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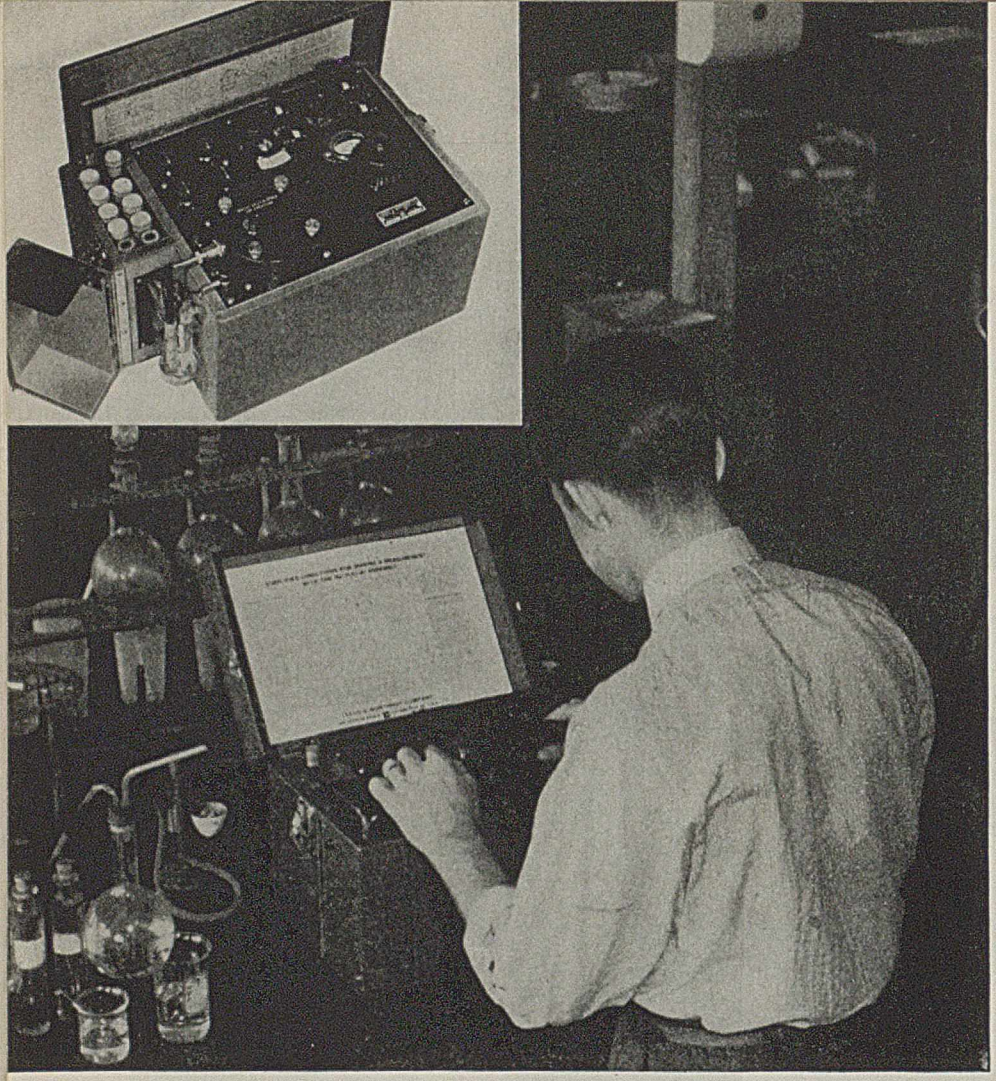

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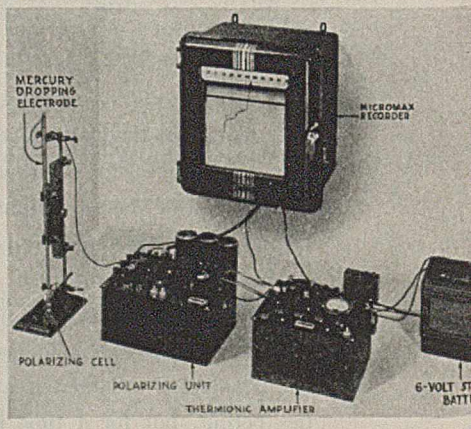

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Determination and Separation of Potassium as Periodate

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THE determination of potassium as periodate, KIO_4, offers a number of advantages. It has a high molecular weight, and can be determined volumetrically by a very exact titration. It is only sparingly soluble, a saturated solution at 25° C. being 0.022 molar (*2*). It contains about the same percentage of potassium as the chloroplatinate and cobaltinitrite and much less than the perchlorate.

The first experiments along this line were made by Greathouse (*1*), who added periodic acid to the solution, 2 or 3 ml. in volume, and completed the precipitation by adding alcohol, free from aldehyde. He carried out a few gravimetric and volumetric determinations, the results being usually slightly low, but did very little on the separation of potassium from other metals. At that time the price of iodine was high, and the preparation of periodic acid was an expensive process. Since that time two satisfactory methods for preparing the acid have been published and the reagent has become less expensive. As the reagent is now available, it seemed desirable to make an extensive investigation of this method of determining and separating potassium, and in particular, to find a better solvent than ethyl alcohol which is so easily oxidized that it is almost impossible to avoid some reduction of periodic acid to iodic acid.

The reaction used in titrating periodate to iodate was first suggested by Müller and Friedberger (*3*):

$$IO_4^- + 2I^- = IO_3^- + I_2$$

and is quantitative only in a neutral solution buffered by carbonic acid–bicarbonate or boric acid–borate, preferably the latter. The free iodine is then titrated by standard arsenite. This method has the advantage that iodate does not interfere, as it would if the periodate were reduced to iodide. In the latter process, however, the equivalent would be much smaller—one eighth of the molecular weight.

The periodate method for potassium is both rapid and accurate, and can be applied to amounts of potassium as low as 0.4 mg.

Experimental

SELECTION OF A SOLVENT. As indicated above, the selection of the proper solvent is a matter of prime importance. It must possess the following properties: (1) It must not be appreciably oxidized by periodic acid during the time required for the analysis, 0.5 to 2 hours. (2) It should be miscible with water at least to the extent of about 10 per cent of the latter by volume. (3) Potassium periodate must be insoluble in the solvent and yet it must dissolve sodium periodate and such other salts as might be present.

As explained above, Greathouse (*1*) used ethyl alcohol, free from aldehyde, since the aldehyde is more easily oxidized than the alcohol. This, however, was not an ideal solvent because some oxidation of the alcohol invariably occurred. Among the solvents which are less affected by oxidizing agents is tertiary butyl alcohol; this was therefore first investigated. It was found, however, to dissolve so little sodium periodate as to be useless for the purpose. Admixture of ethyl acetate did not improve matters. Other solvents tried in which sodium periodate was insoluble were dioxane, the carbitols, Cellosolve, and diethylene glycol.

It was found that if ethyl acetate was added to ethyl alcohol, the resistance of the latter to oxidation was considerably increased, whereas its solvent power was not diminished.

A quantity of 95 per cent ethyl alcohol, free from aldehyde, was prepared by refluxing the alcohol for 2 or 3 hours after the addition of 0.5 gram of sodium hydroxide and 2.5 grams of silver nitrate per liter. The alcohol was then distilled and mixed with an equal volume of anhydrous ethyl acetate. The mixture was miscible with the quantities of water which were required and showed considerable resistance to oxidation by periodic acid. One gram of periodic acid dissolved in 100 ml. of the solvent showed the first traces of free iodine after standing at room temperature for 24 hours. Since in most cases the time required for the separation of potassium was from 15 minutes to 1 hour, it is apparent that only very slight oxidation of the organic solvent could occur. The magnitude of this effect is shown by the fact that the average of a long series of gravimetric determinations (only part of which are recorded in this paper) showed an error of +0.02 mg., and the volumetric determinations −0.05 mg. This would indicate that the precipitates contained a trace of iodate owing to very slight reduction by the solvent, because the percentage of potassium in the iodate is only slightly less than in the periodate, whereas the iodate is not determined at all in the volumetric process. These results are much better than those obtained by Greathouse (*1*). It was also found possible to reduce considerably the concentration of periodic acid.

PREPARATION OF REAGENTS. The periodic acid used was recrystallized from concentrated nitric acid until free from iodic acid. Some of it was prepared by the electrolytic process (*5*) and although originally free from iodic acid, some batches were found to contain a good deal of the latter

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after standing for a month or two. This is probably due to the catalytic effect of traces of colloidal platinum derived from the electrodes. A solution of periodic acid to which considerable colloidal platinum is added evolves oxygen rapidly. The acid prepared by the chemical process (*4*) is always entirely stable. Small amounts of iodate are easily detected as follows:

One-half gram of the periodic acid is dissolved in 25 ml. of water, a few drops of 0.25 *M* silver nitrate are added, and the solution is warmed until the yellowish-brown silver periodate dissolves, which should occur readily at about 80° C. If iodic acid is present, a white flocculent precipitate of silver iodate will form. If the brown silver periodate is not completely dissolved by heating, a drop or two of nitric acid, free from nitrous acid, should be added.

The potassium and sodium periodates used were purified by recrystallization and tested for iodate by the method of Willard and Thompson (*6*). This is similar to the method suggested above, except that most of the periodate in the sample to be tested is first removed by the addition of an excess of potassium nitrate, after which the solution is acidified with nitric acid and tested for iodate by the addition of silver nitrate. In this way it is possible to detect 0.03 per cent of iodate in periodate.

The potassium nitrate used in this work had been carefully purified and dried at 105° to 110° C. for several hours. An attempt was made to fuse the salt at about 300° C., but the resulting material contained considerable nitrite.

Table I. Determination of Potassium as Periodate in Presence and Absence of Sodium

Wt. of K as Nitrate	Na Added as Nitrate	Wt. of KIO_4	Error in K Gravimetric	Error in K Volumetric
Gram	*Mg.*	*Gram*	*Mg.*	*Mg.*
0.0794	...	0.4676	+0.1	...
0.0490	...	0.2887	+0.1	0.0
0.0405	...	0.2391	+0.1	+0.1
0.0382[a]	...	0.2241	−0.1	−0.1
0.0307	...	0.1809	0.0	−0.1
0.0224	...	0.1313	−0.1	...
0.0030	...	0.0171	−0.1	−0.1
0.0600	68	0.3524	−0.1	0.0
0.0428	73	0.2518	0.0	0.0
0.0464	135	0.2746	+0.3	+0.3
0.0420	135	0.2517	+0.8	+0.6
0.0481[a]	140	0.2842	+0.2	+0.2
0.0407[a]	143	0.2406	+0.2	+0.1
0.0440[a]	190	0.2598	+0.2	−0.1
0.0335[a]	190	0.1980	+0.1	0.0
0.0017	54	0.0098	0.0	...
0.0013	54	0.0077	0.0	−0.2
0.0007	54	0.0041	0.0	0.0
		5 ml. of water used		
0.0008	...	0.0045	0.0	+0.2
0.0008	...	0.0043	−0.1	0.0
0.0008	27	0.0045	0.0	0.0
0.0008	27	0.0052	+0.1	+0.1
0.0004	27	0.0025	0.0	−0.1
0.0004	27	0.0020	−0.1	−0.1

[a] 180 ml. of alcohol-acetate mixture used.

General Procedure

A sample weighing 0.1 to 0.2 gram is dissolved in water in a 150-ml. beaker. If potassium chloride is used it must be evaporated to dryness with 10 ml. of concentrated nitric acid; otherwise the chloride will cause reduction of periodic acid. To the nitrate dissolved in 4 to 5 ml. of water 3 ml. of water containing 1 gram of periodic acid are added, the mixture is stirred, and 3 or 4 minutes are allowed for the potassium periodate to precipitate. Ninety milliliters of the alcohol–ethyl acetate mixture are added and the solution is allowed to stand in an ice bath for 0.5 hour with continuous mechanical stirring. It is essential that the precipitation be carried out as indicated. If periodic acid is added to the alcohol–ethyl acetate solution, the precipitate will be gelatinous and difficult to filter, whereas if it is first formed in aqueous solution it is crystalline. The solution is filtered through a Gooch crucible and the precipitate washed with anhydrous ethyl acetate which has been cooled to 0°. It is dried in the oven for 10 minutes at 105° C., cooled, and weighed.

If volumetric results are desired, the crucible with the precipitate is placed in a 250-ml. beaker, to which are added 125 ml. of a solution containing 5 grams of boric acid and 5 grams of sodium tetraborate. The potassium periodate dissolves readily in this solution, which has a pH of about 7.5. It is unnecessary to take the crucible out of the solution.

Table II. Determination of Potassium as Periodate in Presence of Free Acid

Wt. of K as Nitrate	Acid Added	Wt. of KIO_4	Error in K Gravimetric	Error in K Volumetric
Gram		*Gram*	*Mg.*	*Mg.*
0.0524	0.5 ml. concd. HNO_3	0.3092	+0.2	0.0
0.0289	0.5 ml. concd. HNO_3	0.1695	−0.1	+0.1
0.0608	5 drops H_3PO_4, 85%	0.3575	0.0	−0.2
0.0561	5 drops H_3PO_4, 85%	0.3302	0.0	−0.1
0.0572	1 ml. concd. H_3PO_4	0.3377	+0.2	0.0
0.0603	1 ml. concd. H_3PO_4	0.3570	+0.4	+0.1
0.0461	5 drops concd. H_2SO_4	0.2806	+0.5	0.0
0.0580	5 drops concd. H_2SO_4	0.3408	−0.1	+0.1
0.0451	200 mg. H_3BO_3	0.2658	+0.1	0.0
0.0444	200 mg. H_3BO_3	0.2630	+0.3	+0.3
0.0447	5 drops $HClO_4$, 70%[a]	0.2623	−0.1	−0.4
0.0541	5 drops $HClO_4$, 70%[a]	0.3179	−0.1	−0.5

[a] Stirred 45 minutes.

When the potassium periodate has dissolved, 3 grams of potassium iodide are added and the iodine is titrated with 0.1 *N* arsenite solution, prepared by dissolving 4.945 grams of arsenious oxide in a solution of 10 grams of sodium bicarbonate, warmed to about 80° C. The solution is then saturated with carbon dioxide and diluted to 1 liter. Although the solution was made up from arsenious oxide of known purity, it was standardized by titration against pure potassium and sodium periodates and the values obtained in this way were taken as correct. In some cases the normality found in this way deviated from the theoretical value by as much as 0.0012.

Periodic acid was always weighed out in the solid form and dissolved just before use to avoid any danger of decomposition on standing. Gravimetric results were satisfactory only when a nitrate was used, but were high with sulfates, though volumetrically the sulfates did not interfere. The latter procedure is usually preferable because it is not affected by the presence of iodate or of inert materials which may be insoluble in the organic solvent.

When the amount of potassium present is very small, it is desirable to increase the amount of periodic acid to 1.5 or 2 grams. If not much sodium or other metal is present, the initial volume of water should be decreased to 5 ml., although this is not absolutely necessary. The time for precipitation should be increased to 1 or 1.5 hours. If less than 0.4 mg. of potassium is present the results are unreliable, even though the volume of water is reduced and the amount of periodic acid increased.

Results of Analyses

In all the analyses described below, unless otherwise stated, the same conditions were used—namely, 90 ml. of solvent, 1 gram of periodic acid, 7 to 8 ml. of water, and 30 minutes stirring at 0° C. The precipitate obtained was, in all cases, crystalline, and showed no tendency to adhere to the beaker. The results of a series of determinations are shown in Table I.

It is obvious that potassium can be completely separated from 70 mg. of sodium, but that there is a slight error when 140 mg. are present. It was subsequently found that the limit could be raised to 100 mg. By doubling the volume of alcohol-acetate mixture potassium was readily separated from 190 mg. of sodium.

Effect of Temperature, Sulfate, and Free Acid

In previous experiments the temperature was 0° C. A series of experiments was run in which the solutions were maintained at room temperature for 15 and 60 minutes, respectively. In both cases the results were 0.2 to 0.6 mg. too low.

Weights of potassium nitrate varying from 0.05 to 0.15 gram were taken and 40 mg. of sodium added as sulfate. In a series of 12 volumetric determinations the error varied from

−0.2 to +0.2 mg., with an average of practically zero. In this case the gravimetric results were always too high because of contamination with sodium sulfate.

In Table II are shown the results obtained when free nitric, phosphoric, sulfuric, perchloric, and boric acids are added. In the case of perchloric acid, some of the potassium is precipitated as perchlorate and apparently is not quite converted into periodate within 45 minutes. The other acids do not interfere.

Table III. Separation of Potassium from Metals as Periodate

Wt. of K as Nitrate	Metal Added	Wt. of KIO_4	Error in K Gravimetric	Error in K Volumetric
Gram	*Mg.*	*Gram*	*Mg.*	*Mg.*
0.0403	16 Ca (as nitrate)	0.2731	0.0	−0.1
0.0530	32 Ca (as nitrate)	0.3119	0.0	−0.1
0.0486	20 Mg (as sulfate)	....	...	0.0
0.0464	21 Mg (as nitrate)	0.2732	+0.1	0.0
0.0608	50 Al (as nitrate)	0.3579	0.0	−0.1
0.0615	100 Al (as nitrate)	0.3654	+1.1	−0.1
0.0452	102 Al (as nitrate)	0.2718	+1.0	0.0
0.0405[a]	98 Al (as nitrate)	0.2468	+0.5	+0.1
0.0424	100 Al (as sulfate)	0.2530	+0.6	0.0
0.0400	100 Al (as sulfate)	0.2383	+0.3	+0.2
0.0484	62 Zn (as nitrate)	0.2876	+0.5	−0.1
0.0406	62 Zn (as nitrate)	....	...	−0.1
0.0498	92 Co (as nitrate)	0.2959	+0.5	−0.2
0.0406	88 Co (as nitrate)	0.2400	+0.2	+0.2
0.0423	77 Ni (as nitrate)	0.2513	+0.4	+0.2
0.0395[a]	79 Ni (as nitrate)	0.2336	+0.2	+0.2
0.0413	5 Fe^{+++} (as nitrate)	0.2773	+5.8	−0.9
0.0358	5 Fe^{+++} + 5 drops H_3PO_4	0.2355	+4.2	−1.3
0.0579	50 Mn (as sulfate)	0.3757	+6.0	...
0.0341	3 NH_4 (as nitrate)	0.2243	+4.0	+4.6
0.0516	60 Li (as carbonate)	0.3037	0.0	...
0.0401	69 Li (as carbonate)	0.2371	+0.2	−0.1

[a] 180 ml. alcohol-acetate mixture used.

Separation from Other Metals

The results in Table III show that volumetric determination of potassium is satisfactory in the presence of moderate amounts of magnesium, calcium, lithium, aluminum, zinc, nickel, and cobalt, but not ammonium, iron, manganese, and chromium. In the latter cases oxidation to permanganate and chromate occurs and the titration is not possible. The separations are better when 180 ml. of alcohol-acetate mixture are used. The results are somewhat better when 8 ml. of water rather than 7 ml. are present.

The gravimetric results are satisfactory with calcium (in the absence of sulfate), magnesium, and lithium.

Although calcium does not interfere when present as nitrate, there is serious interference when it is present as sulfate. Not only was the reaction at the end point slow, indicating that something was dissolving during the process, but the results were always too low. Apparently this is due to the formation of the insoluble double salt, $CaSO_4.K_2SO_4$, so that it was impossible to convert all the potassium to periodate. This should be kept in mind if silicates have been decomposed by evaporation with hydrofluoric and sulfuric acids. Attempts to decompose feldspar by evaporation with hydrofluoric and phosphoric acids were unsuccessful.

The cobalt solution became green upon the addition of periodic acid. Ferric iron is readily precipitated as periodate and therefore interferes even when present in small amounts. It was thought that the addition of phosphoric acid, forming a complex with the iron, would prevent its precipitation. Although some effect was noticeable in the gravimetric result, there was no improvement in the volumetric. In the absence of phosphoric acid the precipitate was brick-red, whereas in its presence it was white.

Rubidium and cesium behave like potassium, although no quantitative experiments were made.

In the presence of chromium and manganese, which are oxidized by periodic acid, the color was so intense that no titration was possible, although if not over 1 mg. of manganese is present the end point can be seen.

The periodate method is applicable to the determination of potassium in the mixed chlorides obtained by the J. Lawrence Smith method, providing they are converted into nitrates by evaporation with nitric acid.

Summary

Potassium can be quantitatively precipitated as periodate by adding periodic acid to a solution only a few milliliters in volume and subsequently completing the precipitation by the addition of a much larger volume of a mixture of equal parts of aldehyde-free ethyl alcohol and anhydrous ethyl acetate.

The solution must be maintained at 0° C. and stirred for 30 minutes.

The precipitate of potassium periodate may be weighed or it may be dissolved in a boric acid–borax buffer, potassium iodide added, and the free iodine titrated with arsenite. In this reaction the periodate is reduced to iodate.

Potassium may be separated from calcium, magnesium, zinc, aluminum, sodium, lithium, nickel, and cobalt, but not from manganese, iron, chromium, rubidium, cesium, and ammonium. It is possible to separate as little as 0.4 mg. of potassium from seventy times as much sodium. If both calcium and sulfate are present the results are too low, probably because of the formation of a double potassium calcium sulfate.

Small amounts of sulfuric, phosphoric, nitric, and boric acids may be present, but with large amounts the precipitate becomes gelatinous and difficult to filter. In the presence of sulfate the gravimetric determination is impossible, but the volumetric method gives satisfactory results. Chloride must be absent.

The method is rapid and accurate.

Acknowledgment

The authors wish to express their thanks to Robert McNulty, who performed some of the analyses in Table III.

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From a thesis presented by Albert J. Boyle to the Graduate School of the University of Michigan in partial fulfillment of the requirements for the degree of doctor of philosophy.

Correction. The following corrections should be made to Figure 1 of our article, "An Electronic Relay" [Ind. Eng. Chem., Anal. Ed., 12, 757 (1940)]:

1. In the relay circuit of Figure 1, interchange R_1' and R_1''.
2. In the diagram of the tube socket for the OA4G tube, move the cathode (small circle) connection one pin towards the right.

We wish to thank Professors Waddle and Serfass and Dr. Shomate for calling our attention to these errors.

Paul Fugassi
C. E. Rudy, Jr.

Recovery of Asphalts and Liquid Asphaltic Road Materials from Solution

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In evaluating the service behavior of paving asphalts, test samples must be retrieved from admixtures with mineral aggregates. Usually the binder can be extracted with a solvent and the solvent subsequently removed by some method of distillation. In this paper a method of fractionation and carbon dioxide sweeping is described for the separation of carbon disulfide or benzene from penetration asphalts. The method requires a somewhat more complicated apparatus than the Abson method, which is in wide use, but the results are more reliable, especially for cracked asphalts, which may contain light oils, or for asphalts of unknown origin. The apparatus is almost identical with one used by Siegmann for the recovery of liquid road materials. It may be used not only for penetration asphalts but also for liquid asphalts following Siegmann's procedure.

IN EVALUATING the service behavior of paving asphalts, test samples must be retrieved from admixtures with mineral aggregates, and it is important that the properties of the asphalt be changed as little as possible in this process.

Usually the binder can be extracted with a solvent, and the solvent subsequently removed by some method of distillation. Extraction techniques are described by Siegmann (*8*) and Chalk (*2*) for carbon disulfide, by Suida and Hoffmann (*9*) for benzene, and by Kamptner (*4*) for a variety of solvents. A centrifugal extractor (*10*) is approved by the American Association of State Highway Officials. This paper discusses subsequent separation of asphalt and solvent by distillation.

TABLE I. ABSON RECOVERIES ON WEST TEXAS STRAIGHT-RUN AND CRACKED ASPHALTS

	Original Properties	Properties after Recovery by Abson's Method No. 1	No. 2
West Texas straight-run asphalt			
Change in weight[a] (75-gram sample), gram	..	+0.02	0.00
Penetration at 25° C.	99	99	98
Ring and ball softening point, ° C.	43.9	44.4	44.4
Penetration index (*5*)	−1.2	−1.0	−1.0
Ductility at 4° C. (5 cm. per min.)	6.6	6.7	7.0
Ductility at 25° C. (5 cm. per min.)	100+	100+	100+
West Texas cracked asphalt			
Change in weight (75-gram sample), gram	..	−0.04	−0.04
Penetration at 25° C.	105	98	98
Ring and ball softening point, ° C.	42.8	43.1	43.3
Penetration index	−1.4	−1.5	−1.4
Ductility at 4° C. (5 cm. per min.)	6.4	6.4	6.4
Ductility at 25° C. (5 cm. per min.)	100+	100+	100+

[a] Weighings accurate to ±0.01 gram.

TABLE II. PROPERTIES OF WEST TEXAS CRACKED ASPHALTS RECOVERED FROM CARBON DISULFIDE AND BENZENE

(Using modified Siegmann apparatus)

	Recovered from Carbon Disulfide		Recovered from Benzene	
	Original	Recovered	Original	Recovered
Penetration at 25° C.				
Sample 1	107	105	107	98
Sample 2	107	104	107	99
Sample 3	107	105	107	98
Ring and ball softening point, ° C.				
Sample 1	42.5	42.5	42.5	43.6
Sample 2	42.5	42.5	42.5	43.1
Sample 3	42.5	42.5	42.5	43.1
Weight of sample, grams[a]				
Sample 1	80.3	80.4	83.9	84.0
Sample 2	71.2	71.4	70.7	70.7
Sample 3	79.8	80.0	73.9	74.0

[a] Weighings accurate to ±0.2 gram.

TABLE III. HARDENING EFFECT OF CARBON DISULFIDE AND BENZENE ON WEST TEXAS CRACKED ASPHALT

(Using modified Siegmann apparatus)

		Dissolved in Carbon Disulfide		Dissolved in Benzene	
	Original	Recovered immediately	Recovered after standing 22 hours in atmosphere of inert gas	Recovered immediately	Recovered after standing 22 hours in atmosphere of inert gas
Penetration at 25° C.					
Sample 1	107	105	98	99	98
Sample 2	107	105	98	100	98
Ring and ball softening point, ° C.					
Sample 1	42.5	42.5	43.9	43.3	43.1
Sample 2	42.5	42.5	43.6	43.6	43.6
Yield[a], weight %					
Sample 1	...	100.0	100.2	100.0	100.2
Sample 2	...	100.0	100.2	100.0	100.2
Sulfur, weight %					
Sample 1	3.41	3.40	3.39	[b]	[b]

[a] Yields accurate to ±0.3%.
[b] Not determined.

Distillation Methods

A method of fractionation and carbon dioxide sweeping is here described for the separation of carbon disulfide or benzene from penetration asphalts. It requires a somewhat more complicated apparatus than the widely used Abson method (*1*) but the results are more reliable, especially for cracked asphalts which may contain light oils or for asphalts of unknown origin. The apparatus may also be used for the recovery of liquid asphalts.

In Abson's method the solution of asphalt, in benzene, is distilled in a plain balloon flask at atmospheric pressure, and carbon dioxide is used to sweep out the last traces of solvent. The figures of Table I show, however, that with this method in some cases, as with cracked asphalts, asphaltic oils may also be swept out. Often the recovered distillates are colored. Such cases require a greater amount of fractionation than Abson's apparatus provides. The authors have therefore adopted for all asphalts a simple reflux column and flask used by Siegmann (*8*) for the recovery of liquid road materials from carbon disulfide by vacuum distillation, and have adapted it to the recovery of penetration asphalts by adding a capillary inlet to the flask to permit gas sweeping. They have found that gas sweeping is necessary with penetration asphalts to assure removal of the last traces of solvent. With gas sweeping, temperatures as low as 150° C. can be used. Vacuum at lower temperatures offers no advantage, since lower temperatures are usually attended by greatly increased viscosities which make mechanical agitation difficult.

Modified Siegmann Recovery

PROCEDURE FOR PENETRATION ASPHALTS. The modified Siegmann apparatus adopted for the recovery of penetration asphalts is shown in Figure 1. The capacity of the flask is 1 liter, the capillary inlet is 1 mm. in inside diameter and reaches to within

TABLE IV. PROPERTIES OF ASPHALTS BEFORE AND AFTER RECOVERY FROM CARBON DISULFIDE SOLUTIONS

(Using modified Siegmann apparatus)

Source of Crude	Penetration at 25° C. Original	Penetration at 25° C. Recovered	Ring and Ball Softening Point Original	Ring and Ball Softening Point Recovered	Penetration Index Original	Penetration Index Recovered	Yield[a]
			° C.	° C.			Weight %
Straight-Run Asphalts							
California	22	22	55.6	55.6	−1.5	−1.5	99.7
	63	63	47.5	47.5	−1.3	−1.3	100.2
	97	97	43.9	43.9	−1.2	−1.2	100.0
	133	132	41.4	41.1	−1.2	−1.2	100.0
	231	226	36.4	36.4	−1.1	−1.2	99.8
Mexico	31	32	62.8	63.1	+0.5	+0.6	100.0
	57	56	55.8	55.8	+0.5	+0.5	100.0
	102	104	48.3	48.6	+0.4	+0.4	100.1
	159	156	43.6	43.6	+0.4	+0.4	100.0
West Texas	122	122	42.5	42.5	−1.0	−1.0	100.2
	99	98	43.9	44.7	−1.1	−0.9	100.1
Cracked Asphalts							
West Texas	35	33	52.8	54.2	−1.3	−1.1	100.0
	107	105	42.5	42.5	−1.4	−1.5	100.1
California	32	32	51.1	51.1	−1.8	−1.8	100.3
	108	105	42.2	42.5	−1.5	−1.5	100.0
Blown Asphalts, Final Temperature of Recovery 150° C.							
California	15.1	15.7	112.2	115.0	+5.1	+5.4	101.3
Mexico	24	29	94.4	93.3	+4.4	+4.7	102.1
	95	104	63.1	63.3	+3.6	+3.9	101.5
Blown Asphalts, Final Temperature of Recovery 200° C.							
California	15	15	112.2	111.4	+5.1	+5.0	99.5
Mexico	24	23.6	94.4	96.1	+4.4	+4.5	99.7

[a] Yields accurate to 0.3%.

3 mm. of the bottom of the flask, and the fractionating column is 25 cm. long and 8 mm. in inside diameter.

The asphalt solution, which as extracted usually contains 5 to 10 per cent of asphalt in carbon disulfide or benzene, is concentrated without vacuum in a flask provided with a reflux column to a concentration of approximately 25 per cent. About 400 cc. of this solution are introduced into the apparatus (Figure 1), a few silicon carbide boiling chips are added, and the solution is concentrated without vacuum or agitation over a water bath at 55° to 60° C. if carbon disulfide is the solvent; at 90° to 100° C. if benzene is the solvent. The bulk of the solvent is distilled under these conditions, the temperature being adjusted to maintain a distillation rate just short of a steady stream. When the temperature has reached 100° C. a previously heated oil bath is substituted for the water bath. The oil bath is then gradually heated to 150° C. when using either benzene or carbon disulfide and held at this temperature throughout the remainder of the recovery process.

After the distillation rate has dropped off to 5 to 10 drops per minute a stream of carbon dioxide gas is passed through the asphalt mass for 30 minutes at the rate of 900 cc. per minute. If at the end of this time condensed oils are visible in the fractionating column, careful heating will cause them to flow back into the flask. The flask is finally rotated rapidly to remix any oils condensed on its upper surfaces, and the recovered asphalt is ready for analysis.

As a safety precaution a filter flask filled with solid carbon dioxide should be attached to the open end of the apparatus. This will prevent air being accidentally drawn back into the carbon disulfide or benzene system.

TABLE V. COMPARISON OF ABSON AND MODIFIED SIEGMANN METHODS

(Approximately 75 grams of asphalt and 300 cc. of solvent were used throughout these experiments.)

	Original Properties	Properties after Recovery from Benzene by Abson's Method: Recoveries at 150° C. Maximum Temperature				Recoveries at 163° C. Maximum Temperature		Properties after Recovery from Carbon Disulfide in Modified Siegmann Apparatus	
		No. 1	No. 2	No. 3	No. 4	No. 1	No. 2	No. 1	No. 2
West Texas straight-run asphalt:									
Yield, weight %[a]	..	.....	.....	.....	.....	100.0	100.0	100.0	100.1
Penetration at 25° C.	99	.....	.....	.....	.....	99	98	100	98
Ring and ball softening point, ° C.	43.9	.....	.....	.....	.....	44.4	44.4	44.7	44.7
Penetration index	−1.2	.....	.....	.....	.....	−1.0	−1.0	−0.8	−1.0
Ductility at 4° C. (5 cm. per min.)[b]	6.6	.....	.....	.....	.....	6.7	7.0	6.4	7.5
Insoluble in 60–80° C. boiling point aromatic-free naphtha, %	7.6	.....	.....	.....	.....	7.9	7.7	7.6	7.7
California straight-run asphalt:									
Yield, weight %	..	100.1	100.3	100.0	100.0	100.1	100.1	100.0	100.1
Penetration at 25° C.	97	103	103	97	109	102	95	97	97
Ring and ball softening point, ° C.	43.6	43.3	43.3	43.9	42.2	43.1	43.9	43.9	43.9
Penetration index	−1.3	−1.2	−1.2	−1.2	−1.5	−1.4	−1.3	−1.2	−1.2
Ductility at 4° C. (5 cm. per min.)	7.5	12.0	11.0	9.0	11.0	12.3	9.4	8.5	8.0
Insoluble in 60–80° C. boiling point aromatic-free naphtha, %	6.4	6.5	6.6	7.2	6.8	6.6	6.8	6.9	6.6
Mexican straight-run asphalt:									
Yield, weight %	..	100.1	100.0	100.0	.....	99.9	100.0	100.0	100.0
Penetration at 25° C.	103	107	100	105	.....	95	102	100	100
Ring and ball softening point, ° C.	48.3	48.3	48.9	48.3	.....	49.4	48.9	48.9	48.9
Penetration index	+0.3	+0.4	+0.4	+0.4	.....	+0.4	+0.4	+0.4	+0.4
Ductility at 4° C. (5 cm. per min.)	11.5	15.2	14.5	14.8	.....	10.2	13.0	11.5	12.9
Insoluble in 60–80° C. boiling point aromatic-free naphtha, %	19.7	20.8	21.3	20.8	.....	20.4	19.8	19.5	19.8
West Texas cracked asphalt:									
Yield, weight %	..	.....	.....	.....	.....	99.9	100.0	100.0	100.0
Penetration at 25° C.	105	.....	.....	.....	.....	98	98	102	101
Ring and ball softening point, ° C.	42.8	.....	.....	.....	.....	43.1	43.3	42.8	43.1
Penetration index	−1.4	.....	.....	.....	.....	−1.5	−1.4	−1.5	−1.4
Ductility at 4° C. (5 cm. per min.)	6.4	.....	.....	.....	.....	6.4	6.4	6.7	6.8
Insoluble in 60–80° C. boiling point aromatic-free naphtha, %	18.2	.....	.....	.....	.....	..	17.9	17.9	17.8
California cracked asphalt:									
Yield, weight %	..	100.1	100.3	100.0	100.0	99.9	99.9	100.0	100.0
Penetration at 25° C.	108	105	113	106	106	99	100	103	105
Ring and ball softening point, ° C.	42.2	42.8	41.7	42.0	42.8	42.5	42.8	42.5	42.5
Penetration index	−1.5	−1.4	−1.6	−1.6	−1.4	−1.7	−1.5	−1.6	−1.5
Ductility at 4° C. (5 cm. per min.)	8.4	..[c]	13.0	8.5	13.2	9.1	8.1	..[c]	8.4
Insoluble in 60–80° C. boiling point aromatic-free naphtha, %	15.2	15.1	15.4	15.9	15.4	15.2	15.3	15.5	15.3

[a] Yields accurate to 0.3%.
[b] All ductilities at 25° C. were above 100+.
[c] Low temperature ductilities broke at the start for 6 determinations.

TABLE VI. SUMMARY OF SIEGMANN RECOVERIES OF CUT-

Liquid Road Materials Designation	Mixture: Total weight Grams	Mixture: Yield %	Mixture: Viscosity Saybolt Furol At 25° C.	Mixture: Viscosity Saybolt Furol At 50° C.	Mixture: Viscosity Saybolt Furol At 60° C.	Asphalt: Source (all straight-run)	Asphalt: Total weight Grams	Asphalt: Penetration at 25° C.	Asphalt: Ring and ball softening point ° C.	Asphalt: Penetration index	Asphalt: Oliensis test
MC-1 cutback											
Original properties	279.6	...	104	...	..	California	141.4	115	42.8	−1.1	Neg.
Properties after recovery	280.0	100.1	103	...	..		141.8	105	43.3	−1.2	Neg.
MC-4 cutback											
Original properties	366.6	...	...	...	652	California	274.2	115	42.8	−1.1	Neg.
Properties after recovery	367.1	100.1	...	...	649		275.3	105	42.8	−1.4	Neg.
RC-1 cutback											
Original properties	333.7	...	...	94	..	California	212.2	103	45.3	−0.6	Neg.
Properties after recovery	330.5	99.1	...	105	..		214.2	90	45.0	−1.1	Neg.
RC-4 cutback											
Original properties	414.3	...	...	...	951	California	310.0	103	45.3	−0.6	Neg.
Properties after recovery	414.9	100.1	...	...	966		312.0	89	45.0	−1.1	Neg.
RC-1 cutback											
Original properties	332.8	...	...	80	..	Mexico	195.0	86	51.1	+0.5	Neg.
Properties after recovery	325.9	97.9	...	103	..		196.6	82	51.7	+0.6	Neg.
MC-4 cutback											
Original properties	346.6	...	...	...	487	Mexico	246.3	188	40.6	−0.1	Neg.
Properties after recovery	347.1	100.1	...	...	475		249.1	196	40.6	+0.1	Neg.
MC-1 cutback											
Original properties	285.0	...	94.6	...	..	California	149.0	94	44.2	−1.2	Neg.
Properties after recovery	286.3	100.4	88.1	...	..		149.2	96	43.9	−1.1	Neg.
RC-4 cutback											
Original properties	480.2	...	...	...	951	California	358.3	103	45.3	−0.6	Neg.
Properties after recovery	477.9	99.5	...	...	1086		359.7	87	45.0	−1.2	Neg.
SC-1 road oil											
Original properties	274.9	...	47.6	...	..						
Properties after recovery	276.0	100.4	40.3	...	..						
SC-2 road oil											
Original properties	287.6	...	...	285	..						
Properties after recovery	287.6	100.0	...	264	..						
SC-3 road oil											
Original properties	293.8	...	...	303	..						
Properties after recovery	294.5	100.2	...	261	..						
SC-4 road oil											
Original properties	294.9	...	...	...	453						
Properties after recovery	295.6	100.2	...	...	395						

In recovering 100-penetration low flash point cracked asphalt from benzene by this method no differences in yields or properties of the recovered asphalts were obtained by increasing the final bath temperature from 150° to 180° C., by increasing the time of passage of carbon dioxide from 30 to 80 minutes at 900 cc. per minute, or by varying the distillation rate between 100 and 200 drops per minute. Reducing the carbon dioxide rate from 900 to 450 cc. per minute resulted, however, in high yields and in softer recovered asphalts. In recoveries of the same asphalt from carbon disulfide, increasing the final bath temperature from 150° to 180° C. or increasing the time of passage of carbon dioxide from 30 to 80 minutes had no effect.

Data for parallel recoveries from carbon disulfide and from benzene are given in Table II. They show less hardening from carbon disulfide. These recoveries were made immediately after preparing the solutions, but as the solutions become older the differences tend to disappear. This behavior is illustrated by the figures of Table III. The authors have chosen to use carbon disulfide and recover as soon as possible. In many laboratories benzene may be preferred because of the great fire hazard with carbon disulfide. The benzene used may be the C. P. grade, or a commercial grade having a narrow distillation range and containing a negligible amount of high-boiling residue as determined by evaporation in a glass dish on the steam bath. Many commercial grades contain appreciable amounts of residue which will remain in the recovered asphalt and vitiate the results.

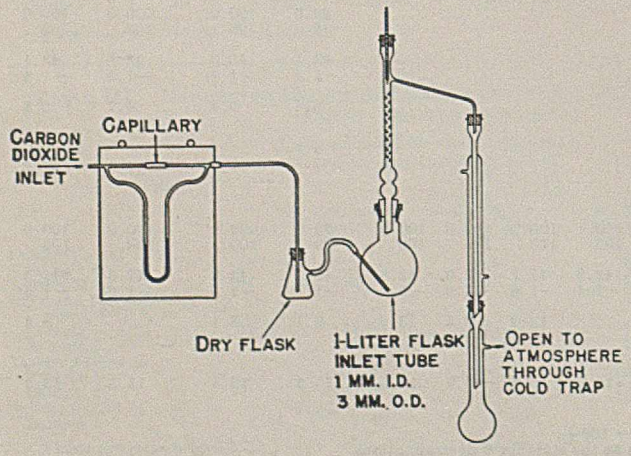

FIGURE 1. MODIFIED SIEGMANN RECOVERY APPARATUS

Greutert (*3*) and Pfrengle (*6*) report hardening in carbon disulfide which is greater if water is present, and Preston and Brandon (*7*) also give data on this point which appear conclusive. Thus, prior to extraction of paving samples, thorough drying is essential. The authors used dry asphalts and Baker's C. P. carbon disulfide in all recoveries here reported.

Chlorinated solvents as a class are considered reactive by most authors. Kamptner (*4*) states that in general chlorinated solvents make asphalts brittle. Solvents tested by him in the order of decreasing hardening effect are: carbon tetrachloride, trichloroethylene, dichloroethylene, chloroform, benzene, and carbon disulfide. Pfrengle (*6*) uses chloroform for recoveries and admits a hardening effect but attempts to apply a correction factor. Suida and Hoffmann (*9*) state that chloroform, carbon tetrachloride, and carbon disulfide but not benzene are reactive in the absence of air. Abson (*1*) on the other hand reports progressive hardening in benzene. In parallel recoveries on fresh solutions of 100-penetration asphalts the authors obtained: from chloroform, a drop in penetration of 22 points; from benzene, 9 points; and from carbon disulfide, 3 points.

Recoveries of Representative Penetration Asphalts

Results for recoveries of 18 straight-run, cracked, and blown asphalts from carbon disulfide by the procedure outlined above are given in Table IV. Of these 18 asphalts only the highly blown asphalts were too viscous at 150° C. to permit complete escape of the solvent. Better results were obtained at 200° C.

Results of parallel Siegmann and Abson recoveries of various grades of penetration asphalts are given in Table V. The recoveries by the Abson method are from benzene; by the authors method, from carbon disulfide.

Abson recommends a final temperature of 150° C. for 100-penetration asphalts and suggests 163° C. for asphalts of

BACKS AND ROAD OILS FROM CARBON DISULFIDE SOLUTION

Nature of fluxing agent	Total weight	Specific gravity 25°/4° C.	Fluxing Agent Distillation (A. S. T. M. Apparatus) D86-35 Used	Initial b. p.	5% over	10% over	20% over	30% over	40% over	50% over	60% over	70% over	80% over	90% over	95% over	Dry point	% over	% residue	Distn. loss
	Grams		*Cc.*	*° C.*															
Kerosene distillate	95.4	0.8377	100	200	204	207	209	212	214	217	220	224	229	236	245	251	97.7	2.2	0.1
	94.1	0.8371	100	191	203	207	209	212	214	217	220	223	228	235	242	258	98.5	1.3	0.2
Kerosene distillate	46.5	0.8377	50	198	203	206	209	212	214	217	221	224	230	238	248	257	97.2	2.6	0.2
	45.3	0.8397	50	195	202	207	210	213	216	218	222	226	231	240	...	257	96.0	3.0	1.0
Naphtha	75.0	0.7975	50	121	134	142	152	161	169	176	183	191	200	214	225	238	98.0	1.7	0.3
	73.3	0.8050	50	120	143	150	159	165	172	178	184	192	200	213	222	234	97.0	2.7	0.3
Naphtha	39.9	0.7975	50	121	134	142	152	161	169	176	183	191	200	214	225	238	98.0	1.7	0.3
	39.3	0.8196	40	64	106	157	162	168	174	180	186	190	203	220	236	238	95.5	3.4	1.1
Naphtha	85.5	0.7720	100	113	125	130	135	141	146	153	160	166	174	186	196	209	98.3	1.2	0.5
	84.1	0.7788	100	116	131	136	141	146	151	156	162	168	178	188	200	216	98.0	1.4	0.6
Cutback cracked gas oil	53.7	0.8312	50	170	180	187	195	200	205	211	218	224	234	245	257	264	96.8	2.7	0.5
	50.8	0.8331	50	168	182	189	195	200	205	212	215	222	229	242	254	264	96.2	2.9	0.9
Kerosene distillate	101.0	0.8355	100	198	203	206	208	211	213	215	218	221	226	234	241	250	98.5	1.1	0.4
	101.2	0.8371	100	181	203	206	208	210	212	214	218	221	225	233	239	246	98.4	1.3	0.3
Naphtha	44.2	0.7975	50	121	134	142	152	161	169	176	183	191	200	214	225	238	98.0	1.7	0.3
	43.4	0.8051	50	129	147	153	159	166	173	180	187	196	202	218	232	240	96.8	2.8	0.4

unknown penetration. Therefore, in Table V data are given for recoveries at both temperatures. At the higher temperature there is a tendency for asphalt oils to be lost, which results in hardening, and at the lower temperature solvent is sometimes retained, which causes softening. The authors have found that retained solvent is shown best by increased low-temperature ductility, and consequently, in those cases where the ductilities of the recovered asphalts are high and yields still not over 100 per cent, it must be concluded that losses of oils are compensated by retained solvent. The discrepancies when using the Abson method are not large and in many cases use of the somewhat more complicated Siegmann apparatus may not be justified. Nevertheless, whenever road oils or cracked asphalts may be encountered, the assurance of fractionation between asphalt oils and solvent seems necessary.

Recoveries of Liquid Road Materials by Siegmann's Method

The apparatus used here (Figure 1) for the recovery of penetration asphalts is Siegmann's apparatus for the recovery of liquid road materials, with the exception of the capillary inlet in the flask.

SIEGMANN'S PROCEDURE (*8*). The concentrated carbon disulfide extract is introduced into the flask of the Siegmann apparatus. The flask is placed in an oil bath, the temperature of which is raised carefully, so that the carbon disulfide passes over slowly (about 20 cc. per 15 minutes). When the greater part of the carbon disulfide has passed over, the apparatus is carefully subjected to vacuum, which is gradually increased to 40 cm. After the bulk of the carbon disulfide has been removed, the temperature of the oil bath is raised in 1 hour to 200° C.; upon continued heating the level of the fluxing agent is seen to rise slowly in the column. As soon as the vapors reach the bottom of the side tube of the fractionating column the vacuum is decreased, to prevent the fluxing agent from distilling over.

In the case of thinly liquid cutbacks, heating is continued until the thermometer reads 60° C., where the distillation is stopped.

With viscous cutbacks the thermometer reads 60° C. before the level of the fluxing agent vapors (visible by mist formation) has risen to the side tube. In such cases heating is then continued until the level of the side tube has been reached, which may take place at a temperature of about 85° C.

With kerosene cutbacks the oil bath has to be heated to at most 250° C., with creosote cutbacks to 300° C. to recover the cutbacks. If cutbacks that have lost nearly all their fluxing agent are to be examined, the oil bath will be at a temperature of 300° C. before the vapors reach the side tube. Distillation is then discontinued.

After cooling, the flask is rotated to distribute the condensed fluxing agent homogeneously in the residue. When this is done the binder is ready for further examination.

Using a flask without a capillary and following this procedure, with domestic RC and MC cutbacks and SC road oils, the authors have obtained the results shown in Table VI. Figures are also given in Table VI for the separation of cutbacks into asphalts and fluxing agents using steam-distillation as described by Siegmann (*8*):

The distillation flask containing 600 grams of cutback is heated until the required temperature (Siegmann specifies 150° to 200° C. for gasoline; 200° to 220° C. for kerosene; 240° C. for gas oil) has been reached; then superheated steam of the same temperature is conducted through the mass in the flask. The distillation is continued until 10 times the amount of steam calculated on solvent (evaporation test) has passed over. It is of importance to ascertain whether, and how much, distillate passes over towards the end of the distillation. The best procedure is to plot a curve of the quantity of distillate against the quantity of water.

Though it is desirable to have an intake of 600 grams in order to make a reliable analysis of the separated solvent, in some cases, especially when analyzing road samples, the available quantity will be much smaller. In these cases the intake may be reduced to 300 grams, a 1-liter distillation flask being used instead of a 2-liter flask. If the cutback contains only a small percentage of volatile products, the total quantity of steam (10 times the amount of distillate) would be somewhat on the low side. Therefore, the minimum quantity of steam should in any case be not less than 200 grams.

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Physicochemical Assay of Vitamin A

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THE vitamin A potencies of various materials are determined in many laboratories by one or both of two well-known physicochemical methods. The method which is often considered to be the more exact depends upon the measurement of the absorption of ultraviolet light at the maximum (328 mμ) of the vitamin A absorption band of an alcohol solution of the material or a purified extract (*1*, *2*, *4*, *8*). The vitamin A concentration is proportional to the value of $E^{1\%}_{1\ cm.}$ (328 mμ).

$$E^{1\%}_{1\ cm.} = \frac{1}{cd} \log \frac{I_0}{I}$$

where c = concentration in grams per 100 ml., d = depth of optical cell in centimeters, I_0 = intensity of the light incident on the solution, and I = intensity of the light transmitted by the solution.

The other method, which is almost as exact, depends upon the measurement of the absorption of light at the maximum (620 mμ) of the absorption band of the blue colored reaction product formed by mixing a chloroform solution of the material with ten times its volume of a saturated chloroform solution of antimony trichloride (*3*, *5*, *6*). The vitamin A concentration is proportional to the value of $E^{1\%}_{1\ cm.}$ (620 mμ). If a spectrophotometer is not available, this color measurement may be made with a photoelectric colorimeter using a sharp-cut filter. In this latter case a calibration curve is made relating the vitamin A potency of the solution being tested to the depth of the blue color.

TABLE I. STABILITY OF SOLUTIONS OF VITAMIN A KEPT IN BROWN GLASS BOTTLES

[Halibut liver oil distillate diluted with cottonseed (Wesson) oil]

Solvent	Location of Maximum Absorption	Concentration	$E^{1\%}_{1\ cm.}$ (Max.) 1 hour	24 hours	7 days	50 days
	mμ	%				
Ethyl alcohol	328	0.0822	15.1	15.1	15.1	13.1
Cyclohexane	327	0.0863	14.6	15.0	14.6	14.5
Hexane	328	0.0844	16.1	15.8	15.6	16.0
Ether	328	0.0848	16.6	16.3	15.8	11.9
Chloroform	333	0.0846	13.2	13.6	14.4	13.2
Benzene	333	0.0846	14.4	14.3	14.3	14.7

When most fish liver oils and concentrates are tested by either of these methods the assays are precise and reproducible, especially in commercial laboratories doing the work as a routine procedure. The vitamin A solutions are prepared and tested within a half hour or, at most, an hour. However, the assays of low-potency fish liver oils, food products, and certain pharmaceutical preparations are not so satisfactory. These assays often involve extractions, saponification, etc., and take from 2 to 6 hours to complete. The potencies determined by these assays are often slightly, and many times surprisingly, low. The poor results are attributed to the instability of vitamin A in dilute solution. The degeneration of the vitamin is often assumed to be due to oxidation, but handling the solutions under inert gas does not improve the results to any great extent.

It has been shown (*7*) that vitamin A is destroyed by ultraviolet light, but it is not generally realized that the annoying instability of dilute solutions of vitamin A is almost entirely due to this effect. In Table I are given data which show the constancy for several days of the ultraviolet absorption of solutions of vitamin A stored in 50-ml. brown-glass-stoppered bottles. The solvents are of the ordinary C. P. grade, and the source of the vitamin A is a halibut liver oil distillate which had been diluted with cottonseed (Wesson oil) oil to a potency of slightly over 30,000 U. S. P. units per gram.

Since solutions of vitamin A are so stable in the absence of ultraviolet light, it seems desirable that they be handled entirely in apparatus which will absorb these harmful radiations. The apparatus, in general, must be transparent, so that the operator may separate layers, look for emulsion formation, etc.

Test tubes made of an amber glass which seemed suitable for the manufacture of such apparatus were furnished to the author by a glass manufacturer. Some tests of the usefulness of this glass were made.

Several solutions of vitamin A which were being assayed in the regular work were split up into two parts. One part was put into the amber test tube, and the other part into a clear test tube of the same dimensions. The tightly stoppered test tubes stood in a glass beaker on the laboratory bench 12 feet from the window of the room. After a few hours of this exposure to daylight, the solutions were again tested and the potencies of the vitamin A concentrates calculated. Cloudy days during which no sun shone were chosen for these tests, in order to help compensate for the higher actinic power of summer daylight. The solutions contained 20 to 25 units of vitamin A per ml.

Table II gives the results of these tests, which show that serious losses of vitamin A potency result from exposure of solutions of vitamin A in clear glass test tubes, while only small losses are found if amber test tubes are used. Contrary to prevalent opinions, solutions in chloroform are about as stable as solutions in alcohol, and solutions of vitamin A in the alcohol form are about as stable towards light as those in the ester form. This last fact should be borne in mind because, owing to the greater resistance of vitamin A esters to autoxidation, solutions of vitamin A esters are sometimes not handled so carefully as those of vitamin A alcohol.

TABLE II. EFFECT OF EXPOSURE TO LIGHT ON SOLUTIONS OF VITAMIN A

Material	Exposure to Daylight	Original Potency[a]	Solvent	Final Potency % by ultraviolet[a]	% by $SbCl_3$[b]	Color of Test Tube
	Hours					
Vitamin A alcohol concentrate A, 8-1-40	5.5 Cloudy day	3,260,000	EtOH	72	..	Clear
				93	..	Amber
Vitamin A alcohol concentrate B, 9-4-40	5.0 Cloudy day	2,989,000	EtOH	92	..	Clear
				100	..	Amber
Vitamin A alcohol concentrate B, 8-28-40	4.0 Cloudy and rain	2,980,000	$CHCl_3$	91	93	Clear
				104	105	Amber
Fish liver oil C, 8-23-40	5.0 Cloudy day	30,000	EtOH	45	..	Clear
				96	..	Amber
Fish liver oil C, 8-23-40	4.0 Cloudy day	30,000	$CHCl_3$	59	63	Clear
				101	101	Amber
Fish liver oil distillate D, 8-27-40	5.0 Cloudy day	216,000	EtOH	73	..	Clear
				98	..	Amber
Fish liver oil distillate D, 8-27-40	4.0 Cloudy day	216,000	$CHCl_3$	80	85	Clear
				101	101	Amber

[a] Taken to be 2000 times $E^{1\%}_{1\ cm.}$ (328 mμ) in alcohol.

[b] Measured with Evelyn photoelectric colorimeter using Rubicon 620 filter.

Unfortunately, no amber laboratory glassware is regularly made by the apparatus manufacturers. The author believes that every laboratory performing assays for vitamin A on food, medicinal, and physiological preparations needs at least the following types of amber glassware: separatory funnels, conical flasks, volumetric flasks, and boiling flasks for Soxhlet extractors.

There are undoubtedly other substances which could well be handled in amber glassware. The Kimble Glass Company, Vineland, N. J., has available the glass used in the work reported here, and is compiling a list of items which will be used in sufficient quantity to permit stocking. The coefficient of expansion is 64 × 10^{-7} per ° C., and apparatus made of it can be heated with reasonable care. The company will welcome suggestions concerning possible listings.

Acknowledgment

The author thanks George H. Wait for the experimental data given in Table II and the Eastman Kodak Research Laboratories for the optical measurements made for Table I.

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COMMUNICATION 20 from the laboratories of Distillation Products, Inc.

Determination of Small Amounts of Zinc in Plant Materials

A Photometric Dithizone Method

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DIPHENYLTHIOCARBAZONE, commonly referred to as "dithizone", has been used by several investigators (*2–4, 8*) as a colorimetric reagent for the determination of small amounts of zinc. The reaction between dithizone and zinc in slightly alkaline solution results in the formation of a red complex which is quantitatively extractable with chloroform or carbon tetrachloride. The reaction is extremely sensitive and the color sufficiently stable to form the basis of an excellent colorimetric method. However, more than a dozen other metals also react with dithizone to form extractable colored complexes. This lack of specificity has been the chief difficulty in the use of the reagent. It is possible to eliminate interferences to some extent by careful regulation of the pH at which the extractions are carried out and by the use of the selective complexing action of certain inorganic anions, but neither of these methods is entirely satisfactory.

Holland and Ritchie (*5*) reported the important observation, for which they give credit to R. H. Caughey, that in 0.02 *N* ammonium hydroxide solution sodium diethyldithiocarbamate, the copper reagent usually referred to as "carbamate", inhibits the reaction of all metals with dithizone except zinc. These workers proposed a colorimetric method for the determination of zinc in foods in which carbamate is used to eliminate interferences by other metals which form dithizone complexes. This method, although it represents an important step toward the solution of the problem, did not give highly reproducible results in the authors' hands when color intensities were measured with a photoelectric colorimeter. It was hoped by an investigation of the action of carbamate in the determination of zinc to develop a method free of interferences and capable of an accuracy comparable with that obtainable in measuring color intensities with modern photoelectric colorimeters.

Effect of Carbamate on Extraction of Zinc as Dithizonate

Zinc can be quantitatively extracted as dithizonate from aqueous solution at a pH between 8 and 9 with carbon tetrachloride containing excess dithizone. In the presence of carbamate, however, complete extraction of the zinc as dithizonate does not occur. The zinc is distributed between the red dithizone complex and the colorless carbamate complex. The result of this effect is a reduction in the color intensity of the dithizone extract, as is shown by a comparison of the curves on Figure 1.

Curve 1 represents the relationship between the amount of zinc present and the per cent light transmission of the carbon tetrachloride extract obtained when no carbamate is present, and curve 2 represents the same relationship when 12.5 mg. of carbamate are present. A comparison of the two curves shows that the presence of the carbamate appreciably reduces the color intensity of the carbon tetrachloride extract obtained for all amounts of zinc.

The effect of varying the amount of carbamate present

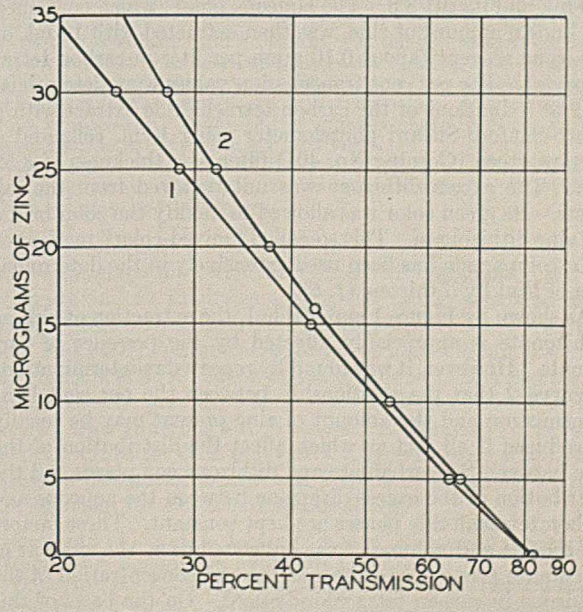

FIGURE 1. EFFECT OF CARBAMATE ON RELATION BETWEEN COLOR INTENSITY OF DITHIZONE EXTRACT AND AMOUNT OF ZINC PRESENT

1. No carbamate present
2. 12.5 mg. of carbamate present

Filter. Sextant green (3.52 mm.)
Cell thickness. 1 cm.

[1] Present address, American Viscose Company, Marcus Hook, Penna.

Table I. Effect of Amount of Carbamate Present on Distribution of Zinc between Dithizone and Carbamate Complexes

(30 micrograms of zinc present in each case)

Carbamate Present	Distribution: Dithizone		Distribution: Carbamate	
Mg.	γ	%	γ	%
0.00	30.0	100	0.0	0
0.63	28.9	96	1.1	4
1.25	29.0	97	1.0	3
2.5	28.1	94	1.9	6
5.0	27.3	91	2.7	9
12.5	25.9	86	4.1	14
25.0	21.9	73	8.1	27

Table II. Effect of Dithizone Complex-Forming Metals on Determination of Zinc

(10 micrograms of zinc present in each case)

Metal		Zinc Found	Error	
	γ	γ	γ	%
Cu^{++}	10	10.0	0.0	0
	20	10.1	0.1	1
	40	10.2	0.2	2
	60	10.3	0.3	3
	80	10.1	0.1	1
	100	10.2	0.2	2
Pb^{++}	20	10.3	0.3	3
	40	10.0	0.0	0
	60	10.2	0.2	2
	80	10.0	0.0	0
	100	10.4	0.4	4
Hg^{++}	100	10.1	0.1	1
Bi^{+++}	100	10.0	0.0	0
Co^{++}	100	10.2	0.2	2
Sn^{++}	100	9.9	−0.1	−1
Ni^{++}	100	10.6	0.6	6
Cd^{++}	5	10.4	0.4	4
	10	11.2	1.2	12
	20	12.2	2.2	22
	50	13.0	3.0	30
	100	16.7	6.7	67

upon the distribution of zinc between the carbamate and dithizone complexes is demonstrated by Table I. As the amount of carbamate is increased from 0 to 25 mg., the percentage of zinc extracted as dithizonate is decreased from 100 to 73 per cent.

In each of the extractions which were carried out in order to obtain the data for Figure 1 and Table I a fixed set of conditions was employed. The volume of the aqueous phase was 100 ml. and its pH 8.8. The aqueous phase, which contained the known amount of zinc, was then extracted with 10 ml. of dithizone reagent (about 0.10 gram per liter in carbon tetrachloride). The per cent transmission values were determined on 1 to 5 dilutions of the carbon tetrachloride extract with a Cenco-Sanford-Sheard photelometer using 1-cm. cells and a Sextant green (Corning No. 401) filter of a thickness of 3.52 mm. The excess dithizone was not removed from the extracts. Its green color was allowed to modify the color of the red zinc dithizonate. This so-called "mixed-color" method of color comparison has been used extensively in the determination of lead by dithizone (*1*, *6*).

As shown by Figure 1 and Table I, the extraction of zinc as dithizonate is appreciably affected by the presence of carbamate. However, it was found by repeated redeterminations of curve 2 that the relationship between the per cent light transmission and the amount of zinc present may be readily reproduced if all factors which affect the distribution of the zinc between the carbamate and dithizone complexes and the distribution of the excess dithizone between the aqueous and carbon tetrachloride phases are kept constant. These factors are the pH and volume of the aqueous phase, the amount of carbamate present, and the volume and concentration of the dithizone in carbon tetrachloride used. On the basis of this relationship a method was devised for the determination of traces of zinc in plant materials in which carbamate is used to eliminate the interference of other metals which also form dithizone complexes.

Outline of Method

1. Ashing of the sample and extraction of the zinc from the ash with hydrochloric acid.
2. Separation of the zinc and other metals which form dithizone complexes from the other constituents of the plant ash solution by repeated extraction with dithizone in carbon tetrachloride at a pH between 8.5 and 9 and in the presence of ammonium citrate to prevent the precipitation of iron and aluminum.
3. Separation of the zinc from copper and excess dithizone and solution of the zinc in a definite volume of standard acid by extraction of the dithizone extracts obtained in step 2 with 50 ml. of 0.02 *N* hydrochloric acid.
4. Extraction of the aqueous phase from step 3 with dithizone in carbon tetrachloride after the adjustment of the pH to a value between 8.5 and 9 with ammonia–ammonium citrate buffer and the addition of sodium diethyldithiocarbamate, employing in this extraction the same pH, volumes of phases, and amounts of carbamate and dithizone as were used in the determination of the standard curve. The amount of zinc present in the plant material is then obtained by comparison of the instrument reading of this extract with the standard curve.

Table III. Recovery of Added Zinc from Plant Materials

Material	Zinc Added	Zinc Present	Zinc Found	Error	
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	%
Dried grapes	0.0	11.4[a]	11.0	−0.4	−4
	0.0	11.4	11.4	0.0	0
	0.0	11.4	12.0	0.6	6
	0.0	11.4	11.4	0.0	0
	0.0	11.4	11.2	−0.2	−2
	10.0	21.4	22.0	0.6	3
	10.0	21.4	21.8	0.4	2
	20.0	31.4	31.4	0.0	0
	20.0	31.4	31.6	0.2	1
	20.0	31.4	30.8	−0.6	−2
	40.0	51.4	50.4	−1.0	−2
	40.0	51.4	51.8	0.4	1
	40.0	51.4	51.4	0.0	0
Dried asparagus	0.0	13.6[a]	13.6	0.0	0
	0.0	13.6	12.6	−1.0	−7
	0.0	13.6	13.8	0.2	2
	0.0	13.6	14.0	0.4	3
	0.0	13.6	14.0	0.4	3
	10.0	23.6	23.2	−0.4	2
	10.0	23.6	22.0	−1.6	−7
	10.0	23.6	23.6	0.0	0
	10.0	23.6	24.6	1.0	4
	20.0	33.6	32.6	−1.0	−3
	20.0	33.6	32.6	−1.0	−3
	20.0	33.6	34.2	0.6	2
	20.0	33.6	34.6	1.0	3
	40.0	53.6	53.6	0.0	0
	40.0	53.6	53.6	0.0	0
	40.0	53.6	53.4	−0.2	0
	40.0	53.6	53.6	0.0	0
Corn mea	0.0	9.3[a]	9.0	−0.3	−3
	0.0	9.3	9.4	0.1	1
	0.0	9.3	9.4	0.1	1
	0.0	9.3	9.4	0.1	1
	10.0	19.3	19.4	0.1	1
	10.0	19.3	19.4	0.1	1
	20.0	29.3	29.8	0.5	2
	20.0	29.3	29.8	0.5	2
	40.0	49.3	50.4	1.1	2
	40.0	49.3	49.0	−0.3	−1

[a] Zinc naturally present in material is assumed to be equal to average amount of zinc found by analysis by this method.

Reliability of Method

The reliability of this method was tested by studies involving (1) the ability to determine zinc accurately in the presence of other metals which form dithizone complexes, (2) the accuracy with which added zinc could be recovered from plant materials, and (3) the ability of the method to give good agreement between duplicate determinations on the same plant material. The results obtained in these three tests are given in Tables II, III, and IV, respectively.

Table II shows that the method is remarkably free of interferences by other metals which form dithizone complexes. It was found that zinc could be determined without serious interference in the presence of ten times as much copper, lead, mercury, bismuth, cobalt, tin, or nickel. The interference of cadmium was greater than any of the other metals studied; however, about half as much of this metal as zinc may be present without causing an appreciable error. Since this

TABLE IV. ZINC IN VARIOUS PLANT MATERIALS

Material	Zinc Found: Sample A	Zinc Found: Sample B	Zinc Found: Av.	Deviation
	P. p. m.	*P. p. m.*	*P. p. m.*	%
Sugar-beet pulp	15.0	14.2	14.6	3
Oats	29.6	30.2	29.9	1
Wheat	29.4	31.0	30.2	3
Brome grass hay	20.6	20.4	20.5	0.5
Fresh alfalfa	32.4	31.8	32.1	1
Spinach[a]	92.0	92.0	92.0	0
Table beets	45.8	44.2	45.0	2
Onions	49.6	49.8	49.7	0
Parsnips	15.8	16.6	16.2	3
Celery	30.0	29.2	29.6	1
Carrots	23.0	24.2	23.6	2
Turnips[a]	84.4	85.0	84.7	0.5

[a] Zinc determined using 5-ml. aliquot of ash solution. A 10-ml. aliquot was used in all other cases. In every case a 5-gram sample of ground, air-dry material was ashed.

amount of cadmium is seldom encountered in the analysis of plant materials, its interference would rarely be significant.

Table III shows satisfactory recoveries of zinc added before ashing to dried grapes, dried asparagus, and corn meal.

Table IV shows that the method gives excellent agreement among duplicate determinations on the same material for a wide variety of plants. In this study two samples of each plant material were ashed, and the zinc was determined on one aliquot from each ash solution.

These three tests show that by the use of carbamate the interference of other metals in the determination of zinc is practically eliminated, and that the accuracy obtainable is consistent with the best photometric method for trace amounts.

The plant materials examined were dry-ashed and the zinc was extracted from the ash by means of hydrochloric acid. By means of the spectrograph Rogers and Gall (*7*) found that extraction of the ash of some plants with hydrochloric acid did not remove all the zinc. It is possible that this hydrochloric acid–insoluble zinc is from soil which contaminated the samples of plant material, or is zinc assimilated by the plant and either rendered insoluble by some reaction during ashing or occluded by hydrochloric acid–insoluble constituents of the ash. This problem is sufficiently important, particularly where plant materials containing large amounts of insoluble matter are analyzed, to warrant future attention.

Apparatus

All glass apparatus used in the application of this method must be carefully cleaned. All apparatus should be rinsed with strong acid, followed by four or five rinses with distilled water, and finally be given a rinse with zinc-free water (redistilled from Pyrex). Separatory funnels may be conveniently cleaned by first dipping them in warm chromic acid cleaning solution, which is removed by three thorough rinsings with tap water. The funnels are then rinsed with distilled water four times, and given a final rinse with zinc-free water. Care must be taken that no chromic acid remains on the funnels, for it will cause trouble by oxidizing dithizone.

All vessels in which aqueous solutions are stored for more than a few hours should be of Pyrex glass. Soda-lime separatory funnels, volumetric flasks, etc., in which solutions are kept for only a short time, were used and found satisfactory. White petroleum jelly was found to be a satisfactory lubricant for the stopcocks of the separatory funnels. A separatory funnel rack is necessary. Convenient racks have been described by Winter *et al.* (*9*) and by Bambach (*1*).

Although a Cenco-Sanford-Sheard photoelectric colorimeter was used exclusively in this investigation, the method is adaptable to any good photoelectric colorimeter.

Reagents

All water used in the preparation of the ash solutions and of reagents should be redistilled from an all-Pyrex glass still, unless it is definitely known that the distilled water available is free of zinc and other dithizone complex-forming metals. The distilled water available in this laboratory did not require redistillation. C. P. carbon tetrachloride may be used without purification. Technical carbon tetrachloride, however, should be dried with anhydrous calcium chloride and redistilled in the presence of a small amount of calcium oxide before use. Used carbon tetrachloride may be reclaimed by distillation in the presence of dilute sodium hydroxide solution containing a small amount of sodium thiosulfate, followed by drying with anhydrous calcium chloride and fractional distillation in the presence of a small amount of calcium oxide.

Most of the necessary reagents contain appreciable amounts of zinc and other metals that form dithizone complexes, which must be removed if good accuracy is expected by use of this method. Chemicals of the highest grade should be used and purified as follows:

STANDARD ZINC SOLUTIONS. Stock solution (1000 micrograms of zinc per ml.). Place 0.25 gram of pure zinc in a 250-ml. volumetric flask. Add about 50 ml. of water and 1 ml. of concentrated sulfuric acid, then heat on the steam bath until all zinc is dissolved. Dilute to 250 ml. and store in a Pyrex vessel.

Standard solution (10 micrograms of zinc per ml.). Dilute 10 ml. of the above stock solution to 1 liter. Store in a Pyrex vessel.

N AMMONIUM HYDROXIDE SOLUTION. Distill into water one-half volume of concentrated ammonium hydroxide by use of an all-Pyrex glass distillation apparatus, then dilute to the proper concentration. Store in a glass-stoppered Pyrex vessel.

N HYDROCHLORIC ACID. Displace hydrogen chloride gas from a volume of concentrated hydrochloric acid in a Pyrex flask by the slow addition of an equal volume of concentrated sulfuric acid by means of a dropping funnel which extends below the surface of the concentrated hydrochloric acid. Absorb the displaced hydrogen chloride gas by conducting it by means of a delivery tube to the surface of a volume of water. No heat is necessary. Dilute to the proper concentration. One hundred and fifty milliliters of each of the acids will yield 1 liter of purified hydrochloric acid solution of a concentration greater than normal.

DITHIZONE REAGENT. Dissolve 0.20 gram of diphenylthiocarbazone in 500 ml. of carbon tetrachloride and filter the solution to remove insoluble matter. Place the solution in a glass-stoppered bottle or large separatory funnel, add 2 liters of 0.02 *N* ammonia (40 ml. of *N* ammonia diluted to 2 liters), then shake to extract the dithizone into the aqueous phase. Separate the phases, discard the carbon tetrachloride phase, and extract the ammoniacal solution of dithizone with 100-ml. portions of carbon tetrachloride until the carbon tetrachloride extract is a pure green color. Discard the carbon tetrachloride phase after each extraction. Add 500 ml. of carbon tetrachloride and 45 ml. of *N* hydrochloric acid and shake to extract the dithizone into the carbon tetrachloride. Separate the phases and discard the aqueous phase. Dilute the carbon tetrachloride solution of dithizone to 2 liters with carbon tetrachloride. Store the dithizone reagent in a brown bottle in a dark cool place.

AMMONIUM CITRATE SOLUTION (0.5 molar). Dissolve 226 grams of ammonium citrate, $(NH_4)_2HC_6H_5O_7$, in 2 liters of water. Add concentrated ammonia (80 to 85 ml.) until the solution has a pH of 8.5 to 8.7. Add an excess of dithizone reagent (orange-yellow coloration in aqueous phase after shaking and separation of phases), and extract with 100-ml. portions of carbon tetrachloride until the extract is a full green color. Add more dithizone if necessary. Separate the aqueous phase from the carbon tetrachloride, and store in a Pyrex vessel.

CARBAMATE REAGENT. Dissolve 0.25 gram of sodium diethyldithiocarbamate and dilute to 100 ml. with water. Prepare a fresh solution just before use.

Three solutions are prepared in large quantities from the above reagents in order to reduce the measuring out of reagents and to minimize errors due to variations in their composition. These solutions should be stored in Pyrex vessels, and care must be taken to avoid loss of ammonia from Solutions A and B. The amount of zinc in these reagents increases slowly with time of storage, and they should be discarded after standing 6 to 8 weeks. A standard curve must be determined for each new set of reagents. The amounts of reagents designated below and 2 liters of dithizone reagent are sufficient for 100 zinc determinations.

SOLUTION A. Dilute 1 liter of 0.5 *M* ammonium citrate and 140 ml. of *N* ammonium hydroxide to 4 liters.

SOLUTION B. Dilute 1 liter of 0.5 *M* ammonium citrate and 300 ml. of *N* ammonium hydroxide to 4.5 liters. Just before use add 1 volume of freshly prepared carbamate reagent to 9 volumes of the ammonia–ammonium citrate solution to obtain the volume of Solution B immediately required.

0.02 *N* HYDROCHLORIC ACID. Dilute 100 ml. of *N* hydrochloric acid to 5 liters.

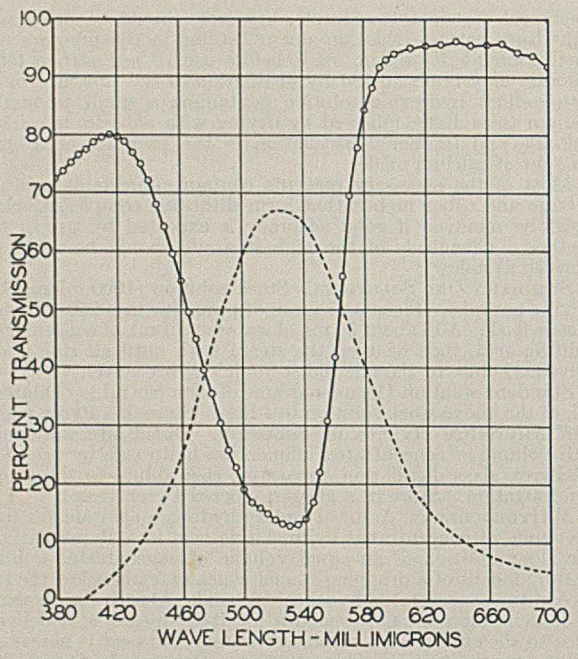

FIGURE 2. LIGHT TRANSMISSION OF SEXTANT GREEN FILTER AND ZINC DITHIZONE COMPLEX

Broken line. Sextant green filter
Solid line. Zinc dithizonate in carbon tetrachloride

Once a set of reagents purified from zinc has been prepared, these reagents can be used to test chemicals for zinc. Certain lots of ammonium hydroxide and hydrochloric acid are sufficiently free of zinc to be used in this method without purification.

Procedure for Zinc in Plant Materials

ASHING. Ash a 5-gram sample of the finely ground, air-dry plant material in a platinum dish in an electric muffle at 500° to 550° C. Wet the ash with a little distilled water, then add 10 ml. of *N* hydrochloric acid (more if necessary) and heat on a steam bath until all substances soluble in hydrochloric acid are brought into solution. Add 5 or 10 ml. of hot water. Filter off the insoluble matter on a 7-cm. filter paper (Whatman No. 42 or equivalent) which has been previously washed with two 5-ml. portions of hot *N* hydrochloric acid, then washed with hot water until free of hydrochloric acid, and collect the filtrate in a 100-ml. volumetric flask. Wash the filter with hot water until washings are no longer acid to methyl red. Add a drop of methyl red indicator to the filtrate in the 100-ml. flask, add *N* ammonium hydroxide until neutral to methyl red, then add 4 ml. of *N* hydrochloric acid. Allow the contents of the flask to cool, then make to volume with water.

FIRST EXTRACTION (separation of dithizone complex-forming metals from the ash solution). Pipet an aliquot of the ash solution containing not more than 30 micrograms of zinc into a 125-cc. Squibb separatory funnel. Add 1 ml. of 0.2 *N* hydrochloric acid for each 5 ml. of ash solution less than 10 ml. taken, or 1 ml. of 0.2 *N* ammonium hydroxide for each 5 ml. over 10 ml. taken. A 10-ml. aliquot has usually been found satisfactory in the analysis of plant materials. Add 40 ml. of Solution A and 10 ml. of dithizone reagent. Shake vigorously for 0.5 minute to extract from the aqueous phase the zinc and other dithizone complex-forming metals which may be present, then allow the layers to separate. At this point an excess of dithizone, which is indicated by an orange or yellow-orange coloration of the aqueous phase, must be present. If excess dithizone is not present, add more of the reagent until after shaking an excess is indicated.

Shake down the drop of carbon tetrachloride extract from the surface, and draw off the carbon tetrachloride extract into a second separatory funnel as completely as possible without allowing any of the aqueous layer to enter the stopcock bore. Rinse down the carbon tetrachloride extract from the surface of the aqueous layer with a 1- to 2-ml. portion of clear carbon tetrachloride, then run off this carbon tetrachloride into the second funnel without permitting the aqueous phase to enter the stopcock bore. Repeat this rinsing process as many times as is necessary to flush the extract completely into the second funnel. Add 5 ml. of clear carbon tetrachloride to the first funnel, shake 0.5 minute, and allow the layers to separate. The carbon tetrachloride layer at this point will have a clear green color if metals which form dithizone complexes have been completely extracted from the aqueous phase by the previous extraction. Run off the carbon tetrachloride layer into the second funnel, then flush down the extract from the surface and out of the funnel as was done before. If the last extract does not possess a distinct clear green color, repeat the extraction with a 5-ml. portion of clear carbon tetrachloride and the flushing out process until complete extraction of the dithizone complex-forming metals is assured, then discard the aqueous phase.

SECOND EXTRACTION (separation of copper by extraction of zinc into 0.02 *N* hydrochloric acid). Pipet 50 ml. of 0.02 *N* hydrochloric acid into the separatory funnel containing the carbon tetrachloride solution of metal dithizonates. Shake vigorously for 1.5 minutes, then allow the layers to separate. Shake down the drop from the surface of the aqueous phase, and run off as completely as possible the carbon tetrachloride phase, which contains all the copper as dithizonate, without allowing any of the aqueous phase, which contains all the zinc, to enter the stopcock bore. Rinse down the carbon tetrachloride extract from the surface of the aqueous phase and rinse out the stopcock bore with 1- to 2-ml. portions of clear carbon tetrachloride (in the same manner as was done in the first extraction) until all traces of green dithizone have been washed out of the funnel. Shake down the drop of carbon tetrachloride from the surface of the aqueous phase, and run off the carbon tetrachloride as completely as possible without allowing any aqueous phase to enter the stopcock bore. Remove the stopper from the funnel and lay it across the neck of the funnel until the small amount of carbon tetrachloride on the surface of the aqueous phase has evaporated.

FINAL EXTRACTION (extraction of zinc in the presence of carbamate reagent). Pipet 50 ml. of Solution B and 10 ml. of dithizone reagent into the 50 ml. of 0.02 *N* hydrochloric acid solution. Shake for one minute, then allow the phases to separate. Flush out the stopcock and stem of the funnel with 1 ml. or so of the carbon tetrachloride extract, then collect the remainder in a test tube. Pipet 5 ml. of the extract into a 25-ml. volumetric flask and dilute to the mark with clear carbon tetrachloride, and then determine the per cent light transmission of the diluted solution with a photoelectric colorimeter, equipped with a Sextant green (Corning No. 401) filter, or equivalent. Readings should be taken not later than 2 hours after the final extraction. Protect extracts from light as much as possible.

A blank determination must be run with each series of zinc determinations in order to determine the correction to be applied for the zinc present in the hydrochloric acid and ammonium hydroxide in the ash solution and the zinc picked up by the other reagents from their containers since the determination of the standard curve. The blank determination is carried out exactly as is done in the case of the plant material, using as a starting point an empty platinum dish that is placed in the muffle along with the samples of plant material.

The amount of zinc on the standard curve corresponding to the per cent light transmission obtained for the unknown less the amount of zinc corresponding to the per cent transmission obtained for the reagent blank gives the amount of zinc present in the aliquot. From this the amount of zinc in the sample is easily calculated. As many as 12 determinations may conveniently be carried simultaneously through this procedure.

Standard Curve

The data for the standard curve are obtained by determining the per cent transmission values for each of a series of solutions containing known amounts of zinc. These zinc solutions are prepared as follows:

Place 0, 5, 10, 15, 20, 25, 30, and 35 ml. of the standard zinc solution containing 10 micrograms of zinc per ml. in 100-ml. volumetric flasks. To each add one drop of methyl red indicator, *N* ammonium hydroxide until neutral, and 4 ml. of *N* hydrochloric acid, and make to volume. Proceed from this point in exactly the same manner as in the case of ash solutions, beginning with the first extraction and using 10-ml. aliquots of each of the zinc solutions. The 10-ml. aliquots contain 0, 5, 10, 15, 20, 25, 30, and 35 micrograms of zinc, respectively.

The standard curve is constructed by plotting micrograms of zinc against per cent light transmission.

A One-Color Method

An investigation was carried out to determine whether the method was reliable if the excess dithizone was removed from the final carbon tetrachloride extract, and the color comparisons were made on the red zinc dithizonate solutions obtained. The color comparisons, if the method proved satisfactory, could then be made conveniently by use of either an ordinary visual colorimeter or a photoelectric colorimeter. It was found that the excess dithizone could be readily removed from the final dithizone extract without appreciable loss of zinc by a wash with 50 ml. of 0.01 *N* ammonia, and that the per cent transmission of the solutions obtained was a reproducible function of the amount of zinc present. Copper was not found to interfere when present in amounts up to 100 micrograms, but metals which are extracted along with the zinc into the 0.02 *N* hydrochloric acid solution, such as lead, were found to interfere. It seems that during the removal of the excess dithizone the colorless lead carbamate complex present in the carbon tetrachloride extract is decomposed.

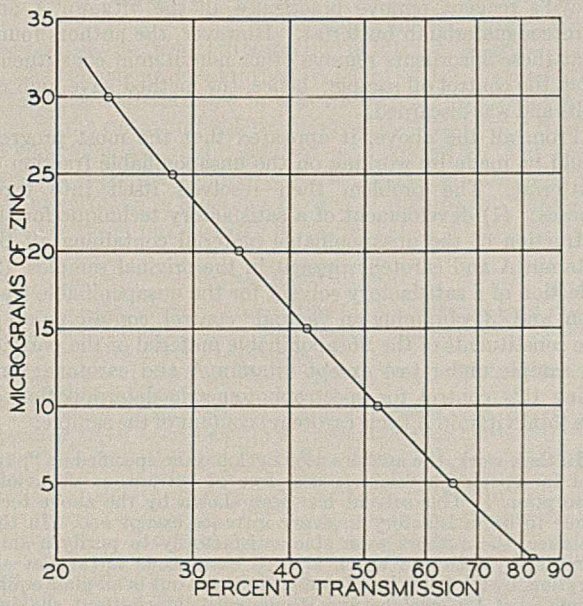

FIGURE 3. STANDARD CURVE OF RELATIONSHIP BETWEEN ZINC PRESENT AND PER CENT TRANSMISSION OF DITHIZONE EXTRACT

Filter. Sextant green (6.00 mm.)
Cell thickness. 1 cm.

The lead then combines with dithizone and forms its red dithizone complex, which causes high results. The one-color method was therefore considered inferior to the mixed color method because of these interferences.

Choice of Color Filter

The light transmission of zinc dithizonate (0.6 mg. of zinc per liter of carbon tetrachloride) for different wave lengths of light was determined by use of a Cenco spectrophotelometer, and is represented by the solid curve on Figure 2. This curve shows that zinc dithizonate has a narrow absorption band between 450 and 575 microns with a maximum absorption at about 535 microns. It is therefore necessary to use in the photoelectric colorimeter a filter that transmits a narrow band of light in this same region of the spectrum, in order to obtain a maximum sensitivity of the instrument to differences in concentration of zinc dithizonate in the solutions compared. These requirements seem to be met best by the Sextant green (Corning No. 401) filter. The light transmission characteristics of this filter (1-mm. thickness) are represented by the dotted curve on Figure 2. A comparison of the two curves indicates the close coincidence of the region of light transmission of this filter with the region of light absorption by the zinc dithizonate solution.

In the preliminary investigation a filter of 3.52-mm. thickness was used. It was found that even greater sensitivity could be obtained by using both the 3.52-mm. and a 2.48-mm. filter, which gave a total filter thickness of 6.00 mm. The standard curve obtained using both Sextant green filters of a total thickness of 6.00 mm. is shown on Figure 3, where per cent transmission is plotted as a function of the amount of zinc present for 1-cm. cells by the mixed-color method. This curve and filter were used in obtaining the data given in Tables II, III, and IV.

Other Applications of Method

Although this method for zinc has been applied here only to the analysis of plant materials, there is no apparent reason why it should not serve equally well for the determination of small amounts of zinc in many other types of materials from which the zinc present may be obtained in solution. If less than one half as much cadmium or less than ten times as much of other dithizone complex-forming metals as zinc are present and substances are not present which are not kept in solution by citrate during the first extraction, one should be able to apply the method directly. Excessive amounts of other dithizone complex-forming metals would be troublesome because of the large volume of dithizone extract that would be obtained in the first extraction. Should trouble be encountered for any of these reasons, a preliminary separation of the substances causing the difficulty would be necessary.

Summary

A photometric, "mixed-color" dithizone method has been developed for the determination of zinc in plant materials in which sodium diethyldithiocarbamate is used to eliminate the interference of other metals which form colored complexes with dithizone. It was found that "carbamate" causes an appreciable reduction in the color intensity of the dithizone extract, but, by keeping conditions constant in all extractions, a reproducible relationship is obtainable between the color intensity of the dithizone extract as measured with a photometric colorimeter and the amount of zinc present.

Tests involving the determination of zinc in the presence of other metals which form dithizone complexes, the recovery of added zinc from various plant materials, and the agreement between duplicate determinations proved the method to be accurate and remarkably free of interferences.

A one-color method for the determination of zinc was tested and found to be inferior to the mixed-color method.

The light transmission curve of zinc dithizonate in carbon tetrachloride was determined with a spectrophotometer. This curve is discussed in relation to filter selection for the photometric determination of zinc with dithizone.

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Determination of Total Vitamin A Content of Dairy Butters

Spectrophotometric Method

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AN ACCURATE and relatively rapid method for the determination of the total—(i. e., carotene plus vitamin A)—vitamin A potency of dairy butter has been desirable, in order to make a more thorough study of the range of vitamin A potency in this food product. It is generally recognized that the biological method is of only limited accuracy, with rather wide variations in results to be expected (*2*).

Literature Review

A review of the literature bearing on the subject reveals that many methods involving various techniques and instruments have been used by different investigators in an attempt to determine the vitamin A potency of dairy butter by physical methods.

Fraps and Kemmerer (*5*) have estimated the vitamin A in butter by determining the absorption at 328 millimicrons (corrected for carotene absorption) of a methanol solution of the unsaponifiable residue, after chilling out light-absorbing impurities, and estimating the carotene content by colorimetric comparison against potassium bichromate. The authors' experience with this method has led them to believe that it gives high results, probably because the chilling does not remove all the nonvitamin material which absorbs light at 328 millimicrons. And, of course, it would be advantageous to determine both vitamin A and carotene in one operation.

Gillam, Heilbron, Morton, Bishop, and Drummond (*7*) attacked the problem by separating the carotene and xanthophyll, and determining these substances separately by spectrographic means, then determining vitamin A by its absorption at 328 millimicrons in the presence of carotene in one fraction and of xanthophyll in the other fraction (correcting for carotenoid absorption). This method is time-consuming and does not take into consideration the natural unsaponifiable materials in oils and fats which absorb some light in the region of 328 millimicrons, but which possess no vitamin A activity. A short time later, Gillam (*6*) improved and shortened the method by showing that about 94 per cent of the light absorption at 455 to 460 millimicrons (in chloroform) is due to carotene, and that the absorption at 328 millimicrons, due to carotene plus xanthophyll, can be corrected for by a constant factor. However, there still remained the problem of accounting for other unsaponifiable materials of no vitamin A potency, which do have general absorption in the region of 328 millimicrons, and hence give high results.

Baumann and Steenbock (*3*) determined carotene and vitamin A separately. For carotene, the absorption values were measured at 460 and 485 millimicrons on melted whole butterfat, and for calculation they compared these values with the absorption of standard solutions of carotene in refined cottonseed oil. The authors' experience over a number of years has indicated that the glyceride composition, free fatty acid content, color components, and other factors such as unsaponifiable content both of refined cottonseed oil and of butterfat are very variable; hence, results which do not take these factors into account may be somewhat variable. Vitamin A was determined on the butterfat unsaponifiable, in methyl alcohol solution, by determining the absorption at 328 millimicrons after first purifying by chilling at −72° C. in a solid carbon dioxide–acetone mixture. These workers recognized that at times excess absorption was found at 328 millimicrons; and attempted to minimize errors that might otherwise be thus introduced by discarding all results in which they found that the absorption at 280 millimicrons exceeded that at 328 millimicrons. However, it is evident from a study of their work that an "actual" control for the spectrophotometric determination of carotene and vitamin A in dairy butter should be very helpful.

Experimental

Attempts were made to determine spectrophotometrically the vitamin A and carotene in whole butterfat, by first removing the vitamin materials by the use of adsorbents and using the devitaminized oil as a control for the untreated oil for direct spectral determination in a suitable solvent. Kraybill and Shrewsbury (*8*, *9*) have shown that charcoal and Lloyd's reagent remove practically all the vitamin A and carotene material in butterfat. However, the authors found that these adsorbents remove other nonvitamin constituents from the control oil sample; hence, the method gave high results and was discarded.

From all the above, it appeared that the most progress could be made by working on the unsaponifiable fraction of butterfat. The problem then resolved itself into three phases: (1) development of a satisfactory technique for the extraction of the unsaponifiable material containing all the vitamin A and carotene present in the original samples, (2) selection of a satisfactory solvent for the unsaponifiable fraction, and (3) obtaining an "actual" control, containing all of the constituents of the unsaponifiable material of the particular sample under test except vitamin A and carotene; and using this control for spectrophotometric determination of the total vitamin A (plus carotene) content of the sample.

In their work, the authors used cyclohexane specified as "purified for spectrophotometric use, free of extraneous ultraviolet absorption". This solvent has been shown by the above technique to be satisfactory in every instance except one. In this instance, the authors were able satisfactorily to purify a shipment of cyclohexane which showed extraneous ultraviolet absorption, by the following treatment, carried out in all-glass equipment: Let the cyclohexane stand, with intermittent shaking, in contact with 10 per cent by weight of concentrated sulfuric acid, for a period of one week, and at a temperature of about 21° to 27° C. (70° to 80° F.); decant the greater part of the solvent from the acid, and distill through a fractionation column.

The most difficult problem encountered in connection with the extraction of unsaponifiable material was that of obtaining solvents in sufficiently pure form. It has been the authors' experience that the ether and alcohol solvents used for this purpose must be carefully purified immediately before use, in order to avoid oxidation of the unsaponifiable material. Details of the purification processes used for these solvents are given under "Method".

Of the optically satisfactory solvents, cyclohexane was found to be the most satisfactory because it is a good solvent for fat and unsaponifiable matter under the conditions employed, it is satisfactory from the standpoint of volatility, and vitamin A and carotene have been demonstrated, in this laboratory, to be stable in a cyclohexane solution for several days, provided that the solutions are stored in the dark and that the cyclohexane is exceedingly pure. It has been the authors' practice to purchase cyclohexane which has been specially purified for spectrophotometric purposes; before using, they compare this solvent spectrophotometrically with a sample known to be satisfactory. Cyclohexane which shows no extraneous absorption in the region between

5000 and 2200 Å. is considered to be sufficiently pure for use; while any sample which shows extraneous absorption within this region is rejected as being impure.

It is well known that vitamin A and carotene are destroyed by ultraviolet light. Demarest (*4*) published the results of his studies on vitamin A determination by destructive irradiation while this present work was in progress.

Preliminary work showed that the vitamin A content of whole butterfat could not successfully be destroyed by ultraviolet light irradiation (from any source of ultraviolet at the authors' command), probably because of the protection afforded to the vitamin compounds by the glycerides of the whole oil. However, it was found that the vitamin A and carotene could be successfully destroyed by ultraviolet irradiation of a cyclohexane solution of the unsaponifiable extract from butterfat. This irradiated solution, containing all of the unsaponifiable fractions of the sample under test (except the vitamin A, carotene, and xanthophyll which have been destroyed by the irradiation), serves as a satisfactory control for the spectrophotometric measurements of the vitamin A and carotene in another portion of the same original cyclohexane solution of the unsaponifiable matter which has not been subjected to the action of ultraviolet light.

The following method, then, is based on the destruction of vitamin A and carotene in a portion of a cyclohexane solution of the unsaponifiable fraction of butterfat, and the use of this "devitaminized" solution as a control for the simultaneous spectrophotometric determination of both vitamin A and carotene in a second portion of the original unsaponifiable solution not exposed to the action of ultraviolet light.

Equipment Used

Spectrophotometer. Adam Hilger, Ltd., intermediate quartz spectrograph with Spekker photometer, equipped with tungsten steel electrodes as a source of light.

Quartz absorption cells, Hilger Type C, 1-cm. Quartz Kjeldahl-shaped flasks, 25-cc.

Ultraviolet lamp. Mercury lamp, rectifier-type quartz arc operated to be equivalent to a 250-watt direct current Uviarc: Uviarc poultry treater, Type RT, Spec. 100, Cooper Hewitt Electric Co., Hoboken, N. J. Operated through an alternating current auxiliary, Code No. 260, 7A4 × 4, 186 to 254 volts, 60 cycles, 2.4 amperes, 450 watts, made by Cooper Hewitt Electric Co., Hoboken, N. J. (The spectral radiation for this lamp can be obtained by taking three fourths of the values given in column 1—i. e., for the 120-volt direct current Uviarc—of data sheet S-201 compiled by the Commercial Engineering Department, General Electric Vapor Lamp Co., Hoboken, N. J.)

Method

Extraction of Unsaponifiable Material. Melt the butter in a water bath at about 60° C., and separate the fat by filtration through a Whatman No. 12 folded filter paper.

Saponify 25 grams of the fat with 30 cc. of a 20 per cent solution of potassium hydroxide in alcohol (specially denatured No. 30) by boiling, with a suitable reflux arrangement, for 5 minutes only.

Dilute the alcoholic solution with water to approximately four volumes and cool in an ice-water bath. Then extract the unsaponifiable matter with cold ethyl ether (usually four extractions with the following successive amounts of ether are sufficient: 180, 150, 100, 50 cc.). Vigorous shaking is required for complete extraction of the unsaponifiable material.

Composite the ether extracts and wash with 150-cc. portions of distilled water until substantially free of alkali, taking due care to avoid troublesome emulsions. Usually six washings are sufficient. The first two washings should be made by merely pouring the water through the ether without shaking.

Filter the ether solution through filter paper and concentrate to 25 to 50 cc. by distillation on a steam bath. Remove the remainder of the ether by evaporating, on a steam bath, under a stream of carbon dioxide to prevent oxidation. When substantially all the ether has been evaporated, cool immediately to about 21° C. (70° F.), and dissolve the unsaponifiable residue in cyclohexane. Make the cyclohexane solution up to 50 cc. (50 per cent solution, weight to volume, on original oil basis). Filter and store in the dark at about 4° to 10° C. (40° to 50° F.) until the sample is examined spectrophotometrically. (The sample should not be stored more than 2 days before its vitamin content is determined.)

Note. Both the ether and alcohol must be carefully purified immediately before using. This is satisfactorily accomplished in the case of ethyl ether (anhydrous C. P.) by letting the ether stand with intermittent shaking, for 3 hours, over a 5 per cent aqueous potassium hydroxide solution, then slowly distilling. The alcohol (specially denatured No. 30) is satisfactorily purified by treating with potassium hydroxide and aluminum grains followed by distillation (*1*).

Spectrophotometric Determination of Carotene and Vitamin A. Divide the cyclohexane solution of unsaponifiable material in two parts, and irradiate one portion, contained in a 25-cc. Kjeldahl-shape quartz flask, under the Uviarc as follows (allowing at least 10 minutes for the lamp to come to full operating temperature before starting irradiations): Stopper the flask with a cork wrapped in aluminum foil, and support the flask in such a position that the cork rests against the rim of the lamp and the bulb of the flask is held about 10 cm. (4 inches) away from the mercury tube of the lamp. Place a sheet of aluminum foil about 7.5 cm. (3 inches) below the flask being irradiated (to reflect the light back toward the sample). Agitate the sample every 15 minutes by gently tapping the flask (for example, with a pencil). Allow the sample to heat up as much as the lamp will heat it, provided that the temperature of the sample does not reach the boiling point of cyclohexane.

Irradiate until the vitamin A and carotene have been destroyed. Under the authors' conditions, 1 to 1.5 hours has proved sufficient time for irradiation. Destruction of carotene and vitamin A can be estimated by the disappearance of the carotene yellow color, and by the Carr-Price test.

After cooling to about 21° C. (70° F.), filter the irradiated solution, which must be clear and colorless, and determine carotene and vitamin A in the nonirradiated solution by means of the spectrophotometer (1-cm. cells) using as a control the ultraviolet irradiated solution. Expose the plates at density settings ranging from 0 to 1.50 in increments of 0.05, with the exposure time graduated up to about two seconds on Eastman 33 plates (D 72, diluted 1 to 2, is a satisfactory developer for these plates).

Read the carotene and vitamin A match points from the same plate. The vitamin A is read at 328 millimicrons (3280 Å.) and the carotene at 460 millimicrons (4600 A.). (These wave lengths as points of maximum absorption are in agreement with published works, *6*, and have been verified in this laboratory for solutions of vitamin A concentrates and carotene in cyclohexane.) Of course, the difference in absorption between the nonirradiated and the irradiated sample, at the wave lengths indicated above, is a measure of the vitamin A and carotene content of the sample.

Calculations. *I. Carotene in Terms of Vitamin A.*

Match point at 4600 A. × 0.94 = corrected match point at 4600 A.

$$\frac{\text{Corrected match point at 4600 Å.} \times 4.54 \times 1000}{0.50 \times 2.1 \times 0.6} = \text{U. S. P. XI}$$

vitamin A units per pound of butterfat, due to carotene

Based on:

1. 94 per cent of the light absorption at 4600 Å. is due to carotene (*6*).
2. $E_{1\text{ cm.}}^{1\%}$ at 4600 Å. for carotene in cyclohexane = 2100. Established for the authors' instrument by the use of a sample of β-carotene containing about 10 per cent of α-carotene, obtained from the S. M. A. Corp., Cleveland, Ohio.
3. By definition, 0.6 microgram of β-carotene equals one International unit of vitamin A (*10*).

II. Vitamin A.

$$\text{Match point at 3280 Å.} - \frac{\text{match point at 4600 Å.}}{6.5} = \text{corrected match point at 3280 Å.}$$

$$\frac{\text{Corrected match point at 3280 Å.}}{0.50} \times (2140 \times 4.54) = \text{U. S. P. XI}$$

vitamin A units per pound of butterfat, due to vitamin A

Based on:

1. The absorption at 328 millimicrons due to carotene plus xanthophyll is given by dividing the observed value of $E_{1\text{ cm.}}^{1\%}$ for these substances at 455 to 460 millimicrons (*6*) by the factor 6.5.

TABLE I. ESTABLISHMENT OF INSTRUMENT FACTOR

(Used at 2140)

Date of Determination	Per Cent Solution (Original Oil Basis)	Match Point at 3280 Å.	$E^{1\%}_{1\ cm.}$ Value	Instrument Factor
2-20-39	0.7000	0.98	1.400	2143
2-24-39	0.7000	0.98	1.400	2143
2-27-39	0.7000	0.99	1.414	2122
3- 4-39	0.8000	1.12	1.400	2143
3- 6-39	0.9000	1.25	1.389	2159
3-13-39	0.7000	0.98	1.400	2143
			Av.	2142

2. 2140 as the instrument factor for vitamin A.

Total. I + II = total U. S. P. units of vitamin A per pound of butterfat due to both carotene and vitamin A.

Notes. The above correction factors have been accepted from the literature (*6*) without verification in the laboratory because they are considered as average figures, and any probable individual variation from these average figures would result in only minor differences in the final results.

As a check on the above irradiation procedure, the authors have adopted the following practice:

A second spectrophotometric determination is made on each (nonirradiated) unsaponifiable solution; however, pure cyclohexane is used instead of the above irradiated solution of the unsaponifiable as a control for this (second) determination. The match point at 3280 Å. is compared with that obtained in the original determination. Of course, the match point obtained by the use of the pure cyclohexane control is numerically greater than that obtained by the use of the irradiated unsaponifiable solution as a control. The results of approximately 100 determinations have indicated that this difference is in the range of 10 to 25 per cent. Any difference greater than 25 per cent between the match points (at 3280 Å.) obtained from the cyclohexane and from the irradiated control would indicate the possibility of incomplete destruction of vitamin materials in the irradiated sample, oxidation, or evaporation of solvent, and would necessitate repetition of the determination.

DERIVATION OF INSTRUMENT FACTOR OF 2140. The instrument factor for converting from the $E^{1\%}_{1\ cm.}$ value for vitamin A, read at 3280 Å. units, to U. S. P. units of vitamin A per gram, was determined for the authors' instrument from the U. S. P. standard of reference cod liver oil.

Results of several instrument factor determinations are listed in Table I. In these determinations the authors used U. S. P. reference cod liver oil containing 3000 U. S. P. XI vitamin A units in 1 gram of the oil. Distribution date was 11-29-38, control 2-38-A, not to be used after 5-29-39.

Note. The method used for the determination of the instrument factor is as follows (the saponification and extraction procedure is a modification of the procedure published by Wilkie, *11*):

Saponify 0.7 to 1.0 gram of U. S. P. standard of reference cod liver oil in a 250-cc. Erlenmeyer flask with 3 cc. of a 50 per cent aqueous solution of potassium hydroxide, after the addition of 30 cc. of purified (see method above) specially denatured alcohol No. 30, by heating on a hot plate for exactly 3 minutes. Suitable reflux arrangements must be provided to prevent loss of solvent during saponification.

Add 20 cc. of distilled water and cool by placing the flask in ice water for 10 minutes.

Transfer the solution to a 250-cc. pear-shaped separatory funnel; extract the unsaponifiable material by shaking vigorously for 1 minute each time, with three 50-cc. portions of freshly purified ethyl ether. (It is good practice to leave the first extraction in the original funnel, and carry out the two subsequent extractions in another funnel.)

Wash the combined ether extracts as follows: Wash twice by pouring 100-cc. portions of distilled water through the ether extract; wash once by pouring 25 cc. of 0.25 *N* potassium hydroxide through the ether extract; wash twice by gently shaking with 30-cc. portions of distilled water.

Filter the washed ether extract into a 250-cc. Erlenmeyer flask. Evaporate the ether on a steam bath, under an atmosphere of carbon dioxide. As soon as the ether has evaporated, cool the flask quickly and dissolve the unsaponifiable residue in cyclohexane.

Make the solution up to 100 cc., shake thoroughly, and filter. Store the sample in the dark, at about 4° to 10° C. (40° to 50° F.), until ready to complete the determination spectrophotometrically. The determination should be completed on the same day that the extraction is made.

Determine the vitamin A content of the unsaponifiable solution by means of the spectrophotometer, using pure cyclohexane as a control and 1-cm. absorption cells.

Calculation of Instrument Factor.

$$E^{1\%}_{1\ cm.}\ \text{value} = \frac{\text{match point at 3280 Å.}}{\%\ \text{concentration (on basis of original reference oil)}}$$

$$\text{Instrument factor} = \frac{\text{U. S. P. XI vitamin A units per gram of reference oil}}{E^{1\%}_{1\ cm.}\ \text{value}}$$

DISCUSSION. In order to establish the fact that the ultraviolet irradiation in the above method actually destroys the biological activity of the vitamin A and carotene in the unsaponifiable extract, the following test was made:

The solvent was vacuum-distilled from a solution of butterfat unsaponifiable which had been irradiated according to the above procedure; the irradiated unsaponifiable material thus recovered was then dissolved in hydrogenated cottonseed oil in an amount equivalent to the unsaponifiable content of the original butterfat. This sample of hydrogenated cottonseed oil, containing the irradiated butterfat unsaponifiable, was submitted to a biological laboratory for determination of its biological activity by the U. S. P. XI method.

The result of this assay, given in Table II, shows that the ultraviolet irradiation of the unsaponifiable material from butterfat actually destroys its biological vitamin A activity. Hence, the use of the irradiated control in the above method may be considered to be justified.

Stability of Extracted Carotene and Vitamin A. In order further to justify the conclusion drawn from Table II, it was necessary to demonstrate that (nonirradiated) carotene and vitamin A, extracted from butterfat by the above method and dissolved in hydrogenated cottonseed oil, would be stable in hydrogenated cottonseed oil for several weeks. At the same time, it was convenient to obtain a confirmation of the method by a biological assay.

A sample of butterfat was analyzed spectrophotometrically by the above method. The solvent was then vacuum-distilled from the nonirradiated sample, and the unsaponifiable material thus recovered was dissolved in hydrogenated cottonseed oil in an amount equivalent to the unsaponifiable content of the original butterfat. This hydrogenated cottonseed oil, containing the above butterfat unsaponifiable, was then periodically analyzed spectrophotometrically over a period of 11 weeks. During this 11-week interval, a portion of the same sample (of hydrogenated cottonseed oil containing the nonirradiated butterfat unsaponifiable) was sent to a biological laboratory for determination of its vitamin A content by the U. S. P. XI method.

TABLE II. BIOLOGICAL ASSAY OF IRRADIATED BUTTERFAT UNSAPONIFIABLE DISSOLVED IN HYDROGENATED COTTONSEED OIL

[Sample: hydrogenated cottonseed oil containing irradiated unsaponifiable from butterfat, in amount equal to unsaponifiable content of original butterfat. Biological (U. S. P. XI) method]

Level Assayed, U. S. P. Units Vitamin A per Pound	Daily Dose		Average Gain in Weight		U. S. P. Units Vitamin A per Pound
	U. S. P. reference oil	Sample	On reference oil	On sample	
	Mg.	*Mg.*	*Grams*		
Determine if sample has any vitamin A activity	0.5	500	27.8	Animals died after 11-20 days	Practically nil

TABLE III. STABILITY OF VITAMIN CONTENT OF BUTTER FAT UNSAPONIFIABLE DISSOLVED IN HYDROGENATED COTTONSEED OIL

Sample	Spectrophotometric Determination No.	Match Point At 4600 Å.	Match Point At 3280 Å.	Carotene[a]	Vitamin A[a]	Total[a]
Butterfat, unsaponifiable in cyclohexane solution	1 (12– 1–39)	0.85	0.95	5700	15,900	21,600
	2 (12–12–39)	0.85	0.95	5700	15,900	21,600
					Av.	21,600
40 Per Cent Solution						
Hydrogenated cottonseed oil containing nonirradiated unsaponifiable from above butterfat in an amount equal to the unsaponifiable in original butterfat	1 (12–12–39)	0.65	0.80	5500	16,600	22,100
	2 (12–26–39)	0.63	0.75	5300	15,800	21,100
	3 (2–1–40)	0.65	0.80	5500	16,600	22,100
					Av.	21,800

Biological (U. S. P. XI) Method

Sample	Level Assayed, U. S. P. Units Vitamin A per Pound	Daily Dose U. S. P. reference oil	Daily Dose Sample	Average Gain in Weight On reference oil	Average Gain in Weight On sample	U. S. P. Units Vitamin A per Pound
		Mg.	*Mg.*	*Grams*	*Grams*	
Hydrogenated cottonseed oil containing nonirradiated unsaponifiable from above butterfat in an amount equal to the unsaponifiable in original butterfat	19,000	0.5	35.8	27.8	27.1	19,000
	24,000	0.5	28.3	27.8	19.4	

[a] As U. S. P. units of vitamin A per pound of butterfat, rounded off to nearest 100 units.

The results presented in Table III show that carotene and vitamin A, extracted from butterfat and dissolved in hydrogenated cottonseed oil, are stable in the hydrogenated cottonseed oil for at least the 11 weeks during which the sample was under test (hence the conclusion drawn from Table II is still further justified). These results also show that the method gives results which are in reasonable agreement with the U. S. P. XI method (on this one sample).

Duplication of Results. The above method has been used for the determination of the total vitamin A content of many samples of dairy butter, produced over a period of more than a year, and has been found to give duplicate results within very good agreement. Typical results are tabulated in Table IV for the purpose of showing the limits of variability which may be expected from this method. From these results it may be concluded that duplicate results obtained by the above method, applied to butters of widely varying vitamin content, agree to within less than ±6 per cent.

Further Confirmation of Results by Biological Assays. In order to determine the agreement between the above spectrophotometric method and the biological method for the determination of vitamin A in dairy butters of widely varying vitamin content, samples 1, 2, 4, and 5 listed in Table IV were sent to a biological laboratory for assay by the U. S. P. XI method. These samples were biologically assayed on the separated butterfat. The results of this comparison (by the two methods) are listed in Table V. Bearing in mind the known variation in results obtainable by biological methods, the data given in Table V may be considered to indicate that results obtained by the spectrophotometric method are in reasonable agreement with those obtained by the U. S. P. XI method.

Effect of Added Coloring Agents. The method is entirely satisfactory for butter colored with annatto, since the caustic treatment and subsequent washings adequately remove the annatto coloring materials. However, azo dyes seriously interfere, and the method is not satisfactory for butter colored with F. D. & C. yellows 3 and 4, formerly known as AB and OB, respectively. These dyes are not removed by the saponification process, and are affected by the ultraviolet light irradiation. Hence samples which show, by chemical test, the presence of these dyes have necessarily been discarded without attempting to determine their vitamin A content by the above method.

Summary

A spectrophotometric method for the determination of the total vitamin A content, including carotene, in dairy butter has been developed. This method is based on the destruction of both the carotene and vitamin A in a portion of the unsaponifiable extract by ultraviolet light irradiation, and the use of this irradiated portion as a control for the simultaneous determination of both carotene and vitamin A by means of the spectrophotometer. The method has been shown to yield results that are in satisfactory agreement within themselves and in reasonable agreement with the U. S. P. XI method.

TABLE IV. LIMITS OF VARIABILITY ON RESULTS FOR A GIVEN SAMPLE

Sample No.	Spectrophotometric Determination No.	Match Point (*vs.* Irradiated Control) At 4600 Å.	Match Point (*vs.* Irradiated Control) At 3280 Å.	Carotene[a]	Vitamin A[a]	Total[a]
1	1	0.87	0.96	5800	16,100	21,900
	2	0.88	0.88	5900	14,400	20,300
	3	0.95	1.0	6300	16,500	22,800
2	1	1.20	1.10	8000	17,900	25,900
	2	1.25	1.05	8400	16,700	25,100
	3	1.25	1.10	8400	17,700	26,100
3	1	0.18	0.30	1200	5,200	6,400
	2	0.18	0.28	1200	4,800	6,000
	3	0.18	0.28	1200	4,800	6,000
4	1	0.22	0.60	1500	11,100	12,600
	2	0.22	0.55	1500	10,100	11,600
	3	0.20	0.55	1300	10,100	11,400
5	1	0.12	0.25	800	4,500	5,300
	2	0.12	0.25	800	4,500	5,300

[a] As U. S. P. units of vitamin A per pound of butterfat, rounded off to nearest 100 units.

TABLE V. COMPARISON BETWEEN SPECTROPHOTOMETRIC AND BIOLOGICAL METHODS

Sample No.	U. S. P. Units Vitamin A per Pound of Butterfat, Range by Spectrophotometric Method	Biological (U. S. P. XI) Method: Level assayed, U. S. P. units vitamin A per pound of butterfat	Daily Dose U. S. P. reference oil	Daily Dose Sample	Average Gain in Weight On reference oil	Average Gain in Weight On sample	U. S. P. units vitamin A per pound of butterfat
			Mg.	*Mg.*	*Grams*	*Grams*	
1	20,300–22,800	21,000	0.5	32.43	24.9	20.2	A little less than 21,000
2	25,100–26,100	25,000	0.5	27.24	24.9	28.0	A little more than 25,000
4	11,400–12,600	10,000	0.67	90.9	42.0	37.1	A little less than 10,000
		12,000	0.67	75.5	43.6	30.3	
5	5,300	4,000	0.66	227.0	32.4	41.4	A little more than 6000
		6,000	0.66	151.3	32.4	34.4	

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Test Formulas for Reclaimed Rubber

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Compounded test formulas are more desirable for testing reclaimed rubber than the reclaim-sulfur mix, because the test results are more consistent and more indicative of the quality of the reclaimed rubber.

Six types of acceleration which might be used in test formulas were investigated, and the combination of dibenzothiazyldimethylthiolurea and diphenylguanidine was found to be satisfactory for most types of reclaimed rubber.

Several specific recommendations of compounded test formulas for reclaimed rubber are made. If the rubber and fillers are master-batched, the test requires no more time and labor than the reclaim-sulfur test.

IN 1934, Palmer (*3*) presented a definite program for the testing of reclaimed rubber in which it was stated that physical tests in a test recipe containing reclaimed rubber were of definite value if they were known to have a direct and accurate interpretation in reference to the tests on the finished product. This procedure was made secondary to an actual factory run with the reclaim mixed in typical compounds. In the latter case, of course, the tests may be run by the consumer of the reclaimed rubber, whereas in the former case they may be run by the consumer or the manufacturer. It is the desire of the manufacturer to test his product in a manner which properly interprets it for the consumer. For this purpose, test formulas have been coming into fairly general use, and inasmuch as there are innumerable formulas which may be used, it is most desirable and essential to have some uniformity for the sake of universal comparison and simplicity of operation. It is the purpose of this paper to point out the greater uniformity of results obtained with compounded test formulas and to make definite recommendations of several such formulas.

The ideal test formula should not only measure the uniformity of reclaimed rubber from lot to lot, but should also predict to some extent its performance when used in the consumers' products. Although the reclaim-sulfur mix is still used by many for this purpose, it is recognized by most of the reclaim manufacturers and many of the larger consumers that this formula gives erratic and undependable results. The reclaim-sulfur mix contains 100 parts of reclaimed rubber and 5 parts of sulfur, and is generally cured from 15 to 35 minutes at 141.7° C. (287° F.). Winkelmann (*7*), Stafford (*6*), Hurleston (*2*), and Palmer (*3*, *4*) have either mentioned or protested the lack of correlation of the reclaim-sulfur test with the compounding value of the reclaim and have pointed out the shortcomings of this test. It has been shown (*3*) that the reclaim-sulfur test is accurate at best to only 10 per cent and that even the best results are not a criterion of the properties of the reclaim in the recipe in which it is to be used. Maximum variations of from 12 to 19.5 per cent of the mean in the tensile strength and from 10 to 11 per cent in elongation in the reclaim-sulfur mix were reported in 1940 by Palmer and Crossley (*5*).

In contrast to the above inconsistencies, the variation involved in the use of a compounded test recipe is definitely less, as shown in Table I. These tests were run in test formulas IV and V (Table VII). Equal parts cut from three slabs of whole tire reclaim A were thoroughly blended on the mill and from this blend six mixes of each formula were made, two

TABLE I. VARIATION IN PHYSICAL TESTS OF TEST FORMULAS

		Formula IV, Cure 6 Minutes at 158° C.		Formula V, Cure 12 Minutes at 148° C.	
Mix No.	Slab No.	Elongation	Tensile strength	Elongation	Tensile strength
		%	*Kg./sq. cm.*	%	*Kg./sq. cm.*
1	1	415	90.7	585	170.8
	2	410	88.6	600	172.2
2	1	410	91.7	590	171.5
	2	390	87.2	605	172.9
3	1	380	88.2	605	181.7
	2	375	87.9	600	175.1
4	1	395	91.0	585	175.8
	2	380	87.5	580	175.8
5	1	380	87.5	585	177.9
	2	395	92.4	575	176.1
6	1	410	91.0	585	175.4
	2	390	88.6	585	178.9
Average		394	89.4	590	175.3
Maximum variation		40	5.2	30	10.9
Per cent of average		10.1	5.9	5.1	6.2

TABLE II. ANALYSES OF RECLAIMS

	A	B	C
Type	Whole tire	Whole tire	Tube
Color	Black	Black	Neutral
Manufacturing process	All by alkali digestion		
Specific gravity, grams per cc.	1.15	1.15	1.25
Chemical tests			
Acetone extract	8.80	10.80	7.50
Ash	16.50	15.70	32.00
Total sulfur	2.10	1.90	2.10
Carbon black	12.70	14.50	0.70
Alkalinity (as NaOH)	0.20	0.50	0.20
Rubber content (by difference)	59.90	57.10	57.70

TABLE III. FORMULAS AND PHYSICAL PROPERTIES

Type	I Mechanical molded goods	II Tire carcass	III Tire carcass
Test Formulas			
Smoked sheets	10.00	...	31.62
Brown crepe	...	17.00	...
Reclaimed rubber	50.00	65.00	50.00
Zinc oxide	2.00	1.50	1.25
Natural whiting	15.40	9.62	12.00
Clay	20.00	2.00	...
Stearic acid	0.60	0.50	0.75
Pine tar	...	1.50	2.00
Sulfur	1.40	2.50	2.00
Diphenylguanidine	0.60	...	...
Mercaptobenzothiazole	...	0.38	0.38
	100.00	100.00	100.00
Range of Physical Properties			
Time of cure, minutes	6	25	25
Temperature of cure, ° C.	158	141.7	141.7
Elongation, per cent	385–435	485–535	600–650
Tensile strength, kg. per sq. cm.	70–100	105–135	140–170
Shore Type A hardness	67–72	52–57	45–50

TABLE IV. RECLAIM-SULFUR MIX *vs.* TEST FORMULAS

Reclaim	Formula	Cure		No. of Tests	Tensile Strength				Elongation			
					Max.	Min.	Av.	Variation	Max.	Min.	Av.	Variation
		Min.	*° C.*		*Kg./sq. cm.*			*% of average*	%	%	%	*% of average*
A	Reclaim-sulfur	25	141.7	36	56.2	39.7	45.7	29	405	300	355	30
B	Reclaim-sulfur	25	141.7	57	91.4	56.2	69.6	51	470	360	405	27
C	Reclaim-sulfur	25	141.7	30	90.3	53.8	66.8	55	595	460	520	26
A	I	6	158	28	98.1	82.3	86.8	18	480	380	425	24
B	II	25	141.7	54	131.8	107.2	114.9	21	530	435	495	19
C	I	6	158	34	103.0	85.4	90.7	19	510	430	460	17

slabs from each mix were cured, and three strips from each slab were tested. All tests were made in accordance with the standard practice as recommended by the A. S. T. M. (*1*) except that the compounds were aged only 4 hours between mixing and curing. The maximum variations in tensile strength from test to test for test formulas IV and V are 5.9 and 6.2 per cent of the mean, respectively, as compared to the figures of 10, 12, and 19.5 per cent previously referred to for the reclaim-sulfur test. The maximum variations in elongation from test to test for formulas IV and V are 10.1 and 5.1 per cent of the mean, respectively, as compared to 10 and 11 per cent previously reported for the reclaim-sulfur test.

Throughout this paper the term "maximum variation from test to test" is used to describe the variation in physical properties of reclaimed rubber, rather than the more mathematically precise "root mean square error" or "standard deviation", because it is believed that the actual extent to which any one test might differ from any other is of more interest than a mathematical expression of the probable error of any one test. From the point of view of control testing, it is the extreme variations from test to test in the reclaim-sulfur mix which have been most confusing, and these extremes are not emphasized if the variations are expressed as "standard deviations".

As further evidence of the unreliability of the reclaim-sulfur test, a comparison was made of three typical reclaims in the reclaim-sulfur mix and in test formulas I, II, and III (Table III). The characteristic analyses of the reclaims used are given in Table II. A and B are whole-tire reclaims, while C is a tube reclaim of neutral color, all of which are manufactured by the alkali digestion process.

Over a period of 9 months, a considerable number of lots of each reclaim were tested, using a blend of five random samples from each lot for each test. Each blend was simultaneously tested in the reclaim-sulfur mix and in test formulas I or II, as indicated in Table IV. There is up to three times as much variation in tensile strength and up to one and one-half times as much variation in elongation from test to test in the reclaim-sulfur mixes as in the corresponding test formula mixes.

In Table V a comparison has been made of the elongations and tensile strengths of the reclaim samples displaying the greatest variation in reclaim-sulfur tensile strength, with the corresponding tests obtained when the same sample of reclaim is tested in test formulas I, II, and III. The variations in reclaim-sulfur tensile strengths and elongations are large and are not reflected in the results obtained from the test formulas. Consequently, the reclaim-sulfur test cannot be used as an indication of reclaim quality.

These are only a few of the many instances of the unreliability of the reclaim-sulfur test which have been experienced. In most cases the variation in reclaim-sulfur tensile strength is not reflected in the final compound. However, in certain types of compounds where reclaim is the main ingredient, variations in tensile strength of reclaim in the reclaim-sulfur test may carry over to the compound, showing a trend, but the differences are not proportional to those observed in the reclaim-sulfur test.

Throughout this paper, tensile strength and elongation have been considered the criteria of quality by which a compound is judged. In some cases these properties are not entirely indicative of the performance of the ultimate product, and it frequently becomes desirable to obtain tests which more nearly duplicate the service conditions of the final product. For example, such tests may be abrasion and flexing resistance, cold and hot compression set, resistance to heat build-up, resistance to artificial aging, and many others, any of which may be obtained from test formulas, and which serve as definite indications of what may be expected in service, whereas such tests run on the reclaim-sulfur mix would have very little meaning. The authors do not mean to imply that the use of test formulas is the panacea to cure all testing ills, but it is believed that by their use the manufacturer and consumer of

TABLE V. RECLAIM-SULFUR MIX *vs.* TEST FORMULAS

Reclaim	Formula	Cure		Tensile Strength			Elongation		
				Sample having highest tensile	Sample having lowest tensile	Difference	Sample having highest tensile	Sample having lowest tensile	Difference
		Min.	*° C.*	*Kg./sq. cm.*			%	%	%
A	Reclaim-sulfur	25	141.7	56.2	39.7	16.5	405	320	85
	I	6	158	92.5	92.1	0.4	425	435	10
B	Reclaim-sulfur	25	141.7	91.4	56.2	35.2	440	370	70
	II	25	141.7	128.7	115.2	13.5	480	505	25
B	Reclaim-sulfur	25	141.7	82.6	52.7	29.9	685	440	225
	III	25	141.7	163.1	163.8	0.7	645	605	40
C	Reclaim-sulfur	25	141.7	90.3	53.8	36.5	595	460	135
	I	6	158	92.8	91.8	1.0	460	460	0

TABLE VI. ACCELERATION FOR TEST FORMULA

Type of Acceleration	Number of Different Reclaims Tested	Number of Reclaims Reaching Optimum Properties, at 158° C.		
		4 Min.	*6 Min.*	*8 Min.*
Diphenylguanidine	13	None	5	8
Dibenzothiazyldimethylthiolurea	6	2	2	2
Zinc salt of mercaptobenzothiazole and di-*o*-tolylguanidine	4	None (All tensile strengths low)	4	None
Dibenzothiazyldimethylthiolurea and diphenylguanidine	13	2	10	1
Zinc salt of mercaptobenzothiazole	4	None (All tensile strengths low)	2	2
Benzothiazyl disulfide	7	3	2	2

reclaimed rubber may more nearly approach the perfect test, which is, of course, an actual production trial in the consumer's factory, with service tests on the resultant product.

Accelerator Combinations

It has been suggested (*3*, *4*, *5*) that the most desirable test formula to use is one typical of that in which the reclaim will be used. For a routine production control test, however, this is not practical, as it would involve a great number of formulas and considerable laboratory work. Accordingly, it seems desirable to standardize on one or two simple formulas of general nature, which could be used to control the uniformity of all reclaims. Formula I (Table III) answers this requirement, except that reclaims having different rates of cure, when tested in this formula, fail to attain their optimum properties in the same curing time.

For example, reclaim highly alkaline in reaction might reach its optimum cure in 5 to 6 minutes, while a neutral reclaim might require 8 to 10 minutes. Thus, it would be necessary to have a special cure for each type of reclaim. It seems desirable from an efficiency standpoint to standardize on one cure for all reclaims if possible; accordingly, a study was made of a number of different accelerator combinations in an effort to find one which would be flexible enough to meet this condition. These results are shown in Table VI. The reclaims used in this work were typical of all the different types commonly produced. While no one accelerator combination was found to be completely satisfactory, the combination of dibenzothiazyldimethylthiolurea and diphenylguanidine appeared to be the best, since it showed 10 optimum tensiles at 6 minutes out of the 13 reclaims tested.

As only a few of the more common accelerator combinations were investigated, there are doubtless many others which would prove equally satisfactory.

Recommended Formulas

In Table VII are listed three test formulas which are recommended for testing reclaimed rubber. Formulas IV and V employ the dibenzothiazyldimethylthiolurea-diphenylguanidine accelerator combination, and are suggested for routine control testing purposes to replace the reclaim-sulfur test. For simplification in handling, and for time and labor saving, the rubber and fillers in these formulas can be master-batched so that it is only necessary to weigh reclaim and master batch for the final mix. In some cases, it might be desirable also to omit the sulfur from the master batch and add it to the final mix, in which case, three weighings would be required, which compares to two weighings for the reclaim-sulfur mix. If the master batch method is used, the final mix can be made in 5 to 6 minutes, which compares favorably to the time required to mix a reclaim-sulfur batch. The curing time for the test formulas (6 to 12 minutes) is less than for the reclaim-sulfur mix (generally 15 to 35 minutes). The point to be emphasized here is that in replacing the reclaim-sulfur test with a compounded test formula, there need be no increase in the time and labor of testing.

Formula III (Table III) and formula VI (Table VII) are suggested as typical of compounds to be used in testing reclaims for use in carcass or tread compounds. Various tests may be obtained, such as stress, elongation, tensile strength, hardness, artificial aging, abrasion and flexing resistance, and any others which aid in predicting the performance of the reclaim in service.

At the request of the consumer, the manufacturer can and does test reclaimed rubber in a specific test formula which the consumer may submit for this purpose. On the other hand, the consumer may agree to be guided by the results of tests from a test formula suggested by the manufacturer. It is hoped that the suggestion of formulas IV and V particularly, as well as formulas III and VI, will aid in the development of this more practical method of testing reclaimed rubber.

TABLE VII. FORMULAS AND PHYSICAL PROPERTIES

Type	IV General purpose molded goods	V General purpose molded goods	VI Tire tread
Test Formulas			
Smoked sheets	10.00	36.00	36.00
Reclaimed rubber	50.00	50.00	40.00
Zinc oxide	2.00	3.30	2.50
Clay	20.00	4.00	..
Whiting	15.30	2.60	..
Carbon black	..	..	17.70
Stearic acid	1.00	1.30	1.70
Sulfur	1.40	2.31	1.75
Mercaptobenzothiazole	..	..	0.35
Diphenylguanidine	0.10	0.16	..
Dibenzothiazyldimethylthiolurea	0.20	0.33	..
	100.00	100.00	100.00
Range of Physical Properties			
Time of cure, minutes	6	12	35
Temperature of cure, ° C.	158	148	134.5
Elongation, per cent	375–425	575–625	550–600
Tensile strength, kg. per sq. cm.	70–100	160–190	195–225
Shore Type A hardness	70–75	48–53	65–70

Conclusions

The maximum variation from test to test obtained when testing a sample of reclaimed rubber in compounded test formulas is approximately half that experienced when the reclaim-sulfur test is used.

When tested with sulfur alone, reclaimed rubber may show a widely varying tensile strength and elongation from lot to lot, whereas much less variation in results is observed if the reclaimed rubber is tested in a compounded formula. For this reason, and also because the results obtained are more indicative of final performance, test formulas of a general nature are preferable to the reclaim-sulfur test.

When test formulas are used in preference to the reclaim-sulfur mix, there is no increase in the work of testing.

A combination of dibenzothiazyldimethylthiolurea and diphenylguanidine gives very satisfactory results for most types of reclaim.

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Chemical Constituents of Cottonseed Hulls

A Partial Separation by Nonchemical Methods

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Crushed cottonseed hulls, extracted in alcohol-benzene, were ground for a suitable time in a ball mill and the less fibrous portion was separated by sieves and by sedimentation from water into coarse and fine fractions. These were analyzed by standard methods for pentosan, uronic anhydride, and "lignin" (no assumptions were made concerning the chemical nature of the hull constituent responsible for the analytical data). The original hulls contained pentosan, uronic anhydride, and "lignin" to the extent of 27, 6.5, and 25 per cent; the corresponding figures were approximately 3, 9, and 50 per cent for the finest fraction and 40, 6, and 24 per cent, for coarser ones. Thus, unusually rich concentrates of either pentosan or "lignin" are potentially available to industry, although large-scale uses for them have yet to be discovered. The possibility that some other species of plant material, particularly other types of hulls, would respond to the new analytical technique was not investigated.

The detailed results on cottonseed hulls were in agreement with the hypothesis that the pentosan and the "lignin" were present as separate chemical complexes, both of which included uronic anhydride. Klason "lignin" prepared from the hulls contained no methoxyl groups and differed sharply, in this respect at least, from wood lignins.

A MAJOR difficulty in studying the chemistry of plant tissue is caused by the sensitiveness of the components to chemical change during isolation, usually by extraction of the plant material with acid or alkaline solutions or by hydroxylic organic solvents. Lignin, for example, has never been separated in substantial amount in a native condition and the question as to whether it exists as a chemical individual in the plant or is combined with other constituents is still undecided. While studying the hull of the cottonseed, routine estimations led to the conclusion that the dust from the hulls differed markedly in composition from coarser fragments. This foreshadowed a new method of separation which could be conducted without the possibility of chemical change, although the efficiency would probably vary with the botanical structure and chemical composition of the various species of plants. The present study describes the nature and extent of such nonchemical separations of cottonseed hull constituents. Published work on these hulls has been restricted to analytical data (*10*), attempts to find economic uses for them (*11*), and detailed investigations of hemicellulose fractions (*3*, *4*).

The hulls, which had not been treated in any way with chemicals, consisted of oily, hard, cup-shaped brown fragments covered with short fiber. They were exhaustively extracted with hot alcohol-benzene and dried in the air. A qualitative test for nitrogen was negative.

Methods of Analysis

All "lignin" (material insoluble in sulfuric acid), pentosan, and uronic anhydride estimations were made on samples ground to pass completely through a 100-mesh screen, as the impermeable nature of the hulls (*18*) caused very erratic results with particles of larger size. Concordant duplicates were obtained throughout and the data were corrected for moisture, determined by heating separate samples at 105° C.

Samples of weight sufficient to give 0.1 to 0.5 gram of residue were used in the "lignin" determination. They were dispersed in 2 cc. of alcohol, 25 cc. of cold 72 per cent sulfuric acid were added, and, after keeping the mixture at 20° C. for 24 hours, the estimation was completed by a standard method (*15*). The presence of the alcohol prevented caking of the finely divided material on addition of the acid and did not affect the result seriously. Found: no alcohol, "lignin" 25.57, 25.25; with alcohol, 25.27, 25.00 per cent. Klason "lignin" prepared in this way had the composition: carbon, 60.7; hydrogen, 5.23; sulfur, 0; methoxyl, 0 per cent (corrected for 0.4 per cent of ash).

No correction was made in the uronic anhydride analyses for the small amount of carbon dioxide evolved from hexoses (*19*). A sample weight of about 2 grams in 100 cc. of 12 per cent hydrochloric acid was heated in a bath kept at 135° for 5 hours in all cases. Whenever possible, the customary estimation for pentosan (*1*) was corrected for uronic anhydrides by means of a relationship derived from artificial mixtures of xylose and glucuronic acid (euxanthic acid) (*5*). The validity of this proceeding was doubtful, but no more appropriate correction was found.

Cross and Bevan cellulose was prepared generally as described by Doree (*6*), chlorine being passed for a few minutes through an aqueous suspension of the finely divided hulls to minimize local heating. Three chlorinations, alternated with hot sodium sulfite extractions, left a white powder containing 0.4 to 3.5 per cent of "lignin".

An attempted isolation of holocellulose (*14*) from finely ground hulls gave a product containing 17 per cent of "lignin" after four chlorinations and extractions with alcohol-pyridine at room temperature. Still less success was obtained with unground samples.

TABLE I. MILLING CONDITIONS AND COMPOSITION OF HULL FRACTIONS

Milling	Fiber Yield	Screen	Nonfibrous Fractions Yield	"Lignin"	Pentosan[a]	Per Cent of Total "Lignin"	Per Cent of Total Pentosan[a]
Hours	%	*Mesh*	%	%	%		
12	0	<100	100	25.1	32[b]	100	100
6	11	>60	38	20	46	44	77
		60–100	16	21	44	..	..
		<100	35	33	13	46	14
4	12	>60	43	22	45	46	72
		60–100	9	22	42	..	..
		<100	29	30	19	35	17
2	31	>60	49	23	45	45	69
		60–100	0	..	..	..	..
		<100	20	36	10	29	6

[a] Not corrected for uronic anhydride.
[b] 27.3% after correction for uronic anhydride.

MILLING CONDITIONS AND SCREENING EXPERIMENTS. The extracted hulls plus attached fiber, 200 grams, were disintegrated in a rotating ball mill (3.785-liter, 1-gallon, capacity) three quarters full of pebbles, and the product was separated into fractions by shaking through sieves of different mesh. Much of the visible fibrous material survived the shorter periods of milling and formed mats upon the screens from which it was readily removed by hand. As appreciable quantities of adhering hull dust and fragments were entrapped in these mats, the yields of fibers quoted in Tables I and II are high.

Table II. Analysis of Hulls Milled for 2 Hours

Hull Fraction	Screen Analysis[a]	"Lignin"	Uronic Anhydride	Pentosan[b]	Per Cent of Total "Lignin"	Per Cent of Total Uronic anhydride	Per Cent of Total Pentosan[b]
	%	%	%	%			
A. Coarse fiber	32.2	24.0	6.14	24.9	30.8	30.7	29.4
B. Fine fiber	9.8	23.4	5.82	15.6	9.1	8.8	6.0
C. Hulls (fiber-free) >30-mesh	30.7	23.9	6.63	34.0	29.2	31.4	38.2
D. Hulls (fiber-free) 30- to 60-mesh	20.6	25.1	7.25	33.4	20.6	23.0	25.2
E. Hulls <60-mesh	6.8	39.7	8.20	8.1	10.8	8.6	2.0
	100.1				100.5	102.5	100.8
Original ground hulls[c]		25.1	6.48	27.3	100	100	100

[a] Based on air-dry weights.
[b] Corrected for uronic acid.
[c] 2.34 kg.

Twelve hours of milling resulted in a powder, all of which passed through a 100-mesh screen. The "lignin" and pentosan contents of 25.1 and 27.3 per cent, respectively, were therefore the average for the original material These values were in general accord with published data (*2*, *9*, *10*, *20*). With 6 hours or less of grinding, nonfibrous fractions which failed to pass the 100-mesh screen were very similar in composition and were combined in the calculation of the over-all "lignin" and pentosan yields quoted in Table I. While 4 to 6 hours of grinding gave the maximum yield and concentration of pentosan in coarse fractions, less drastic comminution for 2 hours resulted in a fraction passing the 100-mesh screen and containing no less than 36 per cent of "lignin". The same short grinding period was adopted for a larger scale experiment in which the whole of the original material, including fiber mats from the coarser and finer screens, was finally obtained as fractions A to E of Table II. The three right-hand columns of Table II give the amounts of "lignin", uronic anhydride, and pentosan contained in each fraction as a percentage of the total amount. In each case the recovery approximated 100 per cent with an accuracy which probably owed something to a fortuitous cancellation of experimental errors. The fine powder, E, contained 10.8 per cent of all the "lignin" in a state of 39.7 per cent purity. If "lignin" associated with the fibrous fractions was not considered, the above yield became 17.9 per cent. This was not necessarily the maximum possible, because a 2-hour regrinding of the coarse hull fraction, C, with 23.9 per cent of "lignin", reduced one fifth of it to fines with 31.9 per cent. The amount and composition of the various fractions naturally depended greatly on the milling conditions.

Separation by Air Blast. About 50 grams of the powdered, fiber-free hulls were placed in a vertical glass column 90 cm. (3 feet) high and about 6.25 cm. (2.5 inches) in diameter. The lower end was fitted with a 5-cm. (2-inch) filter funnel held in place by a rubber stopper and with the dead space between funnel and column packed with cotton. Compressed air, introduced through the funnel stem, continuously removed the center portion of the charge and was regulated to blow the largest particles one half to two thirds of the way up the column. These fell back to the center of the funnel, while the finer particles issuing from the top of the column as a smoke were led through a tube 2.5 cm. (1 inch) in diameter to a series of water traps. Four were sufficient if the smoke was directed against the surface of the water. In the authors' experiments, 15 to 20 per cent of the original charge was collected and contained 34.4 per cent of "lignin" and 18.1 per cent of pentosan (uncorrected). The efficiency of the process naturally varied greatly with the experimental conditions and there seems to be no good reason why suitable equipment would not recover the smoke without wetting it.

Separation by Sedimentation in Water. The glass column was nearly filled with distilled water and 100 to 200 grams of powdered, fiber-free hull fractions approximating in composition 40 per cent "lignin" and 8 per cent pentosan were added. A few seconds of agitation with the air blast gave a uniform suspension which settled slowly to form a deposit consisting of visibly distinct, horizontal layers. A tedious filtration of the supernatant suspension through these layers increased their definition and made it easy to separate them mechanically for analysis. In most cases, however, the supernatant liquor was decanted. Material still in suspension was recovered in a separate filtration, steeped in acetone, and dried *in vacuo*. The observed "lignin" contents were 41.0, 50.8, and 53 per cent after sedimentation for 2, 6, and 12 hours, respectively. An accumulation of about 100 grams averaging 50 per cent "lignin" was fractionated by resuspension in water for 6 hours. Found, "lignin", 51.2, 50.8, 50.0; pentosan (uncorrected), 6.4, 5.4, and 6.2 per cent for the second, third, and the deposit from the third suspension, respectively. Resolution by means of fractional sedimentation from water had therefore reached its limit. A portion of the original, alcohol-benzene-extracted hulls, ground in the ball mill in presence of water, also gave a similar 50 per cent "lignin" fraction. This experiment eliminated the possibility that the concentrate originated through local heating in the dry milling which was usually employed to disintegrate the hulls.

Examination of Suspensoid Fraction

A very rough calculation from the rate of settling in water and from Stokes' law gave an average particle radius of the order of 50 microns for the suspensoid (50 per cent "lignin") fraction in its original state. It consisted of a brown, moist cake which formed a very hard mass when dried directly from water and a very fine powder when the water was removed by solvent exchange with acetone. The fraction was a mixture whose composition varied somewhat in different preparations. The analyses (Table III) were typical and it will be noted that about 15 per cent of the material escaped estimation. The cause of this discrepancy requires further investigation, although it is possible that the Cross and Bevan determination failed to include all carbohydrate not already estimated as pentosan or uronic anhydride.

A 0.215-gram sample was tested for pectic substances by heating it on the steam bath for 16 hours with 50 cc. of aqueous 0.5 per cent ammonium oxalate at pH 5. The almost colorless filtrate was discarded and the residue, washed with water and dried at 105°, weighed 0.198 gram or 92 per cent of the original. Another sample lost 2 per cent by weight, including some ash, when extracted with glacial acetic acid at room temperature.

Table III. Analysis of Suspensoid Fraction

	%
C[a]	50 ± 1[b]
H[a]	6.2 ± 0.3
OCH_3[a]	0.0
"Lignin"[a]	50.5
Pentosan[c]	3
Uronic anhydride	9.1
Cross and Bevan cellulose[d]	15.3
Ash	6.5
Total	84.4

[a] Corrected for ash.
[b] Individual carbon analyses were 46.3, 47.6, 50.0, 49.1 (3.6% ash), and 48.9, 47.7, 50.2, 49.2 (2.0% ash).
[c] Corrected for uronic anhydride. Uncorrected, 6.4%.
[d] Corrected for ash, pentosan, and "lignin".

Discussion

Cottonseed hulls, digested with cold 72 per cent sulfuric acid, gave a methoxyl-free residue which is called "lignin" to emphasize its undetermined relationship to the methoxyl-containing lignins similarly prepared from woods. This lack

TABLE IV. RATIONS OF "LIGNIN" AND PENTOSAN TO URONIC ANHYDRIDE

Fraction	% "Lignin" / % Uronic Anhydride	% Pentosan / % Uronic Anhydride	Moles Pentosan / Moles Uronic Anhydride Corrected
A. Coarse fiber	3.9	4.1	18.0
B. Fine fiber	4.0	2.7	15.3
C. Hulls >30-mesh	3.6	5.1	19.2
D. Hulls 30- to 60-mesh	3.5	4.6	16.1
E. Hulls <60-mesh	4.8	1.0	9.9
Suspensoid (50% "lignin")	5.6	0.3	..
Original hulls plus fiber	3.9	4.2	18.3

of the methoxyl group, in conjunction with the failure to account for 16 per cent of the fine hull fraction (Table III), suggested that the 50 per cent yield of residue obtained in this case was an artifact produced by the action of strong acid upon sensitive hull constituents. The latter were probably not tannins (*20*) because these are usually extracted from plant materials by water or acetone (*16*), and repeated treatment of the very finely divided, suspensoid hull fraction with these solvents failed to diminish the yield of "lignin".

The pentosan, uronic anhydride, Cross and Bevan cellulose (Table III), and also the substances extracted by a hot pectic solvent (8 per cent), were insufficient in amount to account for the 50 per cent residue obtained with sulfuric acid. Thus, if the latter originated with sensitive carbohydrates (*7*), these were not detected by the usual carbohydrate analyses. Although individual microcombustions were somewhat discordant, perhaps owing to sampling errors, the average carbon content of the fine hull fraction was about 50 per cent. Assuming 44 to 45 per cent carbon for the 30 per cent of the hulls which analyzed as carbohydrate, the remaining 70 per cent (on an ash-free basis) had a calculated carbon content of 51 to 52 per cent. This was sufficiently high to eliminate sensitive carbohydrates as precursors of the Klason residue. Whatever the chemical nature of these precursors might be, they reacted like ordinary lignin in the Cross and Bevan determination and gave a similar color change during the operation. They were doubtless related to the "Body X" extracted from the hulls by alkali (*4*) and their zero methoxyl content placed the cottonseed in an extreme position with respect to hulls of other species, which sometimes give Klason residues with low methoxyl values (*8*). Young cereal shoots are in the same category and it has been suggested (*13*) that in them the lignin structure is first laid down and then methylated at a later stage. Perhaps the latter step is partially or completely inhibited in the metabolism of hulls. But speculation concerning the nature of the residues obtained from hulls by strong acid is not germane to the following discussion. The reasoning is based entirely on data from standardized estimations and is independent of assumptions concerning the structure of the hull constituents which respond to the conventional procedures for determining lignin, pentosan, and uronic anhydride.

Consideration of Tables I, II, and III shows very clearly that purely physical methods, based on particle size and density, separated ground cottonseed hulls into fractions containing about 40 to 3 per cent of pentosan, 20 to 50 per cent of "lignin", and 5.8 to 9.1 per cent of uronic anhydride. The same technique, when applied to groundwood of spruce and maple, produced analytical fluctuations of only a few per cent. Other workers with groundwood had a similar experience and found that both pentosan and lignin accumulated very slightly in the finest fractions, which amounted to 42 to 69 per cent of the entire material (*12, 17*). The fine hull fraction, E, 6.8 per cent of the total, was rich in "lignin" and poor in pentosan (Table II). This contrast between hulls and groundwood may depend to an unknown degree upon the milling conditions and upon the particular species examined. Uronic anhydride accumulated with the "lignin" in the finer fractions of the hulls and the ratio between the two was much more constant for various sized particles than the pentosan–uronic anhydride ratio. This is illustrated in the second and third columns of Table IV, which were derived from the data of Tables II and III.

If the assumption is made that the uronic anhydride was in part chemically associated with pentosan and in part with "lignin", the ratio of the components in the latter complex could be taken as 1 to 5.6, since the suspensoid fraction (Table III) was almost pentosan-free. If this ratio was also true for the other fractions, it was possible in each case to calculate the molar ratio of pentosan to that anhydride not assumed to be associated with "lignin". The results (Table IV, column 3) varied between 9.9 and 19.2 to 1 and may be compared with the pentosan–uronic anhydride ratios of 10 and 16 to 1 found from estimations of hemicelluloses isolated from the hulls (*3*). Thus, although the above assumptions lack experimental proof, they are not in conflict with the data at hand.

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CORRECTION. In the article on "Determination of Methionine in Certain Mixtures" [IND. ENG. CHEM., Anal. Ed., 12, 723 (1940)] references (2), (3), (4), and (5) are all to the *Journal of Biological Chemistry*.

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Determination of the Proportion of *d*- and *l*-Isomers in Samples of Lactic Acid

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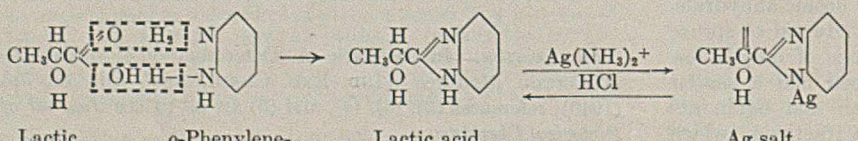
A PRACTICAL method, based on the properties of the benzimidazole derivative of lactic acid, is described for the quantitative measurement of the relative amounts of the two optical isomers in preparations of lactic acid. In the development of the present procedure, the immediate interest has been the need in research and commercial analysis for accurate optical characterization of samples of lactic acid obtained by fermentation processes.

Qualitative identification of the *d* (dextro) or "Sarco" lactic acid, *l* (levo), and *dl* (racemic) forms of lactic acid has generally been based on isolation of the zinc-*d*(or *l*)-lactate dihydrate and zinc-*dl*-lactate trihydrate, with determination of water of crystallization and optical rotation. [For clarity, and in agreement with the more general use of the term dextrolactic acid in commercial practice, the optical notations *d* and *l* are used in this paper to refer in the rotational (not configurational) sense to the forms of the acid which are dextro- and levorotating in aqueous solution.] While the zinc salt procedure is relatively satisfactory in the case of the pure isomers, or for qualitative information on mixtures, the properties of the salts are not favorable from the quantitative standpoint. Under practical conditions, if analytically pure hydrated zinc lactates are to be obtained, the crystallization of the salts from water by the addition of alcohol can rarely be carried to the point of quantitative isolation. Active-racemic fractionation is involved in the crystallization process and it is known that the *d*-*dl* composition of the isolated salt may differ more or less from that of the original lactic acid sample.

From the polarimetric standpoint, the specific rotation of zinc-*d*-lactate dihydrate ($[\alpha]_D = -7.9$, $c = 3$), although more satisfactory than that of the calcium salt, is still of relatively low magnitude for accurate measurement. This becomes especially apparent in consideration of *d*-*dl* mixtures. The previously reported appreciable variation of rotation with concentration of zinc lactate has been reinvestigated by one of the authors (R. J. D.), as reported and confirmed by Maclay, Hann, and Hudson (*7*).

In the case of the benzimidazole derivative of lactic acid, it has proved possible to isolate the benzimidazoles from *d*- and *l*-lactic acid mixtures without fractionation to obtain a pure derivative in which the ratio of *d*- to *l*-isomer is identical with that in the original lactic acid sample. The specific rotation of the active derivative ($[\alpha]_D = -32.6$, $c = 3.8$) shows a fourfold increase over the rotation of the zinc salt and is in the range which permits determination of the *d*-*l* composition by direct polarimetric measurement with an error of less than 1.0 in the percentage composition.

The reaction upon which this method is based is the condensation of lactic acid with *o*-phenylenediamine to give 2-(α-hydroxyethyl)-benzimidazole. Isolation of the derivative is facilitated by the insolubility of the silver salt in ammoniacal solution and regeneration of the benzimidazole by hydrochloric acid, according to the following equations:



Lactic acid — *o*-Phenylenediamine — Lactic acid benzimidazole — Ag salt

The reaction of racemic lactic acid and other α-hydroxy acids with *o*-phenylenediamine has been described by several authors (*3*, *4*, *5*, *10*). The optically active benzimidazole derivatives have not been reported. The earlier method of preparation by the heating of lactic acid with the organic base, without the addition of mineral acids, is not applicable to the *d*- and *l*-isomers because of the racemization (unpublished data) which may take place under these conditions. The satisfactory procedure for the condensation used in the following experiments, employing phosphoric-hydrochloric acid at 135° C. has been developed in conjunction with experiments on *d*-lactic acid and on carbohydrate acids (*8*, *9*). Summarized briefly, it has been proved that the stereoisomeric structure of every optically active acid studied is stable in the range from 100° to 160° C. in the presence of mineral acids. For lactic acid, with which we are concerned here, the data in Table II on known samples verify the fact of stability over a range of optical compositions.

Samples for Analysis

An aqueous sample having a lactic acid concentration of 50 per cent or more is to be preferred. For this reason, as well as because of the purification effected, an ether extraction is generally advisable as the final step in the preparation of a sample for analysis. Research preparations or commercial samples, from which major quantities of interfering substances have been eliminated by suitable methods (*1*, *2*), are acidified to thymol blue with sulfuric acid (pH approximately 2.0) and subjected to continuous extraction with ether for 24 hours. When the starting aqueous solution of lactic acid is dilute, it is convenient to add ammonium sulfate (30 grams per 100 cc.) to decrease extraction of water and to give extracts which, after removal of the ether, approximate 90 per cent lactic acid.

For the condensation it is essential that the millimoles of lactic acid present be at least equal to (preferably greater than) the millimoles of *o*-phenylenediamine. Any sample volume containing lactic acid equivalent to from one to three times the amount of *o*-phenylenediamine used will give satisfactory results. Only the approximate lactic acid titer (millimoles per cubic centimeter by alkali titration) is required and in the case of a series of similarly prepared samples it is possible to dispense with titration after experience with the first few samples in the group.

Relatively pure commercial samples, in some cases, may give a correct analysis without further purification. Ether extraction was not necessary on sample B (Table IV), the same result being obtained before and after ether purification. Also small amounts of unremoved carboxylic acids (other than lactic) do not cause large deviations in the results (Table III). In all cases the possibility of significant error due to impurities can be checked, if necessary, by analysis of the isolated benzimidazole salt for silver (40.1 per cent) or nitrogen (10.4 per cent).

Reagents

o-PHENYLENEDIAMINE (*o*-diaminobenzene), m. p. 100–102°. Photographic grades—e. g., Orthamine—are satisfactory.

Silver-Ammonia Solution. For the 5-cc. procedure, the requirement for each precipitation is 0.3 gram of silver nitrate dissolved in 0.3 cc. of water, to which are added 0.6 cc. of concentrated ammonium hydroxide in excess of the amount required to dissolve the silver oxide formed, and 0.6 cc. of 10 per cent potassium hydroxide. For a series of determinations enough silver-ammonia solution may be prepared to meet several hours' requirements (but not several days, since it may become mildly explosive) and aliquots used for each precipitation. For the 25-cc. scale five times these quantities are used.

Alcoholic Hydrochloric Acid. A solution of 8 cc. of concentrated hydrochloric acid in 92 cc. of 95 per cent ethanol.

Analytical Procedure

Two scales of operation have been found convenient. The first is based on the use of 5-cc. volumetric flasks (0.2-gram samples of lactic acid) for problems in which conservation of material is important. In the second procedure, which may be preferred in control analysis, the final volume is 25 cc.

The 5-Cc. Scale. To 0.15 gram (1.4 millimoles) of *o*-phenylenediamine in a 100 × 12 mm. test tube, add 0.2 cc. of water, 0.1 cc. of concentrated hydrochloric acid, 0.1 cc. of phosphoric acid (sp. gr. 1.7), about 0.2 cc. of ethanol, and a boiling chip. After the mixture has been brought into solution add 2 to 3 millimoles of lactic acid (about 0.25 cc. of 90 per cent acid).

Heat the reaction tube, immersed to the level of the contained solution, for 2 hours at 135° ± 5° C. (oil bath), during which time water and alcohol boil off to give a thick sirup as residue. Stir about 1 cc. of water into the warm viscous reaction mixture and transfer into a 25-cc. flask with about 5 cc. of water. Add 3 cc. of ethanol, 9 cc. of 10 per cent potassium hydroxide, and a little (about 0.05 gram) decolorizing carbon (Nuchar C-250 or Darco). Filter with suction through an asbestos pad (Woolly C) and wash the pad twice with 0.5-cc. portions of ethanol.

Transfer the filtrate to a 50-cc. flask and add 8 cc. of concentrated ammonium hydroxide. Allow the solution to stand for 20 minutes before precipitating the lactic acid benzimidazole as the anhydrous crystalline silver salt by the dropwise addition of silver-ammonia solution equivalent to 0.3 gram of silver nitrate.

Shake the flask for about 1 minute to decompose any aggregates present and allow the salt to settle for 10 minutes. Discard the supernatant solution by decantation and filter the salt on a small Büchner funnel with gentle suction. Wash three times with dilute ammonium hydroxide (10 cc. of concentrated ammonium hydroxide in 200 cc. of water), three times with ethanol, and once with ether. During the washing the suction should be applied and released in such a manner that the salt is not pulled dry (filter cake should not crack) until the final ether wash.

Dry the salt in a vacuum desiccator for about 30 minutes (with well-washed samples, air-drying for the same period of time gives satisfactory results). Transfer the dried salt to a weighed 5-cc. volumetric flask and reweigh. Add 2 cc. of ethanol, shake gently (in upright position) to suspend the silver salt uniformly, and add to the suspension 2 cc. of alcoholic hydrochloric acid solution. Shake well for about a minute. Add 0.15 cc. of concentrated ammonium hydroxide, make up to volume with ethanol, stopper the flask, and shake. After the halide has settled for a few minutes, shake the supernatant solution into the neck of the flask once or twice to wash down any loose particles of silver chloride. Centrifuge the flask for 5 minutes at about 2000 r. p. m. (standard cups for 50-cc. tubes, with cotton packing, are satisfactory). Without disturbing the residue, pipet a sample of the clear supernatant solution into a semimicro 2-dm. polariscope tube (capacity of 2 to 3 cc.). From the rotation (angular degrees) of this solution and the weight of silver salt, w, the per cent of *d*-lactic acid in the original sample is obtained as follows:

$$\text{Per cent } d = 50 - \frac{6.37\alpha}{w}$$

The rotation of the benzimidazole derivative is in the opposite direction to that of the free acid, as is the case with other derivatives of the isomeric lactic acids.

Reprecipitation. If measurement of the rotation is rendered difficult by the presence of color in the alcoholic solution, two possible causes may be considered. The presence of unreacted *o*-phenylenediamine, due to failure to add sufficient lactic acid for the condensation, will give rise to interfering color as a result of oxidation by the silver-ammonia solution. Or failure to remove color-producing impurities from the original lactic acid sample may be involved. A repetition of the condensation, using a correct amount of lactic acid, will eliminate the first effect. Purification of the acid sample by ether extraction, in general, prevents possible interference due to the second cause.

If for any reason a second condensation is not convenient, a rotation with the colored benzimidazole preparation can usually be realized by carrying out a reprecipitation of the silver salt Decolorize the alcohol solution of the benzimidazole (about 5 cc.) with a little carbon, using 1 cc. of ethanol in washing, and add 2 cc. of 10 per cent potassium hydroxide, 8 cc. of water, and 5 cc. of concentrated ammonium hydroxide. Precipitate with silver-ammonia solution (0.3 gram of silver nitrate) and proceed as in the first determination.

The 25-Cc. Scale. Use 10 to 15 millimoles of lactic acid (about 1 cc. of 90 per cent acid), with five times the quantities of reagents and solvents prescribed above, 15-cm. (6-inch) test tubes, and correspondingly increased flask sizes. Standard 2-dm. saccharimeter tubes (10- to 15-cc. capacity) can be used. The factor in the equation for per cent *d*-lactic acid becomes 31.85 instead of 6.37. Centrifuge cups designed for Babcock bottles are suitable for the usual 25-cc. volumetric flasks. If centrifuging is not convenient, the silver chloride separation may be accomplished on this larger scale, after the solution has been made to volume, by rapid filtration through a dry paper directly into the saccharimeter tube. Table IV presents results obtained on the same sample by both procedures. For most practical purposes on this scale satisfactory readings may be obtained with a saccharimeter and converted to angular degrees.

Example of Data and Calculation

Lactic acid benzimidazole, m. w. 162
Ag-salt of benzimidazole, m. w. 269
Conversion factor = 0.602

Weight of flask + Ag-salt = 16.6564
Weight of flask = 15.1075
Weight of silver salt, w = 1.5489

d-benzimidazole $[\alpha]_D = -32.6°$
Volume = 25 cc.
Tube length = 2 dm.
Polarimeter: $\alpha = -1.45$ A.
Saccharimeter:
$\alpha = -4.2°$ Ventzke
$= -4.2 \times 0.3466$
$= -1.46$ Å.

$$[\alpha]_D = \frac{100\alpha}{lc} = \frac{100\alpha}{2 \times \frac{w}{25} \times 0.602 \times 100} = \frac{20.76\alpha}{w}$$

$$\text{Per cent } d = 100\left[\frac{[\alpha]_D}{-32.6} + \frac{1}{2}\left(1 - \frac{[\alpha]_D}{-32.6}\right)\right] = 50 - \frac{31.85\alpha}{w}$$

$$\text{Per cent } d = 50 - \frac{31.85\ (-1.45)}{1.549} = 79.8 \text{ (per cent } l = 20.2)$$

The per cent *d* calculated in these equations represents the total *d*-isomer content of the lactic acid under analysis. This calculation is to be distinguished from the expression of the composition of a sample in terms of *d* (or *l*) and *dl* (racemic). The total *d*-isomer content, as expressed in the above example, is equal to the excess *d*-isomer present plus one half of the inactive *dl*. Observance of the correct sign for α in the above expression consistently gives the result in terms of per cent *d* (per cent $l = 100 - d$).

Discussion

The benzimidazole derivative of lactic acid shows the general amphoteric properties of this type of heterocyclic compound (*6*, *9*). With acids it forms salts, most of which are water-soluble—e. g., hydrochloride, acetate, etc. It also forms soluble salts with strong alkalies, but insoluble salts with silver, zinc, and copper. The free base crystallizes readily (but not quantitatively) from water and may be isolated by the addition of a weak base (ammonium hydroxide) to the acidic aqueous solution obtained in the condensation. The lacto-benzimidazole is soluble in many organic solvents, and the ethanol added in this procedure prevents crystallization of the free base from the ammoniacal solution.

Solubility of Silver Salt. The benzimidazole is isolated as the crystalline silver salt rather than the free benzimidazole in this procedure because of the advantage of essentially quantitative yield and consequent absence of *d-dl* fractionation. Selective precipitation of the silver-benzimidazole in the presence of chloride ion is made possible by the insolubility of the salt of the heterocyclic base in the excess of ammonium hydroxide which prevents silver chloride pre-

cipitation. It has been observed that the ammonium salts of acids—e. g., ammonium chloride—have a solubilizing effect. In order to prevent fractionation of the isomers by incomplete precipitation, the solvent effect of ammonium salts has been blocked by the addition of an excess of potassium hydroxide for neutralization of mineral acids and excess lactic acid.

TABLE I. VARIATION OF $[\alpha]_D^{24}$ WITH CONCENTRATION

(K_c represents specific rotation of pure *d*-benzimidazole from lactic acid at a given concentration, under conditions used in preparation of solution from a sample of silver salt.)

Silver Salt, 5-Cc. Procedure	Silver Salt, 25-Cc. Procedure	Benzimidazole Concentration	K_c[a]
Gram	*Grams*	%	
0.25–0.26	1.25–1.30	3.1	−32.3
0.27–0.28	1.35–1.40	3.3	−32.4
0.29–0.30	1.45–1.50	3.6	−32.5
0.31–0.32	1.55–1.60	3.8	−32.6
0.33–0.34	1.65–1.70	4.0	−32.7
0.35–0.36	1.75–1.80	4.3	−32.8

[a] Values of K_c have been found essentially constant over temperature range 15° to 25° C. Rotations can therefore be taken over normal ranges of room temperature and thermostatic control is not necessary.

Under the conditions given in the experimental section, without attempt for absolute manipulative recovery, the isolated yields of silver salt from a standard solution of crystalline benzimidazole were, in triplicate, 97.6, 97.4, and 98.0 per cent of theory (analysis for silver: calculated, 40.1; found, 40.3). The optical composition of this sample was 70.6 per cent *d* and the found values for the three precipitates were 71.0, 70.8, and 70.0.

The principles for the solubility of the silver salt find application again in the final solution of the sample for polarization. In this case the solubility of the silver salt in the presence of ammonium chloride permits neutralization of hydrochloric acid by an excess of ammonium hydroxide in the presence of silver chloride without precipitation of silver-benzimidazole. The trace of silver ammonia which may be formed is without effect.

SPECIFIC ROTATION. In the case of the benzimidazole derivative of *d*-lactic acid, the variation of specific rotation with concentration is measurable but relatively small (Table I). In the equations for calculation of per cent *d*, an average value of 32.6 has been used and the error thus introduced is within the experimental error of the method, provided the sample weights are kept within the listed range (benzimidazole concentration 3 to 4.5 per cent).

When the weighed sample of silver salt contains a mixture of the *d*- or *l*-isomer with the *dl* form, the concentration of the active component may be less than 1 per cent. However, in the calculation of the composition, the rotation of the pure isomer at the 4 per cent concentration (−32.6) is still applicable. In such mixtures the specific rotation of the excess *d*- or *l*-isomer is essentially a function of the total benzimidazole concentration. If the concentration of *d*-lacto-benzimidazole (from the silver salt) is varied while the total benzimidazole concentration is held constant at 4 per cent by the addition of the inactive *dl*-derivative, the specific rotation of the *d*-benzimidazole used remains nearly constant. For example:

$[\alpha]_D = -32.7$, *d*, $c = 4$ and *dl*, $c = 0$; $[\alpha]_D = -32.3$, *d*, $c = 1$ and *dl*, $c = 3$; but $[\alpha]_D = -31.3$ when *d*, $c = 1$ and *dl*, $c = 0$

If desired, the concentration factor may be incorporated in the calculations to compensate for variations in sample weight. The value for K_c corresponding to the weight of silver salt used may be taken from Table I (or an extrapolation thereof).

$$\text{Per cent } d\text{-lactic acid} = \frac{100}{2}\left[1 + \frac{[\alpha]_D}{K_c}\right]$$

The values in Table I represent the rotations of the active derivative in the particular solvent (approximately 90 per cent ethanol) obtained under the experimental conditions of this method. In pure 95 per cent ethanol the rotation is of similar magnitude ($[\alpha]_D = -32.8$, $c = 3$). In aqueous acids the rotation is lower ($[\alpha]_D = -14.7$, $c = 2$, in 5 per cent citric acid).

The technique of precipitating the silver chloride in the volumetric flask and making to volume before filtering (or centrifuging) is preferable to the alternative order of the operations. Experience with silver chloride precipitation followed by filtration, washing of the precipitate, and adjustment of the combined filtrates to the desired volume, has invariably shown significantly low recoveries. It does not appear possible, within the practical limits of solvent volume for polarimetric measurement, to obtain a quantitative washing of the silver chloride precipitate. The centrifuge technique has regularly given recoveries of 99 per cent or higher.

The actual minor volume error (approximately 0.7 per cent) introduced by the presence of the solid phase in the volumetric flasks is present in both the analysis and in the preparation of Table I. This factor therefore cancels out in the equation for per cent *d*-lactic acid and does not require consideration.

TABLE II. ANALYSES OF KNOWN MIXTURES OF LACTIC ACID FOR OPTICAL FORM

Lactic Acid	Benzimidazole Ag-Salt	α, Angular Degrees	*d*-Lactic Found	Deviation
	Gram		%	
Pure *d*-lactic (100% *d*)	0.3151	−2.46	99.7	−0.3
	0.3335	−2.63	100.2	+0.2
	0.3117	−2.44	99.9	−0.1
	0.3326	−2.62	100.2	+0.2
	0.2886	−2.26	99.9	−0.1
Known mixture No. 1 (74.7% *d*)	0.3263	−1.30	75.4	+0.7
	0.3430	−1.38	75.6	+0.9
	0.3159	−1.26	75.4	+0.7
	0.3436	−1.36	75.2	+0.5
Known mixture No. 2 (40.0% *d*)	0.3324	+0.51	40.2	+0.2
	0.3221	+0.49	40.3	+0.3
	0.3284	+0.50	40.3	+0.3
	0.3054	+0.48	40.0	0.0
	0.3177	+0.47	40.6	+0.6
	0.3322	+0.50	40.4	+0.4
Known mixture No. 3 (14.0% *d*)	0.3112	+1.74	14.4	+0.4
	0.3181	+1.76	14.8	+0.8
	0.3171	+1.76	14.6	+0.6
	0.3103	+1.74	14.3	+0.3
	0.3147	+1.75	14.6	+0.6

ACCURACY. The analyses in Table II show application of the method to lactic acid samples of known optical composition. Known mixtures were prepared gravimetrically from zinc-*d*-lactate dihydrate ($[\alpha]_D = -7.6$; *c*, 4; $H_2O = 12.83$ per cent), zinc-*l*-lactate dihydrate ($[\alpha]_D = +7.5$; *c*, 4; $H_2O = 12.84$ per cent), and zinc-*dl*-lactate trihydrate ($[\alpha]_D = 0.0$, *c*, 1.5; $H_2O = 18.29$ per cent). The salt mixtures were dissolved in water, acidified, and extracted with ether as in the experimental procedure. The values found for per cent *d*-isomer indicate that, in general, the observed per cent will not vary by more than 1.0 from the actual composition. In Table II the observed percentage composition shows a maximum deviation of 0.9 and mean deviation of 0.4 for the representative series of samples listed.

TABLE III. EFFECT OF PRESENCE OF ACETIC AND SUCCINIC ACIDS

Lactic Acid	Benzimidazole Ag-Salt	α, Angular Degrees	*d*-Lactic Found
	Gram		%
Commercial edible	0.3153	−2.44	99.3
	0.3029	−2.34	99.2
10% HOAc added	0.3395	−2.52	97.3
	0.3375	−2.49	97.0
50% HOAc added, steam-distilled before analysis	0.3423	−2.64	99.1
	0.3235	−2.50	99.2
	0.3096	−2.39	99.2
10% succinic acid added	0.3049	−2.24	96.8
	0.2767	−2.07	97.7

EFFECT OF PRESENCE OF OTHER CARBOXYLIC ACIDS. Under the conditions used in this method the condensation with *o*-phenylenediamine is general for the carboxyl group in the aliphatic series. The samples should be free, therefore, from major quantities of acids other than lactic. Small amounts of acetic acid or succinic acid, however, can be present without significantly altering the results. It may be noted that 10 per cent of either acid added to a commercial sample lowered the analytical figures by only 1 to 2 per cent (Table III). The small magnitude of this interference is due to the difference in the rates at which α-hydroxy acids and unsubstituted aliphatic acids react with *o*-phenylenediamine. The reaction rate in the case of lactic acid is relatively high.

TABLE IV. ANALYSES OF COMMERCIAL AND EXPERIMENTAL SAMPLES

Lactic Acid	Benzimidazole Ag-Salt	α, Angular Degrees	*d*-Lactic Found
	Grams		%
Commercial edible, ether extracted	0.3380	−2.53	97.7
	0.3494	−2.60	97.4
Without ether extraction	0.3333	−2.46	97.0
	0.3260	−2.41	97.1
25-cc. procedure	1.7160	−2.57	97.7
	1.6601	−2.48	97.6
Experimental fermentation	0.3230	−1.53	80.2
	0.3213	−1.54	80.5
25-cc. procedure (centrifuged)	1.5489	−1.46	80.0
	1.3507	−1.25	79.5
25-cc. procedure (filtered)	1.6597	−1.55	79.7
	1.4786	−1.39	79.9
Experimental fermentation	0.3191	−1.08	71.6
	0.3211	−1.09	71.6
25-cc. procedure	1.5623	−1.08	72.0
	1.5698	−1.08	71.9
Experimental fermentation	0.2978	−0.32	56.8
	0.3080	−0.32	56.6
	0.3024	−0.32	56.7
Experimental fermentation	0.3479	+2.18	10.1
	0.3446	+2.16	10.1

COMMERCIAL AND EXPERIMENTAL SAMPLES. Table IV lists representative analyses on a variety of lactic acid preparations. In several cases analyses have been made by different operators employing different scales of procedure and the results are found to be in agreement.

Summary

A quantitative procedure for the measurement of the relative amounts of the two stereoisomers in preparations of lactic acid is described. It is applicable, with an error of less than 1.0 in the percentage composition, to samples containing as little as 0.2 gram of lactic acid. To meet the requirements of variations in the available sample size and equipment, two scales of operation are given. The procedure depends on the condensation of lactic acid with *o*-phenylenediamine at 135° C. in the presence of phosphoric and hydrochloric acids to form 2-(α-hydroxyethyl)-benzimidazole. The derivative is isolated by quantitative precipitation as the crystalline silver salt. Regeneration of the benzimidazole gives a solution whose specific rotation is used for the calculation of the *d-l* composition of the original lactic acid sample. The use of the benzimidazole derivative of lactic acid offers the following advantages over the zinc salt: a fourfold increase in rotation ($[\alpha]_D = -32.7$), negligible variation of rotation with concentration, and absence of fractionation of isomers during the preparation and isolation of the derivative.

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Fastness to Atmospheric Gases of Dyes on Cellulose Acetate Rayon

AT A MEETING of the Research Committee of the American Association of Textile Chemists and Colorists the following method was adopted as a tentative method.

TEST SPECIMENS. Four specimens shall be cut from the fabric to be tested. One specimen shall be washed in a soap solution (containing 5 grams of 88 per cent neutral soda soap per liter of water of approximately zero hardness) for 15 minutes at 37.78° C. (100° F.), rinsed in warm water, and allowed to dry in the air. One specimen shall be immersed in cold Stoddard solvent for 15 minutes, squeezed, and allowed to dry in the air. One specimen shall be immersed in cold perchloroethylene for 15 minutes, squeezed, and allowed to dry in air. The fourth specimen shall be retained in its original condition.

APPARATUS. The apparatus shall consist of an enclosed metal chamber, connected by a metal pipe to a lighted gas burner in such a manner that the burnt gases from the burner pass directly through the chamber. Either natural or manufactured gas may be employed. It is strongly recommended that a motor-driven fan be installed in the chamber to keep the fumes well distributed, and that a grid of iron wire be placed over the gas burner, as this will accelerate the production of oxides of nitrogen, which are responsible for the fading of the dyes.

PROCEDURE. Samples of the four specimens, each measuring at least 2.5 cm. (1 inch) square, shall be suspended freely in the chamber together with a "control sample" of a standard acetate rayon dyeing of known sensitiveness to gas fumes. [The control sample shall consist of a 1 per cent dyeing of Celanthrene Brilliant Blue FFS (or its equivalent) on acetate rayon satin.] The gas burner shall be lighted, and the flame adjusted so that the temperature in the chamber does not exceed 60° C. (140° F.). The sample shall remain in the chamber until the control sample shows a change of shade corresponding in degree with that of a "standard fading". [The standard of fading shall consist of a silk fabric dyed with stable dyes to imitate the color of the control sample after it has been exposed to average atmospheric conditions for a period of 6 months. Samples of both the control sample and standard of fading are available from C. A. Seibert, P. O. Box 386, Wilmington, Del.] The samples shall then be removed from the chamber and immediately compared with the respective unexposed portions.

EVALUATION. A sample which shows no alteration of shade shall be considered to have satisfactory resistance to atmospheric gases. A sample which shows as much alteration of shade as the control sample must be regarded as definitely sensitive to atmospheric gases.

Rapid and Accurate Determination of Cadmium

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THE separation of zinc and cadmium has long been a stumbling block in rapid and accurate analyses in the nonferrous metallurgical fields, and laboratories having a large number of cadmium determinations have sought a more rapid and accurate method. The standard procedures of the present day call for the separation of zinc and cadmium by repeated precipitations with hydrogen sulfide or by electrolysis under carefully controlled conditions of pH and current density, involving the use of various addition agents. Because of the consanguinity of zinc and cadmium, these methods are not satisfactory.

Studies have shown that the precipitation of cadmium cannot give a complete separation at any pH (*5*, *7*). Repeated precipitations can only give a closer approximation, with each handling giving greater chance for error. Other objections to this method are the length of time and amount of manipulation required, the toxic nature of the hydrogen sulfide gas, and its destructive effect on the laboratory equipment.

The separation of cadmium and zinc by electrolysis has been extensively studied. The mass of variant literature gives a picture of the confusion in this field. Present methods have been shown to give only partial separation (*1*). As yet no electrolytic method has been found completely satisfactory to the large routine laboratory.

Recent developments in the organic-metallurgical field have uncovered a number of substances that quantitatively precipitate cadmium with no effect on zinc (*2*, *4*). Most of these are very expensive. However, in 1937 Korenman (*3*) suggested the precipitation of cadmium from a cadmium-zinc solution by a brucine–potassium bromide mixture, its subsequent filtration, washing, drying, and weighing. The objectionable features of the method were the long period of precipitation, inability to wash clean, and the tendency of the precipitate, due to its great bulk, to decompose, volatilizing the bromine, before completely drying. Its advantages were the completeness of separation and the cheapness and cleanliness of the chemical.

Experiments have shown that if the cadmium is precipitated from the cadmium-zinc solution with a brucine sulfate–potassium iodide mixture and then washed by special means, the precipitate can be dissolved and the cadmium determined by titrating the iodine present, giving accurate and rapid results if carefully carried out.

Experimental Work

A solution containing cadmium-zinc salts, and freed from lead, copper, and iron, is reduced to a volume of 50 cc., if previous manipulations have resulted in excess volume. The original sample is so taken as to give 20 to 50 mg. of cadmium in the solution. It may be necessary to take aliquot portions of the original solution to get this correct cadmium content. For every milligram of cadmium believed present, 1.5 cc. of a 1 per cent brucine sulfate solution are added, followed immediately by 1.5 cc. of a 10 per cent potassium iodide solution. The solution is well stirred and allowed to stand for 10 minutes, then filtered rapidly through a Büchner funnel using suction. The precipitate is first washed with a 50–50 mixture of the brucine and iodide solutions, then with an organic solvent such as a 1 to 4 mixture of ethanol and toluene until free from potassium iodide. It is then dissolved by heating in water and the iodine content of the organic iodide is determined in any convenient manner. The cadmium content of the sample may be determined by comparison with the iodine value of standards previously determined.

Elements, usually present in nonferrous metallurgy, that interfere with the determination are iron, copper, lead, and antimony. These elements are normally removed during any analysis and will cause no additional work when this method is used. Precipitation should be carried out in a neutral or slightly acidic solution. However, no free nitric acid or other strong oxidizing agent should be present, as it would liberate free iodine from the iodide as well as destroy the brucine. Free ammonium hydroxide should not be present, though ammonium salts do not interfere.

Unlike many other methods, the brucine-iodide precipitation method covers the entire range of zinc-cadmium ratios. Table I shows the results obtained by analysis of mixtures made up to represent various samples in the processes of the zinc and cadmium smelters.

TABLE I. SEPARATION OF ZINC AND CADMIUM

Zn Present *Grams*	Pb Present *Gram*	Cd Present *Gram*	Cd Found *Gram*	Error *Mg.*	Ratio of Zn to Cd
40.005	0.500	0.0200	0.0201	+0.1	1000–1
0.0200	...	0.0201	0.0201	0.0	1–1
0.100	...	0.0250	0.0249	−0.1	4–1
0.270	...	0.0302	0.0302	0.0	9–1
0.003	0.300	0.0301	0.0302	+0.1	1–10
2.000	...	0.0400	0.0402	+0.2	50–1
0.100	0.075	0.0502	0.0503	+0.1	2–1
0.0025	0.022	0.0500	0.0500	0.0	1–20

Standards consisted of commercial cadmium metal running 99.8 per cent cadmium and 0.1 per cent zinc. They were determined in about half an hour. Zinc spelter of the ordinary grades may be run in less than 2 hours, compared to a previous time required of approximately 7 hours. Below is given the detailed procedure for the analysis of a raw ore running 60.0 per cent zinc and 0.4 per cent cadmium with many impurities. The time required is one third that of a hydrogen sulfide determination of the same accuracy with the same sample.

The procedure includes steps made necessary by the complex nature of the raw ores. Procedures for ordinary cadmium samples are normally simple and rapid.

Procedure for a Sulfide Ore

SOLUTION OF SAMPLE. Weigh a 5.00-gram sample into a 400-cc. beaker, add 15 cc. of concentrated hydrochloric acid, and boil until all hydrogen sulfide has passed off. Add 15 cc. of concentrated nitric acid and bake. Cool, add 30 cc. of concentrated hydrochloric acid and 5 grams of ammonium chloride, and boil until the volume is reduced to 5 to 10 cc.

REMOVAL OF IRON. Dilute to 200 cc. with water and add ammonium hydroxide until the iron is all precipitated, adding sufficient to redissolve the cadmium and zinc. Boil to granulate the precipitate, then filter, washing the beaker three times, using a drop of ammonia with the wash water each time. Wash the precipitate four times with hot water. (If the iron content is above 5 per cent, dissolve, reprecipitate, filter, and wash as above.) Neutralize the filtrate with concentrated hydrochloric acid to the methyl orange end point, adding 7 cc. in excess.

REMOVAL OF COPPER. Add 3 to 5 grams of granulated C. P. lead and boil for 10 minutes to precipitate out the copper. Add another full portion of lead and again boil, then test for completeness by adding a few fresh grains of lead. Remove from the heat and decant the liquor. Wash the lead and copper three times by decantation with hot water, adding the washings to the liquor.

SEPARATION OF THE CADMIUM. Add 5 grams of pure magnesium dust to the solution and boil for 3 minutes. Filter through a No. 4 Whatman paper, washing paper and precipitate until free from acid. Repeat the precipitation by adding another portion of magnesium to the filtrate, again boiling, filtering, and washing. Transfer the precipitate to a 150-cc. beaker using 1 to 1 nitric acid and a fine spray of water. Dissolve the sponge in 5 cc. of concentrated nitric acid and take to dryness. Add 15 cc. of concentrated sulfuric acid and again take to dryness. Dissolve with 25 cc. of water and filter off the lead sulfate, using a fine paper. Wash the beaker twice and the paper four times, using only a small volume of water.

Add 30 cc. of the brucine sulfate solution followed immediately by 30 cc. of the potassium iodide solution. Stir well and let settle for 10 minutes. Filter through a 5-cm. (2-inch) Büchner funnel, using moderate suction and a very fine paper. Wash the beaker with a 50–50 mixture of the stock solutions (mixed immediately before use) three times and the paper three times with a 1 to 4 mixture of ethanol and toluene.

ESTIMATION. Wash the precipitate and paper back into the beaker with water, making up to 100 cc. Heat until the precipitate has dissolved. Add 5 cc. of a 0.5 per cent solution of eosin Y as an indicator and titrate to the absorption end point with 0.03 *N* silver nitrate (*6*). The titration value is compared to that of a standard test to give the amount of cadmium in the sample.

Notes

In making up the brucine sulfate solution, the addition of sulfuric acid up to 5 per cent is helpful in the solution of the brucine and in the adjustment of the acidity during the precipitation. Care must be taken, however, not to oxidize the brucine. For best results, a fresh solution of brucine sulfate should be made every day.

Excessive volume for the precipitation may cause a colloidal precipitate. Difficult filtrations may be aided by judicious choice of filter papers and filter aids.

If there is a large mass of precipitate, it may hold some of the iodide solution, raising the titer and affecting the factor. It may be necessary to dissolve and reprecipitate the cadmium. The work requires only a few minutes and may be well worth the time

As in most analytical procedures, directions must be minutely followed, and a definite technique established. Careless work or minor differences in procedure may vitiate all results. Short cuts or the removal of additional elements will doubtless cause a different titer value. Careful work and an established technique, always qualities of a good analyst, are fully repaid by extremely accurate and rapid determinations of cadmium that will effect savings in time and money, especially in the large routine laboratories of smelters and metal-working plants.

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Detection of Sodium Alginate in Dairy Products

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AMONG the thickening agents being used at the present time in the dairy industry is the sodium salt of alginic acid, derived from certain seaweeds and put on the market in various forms. The product which is probably most widely used in this country is sold under the trade name of Dariloid, in which sodium alginate is mixed with other substances including dextrin, sucrose, and a phosphate. Other commercial products may consist of the pure alginate.

The present investigation was undertaken because of a lack of a specific qualitative test for alginic acid. The test is needed because of the widespread use of alginates in several dairy products, especially ice cream; and because of the possibility of illegal use in milk, cream, or other dairy products.

Inasmuch as the commercial product is likely to contain dextrin, a quick preliminary test for dextrin may be used to establish the probable presence of alginic acid in milk or cream. Likewise, milk or cream containing the commercial product is likely to give a positive Rothenfusser test (*1*) for sucrose.

Tests

OPTIONAL PRELIMINARY TEST FOR DEXTRIN. Take 10 cc. of milk or cream, dilute with 10 cc. of distilled water, and add 10 cc. of a 20 per cent solution of trichloroacetic acid. Shake vigorously for 1 minute, let stand for 5 minutes, and filter. Make the filtrate alkaline with ammonium hydroxide, evaporate on a steam bath to a volume of about 1 cc., filter into a test tube, cool to room temperature, and add 0.05 *N* iodine solution cautiously to the filtrate, a small drop at a time. A violet to reddish-violet color indicates the possible presence of 0.1 per cent or more of Dariloid.

SPECIFIC TEST FOR ALGINIC ACID. To 20 grams of milk, cheese, cream, or ice cream add a volume of concentrated hydrochloric acid approximately equal to the water content of the sample taken. Shake thoroughly, add a little sand (acid-washed and ignited), bring to a boil, and boil 30 seconds with frequent shaking. Transfer to a 50-cc. centrifuge tube with the aid of 10 to 20 cc. of alcohol. Centrifuge 10 minutes, or until the solid matter forms a compact cake at the bottom of the tube, and decant as much of the supernatant liquid as possible from the solid matter, taking care not to lose any of the solids. The centrifuge should be warm enough to keep the fat liquid and should be allowed to slow down without braking, to avoid stirring up the solids. In some cases, the fat mixed with part of the solids forms at the surface a dense cake which must be punctured with a stirring rod to allow removal of the liquid. The decantation is best carried out against a white background to facilitate observation of the solids through the dark solution.

Wash the solids repeatedly with 75 per cent ethyl alcohol by shaking, centrifuging, and decanting as above, until the wash solution is neutral to litmus paper and is colorless. Then wash twice with ether in the same manner.

Evaporate the last traces of ether by warming the tube in a beaker of hot water and directing a current of air into it. Dissolve the residue so far as possible in 10 cc. of 0.1 *N* sodium hydroxide by shaking a few seconds. Filter, wash the tube and paper with a few cubic centimeters of water, and to the filtrate add an equal volume of 95 per cent ethyl alcohol. Centrifuge 10 minutes, decant the supernatant liquid, and wash the solids with 75 per cent alcohol by centrifuging and decantation until the wash solution is neutral to litmus paper. The solution should be centrifuged and decanted even when it appears clear, as small amounts of gum are invisible beforehand. If the separated gum is now white and free from any yellow or brown color, pass over the next paragraph.

Suspend the gum in 10 cc. of distilled water and add 0.1 *N* sodium hydroxide dropwise, with shaking, until the solution is just alkaline to litmus paper. Add 6 cc. saturated magnesium nitrate solution and shake thoroughly. Centrifuge 10 minutes and decant the supernatant liquid from any precipitate into another centrifuge tube. Discard the precipitate and make the solution acid with a drop of concentrated hydrochloric acid.

Centrifuge, decant, and wash the precipitate with 75 per cent alcohol as before, until neutral.

Dry the gum by warming the tube with hot water and blowing air into it until no odor of alcohol is perceptible. Dissolve as far as possible by shaking with 0.15 cc. of 0.1 *N* sodium hydroxide, add 1 cc. of sulfuric acid reagent, shake thoroughly, and let stand at room temperature.

[The sulfuric acid reagent was prepared by precipitating ferric hydroxide from ferric chloride solution with ammonium hydroxide, washing the ferric hydroxide until neutral, drying on the steam bath, and finally saturating concentrated sulfuric acid with the dry ferric oxide by allowing the two to stand in contact for several days. The clear acid solution was then decanted from any excess ferric sulfate.]

Within a few minutes to several hours, depending on the amount of alginic acid present, the solution will develop a pink color deepening through cherry red to magenta, and finally becoming a deep purple. When the amount of gum is 0.5 mg. or less, the purple color is permanent for at least 1 week, but with larger amounts the color eventually changes to a brown-black.

Table I shows the time required for development of the color with various amounts of the gum.

Discussion

By means of this test, it is easily possible to detect 0.2 per cent of sodium alginate or Dariloid in dairy products.

The following substances do not interfere with the test: starch, gelatin, Irish moss, agar, gum tragacanth, India gum, locust bean gum, gum arabic, and formaldehyde. The test has been found satisfactory with a 0.2 per cent solution of Dariloid in milk, heavy cream, cream cheese, and vanilla, strawberry, chocolate, and maple walnut ice cream.

TABLE I. DEVELOPMENT OF COLOR

Weight of Dry Gum *Mg.*	Time for Development of Violet Tinge	Time for Development of Deep Purple Color
0.04	No color developed	No color developed
0.1	Overnight	Not reached after standing one week
0.3	4 hours	Overnight
0.5	4 hours	Overnight
1.0	10 minutes	3 hours
3.3	2 minutes	1 hour

In order to make sure that the substance which gives the color test with the sulfuric acid reagent was derived from the sodium alginate and not from any accompanying impurity, a sample of sodium alginate, which was represented as being substantially pure, was obtained from the Kelco Company. This product was dissolved in water and precipitated by the addition of an equal volume of concentrated hydrochloric acid. The mixture was then boiled for 1 minute and the residue washed free of acid by repeated centrifugalization and decantation. The residue was then dissolved in just sufficient 0.1 *N* sodium hydroxide and the whole process repeated five more times. The residue from the final acid precipitation was assumed to be free of any contaminants and still gave the color test.

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Determination of Menthol in Oil of Peppermint

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EACH year it is necessary for the author's laboratory to analyze several hundred samples of oil of peppermint within the short period which comprises the harvesting season. It has, therefore, been the constant aim to devise a method which would permit more rapid analyses without sacrificing accuracy. The method of Power and Kleber (*6*), which is generally used in the aromatics industry and is the official procedure of the United States Pharmacopoeia, is known to be erroneous and to give variable results in different laboratories; however, none of the modifications proposed thus far has been entirely satisfactory. This procedure is known to indicate as alcohols such nonalcohol constituents as aldehydes, amines, phenols, esters, and some of the more unstable terpenes which are acetylated along with the alcohols when that method is used. The present are included during saponification. During the course of research this method has been thoroughly investigated and, as a basis for further conclusions, it is believed advisable to review briefly the possible sources of error in the order in which they occur during the analysis of an oil. These discrepancies include those observed in the author's laboratory as well as those reported in the literature.

In a report upon the analysis of oil of peppermint, Nelson (*5*) states that "the proper care of the sample previous to analysis is highly important: all reagents used must be of the best quality and the sodium acetate used as a catalyst must be absolutely anhydrous."

It has been observed here that acetic anhydride must be used within a short time after the container is opened; otherwise, deterioration of the anhydride, due to its hygroscopic nature, will be evidenced by low results in alcohol analyses. Frequent standardization of solutions, especially of the alcoholic potassium hydroxide, is necessary to ensure consistent results. It has been found necessary to use hot solutions when washing the acetylated oil to hydrolyze the excess acetic anhydride completely. No evidence of hydrolysis of the acetylated oil has been found if this procedure is used and the dried acetylated oil has always been neutral.

The author has long stressed an exact 1-hour saponification time, having observed that variations in this factor caused marked differences in results. Recently Baldinger (*1*) conducted a time study, varying the length of both acetylation time and saponification time with each sample. The interpretation of the results of this study is summarized as follows: "It would seem that the time of acetylation may vary within broad limits, while the time of saponification had best be varied between 45 and 60 minutes. While experimental evidence is not yet available to prove the contention, it is believed that resinification or polymerization of certain constituents is induced by prolonged heating with potassium hydroxide and that some base is used up, thereby leading to erroneous results."

Redemann and Lucas (*7*) have observed that more rapid hydrolysis of esters results if diethylene glycol is substituted for ethyl alcohol as a solvent for potassium hydroxide. This has been verified by Hall, Holcomb, and Griffin (*4*), who applied this method to the analysis of the isomers of menthol.

In the titration of the saponified oil, variations occur due to differences in the estimation of the end point by different analysts. Badly oxidized or poor quality samples are often difficult to analyze consistently, owing to discoloration during saponification. This interferes with the observation of the end point when phenolphthalein is used as an indicator.

The method of Delaby, Sabetay, and Breugnot (*2*) for the determination of free alcohols in the sandalwood oils gives results from 5 to 8.5 per cent lower than those obtained by Power and Kleber. These authors traced this divergence to the fact that

TABLE I. FREE MENTHOL DETERMINATIONS, SHOWING EFFECTS OF VARIATIONS IN ACETYLATION AND SAPONIFICATION PERIODS

Sample	Free Menthol Present	Acetylation Period	Free Menthol Found in Saponification Period of: 0.5 hour	1 hour	1.5 hours	2 hours	Av.
	%	*Hours*	%	%	%	%	%
		0.5	0.2	0.1	0.1	0.5	0.2
		1	0.1	0.1	0.1	0.2	0.1
1	0.0	1.5	0.1	0.1	0.3	0.3	0.2
		2	0.2	0.1	0.1	0.3	0.2
		Av.	0.1	0.1	0.2	0.3	0.2
		0.5	13.2	13.1	13.3	13.9	13.4
		1	13.0	13.0	13.3	13.3	13.1
2	12.6	1.5	13.1	13.3	13.3	13.5	13.3
		2	13.0	13.3	13.3	13.7	13.3
		Av.	13.1	13.2	13.3	13.6	13.3
		0.5	26.2	25.9	26.2	27.3	26.4
		1	25.9	26.0	26.3	26.6	26.2
3	25.5	1.5	26.2	26.6	26.0	26.7	26.4
		2	26.1	26.3	26.4	26.7	26.4
		Av.	26.1	26.2	26.2	26.8	26.3
		0.5	37.9	38.1	38.8	28.7	38.4
		1	38.2	38.4	38.6	38.4	38.4
4	37.5	1.5	37.9	38.2	38.1	39.3	38.4
		2	38.2	38.4	38.4	38.7	38.4
		Av.	38.0	38.3	38.5	38.8	38.4
		0.5	50.7	50.9	51.0	52.3	51.2
		1	50.6	51.0	51.3	51.5	51.1
5	50.1	1.5	50.7	51.0	51.0	51.5	51.0
		2	50.8	51.2	51.3	52.4	51.4
		Av.	50.7	51.0	51.1	51.9	51.2
		0.5	62.8	63.1	63.5	63.7	63.3
		1	62.8	63.3	63.8	64.1	63.5
6	62.5	1.5	62.9	63.3	63.4	65.2	63.7
		2	63.1	63.6	63.4	65.4	63.9
		Av.	62.9	63.3	63.5	64.6	63.6
		0.5	75.4	75.9	76.2	77.3	76.2
		1	75.6	75.7	76.5	76.7	76.1
7	75.1	1.5	76.1	76.3	76.8	77.9	76.8
		2	75.5	75.9	76.5	76.9	76.2
		Av.	75.6	76.0	76.5	77.2	76.3
		0.5	99.6	100.0	100.1	101.2	100.2
		1	99.5	99.6	100.7	101.4	100.3
8	100.0	1.5	99.3	100.0	100.1	101.5	100.2
		2	99.4	100.3	100.5	100.5	100.2
		Av.	99.4	100.0	100.4	101.1	100.2
		0.5	45.8	45.9	46.2	46.9	46.2
		1	45.7	45.9	46.3	46.5	46.1
Averages	45.4	1.5	45.8	46.1	46.1	47.0	46.2
		2	45.8	46.1	46.2	46.8	46.2
		Av.	45.8	46.0	46.2	46.8	46.2

the santalenes present were esterified along with the alcohols by the latter method.

With these facts in mind, the following experimental work was performed in an effort to reduce to a minimum the errors in the present procedure, or to find a new and more accurate method of assay.

Experimental Procedure

Samples containing known amounts of free menthol were prepared.

A 1-kg. sample of alpha-pinene was redistilled in vacuum; the major portion of the distillate consisted of a fraction with a constant boiling point of 14° C. at 2 mm. By the regular method of analysis it was found to contain negligible quantities of esters and alcohols. From a 2-kg. lot of natural oil of peppermint the menthol was separated, using the fraction boiling above 70° C. at 2 mm. This crude menthol was purified by centrifuging and by repeated recrystallization from benzene until a constant melting point of 43° C. was obtained for the product. This menthol was dissolved in varying proportions in the redistilled pinene to give samples having definite percentages of free menthol. Using these samples, a time study similar to that of Baldinger (*1*) was conducted.

Pyrex-resistant glassware, with ground-glass joints and interchangeable connections, was used throughout. Electric heaters equipped with sand baths were used and preheated so that samples placed upon them began to boil almost immediately. Acetylation and saponification periods were varied by half-hour intervals up to and including 2 hours. Except for the introduction of these variables, the method described in the U. S. P. XI was employed.

The results of this study (Table I) substantiate the previously mentioned contentions of Baldinger regarding the reaction of alkali during saponification with components in the oil other than esters. The amount of base entering into these side reactions increases sharply if the acetylated oil is permitted to saponify over 1.5 hours. This contributing factor obviously depreciates the classical method by indicating a higher than actual alcohol content.

Several methods (*2*, *3*, *8*), using an acetylant mixture of pyridine and acetic anhydride, have been proposed for the analysis of free primary or secondary alcohols. Because of the objectionable odor of pyridine, an investigation was conducted for solvents that might be substituted in place of this reagent.

Analytical Procedure

One gram of the sample, accurately weighed in a tared acetylation flask, is treated with 5 ml., accurately measured by means of a Koch microburet, of a freshly prepared acetylant mixture consisting of four parts by volume of *n*-butyl ether and one part of acetic anhydride. A blank is prepared in an identical manner, omitting the oil. The flasks are connected to air condensers and the contents boiled gently for 1 hour on a sand bath. Without removing the flasks from the bath, 20 ml. of hot distilled water are added through the condensers and the contents are boiled vigorously for an additional 30 minutes to convert the excess anhydride into acetic acid. After removing the flasks from the bath and allowing the contents to cool to room temperature, 20 ml. of cold distilled water are added as before.

TABLE II. FREE ALCOHOL DETERMINATIONS USING MIXTURES CONTAINING KNOWN AMOUNTS OF FREE MENTHOL

Sample	Calculated (From Table I)	Power and Kleber's Method	Author's Method Mean	Author's Method Average deviation
	%	%	%	%
1	0.0	0.1	0.1	0.1
2	12.6	13.0	12.5	0.2
3	25.5	26.0	25.8	0.3
4	37.5	38.4	37.6	0.5
5	50.1	51.0	50.1	0.2
6	62.5	63.3	62.4	0.3
7	75.1	75.7	75.0	0.5
8	100.0	99.6	99.8	0.4
Av.	45.4	45.9	45.4	0.3

TABLE III. ANALYSES OF FRACTIONS FROM A VACUUM DISTILLATION OF OIL OF PEPPERMINT

Fraction	Distilled	Power and Kleber's Method: Esters	Total alcohols	Free alcohols	Free Alcohols by Author's Method: Mean	Average deviation
	%	%	%	%	%	%
Light fraction	0.8	...	...	...	...	...
Water	0.7	...	...	...	...	...
1	5.0	1.7	4.9	3.6	1.3	0.2
2	5.0	0.9	2.5	1.8	0.6	0.2
3	5.0	1.1	3.0	2.1	1.2	0.5
4	2.5	4.3	19.0	15.6	15.8	0.0
5	10.0	2.3	10.5	8.7	6.9	0.2
6	10.0	1.6	18.0	16.7	15.4	0.2
7	5.0	3.0	43.4	41.1	40.5	0.5
8	5.0	4.2	62.4	59.0	59.4	0.1
9	10.0	5.8	80.4	75.8	75.3	0.2
10	32.5	8.1	91.3	84.9	84.8	0.3
11	2.0	17.2	86.8	73.2	71.2	0.6
Residue	3.5	10.1	27.4	19.4	15.0	0.4
Loss	3.0					
	Av.	5.2	51.9	47.8	46.9	0.3

The flasks are removed from the condensers and the ground-glass connections rinsed by means of a water wash bottle, allowing the rinsings to flow into the flasks. At this point, the acid strength of the blank is approximately 0.5 *N*. Eight or 10 drops of phenolphthalein test solution (dissolve 1 gram of phenolphthalein in 100 ml. of alcohol) are added and the excess acid is neutralized with 0.5 *N* alcoholic potassium hydroxide solution. The blank is titrated to the full red color of the indicator and the oil sample matched with the blank. It is necessary to conduct this titration on a 2-phase system, but this presents no difficulty. The difference in the amount of potassium hydroxide indicates the per-

centage of free alcohols present in the original sample; the calculation of these results is made by the following formula:

$$\% \text{ free alcohol} = \frac{\frac{\text{molecular weight of alcohol}}{20} \times \text{ml. } 0.5\ N \text{ KOH}}{\text{weight of sample}}$$

If the sample to be analyzed is not perfectly anhydrous, it should be dried; allowance should be made for the acid value of those oils containing free acids.

If the percentage of total alcohols is desired, an ester determination is made by the regular method and the following formula will effect the necessary conversion:

$$\% \text{ total alcohols} = \% \text{ free alcohols} + \frac{\text{molecular weight of alcohol}}{\text{molecular weight of ester}} \times \% \text{ esters}$$

Results obtained by the method of Power and Kleber and the procedure outlined above are given in Tables II to VI. To conserve space all figures are averages of several determinations. If the above procedure is carefully followed, the results obtained are very consistent, as indicated by the average deviations from the mean.

The data in Table IV indicate that in the case of oil of peppermint free alcohol determinations by the author's method average about 3 per cent lower than those done by the method of Power and Kleber. In Table III this divergence is traced to small quantities of aldehydes contained in the lower boiling fractions, which aldehydes, it is believed, are acetylated by the pharmacopoeial process, and to polymerization during saponification of the higher boiling resinous constituents, with a subsequent utilization of alkali. The data on redistilled and badly oxidized oil of peppermint indicate that the amount of resinous material affects the extent of the divergence.

The data presented would indicate that a free alcohol content of 43 per cent for oil of peppermint, when analyzed by the method herein described, would very closely approximate the U. S. Pharmacopoeia 50 per cent total alcohol minimum or the British Pharmacopoeia 46 per cent free alcohol minimum if the analysis were made by the classical procedure.

The pure alcohols included in Table VI give harmonious results regardless of the method used, except that *d*-neomenthol must be saponified for more than 3 hours if the U. S. P. XI procedure is used (*5*).

Differences obtained when assaying the impure alcohols included in Table VI or the essential oils shown in Table V are explained by the presence of nonalcohol constituents which are acetylated by the classical method but are not attacked by the acetylant mixture proposed in this paper.

TABLE IV. ANALYSES OF SAMPLES OF PEPPERMINT

Sample	Power and Kleber's Method: Esters	Total alcohols	Free alcohols	Free Alcohols by Author's Method: Mean	Average deviation
	%	%	%	%	%
Choice Natural Peppermint					
1	3.7	43.6	40.7	36.4	0.2
2	4.5	47.1	43.6	41.9	0.4
3	5.6	50.0	45.6	42.6	0.3
4	5.2	50.4	46.3	44.5	0.4
5	5.2	51.9	47.8	45.2	0.2
6	5.9	54.6	50.0	47.4	0.2
7	7.5	57.1	51.2	47.7	0.3
8	7.2	59.7	54.0	50.8	0.3
9	8.4	63.9	57.3	53.4	0.4
Av.	5.9	53.1	48.5	45.5	0.3
Redistilled Oil of Peppermint					
1	5.3	52.0	47.8	45.3	0.3
2	5.6	52.8	48.4	45.5	0.2
3	5.5	52.6	48.3	45.6	0.2
4	6.3	54.4	49.5	46.4	0.1
5	5.7	57.0	52.5	50.2	0.5
6	5.8	57.1	52.5	49.8	0.4
Av.	5.7	54.3	49.8	47.1	0.3
Badly Oxidized Oil of Peppermint					
1	9.5	54.8	47.4	43.0	0.3
2	10.7	59.4	51.0	46.8	0.1
3	7.8	58.9	52.7	50.5	0.2
4	11.0	62.9	54.3	52.0	0.8
5	8.5	63.9	57.2	52.7	0.2
6	9.0	63.5	56.4	53.2	0.2
Av.	9.4	60.6	53.2	49.7	0.3

TABLE V. ANALYSES OF OTHER ESSENTIAL OILS

Sample	Power and Kleber's Method: Esters	Total alcohols	Free alcohols	Free Alcohols by Author's Method: Mean	Average deviation
	%	%	%	%	%
Citronella	10.1	81.8	73.8	37.3	0.4
Geranium	25.8	69.3	52.5	38.9	0.3
Lavender	41.5	63.2	30.6	14.1	0.2
Rosemary	5.1	19.0	15.0	9.9	0.3
Sandalwood	2.7	92.9	90.6	81.2	0.4

TABLE VI. ANALYSES OF STOCK SAMPLES OF ALCOHOLS

Sample	Power and Kleber's Method: Esters	Total alcohols	Free alcohols	Free Alcohols by Author's Method: Mean	Average deviation
	%	%	%	%	%
d-Borneol	0.1	100.1	100.0	99.6	0.2
Cinnamic alcohol[a]	3.6	97.5	94.8	87.7	0.2
Citronellol[a]	4.1	101.8	98.6	91.6	0.3
Geraniol	0.3	94.9	94.7	95.9	0.6
l-Menthol	0.1	99.7	99.6	99.8	0.4
d-Neomenthol[b]	0.7	...	...	98.6	1.2
Phenyl ethyl alcohol	0.3	99.9	99.7	99.4	0.4
Isopulegol[a]	0.5	100.1	99.7	94.2	0.5

[a] No attempt was made to purify these samples. Their aldehyde content was found sufficient to account for differences in results by the two methods.
[b] Sample kindly furnished by Swann and Co., Birmingham, Ala. Hydrolysis of acetylated oil is incomplete in 1-hour saponification limit specified in U. S. P. XI (*5*).

Conclusions

The method of Power and Kleber has been thoroughly investigated. Sources of discrepancies have been found and suggestions made to prevent their occurrence.

A new simplified procedure, utilizing an acetylant mixture of *n*-butyl ether and acetic anhydride, permits a more selective esterification of alcohols than the pharmacopoeial method. This procedure is apparently applicable, without variations, for the analysis of free primary or secondary alcohols, regardless of content, in any of the essential oils. Since saponification, shown to be a major source of error in the method of Power and Kleber, is eliminated, the procedure described gives more satisfactory results with those alcohols whose esters hydrolyze slowly or with difficulty and permits the determination of the alcohol content of those oils whose specific gravities are greater than one. This method is more economical and less time-consuming than that of Power and Kleber. The water present in the titration mixture enables the analyst more easily to distinguish a very sharp end point and prevents the formation of an interfering precipitate.

The procedure described also seems to be more satisfactory than those using a pyridine–acetic anhydride mixture. The odor of *n*-butyl ether is not objectionable and this solvent possesses the added advantage over pyridine that its boiling point (142° C.) coincides well with that of acetic anhydride (140° C.), whereas pyridine boils at 115° C. More rapid and complete acetylation would therefore be indicated in an ether mixture than in a pyridine–acetic anhydride mixture. Greater dilution of the acetic anhydride is possible if *n*-butyl ether is used as a solvent, decreasing the percentage of error in the results.

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Methods for Estimation of Some Amino Acids in Corn Grain

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DURING investigations on the chemical composition of inbred and hybrid lines of corn, it was found desirable to determine some of the amino acids present in the corn protein. Similar work on corn has been carried out by Csonka (*3*), Hamilton *et al.* (*8*), and Showalter and Carr (*29*). Detailed investigations on various purified corn proteins have been carried out by numerous workers. The classical work of Osborne and co-workers (*20*, *21*, *22*) as well as the work of Dakin (*4*) and Kossel and Kutcher (*14*) should be recalled in this connection.

The methods used by these workers give very good results but are too long and tedious to be applied to protein studies on large numbers of samples. The work reported here had as its object developing relatively short and accurate methods for the determination of some of the nutritionally important amino acids present in corn grain.

Preparation and Hydrolysis of Protein Fraction

Dry corn grain normally contains 7 to 11 per cent protein, 1 to 2 per cent ash, 3 to 5 per cent fat, 1.5 to 3 per cent crude fiber, and 80 to 85 per cent starch, sugars, and other nitrogen-free materials. In order to obtain the corn protein relatively free from the other constituents of corn grain, it is necessary either to extract the protein or remove the other compounds from the protein.

Hamilton *et al.* (*8*) removed fat and other materials by extraction with dry ethyl ether and absolute ethanol, removed starch by extraction with hot 2 per cent trichloroacetic acid, and then removed the protein by successive treatment with 20 per cent hydrochloric acid and 5 per cent sodium hydroxide.

Csonka (*3*) extracted some of the protein with cold 1 per cent sodium chloride and 80 per cent ethanol. Starch was removed from the residue with cold 21 per cent hydrochloric acid and precipitated from solution with ethanol. The starch-free residue, the acid alcohol solution from the starch precipitation, and the sodium hydroxide and 80 per cent alcohol extracts were hydrolyzed and the hydrolyzate was used as material for the determination of amino acids.

These procedures are both rather long and cumbersome. In an effort to simplify them the following procedure was developed and found satisfactory:

Grind the air-dry corn grain to 1 mm., dry in a vacuum oven at 100° C. for 5 hours, and extract with anhydrous ethyl ether in a continuous Soxhlet extraction apparatus for 48 hours. The ether extract contains only very slight amounts of nitrogen and may be discarded. Grind the dry fat-free residue to 100-mesh in a ball mill. Place 5 grams of the dry finely ground residue in a 200-ml. Erlenmeyer flask, add 150 ml. of distilled water, and heat for 30 minutes on the steam bath to gelatinize the starch. Cool, add 2 ml. of saliva and a few milliliters of toluene, stopper, and incubate at 38° C. for 72 hours with occasional shaking. At the end of the incubation period, heat on the steam bath to inactivate the enzyme and drive off the toluene. Filter through Whatman No. 42 filter paper and wash thoroughly with hot water. Discard the filtrate.

Transfer the residue to a 250-ml. Kjeldahl flask and add 20 ml. of hydrochloric acid, specific gravity 1.10. Add a few drops of capryl alcohol, place a test tube through which water is flowing in the neck of the flask to act as a reflux, and boil gently on a sand bath for 20 hours. Remove the condenser and boil off the capryl alcohol. Filter off the humin and wash thoroughly, catching the filtrate and washings in a 250-ml. Claisen flask. Evaporate the filtrate and washings to a thick paste on a boiling water bath under reduced pressure, keeping the temperature below 60° C. Add 50 ml. of distilled water and again evaporate to a thick paste. Repeat twice more to remove as much of the hydrochloric acid as possible. Add 25 ml. of water to the paste and make just alkaline to litmus with solid calcium hydroxide. Add 25 ml. of ethanol to prevent foaming and distill off the ammonia into an excess of 0.1 *N* hydrochloric acid. Thirty minutes under reduced pressure on a boiling water bath is sufficient to remove the ammonia.

Back-titrate the solution in the receiving flask with 0.1 *N* sodium hydroxide and calculate the amount of ammonia nitrogen present. (Run a blank on the reagents used and deduct this from the value obtained.) Filter off the material remaining in the Claisen and wash with hot water until free from chlorides. Acidify the filtrate and washings with hydrochloric acid and concentrate under reduced pressure to about 10 ml. Transfer to a 50-ml. volumetric flask and make to volume with distilled water. Pour into a 150-ml. beaker, add a few grams of Norit A (which has been washed with acid and alkali), stir thoroughly, and filter. This treatment was found to decolorize the solution satisfactorily. Determine cystine and cysteine on aliquots of this solution according to the directions given below.

Pipet another aliquot (25 ml.) of the solution into a 50-ml. centrifuge tube and precipitate the basic amino acids with phosphotungstic acid according to the directions given by Cavett (*2*). When washing the phosphotungstic acid precipitate, keep the wash solution, the centrifuge tubes, and the tube holders as nearly ice cold as possible. To the washed phosphotungstic acid precipitate add a thick suspension of barium hydroxide with stirring until the solution is just alkaline to litmus. Centrifuge off the barium phosphotungstate and wash several times by dispersing the precipitate in hot distilled water and centrifuging. Combine the filtrate and washings and precipitate the excess barium by the addition of a slight excess of sulfuric acid. Filter off the barium sulfate and wash with hot distilled water. Concentrate the filtrate and washings to a small volume under reduced pressure, transfer to a 25-ml. flask, and make to volume. Use aliquots of the solution for the determination of arginine and histidine according to the directions given below.

For the determination of tyrosine and tryptophan another sample of corn is hydrolyzed with alkali, since tryptophan is partially destroyed by acid hydrolysis in the presence of carbohydrate material (*9*).

Transfer the residue from the saliva digestion of 5 grams of dry, fat-free corn to a test tube and add 20 ml. of 5 *N* sodium hydroxide. Insert an air condenser (a glass tube about 30 cm. long through a stopper covered with tin foil) and digest for 24 hours in the steam bath. Wash the hydrolyzate into a 50-ml. volumetric flask with water. Add 10 ml. of 15 *N* sulfuric acid

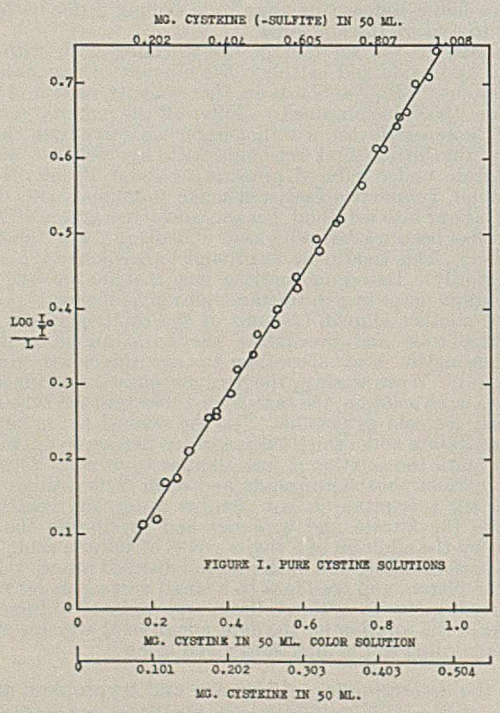
TABLE I. NITROGEN DISTRIBUTION OF FRACTIONS FROM A SAMPLE OF DRY, FAT-FREE CORN GRAIN

Fraction	Equivalent Weight of Corn	Nitrogen	% of Total Nitrogen in Fat-Free Corn
	Grams	*Mg.*	
Dry, fat-free corn grain	1	17.4	100.00
Filtrate from saliva digestion	2	2.80	8.04
Acid-insoluble humin	5	3.12	3.59
Acid-soluble humin	5	1.26	1.45
Ammonia nitrogen	5	11.07	12.73
Acid hydrolyzate	1	12.58	72.30

mix thoroughly, cool, and make to volume. Filter or centrifuge the solution. Decolorize the filtrate with Norit A (previously washed with acid and alkali) and use an aliquot for the determination of tryptophan and another aliquot for the determination of tyrosine according to the procedure given below.

For tryptophan a longer hydrolysis period may be necessary or the determination may be carried out on the unhydrolyzed residue by the original May and Rose procedure (*19*).

These hydrolysis procedures are not new. The acid hydrolysis is essentially that described by Cavett (*2*), while the alkaline hydrolysis is similar to that described by Folin and Marenzi (*6*) and Lugg (*18*). The author is not aware of any previous use of saliva to remove starch in protein purification. Some nitrogen compounds, the nature of which is not known, are lost in the filtrate from the saliva digestion. Acetic acid, trichloroacetic acid, and basic lead acetate do not cause the precipitation of any nitrogen-containing compounds from this filtrate, indicating that the nitrogen is not protein nitrogen. The solution gives only a faint ninhydrin reaction, which indicates that only small amounts of proteins or amino acids are present.

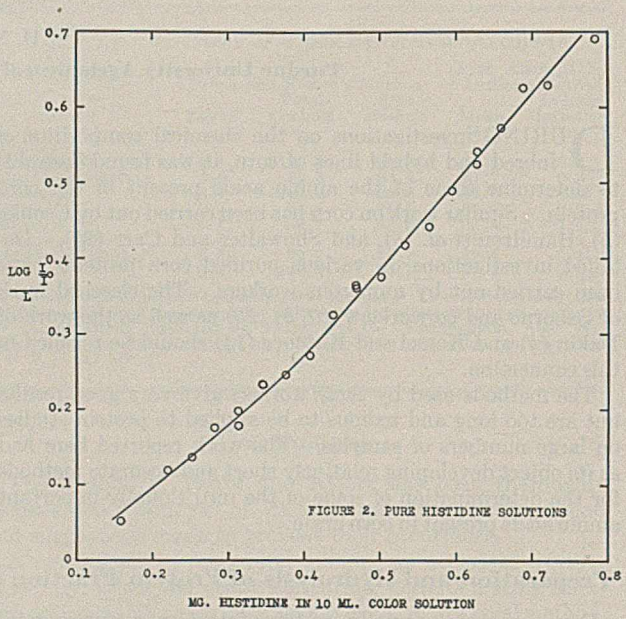


Approximately 20 per cent of the original weight of the ground fat-free corn remains in the residue after saliva digestion. On the basis of total nitrogen determinations, about 45 per cent of this residue is protein, the remainder being cellulose and other complex carbohydrate material.

Table I shows the nitrogen distribution in the fractions through the acid hydrolysis.

Colorimetric Determination of Amino Acids

Colorimetric methods for the determination of some of the amino acids have been carefully studied and shown to give as accurate results as direct isolation methods. In general, colorimetric methods are less cumbersome and time-consuming than direct isolation or gravimetric methods. Colorimetric methods may often be simplified and improved by using some type of photometer, so that reference data can be obtained from pure solutions of the substance to be determined. These reference data may then be used in the analysis of unknown samples, thus obviating the necessity of carrying standard solutions through the procedure with each set of unknowns.



In this work the KWSZ photoelectric photometer described by Withrow, Shrewsbury, and Kraybill (*30*) has been applied to the colorimetric determination of tyrosine, tryptophan, cystine, arginine, and histidine in pure solutions.

In order to determine what light filters to use in the KWSZ photometer for the estimation of the various amino acids, the colored compounds were formed and the absorption spectra determined with a visual Bausch & Lomb spectrophotometer. For cystine, which is reduced with sulfite and then reduces Folin's uric acid reagent to give a blue compound that shows maximum light absorption above 7000 A., Corning filters Nos. 246 and 585 were used in the KWSZ instrument with 0.5 per cent copper sulfate in the cooling cells. For tryptophan, which forms with *p*-dimethylaminobenzaldehyde a blue compound that shows maximum absorption near 6000 Å., Corning filters Nos. 246 and 428 were used with 0.5 per cent copper sulfate in the cooling cells. For histidine, tyrosine, and arginine whose colored compounds exhibit maximum light absorption in the neighborhood of 5000 Å., Corning filters Nos. 430 and 401 were used with 5 per cent copper sulfate solution in the cooling cells.

CYSTINE. The colorimetric determination of cystine by means of phospho-18-tungstic acid reagent as originally described by Folin and Looney (*5*) has been extensively studied by Folin and Marenzi (*7*), Lugg (*15*, *16*), Shinohara (*25–28*), Schoberl and co-workers (*23*, *24*), and Kassell and Brand (*12*).

The procedure adopted for the determination of cystine is that described by Shinohara (*26*) with some slight modifications suggested by the studies of Lugg and Kassell and Brand. The solutions used are those of Shinohara.

Add 10 ml. of 2 *M* sodium acetate solution and 3 ml. of 2 *M* acetic acid to a 50-ml. volumetric flask. Add an amount of

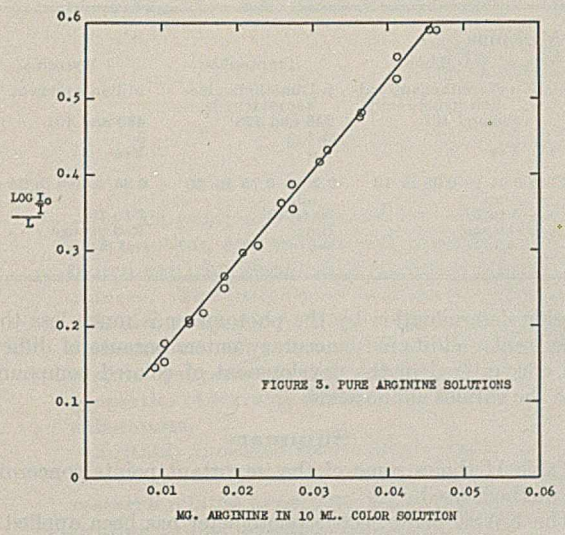


FIGURE 3. PURE ARGININE SOLUTIONS

solution containing 0.2 to 1.0 mg. of cystine, enough sodium hydroxide solution to neutralize the acidity of the added cystine solution (determined by titration of a separate aliquot of the solution using bromophenol blue as the indicator), and 2 ml. of 1 *M* sodium bisulfite solution. Place in a water bath at 18° ± 1° C. for 15 minutes, add 2 ml. of phosphotungstic acid reagent, mix, and allow to stand in the water bath for 15 minutes. Dilute to volume with water and after 5 to 10 minutes determine the light transmission in the KWSZ photometer, with water in the reference cell. Since the sulfite itself develops a small amount of color with the phosphotungstic acid reagent, it is necessary to deduct the value obtained from a sulfite blank determination.

Figure 1 shows the curve obtained with pure cystine solutions. It will be noted that the log values for the light absorption are directly proportional to the cystine concentration within the concentration limits used.

Since cystine with sulfite and cysteine with sulfite develop the same amount of color (mole for mole) and cysteine develops half as much color in the absence of sulfite as in the presence of sulfite, both cystine and cysteine may be determined using this curve. For this the solutions are those as outlined by Kassell and Brand (*12*).

HISTIDINE. For the quantitative determination of histidine the following procedure was found satisfactory:

To a few milliliters of histidine solution (containing 0.15 to 0.75 mg. of histidine) in each of three 10-ml. volumetric flasks add bromine solution (1.0 per cent bromine in 33 per cent acetic acid) dropwise until a deep yellow color persists. Let the solutions stand at room temperature for 10 minutes. Remove the excess bromine by bubbling air through the solution until the yellow color disappears. Add 2 ml. of a solution containing 2 parts of concentrated ammonium hydroxide and 1 part of 10 per cent ammonium carbonate to each flask and immerse the flasks in a boiling water bath for exactly 5 minutes. Remove the flasks from the water bath and immerse in an ice bath for 5 minutes. Allow to stand at room temperature for 10 to 20 minutes, dilute to the mark with 95 per cent ethyl alcohol, and mix. Pour the contents of the three flasks into a small Erlenmeyer flask, mix, and measure the transmission value in the KWSZ photometer within 15 minutes. The mixing of three separate dilutions offers a rapid method for obtaining an average transmission value from three separate determinations. The blank solution used in the reference cell of the instrument is made up and treated as above, except that water instead of a histidine solution is used.

This is an adaptation of the Wooley and Peterson modification (*31*) of the Kapeller-Adler procedure (*11*) for the determination of histidine.

Figure 2 shows the curve obtained using pure histidine solutions. The log values for the light absorption are proportional to concentration when the histidine concentration is between 0.45 and 0.80 mg. of histidine in 10 ml. of color solution.

ARGININE. Arginine was determined as follows:

Place an amount of arginine solution containing 0.01 to 0.05 mg. of arginine in each of four 10-ml. volumetric flasks and add water to make 5 ml. Cool in an ice bath, add 1 ml. of 10 per cent sodium hydroxide solution and 1 ml. of 0.02 per cent α-naphthol, mix, and embed in ice for 1 hour. Add 0.1 ml. of sodium hypobromite solution (2.5 grams of bromine dissolved in 100 ml. of cold 5 per cent sodium hydroxide), shake vigorously, and exactly 15 seconds later dilute to volume with cold 40 per cent urea solution. Pour the solutions from the four volumetric flasks into an Erlenmeyer flask, mix, and measure the transmission in the KWSZ photometer after 5 minutes and in less than 7 minutes after adding the hypobromite, with water in the reference cell.

Four dilutions are made and the four solutions mixed before reading, because even pure solutions of arginine do not always develop the same shade of color under the conditions outlined. The mixing of four separate dilutions is a rapid method for determining the average transmission value of four separate dilutions.

This is an adaptation of the procedure given by Jorpes and Thoren (*10*) for the determination of arginine by the Sakaguchi reaction.

Figure 3 shows the curve obtained with pure solutions of arginine. Within the concentration range used the log values for the light absorption are proportional to the arginine concentration and Beer's law is obeyed.

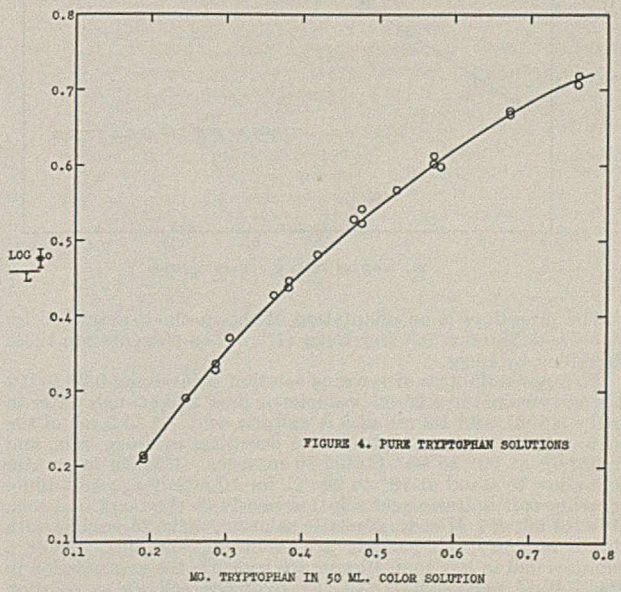


FIGURE 4. PURE TRYPTOPHAN SOLUTIONS

TRYPTOPHAN. The following procedure was used for the colorimetric determination of tryptophan:

Pipet an amount of tryptophan solution containing 0.2 to 0.8 mg. of tryptophan into a 50-ml. volumetric flask. Add 2 ml. of 1 per cent tryptophan-free gelatin solution, 0.2 ml. of 1.5 per cent sodium nitrate solution, 0.5 ml. of 5 per cent *p*-dimethylaminobenzaldehyde solution, and 2.5 ml. of concentrated hydrochloric acid. Mix and allow to stand for 20 minutes at room temperature. Dilute to volume with water, mix, and immerse in an ice bath for 30 minutes. Subject the solution to colorimetry in the KWSZ photometer. The blank solution used in the reference cell should be made up and treated as above, using water instead of a tryptophan solution.

This is an adaptation of methods described by Bates (*1*) and Komm (*13*) who modified the original May and Rose (*19*) procedure.

Figure 4 shows graphically the data obtained with pure tryptophan solutions. The log values for light absorption are not proportional to tryptophan concentration when the color is developed according to the above procedure. How-

TABLE II. DATA ON METHODS

Amino Acid	Cystine	Histidine	Arginine	Tryptophan	Tyrosine
Reagent used	Phospho-18-tungstic acid	Bromine and NH_4OH	α-Naphthol and sodium hypobromite	p-Dimethylaminobenzaldehyde	Millon's reagent
Filter system, Corning filters, No.	246 and 585	430 and 401	430 and 401	246 and 428	430 and 401
Heat filter cell, copper sulfate, %	0.5	5	5	0.5	5
Is concentration proportional to light absorption?	Yes	Yes, between 0.45 and 0.8 mg.	Yes	No	Yes
Concentration range, mg.	0.2 to 0.9 in 50 ml.	0.2 to 0.8 in 10 ml.	0.01 to 0.05 in 10 ml.	0.2 to 0.75 in 50 ml.	0.25 to 0.9 in 25 ml.
Time limit on reading, min.	5 to 10	15	5 to 7	30 to 40	5 to 10
Color of solution	Blue	Blue-violet	Orange	Blue	Red orange
Accuracy, %	±3	±4	±5	±3	±1.5

ever, the method is sensitive for small amounts of tryptophan and with pure solutions gives results that are accurate within ±3 per cent.

TYROSINE. The color developed by Millon's reagent was used for the determination of tyrosine.

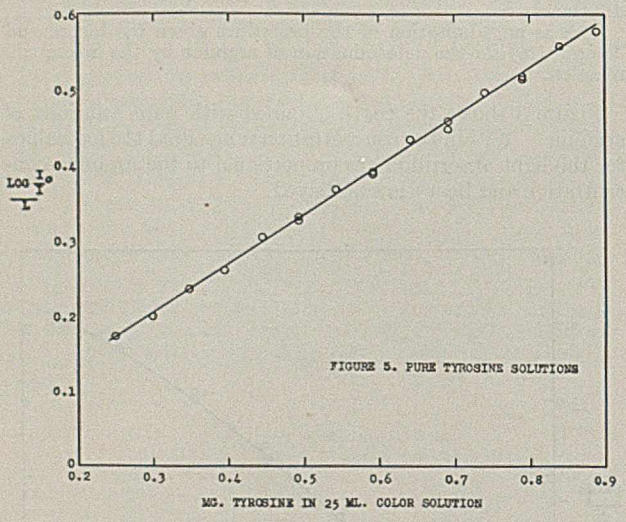


FIGURE 5. PURE TYROSINE SOLUTIONS

The procedure is an adaptation of the methods described by Folin and Marenzi (*6*) and Lugg (*17*). The reagents are those described by Lugg.

To a few milliliters of tyrosine solution (containing 0.25 to 0.9 mg. of tyrosine) in a 25-ml. volumetric flask add enough water to make 3.5 ml. and 1.5 ml. of 5 *N* sulfuric acid. Add 5 ml. of the mercury salt–sulfuric acid solution described by Lugg, mix, and maintain at 60° to 65° C. for 30 minutes. Cool in tap water and allow to stand at 18° to 20° C. for 10 minutes. Add dilute mercury salt–sulfuric acid solution nearly to the mark and mix. Add 0.1 ml. of 1 *M* sodium nitrite solution, make to volume with dilute mercury salt–sulfuric acid solution, and mix. After 5 minutes and in less than 10 minutes measure the transmission in the KWSZ photometer. In the reference cell use a solution prepared in an identical manner except that water replaces the tyrosine solution. If tryptophan is present in the unknown solution, the mercury salt of tryptophan must be centrifuged out and washed as described by Lugg, and the solution must stand at 18° to 20° C. for 1 hour instead of only 10 minutes as recommended above.

Figure 5 shows the curve obtained using pure tyrosine solutions. The log values of light absorption are proportional to tyrosine concentration and Beer's law is obeyed.

The methods outlined above may be applied to the determination of the amino acids in the solutions obtained by the procedure outlined in the early part of this paper. The curves obtained with pure solutions of the amino acids are used as reference data for the determination of the amino acids in the unknown solutions.

The data obtained with the KWSZ photoelectric photometer using pure solutions of the amino acids show that colorimetric determinations can be made with the following accuracy: tryptophan ±3, histidine ±4, tyrosine ±1.5, arginine ±5, and cystine ±3 per cent. The actual error in the transmission determination by the photometer is much less than 1 per cent; additional inaccuracy enters because of difficulties encountered in the development of colored compounds with the various amino acids.

Summary

Table II shows some of the important points concerning the methods used.

The KWSZ photoelectric photometer has been applied to the colorimetric determination of tyrosine, tryptophan, arginine, histidine, and cystine in pure solutions.

The data obtained from pure solutions of these amino acids are being used as reference data in the determination of the amino acid content of corn grain. Starch is removed from the dry fat-free corn by digestion with saliva and the residue hydrolyzed with acid. Cystine is determined in an aliquot of the acid hydrolyzate. Histidine and arginine are determined in a solution from the phosphotungstic acid precipitate of an aliquot of the acid hydrolyzate. Tyrosine and tryptophan are determined in an alkali hydrolyzate of the residue after removal of starch by saliva digestion.

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New Instrument for Rheological Studies of Plastic Substances

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In this paper are discussed the aims, details of construction, accessories, and procedure of a new instrument for rheological studies of plastic substances. The instrument is to be regarded not as a fixed quantity invariable in make-up but as an embodiment of principles for accomplishing a certain purpose—namely, study of mechanical properties observable during deformation of plastic substances.

Suitably modified forms of the instrument and its accessories should be useful in both control and research related to pectins, jellies, gelatins, oil-well drilling muds, ceramic slips, pharmaceutical gels, thermoplastics, and polymerplastics. In fact, the principles may be employed in studying any materials which may be allowed to set under conditions of controlled past history (or path) with a view to measuring the forces required to produce incipient flow or permanent deformation. However, in this paper the instrument is discussed from the point of view of its use as a research tool in studying lyogels.

IF A SOLID substance exists in such a state that it can undergo permanent deformation without fracture, it is said to be a plastic substance (*6*). Because of the increasing importance of such substances in several industries, it has become important to be able to measure some intensive property which would be characteristic of the state of a plastic material. This implies that the property chosen must be reproducibly detectable—for example, by obvious permanent distortion. Concepts based on detectable permanent distortion are proportional limit, yield points, and ultimate strength. These three properties are usually determined for defining the strength of a material (*7*).

Proportional limit has been defined (*5*) as the stress at which the deformation ceases to be proportional to the load; yield point (*5*) as the stress at which marked increase in deformation of specimen occurs without increase in load; and ultimate strength (*8*) as the stress obtained by dividing the maximum load reached, before breaking the specimen, by the initial cross-sectional area, thus giving the dimensions of force divided by length squared. This quantity is often taken as a basis for determining working stresses, and is sometimes used as a characteristic property of a material. If the force applied before reaching the ultimate strength is plotted against displacement, the area under the curve represents work done in producing structural breakdown. It is the purpose of this article to describe an instrument which makes possible the quantitative study of these characteristics of plastic materials.

The instrument has been used in studying the forces required to produce breakage of certain bentonite lyogels. Results and theoretical conclusions resulting from these studies are to be found elsewhere (*2*).

The instrument embodies the torsion principle which has been used previously in certain viscometers and by Garrison (*4*) in his torsion wire gelometer. However, in the instrument described here, the sample is actually sheared and measurement is made of ultimate strength within the sample rather than of forces between interfaces.

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Details of Construction

ESSENTIAL MECHANICAL PARTS. Figure 1 represents a cross section from the front to the rear through the central vertical axis of the instrument. Some of the parts have been overproportioned, so that all the significant details may be shown on one cross-sectional view. Rigid support of the essential parts of the instrument is obtained by mounting them on platforms which slide up and down the vertical race, *RA*, of a floor-type drill stand and may be fixed at any vertical position by tightening the clamp handles, C_1, C_2, C_3.

It seems best to regard the instrument as built around the sample to be tested, which occupies the position designated as *S*. The sample at *S* is supported in a circular trough on a glass disk, *SD*, 4 inches in diameter and 0.25 inch thick (see also Figures 2, 3, and 4). The vertical walls of the circular trough are made of sections cut from beakers and set in concentric grooves on the disk as follows: A circular concentric groove is cut in the top of the disk at a radius of 1.33 inches to a depth of 0.125 inch. Hermetically sealed in this groove is a section, *SB*, cut from a beaker, which serves as the outside container wall. The section of beaker is 2.7 inches in outside diameter, 1.4 inches deep, and has a wall thickness of 0.05 inch. Twelve equally spaced Pyrex fins, *F*, are cemented by means of de Khotinsky cement.

Figure 4 shows an assembly having an outside wall only. The inside wall of the circular trough to contain the sample as shown in Figure 1 is likewise cut from a beaker 0.9 inch in outside diameter and 1.25 inches deep and has a wall thickness of 0.05 inch. It also has Pyrex fins cemented vertically to the outside surface of its walls. These vertical fins on both walls prevent the sample from slipping at the wall interface and thus cause the shearing force to be applied in the limited vertical cylindrical path of the prongs. The disk supporting the sample and forming the bottom of its container is supported inside a water jacket, *J*, made of concentric brass tubing 4 and 4.5 inches in diameter and 18 inches long. This water jacket is movable vertically and is freed or fixed by loosening or tightening clamp handle C_1.

Suspended from above and dipping into the sample are six prongs, *PR*, two of which are shown in cross section. These Pyrex glass rods are 0.2 inch in diameter and 1.7 inches long. They are supported through a glass disk, *D*, of 2.75 inch outside diameter, 0.65 inch inside diameter, and 0.25 inch thickness, drilled with six holes having 0.2 inch diameter equally spaced in a concentric circle of 1.43 inch radius. The prongs are sealed in the six equally spaced holes by means of a sodium silicate cement. Figure 4 shows this disk-prong assembly.

A rigid pointer extends horizontally across the diameter of the top of the glass disk, to indicate the position of the disk as regards rotation. The disk is in turn supported through a grooved rubber gasket tightened by two nuts on a hollow, threaded, cylindrical brass rod having outside diameter of 0.5 inch and a right circular conical segment hole through its center. By means of a six thirty-two screw, 0.5 inch long, this fitting is tightly pressed onto a pinion, *P*, made up of brass in one piece having cylindrical upper and right circular conical lower section. The cylindrical shoulder section is 0.4 inch in diameter and 0.45 inch in length, and the frustrum of right circular conical segment is 0.85 inch long, 0.325 inch in diameter at top, and 0.25 inch in diameter at bottom. This pinion is soldered to the lower end of the torsion wire (piano wire), *TW*, which supports the disk-prong assembly. The wire is 11.4 inches long between fittings.

The upper end of the torsion wire is soldered into a section of a circular brass rod, *R*, 0.25 inch in diameter and 0.25 inch long. Also soldered into *R* is a rigid indicator rod, *I*, made of cold rolled steel 0.125 inch in diameter, 11.2 inches in vertical length, and 2.25 inch in horizontal length at its lower end. The purpose of this pointer is to register in the horizontal plane just above the gel the position of the top of the torsion wire. Torque and deformation in the sample are found by referring the indicating pointer,

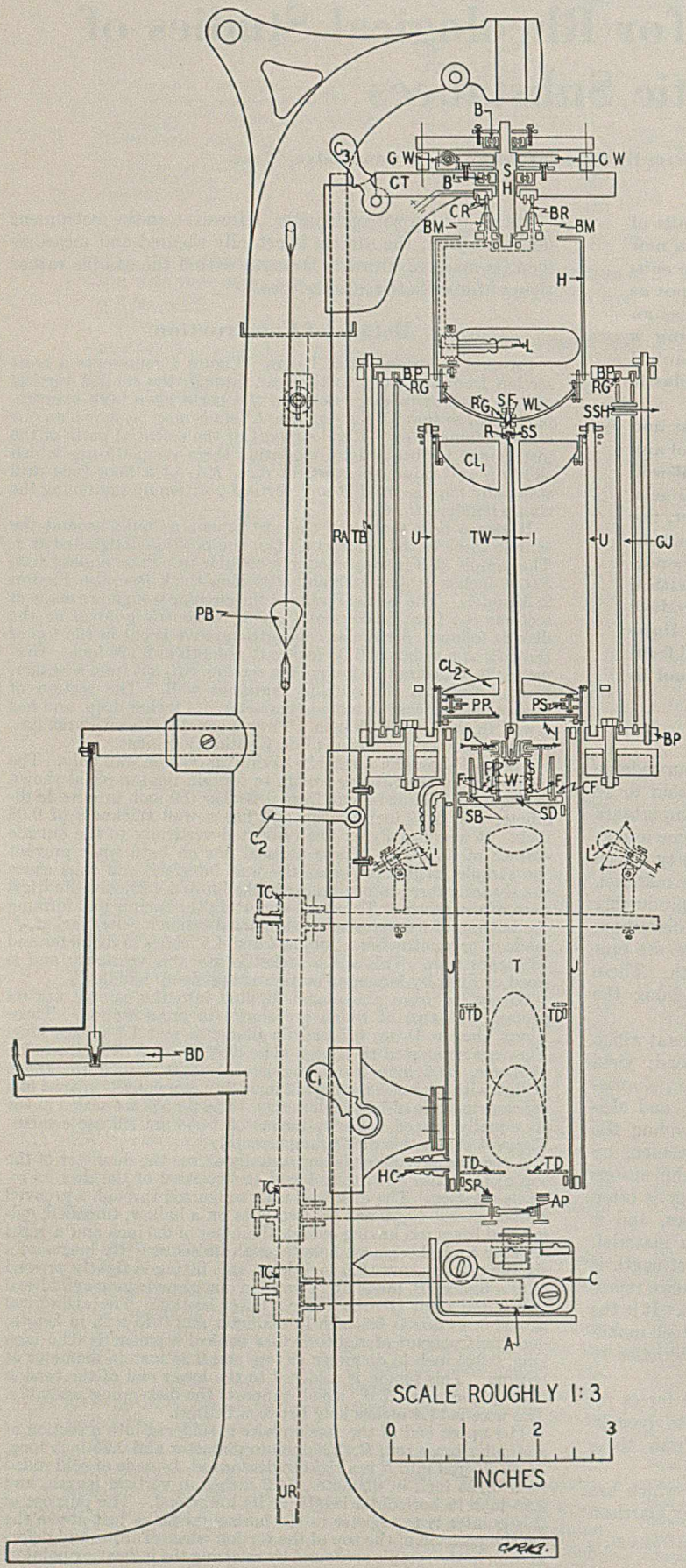

FIGURE 1. VERTICAL CROSS SECTION, SHOWING DETAILS OF CONSTRUCTION

A. Image on film
AP. Analyzer polarizer
B, B'. Ball bearings
BD. Brass disk
BM. Mounts
BP. Brass plates
BR. Brushes
C. Camera
C1, C2, C3. Clamp handles
CF. Circular filters for equalizing actinic value of light through and around sample
CL1, CL2. Condenser lenses
CR. Brass rings
CT. Supporting platform
CW. Cogwheel
D. Disk
F. Pyrex fins
GJ. Glass water jacket
GW. Worm gear
H. Housing
HC. Hose connection in lower water jacket
I. Pointer
J. Water jacket
L, L'. Light sources
P. Pinion
PB. Plumb bob
PP. Polarizer disk
PR. Prongs
PS. Protractor scale
RA. Vertical race
R. Rod fitting to support torsion wire and pointer
RG, RG1. Rubber gaskets
S. Sample
SB. Section of beaker
SD. Glass disk
SF. Fitting
SH. Shaft
SP. Protractor scale referring to analyzer polaroid
SS. Setscrew
SSH. Hole in jacket
T. Tube
TB. Through-bolts
TC. Tee clamps
TD. Tin disks
TW. Torsion wire
U. Brass uprights
UR. Upright rod
W. Stop watch
WL. Watch-glass lens

I, and the pointer on the glass disk-prong assembly to the transparent circular protractor scale, *PS*, which is graduated in percentage degrees and has 4 inch outside diameter and 3.15 inch inside diameter. Below this scale is a polarizer disk, *PP*, having 6 inch outside diameter and 1 inch inside diameter.

The tops of the torsion wire and pointer are supported through the fitting, *R*, by a six thirty-two setscrew, *SS*, in the supporting fitting, *SF*. This fitting passes through a grooved rubber gasket, RG_1, set in a 0.5-inch hole drilled through a watch-glass lens, *WL*, having outside diameter of 5.25 inches, convex radius of curvature 3.5 inches, and thickness of 0.125 inch. This lens is adapted by means of sections of brass tubing to the housing, *H*, also made from brass tubing having diameter of 6.25 inches and length of 6.75 inches. The top of the housing is made from a brass disk 0.125 inch thick and is drilled for ventilation. This housing is held tightly against the shaft, *SH*, by means of a lock nut.

The shaft is supported through the inner races of the ball bearings, *B* and *B'*, having inside diameter of 0.4 and 0.5 inch, respectively, *B'* taking the vertical thrust. The shaft (and thus the entire assembly) may be rotated in the horizontal plane by turning a crank handle which rotates the worm gear, *GW*, 0.6 inch in diameter, 0.8 inch long, 8 threads per inch supported on an axle through two pillow blocks. This gear drives the cog wheel, *CW*, 4.25 inches in diameter, 100 cogs, which is fixed rigidly to the shaft, *SH*.

OPTICAL SYSTEM. To provide means of reading torque displacement and time accurately, an optical system is placed in the same vertical axis as the torsion wire. It is essentially the same as a lantern slide projec-

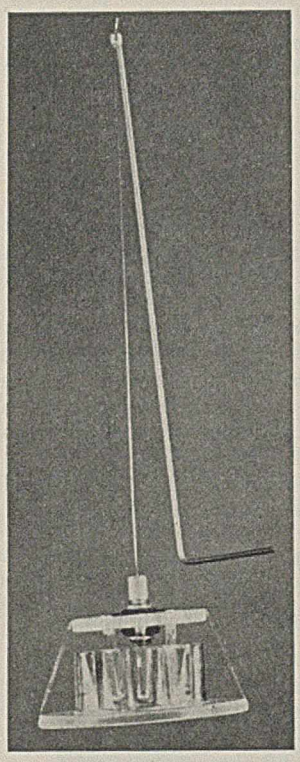

FIGURE 2. PRONGS RIGIDLY HELD IN DISK SUSPENDED ON TORSION WIRE AND FREE TO ROTATE IN SAMPLE CONTAINER

tion system in which the pointers, transparent circular protractor scale, and sample act as a slide. The system is composed of a 6-volt projection lamp "point" source of light, *L*, mounted in the optical axis but free to rotate with the housing, *H*. This point source of light is supplied with electrical energy through two concentric brass rings, *CR*, embedded in Bakelite. This energy is received by phosphor-bronze brushes, *BR*, supported on porcelain mounts, *BM*. Below this source of light is a watch-glass lens, *WL;* 6-inch diameter, 6-inch focal length plano-convex condenser lens, CL_1, drilled with a 0.5-inch hole through the center; 6-inch diameter, 18-inch focal length, plano-convex lens, CL_2, drilled with 1-inch hole through center; plane polarizer, *PP;* and the object to be projected, consisting of the circular protractor scale, pointers, and sample.

The converging light from CL_2 passes through a plane analyzer polarizer, *AP*, whose plane of polarization can be recorded with reference to the circular protractor scale, *SP*. Then the light converges into the f.3.5, 50-mm. lens of a Sept movie camera. This lens causes the formation of an image on the film in the position of the dotted arrow, *A*. The recording camera also looks through the side tube, *T*, and views the stop watch, *W*, which is in about the same horizontal plane as the gel and is illuminated by the lights, *L'*. Actinic value of the light reflected from the watch through the side tube, *T*, can be regulated by rotating a plane polarizer placed in the upper end of the tube. To prevent glare from the sides of the tube and water jacket, tin disks, *TD*, have been supported on brass springs inside the tubes.

FURTHER DETAILS OF CONSTRUCTION. The lenses, CL_1 and CL_2, and transparent circular protractor and polaroid are supported in short sections of aluminum tubing 6.125 inches in inside diameter by means of a circular spring made from 0.25-inch cold-rolled steel bar. These tube sections are supported by three rigid brass rod uprights, *U*, 13.25 inches long and 0.475 inch in diameter. The plane surface of CL_1 is 10.625 inches above that of CL_2, which is 17.5 inches above the lens of the camera.

Surrounding the 6-inch condenser lens system is a glass water jacket, *GJ*, made from sections of Pyrex tubing 10.1 and 8.75 inches in outside diameter, respectively, 0.25 inch in wall thickness, and 16.25 inches long. These tubes are set into rubber gaskets, *RG*, which are set into grooves in brass plates, *BP*, 0.5 inch thick and 10.75 inches square. These header plates are tightened against the ends of the glass tubing by means of long brass through-bolts, *TB*. A hole through the jacket, *SSH*, 0.5 inch in diameter, has rubber tubing cemented in, so that a long slender screwdriver may be inserted for loosening or tightening the setscrew, *SS*. Hose connections for circulating water through this jacket are not shown; however, they are shown, *HC*, in the lower water jacket. A baffle inside the lower jacket causes the water to circulate around the gel.

A vertical upright rod, *UR*, is seen in dotted lines on the opposite side of the drill stand. This rod is 0.75 inch in diameter and 4 feet 2 inches long. The lights, *L'*, polarizer, *AP*, and camera are supported from it by means of tee clamps, *TC*, which permit these accessories to be swung aside so that the jacket sample container, *J*, may be slipped down on the race, *RA*. With the jacket slipped entirely off the end of the race, the torsion wire, prong, and pointer assembly may be removed or inserted as a unit by holding the rigid pointer, *I*, and loosening or tightening the setscrew, *SS*, by means of a screwdriver through the hole, *SSH*. The sample, after being placed in its container jacket, may be pushed up into position and locked there by means of the clamp handle, C_1.

ACCESSORIES FOR USE IN GELATION STUDIES. An accessory shown on the back of the apparatus is a means of suspending a brass disk, *BD*, of known moment of inertia, on the end of the torsion wire to be calibrated. By the torsion pendulum principle (*3*) the coefficient of torsional stiffness of the wire may be found, thus providing a means of calculating torque from any measured angle of deflection.

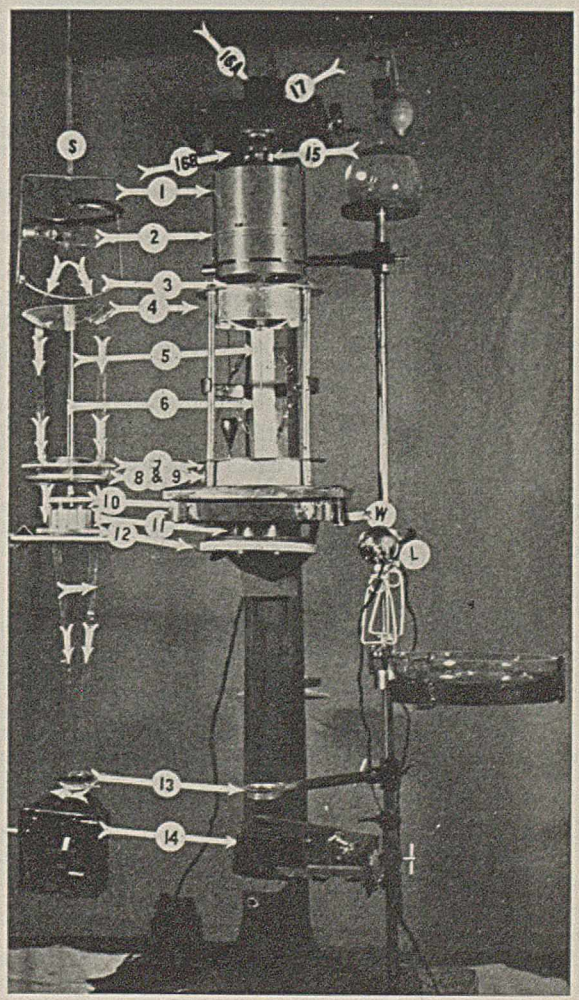

FIGURE 3. ESSENTIAL PARTS OF OPTICAL AND MECHANICAL SYSTEMS

1. Lamp housing
2. Projection lamp bulb
3. Watch-glass lens
4. Condenser lens
5. Pointer indicating position of top of torsion wire
6. Torsion wire
7. Condenser lens
8, 9. Circular protractor and polaroid
10. Disk-prong assembly
11. Wall of sample container
12. Bottom of sample container
13. Analyzer polaroid
14. Camera
15. Phosphor-bronze brush mountings
16*A*. Shaft supporting lamp housing
16*B*. Concentric slip rings for electricity to light filament
17. Gear on shaft for rotating lamp housing
L. Light source
S. Shaft
W. Stop watch

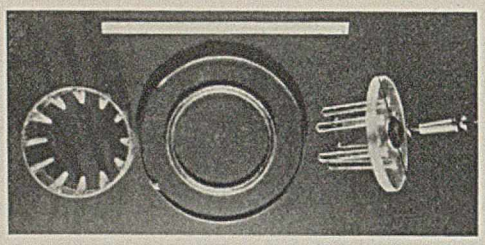

FIGURE 4. PARTS OF SAMPLE CONTAINER AND DISK-PRONG ASSEMBLY

A plumb bob, *PB*, provides a means of leveling the instrument.

Other accessories are shown in Figures 5 and 6. The pump, 16, is used to circulate the water from the constant-temperature tank, 11, through the water jackets. Although the pump is shown in this figure, it was actually isolated from the instrument by using a surge tank to prevent vibration in the water jackets. To prevent mechanical vibration of the floor the pumping mechanism was suspended in a network of rubber tubing.

Submerged in the water in tank 11 is a 2-gallon bottle, the bottom of which has been cut off. In its neck is a rubber stopper in which is inserted a brass tube which makes an arch above and to the right of the tank and enters the water jacket of the instrument. Surrounding this brass tube is a rubber hose. Circulating water passes between the hose and tube. Dry air is blown through a sparger beneath the bottomless bottle in tank 11, becomes saturated by passing up through the water inside, then flows isothermally through the brass tube into the jacket containing the gel sample, thus preventing evaporation while the gel is setting.

Also shown in Figure 5 are a battery, *B*, and transformer, 12, which serve as sources of electrical energy for the lights. An electromagnet for operating the shutter is shown mounted beside the camera.

Consider that a test on a gel has been completed in the instrument. The camera, 6, the lights, *L*, and polarizer, 5, may be swung to the right and the jacket, 7, removed by loosening the clamp handle, 14, and slipping the jacket support down off the race. The dish, 9, is then swung under the prongs, which may be cleansed using the wash bottle, 10, and the brush, 4, the washings dropping into the dish.

Procedure for Use

With reference to Figure 5, consider that the lights, *L*, polaroid, 5, and camera, 6, have been swung to the right and the water jacket, 7, has been lowered off the end of the race as described above. The torsion wire assembly may now be inserted from below through the holes in the lenses into the fitting, *SF* (Figure 1). The setscrew, *SS*, is now tightened by means of a long screwdriver inserted through the opening, 13, in the glass water jacket. The gel to be tested is placed in its container in the brass water jacket, 7, which is then slipped back and clamped in position, so that the prongs suspended from the torsion wire dip into the specimen to the proper depth as indicated by a pointer on the clamp with reference to a scale marked on the race, *RA* (Figure 1). All openings in the jackets are then closed as tightly as possible, and humid air is admitted during gelation. While the gel is setting, isothermal conditions are maintained by circulating water in the jackets.

At the beginning of the test the lights are turned on and a picture is made to record relative zero readings of the pointers, showing the positions of the top and bottom of the torsion wire with reference to the transparent protractor scale. The crank, 17, is then turned, driving the worm gear which causes the top of the torsion wire to rotate. Rotation of the bottom of the torsion wire is restrained by the sample up to a maximum torque at which there is suddenly accelerated rotation of the prongs in the sample. During this rotation of the prongs, the applied force is decreasing.

Pictures or visual readings taken at various intervals during the application of force reveal the maximum torque applied before accelerated motion of the prongs sets in. This may be considered the torque required to break the gel structure.

This measurement is definite and reproducible, because there are no frictional forces to be overcome between the top and bottom of the torsion wire. The force delivered around the top of the wire will be the same as the force overcome at the bottom of the wire and is accurately proportional to the elastic displacement in the wire according to Hooke's law.

Torsion Wires

SELECTION AND CALIBRATION. The design of the instrument and method of calibration described below require that the torsion wires be able to suspend a small weight without change in torsional properties. The tensile properties of piano wire are sufficiently high to meet this requirement. Furthermore, if the stress applied never exceeds 50 per cent of the elastic limit, the properties of piano wire are permanent (assuming prevention of corrosion). It is very difficult to straighten piano wire which has been coiled; therefore, it is desirable to obtain straight lengths from the factory and keep them straight during preparation and use.

The principle of the torsional pendulum (*1*) was employed in calibrating the wires as follows:

A rigid brass disk, *BD*, Figure 1, of known weight and dimensions was attached to the lower end of the torsion wire, thus forming a torsion pendulum. To evaluate the period of this pendulum, the disk is rotated from its equilibrium position

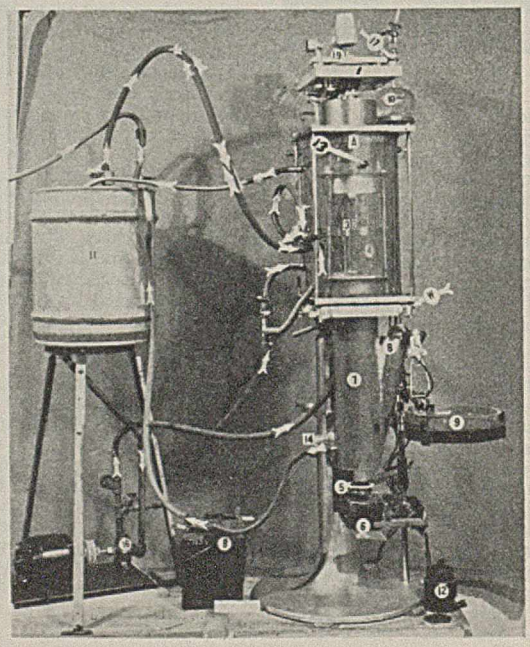

FIGURE 5. APPARATUS USED IN STUDYING BREAKING STRENGTH OF LYOGELS

1. Upper platform supporting lamp housing
2. Torsion wire
3. Pointer
4. Brush
5. Polarizer
6. Camera
7. Water jacket
8. Side tube through which camera sees stop watch, *W*
9. Dish
10. Wash bottle
11. Tank
12. Transformer for operating projection lamp bulb
13. Opening in glass water jacket so that screwdriver may be inserted to remove or replace torsion wires
14. Clamp handle
15. Slip ring and brush assembly
16. Pump
17. Crank for driving worm gear to rotate torsion wire
19. Upper ball bearing housing support

A. Glass water jacket
B. Battery for operating camera shutter
L. Lights
W. Stop watch

Arrows on tubes show directions of flow

about the vertical axis of the torsion wire. When released, the disk will begin to whirl and the number of complete oscillations during a measured interval of time can be counted. The time in seconds divided by the number of complete vibrations is the period, T, of the pendulum.

This period of the torsion pendulum is also given by the equation

$$T = 2\pi \sqrt{I/K'} \qquad (1)$$

where I is the moment of inertia of the disk and K' is the coefficient of torsional stiffness of the wire. I is the product of the mass, M, times the square of the radius, R^2. I is equal to MR^2; hence, it can be found for any given disk. Knowing T and I, the value of K' can be obtained from Equation 1. This value will be characteristic of the particular wire used in its determination.

Although all piano wires of a given diameter, length, and past history as regards tempering, stretching, etc., will have coefficients of stiffness within a certain range, it is necessary to determine the coefficient for each particular wire and tabulate it with reference to that wire only. Furthermore, a similar length of the same wire should be used to determine the elastic limit, which should be tabulated with reference to 50 per cent of its value. This value should not be exceeded in use.

If a wire is twisted about its axis by application of a torque, it has been found experimentally that the angle in radians, Θ, through which the wire is twisted is proportional to the applied torque. That is,

$$T = K'\Theta \qquad (2)$$

in which K' is again the coefficient of torsional stiffness evaluated in Equation 1 and is the characteristic of a given wire mentioned above for tabulation. The value of Θ in radians can be determined for the reading obtained by referring the upper and lower torsion wire pointers to the circular protractor scale in the apparatus. It is the difference between the two readings at any time. The value of T thus calculated by use of Equation 2 has dimensions in centimeter-dynes and is used in calculating the specific ultimate strength as discussed below.

Specific Ultimate Strength

An expression for specific ultimate strength for use with this instrument may be derived as follows:

Consider the plastic set in a circular trough having fins to prevent slippage of the sample at the walls. Consider also that the force is applied by means of a set of prongs made from a section of Duralumin or other suitable tubing, closed at the upper end by a disk to which the torsion wire is fixed and slitted in the lower end to form rounded-edged prongs dipping into the sample. Assume that there is a sufficient number of prongs to produce a circular break in the test specimen and that the surfaces are treated to prevent establishment of forces of adsorption between sample and prongs. The break will be in the form of a vertical cylindrical path or sleeve having a radius equal to that of the circle in which the prongs are set and having a height equal to the depth of the prongs in the gel. The area of this cylindrical break will be equal to the circumference of the circle diminished by the summation of the circular lengths of the prongs, then multiplied by the depth of the prongs in the specimen. The torque required to produce breakage is proportional to this area. Let

T = torque, cm.-dynes
r = radius of circle in which prongs are set, cm.
h = depth of prongs in specimen, cm.
Σl = summation of circular lengths of prongs, cm.
β = specific ultimate strength, dynes divided by sq. cm.

Maximum torque applied in breaking specimen divided by radius at which this torque is acting is equal to the maximum total applied force; and also is equal to the ultimate force which can be resisted by the given vertical cylindrical area of the specimen in which break occurs. This ultimate force is given by the cross-sectional area of breakage times specific ultimate strength of the specimen. Consider the cross-sectional area of breakage to be the summation of the circular areas of the segments removed from the tubing in making the slits mentioned above. That is,

$$T/r = [(2\pi r) - (\Sigma l)]\, h\beta \qquad (3)$$

or

$$\beta = T/r\,[(2\pi r) - (\Sigma l)]\, h = \text{specific ultimate strength of the sample in terms of dynes per square centimeter} \qquad (4)$$

The above derivation assumes no forces of adsorption between prongs and test specimen. This condition might be approached by coating the prongs with a suitable adsorbed film before the test.

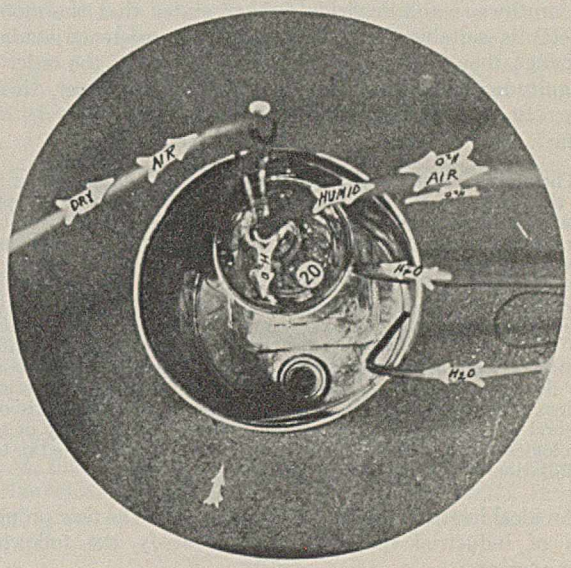

FIGURE 6. LOOKING DOWN ON TANK 11 OF FIGURE 5
20. 2-gallon bottle with bottom removed. Coiled copper tube air sparger may be seen beneath bottle

If the torque is alternately applied and released reversibly, a value of T may be found which is a maximum beyond which the prongs no longer return to their original position with respect to the protractor scale after the applied torque has been released. By substituting this value of T in Equation 4 the proportional limit may be calculated beyond which creep will occur in the sample. However, the ultimate strength is most important and is found by substituting the maximum value of T observable at the start of accelerated rotation of the prongs with simultaneous decrease in torque.

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Measurement of Flow Properties with the Gardner Mobilometer

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THE rheological properties of various plastic soft solids as reported by use of the mobilometer have never been stated in absolute units and, therefore, comparison with the results obtained with other instruments is not easy. It is the purpose of this paper to show how one mobilometer has been calibrated to give results in absolute units. A comparison of the results obtained when several materials are measured with three different types of disk is presented.

Cornthwaite and Scofield (*5*) have stated that the mobilometer is suitable for precise work in consistency studies, although they have not given any indication of the order of magnitude of the precision obtainable. This paper, therefore, also reports a study of the precision that may be obtained.

The mobilometer was first described by Gardner and Parks (*6*) for the control of the flow properties of paints, enamels, and pigmented lacquers. Its dimensions were later standardized in the Gardner laboratory by Sward and Stewart (*12*). It has been used by numerous workers to report the rheological properties of several systems of materials. Gardner and Van Heuckeroth (*7*) have applied the instrument to food products, mineral oils, petrolatum, coal tar, etc. Gray and Southwick (*8*) have used the instrument in their studies of the mayonnaise emulsion. Turnbull (*13*) and McIntyre and Irwin (*11*) have used it to study the flow properties of ceramic clay slips. Baldeschwieler and Wilcox (*1*) have utilized the instrument as a viscometer for viscous mineral oils. The writer has found use for it in a study of the rheological properties of adhesives, particularly those used in the installation of various floor coverings.

An ideal instrument for use in the study of the flow properties of industrial materials should satisfy the following requirements:

1. It should work on the right principle—i. e., it should be possible to vary the rate of shear so that the fundamental flow properties are immediately apparent.
2. It should be relatively precise and the degree of precision must be known.
3. It should be adaptable to a rather wide range of consistencies.
4. From the data collected, it should be possible to calculate the results in absolute units.
5. The instrument should be easily manipulated, so that a relatively inexperienced operator can be trusted to collect data.
6. It should be sturdy in construction.
7. It should be easily cleaned.
8. The speed at which determinations can be made should be high, so that the large amount of data usually necessary can be collected without too much expenditure of time.

In control instruments, the first four requirements are often sacrificed, and the results are of little value if the system being measured is altered more than is slightly incidental to the consistency being controlled. The results so collected are usually of no value as research data.

An industrial research instrument must meet all the above requirements, with emphasis on the first four. The Gardner mobilometer is a control instrument which can be made to satisfy all points if the degree of precision is known and if the results are calculable in absolute units. It can, therefore, be used as an industrial research instrument.

Description of the Gardner mobilometer has been adequately covered in the literature. Essentially, no fundamental changes have been made in the shape or size of the disks or cylinders of the commercially available instruments. Combes, Ford, and Schaer (*4*) have replaced the disks by a perforated cone for use in measuring the consistency of greases.

The writer has found a glass tube fitted in a stopper on the top of the cylinder helpful in dealing with materials containing volatile solvents. Continued exposure of such materials to the air currents of the room causes loss of solvent which sometimes results in deleterious skinning of the exposed surface. High loss of solvent will, of course, produce inaccuracies. The use of the tube decreases this source of error to a large degree. The tube consists of a piece of standard 20-mm. glass tubing 180 mm. long, drawn down at the upper end, and fitted into a No. 9 rubber stopper at the lower end. The clearance between the drawn end of the tube and the moving plunger rod should be about 1 mm. It is important that the drawn end be carefully fire-polished.

In a study of the mobilometer, one is impressed by a similarity between it and an instrument described by Clarvoe (*3*), which was designed for measuring the consistency of roofing putties and fibrous roof coatings, and was developed without knowledge of the existence of the mobilometer. In construction, the Clarvoe instrument varies essentially only in that the diameter of the cylinder is greater and no disk is used on the end of the plunger rod when measuring stiff putties. Materials of lower consistency are measured with a ball on the end of the rod. The perforated disks of the Gardner instrument are not suitable for use with fibrous materials, but it has been suggested that the ball tip principle be borrowed from the Clarvoe instrument, for this purpose.

Procedure

A standard procedure has been employed in all calibrations and observations. All work was performed in a constant-temperature room held at 22 ± 0.5° C., although an effort was made to keep the temperature of the material being measured more closely adjusted. This eliminated the necessity of a water bath, but decreased the accuracy of calibration and observation in varying degrees, depending on the temperature coefficient of the material.

The sample was brought to temperature and poured into the cylinder of the mobilometer to a height of within 2 cm. from the top. In the case of a viscous liquid the air bubbles were expelled by merely allowing the cylinder to stand undisturbed for a time. In the case of highly plastic materials, the entrapped air was eliminated by gently and steadily tapping the bottom of the cylinder on a resilient surface for several minutes. The cylinder was screwed into the base and the rod-guide was clamped in place. The top of the guide clamp in contact with the cylinder was kept at approximately the same height as the level of the contents of the cylinder. In adjusting the weights producing the flow (the weight of disk, rod, and weight pan plus the added weight) no attempt was made to keep the minimum weight of the system 100 grams, as is usually done. In fact, an effort was made to have available a lower minimum shearing weight in some cases, so that the instrument could be applied to more fluid materials. This was done by removing the weight shot from the hollow rod (the shot is placed in the rod by the maker in order to standardize the weight of disk, rod, and weight pan, so that this combination will weigh 100 grams). In this manner, a shearing weight of less than 50 grams was available. At a chosen shearing weight, the time for several plunges was recorded, having the plunger in a different position radially for each determination. At certain positions, the frictional forces of the instrument appear to be at a minimum. Reference is made below to means of minimizing the effects of friction due to the crudity of construction. The position of minimum time was chosen and an effort was made to keep this same radial position in subsequent calibration and

observation. The time was recorded for a fall of 10 cm., this 10 cm. being chosen so that the disk will fall through that volume of material in approximately the middle section of the cylinder, in order to minimize any end effects which might be inherent.

In the case of true liquids, after each plunge the rod was not wiped clean of adhering material, but at least 2 minutes' draining time was allowed, so that the material could flow from the rod before the next plunge was made. This practice was adopted because the use of the glass air current shield in certain cases made wiping after each plunge impractical. The amount of liquid adhering to the rod after 2 minutes' draining time was usually less than 1 gram for 10 cm. of exposed rod. In the case of plastic soft solid materials of considerable yield value, frequent wiping was necessary, as layers of the material tended to build up with successive plunges and withdrawals. This point of procedure was not particularly desirable but seemed practical. An average time for at least three falls for each shearing weight was used in the calculations.

Theoretical

The Gardner mobilometer in its operation applies the multiple orifice extrusion principle. Each hole in the disk may be considered to be a short tube of large diameter. The clearance between the disk and the cylinder wall is wholly operative in flow in the case of the blank disk and only partially operative in the cases of the disks in which there are holes. We may consider the operation of the blank disk, as applying flow between cylinders. Observation shows that the disk, however, does not travel coaxially to the cylinder, but rather travels so that the disk is closer to the cylinder at one point. The flat face surfaces of the disk will also offer resistance to flow. The rod may be considered to apply approximately the coaxial cylinder type of fall. The resistance offered by the passage of the rod is negligible, however, as will be noted if we try to time the fall of the rod on which there is no disk. Unless we are dealing with materials of very heavy consistency, the time is so small that it is not measurable by means of ordinary stop-watch technique.

It can be seen that in the mobilometer the types of flow are complicated, and the results of calculations based purely on the dimensions of the instrument would probably mean very little.

Newton's fundamental law of viscous flow states that when two parallel planes separated by distance s are sheared by a force, F, per unit area, the velocity, v, which one plane travels with respect to the other will be proportional to the coefficient of viscosity, η, of the material separating the planes.

Thus,

$$\eta = \frac{Fs}{v} \tag{1}$$

or

$$\eta = Fst \tag{2}$$

where t = time for constant amount of shear $= \frac{1}{v}$. Here s may be considered to be an instrumental constant, K, since the value of s depends only on an instrumental adjustment. K, in this case, can be evaluated either by calibration against a liquid of known viscosity or from the dimensions of the instrument. The general expression

$$\eta = KFt \tag{3}$$

or

$$\frac{1}{t} = \frac{KF}{\eta} \tag{4}$$

may be derived. Equation 3 is a general expression into which the more complicated formulas of all instruments may be transformed.

Thus in the case of the more complicated type of flow, the flow through a capillary tube, Poiseuille has given the law

$$\frac{V}{t} = \frac{\pi PR^4}{8l\eta} \tag{5}$$

where V = volume of flow, t = time of flow, P = pressure, R = radius of capillary, and l = length of capillary, which yields

$$\frac{1}{t} = \frac{KF}{\eta} \quad \text{or} \quad \eta = KFt \qquad \text{(4) or (3)}$$

where

$$F = \frac{RP}{2l} \tag{6}$$

and

$$K = \frac{\pi R^3}{4V} \tag{7}$$

All the terms of K depend on dimensions of the instrument, so here again K may be regarded as an instrumental constant. Dimensional analysis shows the value of KF to be dynes per sq. cm., in both examples. Here again, the value of K can be obtained either by calibration against a liquid of known viscosity or from the dimensions of the instrument.

In the case of the mobilometer where the type of flow is much more complicated than in parallel plate or capillary flow, the expression

$$\frac{1}{t} = \frac{KF}{\eta} \quad \text{or} \quad \eta = KFt \qquad \text{(4) or (3)}$$

also applies, although the value of K cannot be obtained from dimensions of the instrument but must be evaluated by calibration against a standard liquid. Here again, however, the dimensions of KF are dynes per sq. cm.

The mobilometer has been designed to measure the flow properties of materials which do not behave as true liquids—i. e., those materials which behave as plastic soft solids or non-Newtonian liquids. In the case of plastic solids, Bingham (*2*) has given an equation which expresses their behavior.

$$\frac{V}{t} = \frac{\pi R^4 \mu}{8l}(P - p) \tag{8}$$

where μ = mobility (sq. cm. per dyne-second) and p = yield value due to structure (dynes per sq. cm.). The values of μ and p are, respectively, the slope and intercept at the abscissa of the straight line asymptotic to the curve obtained when the rate of shear, V/t, is plotted as the ordinate against the shearing stress, P, as the abscissa. The yield value, p, has the same dimensions as P. The mobilometer gives curves such that the points measured, for the most part, fall on the section of the curve which is coincident with the asymptote. In fact, it is impossible in most cases to detect curvature graphically in this section of the curve, so it may be considered to be a straight line.

Equation 8 may be given the same treatment as Equation 5 to give

$$\frac{1}{t} = \mu(KF - Kf) \tag{9}$$

Equation 4 is specific for application in the measurement of the viscosity of a liquid. Equation 9 is for application to the mobilometer in the case of a plastic solid. The value of K for the mobilometer, obtained from calibration with a standard liquid, is an instrumental constant and it can, therefore, be used in Equation 9. The actual values of K obtained in the cases of the different instruments (parallel plates, capillary tube, or mobilometer), of course, will not be equal.

The values of μ and Kf, necessary as physical constants to express the flow characteristics of a plastic solid, can be calculated by the method of averages (*10*) using values of $1/t$ and KF obtained experimentally. Only those values which occur

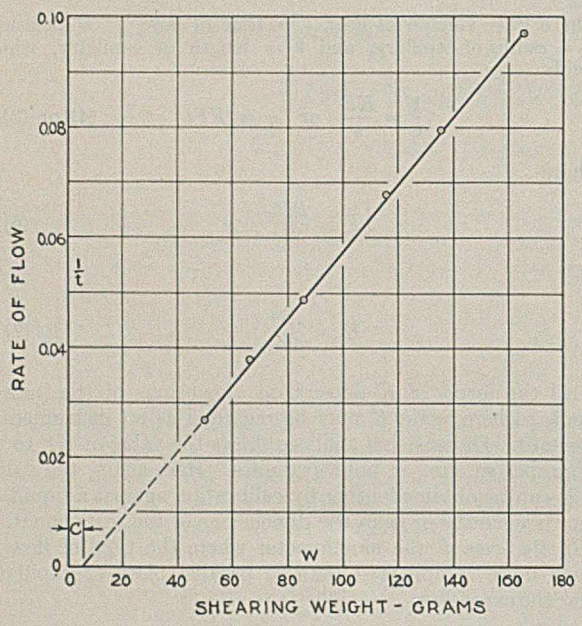

FIGURE 1. CURVE OF TABLE I
Viscosity standard 0, η = 35.74 poises at 22.0° C., N cylinder, and 0–4 disk

on the linear portion of the curve should be used in the calculations.

Calibration

A Bureau of Standards calibrated viscosity standard liquid having a viscosity of 35.74 poises at 22.0° C. was used to calibrate the instrument and thus obtain the instrumental constants for various combinations of disks and cylinders. Two sets of three disks—i. e., one blank, one 51-hole, and one 4-hole disk in each set—were calibrated. One set had been in use for some time and one set was recently purchased from the Gardner laboratory; the former set is identified by the prefix O- and the latter, by N-. Five cylinders were calibrated, four old and one recently obtained, identified by the symbols I, II, III, IV, and N. The practice followed has been outlined above under the heading of Procedure.

Corrections

In Table I are given the times necessary for a 10-cm. fall of the plunger rod fitted with a four-hole disk, using various weights to produce the shear. It was found that the straight line obtained when the rates of fall—i. e., the values of $1/t$—were plotted against the shearing weights in grams, did not go through the origin, but instead had an intercept at a small value on the abscissa. Inspection shows that the total weight producing the shear is not just overcoming the viscous resistance of the liquid, but a portion of the weight is being used to overcome the buoyant force of the submerged disk and rod. In order to have the shearing weight corrected so that it will equal the weight producing shear, the buoyant effect correction must be subtracted from the shearing weight. Equation 3 can be expressed

$$\eta = K(W - C)t \qquad (10)$$

where $F = W - C$

Figure 1 shows the curve obtained when the values given in Table I are plotted. Constants A and C of the equation representing the curve were calculated by the method of averages.

$$W = A\frac{1}{t} + C \qquad (11)$$

where W = shearing weight—i. e., the weight of disk, rod, and weight pan plus added weights; C = correction constant, the need of which is attributed mainly to the buoyant effect; and A = straight line equation constant.

It will be noted that

$$K = \frac{\eta}{A} \qquad (12)$$

The value of C can be approximated by calculation from the dimensions of the submerged parts and the specific gravity of the material under measurement.

The above statement has not taken into consideration any frictional forces due to contact of the moving disk and the inside of the cylinder, which are in opposition to the weight and are therefore in the same direction as the buoyant force. These frictional forces cannot be calculated from dimensions of the instrument and may be disregarded in the cases of well-machined disks and cylinders where the friction, if effective at all, is very small. The frictional forces arise from poorly machined working parts; those disks and cylinder walls which appeared to be rough had higher values of C than the smoother appearing ones. If accurate results are desired, the moving parts should be accurately machined and carefully finished. It was noted that those disks which had been used for some time had lower values of C than the newer ones. If careful technique is practiced, it is logical to conclude that any gross discrepancies in measurement or low precision may be accredited to large values of frictional forces.

TABLE I. CALIBRATION WITH BUREAU OF STANDARDS VISCOSITY STANDARD

(η = 35.74 poises at 22° C.; t = time of fall of 10 cm. with weight W, using a 4-hole disk)

A = 1.645, C = 5.4, K = 0.02173

W Gram	t Sec.	$1/t$	$F = W - C$	η Calcd.	Error %
49.3	37.3	0.02681	43.9	35.58	0.4
65.9	27.2	0.03676	60.5	35.76	0.1
85.9	20.5	0.04878	80.5	35.86	0.3
115.9	14.8	0.06757	110.5	35.54	0.6
135.9	12.6	0.07937	130.5	35.73	0.0
165.9	10.3	0.09709	160.5	35.92	0.5
			Av.	35.73	0.3

TABLE II. VALUES OF INSTRUMENTAL CONSTANTS

Cylinder No.	Disk No.	A	C	$K = \frac{\eta}{A}$	$k = \frac{V\eta}{A}$	Average Deviation %
N	N-B	220,600	16.6	0.0001620	0.01925	1.9
N	N-51	16,120	16.7	0.002217	0.2634	1.0
N	N-4	1,931	6.4	0.01851	2.200	1.5
N	O-B	57,820	−0.2	0.0006181	0.07345	0.4
N	O-51	14,600	2.1	0.002448	0.2909	0.3
N	O-4	1,645	5.4	0.02173	2.583	0.3
I	O-4	1,702	2.9	0.02100	2.495	0.5
II	O-4	1,705	3.3	0.02096	2.491	0.9
III	O-4	1,639	4.7	0.02180	2.591	0.4
IV	O-4	1,691	3.0	0.02113	2.511	0.1
Average	O-4 Disk	1,676	3.9	0.02132	2.534	0.4

The error introduced by not wiping the rod clean of adhering liquid between determinations is not large if sufficient time is allowed for drainage. In the case of the calibrating liquid less than 1 gram of material adhered after 2 minutes' draining time. As plastic soft solids do not drain properly and consequently add considerably more weight, they should be wiped after each determination if high precision is desired. The percentage error introduced by not removing the adhering film, however, in the case of these plastic materials, may not be very large, since the weights necessary to produce flow in these materials are usually rather high.

There is a different value of the instrumental constant for each combination of disk and cylinder. Table II gives the values of the instrumental constants, K, along with the value

of A from Equation 11 which is used in calculating K and k. In order to calculate k it is necessary to know the value of V, the volume of flow. This value, calculated from the dimensions of the instrument, is 118.8 cc. for 10-cm. fall.

The constants are given for the combination of one cylinder and different disks and for one disk and several cylinders. The wide variation in the values of K, for the disks which are supposed to be interchangeable, makes the necessity of calibration of the various disks immediately apparent, if results of measurement of flow properties with one mobilometer are to be compared with those of another. The values of K obtained when the different cylinders are used interchangeably do not vary so widely as do the values of K using the "interchangeable" disks.

The average value of the buoyant force correction, C, for disk O-4 in combination with the various cylinders is 3.9 grams. This value compares very well with the value calculated from the dimensions of the submerged parts, the mean volume of the submerged parts being 5.7 cc. This would give 4.9 grams' buoyant force, since the specific gravity of the calibrating liquid is 0.86. The 1.0-gram difference between average C and buoyant force calculated from the dimensions is attributed to the weight of calibrating liquid adhering to the rod.

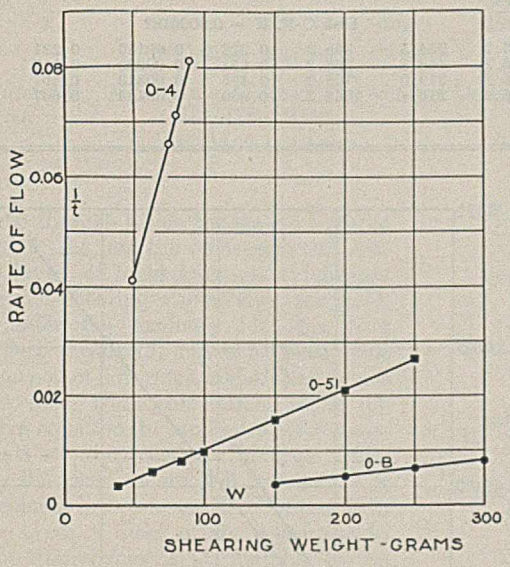

FIGURE 2. MANILA COPAL RESIN SOLUTION IN ALCOHOL
58.4% by weight

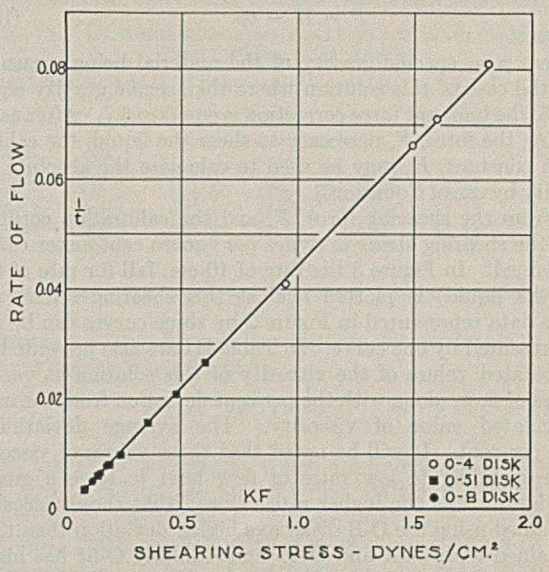

FIGURE 3. MANILA COPAL RESIN SOLUTION IN ALCOHOL
58.4% by weight, plotted in absolute system units

TABLE III. GARDNER MOBILOMETER DATA FOR 58.4 PER CENT ALCOHOLIC SOLUTION OF MANILA COPAL RESIN

(O-4, O-51, and O-B disks with N cylinder. Temperature, 22° C. C, 5.7)

W	t	F	KF	$1/t$	$\eta = KFt$	Deviation from Mean Value of η
Grams	*Sec.*		*Dynes/ sq. cm.*			%
			Disk O-4, K = 0.02173			
49.3	24.3	43.6	0.947	0.041	23.0	0.0
74.2	15.2	68.5	1.489	0.066	22.6	1.8
79.2	14.1	73.5	1.597	0.071	22.5	2.2
89.2	12.4	83.5	1.814	0.081	22.5	2.2
						Av. 1.5
			Disk O-51, K = 0.002448			
39.1	286.7	33.4	0.0818	0.00349	23.4	1.7
64.0	164.1	58.3	0.143	0.00609	23.4	1.7
84.0	123.2	78.3	0.192	0.00812	23.6	2.5
100.0	101.2	98.3	0.241	0.0099	24.3	5.3
150.0	64.6	148.3	0.363	0.0155	23.4	1.7
200.0	47.9	198.3	0.485	0.0209	23.2	0.9
250.0	37.5	248.3	0.608	0.0267	22.8	0.9
						Av. 2.1
			Disk O-B, K = 0.0006181			
150.2	262.8	144.5	0.0893	0.00380	23.5	2.1
200.2	188.1	194.5	0.1202	0.00531	22.6	1.8
250.2	148.3	244.5	0.1511	0.00674	22.4	2.7
300.2	122.2	294.5	0.1820	0.00818	22.2	3.6
						Av. 2.5
				Mean value	23.0 poises	2.0

The frictional force is negligible in the case of disk O-4, as it is with the other older, smoother-surfaced disks O-B and O-51. Higher frictional force is, of course, associated with rougher working surfaces. Also, as summarized in Table II, the average percentage deviations between the known viscosity of the calibration liquid and the values of viscosity, calculated using Equation 3, are higher for the new disks than for the smooth ones. It will be noted that the value of C for disk O-B is −0.2. This out-of-line value is attributable to calibration inaccuracy, but since the shearing weights used in this case were in the range of 350 to 1100 grams, the resulting percentage deviation between −0.2 and 3.9 values of C was not excessive. The percentage deviation in this case was of the same order as for the other O-set disks.

Determinations

The viscosity of a clean, concentrated solution of Manila copal resin in denatured alcohol (Special Denatured Formula No. 1) was measured. The solution was prepared by dissolving the CNE grade of this natural resin in the alcohol, allowing the dirt and other insoluble material to settle, and using the clear supernatant liquid. Analysis of this solution showed its concentration to be 58.4 per cent by weight. Table III presents the data obtained when the viscosity was measured using O-4, O-51, and O-B disks and N cylinder. In Figure 2, the reciprocal of the time required for 10-cm. fall of the plunger is plotted against the weight producing the fall. Each numerical value of t is an average of the times of at least three falls at the same weight. In order to calculate the viscosity from the data, it is necessary to subtract from the shearing weight a correction for the buoyant force. For purposes of calculation, the average submerged volume will be taken as 6 cc. To calculate the shearing force—i. e., the weight overcoming viscous resistance—from the shearing weight, Equation 13 is used.

$$F = W - 6\rho \qquad (13)$$

where ρ = specific gravity of the material being measured. In the case of this solution where the specific gravity equals 0.95, the buoyant force correction is equal to 5.7. After calculating the force, F, necessary to shear the liquid, the calibration constant, K, may be used to calculate the absolute viscosity by use of Equation 3.

From the shearing force, F, and the calibration constant K, the shearing stress in dynes per square centimeter can be obtained. In Figure 3 the rate of 10-cm. fall (or rate of flow of the liquid) is plotted against this shearing stress, KF. The data represented in Figure 2 by three curves can be now represented by one curve. In Table III are also presented the calculated values of the viscosity of this solution at various rates of flow, along with the per cent deviation from the mean calculated value of viscosity. The average deviation is 2.0 per cent. It will be noted that those values of viscosity corresponding to low rates of flow tend to have a greater deviation than the higher rate values; that those viscosities obtained using the O-B disk have higher deviation than those obtained with O-51 and O-4; and similarly O-51 has higher values than O-4. The explanation of this is that probably the incalculable frictional forces are present and are exerting a greater influence at the lower rates of fall.

If to this Manila copal resin solution is added some considerable quantity of a mineral filler, the flow properties will change from those of a viscous liquid to those of a plastic soft solid and the rate of shear will no longer be proportional to the shearing stress.

To 100 parts by weight of this 58.4 per cent resin solution 80 parts of kaolin were added. This resulted in a mixture which was 21.8 per cent by volume of filler. Table IV contains the data collected using the three types of disk. The specific gravity of this mixture is 1.33, making the calculated buoyant force correction, C, equal to 8.0. Figure 4 shows curves resulting when the rate of 10-cm. fall is plotted against the shearing weight. If by using the calibration constants we calculate the shearing stress and plot this against the rate of fall, we obtain the curves shown in Figure 5.

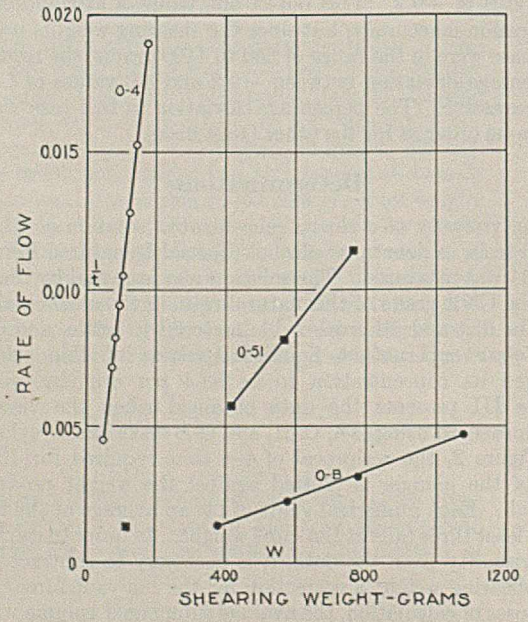

FIGURE 4. MIXTURE OF KAOLIN AND MANILA COPAL RESIN SOLUTION

21.8% kaolin by volume

TABLE IV. GARDNER MOBILOMETER DATA FOR KAOLIN–MANILA COPAL RESIN SOLUTION

(21.8% kaolin by vol., O-4, O-51, and O-B disks with N cylinder. C, 8.0)

W	t	F	KF	$1/t$	KF (Calcd.)	Deviation
Grams	*Sec.*		*Dynes/ sq. cm.*			%
			Disk O-4, K = 0.02173			
49.3	222.2	41.3	(0.897)	0.00450	(0.967)	(8.1)
74.2	139.9	66.2	1.438	0.00715	1.457	1.3
84.2	121.5	76.2	1.656	0.00823	1.656	0.0
94.2	106.5	86.2	1.873	0.00939	1.870	0.2
104.2	95.4	96.2	2.090	0.0105	2.074	0.8
124.2	77.9	116.2	2.525	0.0128	2.498	1.0
144.2	65.4	136.2	2.960	0.0153	2.959	0.1
174.2	52.7	166.2	3.612	0.0190	3.640	0.8
					Av.	0.7
			Disk O-51, K = 0.002448			
114.0	775.4	106.0	(0.259)	0.00129	(0.327)	(26.3)
414.0	174.2	406.0	0.994	0.00574	0.998	0.4
564.0	123.1	556.0	1.361	0.00812	1.357	0.3
764.0	87.7	756.0	1.851	0.01140	1.851	0.0
					Av.	0.2
			Disk O-B, K = 0.0006181			
376.2	734.5	368.2	0.228	0.00136	0.231	1.3
576.2	448.8	568.2	0.351	0.00223	0.347	1.1
776.2	319.0	768.2	0.475	0.00313	0.467	1.7
1076.2	216.1	1068.2	0.660	0.00463	0.667	1.1
					Av.	1.3

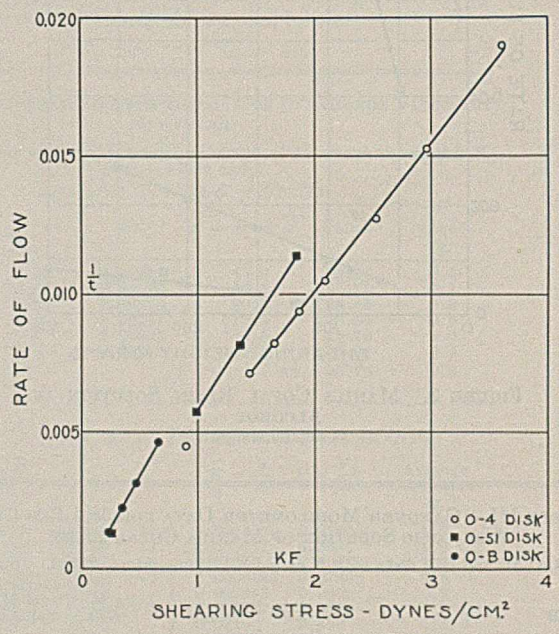

FIGURE 5. MIXTURE OF KAOLIN AND MANILA COPAL RESIN SOLUTION

21.8% kaolin by volume, plotted in absolute system units

The curves for the three disks, while apparently straight lines, do not coincide as with the resin solution without filler. The explanation of this phenomenon is that the rate of fall of the plunger rod (or rate of flow of the material) and the rate of increase of shear of the material are the same only in the case of true liquids. In the plastic soft solids, "plug flow" is exhibited. Plug flow is an extrusion mechanism with a high shear rate in the region near the wall of a tube, but little or no shear in the central section of the material being forced through the tube. The structure of the material near the wall is being violently disturbed while the center or "plug" suffers little change. The layer in a high rate of shear acts as a lubricant for the central plug. The larger the diameter of

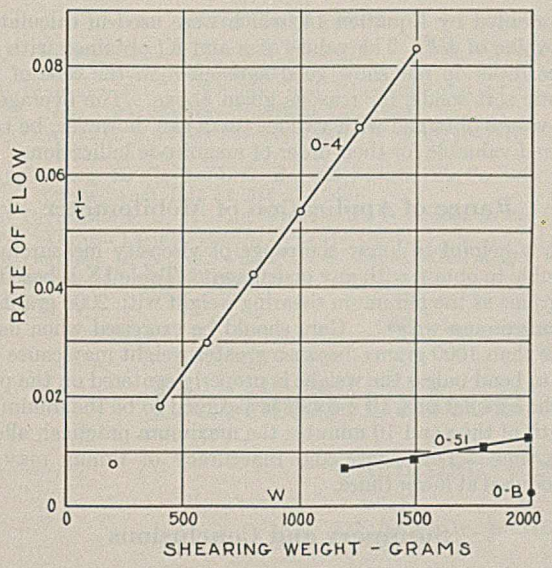

FIGURE 6. MIXTURE OF KAOLIN AND MANILA COPAL RESIN SOLUTION
26.8% kaolin by volume

TABLE VI. GARDNER MOBILOMETER DATA FOR KAOLIN–MANILA COPAL RESIN SOLUTION

(31.2% kaolin by volume, O-4 disk with N cylinder. C, 9.0. K, 0.02173)

W	t	F	KF	$1/t$	KF (Calcd.)	Deviation
Grams	*Sec.*		*Dynes/ sq. cm.*			%
400.0	210.8	391.0	8.50	0.0047	8.66	1.8
600.0	122.1	591.0	12.87	0.0082	12.77	0.8
800.0	84.4	791.0	17.19	0.0118	17.13	0.3
1000.0	64.8	991.0	21.53	0.0154	21.39	0.6
2000.0	29.5	1991.0	43.26	0.0339	43.39	0.3
					Av.	0.8

Equation 14 can be used to calculate the constants of the straight lines shown in Figure 5.

$$KF = R\left(\frac{1}{t}\right) + Kf \qquad (14)$$

The low shear rate values, which are represented by points that do not fall on the straight line, were not used in the calculation of the constants of the straight line. From the calculated constants R and Kf, the calculated values of KF can be obtained. These data are given in Table IV, with the values of deviation between observed and calculated values of KF. The average value of the deviation is 0.7 per cent,

the tube through which the plastic material is flowing, the less the cross-sectional area of the layers of breakdown on application of a given shearing stress, and, therefore, the greater the tendency for this plug flow effect. Hall (*9*) has experienced this phenomenon of flow rates, higher than should be the case, from calculations using the Bingham equation, in capillaries in his measurements of the plasticity of clays. Obviously this phenomenon will be a source of disagreement between results obtained with various types of disks of the mobilometer, but the discrepancy is attributable to the structural properties of the material and lack of complete applicability of the Bingham equation, and not necessarily to any correctable instrumental deficiencies.

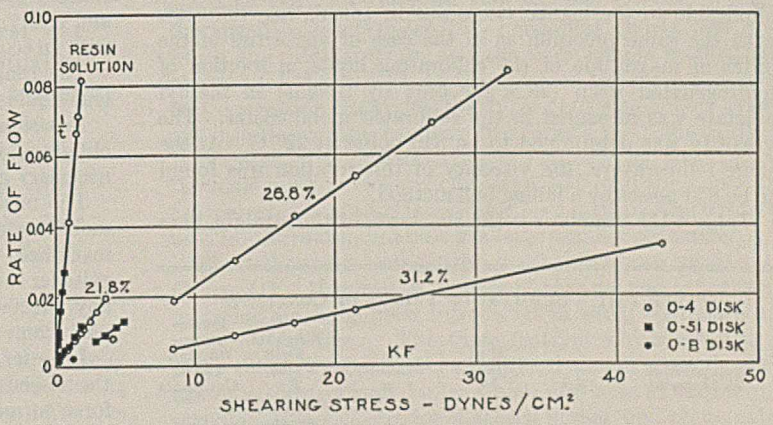

FIGURE 7. CALIBRATED GARDNER MOBILOMETER RESULTS
Manila copal resin solution and kaolin resin solution mixtures, plotted in absolute system units

TABLE V. GARDNER MOBILOMETER DATA FOR KAOLIN–MANILA COPAL RESIN SOLUTION

(26.8% kaolin by volume, O-4, O-51, and O-B disks with N cylinder C, 8.5)

W	t	F	KF	$1/t$	KF (Calcd.)	Deviation
Grams	*Sec.*		*Dynes/ sq. cm.*			%
			Disk O-4, K = 0.02173			
200.0	129.4	191.5	(4.16)	0.0077	(4.58)	(10.1)
400.0	54.5	391.5	8.51	0.0183	8.50	0.1
600.0	33.4	591.5	12.85	0.0299	12.78	0.5
800.0	23.7	791.5	17.20	0.0421	17.28	0.5
1000.0	18.7	991.5	21.55	0.0535	21.49	0.3
1250.0	14.6	1241.5	26.98	0.0686	27.06	0.3
1500.0	12.0	1491.5	32.41	0.0830	32.38	0.1
					Av.	0.3
			Disk O-51, K = 0.002448			
1189.8	143.7	1181.3	2.89	0.0069	3.00	3.8
1489.8	118.1	1481.3	3.63	0.0084	3.52	3.0
1789.8	93.7	1781.3	4.36	0.0107	4.31	1.1
1989.8	80.6	1981.3	4.85	0.0124	4.90	1.0
					Av.	2.2
			Disk O-B, K = 0.0006181			
2000.2	435.0	1993.5	1.232	0.0023	...	..

not, of course, including in the deviation the nonlinear, low-shear rate, calculated value of KF, enclosed in parenthesis in Table IV.

Table V gives the data obtained when a mixture of 100 parts of the 58.4 per cent resin solution and 105 parts of kaolin, to give a 26.8 per cent by volume mixture (specific gravity = 1.42), is measured with the three disks. This material could be handled with the O-4 and O-51 disks; with the O-B disk the material was apparently too heavy in consistency to handle practically. The curves for rate of fall *vs.* shearing weight and rate of fall *vs.* shearing stress are given, respectively, in Figures 6 and 7. The calculated values of KF and the per cent deviation of these calculated values from the observed values are given in Table V, the average being 1.1 per cent. Here again, the low shear rate results obtained with the O-51 disk have a greater average deviation than those obtained with the O-4 disk; also, as shown in Figure 7, the curve obtained with the O-51 disk does not coincide with that obtained with the O-4 disk.

Table VI contains the data for a mixture of 100 parts of the 58.4 per cent resin solution and 130 parts of kaolin, 31.2 per cent by volume (specific gravity = 1.50), measured with the

TABLE VII. GARDNER MOBILOMETER DATA FOR 76.8 PER CENT ALCOHOLIC SOLUTION OF MANILA COPAL RESIN

(O-4 disk with N cylinder. C, 6.0. K, 0.02173)

W	t	F	KF	$1/t$	$\eta = KFt$	Deviation from Mean Value of η
Grams	*Sec.*		*Dynes/ sq. cm.*			%
800.0	149.1	794.0	17.28	0.00670	2573	0.8
1100.0	110.2	1094.0	23.77	0.00908	2619	2.5
2128.1	53.5	2122.1	46.11	0.01869	2467	3.5
				Mean value =	2553 poises	2.3

mobilometer. Here it was practical to use only the O-4 disk, the material being too heavy to be accommodated by the O-51 and O-B disks. Figure 7 also shows the curve of this material. The average deviation for this material is 0.8 per cent.

A more viscous solution of Manila copal resin was prepared by allowing some of the alcohol of the 58.4 per cent solution to evaporate. Analysis of the resulting solution showed its concentration to be 76.8 per cent solids. Table VII gives the mobilometer data and the calculated viscosity, the average value being 2553 poises, with an average deviation of 2.3 per cent.

In order to ascertain whether the instrumental constant is applicable to the higher viscosities in calculating viscosity, with the same precision as in the case of viscosities of the order of magnitude of the calibrating liquid, a solution of hydrogenated rosin (28.5 per cent by weight) in methyl abietate was measured in the calibrated mobilometer. The viscosity was determined to be 840 poises at 22° C. At the same temperature, the viscosity of this solution was found to be 830 poises by a falling ball method.

Table VIII summarizes the constants of the straight lines

TABLE VIII. SUMMARY OF VALUES OF CONSTANTS

Composition of Material	Disk	R of Equation 14	Mobility, μ	Bingham Yield Value, Kf	Deviation through Use of Constants
				Dynes/ sq. cm.	%
58.4% alcoholic solution of manila copal resin	O-4	22.6	0.04415	..	1.5
	O-51	23.4	0.04266	..	2.1
	O-B	22.7	0.04411	..	2.5
	Mean value	23.0[a]	0.04348	0.00	2.0
Above solution plus kaolin (21.8% by volume)	O-4	184.2	0.00543	0.14	0.7
	O-51	150.7	0.00664	0.13	0.2
	O-B	133.3	0.00750	0.05	1.3
	Av.	156.1	0.00641	0.09	0.8
Solution plus kaolin (26.8% by volume)	O-4	369.2	0.00271	1.74	0.3
	O-51	344.9	0.00290	0.62	2.2
	Av.	357.0	0.00280	1.18	1.1
Solution plus kaolin (31.2% by volume)	O-4	1191.0	0.00084	3.01	0.8
76.8% alcoholic solution of manila copal resin	O-4	2553.0[a]	0.00039[a]	0.00	2.3

[a] In case of true liquid where Kf = O, $R = \eta$, and mobility (μ) is expressed as the fluidity, ϕ.

represented by Equation 14, which were used in calculating the value of KF. The values of μ and Kf obtained with the three disks do not show good agreement in the case of the plastic soft solids, for reasons given above. The average of the values obtained with various disks can, however, be considered valuable for their order of magnitude indications.

Range of Application of Mobilometer

It is helpful to know the range of viscosity measurement possible to obtain with any instrument. Table IX is based on 50 grams as the minimum shearing weight with 2000 grams as the maximum weight. Care should be exercised when using more than 1000 grams, because greater weight may cause the rod to bend unless the weight is properly centered on the pan. In the calculations, 10 seconds is assumed to be the minimum length of time and 10 minutes the maximum practical, allowable time. Turbulence and inaccuracy of timing may be introduced at lower times.

Summary and Conclusions

The Gardner mobilometer is a control instrument which can be used in industrial research, since in its operation it employs the varying rate of shear principle, it is precise enough for industrial work, it is adaptable to a wide range of consistencies, it is easily manipulated, sturdy in construction, and easily cleaned, and the necessary data can be collected with an economy of time.

The range of application in determining the viscosity of true liquids with the mobilometer is 10^{-1} to 10^{4} poises.

Precision of the order of magnitude of 2 per cent can be obtained, if proper precautions are exercised and certain necessary corrections are employed.

The most urgent precaution to be exercised in use of the mobilometer is to be sure that the working parts are well machined. The surfaces of the disk and of the inside of the cylinder should be smooth and well polished, so as to minimize any effects of the friction set up when the surfaces of these parts come in contact.

In order to obtain the true value of the force overcoming the viscous or plastic resistance of the material, a buoyant force correction must be subtracted from the weight producing the shear. This correction is especially important where the shearing weight is relatively small.

The instrument can be calibrated with a standard liquid, so that the results can be given in absolute units by the equations:

$$\eta = K(W - 6\rho)t, \text{ for a true liquid}$$

$$\frac{1}{t} = \mu(KF - Kf), \text{ for a plastic soft solid}$$

Acknowledgment

The author wishes to thank his co-workers of the Central Technical Laboratory for helpful suggestions and valuable criticism.

TABLE IX. RANGE OF APPLICATION OF GARDNER MOBILOMETER USING THREE STANDARD DISKS

(Limits of time of 10-cm., fall of plunger rod = 10 seconds to 10 minutes, limits of shearing weight = 50 to 2000 grams. Application is to true liquid.)

Disk	K (Approx.)	t = 10 Seconds			t = 60 Seconds			t = 180 Seconds			t = 600 Seconds		
		W, 50 grams	W, 100 grams	W, 2000 grams	W, 50 grams	W, 100 grams	W, 2000 grams	W, 50 grams	W, 100 grams	W, 2000 grams	W, 50 grams	W, 100 grams	W, 2000 grams
		Poises			*Poises*			*Poises*			*Poises*		
4	0.02	10	20	400	60	120	2400	180	360	7200	600	1200	24,000
51	0.002	1	2	40	6	12	240	18	36	720	60	120	2,400
B	0.0002	0.1	0.2	4	0.6	1.2	24	1.8	3.6	72	6	12	240

4. Disk range = 10 to 24,000
51. Disk range = 1 to 2400
B. Disk range = 0.1 to 240

or

10^{1} to 10^{4} poises
10^{0} to 10^{3} poises
10^{-1} to 10^{2} poises

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Determination of Blood Magnesium

Quantitative Spectrochemical Method

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A spectrochemical method for the determination of magnesium in blood which is accurate, rapid, and simple to perform and requires only 1 ml. of sample is described. The blood samples are first diluted with a potassium alum solution which acts both as a spectroscopic buffer and internal standard and then atomized into a spark between graphite electrodes. Analysis is made by measurement of relative intensities. The average error obtained by this method is less than 3 per cent.

IN GENERAL clinical practice biological fluids are only occasionally analyzed for magnesium. However, during recent years it has become desirable to be able to determine accurately the magnesium content of blood as well as of other biological fluids in order to ascertain its possible relationship to certain pathological conditions. A comprehensive review of the work reported in this field, including the most reliable values for blood magnesium, is given by Myers and Muntwyler (*10*). As stated in the original articles (*5, 6, 14, 15*), the magnesium determinations were made by chemical methods of analysis. However, because of the limited amount of sample available, it is necessary to have a method requiring only a few milliliters for the analysis. For this reason the possibilities of a spectrochemical method embodying speed, accuracy, and minimum sample requirement have been investigated.

A number of methods for the spectrochemical analysis of biological fluids for various elements have been reported in the literature. Among these are the methods described by Hess, Owens, and Reinhardt (*7*) and by Thomson and Lee (*12*), both of which make use of the well-known internal standard method of Gerlach (*4*), intensity calibration of each plate, and photometric measurement of the relative intensities of spectral lines as described by Duffendack, Wolfe, and Smith (*2*). Both methods require wet-ashing of the sample, a time-consuming step which the method here described successfully eliminates.

The method used is that of atomizing a solution into a spark between graphite electrodes, the atomizer being similar to the one employed by Lundegårdh (*9*) in his flame method. Several other methods have been described for introducing solutions into a spark: the Hitchen (*13*) sparking tube for solutions, the source used by Thomson and Lee (*12*) in which the spark passes between two horizontal quartz jets from which the solution drips, and the more simple method employed by Keirs and Englis (*8*) in which the spark is formed between carbon electrodes, the upper one being hollow to permit inserting a glass capillary from which the solution drips. While each of these methods has a number of good points, the manipulations required to clean and refill the apparatus are more involved than is desirable for a method which is to be used for the routine analysis of a large number of samples.

The preliminary work on this method has been reported by Cassen (*1*). However, a number of changes and refinements in technique have been introduced in order to obtain the necessary accuracy. The three features which combine to make the method accurate, rapid, and simple to perform are use of a spectroscopic buffer which also acts as an internal standard, use of electrodes of special design, and photometric measurement of relative intensities of spectral lines.

The blood is diluted with a solution of potassium aluminum sulfate which serves both as a spectroscopic buffer and as an internal standard. It has been established by experiment that when aluminum is present in a concentration corresponding to 47 grams of $Al_2(SO_4)_3.K_2SO_4.24H_2O$ per liter of solution (using the conditions of exposure standardized upon and described under procedure for analysis) the densities of the aluminum line at 2816 Å. and the magnesium line at 2795 Å. are equal for a solution containing 0.35 mg. of magnesium per 100 ml. and also fall near the center of the straight-line portion of the characteristic curve of the photographic emulsion. Both conditions are ideal for obtaining reproducible results, and are especially desirable since this figure corresponds to 3.50 mg. of magnesium per 100 ml. of blood, which is about the average value reported for normal whole blood, the range being 2.75 to 5.00 mg. of magnesium per 100 ml. (*6*). The compound containing this internal standard, in the concentration used, is also a very effective spectroscopic buffer. The advantages of the use of spectroscopic buffers in analyzing biological materials are fully discussed elsewhere (*3, 7*). Briefly, in this case, the problem is reduced to the determination of small amounts of magnesium in a potassium aluminum sulfate base solution; and any effects which might be introduced by the comparatively small amount of organic material present in blood or other biological fluids have been

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found by experiment to be negligible. Proof of this statement is shown by the experimental results given in Table I. Thus it is possible to analyze the samples directly after proper dilution with the internal standard stock solution. This results in a marked saving of time as compared with the time required for wet-ashing of samples and also makes it possible to adapt the method easily to the analysis of other biological fluids.

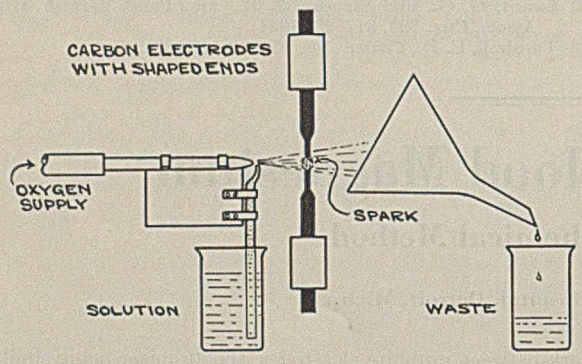

FIGURE 1. APPARATUS

The electrodes used are shaped as shown in Figure 1. These were found greatly to increase the uniformity of exposure since, as the electrodes burn away, the cross-sectional area (6.25 × 2.5 mm., 0.25 × 0.10 inch) remains constant, making it possible to use the same pair of electrodes for a large number of samples. This narrow rectangular electrode also prevents wandering of the spark, for the source of illumination is at all times in optical alignment with the slit of the spectrograph. Further, it has been established experimentally that by sparking the electrodes in a spray of distilled water for 60 seconds after each sample all traces of aluminum and magnesium are removed. This fact, together with the shape of the electrode, makes it possible to use one pair of electrodes for all the exposures on one plate. This greatly reduces the number of manipulations required and is a great saving in time.

The method of measuring relative intensities of spectral lines (*2*) has been used in order to obtain more reproducible results and to eliminate the necessity of repeating standard solutions on each plate. While it is necessary to place a calibration pattern on each plate, which is accomplished by means of a step sector, yet it is possible to run sixteen separate or eight samples in duplicate on a single plate.

Apparatus

For this work a Zeiss quartz spectrograph (chemist's model) was used. A rotating step sector (ratio 1.5) was placed directly in front of the slit for obtaining the plate calibration patterns. The atomizing unit (Figure 1) was placed 1 meter from the slit with no intervening lens system. A Hilger nonrecording microphotometer was used for making the photometric measurements. The spark source used was a condensed spark with synchronous interrupter.

Method of Analysis

PREPARATION OF SOLUTIONS. Standard solutions containing 47 grams of $Al_2(SO_4)_3.K_2SO_4.24H_2O$ per liter and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 mg. of magnesium as magnesium sulfate heptahydrate per liter are prepared. An internal standard stock solution for dilution of blood samples containing 52.22 grams of potassium aluminum sulfate per liter is prepared.

DETERMINATION OF ANALYTICAL CURVE. A number of plates were made from the standard solutions using the technique described below. The blackenings of the aluminum line at 2816 Å. and the magnesium line at 2795 Å. were measured photometrically. The logarithms of the ratios of the intensities of these two lines, obtained by application of the measured blackenings to the charateristic curve drawn for each plate, were plotted against the logarithms of the corresponding concentrations of magnesium. Figure 2 gives the resulting analytical curve for the determination of magnesium in a potassium aluminum sulfate base solution.

PROCEDURE FOR ANALYSIS. The atomizer is adjusted to a rate of 15 ml. per 120 seconds. Before the first exposure and after each succeeding exposure the electrodes are cleaned by a preliminary sparking for 1 minute in a spray of distilled water. The distance between the electrodes is set at 2.35 mm. (0.094 inch) by a spacer. The blood sample is diluted by pipetting 1 ml. of blood into 9 ml. of the internal standard stock solution, and then carefully mixed until a stable uniform suspension results. The diluted blood sample or a standard magnesium solution, as the case may be, is placed in the atomizer, the stop watch being started at once; after the solution has atomized for a period of 30 seconds, the spark is started and a 30-second exposure made. The graphite electrodes are then cleaned by sparking in distilled water for 60 seconds and the procedure is repeated for a series of samples. For producing a calibration pattern on each plate (Eastman process) the step sector is rotated in front of the slit and a solution of ferrous sulfate is sprayed into the spark in order to produce lines of suitable blackening for determining the characteristic curve of the plate. The plates are rapidly processed by the procedure described by Sawyer (*11*) except that Eastman developer D-19 is substituted for D-8. The blackening of the spectral lines is then measured and the ratios of the relative log intensities of the selected pair of aluminum and magnesium lines are determined. By referring to the analytical curve the amount of magnesium present is quickly obtained.

Results and Discussion

In order to determine the accuracy of the method a number of magnesium determinations were made on whole blood, followed by redeterminations after known amounts of magnesium were added. Two different samples of blood were run in triplicate. Sample 1 was found to contain 3.10 mg. of magnesium per 100 ml. and sample 2, 3.51 mg. of magnesium per 100 ml. of whole blood. To each sample 1 mg. of magnesium as magnesium sulfate heptahydrate was added per 100

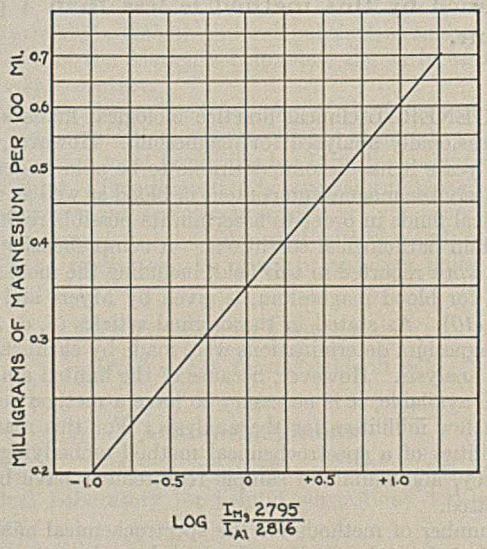

FIGURE 2. ANALYTICAL CURVE FOR DETERMINATION OF MAGNESIUM

ml. of blood. These additions were made on three separate portions of each sample, and the total magnesiums redetermined spectrographically. In a similar manner 2 mg. of magnesium were added per 100 ml. to three separate portions of each sample and again the total magnesium contents redetermined. The results of these determinations are given in Table I. It is noted that the maximum error in these twelve determinations is 2.44 per cent, with an average error of 1.5 per cent.

TABLE I. TEST DATA FOR ACCURACY OF ANALYSIS OF BLOOD FOR MAGNESIUM

Found per 100 Ml. of Original Blood	Found per 100 Ml. of Original Blood + 1 Mg. of Mg per 100 Ml.	Error[a]	Found per 100 Ml. of Original Blood + 2 Mg. of Mg per 100 Ml.	Error[a]
Mg.	*Mg.*	%	*Mg.*	%
3.10[b]	4.00	2.44	5.00	1.96
3.10	4.10	0.00	5.05	0.98
3.10	4.20	2.44	5.20	1.96
3.51[c]	4.48	0.66	5.60	1.63
3.51	4.40	2.44	5.50	0.18
3.51	4.60	1.99	5.60	1.63

[a] Per cent error based upon total amount of magnesium present in mg. per 100 ml.
[b] 3.10 is average value of three determinations of original blood sample 1.
[c] 3.51 is average value of three determinations of original blood sample 2.

TABLE II. MAGNESIUM DETERMINATION IN NORMAL BLOOD

Sample	*Mg. per 100 ml.*			Av.	Deviation %	Deviation %
1	2.90	2.75	2.85	2.83	+2.4	−2.9
2	3.20	3.20	3.35	3.25	+3.1	−1.5
3	3.15	3.20	2.95	3.10	+3.2	−4.8
4	2.80	2.85	..	2.83	+0.7	−1.0
5	3.25	3.35	3.35	3.32	+0.9	−2.2
6	3.55	3.65	3.75	3.65	+2.7	−2.7
7	3.65	3.90	3.80	3.78	+3.2	−3.4
8	4.25	4.30	..	4.28	+0.5	−0.7
9	4.55	4.65	..	4.60	+1.1	−1.1
10	3.30	3.15	..	3.23	+2.2	−2.5
11	4.80	4.40	4.55	4.58	+4.8	−4.0
12	4.15	3.75	3.90	3.93	+5.5	−4.6
13	3.10	3.00	3.25	3.12	+4.2	−3.8
14	3.10	3.40	3.40	3.30	+3.0	−6.1
15	3.50	3.60	3.55	3.55	+1.4	−1.4
16	3.25	3.40	3.10	3.25	+4.6	−4.6

Table II presents the individual values, average values, and per cent deviation obtained on 16 normal blood samples run in regular routine practice. The values range from 2.83 to 4.60 mg. of magnesium per 100 ml. of whole blood, which agrees very well with the most reliable values reported in the literature.

Work is now in progress on the analysis of a large number of blood samples from patients having malignant diseases to determine whether or not there is a correlation between the amount of magnesium present in the blood and the progress of the disease which would be of diagnostic importance. A similar study on a variety of diseases was undertaken by Zimmer (*16*), in which no correlation was obtained. However, this lack of correlation may be due to the fact that consistently high results with an average precision of ±13 per cent were obtained by the spectrographic method which she used.

Acknowledgment

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Determination of Ammoniacal and Nitrate Nitrogen in Decomposed Plant Material

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IN STUDIES on the preferential utilization of different forms of nitrogen during the decomposition of plant materials (*5*) the inadequacy of existing methods was apparent. This aspect of the problem of decomposition arises in connection with the practice of incorporating into the soil fresh undecomposed farmyard manure saturated with urine, or, less frequently, straw supplemented by a dressing of ammonium sulfate. In such circumstances, in addition to the ammonia, nitrogen may also be available in the form of soil nitrate. Subsequent changes in the amounts of the various forms of nitrogen were hard to follow, especially when the quantities involved were small, because of inaccuracies in existing procedures when applied to decomposing or decomposed residues and manures.

The common method of estimating ammoniacal and nitrate nitrogen in decomposed vegetable material is that of distillation in presence of magnesium oxide for ammonia, and subsequent reduction of the residue with Devarda's alloy for nitrate. The conditions under which such estimations are made are drastic and some of the ammonia, which is liberated at the abnormally high pH of 10 to 11 produced by the use of magnesia, comes from the plant amides. The results thus obtained are apparently higher than the true ammonia or nitrate content of the samples under examination. Another method which is sometimes employed is the extraction of the residues or manure with sodium chloride solution, thus liberating the ammonium ion by the process of base exchange, and subsequent distillation of this extract with magnesia. This method, being laborious and time-consuming, is still unsatisfactory, as some of the organic nitrogen may be dissolved out

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by the salt solution and hydrolyzed to ammonia at the high pH, giving higher quantities of ammonia and nitrate than are actually present. It thus is evident that the chief defect of the distillation method lies essentially in the use of magnesium oxide, which produces a highly alkaline reaction conducive to the hydrolysis of some of the organic nitrogen. The ammonia so liberated increases the apparent ammonia content of the sample.

Experimental

AMMONIA. Nichols and Foote (*3*) showed that ammonia determinations in sewage and nitrogenous trade wastes were unsatisfactory if carried out by distillation with magnesia or 10 per cent sodium carbonate, because of the high alkalinity, and proposed the use of a phosphate buffer of pH 7.4 which causes no liberation of ammonia from such organic compounds as asparagine, acetamide, glycine, and arginine. Decomposing straw was accordingly distilled in the presence of amounts of phosphate buffers, giving four different reactions. The straw had been allowed to decompose without additional nitrogen having been supplied, and the high positive "nitrogen factor" of the straw (*4*) therefore precluded any possibility of ammonia accumulation in the decomposing residue. To this were added in each case 10 mg. of ammonia just prior to distillation (Table I). These results leave no doubt that the high alkalinity of the magnesia causes high ammonia figures to be obtained, and that by the use of a phosphate buffer at pH 7.4 this disturbing factor can be largely eliminated. Additional proof was provided by examining the effect of distilling 5 grams of undecomposed paddy straw (0.78 per cent nitrogen) with similar amounts of phosphate buffer and magnesia. Progressively increasing amounts of ammonia were obtained as the alkalinity was increased, no doubt as a result of hydrolytic deamination of organic nitrogenous constituents (Table II).

TABLE I. RECOVERY OF AMMONIA ADDED TO DECOMPOSED STRAW

(10 mg. supplied in each case)

pH	NH_3 Mg.
7.0 (phosphate buffer)	9.50
7.4 (phosphate buffer)	10.11
8.0 (phosphate buffer)	10.84
8.5 (phosphate buffer)	11.65
10 (magnesia)	11.96

TABLE II. EFFECT OF CONCENTRATION OF DISTILLING REAGENTS ON HYDROLYSIS OF NITROGENOUS CONSTITUENTS OF AIR-DRIED PADDY STRAW

Phosphate Buffer Cc.	NH_3 Mg./5 g.	MgO Grams	NH_3 Mg./5 g.
40	0.00	4	0.70
50	0.00	6	0.84
60	0.00	8	0.98
70	0.00	10	1.01

The procedure finally adopted consists of distilling about 10 grams of moist residue or manure with 30 ml. of phosphate buffer in 300 ml. of distilled water. The buffer solution is prepared as described by Nichols and Foote (*3*) by dissolving 14.3 grams of KH_2PO_4 and 91 grams of K_2HPO_4 in 1 liter of distilled water. In this way distillation is accomplished at pH 7.4 and the only precautions necessary are those to prevent undue frothing.

NITRATE. The cause of an apparently high yield of ammonia by the magnesia method is also operative in the nitrate determination, and accordingly a modification of the Bengtsson (*1*) procedure was employed.

TABLE III. RECOVERY OF NITRATE NITROGEN ADDED TO MANURE

Nitrate Nitrogen Added Mg.	Nitrate Nitrogen Recovered Mg.	Recovery %
5	5.01	100.2
10	10.06	100.6
15	15.15	101.0
20	20.25	101.3

TABLE IV. RECOVERY OF AMMONIA AND NITRATE NITROGEN FROM DECOMPOSING STRAW

(Comparison of old and new methods. Grams of nitrogen per 100 grams of original straw)

Days	Ammoniacal Nitrogen MgO	Ammoniacal Nitrogen Buffer	Nitrate Nitrogen Old method	Nitrate Nitrogen Modified method
N Added as Ammonium Nitrate				
0	0.57	0.57	0.57	0.57
3	0.42	0.32	0.50	0.49
7	0.21	0.05	0.36	0.15
15	0.28	0.03	0.31	0.04
30	0.19	0.02	0.21	0.01
56	0.13	0.05[a]	0.19	0.03
N Added as Ammonium Carbonate and Sodium Nitrate				
0	0.57	0.57	0.57	0.57
3	0.45	0.36	0.48	0.48
7	0.25	0.04	0.35	0.12
15	0.28	0.03	0.30	0.03
30	0.21	0.02	0.22	0.01
56	0.14	0.04[a]	0.17	0.02

[a] Increase due to ammonification of fungal protein which sets in after about 6 weeks' decomposition.

Ten grams of the fresh sample are extracted with 300 ml. of distilled water in 50-ml. portions. Each fraction remains in contact with the sample for about 10 minutes prior to filtration through a cotton plug. Extraction is continued until no blue coloration is obtained with diphenyl benzidine, a test sensitive to one part in a million (*2*). Suspended colloids are then precipitated by the addition of a few drops of sulfuric acid (20 ml. to 100 ml. of water). The solution is warmed and coagulated matter is removed by suction. By addition of caustic soda the filtrate is made alkaline, and boiled down to 30 ml. A little more caustic soda is added and the volume is made up to 200 ml. before concentrating again to 30 ml. In this way ammonia initially present as such, or capable of being liberated from the small amount of organic nitrogenous material present, is removed. The residue is finally distilled with Devarda's alloy after dilution to 300 ml.

The effect of this improved procedure on the recovery of nitrate nitrogen added to manure is shown in Tables III and IV.

Summary

The magnesia distillation method is unsuitable for the determination of ammonia in decomposed plant residues or manure, because high results are obtained through the concurrent liberation of ammonia from organic nitrogenous substances. The substitution of a phosphate buffer giving a reaction of pH 7.4 is recommended.

Nitrate nitrogen can be determined on the aqueous extract of such materials by the Devarda reduction method after removing free or liberated ammonia by boiling under alkaline conditions.

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THE work described in this paper was commenced at the Rothamsted Experimental Station, Harpenden, Herts, England, under the direction of E. H. Richards, late head of the Fermentation Department, and completed at the Tea Research Institute, Talawakelle, Ceylon.

Machine and Methods for Testing Mechanical Stability of Latex

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Methods and machines have been studied for making mechanical stability tests on latices. The results indicate that a constant-speed machine will give reproducible results at widely separated laboratories when test samples of fixed solids content are used.

THE mechanical stability of a sample is one of the important factors in processing latex. Therefore it is necessary to have a testing method which is simple and reasonably rapid yet gives accurate and reproducible results.

The various methods of testing mechanical stability where latex is used and tested result in a different standard at each place and create confusion when it is necessary to compare results obtained by different procedures.

This paper gives the results obtained with the original standard test and modifications which were introduced in an attempt to establish a universal and more accurate test for determining the mechanical stability. In this work three different methods were used and in each one two different types of stirrers were tried (Figures 1 and 2).

One machine, used as a high-speed stirrer, was a Benedict Indestructo mixer, No. 53, Serial No. 23270, 105 to 120 volts, 70 watts, and 25 to 60 cycles. The other machine was a constant-speed stirrer built at the Firestone Laboratories. It consists of a vertically mounted Bodine synchronous motor of 1800 r. p. m., 110 volts, 7.4 amperes, 0.2 horsepower, and 60 cycles, with a 20.15-cm. (8.06-inch) wooden pulley mounted on the motor shaft, and a rubber fiber belt connecting the pulley to a stainless steel shaft 2.5 cm. (1 inch) in diameter, mounted vertically in a steel tripod. The steel shaft is furnished with a metal propeller attached to the lower end, and this makes up the stirring element. The motor support and steel tripod are permanently mounted on a wooden base plate. This construction is not to be considered the best equipment that could be built, but it did serve its purpose for this work. Plans are being drawn up for a simpler and more permanent piece of apparatus.

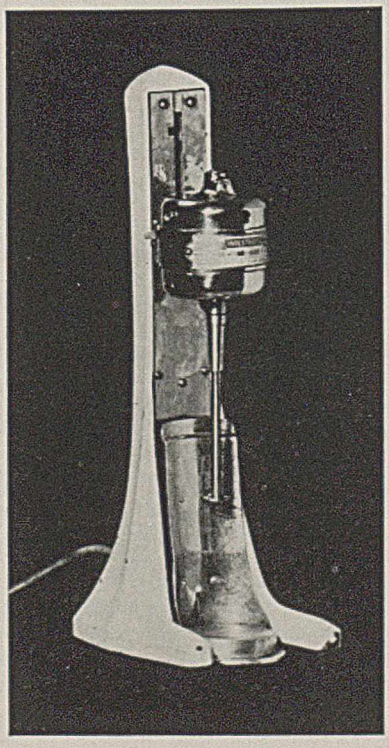

FIGURE 1. BENEDICT STIRRER

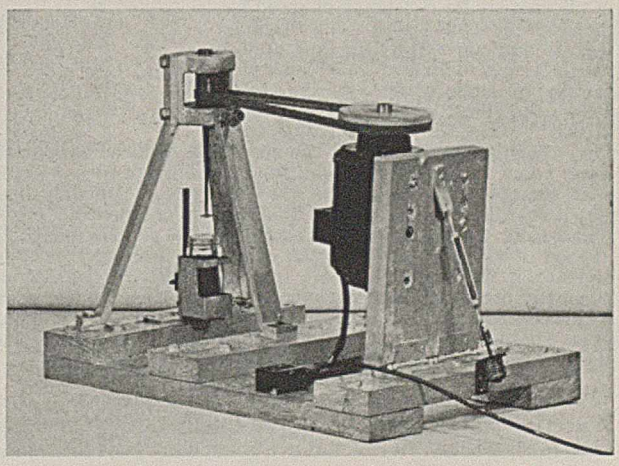

FIGURE 2. CONSTANT-SPEED STIRRER

Methods Used

Method 1A is carried out on the Benedict mixer. A 50-cc. sample of the latex is obtained from the drum and is stirred at 25° C. in a 118-ml. (4-ounce) square bottle with a high-speed stirrer until completely coagulated. The stirrer is equipped with a hexagonal, slotted propeller, as shown in Figure 3.

Method 1B is exactly the same as 1A, except that the test is carried out on the constant-speed stirrer, which is also equipped with the hexagonal slotted propeller.

Method 2A is a modification wherein an 80-cc. sample of latex with 50 per cent total solids and 0.5 per cent ammonia is held at 35° C. and is stirred in a 250-cc. round bottle with the Benedict mixer.

Method 2B is the same as 2A, except that the constant-speed stirrer is used.

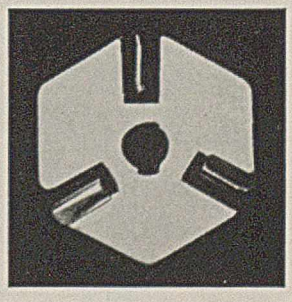

FIGURE 3. PROPELLER

Method 3A is a slight modification of Method 1A, using a sample diluted with distilled water to a total solids content of 50 per cent and 0.5 per cent ammonia. The Benedict mixer is used in this case.

Method 3B is the same as 3A, except that the constant-speed stirrer is employed.

Speed Measurements

The shaft speed in r. p. m. of each stirrer was measured by the use of an Edgerton stroboscope under various operating conditions. This stroboscope was capable of measuring speeds directly, as shown on the scale, up to 3000 r. p. m. Beyond this point, factors had to be used when perfect synchronization was obtained, to calculate the shaft speed.

The constant-speed stirrer was operated in media of different viscosities. When running free, the stirring element of this machine ran at 13,420 r. p. m. On checking the same shaft in such media as water, concentrated latex, glycerol, and Karo sirup that had been cooled to 10° C. in order to make a very viscous medium, this speed remained constant without varying over 15-minute periods. In view of these results, there is no question as to the constant speed of this stirrer during any test.

The new Benedict mixer was tested with various latex samples of high total solids content for constancy of speed by means of the stroboscope. This machine was strongly affected by the loads under which it was running. Under load of a sample of latex of 60 per cent total solids, the speed varied from 17,000 to 20,000 r. p. m. during the actual test; after coagulation took place, the speed was reduced to a much lower value. In samples of total solids contents of 40 and 50 per cent, the speed variations were not very great. Although there was a considerable variation in the speed of the Benedict mixer under load, the variation over actual testing periods tended to average itself, and in some cases the speed was very close to 19,000 r. p. m. during the test. In the samples of latex having 40 and 50 per cent solids, the average was very good under what might be termed normal operating conditions. However, in many cases conditions were not normal, and in these cases the speed of the machine varied considerably and gave different results.

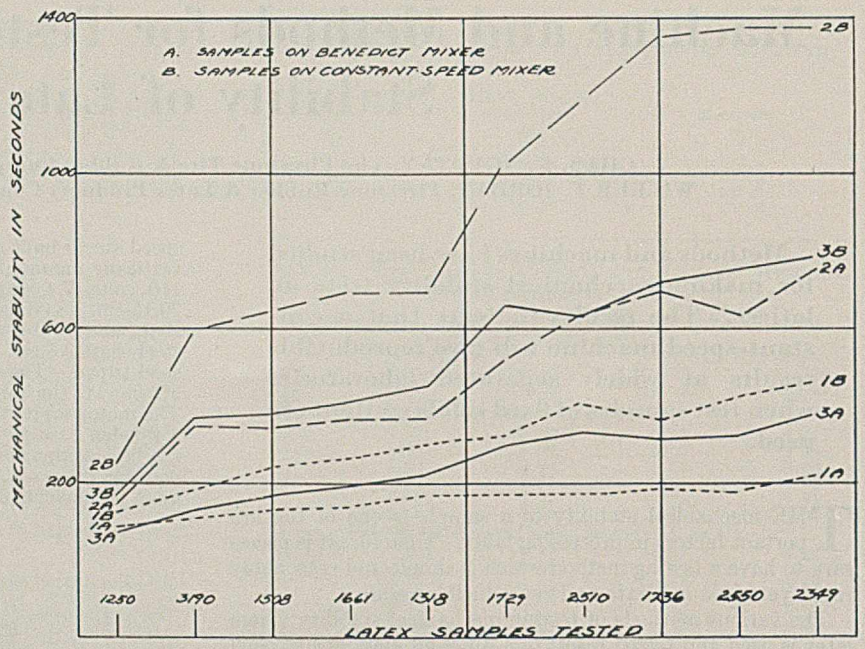

FIGURE 4

TABLE II. MECHANICAL STABILITY TESTS ON LATEX

	Methods 2B and 2A					
	Constant-Speed Mixer			Benedict Mixer		
Sample	Plant 1	Plant 2	Plant 3	Plant 1	Plant 2	Plant 3
1250	160	160	150	165	145	120
1661	520	530	530	540	475	240
1729	450	435	480	440	355	240
2349	480	480	460	550	440	255
3190	315	325	375	325	280	140

The above results indicate that the constant-speed stirrer is very reliable, and that results on the Benedict mixer averaged fairly well over a run, but in a good many tests the results were changed by the inability of the mixer to keep a constant r. p. m. This was due to change in operating conditions which must be expected when handling such materials as latex where coagulum is introduced during the test. If the coagulum comes into contact with the shaft in any way so as to create high friction and thereby a braking action, it upsets the value for this particular test.

TABLE I. DUPLICABILITY OF RESULTS IN DETERMINING MECHANICAL STABILITIES

Drum No.	Total Solids	Method 1A Run 1	Run 2	Av.	Method 1B Run 1	Run 2	Av.
	%						
1250	61.25	92	102	97	130	128	129
1318	60.91	175	166	171	306	295	301
1508	60.92	130	143	137	249	233	241
1661	60.82	128	155	142	253	274	264
1729	59.78	176	170	173	317	326	322
1736	61.42	180	186	183	357	360	359
2349	61.87	208	215	212	436	463	450
2510	62.86	170	159	165	425	390	408
2550	62.81	180	161	171	423	419	421
3190	60.72	110	114	112	217	165	191
		Method 2A			Method 2B		
1250	...	151	135	143	260	251	256
1318	...	355	374	365	698	694	696
1508	...	352	337	345	646	640	643
1661	...	369	374	372	701	695	698
1729	...	529	522	526	1021	1017	1019
1736	...	710	687	699	1350	1339	1345
2349	...	728	760	744	1380	1352	1366
2510	...	627	645	636	1160	1176	1168
2550	...	625	639	632	1380	1358	1369
3190	...	343	355	349	585	583	584
		Method 3A			Method 3B		
1250	...	73	72	73	163	167	165
1318	...	249	210	230	457	442	450
1508	...	163	170	167	389	375	382
1661	...	185	196	191	413	401	407
1729	...	297	300	299	660	672	666
1736	...	313	300	307	704	713	709
2349	...	375	361	368	770	782	776
2510	...	331	326	329	632	626	629
2550	...	333	309	321	755	738	747
3190	...	129	123	126	393	343	368

Experimental Data

Ten samples of latices, selected over a period of one year to give as wide a range as possible, were taken for these tests. Each sample was run at least twice according to the methods described above, and the data obtained are given in Table I. The constant-speed stirrer gives the most accurate results in all cases. In this present setup, the constant-speed stirrer requires a longer time for end points in the test. Methods 1A and 1B give extremely rapid results; because of this, it is impossible to bring out small differences in stability between two samples. Methods 2A and 2B correct this somewhat, but still do not give the desired differentiation between samples. If, however, we consider methods 3A and 3B, there are larger differences between samples.

The results presented in Figure 4 and Table I in many cases hardly seem to show the necessity for using a constant-speed stirrer. However, in some cases it was necessary to run a third sample when using a Benedict stirrer because the original results were too far apart to average.

The following data are very convincing as to the need of a constant-speed stirrer in the latex industry. The Firestone Tire & Rubber Company has need for determining the mechanical stabilities of latices at four widely separated points. Even though the specifications for test were drawn up at the home office and equipment was purchased in each case from the same company under specifications and checked at the

home laboratories with the determination of factors if necessary, there was a good deal of confusion and inconsistency in the results obtained at the various places. These discrepancies brought about the work which is presented in this paper. The following results indicate difficulties which are very apt to be encountered with the use of the malted milk (Benedict) type of mixer.

The test results (Table II) were first obtained at the home laboratories with samples selected and prepared at that point. These results were not furnished to the subsidiary plants, but they in turn determined the stabilities, on both the Benedict mixer and the constant-speed instrument, of the samples which had been prepared at the home plant. Table II shows the wide discrepancies which can be encountered with what at first might seem to be a very reliable and accurate method of determining mechanical stabilities, but with which it seems practically impossible to duplicate results in different laboratories. Here the constant-speed stirrer has shown its reliability.

There probably will be need for further standardization of this test, but it can readily be seen that it merits adoption.

Tachometers or other speed-determining instruments may be attached to the Benedict mixer. In this way a record may be kept of the speed from time to time, an average may be made over a period of time, and a factor may be introduced for correcting the result in seconds. But the observation of the end point in any mechanical stability test requires the attention of the operator at all times. Also, any instrument which must be attached to the malted milk mixer for recording speed, etc., immediately removes its only advantage over the constant-speed mixer: cheapness. The cost of the constant-speed stirrer is slightly higher, but will be less than that of a Benedict mixer with all other instruments attached. The constant-speed stirrer is much simpler to operate and the test in itself is simpler, inasmuch as the operator has only the sample under test to consider.

A point which has not been studied in any detail in this investigation has been the type of propeller used on the stirring element. This should be given some consideration, as the propeller itself can affect the result if it is not readily self-cleaning when pieces of coagulum are picked up on the edges which cause the major portion of the agitation. A propeller should be adopted which can be easily reproduced in any machine shop and not have to be stamped out with a die.

The type of container, the quantity of latex, specifications as to solids content, and ammonia content are all very important in this test. However, the two factors of solids content and ammonia content need not be introduced into a standard specification if the equipment itself is standardized sufficiently to give reproducible results at different laboratories. If such specifications are followed, an ammonia content can be decided by the laboratories using this type of test.

Acknowledgment

The authors wish to express their sincere appreciation to The Firestone Tire & Rubber Company for permission to publish this paper and to J. N. Street for his direction in this work.

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Silica and Pyrex Glass Stills with Automatic Constant-Pressure Feeds

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INVESTIGATIONS on the importance of small quantities of the heavy metals for plant growth and animal health have necessitated the production of water of greater purity than is usually required for analytical work. In the usual types of laboratory still significant amounts of some of the heavy metals may be carried over with the steam or derived from the materials used in the construction of the boiling chamber and the condenser. The ordinary distilled water available in these laboratories, although otherwise of very high quality, contains 13 to 40 micrograms of zinc and 20 to 70 micrograms of copper per liter. To obtain water with a lower content of heavy metals redistillation from silica or Pyrex glass is essential, and the production of small quantities presents no difficulties. When larger amounts are required, as for water culture experiments, some form of continuous distillation is necessary. Several stills for continuous operation have been described (*1–4*), but judging by the relatively complicated arrangements employed, it would seem that the very simple form of continuous feed from a constant-pressure Mariotte's bottle has been overlooked.

Three types of stills for continuous operation which have been used extensively in these laboratories are described below. These stills have been designed to yield from 1 to 2 liters of redistilled water per hour, and have been operated continuously for long periods. One still produced nearly 1000 liters of water per month for 4 months.

In all three stills a Mariotte's bottle maintains a constant level of water in the still. The aspirator bottle may be refilled, and the boiling flask drained, washed, and refilled, merely by the operation of the appropriate stopcocks. No dismantling of any part of the apparatus is necessary.

The supply of ordinary distilled water in the Mariotte's bottle is replenished by connecting the large-bore tube, *E* (11 mm. in diameter), in Figure 1, to a source of distilled water. Stopcocks *B* and *C* are closed, and stopcock *A* is connected to a slowly operating filter pump, giving a reduction of pressure in the bottle not greater than 25 to 30 cm. (10 to 12 inches) of mercury. When the bottle is full, stopcock *A* is closed and *B* is opened. Atmospheric pressure is thus established at the lower end of tube *B*. When stopcock *C* is opened the level of the water in the boiling flask, at atmospheric pressure, rises until it reaches the level of the lower end of tube *B*, also at atmospheric pressure. Air flows into the bottle through tube *B*. So long as the end of tube *E*, within the bottle, is lower than the outlet of tube *B*, no stopcock is required on it. (In laboratories where the supply of distilled water is under pressure, the method of refilling the Mariotte's bottle is essentially the same.) The boiling flask is drained and washed by operating stopcocks *C* and *D*.

Figure 1 (left) shows a still with double surface condenser constructed of Pyrex glass and arranged for gas heating. The flask has a capacity of 3 liters and all ground joints are interchangeable (standard size $\overline{\$}$ 29/42 with minimum inside diameter of 20 mm.). There is no constriction of bore at the joints or in the condenser. The minimum bore of 18 mm. prevents appreciable back pressure being developed in the flask and the level of the water is not lowered on vigorous boiling. While this arrangement is desirable, a

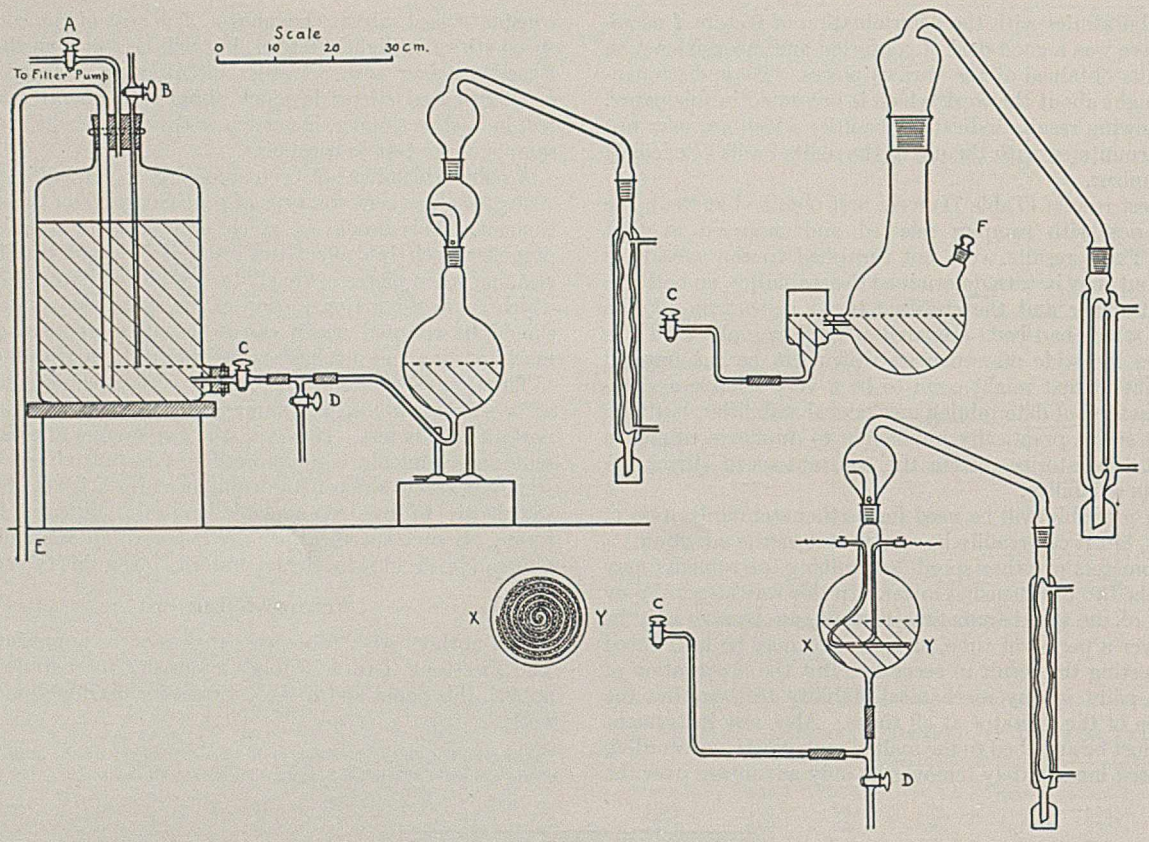

FIGURE 1. SILICA AND PYREX GLASS STILLS

small back pressure in no way affects the constancy of the automatic feed, once equilibrium is reached. It is only necessary to raise the level of the lower end of tube *B* to compensate for this back pressure.

Rubber comes into contact only with the cold water being fed into the still. The feed through the bottom of the flask has been found very satisfactory, since hot water cannot flow downward into this tube and no air locks are formed. Boiling is very even and free from bumping. The double splash head ensures freedom from spray even at high rates of distillation. This still will produce 2 liters of high-grade redistilled water per hour, but it is usually operated at 1.3 to 1.6 liters per hour. A trace of copper and other heavy metals can often be detected in the first 50 ml. of distillate each time the still is started, and it is therefore usual to reject the first 200 ml. of distillate. The redistilled water, as collected, contains not more than 0.2 to 0.3 microgram of copper per liter.

Figure 1 (lower right) shows a similar type of still, the unique feature being the immersion type of electric heater, *x*, *y*. A heating element constructed of a helix of Nichrome wire enclosed in a spiral of snugly fitting thin-walled Pyrex glass tubing combines the high efficiency of an immersion heater with freedom from metallic contamination. The spiral heating element is sealed into the flask after the latter has been cut near its maximum circumference. Heavy-gage copper wire is welded to the ends of the Nichrome wire to form cold leads to the terminals, which are mounted firmly, but not tightly, on the ends of the glass tubes projecting through the neck of the flask. Since the heating element is immersed in water, the Nichrome wire will carry a large current without a big rise in temperature. In the still as constructed 20 B & S gage Nichrome wire is used, and with a current of 6.3 amperes (202 volts, 1270 watts) the temperature of the wire is well below dull red heat. (Calculations show that the inside surface of the tubing is at a temperature of about 105° C. with the outside surface at 100° C.) To ensure a low temperature for the wire, the coils should fit closely against the inside surface of the glass tubing. The wall thickness of the tubing is of minor importance. The low temperature of the wire gives a practically indefinite life for the heating element. This type of immersion heater is suitable only for redistillation of water, since any scale formation, from ordinary water, would lead to overheating the glass tubing.

The high efficiency of the still, without any external thermal insulation, can be appreciated from the following figures: room temperature (temperature of feed water), 17.5° C.; power required for operation, 1270 watts; output of redistilled water, 1560 ml. per hour.

As 0.73 kilowatt-hour is required to raise the temperature of 1 liter of water from 17.5° to 100° C. and to vaporize it, the efficiency of the still is about 90 per cent. A higher room temperature gives a greater efficiency, due mainly to smaller radiation losses from boiling flask and splash trap. Redistilled water from this still has been found to contain total heavy metals of the order of 1 microgram per liter.

The presence of boron in Pyrex glass renders it unsuitable for some work and where freedom from this element is required stills can be constructed of fused silica.

Figure 1 (upper right) shows such a still, the boiling flask being made of translucent Vitreosil, while the splash head and double surface condenser are made of transparent fused silica. A stopper in the body of the flask allows the introduction of a siphon tube for draining the residue from the flask without dismantling. The cold-water feed into the flask is through a capillary tube of suitable bore connecting the feed chamber to the flask. This still is designed for gas or electrical heating. With a 1500-watt radiant type of heater and good thermal insulation, distillation is at the rate of 1.2 to 1.3 liters per hour.

Acknowledgment

Acknowledgment is made to V. A. Stephen, technician at the Waite Institute, who constructed the immersion heater (lower right) and sealed it into the boiling flask.

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A Gelometer for Starch Pastes

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SHEPPARD (*8*) and Bogue (*2*) have reviewed the methods for measuring jelly strength. These fall generally into two classes—those in which the jelly is broken, as in the Delaware jelly strength tester (*1, 11*), and those in which it is deformed only a specified amount, as exemplified by the Bloom gelometer (*4*). Neither type of measurement is entirely satisfactory because the breaking strength of a gel is not always an indication of its resistance to deformation (*7*).

Consequently, there has come into favor a third class of instrument which takes both these properties into consideration. Thus, the methods of Sheppard (*8*) and of Saxl (*5, 6, 7*) give a more complete picture of the rheological properties of a gel than was possible by the older methods. The apparatus described in this paper was designed especially for use with 6 to 9 per cent starch pastes, and will measure on them the same properties that Saxl's gelometer measures on more resistant materials.

Description of Apparatus

The gelometer shown in Figure 1 consists of a regulator, *B*, to give constant head of suction; expansion chamber, *C;* screw clamp, *D*, to regulate rate of flow of air through the system; water manometer, *E*, to read the amount of suction applied to the paste as determined by the height of water column in *H;* brass platform, *J*, with hole in center over which starch paste is placed; graduated pipet, *F*, for reading volume of water displaced by the gel as it is deformed by suction; and constant-head capillary-overflow, *K*, to provide constant rate of increase in suction applied to the paste.

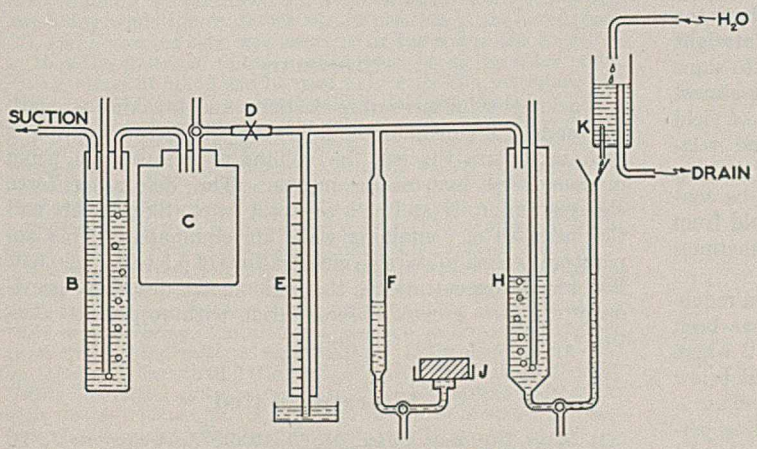

FIGURE 1. DIAGRAM OF GELOMETER

Procedure

The weighed sample mixed with 150 ml. of water is pasted by heating at 99° C. for 0.5 hour in a water bath. In order to prevent excessive evaporation, the tubes are covered by rubber stoppers containing a hole in which a stirring rod is placed. The pastes are stirred by hand from time to time to allow for uniformity in heating. Violent mechanical agitation is avoided, since it increases evaporation losses and may injure the granules. The paste is poured into a 400-ml. beaker, covered with a watch glass, and allowed to stand for 15 hours at room temperature. The gel may then be removed from the beaker and placed bottom down on the brass platform, *J*.

Water is turned on at *K* and suction applied. As water drips from the capillary into the funnel, the level in *H* rises at a constant rate. During this time, readings are taken on the manometer, *E*, and pipet, *F*. Rates of 1 to 4 cm. per minute increase in suction are convenient and allow ample time to make accurate readings. When plotted (Figure 2) the readings of cm. suction *vs.* ml. deformation show a straight-line relationship in the range where the gel is elastic. The breaking point of the gel is indicated by a fairly sharp break in this line.

In order to obtain hysteresis curves, the three-way stopcock below *H* is opened before suction has reached the elastic limit, and readings are made as the force on the gel is gradually reduced. From these three quantities—elasticity, breaking point, and hysteresis—may be calculated the gel factor, Bloom point, elastic recovery, and other values which have been used as expressions of gel strength (*5*).

The slope of the suction-deformation line (indicative of elasticity) is roughly proportional to the area of the hole over which the gel is placed. The amount of suction (centimeters of water) necessary to break the gel, since it is exerted equally on each unit area, is relatively independent of hole size except in cases where the gel is deformed by its own weight.

Starch pastes are extremely sensitive to variations in the method of preparation; before starting routine measurements on this instrument, it is essential that the method of preparation be checked as to duplicability of the product.

Principles

The combination of principles which differentiates this gelometer from others described in the literature may be summarized as follows:

1. Suction is used to deform the gel (*9*). The force is thus transmitted uniformly to all parts of the gel exposed to it. Since the deformed portion is in contact only with water, such factors as abrasion due to mechanical plungers and inconveniences due to the use of elastic membranes are eliminated.
2. The volume of deformation is measured by hydrostatic means (*10*). Using a hole size of 2.6-cm. diameter and a 2-ml. pipet, this volume may easily be measured to 0.02 ml., corresponding to a deformation of 0.04 mm. by instruments which measure depth directly.
3. The deforming force changes slowly, uniformly, and at a constant rate. Having started the flow of water from *K*, the operator may

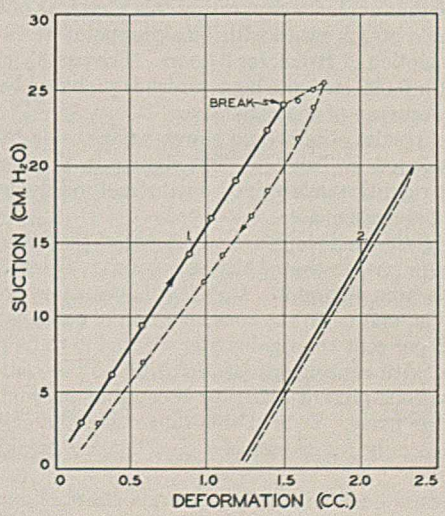

FIGURE 2

TABLE I. COMPARISON OF BREAKING STRENGTH AND ELASTICITY OF 6.5 PER CENT STARCH GELS

Starch	Breaking Point *Cm. H_2O*	Slope *Cm./ml.*	Rigidity × 10^{-1} *Dynes/sq. cm.*
Yellow Creole	18.8	15.0	660
Popcorn	17.5	13.8	590
Mandan	12.5	10.7	440
Commercial corn	10.5	11.0	370
Hegari sorghum	7.8	10.3	378
Modified A	9.5	9.5	320
Modified B	7.0	9.0	270
Modified C	4.5	7.5	205
Country Gentleman	3.5	7.5	175

concentrate on reading the manometer and pipet with no further attention to the mechanics of increasing the load. This uniform change of applied force is especially valuable in measuring properties where the time factor is important and stepwise addition of load would be likely to introduce error.

4. Skin effects and the influence of the walls of the containing vessel are eliminated by removing the mold from the beaker and placing it bottom down on the brass platform. The dimensions of the gel in relation to the area to which suction is applied should be large enough so that deformation occurs chiefly in the part adjacent to the hole without causing the rest of the mold to change its shape appreciably.

Applications

Figure 2 indicates the type of information which may be obtained on this apparatus. Curve 1 is typical for starch pastes. The amount of deformation proceeds in direct proportion to the increasing suction up to the point where the gel breaks, with a corresponding sharp break in the straight line (at 23.5 cm. in this case). Such behavior tends to show that starch gels of this concentration (7 per cent) are almost perfectly elastic, since they do not undergo a period of yield before actual shearing; otherwise the straight-line relationship would not hold at pressures close to the breaking point. The fact that the gel is broken may further be confirmed by direct observation upon removing the mold from the platform. A circular cut is usually produced; sometimes a hemispherical piece is torn from the center.

The dotted portion of curve 1, representing gradual reduction of suction, has no significance since the gel has been broken. It is included for comparison with curve 2 where suction on the paste was increased up to 20 cm. (just below the breaking point) and then reduced to its original value.

The difference between the ascending and descending portions of curve 2 (0.05 ml.) indicates that hysteresis is present to only a limited extent, about 2 per cent of the total deformation. Further evidence supporting this view was obtained by subjecting a starch paste with breaking point at 18 cm. to a constant suction of 16 cm. for 3 hours. The paste yielded only 0.01 ml. (or 0.002 cm.) per hour, which is probably beyond the range of accuracy of the apparatus.

Typical results obtained on starch pastes using this instrument are listed in Table I. The figures in the last column represent rigidities determined by the method described in a previous publication (*3*).

Both rigidity and slope are a measure of the force required to deform or stretch the gel a given amount. This relationship is evident from Figure 3. Rigidity values show greater differentiation, and when the concentration of paste can be kept below 6.5 per cent the rigidometer method is to be preferred. However, with concentrations of 6.5 to 8.5 per cent, rigidity determinations present difficulty because of the high consistency of the paste. It is in this range that the gelometer is useful, not only for values of slope, but for breaking point data.

This paper deals primarily with raw starches and slightly modified products. With more highly converted starches, the method of preparation would have to be altered by increasing the concentration and time of setting. Such gels would be expected to exhibit plasticity and hysteresis as the ratio of gelling to nongelling components becomes increasingly large.

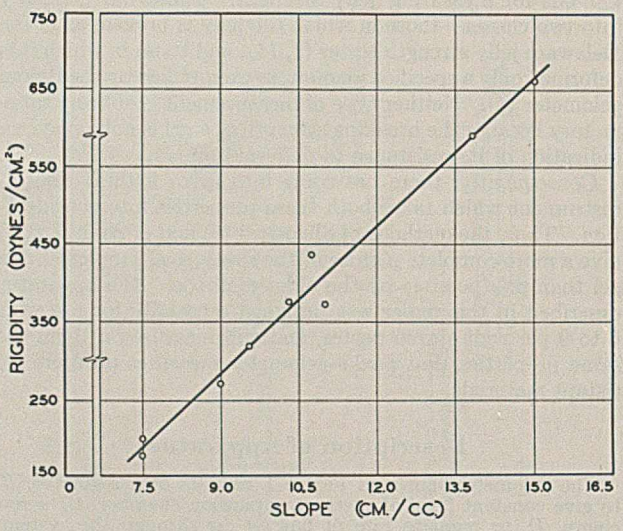

FIGURE 3

Summary

A gelometer for measuring elasticity and breaking strength of starch gels embodies the following principles: Suction is used to deform the gel, the volume of deformation being measured by hydrostatic means. The deforming force changes uniformly and at a constant rate. Skin effects and the influence of containing walls are eliminated. This apparatus is suited to starch concentrations of 6.5 to 8.5 per cent. For lower concentrations, the rigidometer described previously (*3*) gives greater differentiation with respect to elasticity.

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CORRECTION. In the article on "Determination of Bromine Addition Number" [IND. ENG. CHEM., Anal. Ed., 13, 91 (1941)], the second line of the procedure, second column, should read "into a 25-ml. Erlenmeyer flask".

KARL UHRIG

An Improved Thixotrometer

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THIS thixotrometer is an improved design of one that was described in connection with a study of the leveling of paints (*2*). Apparatus of somewhat similar construction has been used by Schofield and Scott Blair (*3*) and by Freundlich and Röder (*1*).

To obtain better insulation from building vibration, the thixotrometer is now mounted on a pair of slings (Figure 1). Rubber bands suspend it so that the base just touches four rubber balls, placed under the corners of the base and flattened on one side to prevent their rolling. The balls furnish a damping effect and prevent swaying of the thixotrometer. The insulation from building vibration is very satisfactory.

To reduce to a minimum the stirring which resulted from the insertion of the sphere (*2*) into the material being examined, several changes were made (Figure 2). The torsion wire assembly was mounted on a precision rack and pinion (Pillor rack and pinion unit, Spencer Lens Company), the sphere was replaced by a steel disk of the same diameter, an electromagnetic clamp to hold the disk prior to release was constructed, and extension arms were made to permit operation of the micrometer (*2*) and the rack and pinion without jarring the thixotrometer. (Two mechanical clamps which were tried before resorting to the electromagnetic clamp were unsatisfactory. A wooden core is used in the electromagnet, since an iron core becomes permanently magnetized. Because alternating current causes a stirring action, direct current must be used in the electromagnet.)

To overcome any irregularities which might result from variations in filling the container (*2*), a funnel was constructed to permit filling while the container was in position in the water bath (*2*), and a vertical tube was mounted at the end of the container so that the source of filling would always be at the same point. This method of filling can be used only with thin materials, and so far no advantage in its use has been demonstrated.

To use the thixotrometer, the torsion wire assembly is raised, the torsion setting is made, and the disk is clamped in position by turning on the electromagnet. A stop watch is started the moment that the pouring of the material into the container is started. The disk is lowered into the material at the end of 1 minute and the disk is released at exactly 10 minutes. The distance traveled by the disk in 5 minutes is recorded.

The true zero position of the disk is that at which buoyant effects do not alter its position. The zero torsion setting is that which gives the true zero position of the disk. In order to keep the disk clear of the clamp, the clamped position is chosen 0.015 inch (0.04 cm.) behind the true zero position. If the distance traveled by the disk is less than 0.015 inch, the distance is determined by noting the initial and final positions on the eyepiece micrometer scale of the microscope. For greater distances of travel the disk is brought back to the true zero position by means of the micrometer and the correction of 0.015 inch is added to the micrometer reading.

The data in Table I were obtained in a series of consecutive measurements on a typical paint. $D_{obs.}$ is the distance traveled by the disk in 5 minutes for a torsion setting of 50°. $D_{calc.}$ is the corresponding value obtained from a line fitted to the data by the method of least squares. (In fitting the line the number of the observation was chosen as the abscissa and the corresponding distance of travel as the ordinate for the plotted points.) In the last column the per cent differences of the calculated from the observed values are given.

TABLE I. MEASUREMENT OF PAINT

No.	$D_{obs.}$	$D_{calc.}$	$\frac{100(D_{obs.} - D_{calc.})}{D_{calc.}}$
1	0.1229	0.1120	+9.75
2	0.1076	0.1140	−5.61
3	0.1075	0.1161	−7.40
4	0.1173	0.1181	−0.68
5	0.1215	0.1202	+1.08
6	0.1242	0.1222	+1.61
7	0.1247	0.1243	+0.32
8	0.1276	0.1263	+1.32

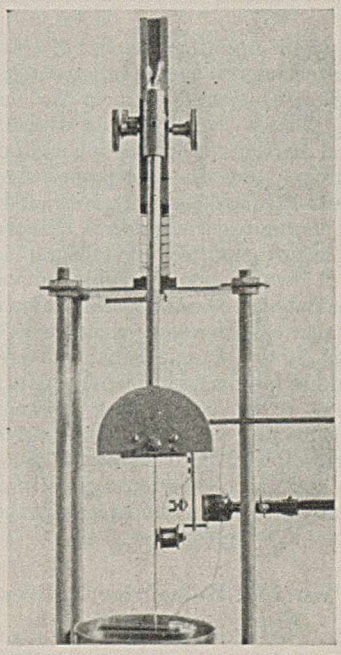

FIGURE 2. RACK AND PINION, AND ELECTROMAGNETIC CLAMP

From the differences in per cent the probable error of a single observation is found to be 3.49 per cent. The probable error of a single observation calculated from the results of 55 pairs of observations on various paints was found to be 3.55 per cent, which is in good agreement with the above value. These values seem large, but since variations of the order of a hundredfold are frequently encountered in comparing one paint with another, the precision of measurement is believed to be satisfactory.

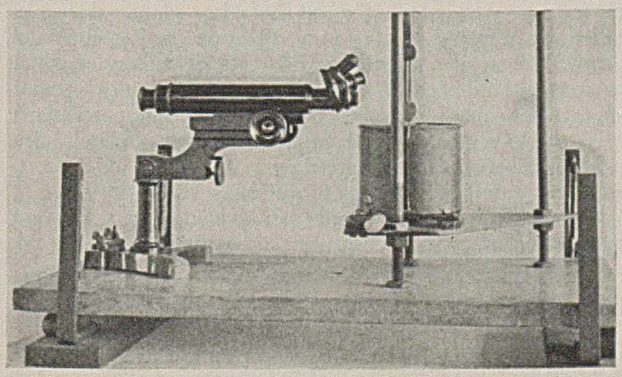

FIGURE 1. THIXOTROMETER IN POSITION ON SLINGS

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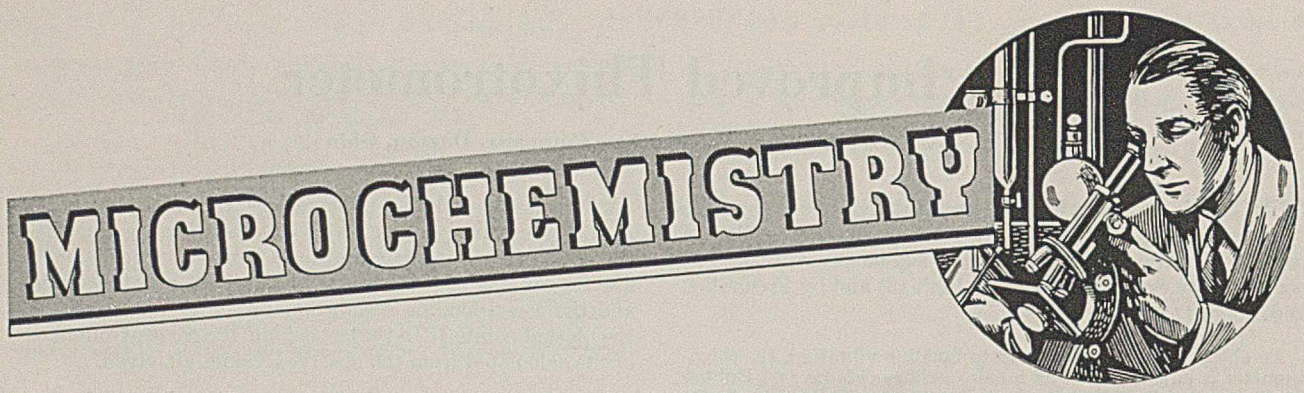

Application of the Grating Microspectrograph

To the Problem of Identifying Organic Compounds

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THE study of the optical properties of crystals has relatively greater importance in microscopic methods of identifying organic compounds than in the identification of inorganic ones. This is partly because with inorganic compounds chemical reactions of both cation and anion may usually be carried out under the microscope, whereas with organic compounds chemical tests are restricted to detecting the presence of certain reactive groups. However, in general, it is not possible to use optical crystallographic methods as the sole means of identification of organic compounds, because the optical constants have been determined for relatively few of the vast number of such known compounds. When, however, the optical data of organic compounds are used in conjunction with other physical and chemical data, they may be of considerable use to the microchemist, particularly where the problem may be reduced to that of finding out if an unknown substance is identical with one of a group of known substances.

The relative usefulness of various optical properties of organic compounds for such comparison purposes may be judged from the following considerations.

Principal Refractive Indices

It is convenient to follow the classification given by Wooster (*8*) and to divide molecular crystals into three main classes:

1. Almost spherical molecules, such as pentaerythritol and some of its esters. Crystals of this class have refractive indices well within the range of organic immersion media. Mixtures of Nujol with α-bromonaphthalene or α-iodonaphthalene have a sufficiently low solvent action to be used as immersion media.
2. Rod-shaped molecules. This classification includes the majority of long-chained aliphatic compounds. Usually the refractive indices of crystals of this class are low, unless the molecule contains substituents of high refractivity, such as bromine, iodine, sulfur, etc. The crystals may have an appreciable solubility in immersion media, so that refractive index measurements are not as a rule easy to make. Increasing the length of carbon chain by one or two carbon atoms does not greatly affect the refractive indices, and as the limit of accuracy of determining these refractive indices is about ± 0.002, it follows that the measurements may have but little analytical value.
3. Planar molecules. This classification includes the majority of aromatic compounds. Unless the molecules are grouped in nonparallel directions, n_γ and often n_β are beyond the range of known immersion media. Solubility of the crystal in immersion media may render it impossible to determine its refractive indices. The position and nature of weakly polar or nonpolar substituents usually have little effect on the refractive indices.

With all three classes of molecular crystal, n_α is usually low enough to be determined by the immersion method, using the light from a sodium vapor lamp for the final adjustment of the refractive index of the immersion liquid. The temperature variation method is not often applicable to organic compounds on account of their solubility in liquids having a suitably high thermal variation of refractive index. The author prefers to use the method of mixing two liquids of very low volatility and widely different refractive index on the microscope slide until the lowest refractive index of the crystal under test is matched. The immersion liquid is then transferred to a microrefractometer or an Abbe refractometer. Mixtures of tri-*N*-butyl citrate and *n*-butyl phthalate cover the range 1.447 to 1.492, and of Nujol and α-iodonaphthalene, cover the range 1.484 to 1.701 (*2*). Kunz and Spulnik have suggested the use of mixtures of heptylic acid and α-bromonaphthalene to cover the range 1.423 to 1.658 (*6*).

Birefringence

The birefringence of a doubly refracting crystal may have any value between the limits of zero for rays traveling along an optic axis, and $n_\gamma - n_\alpha$ (or $n_\omega \sim n_\epsilon$ in the case of uniaxial crystals) for rays traveling normal to the plane of the optic axis or axes. Consequently it is usual to restrict the term to the three constants $n_\gamma - n_\beta$, $n_\beta - n_\alpha$, and $n_\gamma - n_\alpha$ of biaxial crystals, and $n_\omega \sim n_\epsilon$ of uniaxial ones.

The birefringence of organic compounds is due to two factors, molecular anisotropy and structural anisotropy, and in general $n_\gamma - n_\alpha$ is large for rod-shaped or planar molecules which are oriented in the same direction, and is small for roughly spherical molecules and for planar molecules which have several orientations in the crystal. It may also be small for crystals containing solvent of crystallization, particularly when the solvent of crystallization also has planar molecules.

Methods of measuring birefringence with the aid of compensators entail measuring the thickness of the crystal, and as such thickness measurements can be made only with a low order of accuracy, the birefringences so determined are not very accurate.

Dispersion of Birefringence

The dispersion of refraction, $dn/d\lambda$, of a crystal is usually different for each of its principal refractive indices, and as birefringence is the numerical difference between two such

indices, it follows that birefringence varies considerably with wave length. So-called "anomalous" interference colors are caused by extreme dispersion of birefringence, but in no known case does the scale of interference colors given by a crystal exactly correspond to Newton's scale of colors given by an air film. Crystals which are pleochroic in the violet or near ultraviolet show marked differences between the dispersions of the principal refractive indices, and consequently have a strong dispersion of the birefringence, particularly along the acute bisectrix when the optic axial angle is small. By way of an example, the values of $n_\gamma - n_\beta$ of ammonium picrate and *o*-dihydroxybenzene are compared for various wave lengths.

The measurement of dispersion of the principal birefringences is complicated in the case of monoclinic and triclinic crystals which possess an appreciable degree of dispersion of the axes of the optical ellipsoid. In such cases the author prefers to measure the dispersion of birefringence presented by a fixed orientation of the crystal, and to take care that an identical orientation is used in comparisons with other crystals. Whenever possible, dispersion of the maximum birefringence, $n_\gamma - n_\alpha$, is chosen for comparison purposes, as errors of up to 10° in orientation of the crystal do not seriously affect the accuracy of the results. The measurements have considerable practical interest, as it is not necessary to know the thickness of a crystal in order to determine its dispersion of birefringence.

TABLE I. $(n_\gamma - n_\beta)$ OF A COLORLESS AND A YELLOW CRYSTAL

λ, mμ	*o*-Dihydroxybenzene	Ammonium Picrate
513	0.0106	0.044
541	0.0104	0.000
589	0.0099	−0.037[a]
656	0.0093	−0.071[a]

[a] Vibration directions of n_γ and n_β interchange at 541 mμ.

Optic Axial Angle

Observations of the interference figure given by a crystal in convergent polarized light have a well-recognized value in determining whether a crystal is uniaxial or biaxial, and, in the latter case, it is sometimes possible to measure the optic axial angle. Observation of the type of dispersion of the optic axes, when it can be made, renders possible differentiation among rhombic, monoclinic, and triclinic crystal symmetry.

Principal Absorptions

Observations of the principal absorptions of strongly colored crystals are usually qualitative and are confined to the study of dichroism or trichroism. This is understandable, as a great many strongly absorbing organic compounds form monoclinic or triclinic crystals which often have extreme dispersion of the axes of the optical ellipsoid, so that the optical properties of the crystals are not easy to interpret. Nevertheless, it is this complication of optical properties of absorbing crystals which makes them all the more specific and useful for comparison purposes.

The above considerations show the importance, from a determinative standpoint, of studying the optical properties of organic crystals for as wide a region of the spectrum as possible. It is obviously not practicable to repeat a complicated series of observations for many wave lengths, particularly as the eye is neither very sensitive nor very well focused for the extreme blue end of the spectrum.

Grating Microspectrograph

It was in order to simplify the study of crystal optics throughout the visible region of the spectrum that the author turned to the aid of photography and developed a transmission grating microspectrograph for use with the petrographic microscope. The first model of the apparatus used 8.125 × 10.625 cm. (3.25 × 4.25 inch) panchromatic cut film (*5*); the second model was much more compact and used 35-mm. Panatomic film (*3*). In neither of these instruments was the light collimated before its passage through the grating. The achromatic lens which projected the image of the slit onto the film had a very small diameter relative to the projection distance; the astigmatism thereby introduced into the spectrum was not at first considered serious. As experience in applying the apparatus to specific problems was accumulated, it became evident that it would be advantageous to have a spectrum completely free from astigmatism.

The generous gift of a plane metal grating from H. D. Babcock of Mt. Wilson Observatory made it possible for the author to construct a reflection-grating microspectrograph working on a somewhat different principle, which incorporated the improvements suggested by the earlier work. The general principle of the new apparatus is shown in Figure 1.

A Steinheil lens is used to focus the microscope image on the slit. The microscope, which is used with an eyepiece not fitted with cross webs, is focused for an image distance at infinity, so that the Steinheil lens is used at its principal focus from the slit. It is provided with centering screws, in order to bring the center of rotation of the image on the center of the slit. The collimating lens is a cemented achromat which collects light from the slit and gives a collimated beam which is then reflected by a silvered right-angled prism on the plane grating. A Wollaston prism, which is provided with a fine adjustment to its rotation, may be swung into the beam for studies of dichroism. Over half the light incident on the grating is diffracted in one of the first-order spectra, which is collected by a cemented achromatic camera lens and is brought to a focus in the image plane of a reflex camera which uses 35-mm. Panatomic film. It was found that much less coma was obtained when the plane side of the camera lens faced the grating and that all the spectrum was sharply in focus at the same time.

The grating is mounted in a heavily built holder which rotates on spindles fitted with Hoffmann ball bearings. The grating is rotated by a micrometer fitted with an accurately worked face plate of sapphire which presses against a steel ball fixed in the grating housing. In the center of the reflex ground glass, there is a clear patch fitted with adjustable cross webs. The spectrum can be focused on these cross webs which are viewed with a × 12 Ramsden ocular. Wave lengths of emission lines may thus be determined to 0.2 mμ. Spectra of very low intensity are observed through a telescope fitted with a reflecting prism which intercepts the light from the grating before it reaches the camera lens.

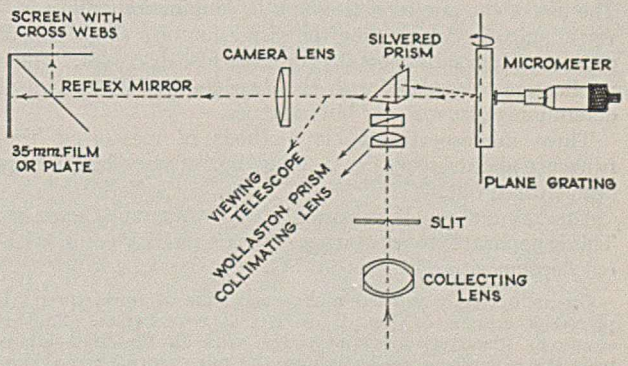

FIGURE 1. DIAGRAM OF APPARATUS

Compared with the transmission grating apparatus described by Chamot and Mason (*1*), the new apparatus is considerably easier to manipulate, as it is merely necessary to place the microscope under the microspectrograph. Accurate centration is not necessary; the microscope is moved on the baseboard until the spectrum is evenly illuminated, a matter of a few seconds' adjustment.

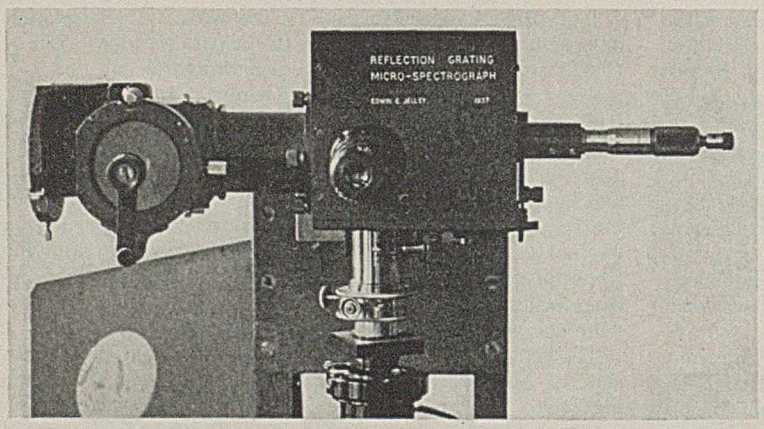

FIGURE 2. REFLECTION-GRATING MICROSPECTROGRAPH

The reflection-grating microspectrograph is shown in Figure 2, and the complete setup, with a Leitz universal polarizing microscope constructed to the author's specification, is shown in Figure 3. A silvered right-angled prism is used over the eyepiece to deflect an image of the crystal onto a screen. When the spectra are to be recorded photographically, the prism is thrown out of the optical axis of the system. There is, however, a disadvantage attendant on the use of a metal reflection grating; the violet end of the spectrum is relatively weaker than that obtained with a transmission grating. This is because the speculum metal on which the grating is ruled has a lower reflectivity in the violet, whereas the violet end of the spectrum is accentuated with a transmission grating by virtue of the higher refractive index in this region of the cellulose nitrate from which it is made.

Applications of Grating Microspectrograph

In the author's first publication on the microspectrograph (*5*), its use in the determination of optic axial angles of orthorhombic crystals for the visible spectrum was discussed. It was proposed to use a calcite plate cut perpendicular to the optic axis for calibration purposes. This plate was calibrated with sodium light (λ = 589.3) by means of a universal stage. In the second publication (*3*), a spectrogram was given which showed the dispersion of the optic axes of *o*-nitroacetanilide. The use of the microspectrograph in conjunction with a universal stage in determining the dispersion of a single axis of praseodymium sulfate octahydrate was also discussed. These publications also dealt with the "wedge-crystal" method of determining dispersion of birefringence.

Three microspectrographic methods of measuring birefringence and its dispersion as a function of wave length have been worked out.

DISPERSION OF BIREFRINGENCE OF WEDGE CRYSTALS. This is applicable to substances having a melting point (without decomposition!) below 300° C.

On an optically worked microscope slide of fused quartz is placed a square of No. 2 or No. 3 cover glass. Another square is placed so that one edge rests on the first square, with the opposite edge resting on the microscope slide. Some crystals of the substance to be studied are placed near the second cover slip, and the preparation is heated over a microburner until the substance melts and is drawn under the sloping cover glass to form a wedge of liquid. On cooling, the substance crystallizes to form wedge-shaped crystals. Refusion may be necessary to get satisfactory results, and the preparation may need to be seeded by touching it with a needle which has been charged with crystals of the substance. Such a wedge preparation shows ascending orders of birefringence when examined with a low-power objective between crossed nicols. The image of the wedge-shaped crystals, which have a slope of about 2°, is thrown on the slit of the microspectrograph and is so oriented that the slit cuts through the ascending orders. Spectrograms are then made on Panatomic-X film, exposures of 1 to 20 seconds being given, according to the width of the slit.

A Philips tungsten arc lamp is used as a light source, as it gives, in addition to the continuous spectrum, neon lines which serve to locate the wave-length calibration. A ribbon-filament lamp can also be used, but in this case an additional exposure is made with the microscope illuminated by a sodium vapor lamp. The simplest way of studying the spectrograms is to enlarge them so that 20 mμ on the wave-length scale is 10 mm. on the bromide enlargement. It is not necessary to use nonshrinking bromide paper, as uniform shrinkage in no way affects the accuracy of computations made from the enlargements.

If the wedge-crystal spectrogram shows several interference bands, it is best to proceed according to Figure 4. A line is ruled through the spectrum in a position corresponding to the thick end of the wedge crystal. Irregularities in the crystal cause many lines to run through the spectrum, and one of these should be chosen as a guide for the ruled line, thereby avoiding errors due to distortion introduced by the camera lens. A wave-length scale is then attached to the enlargement, and the wave lengths λ_n, λ_{n+1}, λ_{n+2}..... at which the nth $(n + 1)$th, $(n + 2)$th..... interference bands cross the line are recorded. The value of n is readily counted if the extreme tip of the wedge appears in the spectrogram. The value of the birefringence for any wave length, λ_n, at which an interference band of the nth order intersects the line, is $n\lambda_n/d$, where d is the undetermined thickness of the wedge at the point corresponding to the ruled line. It is convenient to consider the birefringence at different wave lengths as multiples of the value for sodium light, a procedure whiche liminates the necessity of determining the thickness, d, of the crystal. The calculations are simplified if the line is ruled through the spectrogram at an ordinate where an interference band occurs at λ = 589 mμ. In the diagram, this is the seventh band, which

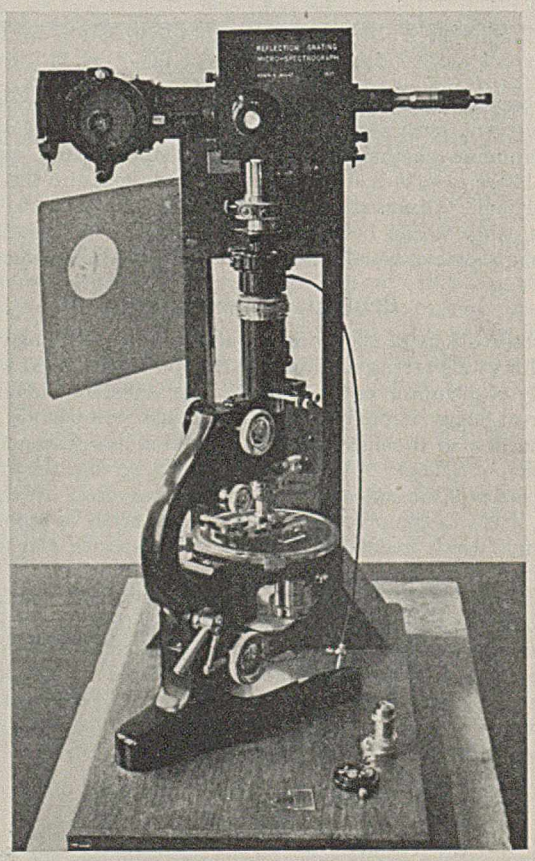

FIGURE 3. COMPLETE MICROSPECTROGRAPH SETUP

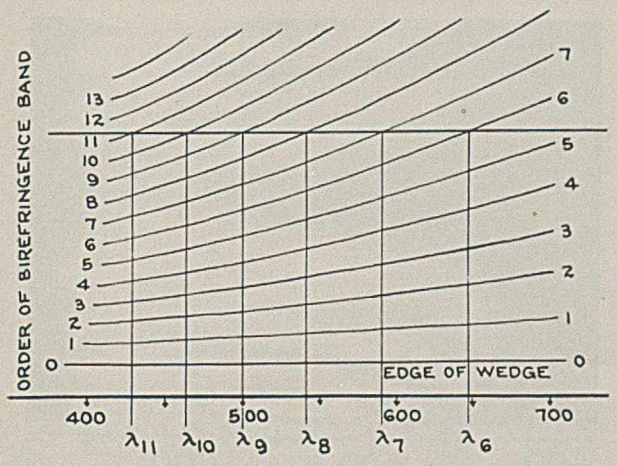

FIGURE 4. COMPUTATION OF BIREFRINGENCE

corresponds to a retardation of 589 mμ × 7 = 4.125μ. Similarly, the retardation at λ_6 is 647 mμ × 6 = 3.922, and at λ_9 is 500 mμ × 9 = 4.5μ (retardation = birefringence × thickness of crystal). The values of the relative birefringence are, therefore, 0.95 for 647 mμ, 1.00 for 589 mμ, 1.09 for 500 mμ, and so on. These values are plotted against wave length.

Some typical curves are given in Figure 5, in which Newton's color scale for an air film is represented by a horizontal line. This method of computing the dispersion of birefringence utilizes the light from a single point on the wedge crystal, and no assumptions are made as to the uniformity of slope of the wedge.

When, however, only a few bands are present, it is necessary to measure the average distance apart of the birefringence bands on the photographic enlargement at a number of wave lengths.

Let the average distance between bands at wave lengths λ_1, λ_2, λ_3..... be d_1, d_2, d_3..... The birefringence at these wave lengths is then represented by $c\lambda_1/d_1$; $c\lambda_2/d_2$; $c\lambda_3/d_3$..... where c is a constant depending on the slope of the crystal wedge and the magnification of its image on the bromide enlargement. If λ_1 is 589 mμ, the relative birefringence at λ_2 is $d_2.\lambda_1/d_1.\lambda_2$, and so on. These values are plotted against wave length, as above.

The value of the birefringence for sodium light may be determined with a moderate degree of accuracy by measuring the slope of the wedge on a goniometer or universal stage and measuring the distance along the wedge between the 2nd and 12th (or higher) interference bands when the microscope is illuminated with a sodium vapor lamp. If the distance between the 2nd and 12th bands, measured with a screw micrometer eyepiece, is $d_{mm.}$ and the slope θ, the birefringence is $0.00589/d \tan \theta$. The value of the birefringence for any other wave length is obtained by multiplying the value for sodium light by the dispersion factor as obtained above.

BIREFRINGENCE AND DISPERSION OF BIREFRINGENCE OF LENS-CRYSTALS. An elegant way of determining both birefringence and dispersion of birefringence may often be applied to readily fusible organic compounds.

A small plano-convex lens, preferably of fused silica, and an optically worked microscope slide of fused silica are required. The substance to be examined is fused on the silica slide, and the curved side of the lens is brought in contact with liquid, which is then allowed to cool and crystallize. Slight pressure is applied to the top of the lens to ensure contact with the slide. If the operation has been successful, a negative lens of crystal is formed, which has zero thickness at the point of contact of the silica lens and silica slide, and which shows circles of increasing birefringence between crossed nicols.

The appearance with sodium light between crossed nicols of a lens-crystal of α-nitronaphthalene is shown in Figure 6 (left). When crystals of more than one orientation are present, birefringence rings corresponding to the different orientations are produced, as in the case of piperonal (Figure 6, right) where the rings of small diameter correspond to $n_\beta - n_\alpha$, and the larger ones to $n_\gamma - n_\beta$.

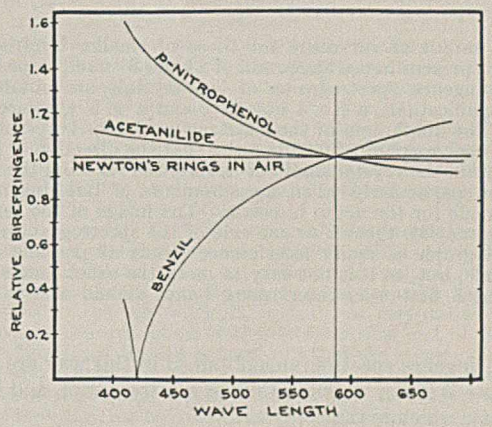

FIGURE 5. TYPICAL CURVES

In actual practice, it is usually necessary to recrystallize the substance by fusion a few times in order to obtain satisfactory lens-crystals. In order to obtain suitable interference figures, it is desirable to have a lens with a relatively large radius of curvature for use with substances of high birefringence, and one of

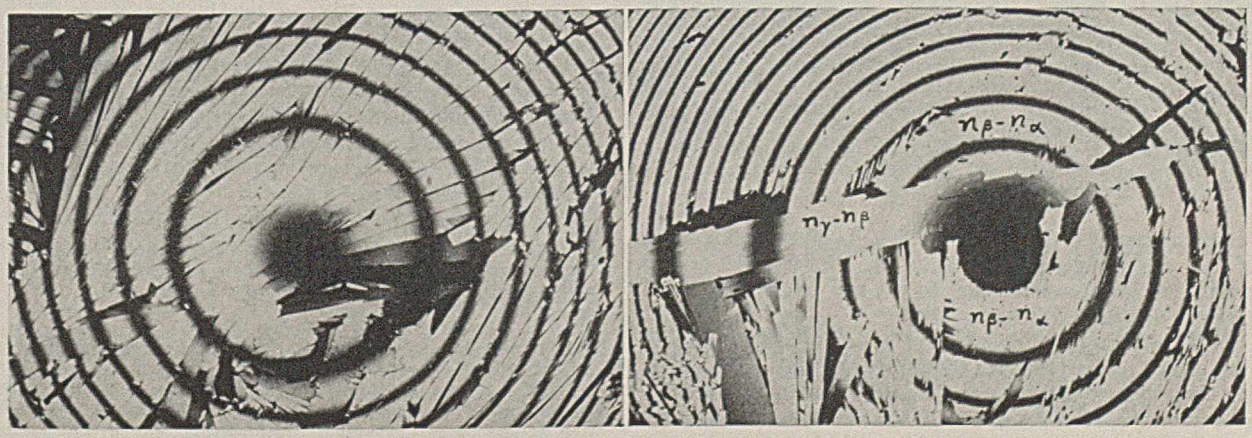

FIGURE 6. BIREFRINGENCE RINGS OF α-NITRONAPHTHALENE AND PIPERONAL

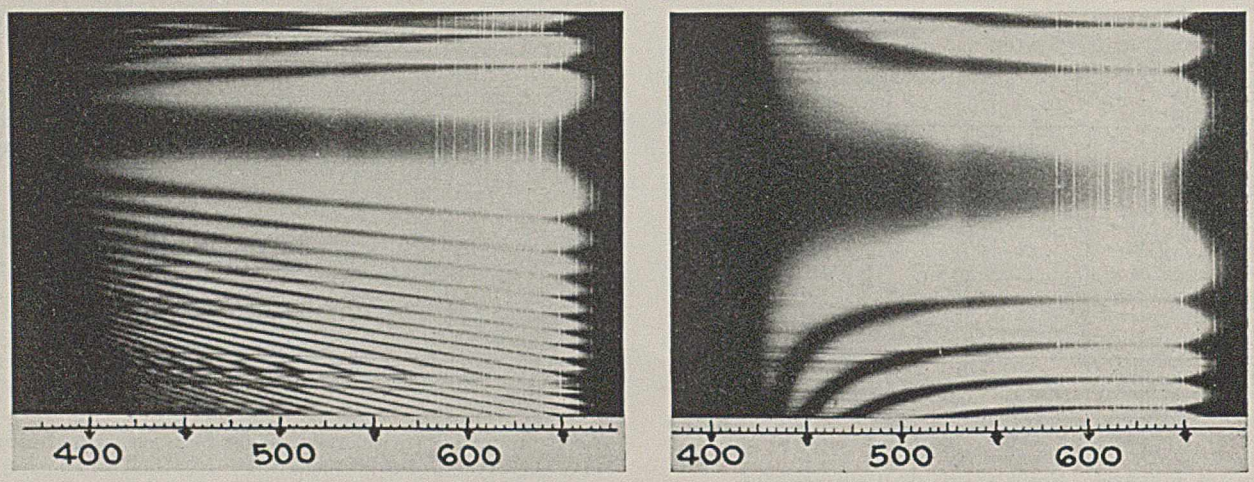

FIGURE 7. BIREFRINGENCE SPECTROGRAMS OF *p*-NITROPHENOL AND BENZIL

smaller radius of curvature for those of smaller birefringence. Those at present in use have radii of 83 and 31 mm., respectively.

Birefringence spectrograms of lens-crystals are obtained at low magnifications, a No. 1 objective and a $\times 5$ eyepiece being used. The diaphragm of the substage condenser is restricted to a numerical aperture of 0.05 in order that the direction of rays of light within the crystal shall not deviate too far from the axis of the lens, particularly when measurements of birefringence are being made for the acute bisectrix. The image of the center of the lens may be thrown to one side of the spectrogram in order to photograph as many interference bands as possible on the other side, but, as it is not easy to mark the exact center of the rings, both first-order interference bands should always be included.

Birefringence spectrograms obtained in this way are shown in Figure 7 (left), which is that of *p*-nitrophenol, and Figure 7 (right), which is that of benzil.

Calibration of the thickness of crystal corresponding to any interference band is best carried out by photographing Newton's rings given in sodium light by the lens in contact with the silica slide. In order to make the rings clearly visible by transmitted light, it is necessary to increase the reflectivity of the lens and slide. The standard way is to half-silver the surfaces, but the author has now found a much simpler method. This consists in applying a drop of a 1 per cent solution of 1,1′-diethyl-ψ-cyanine chloride in methanol to each surface and allowing it to evaporate. The resulting film of dye has an intense narrow absorption band at 578 mμ and, in consequence, a very high refractive index for sodium light. The Newton's rings produced by such treated surfaces are quite as good as those given by half-silvered surfaces, and the dye films are very easily applied and removed, operations which take but a second or so. The Newton's rings are photographed on the same length of 35-mm. Panatomic-X film as is used to record the birefringence spectrograms of the lens-crystals. In order to do this, the slit of the microspectrograph is opened wide, and the grating is rotated to bring either the normally reflected image or the first-order diffracted image to the center of the ground-glass screen. By using the same microscope setup for both the Newton's rings and the lens-crystals, the magnification is unchanged, so that the thickness of crystal necessary to produce any interference band is directly measured in terms of the wave length of sodium light.

A photograph of the Newton's rings is given in Figure 8. As they are produced by transmission, the center is bright, and the air gap corresponding to the *n*th bright ring has a thickness of $n/2 \times 589.3$ mμ.

Measurements of photographs of both Newton's rings and interference bands produced by the lens-crystals have shown that their diameters vary according to the square root of their order within an accuracy of 1 in 2000, so that it is obvious that the microscopic and spectrographic optical systems are not introducing any appreciable distortion.

In practice, these measurements can be made with adequate accuracy from bromide enlargements. The center of the Newton's rings is found by bisecting the diameter of one of the lower-order rings, and the radius of a high-order bright ring is measured. Let the order of the ring be M, and the radius be X. Measurements are then made from the center of the birefringence spectrogram (obtained by bisecting the distance between the two first-order bands) to the Nth order interference band, for some particular wave length, λ. Let this distance be y. The birefringence of the crystal for this particular wave length is then given by the formula $n_1 - n_2 = 589My^2/2N\lambda x^2$, where n_1 and n_2 are the refractive indices of the crystal for this wave length. Measurements of y are made for various values of λ in order to obtain birefringences for the whole spectrum, and these may be plotted in terms of the value for sodium light in order to obtain dispersion curves corresponding to those obtained by the first method.

FIGURE 8. NEWTON'S RINGS

DISPERSION OF BIREFRINGENCE OF DROPLET CRYSTALS. Fragments of the substance, weighing between 0.1 and 10 mg. are fused on a microscope slide, preferably one o optically worked fused silica. On cooling, the droplets crystallize to yield wedge-shaped crystals. As with the first two methods, it is necessary to check the optical orientation of the crystals by conoscopic observation. In order to determine the dispersion of birefringence, a spectrogram is made of the rising orders of interference of one of the wedge-shaped crystals at a suitable magnification. A droplet crystal of *o*-chloroacetanilide gave the spectrogram shown in Figure 9. The method of computing the birefringence–wave-length graph is identical with that of the first method.

One or other of the above methods will work with a great many fusible organic compounds. Failure to obtain satisfactory crystals may be due to one or more of the following causes:

1. The substance is impure. Two or three recrystallizations from a suitable solvent will often put this right.
2. Conditions of cooling the fused substance are not correct. In general, excessive supercooling should be avoided. A defective wedge- or lens-crystal is cautiously heated so that some of the substance remains unfused and is then slowly cooled. This treatment often yields satisfactory crystals.

TABLE II. COLORS OF PLATINOCYANIDE CRYSTALS

$(NH_4)_2PtCy_4.H_2O$	Colorless with blue reflection
$(NH_4)_2PtCy_4.2H_2O$	Yellow with blue reflection
$BaPtCy_4.4H_2O$	Green and yellow dichroic
$MgPtCy_4.5H_2O$	Deep yellow
$MgPtCy_4.7H_2O$	Red with blue and green reflections
$K_2PtCy_4.3H_2O$	Yellow
$Y_2(PtCy_4)_3.21H_2O$	Red, yellow, green, and colorless pleochroic with blue and green reflections
Ag_2PtCy_4	Colorless
$HgPtCy_4$	Colorless

3. The fused substance on cooling forms a mass of small crystals of random orientation. Modifying the conditions of cooling should be tried, and if this fails the method for nonfusible substances should be tried.

DISPERSION OF BIREFRINGENCE OF NONFUSIBLE CRYSTALS. The method of computing the birefringence–wave-length graph described under the first method is readily applied to crystals which possess either a crystallographically or an artificially produced wedge-shaped edge. Such a crystal usually needs to be mounted in some medium having a refractive index reasonably close to that of the crystal in order that the ascending order of interference colors may be seen in the wedge-shaped edge. The mounting medium should be viscous and have as little solvent action on the crystal as possible.

The most generally useful mounting media for crystals of organic compounds are glycerol, glycerol jelly, and "Plasticizer E, 47.7% Cl", supplied by the Hooker Electrochemical Company. The last-named substance has a refractive index, n_D^{25}, of 1.5148. The birefringence spectrogram of the crystal is made to include the wedge-shaped edge, so that the orders of the interference bands given by the thick parallel part of the crystal are readily counted. It often happens that the crystals as grown do not have a wedge-shaped edge and have such strongly defined cleavage planes that they will not give a wedge-shaped fracture. Under such circumstances, a useful procedure is to attack one end of the crystal with a suitable solvent in order to round off the edges. After mounting in glycerol jelly or Plasticizer E, the rounded part of the crystal serves the same purpose as a wedge.

The Color of Crystals

An extensive study of colored crystals has been made by means of the microspectrograph. This work has shown that the spectral absorptions of a crystal depend on both its chemical composition and its crystallographic structure. As a result of this study, it is now proposed to classify the various possible causes of color as follows:

1. Rare-earth elements (Atomic Nos. 57 to 71) give line absorption spectra. Spedding (*7*) has shown that the grouping of lines given by crystals of a rare-earth compound is primarily dependent on the nature of the crystal symmetry. The author (*4*) found that neodymium and praseodymium sulfate octahydrates possess an extraordinary type of pleochroism, characterized by the fact that certain of the absorption bands disappear for specific ray and vibration directions. These directions are different for each absorption band and do not coincide with the axes of the optical ellipsoid.

2. Colored inorganic ions contribute to the color of the crystal. Thus, potassium ferricyanide crystals have a color characteristic of the ferricyanide ion, cupric sulfate pentahydrate crystals have the blue color of hydrated cupric ions, and so on.

When both the anion and cation are colored, their relative contributions may be different for each of the principal absorption spectra, so that the pleochroism of the crystal becomes complicated. In the case of complex cations, it is the arrangement of the coordinating groups, rather than the specific metal atom, which governs the color of the salts. Thus, crystals of the two compounds $[Co(NH_3)_5H_2O]Cl_3$ and $[Cr(NH_3)_5H_2O]Cl_3$ are almost indistinguishable, and both exhibit the same orange and pink dichroism. In the case of rare-earth compounds light is absorbed by electron transitions in the N shell of the rare-earth ion, which is screened by eight electrons of the O shell; hence, the influence of the anion on the absorption is not great.

However, with absorbing anions and cations, the influence of electric fields in the crystal is great, so that the nature of the other ion and of the crystal symmetry may greatly modify the pleochroism of the crystal.

3. The color of the crystal may be characteristic of the molecule as a whole. Under this heading are included inorganic complexes such as $2KNO_2.Cu(NO_2)_2.Pb(NO_2)_2$; metallic coordination compounds such as nickel dimethylglyoxime and the metallic phthalocyanines; organic compounds such as the nitrophenols, nitroanilines, azobenzene, etc. (Iodides, oxides, and sulfides of many metals have a very high absorption over most of the spectrum and may be looked upon as extreme cases of molecular absorption.)

4. Although basic and acidic organic dyes may be considered as colored ions, it is by no means certain that ions exist in crystals of the dye. Organic acids and bases which are colored in solution usually form colored crystals, which means that the absorption-producing mechanism of the dye molecule can contribute to the color of the crystal. The principal absorption spectra of a dye crystal, however, are often unrelated to the absorption spectrum of its solution.

5. Water of crystallization can modify the absorption of a crystal. Sometimes the effect is explainable on the assumption that the water coordinates with a colored ion to yield a complex ion having a different spectral absorption. This, for example, is the case with cobalt chloride; crystals of $CoCl_2$ are blue, and those of $CoCl_2.6H_2O$ are red, the hexahydrate formula probably being more accurately represented by $[Co(H_2O)_6]Cl_2$. This theory receives support from the fact that a saturated solution of cobalt chloride in water is red at room temperature, but becomes blue when the temperature is raised. Furthermore, solutions of $CoCl_2$ in anhydrous organic solvents are blue.

Such an explanation will not account for the extraordinary colors of crystals of platinocyanides which are colorless in solution. That the presence of water of crystallization is responsible in some way for the production of a light-absorbing mechanism is obvious from a study of Table II.

It will be noted that the two anhydrous platinocyanides, those of silver and mercury, are colorless. The ammonium salt with one molecule of water has a strong absorption in the near ultraviolet and has, in consequence, an abnormally high refractive index in the violet which, in turn, is responsible for the selective surface reflection of the extreme blue end of the spectrum. It appears to be a general rule that the greater the molecular proportion of water in a platinocyanide crystal, the lower the vibration frequency of the absorbed light, and it is interesting to note that both the red magnesium and yttrium salts have seven molecules of water to each platinocyanide ion.

The effect of water of crystallization in crystals of basic dyes is usually great, the hydrated crystal being different from the anhydrous crystal in crystal form, birefringence, and principal absorptions. Curiously enough, the color of the nonhydrated crystals often bears most resemblance to the color of the solution, which is an indication that water of crystallization in basic dyes may become part of a new absorption mechanism.

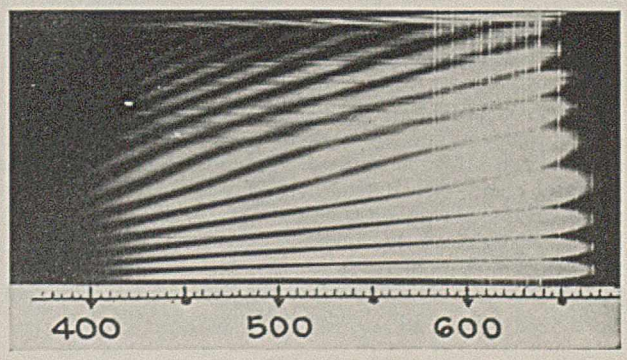

FIGURE 9. SPECTROGRAM OF *o*-CHLOROACETANILIDE

6. The degree and nature of the optical anisotropy of a crystal influence both the intensity and position of the absorption bands. The author has studied anhydrous crystals of 26 different salts of the basic dye 1,1′-diethyl-ψ-cyanine and has found that those of high birefringence have strong absorption and pleochroism, whereas those of low birefringence have not only weak pleochroism but also a very weak depth of color. Crystals of the iodide, 20μ thick, are very deep red in color, whereas a similar

thickness of the trithionate is only very faintly yellowish orange. The dithionate and tetrathionate are rather more birefringent and have slightly more color. The *p*-toluenesulfonate and *β*-naphthalenesulfonate come next in the list and are orange in color. All these crystals give the same magenta-colored solution in methanol. Solvent of crystallization (other than water, which has specific properties discussed above) may increase or decrease the anisotropy and depth of color of the crystal. Thus in the study of the 1,1′-diethyl-ψ-cyanine salts it was found that, whereas crystals of anhydrous chloride had nearly the same depth of color as the iodide, crystals of chloride containing phenol of crystallization had a much lower birefringence and were relatively weak brownish orange in color. It appears to be a general rule that a decrease in anisotropy is associated with a shift of the absorption band towards the shorter wave lengths.

These six factors often operate in combination, particularly with crystals of salts in which both anion and cation are dyes. For example, the chrysophenine salt of 1,1′-diethyl-ψ-cyanine forms yellowish-orange crystals having a relatively weak birefringence. The predominant color is, therefore, that of chrysophenine. A similar effect has been observed with crystals of picrates of basic dyes; the strong yellow of the picrate ion is not much affected by the cation, whereas the light-absorption of the dye cation may be greatly changed in wave length and intensity.

It is evident that considerable care must be exercised in examining unknown dyes and comparing their optical characteristics with those of known dyes, but the above considerations do at least indicate some general rules. Thus both known and unknown should be converted to the same salt, and both should be recrystallized three or four times from the same solvent. So far as basic dyes are concerned, the perchlorates are the most satisfactory salts on account of their low solubility, lack of tendency to take up solvent of crystallization, and freedom from color of the perchlorate ion. As an example, methylene blue perchlorate is precipitated when an excess of sodium perchlorate is added to an aqueous solution of methylene blue chloride. The precipitate is washed and then recrystallized from methanol. Very few technical acid dyes can be induced to form crystals suitable for microscopic examination, but some degree of purification can usually be obtained by precipitation as the free acid and re-solution in ammonia, repeating the cycle several times.

Crystals of dyes must be extremely thin for microscopic examination. The appearance of a colored crystal in ordinary light, with one nicol, and with crossed nicols, may vary very considerably with change of thickness, so it is necessary to study a range of thicknesses. Evaporation of thin films of very concentrated dye solution is one of the easiest ways of preparing crystals for study, suitable solvents for this method being pyridine and benzyl alcohol.

α-Bromonaphthalene and *α*-chloronaphthalene have useful solvent properties. At room temperature they are poor solvents, but at temperatures of 100° to 300° C. they become powerful ones. Consequently, it is possible to perform recrystallizations on microscope slide preparations by heating some finely ground dye under a cover slip with *α*-bromonaphthalene or *α*-chloronaphthalene and then allowing the preparation to cool very slowly. The high index of refraction of these solvents is of particular advantage, as most dyes have very high values of n_β and n_γ.

It has been found that there is only one certain way of studying the pleochroism of strongly absorbing crystals. This consists in confining the area of illumination to within the boundaries of the crystal. If the illuminated area is greater than the size of the crystal, the color is degraded in two ways; intersurface reflections in objective and eyepiece dilute the color of the crystal with white light, and light reflected backwards by the objective is reflected by the surface of the crystal to such an extent that the surface color is often the only one seen in the microscope. A striking example of this effect is shown by crystals of 1,1′-diethyl-ψ-cyanine perchlorate. Examined in the ordinary way, with the microscope slide illuminated with polarized light filling the entire field of view of the microscope, the crystals appear to be colorless and pale brown dichroic, but when the illumination is restricted to the crystal, the dichroism is colorless and very deep crimson.

The swing-out type of condenser, which is usually fitted to petrographic microscopes, is not well adapted for work on very small colored crystals, as it is difficult to illuminate a small enough area of the slide. The author now uses either an achromatic condenser or a 4-mm. achromatic objective (N. A. = 0.65) as a condenser, by means of which the image of a distant tungsten arc lamp or ribbon-filament lamp is focused on the crystal under observation. Double reflections from the microscope mirror have been overcome by having it aluminized. For visual work the dichroism of the crystal is studied by inserting the analyzer in the path of light, no polarizing prism being used. Neither the polarizer nor the analyzer is used for microspectrographic work; instead, the image of the crystal or its conoscopic image is projected on the slit of the microspectrograph, and the Wollaston prism is used to produce two adjacent spectra corresponding to the vibration planes of the crystal. In order to set the crystal so that its vibration planes agree with the vibration planes of the Wollaston prism, the microscope polarizer is temporarily inserted and rotated until it extinguishes one of the images given by the Wollaston prism, and the crystal is set at extinction. The polarizer is then withdrawn and the spectra are photographed.

It was mentioned above that strongly absorbing monoclinic and triclinic crystals may have strong dispersion of the axes of the optical ellipsoid. When this is the case it becomes impossible to make dichroism spectrograms which represent principal absorption spectra. The use of a sodium vapor lamp for the preliminary study of interference figures of colored crystals is strongly recommended, as this greatly simplifies the task of interpreting the usually highly complicated interference figures obtained with white light. Sodium illumination is also of considerable service in studying figures presented by the obtuse bisectrix and normal to the optic axial plane, as even thick crystals give clearly defined "hyperbolic" figures under these conditions.

Surface Color. Colored crystals which possess a strong absorption in some region of the spectrum often have a strong surface color in consequence. If the strong absorption is confined to one vibration direction of the crystal, the selectively reflected light is polarized, and consequently, trichroic crystals may reflect different colored light from different faces and exhibit a "reflection dichroism". The refractive index of an absorbing crystal is abnormally high on the red side of the absorption band and abnormally low on the blue side. In dichroic crystals these abnormalities in refractive index occur only for the ray which is absorbed, and the other ray does not show them. Consequently, the sign of the birefringence may be reversed twice in the visible spectrum. The color of the surface reflection is on the red side of the absorption band for crystals mounted in air, but on the blue side for crystals mounted in a medium of high refractive index, such as *α*-iodonaphthalene; consequently, it is necessary to mount crystals for comparison in the same mounting medium. The surface colors of many basic cyanine dyes have been examined with the microspectrograph. Well-formed faces of the crystals were illuminated with a cover-glass illuminator between the objective and crystal. Reflected light from the crystal was analyzed with the Wollaston prism. No sharp maxima or minima were observed.

The surface reflection of very small crystals is best studied by means of a prism vertical illuminator equipped with a 2-

mm. oil-immersion fluorite objective in a short (metallographic) mount. The objective must be specially selected for freedom from birefringence. An analyzer is used in the microscope for visual examination, and the stage is rotated in order to observe reflection dichroism. For microspectrographic work, the Wollaston prism serves as an analyzer. The vertical illuminator is used without a polarizer for this work. This particular aspect of the optics of strongly absorbing crystals is worthy of further study by the chemical microscopist, as the surface color of such crystals is independent of their thickness.

Acknowledgments

In conclusion the author wishes to thank H. D. Babcock for the gift of a speculum grating, J. L. Houghton and Max Wiedling for valuable assistance in the construction of the microspectrograph, Fred Lee for the gift of a specially worked sapphire disk, and L. G. S. Brooker and Frances M. Hamer for many splendid specimens of strongly absorbing crystals.

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An Electric Heating Mortar

For Use in Carbon and Hydrogen Microcombustions

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THE heating mortar usually employed in carbon and hydrogen microcombustion analyses has not proved entirely satisfactory, as this glass heating device containing boiling cymene with an air-cooled reflux condenser and gas microburner is both fragile and cumbersome. Another disadvantage is insufficient variability of temperature adjustment. This type of heating mortar has been improved upon by Schneider and Van Mater (*2*), who use electrical heating with thermostatic control. A second improved electrically heated and thermostatically controlled heating mortar, known as the "micro thermostatic sleeve", is now commercially available (*1*).

The present discussion has for its object the description of an electric heating mortar, thermostatically controlled, which is compact, simple in construction and operation, and at the same time provides constant temperature control over a comparatively wide range of temperatures. For the most part standard units of laboratory equipment are used. The construction of the remaining parts involves simple machine tool manipulations.

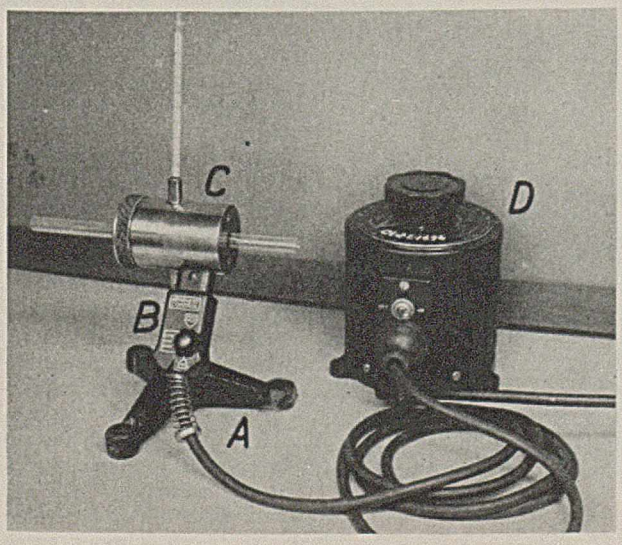

FIGURE 1. HEATING MORTAR

In Figure 1 the heating mortar with thermometer well, thermometer, and a section of standard combustion tubing is shown at *C*. The electrical thermostatic control switch ("Anyheat control", Silex Corporation, Hartford, Conn.) is shown at *B*. The voltage control (Variac Type 200-C, General Radio Corporation, Cambridge, Mass.), shown at *D*, may be replaced by a suitable lamp-bank voltage control. The adjustable support for the heating mortar is mounted on the tripod base, *A*. By its use the heating chamber may be raised or lowered.

The quarter-sectioned diagram (Figure 2) shows details of the construction of the heating mortar. The shaded portion with alternate solid and dotted lines indicates brass. The criss-cross shaded areas indicate Transite. The close-spaced diagonal lines indicate graphite. The circles with diagonals indicate Nichrome.

The barrel of the heating mortar is 8 cm. long with the screw cap shown at the left fully extended and is 48 mm. in outside diameter. The holes at each end and through the graphite disks are 11 mm. in diameter. The electrical contacts for attachment of the thermostatic control switch are 4.5 mm. in diameter and extend approximately 15 mm. from the body of the heating mortar, with a 1-cm. space between.

The casing of the heating mortar serves as one electrical contact, and the brass shoulder at the left of the Transite disk insulating ring at the exit end serves as the other electric contact. The side walls of the barrel of the heating mortar are insulated by use of a Transite tube as shown. The electrical circuit is thus through the casing (using the right-hand lug which is insulated from the casing) into the resistors and out through the right-hand "Anyheat control" lug.

(According to the assembly as described, if the electrical contacts of *B* are not checked a short circuit may result between the operator and a ground connection. This point should be checked, using a voltmeter, and reversing the contacts either at the power supply line or the thermostatic control switch from the transformer, so that no short circuit will be possible.)

The carbon disks (Figure 2) are 37.5 mm. in diameter and 1.5 mm. thick (Allen-Bradley Co., Milwaukee, Wis., Type E-2910 rheostat graphite disks). A sufficient number are slotted as shown, to provide for an opening below the thermometer tube. The Nichrome resistors are 37.5 mm. in diameter and are made from 3-mm. wire cut from a suitable wound helix.

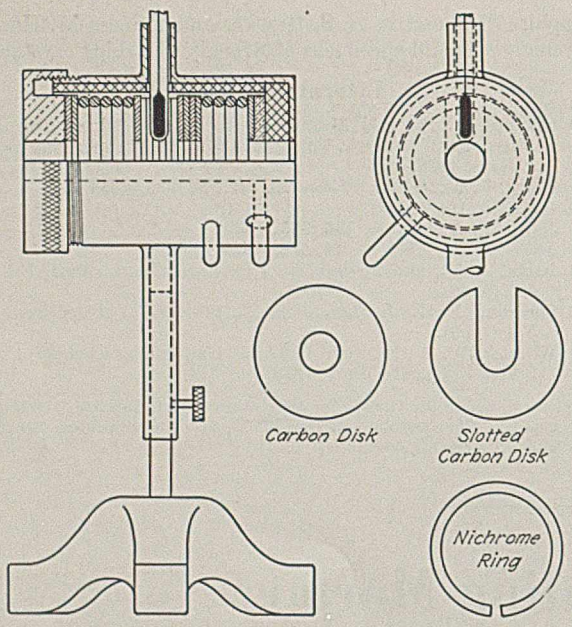

FIGURE 2. DETAILS OF CONSTRUCTION

The thermometer tube is 18 mm. long, 1 cm. in outside diameter, and of 6-mm. bore. The tripod upright is 55 mm. long, and of 6-mm. diameter to provide a slip joint with 55 mm. of the same tubing as that used for the insertion of the thermometer. The heating mortar is supported in contact with it by means of a setscrew as shown, for adjustments in height. All soldered connections are made with silver.

The carbon disk resistors in the middle portion of the mortar provide for the production of less heat energy than that from the Nichrome rings at either end, which brings about an equal heat distribution not attainable if all heating elements are either graphite or Nichrome. The resistance of the whole mortar is diminished or increased in proportion to the tension applied by adjustment of the screw cap. The "make" and "break" of *B* may be adjusted by altering the position of the adjusting knob to higher or lower temperatures. The Variac control is powered by the regular 110-volt laboratory power source. It is used over the voltage range 5 to 15 volts and supplies the third heat control.

The thermometer is an Anschütz type having a range of 150° to 220° C. Temperatures at the thermometer well in the middle of the heating mortar can be readily adjusted at any point between 150° and 220° C. and maintained constant within 1° to 2°. The variation from center to extreme ends can be kept below 5° temperature gradient.

The heating mortar described has been used for 6 months in the authors' microchemical laboratory at the University of Illinois with perfectly satisfactory results and its life span should be practically endless, without need for further adjustments. The temperature at which it is operated does not alter the carbon-to-Nichrome resistance contact in a measurable degree. The heating mortar has been operated continuously for 30 days with no change required in its three temperature-adjusting variables; during this period there was no change in temperature that could not be attributed to changes in room temperature.

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Volumetric Flasks for Microanalysis

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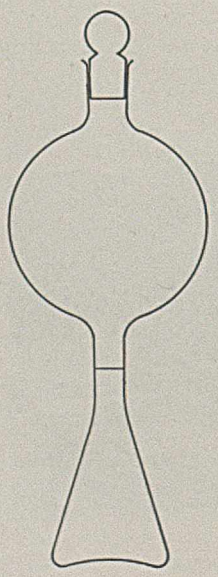
WHEN solutions are mixed in small volumetric flasks of the usual design, the error caused by the trapping of pure solvent or partly mixed solution in the ground glass at the stoppered end of the flask is proportionately much greater than that caused by the same source of error in large flasks.

Moreover, thorough mixing of solutions in conventional flasks of 5- or 10-ml. capacity is difficult to accomplish rapidly. Both this source of error and this lack of convenience in mixing are avoided by the use of flasks of the design shown in the figure.

In such a flask the upper part has a capacity about five times that of the calibrated part, so that after the solution is made up to the mark it may be passed into the upper part by tipping the flask on its side. Then by righting the flask and at the same time giving it a slight circular motion the liquid is caused to swirl as it passes back into the lower part. By repeating this about three times the solution is thoroughly mixed.

The sides of the calibrated lower part should slope more than in flasks of the usual design, in order that the liquid may be entirely, or almost entirely, transferred to the upper part without coming into contact with the upper neck. The neck of the calibrated part may be made slightly smaller in diameter than the neck of conventional flasks, with a corresponding gain in accuracy of measurement. For use with aqueous solutions and micropipets of the usual outside diameter, the internal diameter of the neck cannot, however, be less than about 8 mm. When these flasks are to be used with aqueous solutions there is no need for a ground-glass stopper, but with solutions containing volatile solvents this is, of course, almost necessary.



Though these flasks are considerably taller than conventional flasks of corresponding capacity, they may be completely emptied by micropipets of the bulbless capillary type. The chief disadvantage is the tendency to tip over easily. This disadvantage is of no significance if such a flask is habitually placed while in use in a suitable support such as a small iron ring or the opening in a wooden funnel rack.

Small flasks of this design may be blown without much difficulty from glass tubing.

MODERN LABORATORIES

New Science Building at Kansas State College

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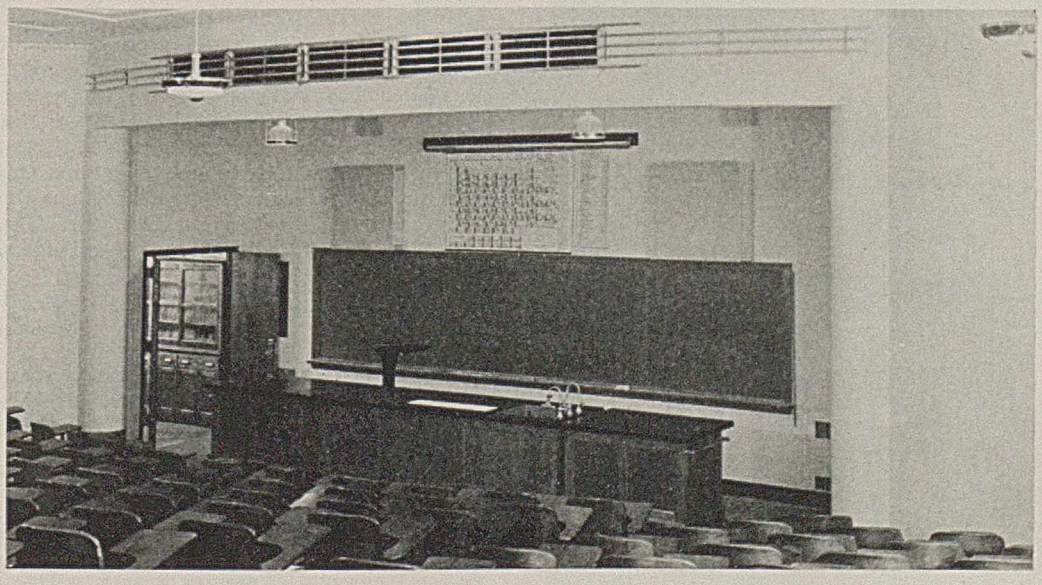
THE Chemistry Department of the Kansas State College of Agriculture and Applied Science is enjoying the use of a new Science Building, after having occupied very cramped quarters since the old building burned in July, 1934. The new building has been named Willard Hall in honor of J. T. Willard, who served as head of the department for many years.

Willard Hall is built of native limestone and trimmed with Bedford limestone. The building is 305 feet long by 65 feet wide with wings measuring 92 by 66 feet placed at each end, giving it the shape of a large E with the center bar missing. The interior is of fireproof steel and reinforced concrete construction; only the doors and furniture are of wood. The floors are of concrete except for the classrooms and offices in which a wood floor has been laid on top of the concrete base. A WPA grant was obtained to aid in the construction and equipment of the building, the total cost of which was about $725,000.

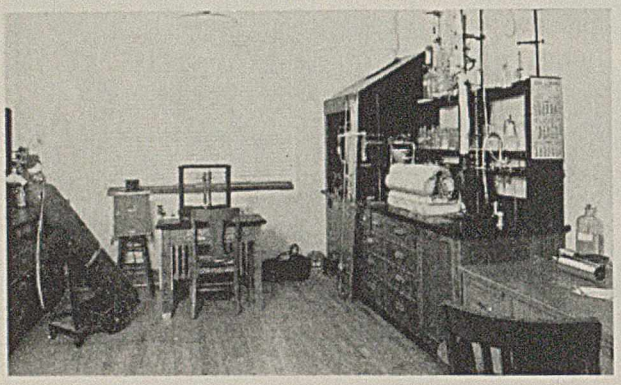
The four floors were carefully planned to make maximum use of the floor space available. An 11-foot corridor runs lengthwise of each floor, dividing it into a 25-foot section toward the front of the building which is used for student work and an 18-foot section toward the rear, in which are placed the remainder of the student laboratories, the service rooms, and the offices and research laboratories of the teaching staff. Stairways are located at each end of the building and in the center rear. Short connecting corridors lead from the center corridor of the main or second floor to the main entrance at the center of the building and to flanking front entrances located toward each end. Other entrances at ground level are located at each end of the building and opposite the main entrance at the rear. The intersection of corridors at the main entrance has been made into a small rotunda, on the floor of which is a mosaic made up by working brass inlays of some of the more important alchemical symbols into a terrazzo background. Large glass-front display cases are recessed into each wall of the short corridor leading to the main entrance.

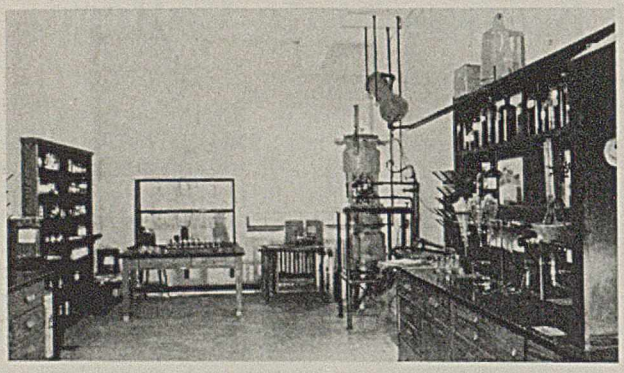
The Department of Chemistry occupies about two thirds of all four floors, while the remainder of the building is given over to the Physics Department.

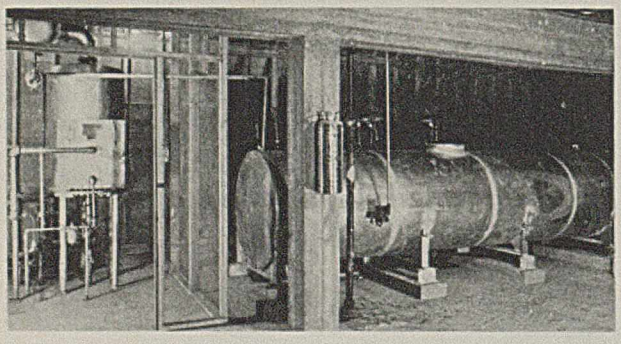


CHEMISTRY LECTURE ROOM, SHOWING LIGHTING SYSTEM AND GRILL FOR VENTILATING SYSTEM OVER LECTURE TABLE

Preparation room is shown through door on left. Right end of lecture table has been detached; left end is of same construction, both ends may be rolled into preparation rooms. White strip on table is illuminated panel. Loud speakers for sound motion pictures are on both sides of periodic chart.







Chemistry Department

First Floor. The first floor houses the laboratories of the Agricultural Experiment Station. In this is included research on starch and other chemurgic problems, soils, animal nutrition, meat, dairy, and poultry, each occupying separate rooms designed and equipped to meet special needs. A suite of two large laboratories, weighing room, and sample grinding and storage room is set aside for the analytical laboratories of the Experiment Station. Here also is done analytical work for the State Board of Agriculture. In another laboratory the analytical work for the State Board of Health is done. A special laboratory which has no gas connections—all heating is done with steam or electricity—is available for work with and the recovery of inflammable solvents. A small animal room; a well-equipped shop with facilities for glass, metal, or wood working; the electrical control panel with its accompanying bank of storage batteries and motor-generator equipment; the compressor and vacuum pumps; the receiving room; and a washroom with shower facilities are all on this floor.

One of the most interesting features is a battery of five constant-temperature and constant-humidity rooms. Each room is separately controlled and the temperature range available is from −30° to 50° C. Any relative humidity between 10 per cent and saturation can be maintained. Another constant-temperature room is connected to the laboratory for soil research. Four laboratories housing special research equipment for physical chemistry, and the Experiment Station Office, with a fireproof vault for the storage of records, complete the facilities on this floor.

Included in the special equipment are several large centrifuges, a supercentrifuge, high-pressure hydrogenation equipment, two large Carver presses, a Kjeldahl digestion rack of 60-flask capacity and a distillation rack capable of handling 36 flasks, a vacuum oven, a large thermostat, a liquid air machine, complete x-ray equipment, a large spectrograph with both glass and quartz prisms and capable of giving a 30-inch spectrum, complete equipment for spectrographic and spectrophotometric work, a photoelectric colorimeter, and a fluorophotometer for absorption in the ultraviolet region. An office desk, desk chair, and filing case have been placed in each research laboratory.

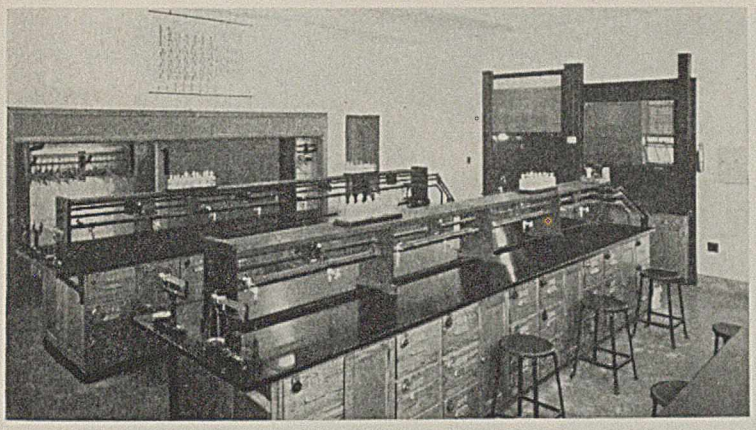
Second Floor. The department offices, located on the second or main floor, consist of a suite of four rooms made up of the business office, with another fireproof vault for records; the general office; and a private office and research laboratory for the head of the department. A two-way buzzer system connects the general office with all the offices for the staff and with the laboratories, both student and research. Communication is further aided by a telephone (intercommunicating and outside) located on each floor. The department library is across the hall from the general offices.

Top. Typical Office-Research Laboratory for Teaching Staff. Left Half of Room Is Similar to Section Shown in Photograph

Second from Top. Laboratory of the Agricultural Experiment Station, Used as an Extraction Room for Work with Volatile Solvents

Third from Top. Water Still and 1000-Gallon Pure Aluminum Storage Tank in Attic. By Using Pure Aluminum in Tank and Distributing Pipes, with Aluminum and Silver Faucets, a Plentiful Supply of Distilled Water with a Conductivity of 10^{-6} Reciprocal Ohm Is Available throughout the Building

Lower. Typical Desk Arrangement in Quantitative Laboratory, Showing Balance Room at Right



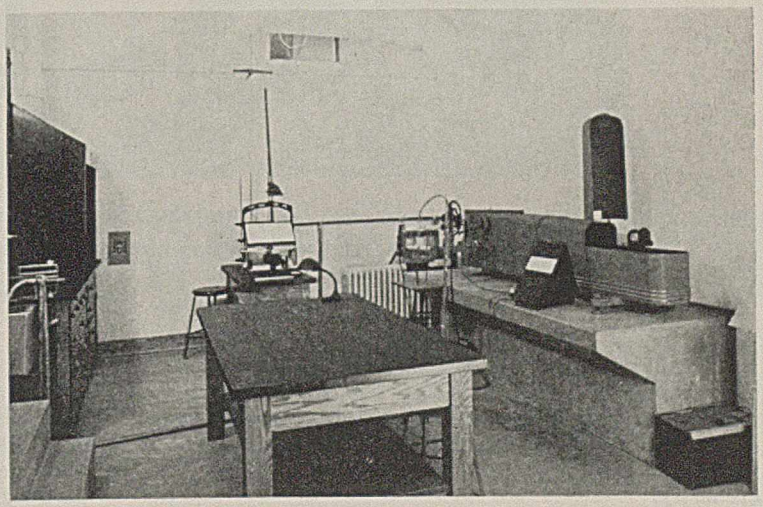
TYPICAL FRESHMAN LABORATORY, SHOWING COMBINATION BLACKBOARD AND CLOTHES LOCKER. EACH STUDENT IS ASSIGNED A DRAWER. CUPBOARD HOLDS EQUIPMENT USED IN COMMON BY SIX SECTIONS

The lecture room occupies the entire wing, with 233 seats arranged in amphitheater fashion. It has indirect lighting and a separate ventilating system. A long, thoroughly modern lecture table occupies the front of this room. The end sections of this table may be detached and rolled into the preparation rooms at each side. These lecture preparation rooms are completely equipped and each may be reached by a spiral stair from the first floor. The lecture table is equipped with hot, cold, and distilled water, gas, air, vacuum, 220-volt alternating current, and variable direct current. The large sink may also be used as a pneumatic trough, and a display plate, made by mounting a ground-glass plate in the top of the table and illuminating it from below, is an aid in making the details of lecture demonstrations plainly visible. A small but powerful table hood removes obnoxious gases. The lighting system of the room, as well as the mechanical shades covering the windows, may be controlled from the lecture table. A 70-slide automatic Baloptican (also controlled from the lecture table) and a motion picture machine for both sound and silent pictures are available for visual education. The sound equipment is so arranged that the lecturer may, if he chooses, operate either the motion picture machine or the projector from the back of the room; yet, by speaking into a small microphone, his voice will reach his hearers from the front of the room.

The facilities for quantitative analysis include a laboratory capable of accommodating two sections of 40 students each. Attached to this laboratory but separated from it by glass partitions are a balance room and a sample preparation room. The advanced inorganic preparations laboratory may be used by two sections of 24 students each, while an electrochemical laboratory with 16 desks and a graduate research laboratory with 32 desks complete the arrangement for student laboratory work on this floor. Here also are a classroom seating 40, and two offices and research laboratories for staff members.

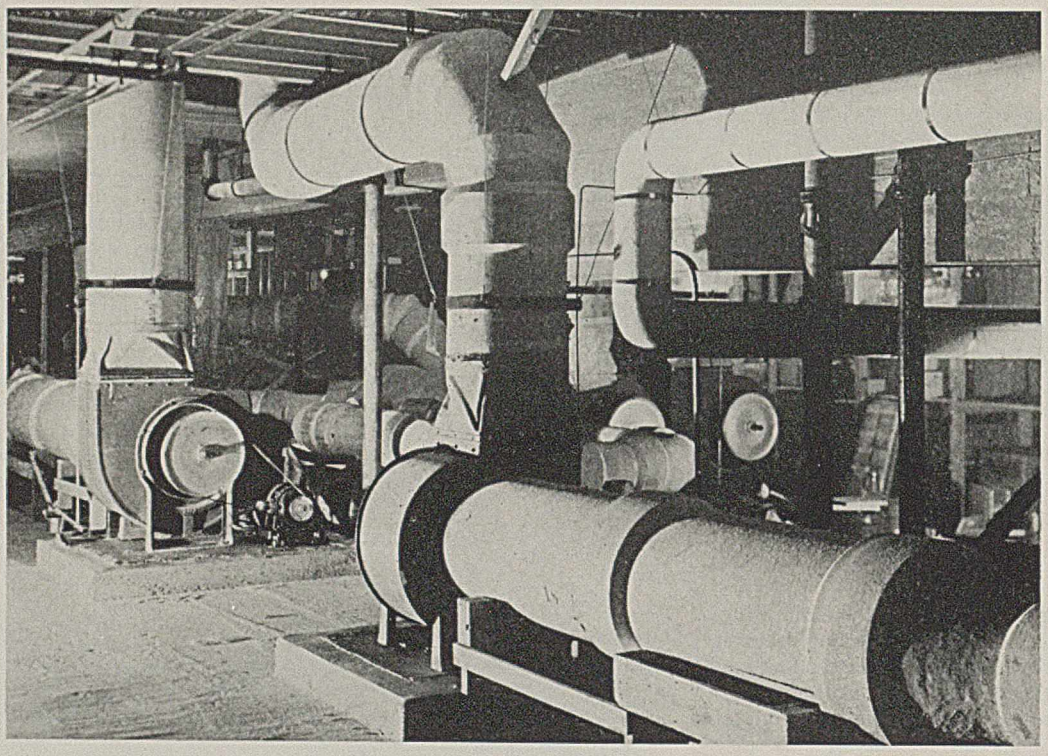
THIRD FLOOR. On the third floor are laboratories for organic chemistry. Two laboratories, used by the elementary courses, will accommodate 416 students working in four sections. A third laboratory, for students majoring in chemistry, can be used by three sections or a total of 111 students. The physiological chemistry laboratory will also accommodate 111 students in three sections. A small laboratory with desks for eight students may be used for organic analysis and organic preparations.

The physical chemistry laboratory will accommodate two sections of 24 students each. Adjoining it are a special supply room and a smaller laboratory in which the more elaborate pieces of apparatus may be set up.



TOP. ONE OF THE RESEARCH LABORATORIES FOR PHYSICAL CHEMISTRY, SHOWING SPECTROGRAPH PLACED ON CONCRETE PIER

BOTTOM. ONE OF THE ORGANIC LABORATORIES. BECAUSE THIS LABORATORY IS LOCATED OVER THE LECTURE ROOM, THE SERVICES ARE DELIVERED TO DESKS FROM CEILING MAINS. IN OTHER LABORATORIES, THE SERVICES COME FROM BELOW



VENTILATING SYSTEM IN ATTIC

In the background, behind network of pipes, is one of the cages for storage of chemicals and equipment.

A classroom seating 40 persons completes the facilities for student work on this floor. Three combination offices and research laboratories and two offices with research laboratories attached are assigned to the nine staff members who work principally with organic, physiological, or physical chemistry.

FOURTH FLOOR. On the fourth floor are five inorganic chemistry laboratories which will accommodate a total of 1308 students working in six sections. Three combination offices and research laboratories and one office with research laboratory attached are used by the nine staff members whose work is with the freshman chemistry.

The laboratory desks are built of oak and were designed to meet the requirements of the classes or individual using them. The desk tops are of a new cement-asbestos composition and are almost impervious to ordinary laboratory reagents. An open drain lined with heavy lead sheeting runs through the middle of each table and empties into a Knight-ware sink at the end. Distilled water is delivered through aluminum pipes to a faucet at the end of each laboratory table. A water-steam mixing bulb is placed over each sink, so that either hot or cold water is always available. Each working place is provided with outlets for water, gas, compressed air, and vacuum. In the organic and physiological laboratories a steam cone is placed at each working place. The laboratories used by upperclassmen have available also a 220-volt alternating current outlet and a variable direct current outlet. Reagent shelves are placed conveniently along the walls of the laboratories. In the freshman laboratories two triple-beam balances with 111-gram capacity are placed at the end of each table opposite the sink. In the other laboratories a triple-beam balance with 1100-gram capacity is mounted on each reagent shelf. Each student laboratory has a large clothes locker occupying one whole end of the room. Blackboards are mounted on the doors of this locker space.

The variable direct current originates with a bank of storage batteries on the first floor, and the necessary motor-generator set is available to keep these batteries charged. From a master control panel board the current selected, which may be anything from 2 to 110 volts, is distributed to subpanel boards located on each floor and from there to the individual laboratories. In the larger student laboratories there are at least two circuits available, so that two widely different experiments may be in progress at the same time.

The laboratory hoods are arranged in vertical banks throughout the four floors, each with its own motor and exhaust fan in the attic. These fans continue to function as long as any hood of a bank is turned on. The individual units are controlled by a system of dampers operated by the motor switch; hence, the exhaust fan is effective only on those hoods which are turned on. In the large student laboratories are placed Univent ventilators which operate simultaneously with the hood fans and in this manner compensate for the air removed through the hoods. Each hood is provided with gas cocks, an electrical power outlet, water, and drain. The larger hoods have electric lights installed toward the top of the hood.

The main storeroom for the department is in the attic. All incoming supplies are unloaded into a receiving room on the first floor, where they are unpacked and entered on stock records. The distribution may be to the service rooms, one on each floor, or to the attic for storage. A freight elevator connects this system of storerooms. The attic also houses a 50-gallon-per-hour Barnstead water still and a 1000-gallon aluminum storage tank for distilled water. The hydrogen sulfide for the qualitative analysis laboratories is bought in commercial cylinders. The pressure is regulated by an oil-filled gasometer, placed in the attic, and from there the dry gas passes through cast-iron pipes to jets located in the laboratory hoods. All volatile and inflammable solvents are stored separately in an underground vault which is reached directly from the receiving room.

Rapid Pretesting for Heavy Metals

REAGENT — Diphenylthiocarbazone

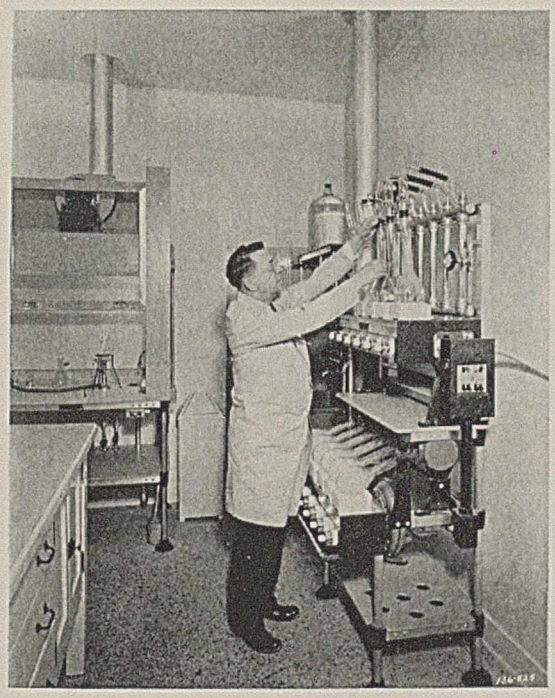
METHOD — Colorimetric

REFERENCE — Fischer and Leopoldi, *Chem. Ztg., 64,* 231 (1940)

PRELIMINARY INVESTIGATION of a solution to determine whether it contains any of the heavy metals may be quickly carried out by the use of diphenylthiocarbazone. Characteristic color changes, depending upon the conditions of the test, indicate the metals that are present.

The sensitivity of the test is within a few hundredths of a milligram. Time-consuming filtrations and re-solution of precipitates are obviated. The method is especially valuable for testing for metals previous to quantitative analysis. The reagent is available as a stock compound, as *Eastman 3092 Diphenylthiocarbazone—10 grams, $2.50.*

Write for an abstract of the article in which rapid pretesting for the heavy metals, with diphenylthiocarbazone, is described. . . . Eastman Kodak Company, Chemical Sales Division, Rochester, N. Y.



(Above) Kjeldahl Nitrogen Apparatus, 6 flask capacity combination unit, distillation decked over digestion and with electric 3 heat control. Shown also (at left) individual section fume hood.

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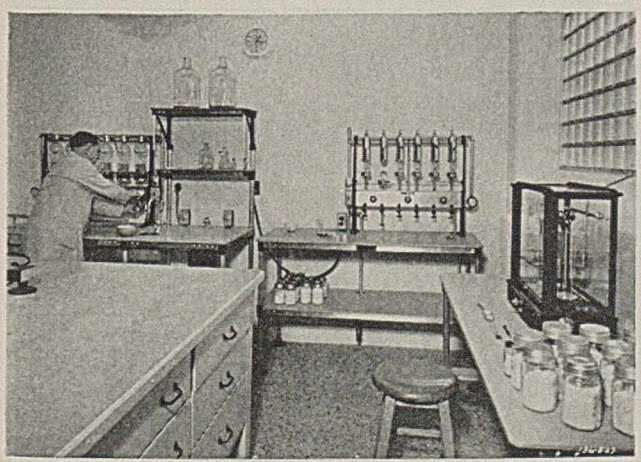
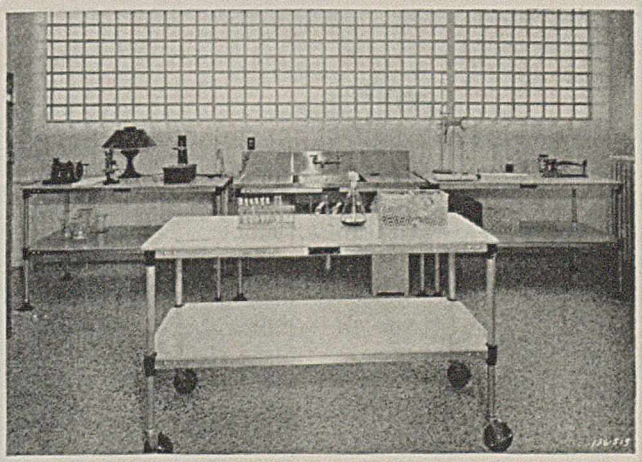


(Above) Reflux condensers mounted on special table for crude fiber determinations. Shown at right in above photo—Goldfisch Fat Extractor for rapid, accurate ether extractions.



(Above) Bacteriology Laboratory. Stainless steel sink and 3 utility work tables, all steel, sturdy, and with tops of highly compressed laminated asbestos.

CATALOG and COMPLETE INFORMATION ON REQUEST—Write now for your personal copy of catalog illustrating and giving important facts concerning Kjeldahl Nitrogen Apparatus and Associated Apparatus and Laboratory Equipment.

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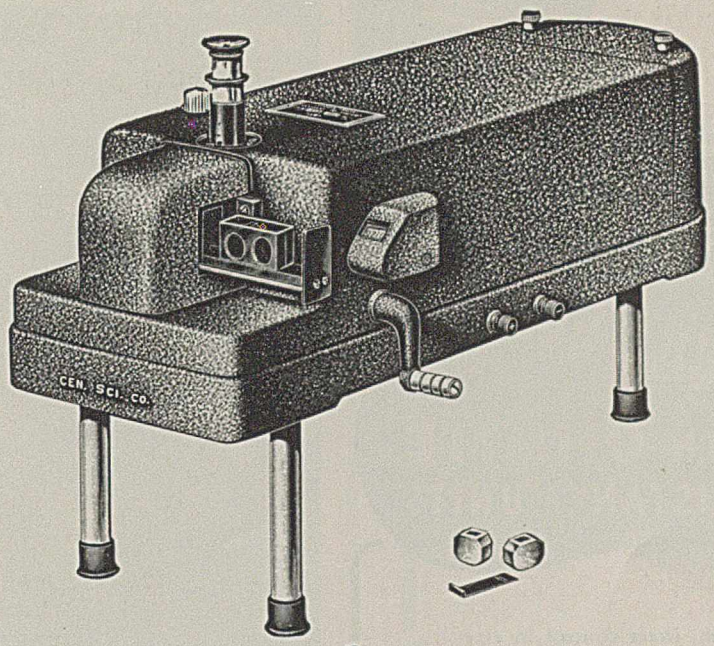

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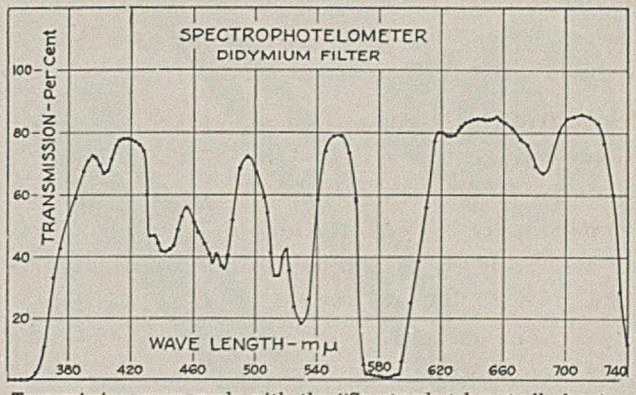
Complete with two slit plates for 2.5, 5, 10, and 20 mμ bands, two glass light filters and two absorption cells, but without illuminator, bench, transformer, condensing lens or galvanometer.....Each $340.00



Transmission curve made with the "Spectrophotelometer" showing high resolving power

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