquinoline turbidities as described in this paper covers the range from about 0.02 to 0.60 mg. of zinc per 50 ml. of final solution and is accurate to about 0.02 mg. Thus larger quantities of zinc can be determined than is possible by most similar methods, without dilution or the necessity of taking aliquot portions. The procedure is simple and extremely rapid, since no filtrations nor extractions with immiscible solvents are required. The method is, however, subject to the many interferences encountered with all procedures involving 8-hydroxyquinoline.

In producing reproducible turbidities, a large number of factors must be considered (*6*), such as the temperature, concentration of other salts present, manner of mixing, time of standing, concentration and order of solutions mixed, presence or absence of protective colloids, etc. All these factors must be controlled and once a method has been established all details of procedure must be adhered to rigidly. The measurement of zinc 8-hydroxyquinolinates by fluorescence of turbidities is further complicated by the fact that the reagent, which must be added in slight excess absorbs the light which excites the fluorescence. Proper filters must be employed in the fluorescence meter and the concentration of reagent must be controlled.

REAGENTS

A standard solution of zinc chloride was prepared by dissolving 4.0982 grams of C.P. zinc metal (low in lead, iron, and arsenic) in 35 ml. of concentrated hydrochloric acid and diluting the solu-

tion to 2 liters with distilled water. A solution containing 0.02049 mg. of zinc per ml. was prepared by diluting 10 ml. of the above standard solution to 1 liter.

A 5% solution of 8-hydroxyquinoline was prepared by dissolving 5 grams of C.P. 8-hydroxyquinoline in 12 grams of glacial acetic acid and diluting to 100 ml. with distilled water.

A 2% solution of gum arabic was prepared by grinding 2 grams of gum arabic in a mortar until fine, and dissolving in water to make 100 ml. The solution was filtered if not clear.

A 2 *N* ammonium acetate solution was obtained by dissolving 154 grams of crystallized ammonium acetate in water to make 1 liter.

A standard dichlorofluorescein (or fluorescein) solution was prepared in the following manner: A 0.1% alcoholic solution of dichlorofluorescein (such as is usually employed as an adsorption indicator) was added drop by drop to 1 liter of water until the resulting solution had a fluorescence approximately the same as that of a turbidity produced from 0.30 mg. of zinc in the manner described below. About 0.35 ml. of dichlorofluorescein solution was required. A more concentrated solution may be employed, if desired. The standard solution is stable for weeks.

PROCEDURE

Amounts of standard zinc solution containing between 0.05 and 0.50 mg. of zinc were placed in a 50-ml. volumetric flask, and 5 ml. of 2 *N* ammonium acetate and 2 ml. of 2% gum arabic solution were added. The mixture was diluted with water to approximately 45 ml. and mixed by swirling the flask. Using a serological pipet, 0.20 ml. of 5% 8-hydroxyquinoline solution was added. The solution was diluted to the mark with distilled water and mixed by gently shaking the flask. The turbid solution was poured into the cell of the fluorescence meter and the per cent fluorescence was measured.

A Lumetron Model 402EF fluorescence meter was employed in this investigation. The 25-ml. cells supplied for this instrument were used for all measurements. The instrument was standardized by setting the fluorescence emitted by the standard dichlorofluorescein solution at 50.0 and that emitted by a blank containing all reagents but no zinc equal to 0.0. If a more concentrated solution of dichlorofluorescein or known zinc turbidity is employed as standard the fluorescence is adjusted to 100.0. A narrow band filter of 420 mμ maximum transmission obtained from the Photovolt Corporation was employed in the primary beam. Since the fluorescent light is greenish-yellow, the yellow secondary filters furnished with the instrument for riboflavin determinations were employed in front of the measuring cells. As can be seen in Figure 1, the secondary filters will effectively absorb any of the primary light which might be scattered or reflected toward the measuring cells.

Measurements were made on the turbidities 2 to 3 minutes after the reagent had been added. The exact time before measurement is not critical. The turbidities remain the same for at least 25 minutes. A calibration curve was constructed by plotting the concentration of zinc against the per cent of fluorescence as read on the instrument. Unknown concentrations are read from this calibration curve.

INFLUENCE OF PRIMARY FILTER AND CONCENTRATION OF REAGENT

The fluorescence of the zinc turbidity is more intense when excited by light of shorter wave lengths in the region 460 to 350 mμ. Above about 460 mμ the fluorescence disappears or is too small to be measurable. The reagent, however, has a strong absorption band in the near ultraviolet and absorption, as measured on a Beckman spectrophotometer, is appreciable even at 460 mμ. The per cent transmission of a 1-cm. layer of 0.002 *M* 8-hydroxyquinoline in acetic acid–ammonium acetate solution (pH = 5.9) is shown in Figure 1. The per cent transmission values for the filters investigated are also shown in Figure 1.

In Figure 2 are plotted the readings of the Lumetron against amount of zinc ion present, using three different primary filters. An intermediate amount of zinc was selected and the effect of adding 0.1 and 0.3 ml. of 8-hydroxyquinoline solution instead of the usual 0.2 ml. was determined. The method of calculation for filter 440 mμ is shown in Figure 2. At 440 mμ the error in milligrams of zinc per 0.1-ml. variation in amount of reagent added is 0.08 mg., at 420 mμ the value is 0.06 mg., and at 390 mμ the value is 0.13 mg. The most suitable filter is the one showing the greatest sensitivity to the amount of zinc present with the least variation due to a change in reagent concentration. The 420 mμ filter is the most suitable and was employed in all further investigations.

INFLUENCE OF TEMPERATURE

Calibration curves were constructed using the standard zinc solution for 15°, 24°, and 35° C. The solutions were allowed to come to the specified temperature before the turbidities were produced. The solutions were diluted to volume with water of the same temperature. The comparisons were all made at the temperature of the instrument, approximately 25° C. The curves are shown in Figure 3. At a level of about 0.3 mg. of zinc a

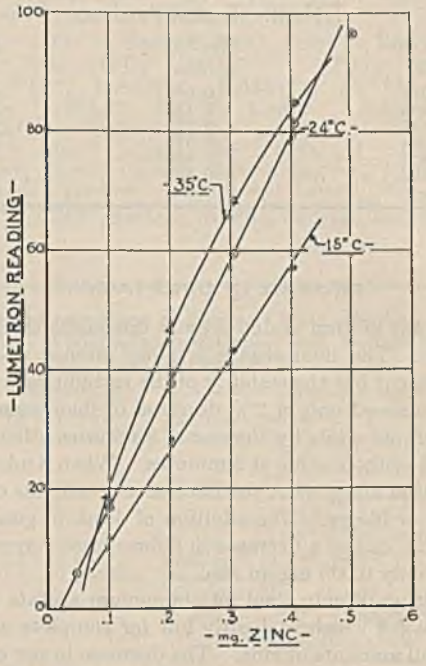

Figure 3. Effect of Temperature

change in temperature, especially a decrease in temperature, of about 2° C. will cause an error of 0.02 mg. of zinc or less. The temperature of the solutions should be kept within ±2° C. of that used in the establishment of the calibration curves. Frequently room temperature will suffice.

INFLUENCE OF ADDED SALTS

Turbidities were prepared containing 0.205 mg. of zinc plus varying amounts of added salts per 50-ml. volume. As little as 0.2 mg. of ferric ion reduces the fluorescence to zero. No more than about 3 micrograms of ferric ion can be tolerated. Aluminum and other ions precipitated in acetic acid–acetate solutions by 8-hydroxyquinoline interfere by increasing the fluorescence and by removing the reagent. The fluorescence is decreased slightly in solutions containing other ions, and calibration curves should be constructed with the same amount of extraneous salts present as are to be expected in the unknowns. The errors in most cases are slight. Calibration curves for various amounts of magnesium ion are shown in Figure 4. Except for the relatively large error when magnesium is first introduced into the sample, slight variations in the total amount of magnesium present cause no appreciable error.

In Table I are shown the variations in the apparent amount of zinc present caused by adding 1 mg. of extraneous salt. The values were calculated by averaging the errors shown when 25 to 500 mg. or more of added salts were present and the amount of zinc was 0.21 mg. In most cases 50 mg. of material will cause an error of 0.02 mg. of zinc or less. Not more than about 3 mg. of fluoride ion should be present.

Table I. Influence of Extraneous Salts

Salt Added	Error, Mg. of Zn per Mg. of Added Salt	Range of Amounts of Added Salt Investigated, Mg.
$(NH_4)_2C_4H_4O_6$	0.0005	50–500
NaCl	0.00004	360–1800
KH_2PO_4	0.0002	27–135
KI	0.00015	50–200
KNO_3	0.00007	50–500
$MgSO_4$	0.0008	27–108
$BaCl_2$	0.0001	200
$Ca(NO_3)_2$	0.0003	70–700
HF	0.0034	2–20
Na_2SiO_3	0.0004	10–100

Table II. Typical Analyses

Zinc Taken *Mg.*	Zinc Found *Mg.*	Error *Mg.*
0.082	0.066	−0.016
0.164	0.153	−0.011
0.247	0.256	+0.009
0.369	0.378	+0.009
0.451	0.450	−0.001
0.492	0.481	−0.011

INFLUENCE OF OTHER FACTORS

The amount of gum arabic present influences the intensity of fluorescence. The fluorescence is more intense when no gum arabic is present but the stability of the turbidities is less. Some turbidities showed only a 2% decrease in fluorescence between 2 and 8 minutes, while by the end of 8 minutes others decreased to one tenth of their value at 2 minutes. When 2 ml. of 2% gum arabic solution are present the fluorescence remains constant for 25 minutes or longer. The addition of 4 ml. of gum arabic instead of 2 ml. causes a decrease in fluorescence corresponding to an error of only 0.005 mg. of zinc.

The addition of only 1 ml. of ammonium acetate results in a pH value of 5.3 which is slightly low for complete precipitation of such small amounts of zinc. The decrease in per cent fluorescence corresponds to an error of about 0.025 mg. of zinc. When 10 ml. of 2 *N* ammonium acetate were used instead of 5 ml. no appreciable variation in the intensity of fluorescence could be noted.

The effect of adding the ammonium acetate last rather than the 8-hydroxyquinoline was investigated. Only a slight change in fluorescence was noted corresponding to an error of 0.007 mg. of zinc.

DISCUSSION

In spite of the above observations, it is strongly recommended that all details of procedure, once established, be carefully followed. The calibration curves should be prepared under conditions as nearly like that of the unknowns as is possible. For more exact work, a final comparison with a standard solution containing the determined amount of zinc should be made.

The method is very rapid. A determination can be carried out, once the calibration curves have been prepared, in about 3 minutes. The accuracy is about 0.01 to 0.02 mg. of zinc. Results of several typical analyses are given in Table II.

Zinc in 1-gram samples of the Bureau of Standards aluminum alloy 86b was separated according to the method outlined by Churchill and Bridges (*12*). The zinc was finally made up to 250-ml. volume and a 5-ml. aliquot portion taken for analysis. Results of several determinations gave 1.4% of zinc. The accepted value is 1.51% of zinc.

Attempts to produce reproducible turbidities with 8-hydroxyquinoline and magnesium and aluminum ions have not been successful, as yet.

A dichlorofluorescein solution may be used for resetting the instrument at the same reading and is quite stable. The method is reproducible. The results in Table II were obtained 9 days after the calibration curve was constructed.

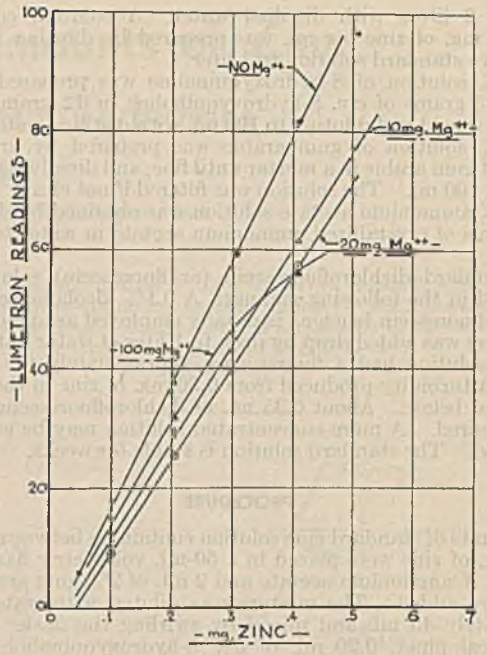

Figure 4. Influence of Magnesium

ACKNOWLEDGMENT

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Cabinet for Boiler Feedwater Testing

The Testmaster cabinet, developed by TruTest Laboratories, Jefferson Bldg., Philadelphia 7, Pa., is an all-metal adjustable cabinet devised to accommodate tests for hardness, alkalinity, chloride, phosphate, pH, sulfite, and dissolved oxygen. Equipped with glareless fluorescent lighting, it presents a flexible and rapid means for testing boiler and feedwater samples to regulate treatment dosage and blowdown requirements.

Detection of Bismuth by Means of Brucine Citrate

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A SURVEY of the literature on methods for the detection of bismuth disclosed several tests that indicated promise either in their present form or with slight modifications. One of the most satisfactory reagents for bismuth is thiourea, which has been considered by a number of investigators (*1*, *4*, *5*, *12*). This reagent provides a highly selective test for bismuth, but is seriously handicapped by its lack of sensitivity. The use of various organic bases in conjunction with potassium iodide has been utilized in a number of procedures. Antipyrine (*7*), quinoline (*6*), cinchonine (*3*), 2-aminopyridine (*11*), 2-methylbenzothiazole (*9*), and numerous other bases have been applied with varying degrees of success. Reichard (*10*) reported that bismuth chloride reacts with brucine to form a red color. Moser (*8*) noted the same effect, but claimed that the reaction was uncertain.

The reported tests were carefully considered. Determination of sensitivities and investigations of interferences indicated that the reagents reported in the literature were generally unsatisfactory. It was, therefore, deemed advisable to undertake the development of new reagents. Different substituted thiourea compounds were investigated, but none was found to possess distinct advantages over the use of the parent compound itself.

A second field of investigation, the bismuth iodide–organic base complexes, was next studied. The reactions in this case seem to involve either the formation of normal salts with the bismuth iodide complex ion, or the formation of double salts. Several organic bases gave promise when used in this manner, but brucine was particularly satisfactory. It was noted that brucine was much more soluble in hot citric acid solution than in water. Other solvents that dissolved large amounts of brucine were ethyl alcohol, chloroform, acetic acid, and hydrochloric acid. None of these gave analytical characteristics comparable to those obtained with citric acid, although Korenman (*7*) had noted the very satisfactory sensitivity of an acetic acid solution of brucine when used in conjunction with potassium iodide to detect antimony, mercury, and bismuth. His studies, however, did not consider possible interferences, and the reactions were merely recorded, without further recommendations concerning the application of the reactions to test procedures. When brucine citrate was added to a solution containing bismuth and an alkali iodide was added, a yellowish orange precipitate was formed which changed after about a minute to an intense brick-red precipitate. The reaction was found to be applicable in any acid solution.

METHOD OF STUDY

The determination of limiting concentration and limit of identification was performed in accordance with the procedures described by Feigl (*2*). Interference studies followed the general procedure discussed by West (*13*), except that the concentration of the bismuth solution was 0.1% while the concentration of the ions studied for interfering effects was 10%. A final check on interferences was made with more dilute solutions (0.01% bismuth to 1.0% interfering ion), so as to determine the reliability of the tests concerned near the sensitivity limit.

The ions investigated in the interference studies are listed below in their more common forms. It is realized that in many instances the ions concerned are present as complexes, but where the structure of such complexes may be in doubt, only the valence of the central atom is indicated.

Li^+, Na^+, K^+, Cu^{++}, Rb^+, Ag^+, Cs^+, Au^{+++}, Be^{++}, Mg^{++}, Ca^{++}, Zn^{++}, Sr^{++}, Cd^{++}, Ba^{++}, Hg^+, Hg^{++}, BO_2^-, $B_4O_7^{--}$, Al^{+++}, Sc^{+++}, Ga^{+++}, Y^{+++}, In^{+++}, La^{+++}, Ce^{+++}, Tl^+, CO_3^{--}, SiO_3^{--}, Ti^{+++}, Zr^{++++}, Sn^{++}, Sn^{++++}, Pb^{++}, Th^{++++}, NH_4^+, NO_2^-, NO_3^-, HPO_4^{--}, $P_4O_{13}^{------}$, $P_6O_{18}^{------}$, PO_3^-, $P_2O_7^{----}$, VO_3^-, $HAsO_3^{--}$, $HAsO_4^{--}$, Sb^{+++}, Sb^{+++++}, $S_2O_3^{--}$, SO_3^{--}, SO_4^{--}, Cr^{+++}, $Cr_2O_7^{--}$, ScO_3^{--}, SeO_4^{--}, MoO_4^{--}, TeO_3^{--}, TeO_4^{--}, WO_4^{--}, UO_2^{++}, UO_4^{--}, F^-, Cl^-, ClO_3^-, ClO_4^-, Mn^{++}, MnO_4^-, Br^-, BrO_3^-, I^-, IO_3^-, ReO_4^-, Fe^{++}, Fe^{+++}, Co^{++}, Co^{+++}, Ni^{++}, Ru^{+++}, Rh^{+++}, Pd^{++}, $Os^{++++++++}$, Ir^{++++}, Pt^{++++}, CN^-, $Fe(CN)_6^{---}$, $Fe(CN)_6^{----}$, CNS^-, acetate, oxalate, tartrate, aniline, pyridine

Table I. General Comparison of Brucine Citrate, Thiourea, Cinchonine, Antipyrine, 2-Methylbenzothiazole, and 2-Aminopyridine Tests for Bismuth

Test	Sensitivity	Interferences	
Brucine citrate	LI = 0.3γ LC = 1:100,000	Positive. Negative. Masking.	None Pb^{++}, Hg^+, Hg^{++}, Ag^+, Cu^{++}, and Cd^{++} (prevent full development of red color) Tl^+ (yellow upon addition of KI), TeO_3^{--} (brown precipitate), Pd^{++} (brown color), Hg^+ (black precipitate)
Thiourea	LI = 1.5γ LC = 1:100,000	Positive. Negative. Masking.	Sb^{+++}, Pd^{++}, VO_3^-, TeO_3^{--} None Hg^+ (black), SeO_3^{--} (red), SeO_4^{--} (red), $Os^{++++++++}$ (brown to pink), and colored ions such as Cr^{+++}, $Cr_2O_7^{--}$, UO_4^{--}, MnO_4^{--}, Rh^{+++}, Pt^{++++}
Cinchonine	LI = 0.3γ LC = 1:80,000	Positive. Negative. Masking.	Sb^{+++}, Sb^{+++++}, Sn^{++++}, Pb^{++} Ba^{++}, Sn^{++} Cu^{++} (brown), Ag^+ (gray-brown), Au^{+++} (black), Hg^+ and Hg^{++} (black), Tl^+ (yellow), Cr^{+++} and $Cr_2O_7^{--}$ (green), SeO_3^{--} and SeO_4^{--} (brown), TeO_3^{--} and TeO_4^{--} (black), Fe^{++} and Fe^{+++} (brown), Pd^{++} (brown to black), and colored ions such as UO_2^{++}, UO_4^{--}
Antipyrine	LI = 0.3γ LC = 1:40,000	Positive. Negative. Masking.	Cd^{++}, Tl^+, Pb^{++}, Sb^{+++++} Sn^{++}, $C_2O_4^{--}$ Cu^{++} (brown), Au^{+++} (brown), Hg^+ (black), Cr^{+++} and $Cr_2O_7^{--}$ (green), SeO_3^{--} and SeO_4^{--} (brown), TeO_3^{--} and TeO_4^{--} (black), Fe^{++} and Fe^{+++} (brown), Rh^{+++} (red-brown), Pd^{++} (brown), $Os^{++++++++}$ (blue to orange gray), and colored ions such as Co^{++}, Ni^{++}, Ir^{++++}
2-Methylbenzothiazole	LI = 0.3γ LC = 1:40,000	Positive. Negative. Masking.	Tl^+, Pb^{++}, Sb^{+++}, Sb^{+++++} Sr^{++}, Ba^{++}, NO_2^-, HPO_4^{--}, CN^-, tartrate, citrate Cu^{++} (brown), Au^{+++} (brown), Hg^+ (black), Hg^{++} (brown), Cr^{+++} (green), $Cr_2O_7^{--}$ (green), TeO_3^{--} (black), Fe^{++} (brown), Fe^{+++} (brown), Pd^{++} (brown), and colored ions such as UO_2^{++}, UO_4^{--}, Ru^{+++}, Rh^{+++}
2-Aminopyridine	LI = 10.0γ LC = 1:10,000	Positive. Negative. Masking.	Be^{++}, Al^{+++}, Ga^{+++}, Y^{+++}, Zr^{++++}, Th^{++++}, Sb^{+++}, Sb^{++++} Sr^{++}, Ba^{++}, Pb^{++}, NO_3^-, $HAsO_3^{--}$, CN^-, $C_2O_4^{--}$ Cu^{++} (brown), Ag^+ (yellow), Au^{+++} (brown), Hg^+ (black), Hg^{++} (black), TeO_3^{--} (black), Fe^{++} (yellow), Fe^{+++} (yellow), and colored ions such as Cr^{+++}, $Cr_2O_7^{--}$, UO_2^{++}, UO_4^{--}

REAGENTS

Brucine citrate. Dissolve 100 grams of citric acid in 100 ml. of water, add 12 grams of brucine and heat until solution is complete.

Borate inhibitor. Mix equal volumes of 1 *M* boric acid and 1 *M* sodium hydroxide.

Sodium bisulfite, saturated aqueous solution.

Potassium iodide, 20% aqueous solution.

PROCEDURE

On a spot plate place one drop of the solution to be tested, and to it add one drop each of borate inhibitor, sodium bisulfite, brucine citrate, and potassium iodide. In the presence of bismuth a brick-red precipitate forms.

DISCUSSION

The brucine citrate reaction, when used as a spot test, has a limit of identification of 0.3 microgram of bismuth at a limiting concentration of 1 part in 100,000. No positive interferences were found. Cadmium, mercury, copper, silver, and lead interfered, inasmuch as they prevented the full development of the red test color and reduced the sensitivity of the test. However, in their presence, a deep orange precipitate was formed which still permitted the absolute identification of the bismuth at a somewhat lessened sensitivity. The brucine citrate solution is very stable, one solution being in use in these laboratories for approximately one year without any change in appearance or reactions. Table I gives a comparison of some of the more important analytical characteristics of the brucine citrate, thiourea, cinchonine, antipyrine, 2-aminopyridine, and 2-methylbenzothiazole tests.

The test procedure followed in the case of thiourea was to add one drop each of 0.1 *N* nitric acid and 5% thiourea to a drop of the solution to be tested. For the cinchonine, antipyrine, 2-aminopyridine, and 2-methylbenzothiazole tests, a drop of saturated sodium bisulfite was added to a slightly acid drop of the solution to be tested, followed by a drop of a 0.1% aqueous or alcoholic solution of the reagent and a drop of 20% potassium iodide. In the case of the four latter reagents many uncertain tests were obtained, owing to the liberation of iodine or the formation of yellow iodide precipitates or complex ions. Such reactions may mask the test for bismuth, or in many cases, the yellow colors formed may be confused with a true test for bismuth. The use of bisulfites does not completely obviate these difficulties.

For spot-test procedures, without prior separation of the bismuth, either the brucine citrate or the thiourea tests are satisfactory. The brucine citrate procedure has an advantage in both sensitivity and selectivity.

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The authors wish to express their appreciation to Yvonne Broussard for her work in checking the interference data.

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Microdetermination of Calcium

By Titration of the Oxalate with Ammonium Hexanitratocerate

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THE limitations of potassium permanganate for microtitrations led several workers to substitute ceric sulfate (*3*, *5*, *7*, *8*) and ceric ammonium sulfate (*6*) as oxidizing agents for the titration of oxalate. Although these two reagents form more stable and more easily prepared solutions than potassium permanganate, they still are not entirely satisfactory. These solutions do not always give either a sharp or a clear end point with the available indicators. In addition, the direct cerate titrations have to be carried out in a hot oxalate solution. At about the same time that the above ceric salts were proposed, Ellis (*2*) reported the use of ammonium hexanitratocerate for the drop-scale titration of calcium oxalate. Smith suggested the use of this salt (*13*) and carried out a series of studies concerning its properties (*1*, *10*, *11*, *12*). Kirk and Tompkins (*4*), however, imply that this reagent is not suitable for microtitration of oxalate.

The need of a precise method for the determination of small amounts of calcium led the authors first to the use of ceric sulfate. The disappointing results with this procedure prompted them to begin an investigation of ammonium hexanitratocerate.

SOLUTIONS

0.01000 *N* Sodium Oxalate, 0.6700 gram of carefully dried Sorensen's sodium oxalate dissolved in 1 liter of distilled water. This solution is stable if kept in a refrigerator with chloroform as a preservative, or if made in 0.1 *N* perchloric acid (*9*). To 4 ml. of this standard, 0.5 ml. of 60% perchloric acid is added before titration.

0.05% Setopaline C, 50 mg. of Eimer and Amend's setopaline C added to 100 ml. of distilled water and warmed on a steam bath or electric hot plate. The dye is only slightly soluble and precipitates on cooling. Therefore, the solution is warmed just before use, and 6 drops of the warm indicator are added to the oxalate solution. Furthermore, when the indicator is added as a cold solution, it is oxidized immediately by the first few drops of the cerate solution rendering it ineffective.

0.01 *N* Ammonium Hexanitratocerate, 6 grams of reagent grade ammonium hexanitratocerate dissolved in about 200 ml. of 1 *N* perchloric acid and diluted to 1 liter with the acid. The solution is kept in a black bottle in the dark.

Wash Solution (cf. *6*), 2 ml. of 28 to 29% ammonium hydroxide, 98 ml. of distilled water, 100 ml. of redistilled ether, and 100 ml. of redistilled ethyl alcohol mixed together. The use of this solution always gave very good settling of the calcium oxalate on centrifugation. The use of 2% ammonium hydroxide, on the other hand, always resulted in the loss of material due to creeping and floating.

PROCEDURE

The sample containing the calcium is pipetted into a 15-ml. conical centrifuge tube, and 2 drops of methyl red indicator and 1 ml. of 4% ammonium oxalate are added. Ammonium hydroxide (1 *N*) is added from a dropper until the indicator just turns yellow, and then 1 *N* hydrochloric acid is added until the

Ammonium hexanitratocerate in 1 *N* perchloric acid is a very good reagent for the microdetermination of calcium by direct titration of the oxalate at room temperature. The indicator setopaline C gives an extremely sharp end point with this reagent. Certain precautions must be observed in the preparation and storage of the ammonium hexanitratocerate solution. The reagent must be dissolved and kept in 1 *N* perchloric acid in order to prevent the irreversible hydrolysis of the salt. It is decomposed by light in either clear or brown glass bottles. The solution generally decreases in normality more rapidly when prepared in increased concentrations of perchloric acid. The solution, however, is stable in a black bottle, in which the decrease in titer is only about 2.5% over a period of 90 days. This change may be further lessened by keeping the bottle in the dark. Finally, the calcium oxalate must be dissolved in at least 1 *N*, but not concentrated, perchloric acid.

pink color reappears. The solutions are stirred with footed glass stirring rods which are removed and hung on a numbered rack. After one hour, the tubes are centrifuged, the supernatant fluid is poured off, and the tubes are inverted on paper or cloth towels to drain for 2 minutes. Then the rims are wiped dry, the stirring rods are placed in their respective tubes, 5 ml. of wash solution are pipetted down the sides of the tubes, and the calcium oxalate precipitate is dispersed by stirring. The tubes are centrifuged and the wash procedures repeated. After the second wash, the precipitate is dissolved in 4 ml. of 1 *N* perchloric acid and titration is carried out in the centrifuge tube at room temperature.

The tube is held by a clamp attached to the buret stand and the buret is lowered into the tube, so that the tip is always above the surface of the solution. The solution is stirred by an up-and-down motion of a narrow glass rod slightly flattened at the end and bent at a 45° to 90° angle at a height just above the lip of the tube. The warm indicator (6 drops) is added and the reagent is delivered from a microburet graduated at 0.02 ml. until the indicator changes from yellow to salmon pink. At the end point this color will persist for only about 15 seconds, after which it changes to a bronze. The color change is extremely sharp and distinct.

PREPARATION OF AMMONIUM HEXANITRATOCERATE SOLUTION

In order to obtain a satisfactory solution of the reagent, the salt must be dissolved directly in 1 *N* perchloric acid or in water, followed by the immediate addition of sufficient perchloric acid to make a 1 *N* solution. The former method is preferred. In the latter procedure, if the perchloric acid is not added immediately after the salt is completely dissolved, a fine insoluble precipitate appears. This precipitate is probably formed by hydrolysis of the salt (*1*). Furthermore, if the solution is warmed even after the above precaution has been observed, a large amount of fine white precipitate appears with a marked loss in titer. This latter fact apparently was not recognized by Kirk and Tompkins (*4*), for they warmed their solutions on the steam bath for 24 hours and filtered. Solutions prepared in the amounts of ammonium hexanitratocerate, stated by these authors to give a 0.02 *N* solution, gave a 0.05512 *N* solution when the heating procedure was omitted.

As a further check on the effect of heat, two solutions, 0.02672 and 0.00880 *N*, were prepared in 1 *N* perchloric acid, and aliquots from each of these were placed on the steam bath for 24 hours, filtered, and restandardized with sodium oxalate solution. The stronger of the two solutions decreased in normality to less than 10% of the original value and the weaker to less than 70%. In addition, the end point was blurred by the formation of a fine white precipitate during the titration. A similar decrease in titer as a result of heating was observed by Smith and Getz (*12*).

STABILITY OF AMMONIUM HEXANITRATOCERATE SOLUTION

The reagent rapidly decreases in titer when exposed to diffuse daylight (Table I). The decrease apparently is due to photochemical reactions, since darkness prevented the change. Brown bottles also protected the ammonium hexanitratocerate solutions, but only when the concentration of the perchloric was not greater than 1 *N*. Black painted bottles provided the greatest protection, especially when placed in the dark.

CONCENTRATION OF PERCHLORIC ACID IN OXALATE SOLUTION

When the concentration of the perchloric acid in the standard sodium oxalate solution was less than 1 *N*, a white cloudy precipitate invariably formed on titration. On the other hand, if the normality of the perchloric acid was greater than 1 *N*, no further increase in accuracy was observed. The titration with a standard 0.02113 *N* sodium oxalate solution of fourteen equal aliquot portions of ammonium hexanitratocerate solution varying from 1 to 4 *N* in perchloric acid gave a mean normality of 0.01831 with an average deviation of only ±0.00004.

SOLUTION OF CALCIUM OXALATE

If the moist precipitate of calcium oxalate was dissolved in 0.5 ml. of concentrated perchloric acid and then diluted with 4 ml. of water, the results always were low and inconsistent (Table II). On the other hand, if the precipitate was previously dried or was dissolved in 1 *N* perchloric acid, theoretical values were obtained. It appears that the concentrated perchloric acid is able to oxidize the moist precipitate.

The figures in Table II also indicate the degree of accuracy of the method. Samples containing as low as 0.5 mg. of calcium have given similarly satisfactory results.

PRECIPITATION OF CALCIUM OXALATE

In the other reported procedures for calcium there is a wide variance in the manner of precipitation of the calcium oxalate; therefore, time of precipitation and digestion were studied. Furthermore, some of the authors' unknown samples required considerable ammonium hydroxide to bring them to the proper pH. Therefore, known samples were made excessively acid by the addition of 0.1 to 0.3 ml. of 6 *N* hydrochloric acid, and neutralized. The results in Table III indicate that one hour is suf-

Table I. Effect of Light and Concentration of Perchloric Acid on Stability of Ammonium Hexanitratocerate Solutions

Bottle	Place of Storage	$HClO_4$ Normality	Duration, Days	$(NH_4)_2Ce(NO_3)_6$ Normality	Decrease, %
Clear glass	On bench	1	25	0.02182	21.1
	In locker	1	124	0.01722	6.8
		1	124	0.05453	1.0
		1	124	0.01795	6.1
		2	124	0.01777	5.0
		3	124	0.01771	4.2
Brown glass	On bench	1	84	0.05434	5.8
		1	84	0.01769	5.8
		2	84	0.01744	14.9
		3	84	0.01742	13.1
Black painted	On bench	1	90	0.06830	2.3
		1	90	0.02253	2.4
		2	90	0.02260	2.1
		3	90	0.02238	6.5
Black painted	In locker	1	80	0.01340	0.0

Table II. Effect of Perchloric Acid on Calcium Oxalate

Series No.	Number of Analyses	$HClO_4$ Normality	Condition of CaC_2O_4 Ppt.	Average Ca Found, Mg.	Average Deviation[a]
1	4	9	Moist	0.968	0.223
2	12	9	Dry	1.203	0.015
3	8	1	Moist	1.208	0.013
4	14	1	Dry	1.217	0.004

Calcium by macroanalysis, 1.217 mg.

[a] Of a single observation.

Table III. Precipitation of Calcium Oxalate under Different Conditions

Series No.	Number of Analyses	Temperature for CaC_2O_4 Pptn.	Hours of CaC_2O_4 Pptn.	Excess NH_4Cl	Average Ca Found, Mg.	Average Deviation
1	10	Room	1	0	1.208	0.011
2	4	Room	20	0	1.211	0.006
3	4	Boiling water bath	2	0	1.208	0.009
4	6	Boiling water bath	3	0	1.200	0.017
5	9	Room	1	+	1.209	0.008
6	6	Boiling water bath	1	+	1.201	0.006

Calcium by macroanalysis, 1.217 mg.

[a] Of a single observation.

ficient time for precipitation and that digestion in a water bath or excess ammonium chloride does not increase or decrease the accuracy.

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Microdetermination of Nitrates by the Devarda Method

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Microquantities (0.05 milliequivalent) of nitrates can be determined with a precision and accuracy of 99.8% by reduction with Devarda's alloy, the ammonia liberated being absorbed in boric acid and titrated with 0.01 *N* hydrochloric acid to bromocresol green–methyl red end point. By following the procedure given below, a single determination can easily be run in 20 minutes. Nitrites and ammonia interfere, but interference of ammonia can be easily overcome. Special apparatus described is convenient, but not essential.

METHODS for the determination of small quantities of nitrates have, in general, been either undesirably time-consuming or rather limited in accuracy. The Devarda method, generally accepted as the most desirable for macrodeterminations (*4*), appeared to be best suited to a microadaptation (*1*, *5*), its only apparent disadvantage lying in the time required for an analysis and the relative complexity of the apparatus. In the hope of eliminating these disadvantages, the work described below was undertaken. Highly satisfactory results were obtained, and more than 200 determinations by the method finally developed have shown no undesirable features.

APPARATUS

The apparatus shown in Figure 1 was constructed to facilitate the use of the method in conjunction with certain other experiments. It is described here, because it is well suited to the method and very convenient to use. Where the construction of such special apparatus is impractical, as for only occasional determinations, a satisfactory substitute can be assembled from ordinary laboratory apparatus.

The reaction and distilling flask, *A*, is a modification of the Parnas-Wagner micro-Kjeldahl apparatus (*2*, *3*), fitted with standard-taper joints, *a*, *c*, and an electrical heating coil, *e*. The heating coil provides a convenient means of boiling the contents of the flask, being unaffected by drafts and capable of accurate control by means of a rheostat. Since the coil has a negligible heat capacity, there is no lag when the current is turned on or off. Expanding air escaping from the stoppered inner tube, *d*, effectively prevents bumping, even when concentrated caustic is being boiled. Alternatively, where the volume of liquid is small, distillation can be accomplished by an outside source of steam entering through the inner tube at *a*.

The spray trap, *B*, and condenser, *C*, are a single unit, with standard-taper joints to fit the distilling and receiving flasks. The spray trap is simply a tube containing a wad of glass wool, *f*. This trap is essential, since the reaction mixture evolves a large amount of fine alkaline spray. It is insulated by a silvered vacuum jacket to eliminate condensation as much as possible. (A steam jacket or electrical heating coil may be substituted for this jacket, as in the macromethod described by Scott, *4*.) It is important that the inner tube of the trap be unconstricted at the ring seal, *g*, so that the glass wool may be readily replaced.

The standard taper joint, *i*, is primarily a matter of convenience, as well as insurance against breakage of the condensate delivery tip, *j*. When the receiver, *k*, is connected by means of this joint, one can be certain that the delivery tip is covered by a maximum depth of liquid, without danger of its pressing against the bottom of the flask. Contamination is also minimized, since only the small vent, *h*, is open to the atmosphere. The receiver, *k*, is a 125-ml. Erlenmeyer flask.

Incidentally, the experience of the writer has been that the entire apparatus may be safely supported by one clamp on flask *A*. The trap-condenser unit is sufficiently strong to allow suspension by joint *c* alone, thus simplifying assembly, and eliminating the danger of breakage through incorrect alignment of clamps.

EXPERIMENTAL

C.P. potassium nitrate was recrystallized three times from double-distilled water, and dried for 3 hours at 110° C. and 3 hours at 200° C. (*4*). The product was assayed by the ferrous sulfate method, and, indirectly, by conversion to the chloride and weighing. Both methods gave a potassium nitrate content of 100%. Probable impurities were tested for, and found absent. Standard solutions were prepared from this salt by weighing and diluting to a definite volume. Fresh solutions were prepared every day.

The procedure followed in all tests was as follows: A definite volume of 0.01 *N* nitrate solution was pipetted into the reaction flask through joint *c*, after which a weighed quantity of Devarda's alloy was added. The inside of the joint was washed down with sufficient water to bring the total volume to 20 ml., and the spray trap and condenser were connected. The receiving flask, containing 10 ml. of 2% boric acid and 2 drops of bromocresol green–methyl red indicator (*2*), was connected to the condenser, 10 ml. of 20% sodium hydroxide were added through funnel *b*, and the funnel stopcock was immediately closed. The mixture was then heated by the electrical heating coil until the reaction proceeded vigorously, when the current was turned off. After a definite length of time, low heat was turned on, and the mixture boiled until foaming had subsided. The heat was then increased, and about 10 ml. were distilled over. The receiver was lowered, and the distillation continued for about 30 seconds, the delivery tip being washed with a stream of water. The distillate was then titrated to a colorless end point with 0.01 *N* hydrochloric acid from a 5-ml. microburet.

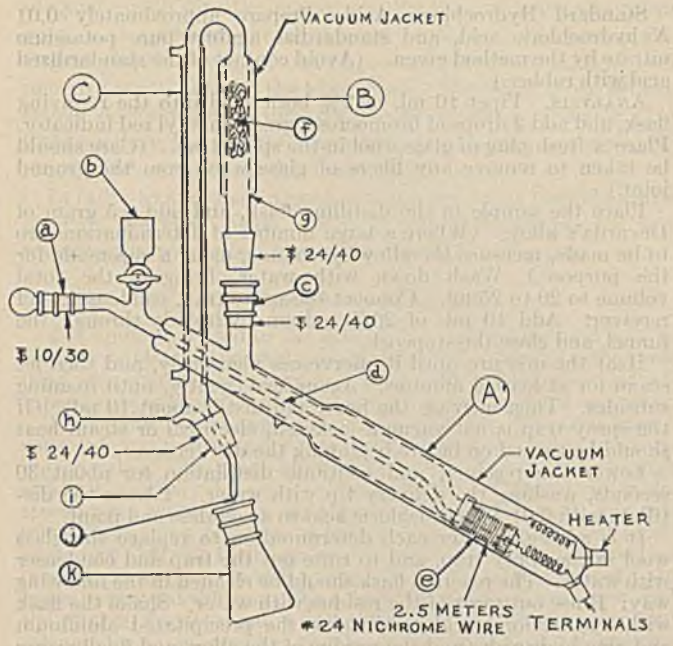

Figure 1. Distillation Apparatus

Table I. Effect of Reaction Time

Reaction Time	Volume of 0.01153 *N* HCl	Weight of KNO_3 Known	Found[a]
Min.	*Ml.*	*Mg.*	*Mg.*
60	4.470	5.055	5.065
	4.460	5.055	5.053
	4.458	5.055	5.051
			Av. 5.056
30	4.462	5.055	5.056
	4.468	5.055	5.063
	4.462	5.055	5.056
			Av. 5.058
0	4.475	5.055	5.071
	4.465	5.055	5.059
	4.450	5.055	5.042
			Av. 5.057
15	0.128	Blank	0.149
	0.124	Blank	0.145
			Av. 0.147

[a] Final figures given in this and following tables are corrected for blank error. All titrations shown are original buret readings.

Considerable difficulty was at first encountered in obtaining consistent results, particularly from tests run on different days. The trouble was finally traced to the rubber tube connecting the microburet to the standard acid reservoir. Tests showed that 0.01 *N* hydrochloric acid standing in the rubber tube for 48 hours was reduced in concentration by 37%. A parallel test run on flexible plastic tubing showed a concentration reduction of only 0.9%. Accordingly, the rubber tube was replaced by the plastic, after which no further trouble was encountered.

It was decided first to determine the length of time actually required to effect a quantitative reduction of the nitrate by the process. In all tests, 5-ml. aliquots of 0.01 *N* potassium nitrate and 0.5-gram portions of Devarda's alloy were used, the reaction mixture being allowed to stand for varying lengths of time between initiation of the reaction and the preliminary boiling. The results of this experiment are given in Table I. Evidently, reaction time preliminary to boiling is not a factor in the accuracy of the method. For practical purposes, however, it is advisable to allow 5 to 10 minutes for the reaction to subside, since the mixture is apt to foam up into the spray trap if boiled immediately. For this reason, an interval of 10 minutes was allowed in all subsequent tests.

The quantity of Devarda's alloy necessary to complete the reaction was another factor open to question. Inasmuch as the alloy itself is the chief source of the blank error, it is desirable to use as little as possible. Using an equal amount of standard potassium nitrate in each case, a series of tests was run with the weight of alloy as the variable (Table II). As was to be expected, a certain minimum weight (0.5 gram) of alloy was found necessary to produce stoichiometric results. Larger amounts merely increased the blank.

Table II. Effect of Weight of Devarda's Alloy

Weight of Alloy	Volume of 0.01153 *N* HCl	Weight of KNO_3 Known	Found	Recovery
Gram	*Ml.*	*Mg.*	*Mg.*	%
0.1	0.060	Blank	0.070	...
	0.060	Blank	0.070	...
	3.240	5.595	3.708	66.3
	3.210	5.595	3.673	65.7
0.2	4.684	5.595	5.353	95.7
	4.700	5.595	5.372	96.0
0.5	0.128	Blank	0.149	...
	0.124	Blank	0.145	...
	4.460	5.055	5.053	100.0
	4.485	5.055	5.081	100.5
1.0	0.275	Blank	0.321	...
	0.265	Blank	0.309	...
	4.600	5.055	5.049	99.9
	4.620	5.055	5.072	100.3

The two uncertain factors involved in the method having been determined, a series of analyses was run on samples of varying nitrate concentrations, in order to determine the method's range of accuracy. In addition to the purified potassium nitrate used in the previous experiments, samples of dried C.P. sodium nitrate were analyzed as an additional check (Tables III and IV).

PRECISION AND ACCURACY OF METHOD

The deviations from the means of the analyses listed in Tables III and IV, expressed as weights rather than percentages, are

Table III. Effect of Size of Sample on Accuracy

Weight of KNO_3 Known	Volume of 0.01153 *N* HCl	Weight of KNO_3 Found	Deviation from Mean	Error
Mg.	*Ml.*	*Mg.*	*Mg.*	%
Blank	0.128	0.149	...	...
	0.124	0.145	...	...
		Av. 0.147		
0.506	0.560	0.506	0.010	0.0
	0.540	0.483	0.013	4.5
	0.555	0.500	0.004	1.2
		Av. 0.496	0.009	1.8
1.011	0.995	1.013	0.002	0.2
	1.005	1.025	0.010	1.4
	0.990	1.007	0.008	0.4
		Av. 1.015	0.007	0.7
2.022	1.900	2.069	0.027	2.3
	1.860	2.022	0.020	0.0
	1.872	2.036	0.006	0.7
		Av. 2.042	0.018	0.9
5.055	4.470	5.065	0.011	0.2
	4.450	5.042	0.012	0.3
	4.465	5.059	0.005	0.1
	4.445	5.036	0.018	0.4
	4.465	5.059	0.005	0.1
	4.470	5.065	0.011	0.2
	4.458	5.051	0.003	0.1
		Av. 5.054	0.009	0.2

Table IV. Analyses of C.P. Sodium Nitrate

Weight of $NaNO_3$ Known	Volume of 0.01153 *N* HCl	Weight of $NaNO_3$ Found	Deviation from Mean	Error
Mg.	*Ml.*	*Mg.*	*Mg.*	%
1.700	1.860	1.699	0.003	0.1
	1.855	1.694	0.008	0.4
	1.875	1.714	0.012	0.8
	1.862	1.701	0.001	0.1
		Av. 1.702	0.006	0.4
4.251	4.458	4.245	0.004	0.1
	4.465	4.252	0.003	0.0
	4.475	4.262	0.013	0.3
	4.450	4.238	0.011	0.3
	4.462	4.249	0.000	0.0
		Av. 4.249	0.006	0.1

fairly constant. They may be largely, if not entirely, attributed to unavoidable errors of measurement, since in only one case is the deviation greater than the equivalent of one drop in the titration.

The accuracy of the method is good: 99.8 to 99.9% for 0.05-milliequivalent samples. Considering the nature of the deviation from the mean, it is obvious that the accuracy of a single determination will decrease with smaller samples, and that the accuracy of the mean value of several determinations will decrease as the size of the sample approaches that of the blank. These facts are corroborated by the last columns of Tables III and IV.

INTERFERENCES

Certain types of nitrogen compounds may be expected to interfere with the method. Of these, ammonium and nitrite compounds are the most likely to be encountered. The former may readily be removed by adding alkali and boiling, before the addition of the Devarda's alloy. (If this procedure is followed, the mixture should be cooled thoroughly before adding the alloy.) Nitrites must be determined separately, and deducted from the total.

As opposed to the macromethod described by Scott, carbon dioxide shows no evidence of interference with this method.

OUTLINE OF PROCEDURE

Reagents. Devarda's Alloy, analyzed reagent.

Sodium Hydroxide, ammonia- and nitrate-free. Dissolve 200 grams of c.p. sodium hydroxide in water, and make up to 1 liter. Add about 1 gram of Devarda's alloy, and boil for about 10 minutes. Cool, replace any water boiled off, and store in a well-stoppered bottle (preferably Pyrex).

Boric Acid, 2% c.p. boric acid crystals in water.

Indicator, 10 ml. of 0.1% bromocresol green plus 2 ml. of 0.1% methyl red, in 95% ethanol.

Standard Hydrochloric Acid. Prepare approximately 0.01 *N* hydrochloric acid, and standardize against pure potassium nitrate by the method given. (Avoid contact of the standardized acid with rubber.)

Analysis. Pipet 10 ml. of 2% boric acid into the receiving flask, and add 2 drops of bromocresol green–methyl red indicator. Place a fresh plug of glass wool in the spray trap. (Care should be taken to remove any fibers of glass wool from the ground joint.)

Place the sample in the distilling flask, and add 0.5 gram of Devarda's alloy. (Where a large number of determinations are to be made, measure the alloy by volume, using a cup made for the purpose.) Wash down with water, bringing the total volume to 20 to 25 ml. Connect the spray trap, condenser, and receiver. Add 10 ml. of 20% sodium hydroxide through the funnel, and close the stopcock.

Heat the mixture until it effervesces vigorously, and then let stand for at least 5 minutes. Again heat, gently, until foaming subsides. Then increase the heat, and distill about 10 ml. (If the spray trap is not vacuum-jacketed, electrical or steam heat should be turned on before beginning the distillation.)

Lower the receiver, and continue distillation for about 30 seconds, washing the delivery tip with water. Titrate the distillate with 0.01 *N* hydrochloric acid to a colorless end point.

It is advisable after each determination to replace the glass wool in the spray trap, and to rinse out the trap and condenser with water. The reaction flask should be cleaned in the following way: Rinse out most of the residue with water. Shake the flask with hydrochloric acid to dissolve the precipitated aluminum and zinc hydroxides and the residue of the alloy, and finally rinse again with water.

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Microdetermination of Nitric Oxide in Gases

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Nitric oxide may be determined in olefin-free gases with an accuracy and precision of 99.0% by the method described. The gas is passed through an alkaline permanganate solution in a specially designed scrubber, after which the solution is analyzed by the micro-Devarda method. Gases containing unsaturated components may be analyzed with a modification of the procedure. If the olefin concentration is low, it is necessary only to increase the permanganate concentration of the absorbent. If it is high, the gas must first be scrubbed with sulfuric acid saturated with silver sulfate. Higher nitrogen oxides interfere. If present, they should be removed by scrubbing the gas with dilute alkali. The method may conveniently be used with nitric oxide concentrations down to 2 parts per million. A semiautomatic sampling apparatus for routine tests is described.

BECAUSE of the insoluble and generally inert nature of nitric oxide, methods for its determination necessarily involve a preliminary oxidation to the more soluble nitrogen dioxide. Three oxidizing agents have been used in the most successful methods, all of which suffer from one or more disadvantages. Hydrogen peroxide, in acid, alkaline, and neutral solution, has been recommended by a number of investigators (*1*, *2*, *3*, *7*, *15*). However, the impurities it inevitably contains produce too high a blank to allow its use with low concentrations of nitric oxide (*6*). Fulweiler (*4*, *5*, *6*) has developed a generally satisfactory method of analysis, using oxygen and a catalyst mixed with the gas before absorption. His apparatus, however, is rather complex, and a rather large correction factor must be applied. Oxygen alone can be used only with certain illuminating gases, which already contain catalysts (*6*, *13*). Potassium permanganate has been used successfully by Shnidman and Yeaw (*14*), its only disadvantage lying in the fact that it is reduced by the olefins usually present in illuminating gases (*6*, *8*). Since the gas to be analyzed by the writer contained negligible quantities of reducing compounds, permanganate appeared to be the oxidizing agent best suited to the purpose.

The nitrogen dioxide obtained from the oxidation of the nitric oxide has, in the past, almost invariably been determined colorimetrically, by absorption in a suitable reagent. Extreme sensitivity is thus obtained: the Griess-Ilosvay reagent is capable of detecting 0.1 microgram of nitric oxide as the nitrite (*6*). However, a rather low order of precision is obtained with all the methods reviewed, the variation in some cases exceeding the necessary correction factor. For this reason, the volumetric micro-Devarda method (*10*) was chosen for use with the method to be described. Alkaline permanganate was used as the oxidizing agent, so that a second scrubber to retain the nitrogen dioxide was unnecessary.

In order properly to develop and evaluate the method, it was necessary to test it against known mixtures of nitric oxide. As mentioned by Shnidman and Yeaw (*14*), the storage of previously prepared dilutions of nitric oxide is an exceedingly dubious proposition. For this reason, it was preferred to store pure nitric ox-

ide, metering it directly into the gas stream during the absorption run. Water appeared to be the best confining liquid for the gas. Organic liquids were, of course, out of the question, and even mercury is attacked by the gas in time. However, nitric oxide is slowly decomposed to nitrogen in the presence of water (*11*). Consequently, the gas was assayed regularly with a gas buret, and renewed when the assay fell below 99%.

In order to avoid additional calculations and a possible source of error, the nitric oxide, which, of course, was saturated with water vapor, was passed through a desiccant before use.

EXPERIMENTAL APPARATUS

The apparatus used in the development of the method was, so far as possible, constructed entirely of Pyrex. Plastic tubing was used wherever flexibility was required, and full-length standard-taper joints were used wherever breaks in the line were necessary.

The layout of the apparatus was as follows: Nitrogen from a constant-pressure reservoir was led through a flowmeter to a mixing chamber, and then, by way of a 3-way stopcock, through either a capillary by-pass or the scrubber. Pure nitric oxide, stored over water in an all-glass chamber (Figure 1), was admitted through a phosphorus pentoxide jar to a calibrated capillary pipet of about 1-ml. capacity, from which it was displaced by mercury into the nitrogen stream in the mixing chamber. In order to prevent diffusion, the connection between the pipet and the mixing chamber was a fine capillary. A thermometer and manometer were provided, for use in calculation of volume corrections.

The nitric oxide content of the gas mixture was determined by the rate at which the mercury was admitted into the nitric oxide pipet. This was controlled by a leveling bulb lifted by a chain passing over a synchronous motor-driven sprocket. Thus, variations in the nitric oxide concentration could be obtained by varying the diameter of the sprocket.

EXPERIMENTAL PROCEDURE

At the start of a run, a capillary tube creating the same effective back pressure as the scrubber to be used was connected to the by-pass port of the 3-way stopcock. The nitrogen flow was started, and adjustments were made with the gas going through the by-pass.

The mercury in the nitric oxide delivery pipet was lowered below the T-connection to the nitric oxide reservoir, and the pipet was flushed with about 5 ml. of the gas. The synchronous motor feed was then started, the nitrogen–nitric oxide mixture being led through the by-pass until the mercury level reached the lower graduation of the pipet. Sufficient time elapsed in this period for the nitric oxide used in flushing the pipet to be swept out of the system.

The 3-way stopcock was then turned to pass the gas through the scrubber, and the time was noted. When the mercury reached the upper graduation of the pipet, the time was again noted, and the gas flow switched back to the by-pass.

The absorbent solution in the scrubber was then washed into the micro-Devarda reaction flask, and an amount of oxalic acid sufficient to reduce the excess permanganate to manganese dioxide was added. The solution was boiled down to about 20 ml., after which the micro-Devarda reaction and distillation were carried out in the usual way (*10*).

In the reduction of the permanganate, excess oxalic acid was required, in order to reduce the pH of the solution to a value where the reaction could occur. The oxalic acid remaining after the reduction had no effect upon the subsequent Devarda reaction. Incidentally, the final titration blank was not appreciably affected by any of these reagents.

DETERMINATION OF FACTORS INFLUENCING ACCURACY OF METHOD

A large number of preliminary runs were made, in the course of which several kinks in the apparatus were found and ironed out.

Table I. Effect of Gas Flow Rate

(Scrubber, 3 fritted-glass bubblers. Absorbent, 0.5% $KMnO_4$–0.5% NaOH. NO concentration, 27 micrograms per liter)

Flow Rate	Recovery	Average	Average Deviation
Ml./min.	%	%	%
300	94.9		
	97.4		
	95.9	96.1	0.9
600	84.7		
	86.4		
	86.7	85.9	0.8

The approximate optimum range of flow rate and absorbent concentration were determined, using as a scrubber a column of three fritted glass bubblers (*9*). A systematic study was then made of the four major variables affecting the accuracy of the method—i.e., the flow rate, the absorbent concentration, the nitric oxide concentration, and the type of scrubber.

The first runs were made with a concentration of 27 micrograms of nitric oxide per liter, since this was known to approximate the actual composition of the gas eventually to be analyzed. (Nitric oxide concentration is expressed in micrograms per liter, since that was the actual relationship measured. To convert approximately to parts per million by volume at 25° C. and 760 mm., multiply by 0.815.) An absorbent solution composed of 0.5% potassium permanganate and 0.5% sodium hydroxide was used, except when the effect of that concentration was being studied.

The first tests made were to determine the effect of the gas flow

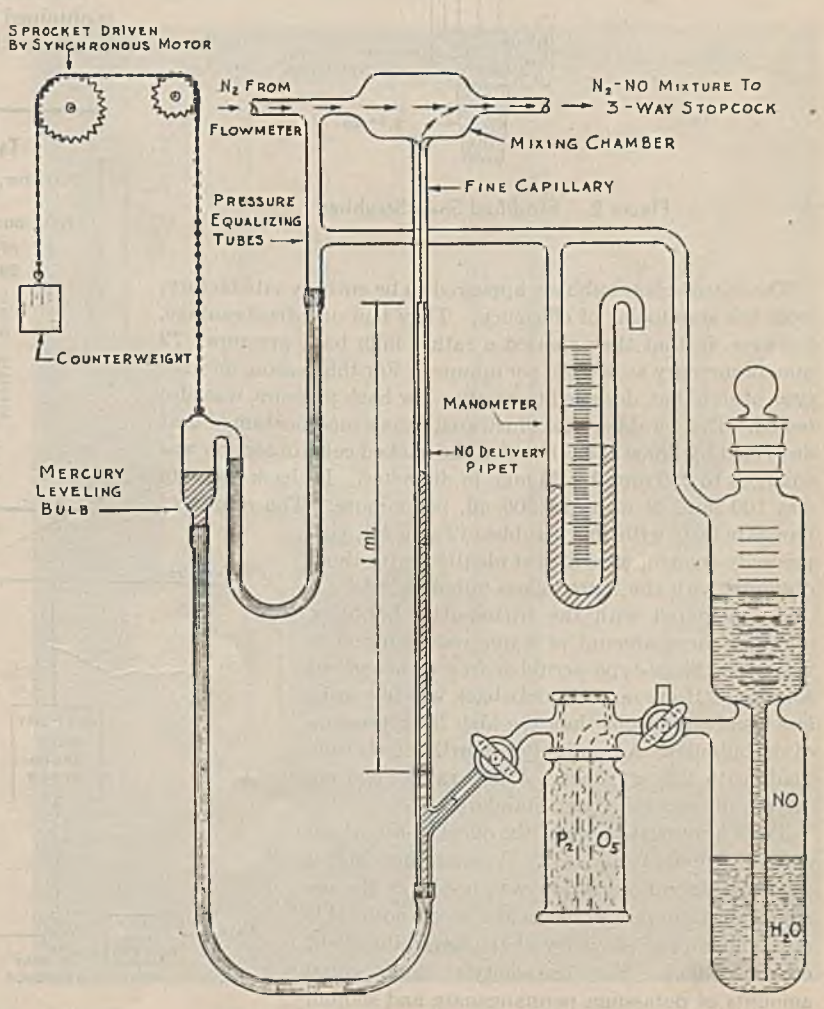

Figure 1. Nitric Oxide Feed Apparatus

rate, using a column of three fritted glass bubblers. The results are given in Table I.

Flow rates lower than 300 ml. per minute were not investigated, since the longer runs that would be required did not seem warranted by the 4% error to be corrected.

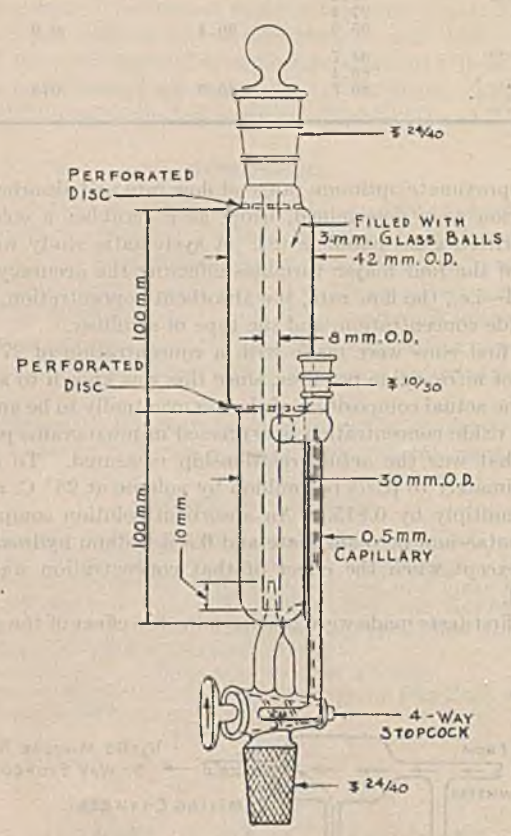

Figure 2. Modified Shaw Scrubber

The fritted-glass bubblers appeared to be entirely satisfactory, from the standpoint of efficiency. They had one disadvantage, however, in that they created a rather high back pressure: 72 mm. of mercury at 300 ml. per minute. For this reason, another type of scrubber, designed to create a low back pressure, was also tested. This scrubber (not illustrated) was a modification of that described by Shaw (*12*), in which the packed column section was enlarged to 200 mm. by 30 mm. in diameter. Its back pressure was 100 mm. of water at 300 ml. per minute. The results of flow rate tests with this scrubber (Table II), surprisingly enough, were almost identical with those obtained with the fritted-glass bubblers.

As compared with the fritted-glass bubblers, a rather large amount of water was required to wash the Shaw-type scrubber free of absorbent solution. However, this drawback was felt to be less disadvantageous than the high back pressure of the bubblers. Accordingly, all further tests were made with this scrubber. A flow rate of 300 ml. per minute was taken as a standard.

Tests were next made of the effect of absorbent concentration (Table III). A somewhat higher nitric oxide concentration was used, on the assumption that this would make more noticeable any falling off of efficiency at the lower absorbent concentrations. For simplicity's sake, equal amounts of potassium permanganate and sodium hydroxide were always used, the percentage indicated in the table referring to the concentration of each component.

The results of these tests demonstrate that maximum recovery is obtained as long as the concentration of the absorption reagents is greater than 0.2% potassium permanganate and 0.2% sodium

Table II. Effect of Gas Flow Rate

(Scrubber, modified Shaw scrubber. Absorbent, 0.5% $KMnO_4$–0.5% NaOH. NO concentration, 27 micrograms per liter)

Flow Rate	Recovery	Average	Average Deviation
Ml./min.	%	%	%
300	97.3		
	95.3		
	94.3		
	95.7	95.7	0.9
600	86.7		
	85.9		
	87.7	86.8	0.6

Table III. Effect of Absorbent Concentration

(Scrubber, modified Shaw scrubber. NO concentration, 55 micrograms per liter. Flow rate, 300 ml. per minute)

$KMnO_4$–NaOH Concentration	Recovery	Average	Average Deviation
%	%	%	%
0.5	97.0		
	96.5		
	97.1	96.9	0.2
0.2	97.3		
	95.3		
	96.0	96.2	0.7
0.1	92.4		
	90.0		
	90.6	91.0	0.9

Table IV. Effect of Nitric Oxide Concentration

(Scrubber, modified Shaw scrubber. Absorbent, 0.5% $KMnO_4$–0.5% NaOH. Flow rate, 300 ml. per minute)

NO Concentration	Recovery	Average	Average Deviation
μg./l.	%	%	%
291	96.7	96.7	
58	97.0		
52	96.5		
54	97.1	96.9	0.2
26	97.3		
28	95.3		
27	94.3		
27	95.7	95.7	0.9
5.5	93.2		
5.7	93.1		
5.7	92.6	93.0	0.2

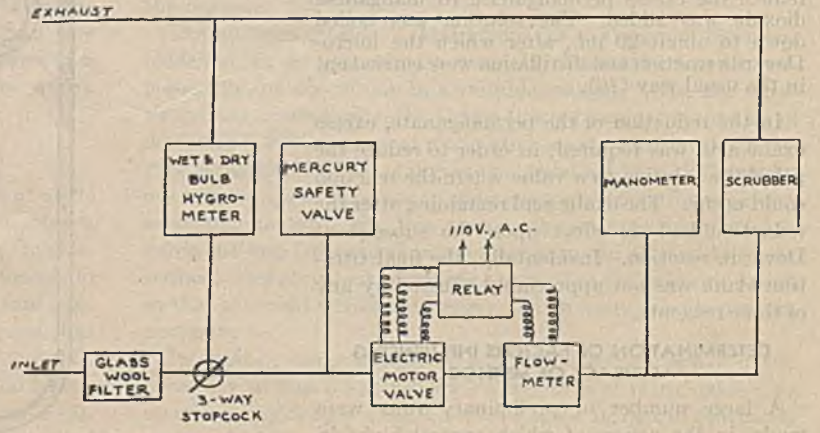

Figure 3. Flow Sheet for Gas-Absorption Apparatus

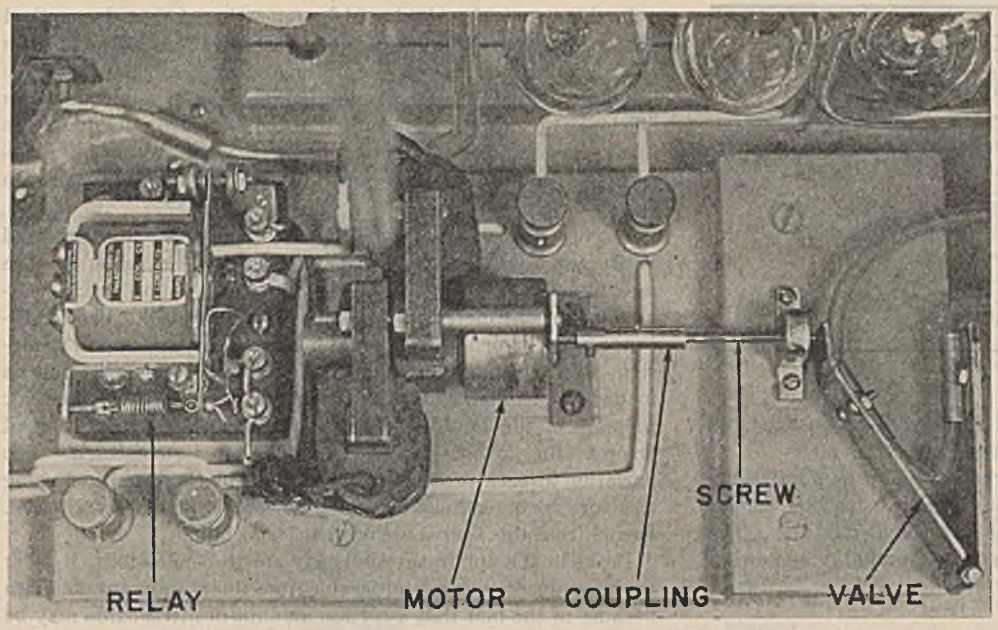

Figure 4. Motor-Driven Valve

hydroxide. To be on the safe side, a 0.5% potassium permanganate–0.5% sodium hydroxide solution was taken as the standard absorbent.

The optimum conditions of operation having been found, it remained only to determine the range of nitric oxide concentration over which the method was effective. A series of tests was accordingly made, in which the nitric oxide content of the gas was varied. The results of these tests are shown in Table IV.

These tests showed a definite falling off of scrubber efficiency at the lower nitric oxide concentrations. For this reason, as well as to make a more compact and convenient unit, the scrubber was redesigned. The final design is shown in Figure 2. The packed section of this scrubber has the same total volume as that of the first model, but is shorter and wider, thus producing a lower gas velocity for a given flow rate. The back pressure at 300 ml. per minute is 70 mm. of water.

Several other modifications were included in the new design, to simplify operation. Perforated glass disks sealed above and below the packed section keep the glass balls in place, and also reduce the probability of breakage of the inner tube. A capillary by-pass is incorporated directly into the unit, while a quarter turn of the special 4-way stopcock at the bottom directs the gas stream through either the by-pass or the scrubber. After a run, a quarter turn of the same stopcock opens the drain at the bottom of the unit. The standard-taper joint at the bottom serves to connect the scrubber to the gas line, or, while draining, to the reaction flask. In addition, it provides a convenient means of support for the apparatus, eliminating the need for a clamp.

The effect of nitric oxide concentration on the efficiency of the new scrubber was tested, as shown in Table V. For practical purposes, the efficiency of the redesigned scrubber was 100%. Since any loss of efficiency would appear at the lower nitric oxide concentrations, tests at high concentrations were not considered necessary.

The low precision encountered in the tests at 2.6 micrograms of nitric oxide per liter requires some explanation. It was obviously desirable to test the scrubber at as low a nitric oxide concentration as possible, in order to detect any falling off of efficiency. However, in order to keep the duration of the test run within reasonable limits, it was necessary to use a smaller total volume of nitric oxide than usual. This was accomplished by raising the mercury in the nitric oxide pipet three-fourths of the pipet's length before starting the run. Because of the shorter distance then to be traveled by the mercury, errors of measurement were magnified. Furthermore, in view of the low velocity of the nitric oxide, diffusion of the gas contained in the capillary connecting the pipet to the mixing chamber became a considerable factor. These two considerations easily account for the apparently poor showing of the scrubber. Inasmuch as the gas to be analyzed would seldom, if ever, contain such low nitric oxide

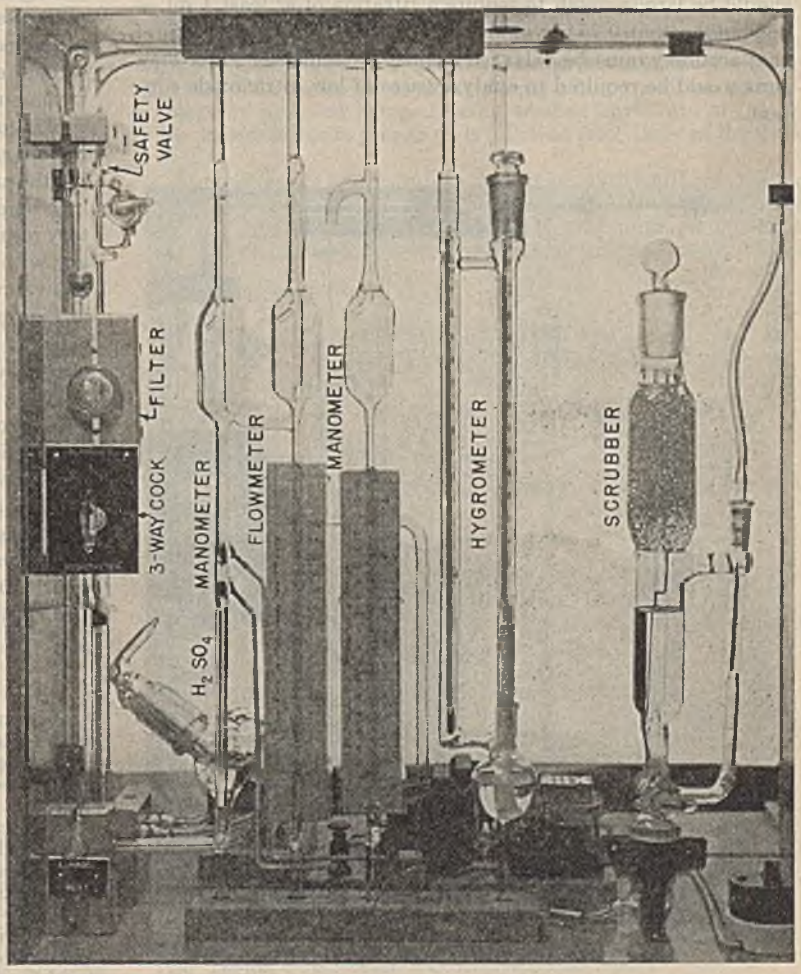

Figure 5. Gas-Sampling Apparatus, Front

Table V. Effect of Nitric Oxide Concentration

(Scrubber, modified Shaw scrubber No. 2. Absorbent, 0.5% $KMnO_4$–0.5% NaOH. Flow rate, 300 ml. per minute)

NO Concentration μg./l.	Recovery %	Average %	Average Deviation %
27	100.0		
26	98.9		
27	99.1	99.3	0.4
5.9	99.5		
5.6	100.0		
5.7	99.5	99.7	0.2
2.6	107		
2.6	98		
2.6	95	100	5

concentrations, it was not considered worth while to construct and calibrate a special pipet for this one test.

The average deviation figures given in the various tables actually represent the over-all precision of the test method, including the apparatus used to prepare the nitric oxide mixtures. The true precision of the analytical method is probably somewhat better than this.

SOURCES OF ERROR AND APPLICABILITY OF THE METHOD

Sources of error in the micro-Devarda procedure used in conjunction with this method have already been discussed (*10*). Obviously, all water and reagents used should be free of nitrates and ammonia. As mentioned above, the reduced absorption reagents have no effect upon the analysis. The only factor requiring special consideration in this application is the size of the sample. For the highest precision and accuracy, the sample should be large enough to require a titration of at least 1 ml.—i.e., not less than 0.25 mg. of nitric oxide. It is obvious, therefore, that accuracy must be balanced against convenience, where long runs would be required to analyze gases of low nitric oxide content.

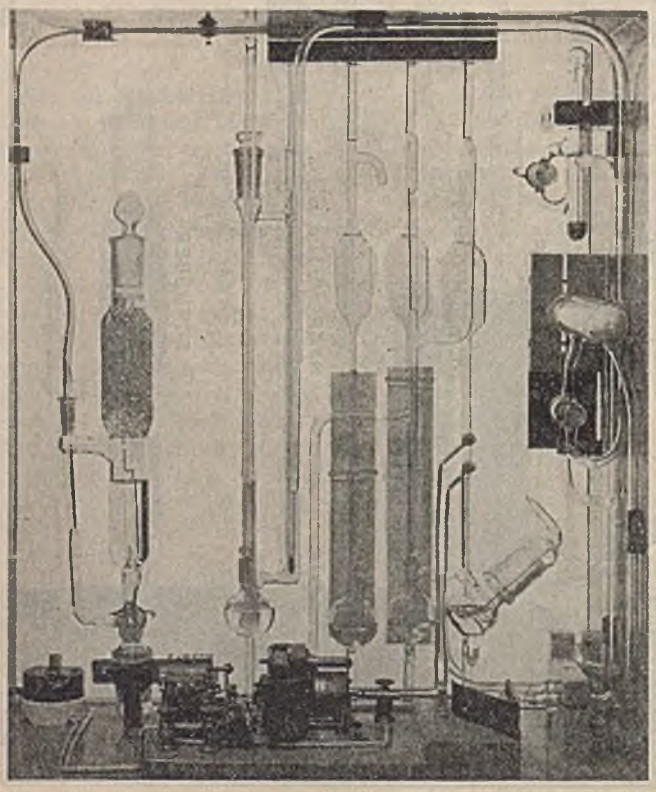

Figure 6. Gas-Sampling Apparatus, Rear

In this connection, it should be remembered that the method has not been checked against nitric oxide concentrations of less than 2.6 micrograms per liter. Judging by the behavior of the original modified Shaw scrubber, it appears possible that the efficiency of the improved scrubber might start to fall off at some lower concentration. This possibility should be checked, if accurate determinations of very low nitric oxide concentrations are required.

Because of its convenience and compactness, a differential flowmeter of the capillary type is used by the writer for the measurement of the gas. (Where only a low gas pressure is available, a wet gas meter may be used to advantage.) The significance of an error in reading the flowmeter depends upon its design. If sufficient gas pressure is available, the differential may be made large enough to make reading errors negligible. The back pressure of the scrubber (70 mm. of water) introduces a small error in the flowmeter reading, which can be corrected. The variation in back pressure is too small to be significant. Timing the run is, of course, necessary, where a flowmeter is used. Errors from this source are ordinarily negligible.

Leaks in the apparatus obviously are to be avoided. Rubber tubing connections are undesirable from this standpoint, and also because of the fact that a certain amount of nitric oxide may be absorbed by the rubber. So far as possible, the apparatus should be all glass, with fused connections. Flexible connections are best made with plastic tubing.

Several of the first trials of the final scrubber design were spoiled by uneven wetting of the packing. This difficulty may be avoided by turning the charged scrubber over, and wetting all the balls with the absorbent solution. If the balls are uniformly wetted at the start, no appreciable channeling will occur during the run.

Excessive reduction of the permanganate absorbent by unsaturated compounds will, as indicated in Table III, give rise to a serious error. The effective potassium permanganate concentration must never fall below 0.2%. To this end, the initial concentration of the absorbent may be raised up to 5% potassium permanganate–5% sodium hydroxide, without otherwise affecting the analysis. If this means is insufficient to handle the reducing compounds, a preliminary scrubbing is necessary. Satisfactory results were obtained by the writer by scrubbing illuminating gas with sulfuric acid saturated with silver sulfate (*16*). The Shaw scrubber used for this purpose was similar to that illustrated in Figure 2, except that the liquid reservoir (lower section) had a capacity of 150 ml. About 18 liters of gas can be handled by 100 ml. of sulfuric acid–silver sulfate before olefins start to come through.

Nitrogen peroxide is likely to be found in any gas containing nitric oxide. Obviously, all the higher oxides will interfere. Unless known to be absent, they should be removed by a preliminary scrubbing of the gas with 0.5% sodium hydroxide. Incidentally, the higher oxides, as a group, may easily be determined by the present method, merely by substituting 0.5% sodium hydroxide for the 0.5% potassium permanganate–0.5% sodium hydroxide absorbent solution.

ROUTINE TEST APPARATUS

A semiautomatic, portable apparatus was constructed, for convenience in sampling gases at various locations. Its flow sheet is given in Figure 3. Gas entering the apparatus is freed of suspended matter by a glass wool filter, and then led by a 3-way stopcock through either a wet and dry bulb hygrometer or the flowmeter-scrubber system. (The hygrometer is used for determining the dew point of the gas. This measurement is required on the gas being analyzed by the writer, but is not related to the nitric oxide determination.)

A flow rate through the scrubber of 300 ± 2 ml. per minute is automatically maintained by means of a motor-driven valve controlled by a differential capillary flowmeter. Dilute sulfuric acid in a manometer in parallel with the water-filled flowmeter manometer makes or breaks contact with fixed platinum con-

tacts. A small magnetic relay is thus operated, which, in turn, operates a reversible Telechron motor to close or open the valve. A bulb on an eccentric dipping into the well of the sulfuric acid manometer facilitates minor adjustments of the zero point.

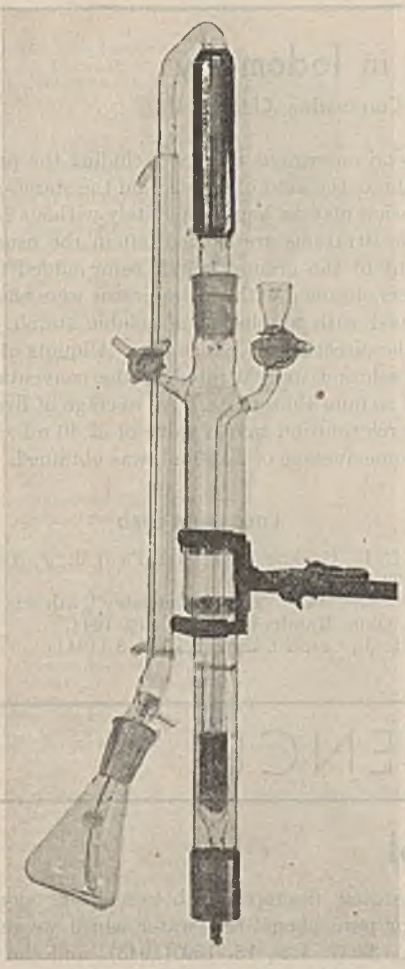

Figure 7. Micro-Devarda Apparatus

The motor-driven valve is shown in Figure 4. Its somewhat unique design is the result of experiments with various types of needle and pinch valves, none of which offered the sensitivity of control required for the purpose. In operation, a kink in a section of soft plastic tubing (3 mm. in inside diameter) is opened and closed by a screw, working against the hinge to which the tubing is clamped. A key and slot coupling connects the screw to the 1 r.p.m. synchronous motor.

The control mechanism operates best with an inlet gas pressure of between 2 and 10 cm. of mercury. Higher pressures are undesirable, in that they cause a wide fluctuation of the flow rate with the oscillation of the automatic valve. Variations in the gas pressure between the above limits have negligible effect, if they are not too rapid for the slow-moving valve to handle. The use of a pressure regulator on the gas line may be desirable, from this standpoint.

A mercury manometer, indicating the gas pressure on the apparatus, is provided with a trap, so that it serves as a safety valve as well. In addition, traps are provided on all the meters, to prevent loss of liquid in case of a sudden rise in pressure, or failure of the control.

A manometer is connected to the line between the flowmeter and the scrubber, indicating the back pressure on the flowmeter. It is calibrated in terms of the correction necessary to be applied to the flowmeter reading. Since the flowmeter was calibrated against a back pressure equal to that created by the scrubber, this correction ordinarily is zero.

Both the flowmeter and manometer are provided with capillary tubes to damp the minor oscillations caused by the bubbling of the gas through the scrubber. Reading of the meters is thus greatly facilitated.

The assembled sampling apparatus is shown in Figure 5 (front view) and Figure 6 (rear view). It is relatively compact and easily portable. The cabinet is provided with removable sliding doors, front and back.

The micro-Devarda flask, used in the analysis of the dissolved sample, has been somewhat simplified (Figure 7). The original flask (*10*) was entirely vacuum-jacketed, to prevent excessive condensation, when distilling with an outside source of steam. This feature is not required for the present application, resulting in a more easily constructed and compact apparatus. The electrical heating coil alone is vacuum-jacketed, chiefly for protection. Electrical connections are made through a standard 2-prong plug, cemented to the flask, to make a base similar to that of a radio tube. The leads are thus protected, and connection to an extension cord is simplified. A stopcock is provided on the left side of the flask, where suction may be applied to facilitate drainage of the scrubber into the flask. As the photograph shows, the flask is used in an upright position, making for a more compact distillation set up.

Operation of the apparatus is very simple. The gas inlet and exhaust lines are connected to the respective ports (lower left corner, Figure 5). The 3-way stopcock (left side, Figure 5) is turned to "Hygrometer", and the gas allowed to flow at a maximum rate until the wet-bulb reading becomes constant. This serves the double purpose of determining the humidity of the gas and flushing out the lines. Meanwhile, the scrubber is charged, and connected in place, its 4-way stopcock being set to the by-pass position.

The 3-way stopcock is then turned to "Scrubber", and the electricity is turned on. When the flowmeter reading settles down at 300 ml. per minute, the scrubber's 4-way stopcock is turned to pass the gas through the scrubber, and the time is noted. The back-pressure manometer reading is checked for any abnormality, such as might be caused by a leak or stoppage in the lines. (It normally shows only slight variation between or during runs.) No further attention is required until the end of the run. The 4-way stopcock is then returned to the by-pass position, the time is noted, and the gas and electricity are turned off.

The scrubber is placed in the standard-taper neck of the micro-Devarda flask, the stopcock turned to the drain position, and the absorbent washed into the flask with water. Ten milliliters of 6% oxalic acid are added, and the mixture is boiled down to about 20 ml. The use of a spray trap during the evaporation is desirable, any material trapped being washed back into the flask. The micro-Devarda procedure is followed from there in the usual way.

It is advisable to wash the scrubber promptly after its use, to prevent etching of the glass balls and freezing of the stopcock by any residual alkali. Hydrochloric acid followed by water serves this purpose, dissolving any deposited manganese dioxide as well.

CALCULATIONS.

$$\frac{(\text{Ml. of HCl} - \text{blank})\,(\text{NF})\,(30{,}000{,}000)}{(\text{Duration of run in minutes})(\text{flowmeter reading} + \text{correction, in ml. per min.})} = \text{micrograms of NO per liter}$$

$$(\text{Micrograms of NO per liter})(0.815) = \text{p.p.m. of NO by volume at 25° C. and 760 mm.}$$

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NOTE ON ANALYTICAL PROCEDURES

Ground Starch as an Indicator in Iodometry

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THE useful life of the solution of soluble starch used as a conventional indicator in iodometry may be prolonged with any one of a number of disinfectants. However, hydrolysis of the starch eventually renders the reagent unsuitable for use, and therefore it has been found advantageous in many cases to replace the solution with a dry preparation of ground starch.

The starch is prepared by a procedure similar to that recommended by Alsberg, Griffing, and Field (*1*) and by Schoch (*3*). A convenient quantity is suspended in about twice its weight of ethyl alcohol and ground in an efficient ball mill for at least 80 hours, at which time a microscopic examination will reveal few, if any, granules that have escaped disintegration. The ground starch is filtered off, dried, and then reground. The resulting preparation, which is almost completely soluble in cold water, is ready for use. An inexpensive salt or pepper shaker has been found to be a convenient container and dispenser for the starch. If desired, a solution of starch may be prepared without the use of heat by simply adding the ground starch to cold water.

Presumably any available starch may be used, but soluble starch is particularly satisfactory. The time required for grinding will depend on various factors, including the particular mill used, the charge, the kind of starch, and the starch-alcohol ratio. The preparation may be kept indefinitely without charge.

Iodometric titrations are carried out in the usual manner, a small amount of the ground starch being added near the end point. Titers obtained with this indicator were checked against those obtained with a solution of soluble starch prepared according to the directions of Lange (*2*). Aliquots of a potassium dichromate solution were titrated in the conventional manner with 0.01 *N* sodium thiosulfate. An average of five titers using a soluble starch solution gave a value of 32.40 ml.; with ground starch the same average of 32.40 ml. was obtained.

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CORRESPONDENCE

o-Cresol in Phenol

SIR: We have subjected the "cloud-point" method of Seaman, Norton, and Foley [IND. ENG. CHEM., ANAL. ED., 15, 159 (1943)] to brief examination and, while we found it an eminently practicable and useful procedure within the limits laid down, we also found that exact confirmation of results was not obtained. The root cause of the discrepancy was discovered in the figure observed for the "cloud-point temperature" of pure phenol and water when mixed in the prescribed proportion; this is quoted as 66.40° in the article, whereas we obtained a figure of 66.10° C.

The phenol used in our short series of experiments had a cloud point (Bell and Herty, Standardization of Tar Products Tests Committee procedure) of 41.0° C. and the *o*-cresol had a cloud point of 31.0° C. The results of our tests are given here.

Observed Cloud-Point Temperature	*o*-Cresol in Mixture with Phenol	*o*-Cresol Calculated by Equations Given Below
° *C.*	%	%
66.10	0.00	0.00
67.95	1.45	1.45
70.40	3.39	3.39
73.00	5.52	5.52

It was found necessary, in order to bring observed and calculated *o*-cresol contents into line, to modify the equations given in the article as follows:

For cloud-point temperatures up to 70.25° C.:

$$\% \text{ } o\text{-cresol} = \frac{\text{cloud point (° C.)} - 66.10}{1.273} \qquad (1)$$

For cloud-point temperatures 70.25° to 73.5° C.:

$$\% \text{ } o\text{-cresol} = \frac{\text{cloud point (° C.)} - 66.25}{1.222} \qquad (2)$$

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GREAT BRITAIN

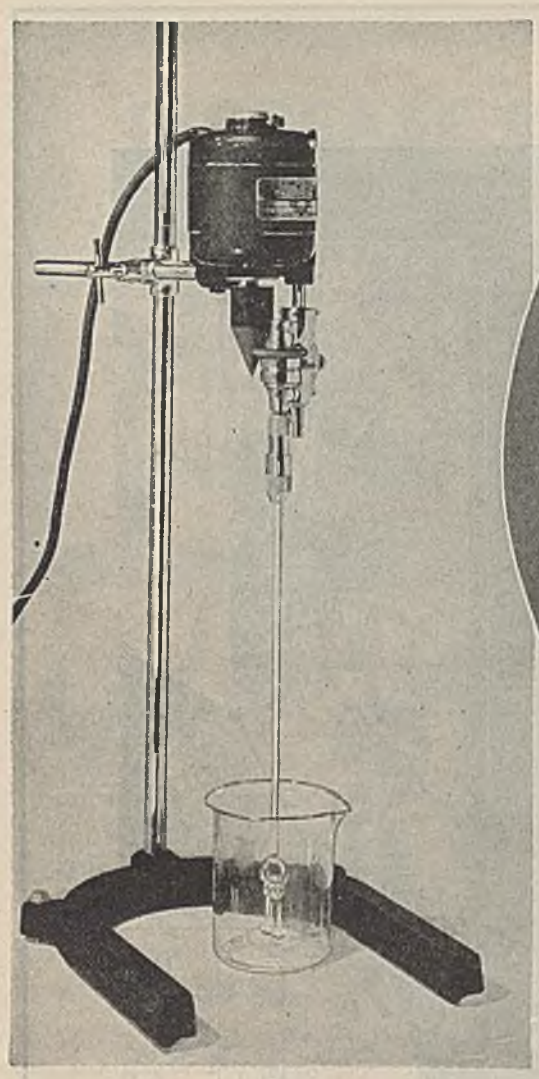
SIR: Regarding discrepancy between the cloud point of 66.40° C. for pure phenol and water which we reported [IND. ENG. CHEM., ANAL. ED., 15, 159 (1943)] and the figure which Kay and Haywood have obtained, 66.10° C.:

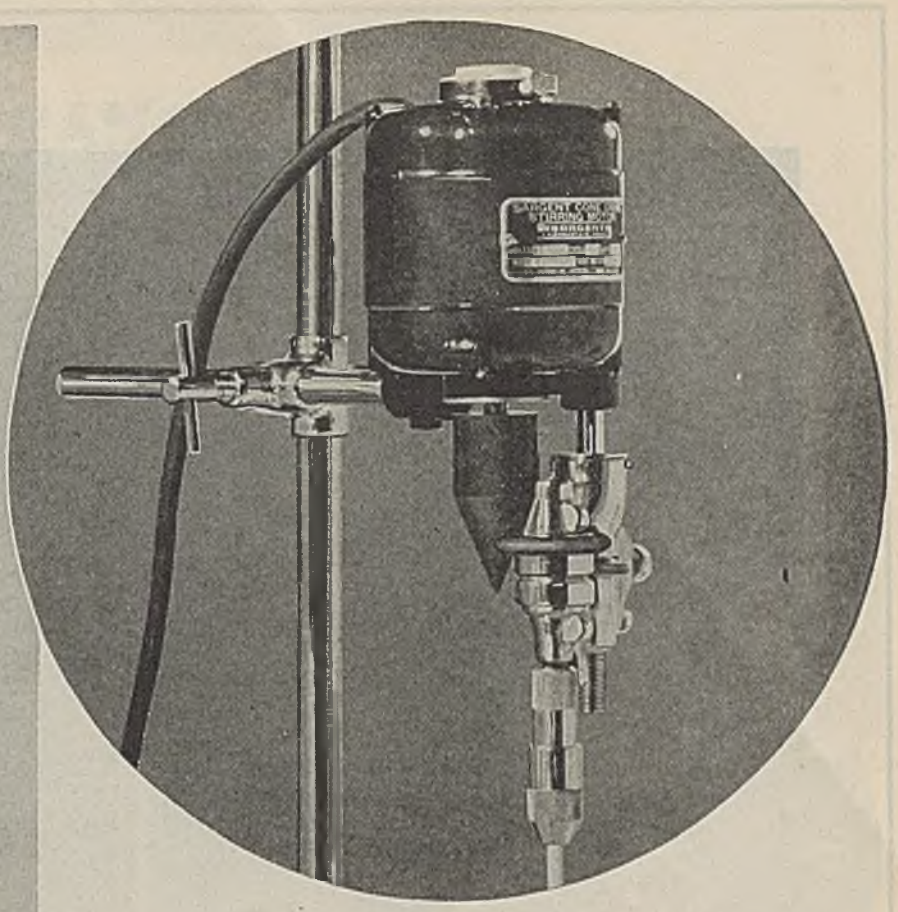
We have repeated some of the experiments and think that we have found the explanation for the discrepancy. Instead of one abrupt change in appearance, there are really two. We took the first one (at 66.40° C.) and evidently they must have taken the second one (at 66.10° C.). It is impossible to describe these points in words, but after one has observed both, one can always detect either point with good precision. Three of us have checked each other quite well at both points. Furthermore, there is a similar difference when determinations are made with phenol containing added *o*-cresol, although we have not done sufficient work to know whether the difference will remain constant or not over a range of concentrations. It may be that a variation in the magnitude of the differences with a variation in *o*-cresol concentration may explain the fact that the slopes of the lines calculated from the four determinations which were sent us differ from those which we reported (1.273 and 1.222 instead of 1.326 and 1.167, respectively).

It would seem likely that either point can be used, provided that the analyst constructs his curve on the basis of that point; but since we have been able consistently to get reproducible values with the point which we had chosen, it might be desirable to have the analyst look for that point, in which case he could use our equations, rather than to use the other point and have to repeat the large number of determinations which would be necessary to establish accurate equations.

WM. SEAMAN

CALCO CHEMICAL DIVISION
AMERICAN CYANAMID COMPANY
BOUND BROOK, N. J.





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