Vol. 17, No. 6 ANALYTICAL EDITION June, 1945

INDUSTRIAL AND ENGINEERING CHEMISTRY

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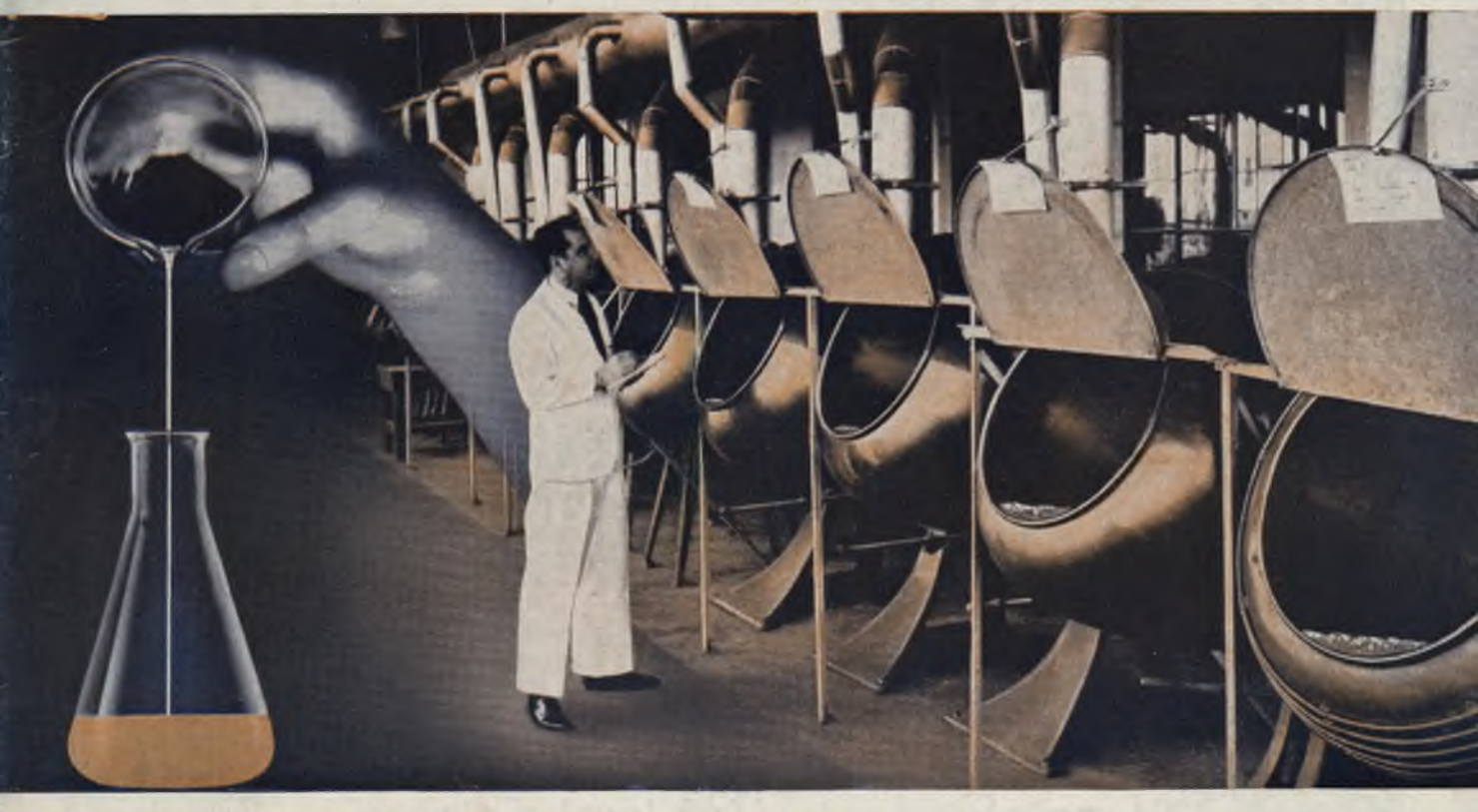

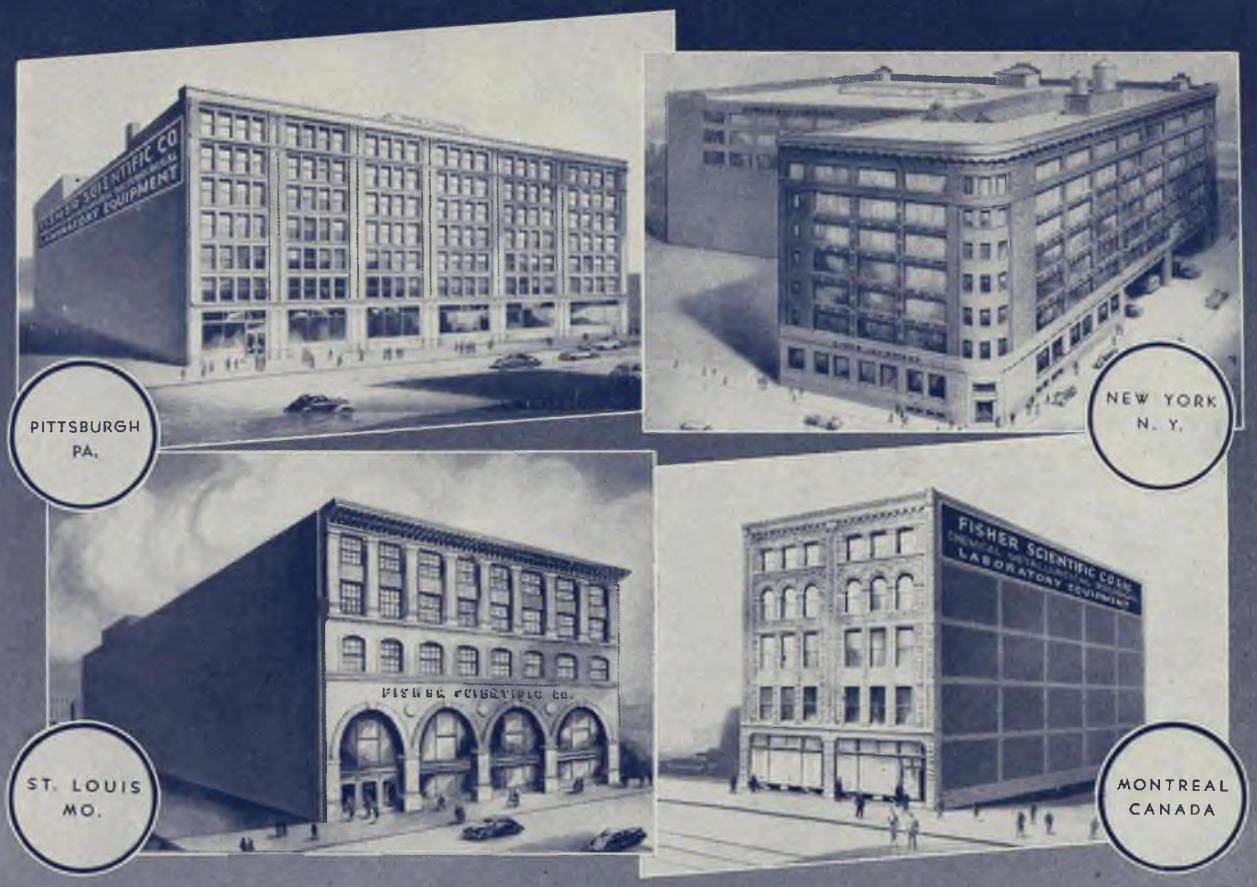

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INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

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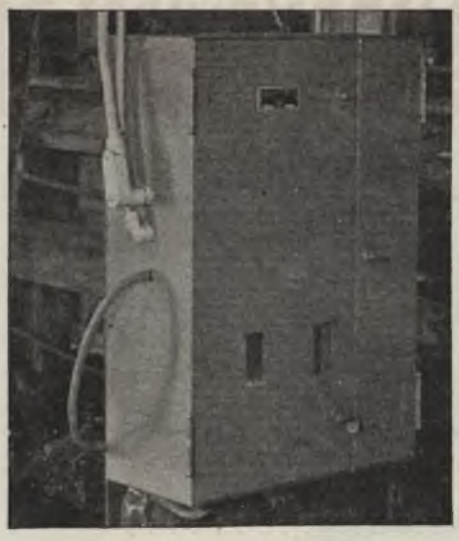

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P. 374 / 59

a Letter to an Advertising Agency*

POROCEL CORPORATION
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N. W. Ayer & Son, Inc.
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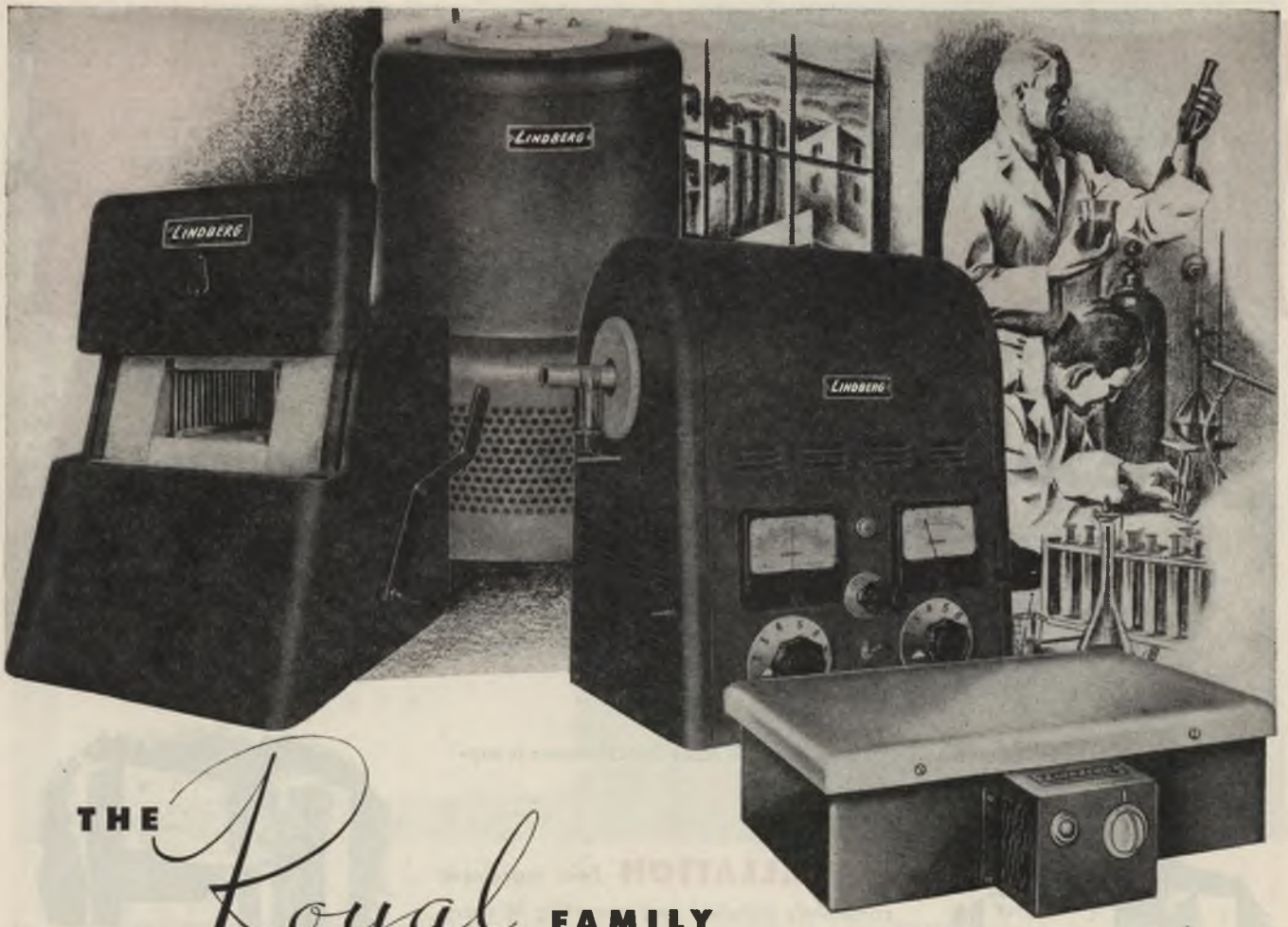
Very truly yours,

C. D. Laughlin

Porocel Corporation

cdl/jc

*This letter was written by an engineer—not an advertising man. It was intended simply to supply information for a Porocel advertisement on this page this month. But the letter was so clear and concise an outline of Porocel's properties that the copywriter said to himself, "Why gild the lily? Why not just run this letter as written and received?" . . . so here it is.



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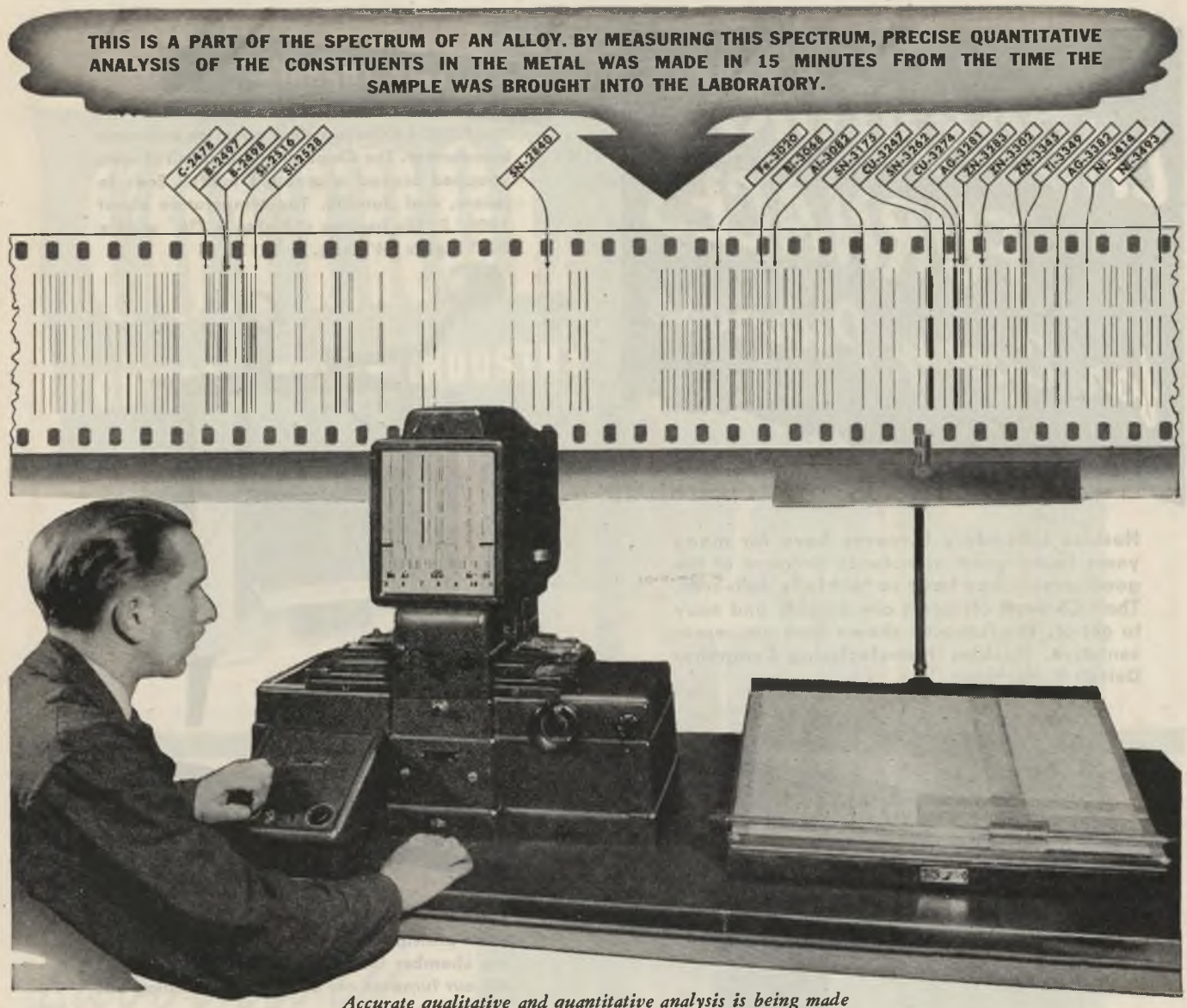

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Spectrographic Analysis of Magnesium Alloys

B. L. AVERBACH[1]
U. S. Radiator Corporation, Geneva, N. Y.

This study describes the spectrography of a magnesium-base alloy containing 6% aluminum, 3% zinc, and 0.20% manganese. A statistical analysis of the procedure indicated that an accuracy of at least ±5% of the contained element was reasonable for this material. In addition, methods of casting a representative sample free from microshrinkage were investigated.

THE spectrographic process is capable of accurately analyzing constituents of fairly high percentage if sufficiently rigid conditions are imposed on the process and on the sampling procedure. This paper describes a procedure for the rapid analysis of a magnesium-base alloy with a nominal composition of 6% aluminum, 3% zinc, and 0.20% manganese. During the establishment of this procedure a study was made of the reproducibility of the process, and of the consistency of the sample itself. As experience with these analyses was gained it was realized that extremely close control was necessary to obtain consistent results.

The spectrographic equipment consisted of a spark generator of the rotary spark type, a high-voltage alternating current arc source, a large grating spectrograph, darkroom equipment for the rapid processing of 35-mm. film, and a photoelectric comparator-densitometer to measure the location and density of the spectral lines. All this equipment was manufactured by the Applied Research Laboratories and the Harry W. Dietert Company.

A flat sand-cast disk was used on a Petrey spark stand. Different types of specimens were found to be subject to extreme variations in the zinc and aluminum contents, and for this reason methods of casting a suitable spectrograph specimen were also investigated.

EQUIPMENT

The spectrograph was a standard ARL-Dietert grating instrument with a dispersion of approximately 7 Å. per mm. in the first order. Thirty-five millimeter Eastman Spectrum Analysis I film was used and was processed in the following manner:

1. Three minutes in D-19 developer at 70° F.
2. Five seconds in acetic acid short stop
3. Three minutes in Eastman liquid x-ray fixer
4. Five minutes in running water
5. Rinse in distilled water
6. Wipe with cellulose sponge
7. One minute in infrared dryer

For excitation, the spark unit was used almost exclusively. Some effects of varying the secondary inductance are described below, but all work was done with the inductance at its lowest possible value, approximately 0.045 mh. There are indications that even lower inductances may be desirable.

The comparator-densitometer was of the type which employs a motor-driven scanning slit 12 microns wide and 1.1 mm. long. This instrument was set so that the transmission reading on clear film was 100. At a density of 1.0, therefore, the transmission reading was 10, and at a density of 2.0 the reading was 1.0. For accurate results, the transmission readings were confined between 80 and 15, so that densities greater than 1.0 could not be used. No background corrections were made.

WORKING CURVES

The preparation of emulsion calibration and working curves has been adequately described (*3*, *4*). For the emulsion calibration, a ground quartz diffusing plate 1 mm. thick was placed in front of the spark to give better uniformity along the length of the line, but this plate was not used during the actual analyses.

The standards which were most suitable for this application were the flat slabs supplied by the Aluminum Company of America. In several instances the standards of the Dow Chemical Company were also used. These standards were in approximately the same metallurgical condition as the samples and were handled in the same fashion. The range of chemical compositions of the various constituents in the standards covered ranges which were similar to the range in the samples, so that errors due to the effects of one element on the emission characteristics of another were minimized. Frequent wet chemical checks were also made. Both standards and samples were sparked under the following conditions:

Power	4/3 kva.
Primary voltage	70 volts
Capacitance	0.014 mfd.
Inductance	0.045 mh.
Prespark	10 seconds
Exposure time	25 to 35 seconds
Slit	60 microns × 2.6 mm.

In the preparation of all magnesium samples and standards the same procedure was used. A 0.16-cm. (0.06-inch) cut was taken from one surface with a lathe, and this surface was sanded with a No. 120 Aloxite belt. This surface was used against a spectrographically pure carbon rod in the Petrey stand, and the conditions mentioned above were maintained.

In the choice of the analysis lines several conditions must be considered:

1. There should be no interfering lines of other elements present. For best results the background should also be low.
2. This line should have a suitable density for the concentration range in which it is used. For maximum accuracy the ratio of the densities of the analysis line and the reference line should be near unity.
3. If possible, the reference and analysis lines should be a homologous pair—that is, they should react similarly to slight changes in excitation conditions. The state of ionization is a valuable guide, but not always a completely reliable criterion of a homologous pair. After a set of lines has been used for some time, this condition may be determined from experience.
4. The standard and the analysis lines should be as close to each other as possible because of the change in the film gamma with wave length.

In practice it was virtually impossible to obtain all these conditions for every line. Since it was desirable to make the entire analysis at one exposure, the lines were split horizontally with a

[1] Present address, Works Laboratory, General Electric Co., Schenectady, N. Y.

rotating sector at the secondary focus. The top half was exposed to four times as much light as the bottom half. With this arrangement aluminum, zinc, magnesium, and manganese lines were read on the bottom half, while iron, silicon, copper, and nickel were read on the upper half.

For each exposure the following lines were read:

	Wave Length, Å.	Mg Reference	Remarks
Al	3587	3330	Not homologous
Zn	3302	3330	Homologous
Mn	2610	3074	Homologous
Si	2516	3074	Homologous
Fe	2382	3074	Not homologous
Cu	3274	3074	Homologous

Unfortunately nickel could not be determined in this exposure, and a separate determination under the following conditions was taken:

Power	4/3 kva.
Prespark	10 seconds
Exposure	50 seconds
Inductance	0.365 mh.

The additional exposure and inductance seemed to sensitize the nickel line, so that the $\frac{\text{Ni } 3415}{\text{Mg } 3074}$ pair was suitable for nickel contents as low as 0.001%. None of the samples contained a detectable amount of nickel, but since the specifications allow only 0.01% nickel, it was necessary to check each heat for its presence.

In some instances plain glass filters made from microscope cover glasses were tried directly in front of the film to reduce the intensity of a particular line. These filters were very satisfactory, since they allowed the exposure condition to be adjusted so that the ratios of the densities of the analysis and reference lines were close to unity. The filter did not affect the shape or slope of the working curves, but for the above procedure no filters were used.

Figure 1 shows some of the working curves for aluminum, zinc, and manganese. Similar curves were used for silicon, copper, iron, and nickel.

The usability of these curves is dependent to a large extent on the slope. If the curve is too steep it is impossible to determine the element concentration with any degree of accuracy, and if it is excessively shallow it is difficult to cover the permissible range with one curve. The accuracy of the aluminum determination, shown in Figure 1, is particularly sensitive to the slope because of the large percentage of contained aluminum. At 3587 Å. the line is broad and diffuse and is actually composed of a number of lines. Experience showed, however, that it followed the aluminum content faithfully and seemed to have the shallowest slope of the available aluminum lines.

Theoretically these curves should be good indefinitely, but they must be frequently checked against standards. For small variations from the curve, small corrections may be made, but if the correction is greater than 5% of the contained element, the entire curve should be redetermined. The exact causes for some of these shifts have not been satisfactorily explained. It is very seldom that the entire set of elements will shift at the same time. In this particular system zinc and aluminum seemed to be the worst offenders, but even then they seldom shifted together.

It is well known that the primary input voltage should be carefully controlled for the maximum accuracy. For the best results a large motor generator set should be used. Unfortunately the 225-volt input to this installation came directly from the line, and this may have been a contributing factor to the curve shifting. When, however, the input voltage was deliberately varied from 200 to 240 it was impossible to reproduce these shifts consistently.

Changes in humidity are often blamed for these variations, and for the best results a constant-temperature, constant-humidity atmosphere should be maintained. Changes in exposure time were necessary to follow the changes in the seasons. A daily record was kept of the humidity, and it was evident that as the relative humidity increased, the exposure time increased.

An ultraviolet lamp was tried at the rotating spark gap. Previous reports had indicated that irradiation of the rotating spark gap should reduce the errors due to variations in excitation. The lamp which was installed was one of very small output supplied by the manufacturer. No difference in reproducibility was discernible with the lamp in or out, and the consistency studies shown later were made without the lamp. With the rotary gap irradiated, exposures had to be increased by about 5 seconds. These limited experiments by no means exclude the possibilities of improving the reproducibility by irradiating the rotating spark gap, since the original experiments were made with a much more powerful ultraviolet lamp (about 250 watts).

In addition to these uncontrollable variables, there was a set of variables which could be controlled. The 4/3 kva. power tap was chosen to give readable line densities with a reasonable exposure time. At the rotating spark gap a voltage of 80 volts was first used. Later trials, however, indicated that 70 volts

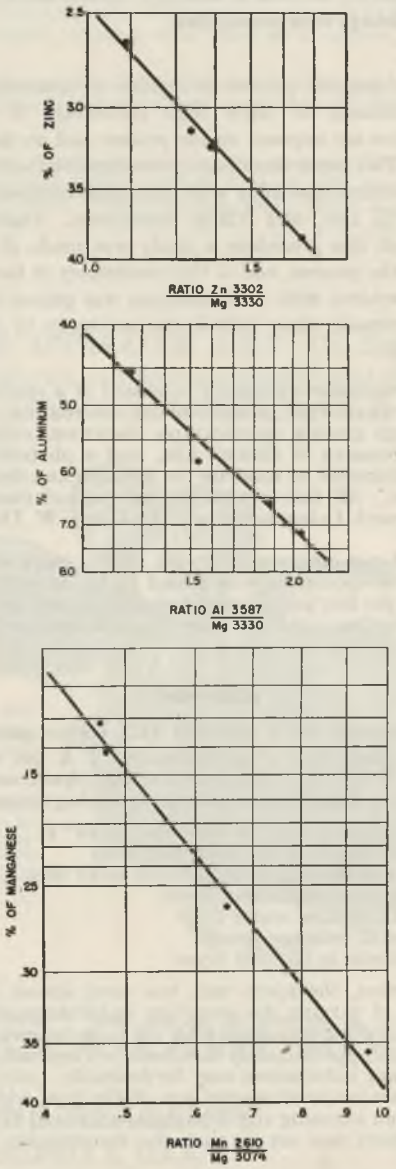

Figure 1. Working Curves for Aluminum, Manganese, and Zinc

Table I. Effect of Inductance on Line Densities and Intensity Ratios

Line		Transmission Readings, Inductance, Millihenrys						
		0.045	0.090	0.180	0.365	0.730	1.095	1.46
Fe 2382	T	66.0	67.0	58.5	73.0	92.5	...	...
	R	0.74	0.61	0.55	0.39	0.35	...	...
Si 2516	T	78.0	72.0	48.0	39.0	64.5	88.0	...
	R	0.58	0.56	0.65	0.68	0.73	0.73	
Mn 2610	T	80.0	79.0	77.0	86.0	96.0	...	...
	R	0.55	0.48	0.40	0.29	0.26	...	...
Mg 3074	T	46.0	36.0	22.5	19.0	44.0	77.0	92.0
Cu 3274	T	58.0	55.0	29.0	22.0	35.0	64.0	78.0
	R	0.83	0.75	0.88	0.95	1.15	1.29	1.61
Zn 3302	T	24.5	11.0	3.5	1.2	2.8	6.9	18.0
	R	1.39	1.69	1.60	1.44	1.51	1.52	1.57
Mg 3330	T	44.0	32.0	10.0	2.9	7.0	19.5	41.0
Al 3587	T	16.5	46.0	70.0	90.0	...	...	...
	R	1.68	0.77	0.31	0.08	...	...	...

T. Average of three transmission readings.
R. Intensity ratio, Zn, Al with 3330; others with 3074. Power 4/3 kva. Line voltage 235 volts; input voltage 70 volts. Aperture 2, Sector 4 to 1, slit 60 microns, prespark 10 seconds, exposure 50 seconds. Inductance was the only thing changed during the experiment.

provided more uniform results, partly because the lower voltage could be maintained at a more constant level.

The spacing between the tungsten points of the rotor and the stator of the rotating gap was very critical. A spacing of 0.25 mm. (0.010 inch) was maintained, and the points were turned and spaced frequently to ensure their accurate alignment. For the best results this adjustment should be made after a number of exposures, so that the entire system is somewhat near its operating temperature. Starting from cold, the same time of exposure produced stronger and stronger lines until the operating temperature was reached. Bringing the points too close together reduced the voltage markedly, and if the back and front gaps were not equally spaced erratic results were produced. Too much emphasis cannot be placed on maintaining the proper spacing at the rotary gap.

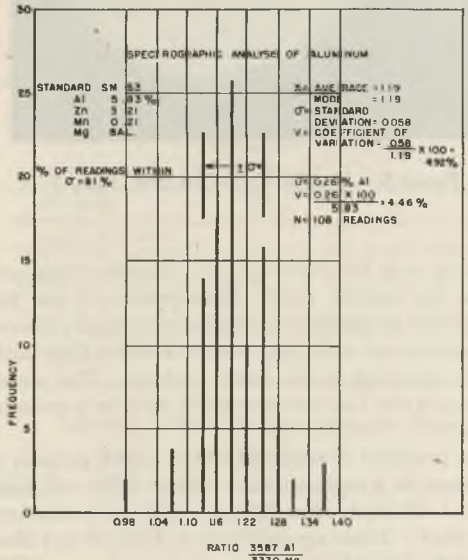

Figure 2. Frequency Distribution Chart for Aluminum

To prevent sparking between the specimen and the stage it was necessary to keep the stage clean with fine emery paper and to place a weight on the disk while it was being sparked. It was also necessary to keep the stage clean at the points where it contacted the carbon rod, and to keep a high pressure on the spring clip holding the carbon rod against the stage. If this was not done, discharges were apt to occur at other places in the circuit.

Attempts to use the high-voltage alternating current arc showed that this method was not so consistent as the spark process for the high percentage elements. For minor constituents such as iron, copper, silicon, and nickel it was suitable, but there was a saving in time by making the complete analysis at one exposure with the spark unit.

Changing the secondary inductance had a selective effect on the density of the individual lines. Table I shows the transmission readings for a series of exposures in which only the inductance was changed. As the inductance increased, the density of the iron line first increased slightly and then became very weak. The intensity ratio, however, showed a steady decrease. The silicon line also went through a maximum density but the ratios showed a steady increase as the inductance increased. Manganese showed only a slight effect, but as the inductance increased it tended to disappear.

Magnesium 3074 and 3330 both went through a maximum intensity at 0.365 mh. Copper and zinc also passed through a maximum intensity at the same value of inductance. Aluminum, however, tended to become extremely light as the inductance increased, and eventually the line disappeared.

A suitable system can probably be worked out for any of these inductance values, but all other work in this report was performed at the lowest possible inductance. In fact, the above data suggest that even lower inductances should be tried.

PROCESS REPRODUCIBILITY

To check the reproducibility of the process a run of 108 determinations was made on a standard disk of the following composition:

	%
Al	5.83
Zn	3.21
Mn	0.21
Mg	Balance

All determinations were made on the same day with all elements of the process carefully controlled. Frequency-dispersion curves were drawn using the standard terminology of the A.S.T.M. (*1*). For convenience, a brief description of the terms used is given.

X = arithmetic average
Mode = value which occurs most frequently
σ = standard deviation = the square root of the average of the squares of the deviations of a set of N number from their average, X
V = coefficient of variation = ratio of the standard deviation to the average = $100\,\sigma/X$
N = number of determinations

Figure 2 shows the frequency dispersion characteristics of the aluminum determination expressed as a function of the intensity ratios.

For aluminum: $\sigma = 0.058$ intensity unit
$V = 4.92\%$

If these deviations are expressed as percentages of aluminum:

$\sigma = 0.26\%$ Al
$V = 4.46\%$ of the aluminum present

To aid in the interpretation of these results the following additional statements may be made:

If X, σ, and N are stated, more than $\left(1 - \frac{1}{t^2}\right)$ of the total number N observations lie within the closed range, $X \pm t\sigma$. This is true even if the results are obtained under uncontrolled conditions. Stated another way, at least 75% of the results must lie within $\pm 2\sigma$.

However, if we can make the additional statement that the data were obtained under controlled conditions, then the results may be expected to fall within the limits expressed by the normal law integral. In this case at least 68.27% are within $X \pm \sigma$ and at least 95.45% are within $X \pm 2\sigma$.

A glance at Figure 2 for aluminum, and Figures 3 and 4 for zinc and manganese, shows that for the conditions used here, the results may be confined within the following limits:

Al	81% within $X \pm \sigma$
Zn	85% within $X \pm \sigma$
Mn	92.5% within $X \pm \sigma$

In other words, conditions were sufficiently standardized to be labeled as controlled conditions.

Figure 3 shows the same data for zinc and Figure 4 for manganese. These data may be summarized as follows:

	σ	V	% of Readings within $\pm \sigma$
Al	0.26	4.46%	81
Zn	0.16	5.0	85
Mn	0.012	5.7	92.5

From these data we can say that for aluminum in this range the accuracy may be expressed as follows:

5.83 $\pm$ 0.26% and 81% of the readings will be within the expressed range. Similar expressions may be written for the zinc and manganese. The manganese determination is the most accurate, since we may say 0.210% Mn = 0.012 and 92.5% of the readings will fall within the expressed range.

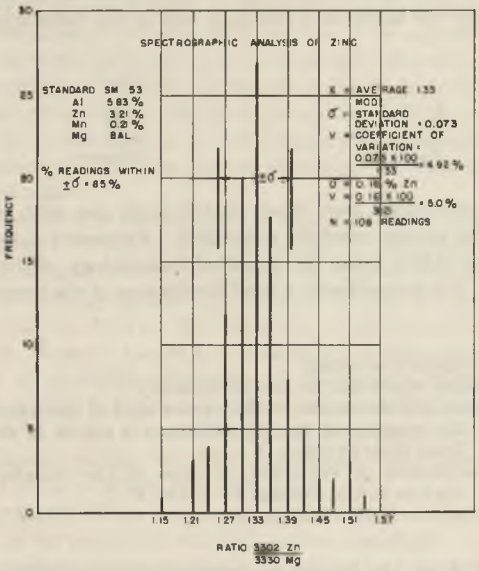

Figure 3. Frequency Distribution Chart for Zinc

These results are based on 108 determinations on the same sample. In practice three closely spaced determinations were taken and the average of the three was used as the result. The average of these three has, of course, a greater possibility of falling within the limits of $\pm\sigma$ than has any single value. On test pieces free from microshrinkage, checks of the spectrograph with wet chemical analyses showed that for aluminum, zinc, and manganese results were accurate within $\pm 5\%$ of the contained element.

SAMPLE CONTROL

The above data on reproducibility included any deviations in the standard disk as well as in the spectrographic process, but

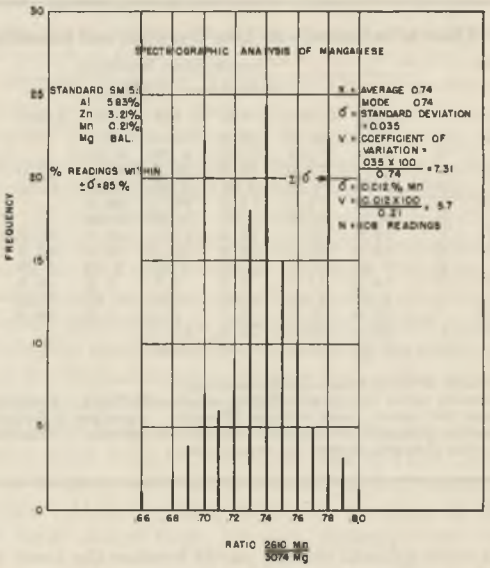

Figure 4. Frequency Distribution Chart for Manganese

Figure 5. Vertical Chill-Cast Disk, Type 1

in the limiting case, the process can be no more consistent or reliable than the sample itself. Radiographs of the standard disks, on which the consistency studies were made, showed that they had substantial areas free from microshrinkage, and could therefore be assumed to be fairly uniform. The consistency studies bore this out, but some standards were far more consistent than others.

When the problems of sampling 950 kg. (2000 pounds) of molten magnesium in a representative fashion arise, the conditions are somewhat different. Six different types of production specimens were tried. These are described in Table II and illustrated in Figures 5, 6, 7, and 8.

Several different types of specimens were made from the same ladle for comparison purposes. The sand-cast disks were all attached to the standard test bar patterns as illustrated in Figures 6, 7, and 8. The chill mold was manufactured by the H. W. Dietert Company and in use was preheated to about 400° F. before pouring. All these specimens were poured from regular production heats at 1350° to 1400° F.

For analysis each specimen was then divided into four equal quadrants, as shown in Figure 9.

Each specimen was radiographed, and then the quadrants were analyzed spectrographically. These determinations were

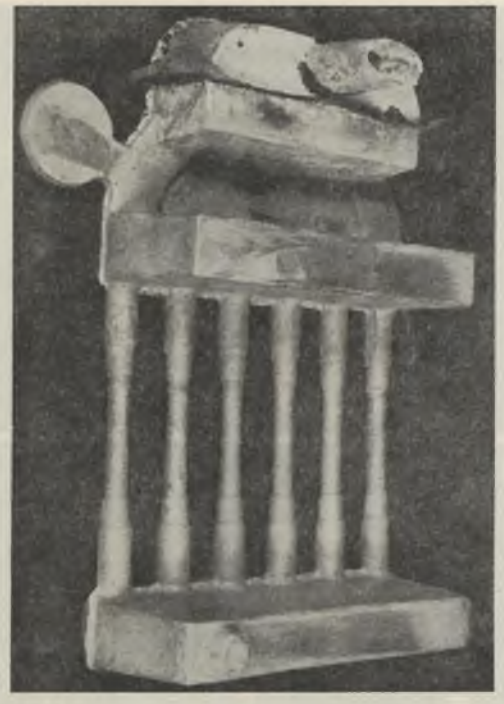

Figure 6. Horizontal Sand-Cast Disk, Type 2

made within a 1.25-cm. (0.5-inch) spot on each quadrant, and the average of these three was then taken to represent the analysis of the quadrant. These quadrants were then sawed out and analyzed for zinc and aluminum by gravimetric methods (*2*).

Table III summarizes the results for the chill-cast disk, which is illustrated in Figure 5. Radiographs of these disks indicated that each had concentrations of heavy microshrinkage in quadrant 1, directly in front of the gate. For this reason quadrant 1 would be expected to be low in zinc and aluminum, and Table III shows that this area was considerably lower in zinc and aluminum than the other three quadrants.

Table IV gives a similar study for the horizontally sand-cast disks shown in Figure 6. These disks were from the same heat as the previous chill-cast disks, and the first quadrant—that is, the quadrant containing the gate—was low in zinc and aluminum to about the same extent as the chill-cast disk. The remaining three quadrants of the sand-cast disk were, however, somewhat more uniform than the corresponding ones in the chill-cast disk. Zinc content was extremely sensitive to microshrinkage and the

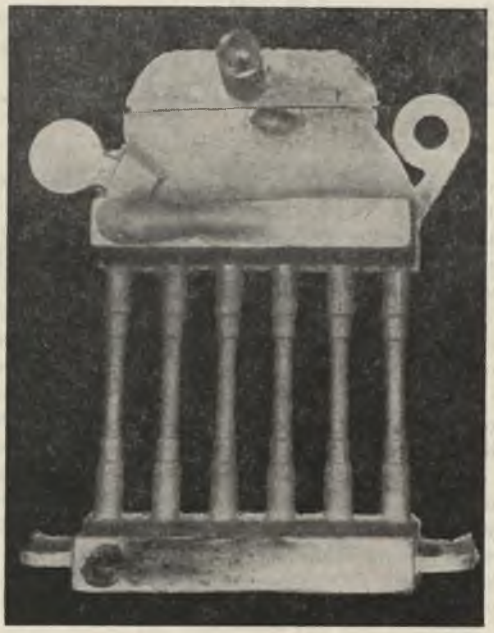

Figure 7. Horizontal Sand-Cast Ring, Type 4
Vertical sand-cast ear attached to riser, Type 6

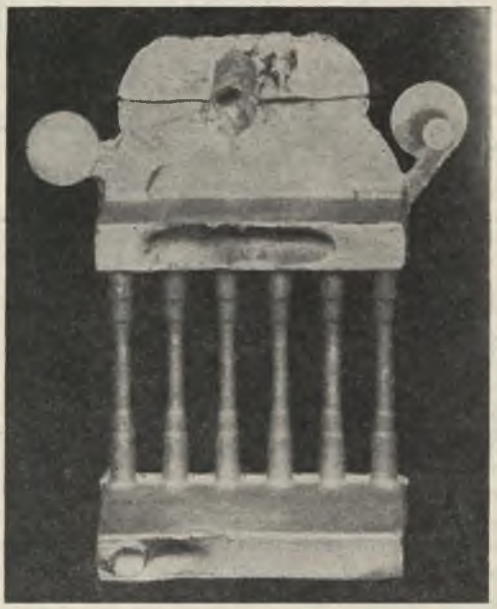

Figure 8. Horizontal Sand-Cast Disk with Blind Riser, Type 5

Table II. Types of Flat Specimen Disks

Type	Description	Figure
1	Vertical chill-cast, 2.5 × 3/16 inches	5
2	Horizontal sand-cast 2 3/16 × 3/16 inches attached to test bar mold	6
3	Horizontal sand-cast, 2 3/16 × 3/16 inches, chilled on one side in the mold	..
4	Horizontal sand-cast ring, 2 3/16 × 3/16 inches, with 1 inch central hole	7
5	Horizontal sand-cast disk 2 3/16 × 3/16 inches, with 1 inch blind riser in center	8
6	Vertical slab, 1.375 inches wide, 2.5 inches high, 0.5 inch thick at bottom, 0.375 inch thick at top, attached to riser of test bar mold	7

Table III. Chill-Cast Spectrographic Disks

(Heat 4384, Type 1)

Method of Analysis	Quadrant	Al	Zn	Mn	Fe	Si	Cu
			Disk Y				
Spectro	1	5.70	2.60	0.197	0.059	0.052	0.006
Wet		5.43	2.62				
Spectro	2	6.07	3.03	0.195	0.064	0.072	0.009
Wet		5.88	3.01				
Spectro	3	6.17	3.08	0.189	0.060	0.070	0.009
Wet		5.85	2.95				
Spectro	4	6.08	2.90	0.193	0.054	0.070	0.008
Wet		5.83	2.71				
			Disk P				
Spectro	1	5.52	2.55	0.195	0.040	0.051	0.006
Spectro	2	5.88	2.90	0.192	0.040	0.070	0.006
Spectro	3	6.30	3.06	0.192	0.037	0.068	0.009
Spectro	4	6.18	3.16	0.188	0.031	0.072	0.009

Table IV. Sand Cast Spectrographic Disks

(Type 2)

Method of Analysis	Quadrant	Al	Zn	Mn	Fe	Si	Cu
		Disk E, Heat 4384					
Spectro	1	6.15	3.05	0.188	0.024	0.053	0.008
Wet		5.50	2.71				
Spectro	2	6.13	3.18	0.192	0.046	0.066	0.009
Wet		5.98	2.92				
Spectro	3	6.13	3.16	0.191	0.038	0.071	0.009
Wet		5.92	3.09				
Spectro	4	6.20	3.20	0.183	0.032	0.063	0.010
Wet		5.98	3.01				
		Disk E, Heat 4411					
Spectro	1	6.18	2.98	0.177	0.022	0.057	0.006
Wet		5.45	2.70				
Spectro	2	6.15	3.10	0.184	0.020	0.060	0.006
Wet		5.80	2.94				
Spectro	3	5.97	2.98	0.178	0.021	0.061	0.005
Wet		5.80	2.98				
Spectro	4	6.25	2.91	0.178	0.022	0.059	0.006
Wet		5.75	2.99				

variation in the zinc analyses was particularly wide. Figure 10 shows radiographs of both types of specimens.

At first glance the spectrographic results seem to be consistently in error on the high side. This is due to the fact that each quadrant contains some microshrinkage. A wet chemical analysis which dissolves this entire quarter might be considerably lower in zinc and aluminum than a spot spectrographic process which is able to hit areas completely free from microshrinkage. As a matter of fact, radiographs on which the spectrographic spots for the previously mentioned disks were located showed that, with the exception of the first quadrant, practically all spots occurred on areas substantially free from shrinkage. Under these conditions, the spectrographic results could be expected to average somewhat higher for zinc and aluminum than the more inclusive wet chemical analyses.

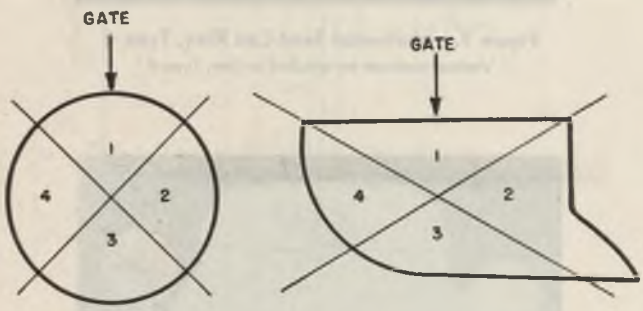

Figure 9. Spectrographic Specimens

Disk and ear divided into quadrants for chemical analysis

A glance at Tables III and IV shows that quadrant 1 was almost 0.5% lower in zinc and aluminum than the remainder of the disk. The radiographs showed that these low areas correspond almost exactly with the areas of heavy microshrinkage. Microshrinkage is caused by the failure of the low-melting ternary eutectic to fill the last voids as the metal solidifies. Since this eutectic is considerably higher in zinc and aluminum than the average analysis, its failure to flow into these last interstices causes the average analysis to be low in zinc and aluminum. Manganese does not enter into the eutectic reaction, and the analyses show that the concentration of this element remains remarkably constant over the entire disk.

Previous to this series of experiments, the entire optical and electrical system of the spectrograph had been checked and realigned. An entire set of new analysis and emulsion calibration curves had been drawn, and after the runs had been made, a standard was sparked again with the following results:

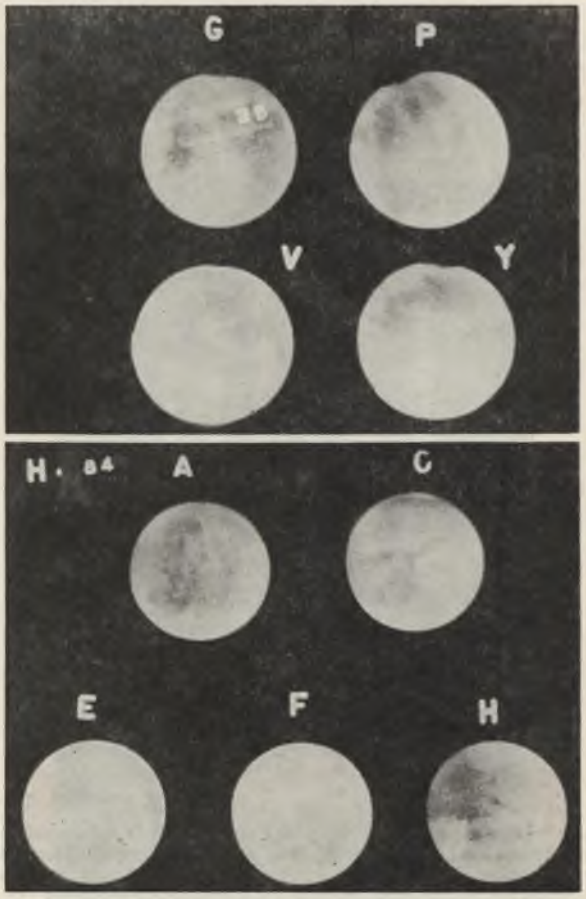

Figure 10. Radiographs of Disks

Above. Chill-cast. *Below.* Horizontal sand-cast

	Al	Zn	Mn	Fe
Standard certified analysis	5.83	3.21	0.210	0.040
Check results	5.82	3.25	0.210	0.051

Apparently conditions had been maintained with reasonable uniformity. The wet chemical analyses checked reasonably well with the spectrograph on sound samples. When microshrinkage was present the two methods of analysis varied to a somewhat greater extent.

Table V. Chilled Sand-Cast Spectrographic Disks

(Heat 4384, Type 3)

Method of Analysis	Quadrant	Al	Zn	Mn	Fe	Si	Cu
		Disk M					
Spectro	1	5.65	3.10	0.184	0.028	0.070	0.009
Spectro	2	5.94	3.20	0.193	0.029	0.071	0.009
Spectro	3	5.78	2.98	0.187	0.028	0.054	0.007
Spectro	4	5.87	3.01	0.191	0.029	0.059	0.007
		Disk O					
Spectro	1	5.94	2.94	0.197	0.026	0.061	0.007
Spectro	2	6.20	3.04	0.182	0.021	0.050	0.008
Spectro	3	5.81	2.88	0.199	0.041	0.059	0.007
Spectro	4	6.08	3.12	0.195	0.023	0.063	0.009

From these experiments it became evident that for uniform results it was necessary either to obtain a sample free from microshrinkage, or to obtain a specimen in which the microshrinkage was always present in the same region, so that it could be avoided. Until a better specimen was developed, all analysis was performed on quadrant 3 in the horizontally sand-cast disk. By confining the work to this quadrant the extreme variations shown in the first quadrant could be avoided.

First the gate on the sand-cast specimen was increased from 1.25 × 0.47 cm. (0.5 × 0.19 inch) to 0.3 × 5 cm. (0.125 × 2 inches). This flood-gating produced spotty microshrinkage throughout the disk.

The gate was then reduced back to 1.25 × 0.47 cm. (0.5 × 0.19 inch) and the disk was moved from the cope to the drag. This specimen was substantially the same as the original sand-cast disk. A blind riser, 2.5 cm. (1 inch) round at the base and 5 cm. (2 inches) high, was then placed at the center. This type of disk is shown in Figure 8. Radiographs showed that there was heavy microshrinkage in the first quadrant. The rest of the disk was sound.

Table VI shows that the results were very uniform for quadrants 2, 3, 4, and a large number of observations showed that the microshrinkage was always located at the same point. When the wet chemical analyses in Table VI were made, therefore, the portion in the center with the heavy shrinkage was drilled out, since this area could always be avoided with the spectrograph. Specimens of this type were very satisfactory if precautions were taken to stay out of the center and the first quadrant. This specimen checked closely with the wet chemical analyses.

Table VI. Sand-Cast Disk with 1-Inch Blind Riser in Center

(Type 5, Heat 4631)

Method of Analysis	Quadrant	Al	Zn	Mn	Fe	Si	Cu
			Disk K				
Spectro	1	6.00	2.86	0.193	0.025	0.067	0.010
Wet		5.98	3.01				
Spectro	2	5.87	3.00	0.194	0.021	0.066	0.012
Wet		5.95	3.04				
Spectro	3	5.84	2.98	0.193	0.022	0.064	0.014
Wet		5.90	3.08				
Spectro	4	5.92	3.06	0.197	0.019	0.062	0.012
Wet		5.98	3.07				
			Disk L				
Spectro	1	5.86	3.04	0.198	0.022	0.069	0.013
Wet		5.91	3.04				
Spectro	2	5.85	2.98	0.201	0.024	0.067	0.013
Wet		5.77	2.88				
Spectro	3	6.03	3.04	0.194	0.024	0.064	0.012
Wet		5.89	3.07				
Spectro	4	5.93	3.07	0.194	0.020	0.062	0.012
Wet		5.88	3.06				

A 0.75-inch hole was drilled from the center of the disk before dividing for wet chemical analyses.

Table VII. Ring Sample with 1-Inch Hole in Center

(Type 4, Heat 4675)

Method of Analysis	Quadrant	Al	Zn	Mn	Fe	Si	Cu
Spectro	1	6.03	2.42	0.172	0.046	0.051	0.005
Wet		5.79	2.95				
Spectro	2	5.65	2.68	0.175	0.021	0.065	0.008
Wet		5.71	2.90				
Spectro	3	6.45	2.74	0.169	0.015	0.065	0.005
Wet		..[a]	2.98				
Spectro	4	6.02	3.16	0.194	0.025	0.073	0.009
Wet		..[a]	2.94				

[a] Determinations erratic.

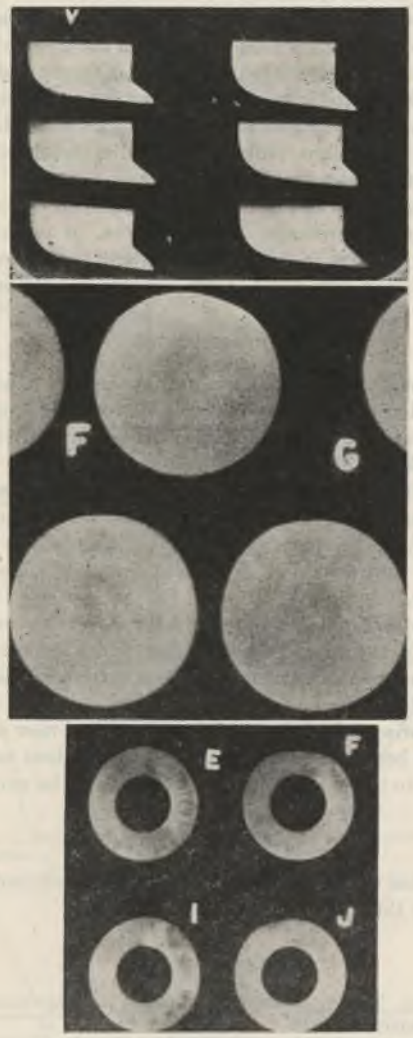

Figure 11. Radiographs

Above. Vertical sand-cast sample
Center. Horizontal sand-cast disks with 1-inch blind riser in center
Below. Sand-cast rings

In an attempt to eliminate the shrinkage from this disk a large cast-iron chill was molded in the drag directly below the gate and riser. The shrinkage disappeared from the gate and center but reappeared as a fringe of microshrinkage around the edge of the chill. This was undesirable, and this type of specimen was abandoned.

Next a ring was cast, as shown in Figure 7. A 1.25 × 0.47 cm. (0.5 × 0.19 inch) gate was used and the central hole was 2.5 cm. (1 inch) round. Radiographs showed that there was radial microshrinkage distributed throughout the entire specimen. Wet chemical analyses, shown in Table VI, were uniform because of the evenly distributed microshrinkage, but it was difficult to check spectrographic results against chemical results, since there were many spots of extreme shrinkage randomly distributed through the sample. On this basis, the specimen was considered unsuitable for analysis. Radiographs of this disk are shown in Figure 11.

Departing on a radically different approach, a vertical specimen was attached to the riser of the test bars as shown in Figure 7. This specimen was 1.25 cm. (0.5 inch) thick at the base and 0.9 cm. (0.375 inch) at the top. It was 3.44 cm. (1.375 inches) wide and 6.25 cm. (2.5 inches) high. With this method the metal was fed progressively to the top and there was an additional pressure from the large riser next to the ear. Radiographs shown in Figure 11 showed that there was little microshrinkage in this specimen.

Table VII shows the analyses for some of these specimens, divided for analysis as shown in Figure 9.

The vertical ears showed surprisingly good consistency within each quadrant, but the variations from quadrant to quadrant were surprisingly large, even though no microshrinkage had been detected. Possibly the specimen was too thick to prevent gross

segregation, but at any rate, the possibility of obtaining a very low reading on zinc or aluminum was virtually eliminated.

Figures 12 and 13 show the striking effect of microshrinkage on the aluminum and zinc contents. A longitudinal slice was taken from a commercial ingot, radiographed, and then spot-analyzed by the spectrograph. Each point on the chemical traverse is the average of three determinations. The area of microshrinkage shown in Figure 12 is outlined on 13, and within this area the zinc and aluminum contents are 0.5 to 0.75% lower than in the sound areas. Wet chemical analyses confirmed these results. This is a striking argument for avoiding areas of heavy shrinkage.

Figure 12. Radiograph of Longitudinal Slice through an Ingot

The choice of an analytical specimen is one of individual preference. Certainly, areas of microshrinkage should be avoided, since they are in no way representative of the sound metal in the heat. Precautions should be taken to use a specimen, or a part of a specimen, which has been demonstrated to be consistently sound, to ensure that metal free from shrinkage is used for analysis. Only then can the spectrograph be expected to check with the wet chemical methods.

In this laboratory, the chill-cast specimen was rejected as entirely unsuitable, and for a long period satisfactory analyses were made on the second and third quadrants of the sand-cast disk shown in Figure 12. This specimen also had the advantage of being automatically obtained for each heat, since test bars were required for every heat. The sand-cast vertical specimen was free of shrinkage but needs further exploration. For the greatest absolute accuracy, the horizontal sand-cast disk with the blind riser was the best, providing the center and first quadrant were avoided.

Several sand-cast disks from the same heat were compared in the as cast, solution heat-treated, and solution heat-treated and aged conditions. No improvement was apparent from the heat treatment, and all specimens were used as cast.

Table VIII. Vertical Sand-Cast Specimen Attached to Riser

(Heat 4691, Type 5)

Method of Analysis	Quadrant	Al	Zn	Mn	Fe	Si	Cu
			Disk SV				
Spectro	1	6.21	2.92	0.193	0.036	0.072	0.009
Wet		5.78	2.96				
Spectro	2	6.13	3.15	0.191	0.036	0.072	0.009
Wet		5.78	3.04				
Spectro	3	6.35	3.25	0.177	0.032	0.073	0.010
Wet		5.95	3.22				
Spectro	4	6.15	3.08	0.182	0.031	0.072	0.009
Wet		5.97	3.20				
			Disk S				
Spectro	1	5.95	2.88	0.184	0.035	0.070	0.009
	2	6.23	2.92	0.192	0.031	0.072	0.009
	3	6.20	3.18	0.195	0.034	0.075	0.010
	4	6.45	3.13	0.187	0.034	0.070	0.009

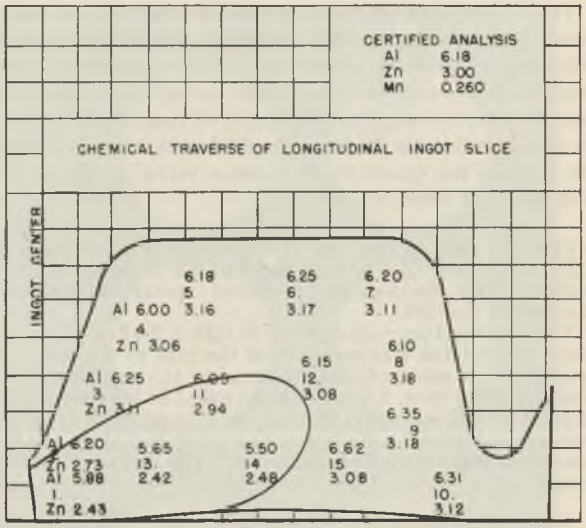

Figure 13. Chemical Traverse of Ingot Slice Shown in Figure 12

Several attempts were made to use a set of pins or rods as specimens. First, rods were drawn from the melt in Pyrex tubes by suction. This produced a very satisfactory rod but it was unusually high in silicon, probably owing to a reaction between the molten magnesium and the glass. Pins were then cast in a permanent mold, but preliminary results indicated that they were no better than the sand-cast flats.

Radiographs showed that it was difficult to cast a sound rod free from microshrinkage in sand, Pyrex, or permanent mold. Since none of these pins was as consistent as the best technique on the flat disks, this series of experiments was abandoned.

CONCLUSIONS

These results are preliminary, in that they are based on what is still a limited experience. It is hoped that subsequent observations by a large number of investigators will improve the accuracy and reliability of the spectrographic analysis.

1. The spectrograph is capable of analyzing aluminum, zinc, and manganese in a magnesium-base alloy with an accuracy of at least ±5% of the contained element. This is true for an alloy containing approximately 6% aluminum, 3% zinc, and 0.2% manganese.

2. Spectrographic analyses should never be made on the quadrant of the separately cast disk which contains the gate. The presence of microshrinkage causes extreme variations in the zinc and aluminum contents.

3. A horizontal sand-cast disk with a blind riser and attached to the test bar mold provides the most consistent sample. The areas next to the gate and in the center should be avoided.

ACKNOWLEDGMENT

The author would like to thank H. C. Robinson and R. Hebblethwaite for their help in collecting this data.

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Neopentane in Refinery Butanes

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Qualitative study of a straight-run refinery butane stream by infrared spectrometry has established the presence of a small amount of neopentane. A spectrophotometric method is described for the quantitative analysis of the system propane, *n*-butane, isobutane, neopentane, and isopentane. Determinations by this method show that neopentane occurs in various Texas crudes to the extent of about 0.03% by volume. The reference spectra necessary for this analysis are included.

THE occurrence of neopentane in natural gasoline has been reported (*5*) but no definite evidence of its presence in crude oils has been published. The analysis of several crude oils by the National Bureau of Standards (*6*) has failed to indicate the presence of the hydrocarbon in the samples studied. Furthermore, low-temperature distillations of butane and pentane streams obtained in topping operations show no trace of neopentane. It is generally believed, therefore, that the compound is not met in refinery operations. The study of refinery butanes by infrared spectrophotometry in this investigation has shown, however, that this is not the case.

QUALITATIVE DETERMINATION OF NEOPENTANE

An examination of a portion of the infrared spectrum (*A*, Figure 1) of a sample from a *n*-butane stream revealed the presence of a weak absorption band near 7.9 mu which could not be attributed to *n*-butane or to either of the known impurities, isobutane and isopentane, but which corresponded closely to one of the strong absorption bands for neopentane, suggesting the presence of that hydrocarbon in low concentrations. The absorption spectra of the pure compounds, presented in Figure 1, for purposes of comparison, were obtained using a recording infrared spectrometer of high resolution (*2*).

In order to obtain more conclusive evidence, the neopentane of the *n*-butane sample mentioned above was concentrated by distillation. The sample was fractionated in a column of 30 theoretical plates at a reflux ratio of 24 to 1. Calculations based on Smoker's equation (*7*) indicated that under these conditions less than 0.1% of neopentane (on a charge basis) would be lost in the overhead product, *n*-butane, when the liquid in the kettle consisted of 80% neopentane. Actually, operational difficulties prevented the attainment of this degree of enrichment.

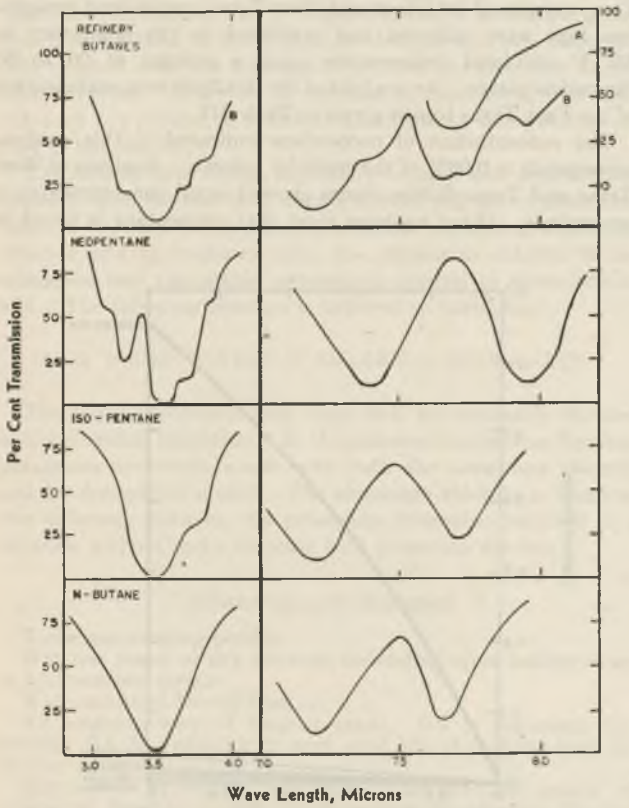

Figure 1. Spectra of Refinery Butanes and Pure Hydrocarbons in 2.8 to 4.2 mu and 7.0 to 8.2 mu Regions

All curves at 200-mm. pressure in 30-cm. cell. *A*, unfractionated refinery butanes. *B*, concentrate from distillation

Table I. Operating Conditions for Spectrophotometric Analysis of Butanes

Principal Absorber	Wave Length *Microns*	Slit Width *Mm.*	Standard Pressure (10-Cm. Cell) *Mm.*	Shutter	Filter
Propane	9.35	0.50	650	Metal	MgO
Isobutane	8.46	0.45	200	Metal	MgO
n-Butane	13.70	1.50	550	LiF	None
Neopentane	7.96	0.30	100	Metal	MgO
Isopentane	9.65	0.50	420	Metal	MgO

B, Figure 1, shows the spectrum of the concentrate obtained by this procedure. The 7.40- and 7.96-mu bands of neopentane are both clearly present in the spectrum, as is also the highly characteristic neopentane band structure in the 3.5-mu region.

As a matter of interest the complete spectrum of neopentane is presented in Figure 2. Attempts to purify the very small amount of neopentane on hand were unsuccessful, so the spectrum given is one previously obtained in this laboratory using a sample of about 90% purity. The agreement with an unpublished neopentane spectrum from another laboratory is satisfactory.

QUANTITATIVE DETERMINATION OF NEOPENTANE

The method developed for the analysis of the system propane, *n*-butane, isobutane, neopentane, and isopentane is an application of the general method described by Brattain *et al.* (*3*). The routine infrared spectrophotometer used was designed by the Shell Development Company and is manufactured by National Technical Laboratories. Operating conditions for the analysis are given in Table I.

All the hydrocarbons other than neopentane used in the calibration were of 99.5+% purity as determined by isothermal distillation (*4*). The best neopentane concentrate available was analyzed qualitatively on the recording spectrometer mentioned above and found to contain only isobutane as an impurity. An isothermal distillation at 0° C. was performed and the mole per cent of isobutane calculated from a plot of vapor pressure *vs.* per cent sample evaporated, using the vapor pressure data of Aston and Messerly (*1*). The amount of isobutane thus determined was 11.5 mole %. Optical densities of the standards were corrected for the effects due to the impurities in all cases. The corrected working curves at the 7.96-mu neopentane absorption band are shown in Figure 3.

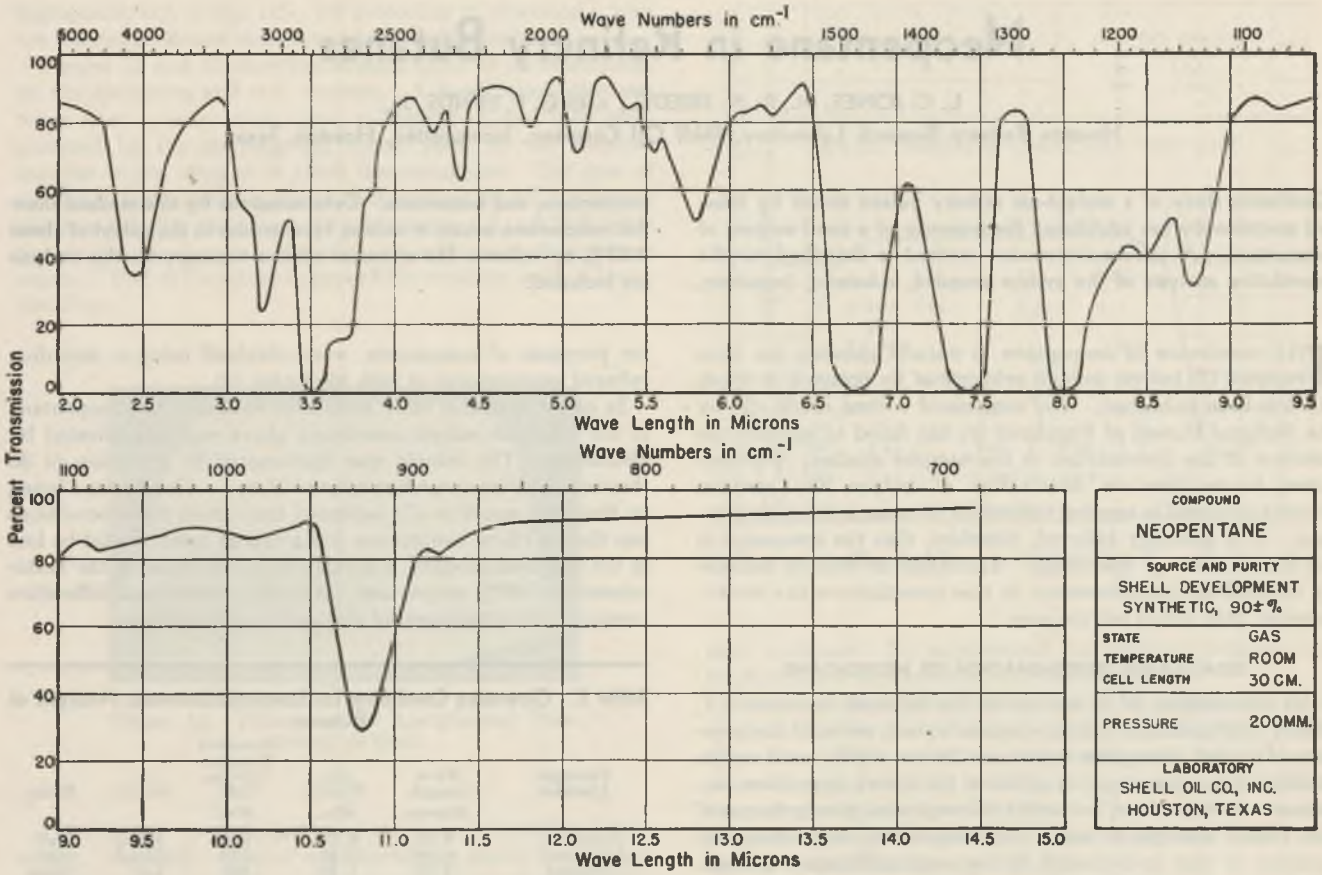

Figure 2. Infrared Spectrum of Neopentane from 2 to 14.5 mu

Brattain's graphical method of calculation (*3*) was employed with one modification: Since the deviations from ideal behavior are different for C_3 and C_5 hydrocarbons, it was necessary to recorrect the observed pressures on the basis of an approximate analysis obtained using the average deviations for the butanes.

RESULTS

The results of the analyses of two typical synthetic blends are given in Table II. The analysis of several such synthetics indicated that the neopentane could be determined to ±0.1% volume if present in low concentrations (up to 8 mole %).

The development of this method made it possible to determine the source of the neopentane and the extent of its occurrence. Thus, samples of both East and West Texas unstabilized straight-run tops were collected and stabilized in the laboratory to 65° F. still-head temperature using a column of 20 to 30 theoretical plates. An analysis of the distillate from stabilization of the East Texas tops is given in Table III.

The concentration of neopentane indicated in this analysis corresponds to 0.03% of the crude by volume. Analyses of West Texas and Texas-Hobbs crudes showed equal concentrations of neopentane. Other analyses show that neopentane is found in

Table II. Analyses of Synthetic Blends

Component	Blend I Known	Blend I Found	Blend II Known	Blend II Found
	Mole Per Cent			
Propane	3.6	3.5	0.0	0.0
Isobutane	14.8	15.7	6.9	6.6
n-Butane	59.8	59.8	85.3	85.6
Neopentane	8.2	8.3	3.9	4.0
Isopentane	13.7	12.7	3.9	3.8

Table III. Analysis of Low-Boiling Fraction from East Texas Crude

Hydrocarbon	Mole %
Propane	7.3
Isobutane	13.1
n-Butane	67.8
Neopentane	0.6
Isopentane	11.2
	100.0

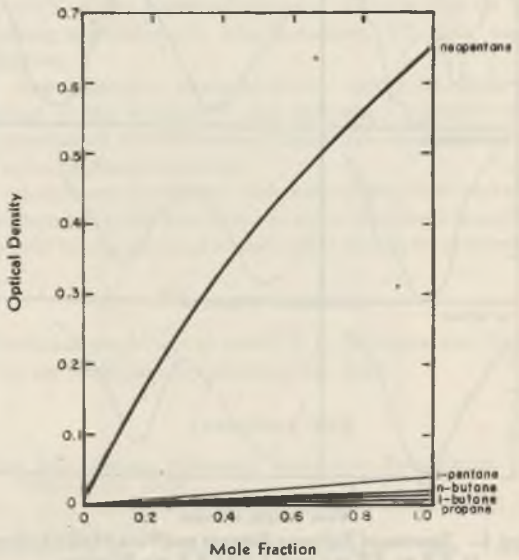

Figure 3. Optical Densities

At 7.96 mu and 100-mm. pressure in 10-mm. cell

other straight-run C_4 streams in the refinery to the extent of about 0.4 mole %.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to K. E. Train of this laboratory who performed the isothermal distillations of the reference standards, and to Edward Gelus for his advice and aid in the concentration by distillation of the neopentane samples and also in the interpretation of the isothermal distillation curves. The authors are further indebted to the Shell Oil Company for granting permission to publish this work.

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Determination of Aluminum Chloride and Hydrochloric Acid in Hydrocarbon Streams

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THE analytical method presented here does not depend on a new principle, since Craig (*1*) published a similar method in 1911 for the determination of free acid or aluminum oxide in alum and Scott modified this method to some extent (*3*, *4*). Martin published a method for the determination of aluminum in ore which makes use of the reaction between aluminum salts and potassium fluoride. Other methods of analysis are discussed by Willard and Diehl (*5*). The method of Malaprade (*2*) probably could be adapted to the analysis of aluminum chloride and hydrogen chloride mixtures but requires the use of a potentiometer. The advantage would be that iron would not interfere.

The author's method is a new application of the use of potassium fluoride, inasmuch as hydrogen chloride or alumina is determined in addition to the aluminum chloride and iron chlorides. The method does not distinguish between the iron and aluminum chlorides. However, in the present application, iron chlorides are not present in sufficient quantities to cause serious errors in the results.

The method used in this laboratory is rapid and accurate and depends upon the fact that when a neutral solution of potassium fluoride is added to an aqueous solution containing aluminum chloride and hydrochloric acid, the aluminum chloride is decomposed into two stable compounds, neutral to phenolphthalein. The following reaction is believed to take place:

$$AlCl_3 + 6KF + XHCl = AlF_3.3KF + 3KCl + XHCl$$

The free hydrochloric acid may then be accurately titrated with potassium hydroxide. In the absence of potassium fluoride, potassium hydroxide reacts with both the aluminum chloride and the hydrochloric acid. The aluminum chloride is found as the difference between the potassium hydroxide required in a titration without and a titration with potassium fluoride.

APPARATUS AND REAGENTS

Three gas-washing bottles.

Wet test meter or dry ice trap, depending upon boiling range of hydrocarbon stream.

Wide-mouthed Dewar flask.

All reagents were of reagent grade. 0.5 *N* potassium hydroxide, 0.5 *N* hydrochloric acid, and 1% phenolphthalein indicator solution.

POTASSIUM FLUORIDE SOLUTION. Dissolve 200 grams of potassium fluoride in 400 ml. of carbon dioxide–free distilled water, which has been neutralized with hydrochloric acid or potassium hydroxide, using phenolphthalein as indicator. This solution should be kept in a paraffin-coated bottle.

SAMPLING PROCEDURE

The following sampling procedure is designed for sampling butane streams containing aluminum chloride and hydrogen chloride.

Construct a sampling arrangement similar to that shown in Figures 1 and 2, so that a sample may be taken from a circulating, steam-trace line. Do not attempt to warm up the sampling

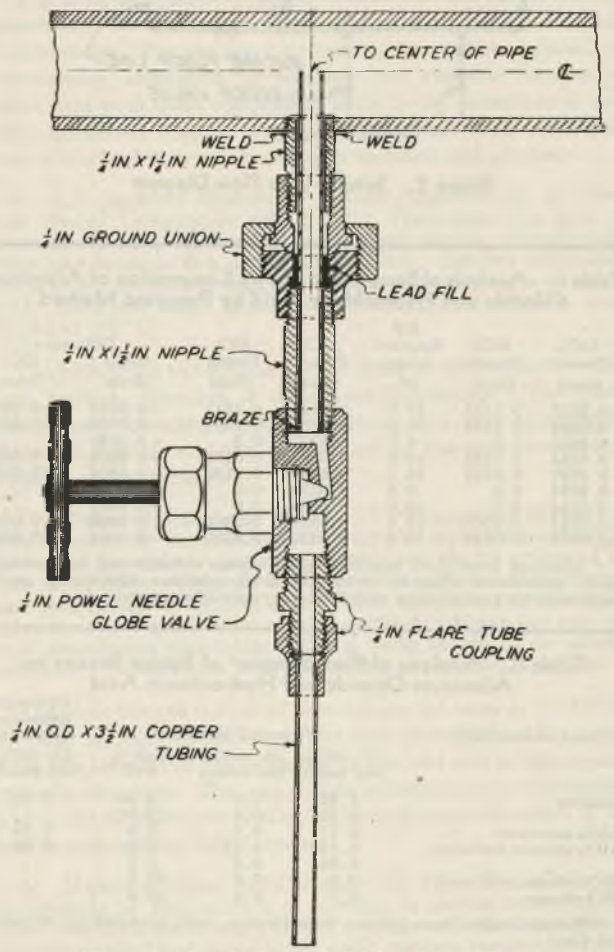

Figure 1. Detail of Sample Valve Assembly

connection during sampling, as this may cause volatilization of the sample before it reaches the valve.

With the needle valve closed, screw the sample connection in place and attach to its exit three gas-washing bottles followed by a wet test meter. Place 100 ml. of distilled water in each bottle. Open the master valves, so that the flow will be partially diverted to pass through the sample line shown, and then carefully open the needle valve sufficiently to allow a moderate rate of flow. Pass between 1.416 and 2.832 $\times 10^4$ cc. (0.5 and 1 cu. feet) of gas through the meter, close the needle valve, and disconnect the absorbers from the sample connection. Close the master valves, open the bleeder valve to drop the pressure, and remove the sample connection.

Wash the aluminum chloride from the needle valve and entry tube into a beaker and combine the washings with the contents of the gas-washing bottles.

METHOD OF ANALYSIS

Dilute the above washings to a definite volume and take aliquots.

To one aliquot, A, add 10 ml. of 0.5 N hydrochloric acid and 10 ml. of potassium fluoride solution. Stir vigorously and titrate with 0.5 N potassium hydroxide, using phenolphthalein as indicator until a pink end point, permanent for one minute, is reached. The difference between the potassium hydroxide titration and the hydrochloric acid added will be due to free hydrochloric acid or alumina. If the potassium hydroxide titration minus the hydrochloric acid titration is positive, the sample contains free acid and no alumina. If this difference is negative, the reverse is true.

To another aliquot, B, add phenolphthalein and titrate with 0.5 N potassium hydroxide until a pink end point, permanent for one minute, is reached. This titration will show the total hydrogen chloride, made up of hydrogen chloride from aluminum chloride and any originally free hydrogen chloride if it has been present.

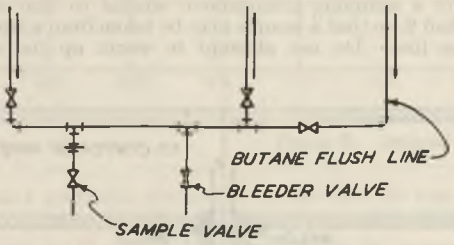

Figure 2. Sample Line Flow Diagram

Table I. Analysis of Samples[a] of Known Composition of Aluminum Chloride and Hydrochloric Acid by Proposed Method

$AlCl_3$ Present	HCl Present	KF Solution Added	$AlCl_3$ Found	HCl Found	Difference $AlCl_3$	Difference HCl
Gram	*Gram*	*Ml.*	*Gram*	*Gram*	*Gram*	*Gram*
0.2237	0.1825	10.0	0.2224	0.1811	−0.0013	−0.0014
0.0224	0.1825	10.0	0.0224	0.1808	0.0000	−0.0017
0.2237	0.0	0.0	0.2207	0.0	−0.0030	....
0.0224	0.0183	10.0	0.0221	0.0180	−0.0003	−0.0003
0.2237	0.0183	10.0	0.2232	0.0186	−0.0005	+0.0003
0.0224	0.0	0.0	0.0220	0.0	−0.0004	....
0.0224	0.0	10.0	0.0225	0.0	+0.0001	....
0.0224	0.2007	10.0	0.0221	0.2000	−0.0003	−0.0007
0.0224	0.0183	10.0	0.0220	0.0180	−0.0004	−0.0003

[a] Made up from stock solutions of aluminum chloride and hydrochloric acid. Aluminum chloride content of stock solutions determined gravimetrically by precipitation with 8-hydroxyquinoline reagent.

Table II. Analysis of Plant Samples[a] of Butane Streams for Aluminum Chloride and Hydrochloric Acid

Point of Sampling	Proposed Method			Hydroxyquinoline Method
	Lb. $AlCl_3$/ bbl. butane	*Lb. Al_2O_3/ bbl. butane*	*Mole % HCl*	*Lb. $AlCl_3$/ bbl. butane*
Reactor	2.42	0.0	8.40	2.31
	1.22	0.0	10.00	..
$AlCl_3$ saturator	2.17	0.0	0.0	2.08
$AlCl_3$ column bottoms	4.94	0.19	0.0	5.09
	3.04	0.0	0.0	..
HCl column overhead	0.0	0.0	85.8	..
HCl charge	0.0	0.0	10.5	..

[a] Samples taken from butane isomerization unit, operated by Wilshire Oil Co.

CALCULATIONS

Aliquot A, titration of free hydrogen chloride

1. Ml. of KOH $\times N$ − ml. of HCl $\times N$ = ml. of N KOH equivalent to free HCl
2. $$\text{Gram moles of HCl} = \frac{\text{ml. of KOH} \times N - \text{ml. of HCl} \times N}{1000 \times F}$$
3. $$\text{Gram moles of butane} = \frac{\text{cu. ft.} \times (P_B - P_w) \times 0.851}{460 + T}$$
4. $$\text{Mole \% HCl in butane (neglecting } AlCl_3) = \frac{\text{gram moles of HCl} \times 100}{\text{gram moles of HCl} + \text{gram moles of butane}}$$
5. $$\text{Pounds of } Al_2O_3 \text{ per barrel of butane at } 60^\circ \text{ F.} = \frac{(\text{ml. of HCl} \times N - \text{ml. of KOH} \times N) \times (460 + T)\ (0.0702)}{\text{cu. ft.} \times F \times (P_B - P_w)}$$

Aliquot B, titration of both $AlCl_3$ and free HCl

$$\text{Pounds of } AlCl_3 \text{ per barrel of butane at } 60^\circ \text{ F.} = \frac{[(\text{ml. of KOH} \times N) - C] \times (460 + T) \times 0.183}{\text{cu. ft.} \times (P_B - P_w) \times F}$$

N = normality of reagent used.
P_B = barometric pressure, mm. of mercury.
P_w = vapor pressure of water at T° F.
T = temperature of water in meter, ° F.
C = ml. of N potassium hydroxide equivalent to free hydrochloric acid from A 1.
F = fraction of total sample taken for analysis. It should be the same for A and B.

Carbon dioxide–free water should be used with the phenolphthalein indicator. If iron is present in more than traces, a separate determination should be made and deducted from aluminum chloride content found.

REPRODUCIBILITY

Duplicate determinations should agree within ±1.0%, providing the iron content is negligible or corrections are made for it.

Table I indicates the accuracy obtainable. The work was done on synthetic samples, but plant samples have been found to give equally good results (Table II).

DISCUSSION

Large errors may result from incorrect sampling, since either excessive heating or cooling of the sample lines will result in deposit of aluminum chloride.

Hot butane should be used for flushing sample lines, since the solubility of aluminum chloride is very low in cold butane. Large pressure drops in the sampling lines should be avoided, as they will cause the butane to vaporize and deposit aluminum chloride especially if the stream is saturated. The sample lines leading from the main lines should be maintained at a temperature only slightly higher than that of the main lines to avoid separation of aluminum chloride from a saturated stream. This can usually be accomplished by use of steam tracing and insulation.

ACKNOWLEDGMENTS

The authors wish to acknowledge the helpful suggestions of Mr. Iverson and other members of the Universal Oil Products Company in the design of the sampling system used.

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Determination of Tetraethyllead in Gasoline

Versatile Direct Evaporation Methods

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Two methods of analysis involving direct evaporation of sample with decomposition agent allow accurate quantitative analysis of lead in all types of gasoline, special motor fuel blends, and tetraethyllead fluid, regardless of the chemical constituents present. These methods, which require no elaborate apparatus, are the result of a systematic review and investigation of existing methods for the decomposition of tetraethyllead in gasoline and for the determination of lead in soluble inorganic residues. The proposed methods convert the tetraethyllead to inorganic lead by mixing with cold hydrochloric acid or iodine, evaporating the resulting mixture to dryness, and removing the organic matter by oxidation with nitric, perchloric, or sulfuric acids, depending on the nature of the decomposition used. The lead in the inorganic residue is determined by precipitating with a measured amount of dichromate solution and determining the excess dichromate by iodometric titration. For commercial gasolines, the reproducibility (different operators) of the methods is within 0.02 ml. of tetraethyllead per gallon; for commercial tetraethyllead fluids, it is 0.4% lead or better.

SINCE the advent of tetraethyllead antiknock gasoline blends some 20 years ago, there has been an increasing necessity for analyzing all sorts of motor fuel for small amounts of lead. At the outset, most analytical needs were satisfied by methods that gave a good approximation of the total lead content; but with the scientific development of modern motors and the consequent production of fuels requiring closer regulation of antiknock qualities, greater analytical accuracy was desired. This situation has led to the development of a number of methods which allowed the accurate determination of the lead contained in the more volatile type of saturated gasolines, such as straight-run aviation gasoline. However, these methods were not always of general applicability to all varieties of leaded fuels, particularly to cracked gasolines, alcohol-blend fuels, and modern motor fuels containing nonhydrocarbon additives. As a result, considerable difficulties have been encountered in obtaining accurate results when using most of the established methods regardless of their mode of decomposition of the tetraethyllead (such as precipitation with bromine, extraction with cold nitric acid, and extraction with hot hydrochloric acid).

Because of the uncertainty regarding the relative merits of the existing procedures, work was undertaken to compare them under comparable conditions. The problem was considered on the basis of four natural classifications: reproducibility and relative accuracy of the common methods in use at the present time; satisfactory determination of the expected amount of lead in inorganic residues with special consideration of rapid methods; study of the decomposition of the organo-lead compounds with hydrochloric acid and iodine; and study of satisfactory methods for the removal of the organic matter remaining after the evaporation of gasoline–hydrochloric acid or gasoline-iodine mixtures.

REVIEW OF LITERATURE

METHODS OF DETERMINING TETRAETHYLLEAD IN GASOLINE. The following representative methods were chosen for study in that they were either outstanding or commonly used. A brief of the essential procedure, chemistry, and technique is given with each method, followed by an initial evaluation of its relative merits.

1. *Early Shell Method.* Precipitate the lead as lead bromide by the addition of 50% bromine in carbon tetrachloride; dissolve the bromide (without filtering) by extracting with concentrated nitric acid; determine the lead in the acid layer by evaporating to fumes with sulfuric acid; precipitate the lead sulfate from 50% ethanol, filter, and weigh in a Gooch crucible (after heating to faint red heat).

The use of the 50% bromine solution is objectionable because the vigorous reaction of bromine with unsaturated materials gives rise to incomplete separation of lead bromide, particularly when large amounts of bromine are required. Furthermore, the use of bromine should be avoided because of its corrosive action on the skin and nasal membrane if spillage should occur. The repeated handling necessary to effect complete extraction of the lead bromide with nitric acid is cumbersome and is apt to be accompanied by handling losses. The gravimetric determination of the inorganic lead is slow but accurate and precise.

2. *Method of Baldeschwieler* (*2*). Extract the lead by direct action, with concentrated nitric acid and water, as lead ion; evaporate the acid layer to fumes with sulfuric and nitric acids, precipitate lead sulfate by adding water and alcohol; filter and weigh lead sulfate in a Gooch crucible, after heating to a faint red heat.

The nitric acid extracts organic matter especially from cracked gasolines; the extraction is generally slow, incomplete, and cumbersome. For gasolines containing appreciable amounts of unsaturates, aromatics, or alcohols, the vigorous reaction of nitric acid with these compounds leads to the formation of possibly dangerous organic nitrogen compounds. The determination of the lead is slow but generally accurate and precise.

3. *I.P. Standard Methods for Testing Petroleum and Its Products* (Serial Designation 68/42, *15*). Precipitate the lead as bromide by the addition of bromine in carbon tetrachloride; filter lead bromide in a dry Gooch crucible; dissolve bromide in nitric acid; precipitate lead chromate in solution acid with acetic acid; filter and weigh lead chromate in a Gooch crucible, after drying at 105° C.

The objections to bromine are the same as in Method 1. The lead bromide filtration is sometimes incomplete. The gravimetric lead chromate method is tedious, slow, and occasionally gives low results, and the composition of the lead chromate is variable and uncertain.

4. *Method of Catlin and Starrett* (*7*). Precipitate the lead as lead bromide by the addition of bromine in carbon tetrachloride; filter lead bromide on paper; dissolve bromide by action of dilute nitric acid; evaporate to fumes with sulfuric acid; precipitate lead sulfate by adding water; filter the lead sulfate and dissolve it in acid ammonium acetate solution; titrate the lead ion with ammonium molybdate solution using tannin as an outside indicator. Standardize molybdate solution against pure lead.

The objections to the use of bromine are the same as in Method 1. The recovery of lead ions from lead bromide tends to be incomplete because of the occlusion of the lead salt in the organic material obtained. The lead sulfate precipitation is unnecessary and is apt to yield low results. The molybdate titration of the lead is accurate and fairly precise.

5. *Method of Edgar and Calingaert* (*9, 11*). Precipitate lead as bromide by the addition of bromine in carbon tetrachloride; filter lead bromide in a dry Gooch crucible and dissolve bromide in concentrated and dilute nitric acid; remove excess nitric acid

and make acid with acetic acid; titrate lead ion with ammonium molybdate solution using tannin as outside indicator.

Same comments as given for Method 4 except that the objectionable lead sulfate precipitation is omitted.

6. *Method of von Ulrich* (*18*). Precipitate lead as bromide by addition of bromine in carbon tetrachloride; dissolve the lead bromide (without filtering) by extraction with concentrated nitric acid; evaporate acid layer to fumes with sulfuric acid in a weighed crucible; remove sulfuric acid by evaporation; weigh lead as sulfate after igniting at faint red heat.

The objections to the use of bromine and nitric acid are the same as in Method 1. The lead determination is fast but lacks accuracy and precision.

7. *Method of Epler* (*10*). Precipitate lead as bromide by addition of bromine in carbon tetrachloride to sample diluted with kerosene; filter lead bromide in glass crucible, dissolve bromide in concentrated and dilute nitric acid; remove excess nitric acid and make acid with acetic acid; add excess of standard potassium dichromate solution, digest, and filter the lead chromate; titrate excess dichromate ions iodometrically.

Same objections to the use of bromine as in Method 4. The lead determination is accurate and precise, provided the thiosulfate solution is standarized against the standard dichromate solution.

8. *Method of Dosios and Pierri* (*8*). Extract the lead from gasoline by shaking with excess bromine water; evaporate the water layer to fumes with sulfuric acid; precipitate lead sulfate by addition of 50% ethanol, digest; filter lead sulfate in a Gooch crucible; weigh, after igniting at dull red heat, as lead sulfate.

The bromine water extraction is slow and fails entirely in the presence of cracked gasolines. The lead determination is generally satisfactory. The method lacks precision owing to the small volume of sample used.

9. *Method of Birch* (*3*). Shake gasoline with concentrated sulfuric acid; distill gasoline off; remove organic matter by digesting with concentrated sulfuric acid and potassium nitrate; precipitate lead sulfate with cold water; filter in a Gooch crucible and weigh after ignition at a dull red heat.

This method yields a very large amount of organic residue that is impossible to handle unless the sample is entirely composed of low-boiling saturated hydrocarbons. Same comments on the lead determination as in Method 2.

10. *Method of Ferreri* (modified, *12*). Reflux gasoline with concentrated hydrochloric acid in an Erlenmeyer flask containing a loose-fitting water-cooled glass tube hanging in the neck of the flask; evaporate the acid and gasoline to dryness; oxidize organic matter with concentrated nitric acid; determine lead by one of two methods: (1) evaporate to fumes of sulfuric acid, precipitate lead sulfate by adding 50% ethanol; filter in Gooch crucible and weigh after igniting at faint red heat; or (2) remove excess of nitric acid, make acid with acetic acid, and titrate lead ion with standard molybdate solution using tannin as outside indicator.

This method is applicable only to saturated gasoline. With highly unsaturated gasolines, evaporation of the gasoline-acid mixture leaves an organic residue which nitric acid will not completely remove. Comments on the lead determinations are found in Methods 1 and 4.

11. *Method of Calingaert and Gambrill* (standardized by A.S.T.M. as serial designation D526–42, *5*). Reflux gasoline in a special all-glass combination refluxing vessel and separatory funnel with concentrated hydrochloric acid; evaporate the acid layer to dryness, oxidize the organic matter with nitric acid, and remove excess acid; make solution acid with acetic acid and titrate lead ion with molybdate using tannin as outside indicator (or determine lead gravimetrically as chromate or sulfate).

Although this method is generally applicable to all types of gasoline, low results have been found when it is applied to highly volatile gasolines and cracked gasolines owing to loss of lead by volatilization and interference of organic matter, respectively. This method requires special extraction apparatus and is slow, especially in the presence of organic materials soluble in the hot acid. Same comments on the lead determinations as in Methods 4, 3, and 1.

Table I. Precision of Selected Existing Methods for Determining Lead in Gasoline (Saturated)

Method No.	Repeated Determinations	Mean[a]	Standard Deviation[b]
	Grams of Pb per U. S. gallon		
1	2.30, 2.32, 2.28, 2.30, 2.28, 2.29, 2.30, 2.29, 2.30	2.296	0.012
2	2.29, 2.23, 2.27, 2.25, 2.23, 2.21, 2.26	2.249	0.025
5	2.20, 2.25, 2.24, 2.26, 2.24, 2.22, 2.23, 2.20, 2.26, 2.21, 2.26	2.234	0.022
7	2.29, 2.32, 2.33, 2.29, 2.28, 2.28, 2.32	2.301	0.020
10	2.35, 2.34, 2.36, 2.35, 2.32, 2.33, 2.35, 2.32	2.340	0.014
11	2.25, 2.33, 2.33, 2.30, 2.32, 2.33, 2.32, 2.25	2.304	0.032
12	2.29, 2.28, 2.27, 2.29, 2.28, 2.28, 2.27, 2.29	2.281	0.008
13	2.31, 2.30, 2.31, 2.30, 2.31, 2.31, 2.31, 2.31	2.308	0.004

1 Br_2 in CCl_4, gravimetric, sulfate (early Shell)
2 HNO_3, volumetric, molybdate (Baldeschwieler)
5 Br_2 in CCl_4, volumetric, molybdate (Edgar and Calingaert)
7 Br_2 in CCl_4, volumetric, chromate (Epler)
10 HCl, volumetric, chromate (Ferreri)
11 HCl, volumetric, molybdate (Calingaert and Gambrill)
12 I_2 in CCl_4, evaporate, acidimetric (Gonick and Milano)
13 $HNO_3 + KClO_3$, volumetric, chromate (Schwartz)

[a] Mean not intercomparable; several different batches of gasoline used in tests.
[b] Square root of the mean of the squared deviations.

12. *Method of Gonick and Milano* (*14*). Mix the gasoline with a solution of iodine in carbon tetrachloride, evaporate the mixture to dryness with the aid of a hot air jet, and oxidize organic matter with mixture of nitric acid and potassium chlorate. Dissolve the lead salts in water, neutralize carefully, add increments of 8-hydroxyquinoline solution, and titrate the acidity liberated by the formation of lead hydroxyquinolate.

The iodine decomposition is complete and fast, but it produces considerable quantities of carbonaceous matter with unsaturated gasolines. In the latter case, the oxidation with nitric acid and potassium chlorate is sometimes hazardously rapid. The lead determination is quick and direct, but the alternate addition of 8-hydroxyquinoline and base solutions is cumbersome and likely to give erratic results, generally low.

13. *Method of Schwartz* (*16*). Extract the gasoline with cold dilute nitric acid solution saturated with potassium chlorate. Evaporate the extracts to dryness, dissolve residue in water, precipitate lead as lead chromate, and wash with acetone and ether. Dissolve precipitate in acid salt solution, add an excess of iodide solution, and titrate the iodine liberated.

This method is rapid and precise. However, the extraction procedure is somewhat cumbersome and the ease of extraction appears to be a function of the chemical nature of the sample.

In many of the above procedures it is necessary to make a separation between an aqueous and a hydrocarbon phase before proceeding with the determination of the inorganic lead extracted in the aqueous phase. This requires a transfer between vessels which may not be entirely quantitative unless special care is taken; there is also the necessity of effecting a complete extraction of a lead halide from a hydrocarbon solution in which the lead salt is appreciably soluble.

Most of the methods for the determination of the amount of extracted inorganic lead salt, particularly the volumetric methods, are applicable only in the complete absence of organic matter. A perfectly clear solution is not sufficient evidence of complete decomposition of organic material, and in many of the described procedures insufficient decomposition results. Generally, it is necessary that the dry lead salt residue be free of more than traces of brown or black carbonized material.

METHODS OF DETERMINING LEAD IN INORGANIC RESIDUES. A search of the literature for gravimetric and volumetric methods

suitable for determining lead, in inorganic residues yielded no methods of promise that had not already been incorporated in the various methods for determining the lead content of gasolines. Practically all the better methods, not already associated with this field, were inapplicable to the amounts of lead that might be found in 100 ml. of gasoline for the accuracy required.

EXPERIMENTAL

Evaluation of Typical Existing Methods for Determining Lead in Gasolines. In order to determine the possible merits of the various methods listed above, preliminary quadruplicate determinations were carried through each procedure following the details given in the original reference; no attempt at improvements was made. A regular commercial grade of Ethyl gasoline (saturated) was used as sample. Based on the experience gained in the preliminary tests, the methods were evaluated according to the following considerations: (1) common usage at the present; (2) apparent reproducibility; (3) apparent relative accuracy; (4) ease of manipulation; (5) time required for analysis. Those methods that were judged important from these considerations were selected for further comparison on the basis of their ultimate precision. Repeated determinations were made by the eight chosen procedures (Table I).

In order of decreasing precision, the best results were obtained by the method of Schwartz (*16*), Gonick and Milano (*14*), Ferreri (modified, *12*), and the early Shell method (No. 1). It was concluded that these four methods give satisfactory precise results for straight-run gasolines. No significance was attributed to the variations of the mean results (Table I), since the work was done over a period of several months on different batches of gasoline. However, all tests by a given procedure were made simultaneously on samples taken from the same batch of gasoline.

Determination of Lead in Inorganic Residues. In order to establish the accuracy and precision of the determination of inorganic lead, several accepted methods used for lead analysis were compared. The standard deviation and systematic error of each method were determined by quadruplicate analysis of portions of a standard lead nitrate solution.

The lead nitrate solution was carefully prepared from a weighed amount of C.P. "test lead". A sample of the test lead was found by spectrographic analysis to be free from noticeable traces of any impurity; the lead granules had a very bright luster and gave no appreciable loss in weight upon heating at 120° C. for several hours. From 280 mg. of test lead ignited lead sulfate precipitates equivalent to 99.83% lead were obtained, this figure being the result of the analysis of eight samples.

Method A. Evaporate to fumes with sulfuric acid; precipitate lead sulfate with 50% ethanol, digest at room temperature; filter in Gooch crucible and weigh after ignition at 650° C. (*8*).

Method B. Evaporate to fumes with sulfuric acid (twice); precipitate lead sulfate with ice water, let stand for several hours in a bath of ice water; filter in Gooch crucible and ignite at 650° C. (*2*).

Method C. Evaporate to dryness, add 3 ml. of concentrated nitric acid; neutralize with ammonium hydroxide and make acid with acetic acid, dilute to 450 ml.; precipitate lead chromate in boiling solution, digest while hot; filter while warm through Gooch crucible and dry at 120° C. (*15*).

Method D. Evaporate to fumes with sulfuric acid; precipitate lead sulfate with water at room temperature, digest at room temperature; filter, dissolve lead sulfate in dilute acid ammonium acetate; dilute to 150 ml., titrate hot solution with ammonium molybdate solution, using tannin as indicator (spot plate); determine blank; standardize molybdate solution directly against pure lead (*7*).

Method E. Evaporate to dryness; add 3 ml. of concentrated nitric acid; neutralize with ammonium hydroxide and make acid with acetic acid, dilute to 150 ml.; titrate hot solution with molybdate solution as in Method D (*5*, *11*).

Method F. Evaporate to fumes in a large crucible with sulfuric acid; fume off the sulfuric acid leaving lead sulfate; ignite at 650° C. (*18*).

Method G. Evaporate to dryness; dissolve and make acid with acetic acid; add excess standard dichromate at room temperature; boil to precipitate lead chromate; filter; add iodide and hydrochloric acid to filtrate; titrate with standard thiosulfate; standardize thiosulfate against potassium dichromate as primary standard (*10*).

Method H. Evaporate to dryness, add 3 ml. of concentrated nitric acid; neutralize with ammonium hydroxide and make acid with acetic acid; add ammonium acetate, dilute to 150 ml., precipitate lead chromate in boiling solution with 15 ml. of 10% potassium dichromate, filter through Gooch crucible at room temperature; dry at 120° C. (*5*).

Method I. Evaporate to dryness, dissolve in water, and add sodium chloride; neutralize carefully to methyl red indicator, titrate with alternate portions of 8-hydroxyquinoline solution and standard base solution until a permanent end point is obtained with phenol red indicator (*14*).

Table II contains the results obtained by analyzing samples containing 0.0700 gram of test lead. Apparently all methods except B, C, and D are satisfactory for the amount of lead realizable from 100 ml. of gasoline.

In order to clarify the unexpected poor precision found for the molybdate-tannin direct titration (Method E), standard lead solutions containing varying amounts of lead were titrated by this method. It was found (Table III) that, although the method tends to be erratic, it gives reasonably reproducible results when dealing with 0 to 70 mg. of lead. However, the spot-plate color indication of the end point was difficult and tedious. Experiments with various suggested substitutes for tannin as indicator (*19*, *20*) failed to reveal any with advantages over the usual 0.5% tannin solution.

Brunck (*4*) has proposed the analysis of lead solutions by precipitating and weighing the lead as lead sulfide. Experiments with this method indicated that it lacks accuracy and precision, even when applied under carefully controlled conditions.

Volumetric methods for determining lead, using internal indicators, have been developed making use of hydrolytic (*22*) reactions and precipitation (*6*) reactions. Extensive work in this field has indicated that an accurate, precise, and quick determination of lead may be made by hydrolytic and precipitation titrations, with one of several reagents, provided solutions of pure lead salts are used. However, attempts to apply these methods to the oxidized inorganic residues obtained from gasoline have met with failure; this failure is generally caused by the presence of small amounts of some material that combines with and precipitates some of the lead under the conditions of the test or that interferes with the means for determining the end point.

Table II. Standard Deviation and Systematic Error of Various Lead Methods

Method No.	Lead Found, Gram[a]	Mean	Standard Deviation	Systematic Error
A	0.0700, 0.0697, 0.0697, 0.0701	0.0699	0.00018	−0.0001
B	0.0689, 0.0674, 0.0677, 0.0682	0.0682	0.00062	−0.0018
C	0.0691, 0.0688, 0.0691, 0.0692	0.0691	0.00016	−0.0009
D	0.0674, 0.0684, 0.0674, 0.0679	0.0678	0.00042	−0.0022
E	0.0704, 0.0704, 0.0694, 0.0704	0.0702	0.00046	+0.0002
F	0.0700, 0.0704, 0.0704, 0.0709	0.0704	0.00034	+0.0004
G	0.0698, 0.0694, 0.0694, 0.0690	0.0694	0.00028	−0.0006
H	0.6097, 0.0701, 0.0696, 0.0695	0.0697	0.00023	−0.0003
I	0.0700, 0.0704, 0.0703, 0.0706	0.0703	0.00021	+0.0003

[a] Lead taken, 0.0700 gram.

Table III. Applicability of Molybdate-Tannin Titration to Varying Quantity of Lead

Lead Taken	Lead Found	Mean	Standard Deviation
Gram	*Gram per ml., molybdate solution*		
0.035	0.005036, 0.005036, 0.005000, 0.005073	0.005036	0.000025
0.070	0.005036, 0.005053, 0.005020, 0.005053	0.005040	0.000014
0.070[a]	0.005020, 0.005036, 0.005073, 0.005020	0.005036	0.000021
0.100	0.005076, 0.005051	0.005063	
0.0	0.00010, 0.00010, 0.00010[a], 0.00010[a]	0.00010	

[a] Run by artificial light in a dark room.

Volumetric methods depending on the indirect determination of lead by oxidimetric titration of the precipitate (*13*, *21*), or excess precipitant (*10*, *13*, *17*), have been applied to pure inorganic residues. The authors' experiments indicate that these principles may be applied to pure lead salt solutions by precipitating lead iodate and lead chromate, and that the precision and accuracy of the methods are satisfactory. When these methods are applied to inorganic residues from gasolines, the lead chromate method gives the most reproducible and accurate results. It has been found that it is better to titrate the excess dichromate ions than to titrate the lead chromate precipitate because of technical difficulties in preparing the precipitate for a titration of the desired accuracy.

With the method suggested by Epler (*10*) as a basis, a procedure was developed for determining lead; an excess of standard potassium dichromate is used to precipitate the lead, the lead chromate is filtered off, and the excess dichromate ions are titrated iodometrically. The details of this method, referred to below as the "excess-dichromate" method, are given in the following proposed methods for determining the lead content of gasoline. The standard deviation and systematic error of the excess-dichromate method are given in Table IV. This method gives reliable and accurate results when applied to any gasoline residue that has been treated with nitric, perchloric, or sulfuric acids, provided the residue is completely soluble in ammonium acetate solution just acid with acetic acid. It is applicable in the presence of 5% of ammonium acetate, or alkali salts, but low results may be found in the presence of appreciable amounts of ammonium salts other than ammonium acetate. The accuracy of the excess-dichromate method is independent of the amount of excess dichromate present. The iodometric titration of the lead chromate precipitate, when dissolved in sodium chloride–hydrochloric acid solution, gives less precise results that tend to be 0.0002 to 0.0006 gram high.

Table IV. Precision, Accuracy, and Applicability of Excess-Dichromate Method for Lead

Lead Taken	0.15 *N* $K_2Cr_2O_7$	Lead Found	Mean	Standard Deviation	Systematic Error
Gram	*Ml.*	*Gram*	*Gram*	*Gram*	*Gram*
0.0700	10.00	0.0702, 0.0700, 0.0700, 0.0700 0.0700, 0.0701, 0.0701, 0.0701 0.0700, 0.0700, 0.0700, 0.0700 0.0700, 0.0700, 0.0696, 0.0696	0.0700	0.00016	0.0000
0.0700[a]	10.00	0.0702, 0.0698	0.0700	.....	0.0000
0.0280	10.00	0.0274, 0.0284	0.0279	.....	−0.0001
0.0560	10.00	0.0562, 0.0559	0.0561	.....	+0.0001
0.0700	10.00	0.0701, 0.0701	0.0701	.....	+0.0001
0.0840	10.00	0.0839, 0.0840	0.0840	.....	0.0000
0.0700[b]	10.00	0.0702, 0.0700, 0.0698, 0.0700	0.0700	.....	0.0000

[a] Determination made in presence of $KClO_4$.
[b] Determination made in presence of $PbSO_4$ and K_2SO_4.

The excess-dichromate method also has distinct operational advantages over the molybdate titration method. The time per analysis for single tests is approximately the same as for the molybdate titration; for multiple analyses there is a distinct advantage in time for the excess-dichromate method. In addition, the starch end point is generally preferable to the tannin spot-plate end point used for the molybdate titration. Generally, less experience is required for dependable results by the excess-dichromate method than by other volumetric methods.

The gravimetric determination of lead as lead chromate is not recommended for the small amounts of lead usually found in 100 ml. of gasoline. The principal objection is the varying composition of the dried or ignited lead chromate. The precipitate theoretically contains 64.11% lead; in some cases 63.9% lead (*1*) has been taken as a more correct figure. The conditions of precipitation and ignition seem to have some influence on the composition of the precipitated lead chromate. Various common procedures used in precipitating and igniting the lead chromate are compared in Table V. Details of precipitation procedures have been given above.

Table V. Comparison of Gravimetric Chromate Methods for Lead[a]

Method	Lead Taken	Lead Found: $PbCrO_4$ (dried)	Lead Found: $PbCrO_4$ (ignited)
	Gram	*Gram*	*Gram*
C	0.0700	0.0692, 0.0696	.....
G	0.0700	0.0696, 0.0698	.....
H	0.0700	0.0697, 0.0701, 0.0696, 0.0695	0.0696, 0.0693
H	0.1400	0.1401, 0.1402, 0.1401	0.1398, 0.1396

[a] Drying at 110° C.; ignition at 600° C. Factor for Pb in $PbCrO_4$ taken as 0.6411.

These results and the data given in Table II indicate that whenever a gravimetric method for lead is required, the lead is best precipitated and weighed as lead sulfate. The precipitation is made in a 50% ethanol solution containing excess sulfuric acid. The procedure (based on Method A) is essentially as follows:

Add 12 ml. of concentrated sulfuric acid to the lead solution that contains no acid-insolubles. Evaporate to fumes of sulfuric acid and continue the fuming until a water-clear liquid results. Use nitric acid or hydrogen peroxide to aid in the removal of organic matter. Cool to room temperature and quickly add 100 ml. of 50% ethanol. Allow the mixture to stand for several hours, and filter through a tared Gooch crucible or porous-bottomed porcelain crucible. Transfer and wash the precipitate with 50% ethanol containing 4 ml. of concentrated sulfuric acid in 100 ml. of solution. Finally wash once with absolute ethanol, dry for 15 minutes at 100° C., and ignite to constant weight at 600° C.

The standard deviation of this lead sulfate method (Table II, Method A) is ±0.00018 gram; the systematic error is −0.0001 gram.

Decomposition of Organic Lead Compounds with Hydrochloric Acid. Refluxing a gasoline sample with concentrated hydrochloric acid was successful in extracting the lead from the gasoline. Two methods were used: (1) A.S.T.M. method D526 (*5*) (Method 11) in which the gasoline is extracted with boiling concentrated hydrochloric acid and boiling water; and (2) the modified method of Ferreri (*12*) (Method 10) in which the gasoline is refluxed with concentrated hydrochloric acid and the resulting mixture is evaporated to dryness. The success in obtaining results of good reproducibility with these methods suggested that more simple conditions might be used to decompose tetraethyllead dissolved in gasoline.

Further study of A.S.T.M. method D526 (*5*) showed plainly that the decomposition of the organic lead compounds took place quickly and that the prolonged time of reflux is necessary only for extracting the inorganic lead compounds from the gasoline phase. These findings were further substantiated by the fact that more hydrogen chloride than would be required to react with the tetraethyllead present would quickly dissolve in the gasoline layer when the gasoline was refluxed with concentrated hydrochloric acid. For comparison, a solution of sulfuric acid (equivalent in acid strength to concentrated hydrochloric acid) was found to extract only a small fraction of the lead when it was refluxed with the gasoline in a similar manner. Complete extraction was also achieved using 6 *N* hydrochloric acid. Apparently contact with an acid in itself does not cause decomposition of tetraethyllead under these conditions.

The foregoing observations seem to indicate that tetraethyllead in gasoline can be completely decomposed by shaking the gasoline, at room temperature, with concentrated hydrochloric acid. This contention was supported by successful extraction, at room temperature, of all the lead from gasoline that had been shaken with concentrated hydrochloric acid, by the use of 6 *N* hydrochloric acid in one instance, and of dilute acetic acid–ammonium acetate solution in another instance. In these experiments 100

ml. of gasoline were shaken with 50 ml. of concentrated hydrochloric acid before the extraction trials were made. The extractions were time-consuming when carried out at room temperature. It was also found that the lead chloride compound, formed by the reaction of the hydrogen chloride and tetraethyllead, has a marked tendency to adhere tenaciously to glass surfaces.

To indicate further that the decomposition of tetraethyllead with hydrogen chloride was rapid, 100 ml. of gasoline and 50 ml. of concentrated hydrochloric acid were placed in a 500-ml. Erlenmeyer flask, and the contents of the flask were immediately evaporated to dryness on a hot plate with a surface temperature of 250° C. Analysis of the residue showed that none of the lead was lost by volatilization. If the decomposition of the tetraethyllead were slow, some of the lead should have been lost through the volatilization of unchanged tetraethyllead. However, further tests indicated that it was necessary to mix the gasoline and acid prior to evaporation in order to ensure complete decomposition.

These observations indicate that complete conversion of the tetraethyllead to inorganic lead compounds can be expected after thoroughly mixing the gasoline with concentrated hydrochloric acid, and evaporating the mixture to dryness. After many experiments, the following conditions were found to be adequate: Shake 100 ml. of gasoline with 50 ml. of concentrated hydrochloric acid for 5 minutes in a glass-stoppered Erlenmeyer flask; allow the mixture to stand 10 minutes, and evaporate the mixture to dryness. The residue is analyzed for lead by any appropriate procedure. This method decomposes tetraethyllead that is dissolved in any saturated, cracked, or blended gasoline motor fuel.

Attempts were made to decompose the tetraethyllead in gasoline by addition of glacial acetic acid, trichloroacetic acid, acetyl chloride plus water, or gaseous hydrogen chloride; after thorough mixing, the mixtures were evaporated to dryness. In no case did the recovery of lead exceed 75%.

Removal of Organic Matter Formed by Evaporation of Gasoline–Hydrochloric Acid Mixtures. The evaporation of saturated gasolines in the presence of concentrated hydrochloric acid leaves only small amounts of organic matter associated with the lead chloride residue. Oxidation with concentrated nitric acid suffices to remove the organic material, so that there is no appreciable interference with the subsequent lead determination. However, when cracked gasolines are similarly treated, polymerized and charred materials are found in the lead chloride residue. These polymerized materials are, as a rule, incompletely removed by several evaporations with concentrated or fuming nitric acid. The oxidized organic matter remaining after the nitric acid treatment precipitates lead ions in a water solution containing ammonium acetate and a small amount of excess acetic acid. Therefore, the lead cannot be accurately determined either by the molybdate titration method or by the excess-dichromate method unless these organic substances are completely removed.

Interfering organic material can be completely oxidized by fuming with perchloric acid after the usual nitric acid oxidation. When the fuming is carried out in an Erlenmeyer flask, so that a steady reflux of perchloric acid takes place down the sides of the flask, all the organic matter is removed in 1 or 2 hours. Concentrated nitric acid added to 70% perchloric acid is helpful where the organic residue resists decomposition. The flask must be covered by an efficient spray trap to avoid mechanical losses during the fuming periods. The evaporation should not go to dryness; optimum results are obtained when the flask contains approximately 0.5 ml. of liquid at the end of the fuming period. It is necessary to neutralize the excess perchloric acid with a strong base before the lead analysis is made; neutralization with ammonium hydroxide often produces low results in the subsequent lead analysis. This drastic treatment is necessary only with highly cracked gasolines.

The interfering organic matter is completely removed by fuming with sulfuric acid along with occasional additions of nitric acid or hydrogen peroxide, or both. This is a desirable and satisfactory means when determining the lead gravimetrically as lead sulfate. It is also usable in conjunction with the excess-dichromate method but only after carefully removing almost all the sulfuric acid by evaporation, a slow and tedious process. For this reason, the perchloric acid oxidation is preferred.

Decomposition of Organic Lead Compounds by Halogens. Halogens like bromine and iodine give almost instantaneous decomposition of tetraethyllead with the formation of a lead halogen compound insoluble in gasoline. Bromine is unsatisfactory for unsaturated gasoline because it reacts energetically with olefins, producing very undesirable bromination products and tending to prevent reaction with tetraethyllead. Evaporation of an unsaturated gasoline treated with excess bromine gives a residue containing an excessive amount of carbon (often several grams). Difficult oxidation renders this method impractical.

It was found that iodine behaves in a manner similar to bromine but with considerably less reaction with unsaturated hydrocarbons, a fact that was independently observed by Gonick and Milano (*14*). Thus, when a mixture of unsaturated gasoline and excess of iodine is evaporated, all the lead is found in the residue which is readily oxidized with sulfuric and nitric acids. However, iodine produces considerably more organic residue than is produced by hydrochloric acid under comparable conditions of unsaturation; so much so that it precludes the safe application of perchloric acid even after several treatments of nitric acid.

The use of iodine for decomposing tetraethyllead followed by evaporation of the mixture was investigated for two reasons: (1) petroleum laboratories are reluctant to use perchloric acid even in carefully worked out procedures; and (2) the mixture of iodine and gasoline can be evaporated to dryness faster and with less tendency to spatter than a two-phase mixture of hydrochloric acid and gasoline. However, decomposition by direct evaporation with hydrochloric acid was found preferable to decomposition by iodine because it produces a smaller organic residue under comparable conditions.

RECOMMENDED METHODS FOR DETERMINATION OF TETRAETHYLLEAD

The following two methods have been thoroughly tested and found to give satisfactory results on all types of aviation and automotive gasoline or motor fuel. Unsaturated hydrocarbons, acids, bases, and other common organic compounds do not interfere and do not require preliminary treatment. Both methods are applicable to tetraethyllead fluids and concentrates.

The direct hydrochloric acid decomposition method is recommended for general application because, except for gasolines unusually high in olefins, it leaves a residue that can be freed from organic matter by oxidation with nitric acid alone. It is also recommended for highly unsaturated gasolines, or other fuel blends leaving appreciable organic residues, because the perchloric acid oxidation requires less attention than oxidation with nitric and sulfuric acids. The iodine decomposition–iodometric method is recommended in those cases in which the hydrochloric acid decomposition method leaves a residue requiring use of perchloric acid, when the use of this acid is not possible or allowable.

DIRECT HYDROCHLORIC ACID DECOMPOSITION METHOD

The gasoline is shaken with cold hydrochloric acid and the mixture evaporated to dryness. Organic material remaining in the residue is removed by digesting with nitric acid and, if necessary, perchloric acid. After the lead chloride residue is dissolved, the lead is determined volumetrically by adding a known volume of dichromate solution, filtering out the precipitated lead chromate, and determining the excess dichromate iodometrically.

Apparatus. Volumetric flasks, 100-ml. and 50-ml., calibrated for gasoline delivery at 15.6° C.

Electric hot plate, with a thin asbestos covering, adjusted to maintain a temperature of 230° to 260° C. as measured by means of a thermometer placed so that it lies flat on the heating surface with its bulb near the center of the surface.

Air-jet evaporator, having an orifice 3 to 5 mm. in diameter and so constructed that a stream of clean, filtered air may be introduced at a rate of not more than 5 liters per minute into a 500-ml. Erlenmeyer flask at a point at least 30 mm. above the surface of the liquid in the flask.

It is desirable that the evaporator also be equipped with a means of heating the air stream to 150° to 175° C. (measured with a thermometer held with its bulb in the air stream at a point 10 mm. below the orifice).

Special Reagents. Sodium thiosulfate solution, standard 0.1 *N*. For highest accuracy, standardize according to the procedure given below in Procedure against 25 ml. of a standard lead nitrate solution which contains 4 mg. of lead per ml.

Starch Solution. Prepare a paste of 5 grams of arrowroot starch and add to 2 liters of boiling water. Boil for 5 to 10 minutes. To preserve this solution, add 5 to 10 mg. of C.P. mercuric iodide to the boiling solution, allow to settle, and use only the clear supernatant liquid.

Gasoline diluent, lead-free, saturated, aviation-gasoline base stock.

Sample. Obtain the sample in a dark-colored glass bottle whenever possible. Fill the container as full as possible consistent with safety, and keep tightly stoppered to avoid loss of volatile constituents and decomposition of the tetraethyllead by acid vapors in the atmosphere. Do not allow the sample to stand more than one or two days before analyzing.

Measure all samples within 2 hours after opening the container; do not sample from partly filled containers except for approximate work. In sampling, it is recommended that the temperature be adjusted to 15.6° ± 2° C. by placing the container in a bath at that temperature for 30 minutes or longer before opening. For convenience, samples may be measured at moderate room temperatures and the volumes corrected to 60° F. (see Table VII); however, to avoid excessive evaporation losses during sampling, samples must not be taken at temperatures above 30° C. (86° F.).

Procedure. Into a 500-ml. glass-stoppered Erlenmeyer flask, introduce quantities of sample and gasoline diluent as indicated in Table VI. For gasoline samples, measure and record the temperature (to 1° C.) or, preferably, adjust the temperature to 15.6° ± 2° C. Measure gasoline samples by means of volumetric flasks which have been calibrated for gasoline delivery at 15.6° C.; weigh tetraethyllead fluid in a Lunge pipet.

Table VI. Recommended Sample Sizes

Type of Sample	Range, Ml. of TEL per Gallon	Sample, Ml.	Diluent, Ml.
Gasoline	0 to 3	100	.....
	3 to 6	50	45 to 50
TEL fluid	39 to 45% Pb	0.15 to 0.25 gram[a]	90 to 100

[a] Weights based upon present commercial tetraethyllead fluids and an optimum of 70 to 100 mg. of lead present in analysis.

Add 50 ml. of 12 *N* hydrochloric acid, stopper tightly, and swirl briefly. Loosen the stopper to release any pressure generated, restopper tightly, and shake vigorously for 5 to 6 minutes, keeping the stopper firmly in place to avoid any loss of liquid. Allow the mixture to stand for 10 to 15 minutes. Carefully remove the stopper and rinse it with a small quantity of water. Evaporate the contents of the flask to complete dryness on the hot plate, aiding the evaporation by use of the air jet. If a sublimate appears on the neck of the flask, remove by carefully heating with a Bunsen flame.

Note. Use of the air jet appreciably shortens the evaporation time, aids in removing the heavy ends, and reduces spattering when analyzing unsaturated gasolines. When analyzing saturated aviation gasolines, satisfactory results can be obtained without use of the air jet.

Allow the flask to cool, add 5 ml. of 16 *N* nitric acid, and without using the air jet evaporate slowly to dryness on a hot plate. If the resulting residue contains more than traces of brown or black charred material, repeat the nitric acid evaporation, taking care that the acid contacts any residue which had spattered onto the sides of the flask. If the resulting residue still shows more than traces of carbonized materials, add 5 ml. of 16 *N* nitric acid and 2 ml. of 70% perchloric acid, place a short-stemmed funnel covered with a ribbed watch glass in the neck of the flask, and evaporate to fumes. (*Caution.* If improperly carried out, the use of perchloric acid may lead to an explosion; therefore, the face and body should be protected by a mask or shield during the fuming with perchloric acid.) Fume for 1 hour or until the liquid in the flask becomes colorless.

Note. Sufficient heat must be supplied by the hot plate to cause the perchloric acid to reflux freely down the sides of the flask. If this refluxing does not take place, the oxidation may be incomplete. Do not evaporate to dryness, but regulate conditions so that approximately 0.5 ml. of liquid remains in the flask at the end of the perchloric acid treatment. The covered funnel is necessary to trap any spray losses.

Wash down the flask (and funnel if used) with approximately 75 ml. of water and boil the solution for several minutes. Neutralize the solution to litmus with 6 *N* sodium hydroxide solution and make the solution just acid to litmus with 50% acetic acid solution. (Presence of a white precipitate at this point may indicate incomplete oxidation of the organic residue; more than a mere cloudiness will cause the results to be in error.)

Add 2 grams of ammonium acetate and, from a pipet, 20 ml. of 0.1 *N* potassium dichromate solution. Heat the solution just below boiling until the precipitate changes to an orange color and settles out readily. Cool to room temperature; filter through a No. 42 Whatman filter paper into a 500-ml. Erlenmeyer flask and wash the flask and the filter with three portions of cold 2% ammonium acetate solution. To the combined filtrate and washings add 10 ml. of 12 *N* hydrochloric acid and 10 ml. of 10% potassium iodide solution. Allow the solution to stand 3 to 4 minutes and titrate with standard 0.1 *N* sodium thiosulfate solution until the solution turns yellow; add 3 to 5 ml. of starch solution and continue the titration, drop by drop, until the dark blue color changes to a clear green. Mix well by swirling after each increment of thiosulfate solution.

Make duplicate blank determinations by repeating the entire procedure but omitting the sample; use the same amount of potassium dichromate solution as added in analyzing the sample. Use the average of the blank titrations in calculating the results.

Calculation. *Gasoline Samples.* Calculate the lead content as milliliters of tetraethyllead per U. S. gallon at 60° F. by means of the following equation:

$$\text{Tetraethyllead, ml. per gal. at 60° F.} = \frac{(B - S)(N)(247.4)}{V}$$

where

S = ml. of sodium thiosulfate solution used in analysis
B = average volume of sodium thiosulfate solution used in blank determinations
N = normality of sodium thiosulfate solution
V = volume of sample at 60° F., ml. If sample was measured at a temperature other than 15.6 ± 2° C., use for V the appropriate corrected volume given in Table VII.

Tetraethyllead Fluid. Calculate the lead content by means of one of the following equations:

$$\text{Lead content, \% by weight} = \frac{(B - S)(N)(6.91)}{W}$$

$$\text{Tetraethyllead, ml. per gram} = \frac{(B - S)(N)(0.06535)}{W}$$

where

S = ml. of sodium thiosulfate solution used in analysis
B = average volume of sodium thiosulfate solution used in blank determinations
N = normality of sodium thiosulfate solution
W = weight of sample, grams

IODINE DECOMPOSITION-IODOMETRIC METHOD

The tetraethyllead is decomposed by mixing the gasoline with a solution of iodine in carbon tetrachloride. The mixture is evaporated to dryness and any remaining organic material de-

Table VII. Gasoline Volumes Corrected to 60° F.

Temperature, ° C.	Volume at 60° F., Ml.	
10	50.4[a]	100.7[b]
15	50.1	100.1
15.6	50.0	100.0
20	49.8	99.5
25	49.4	98.8
30	49.1	98.2

[a] 50-ml. sample measured.
[b] 100-ml. sample measured.

Table VIII. Precision and Accuracy of Recommended Direct Evaporation Methods Applied to Leaded Gasoline and Tetraethyllead Fluid

(All results for gasoline in ml. of TEL per U. S. gallon at 15.6° C.; results for fluid in % by weight)

Material	Lead Content: True	Lead Content: Found	Mean	Standard Deviation	Systematic Error
Hydrochloric Acid Decomposition Method					
Saturated aviation gasoline[a]	0.00	<0.01, <0.01, <0.01	<0.01	...	.....
	....	2.06, 2.07, 2.05, 2.07, 2.05, 2.06, 2.07	2.06	0.008	.....
	....	3.08, 3.05, 3.06, 3.07	3.065	0.011	.....
	3.08	3.07, 3.09	3.08	...	0.00
	3.08	3.08, 3.08, 3.06	3.075	...	−0.005
	0.05	0.06, 0.06, 0.06, 0.06	0.06	0.00	+0.01
	0.27	0.28, 0.28, 0.28, 0.28	0.280	0.00	+0.01
Unsaturated motor gasoline[b]	1.02	1.02, 1.02, 1.02, 1.03	1.023	0.024	+0.003
	2.14	2.12, 2.14, 2.15, 2.16, 2.16, 2.13, 2.13	2.14	0.015	0.00
	2.03	2.04, 2.03, 2.02, 2.04	2.03	0.009	0.00
	3.03	3.01, 3.01, 3.02, 3.01	3.013	0.004	0.017
	3.63	3.63, 3.64, 3.63, 3.63	3.63	0.005	0.00
	3.63	3.62, 3.61, 3.62, 3.60, 3.62, 3.62, 3.61, 3.62	3.62	0.009	−0.00
	3.94	3.95, 3.94, 3.93, 3.95	3.94	0.009	0.01
	4.64	4.64, 4.64, 4.65, 4.63	4.64	0.007	0.00
	5.81	5.81, 5.79, 5.82, 5.76	5.795	0.023	−0.015
Tetraethyllead fluid	40.35[d]	40.30, 40.36	40.33	...	<0.1
	39.68[e]	40.00, 40.00, 39.90, 39.90	39.95	0.05	+0.27
Iodine Decomposition—Iodometric Method					
Unsaturated motor gasoline[b]	3.63	3.62, 3.64, 3.63, 3.63, 3.64, 3.63, 3.63	3.63	0.007	0.00
	3.94	3.95, 3.94, 3.94	3.94	...	0.00

[a] 5% or less of olefins.
[b] Approximately 50% olefins.
[c] Oxidation by HNO_3 and H_2SO_4 instead of $HClO_4$.
[d] By A.S.T.M. D526.
[e] By bromine–lead sulfate gravimetric method (4 tests).

stroyed by treatment with sulfuric and nitric acids. The lead salts are dissolved in ammonium acetate solution and the lead is determined volumetrically by adding a known volume of dichromate solution, filtering out the precipitated lead chromate, and determining the excess dichromate iodometrically.

Apparatus, Special Reagents, and Sample. Same as in preceding method.

Procedure. Into a 500-ml. Erlenmeyer flask, introduce quantities of sample and gasoline diluent as indicated in Table VI. For gasoline samples, measure and record the temperature (to 1° C.) or, preferably, adjust the temperature to 15.6° ± 2° C. Measure gasoline samples by means of volumetric flasks that have been calibrated for gasoline delivery at 15.6° C.; weigh tetraethyllead fluid in a Lunge pipet.

Add 50 ml. of the iodine solution (saturated solution in carbon tetrachloride), swirl to mix, and allow to stand for 2 to 3 minutes. Evaporate to complete dryness on the hot plate, aiding the evaporation by use of the air jet. If a sublimate appears on the neck of the flask, remove by carefully heating with a Bunsen flame.

Note. Use of the air jet appreciably shortens the evaporation time, aids in removing the heavy ends, and reduces spattering when analyzing unsaturated gasolines. When analyzing saturated aviation gasolines, satisfactory results can be obtained without use of the air jet.

To the residue, add 2 ml. of 18 *N* sulfuric acid and 20 ml. of 16 *N* nitric acid. Evaporate to strong sulfuric acid fumes on a hot plate without using the air jet. Allow the flask to cool for a few minutes and repeat the evaporation with 3- to 5-ml. portions of 16 *N* nitric acid until a light yellow-colored solution is obtained. Add 1 to 2 ml. of 30% hydrogen peroxide with swirling and evaporate to sulfuric acid fumes. If the solution is not colorless (or nearly so), repeat the treatment with nitric acid and hydrogen peroxide. Should additional sulfuric acid be required during the oxidation procedure, replenish with not more than 2 ml. of the 18 *N* acid. After a colorless solution has been obtained, remove the excess sulfuric acid by evaporating to dryness with the aid of an air stream, using only enough air to keep the sulfuric acid fumes coming off at a steady rate. If the residue is dark in color, add 3 to 5 ml. of 16 *N* nitric acid and evaporate to dryness. Add 70 to 75 ml. of distilled water, boil the solution for several minutes, make the solution neutral to litmus with 6 *N* sodium hydroxide, then make just acid with acetic acid. Add 2 grams of ammonium acetate, heat to boiling, and boil for 2 to 3 minutes. If the residue does not dissolve, add another 2 grams of ammonium acetate (but no more) and boil until solution occurs.

By means of a pipet, add 20 ml. of 0.1 *N* potassium dichromate solution. Heat the solution just below boiling until the precipitate changes to an orange color and settles out readily. Cool to room temperature; filter through a No. 42 Whatman filter paper into a 500-ml. Erlenmeyer flask and wash the flask and the filter with three portions of cold 2% ammonium acetate solution. To the combined filtrate and washings add 10 ml. of 12 *N* hydrochloric acid and 10 ml. of 10% potassium iodide solution.

Allow the solution to stand 3 to 4 minutes and titrate with standard 0.1 *N* sodium thiosulfate solution until the solution turns yellow; add 3 to 5 ml. of starch solution and continue the titration, drop by drop, until the dark blue color changes to a clear green. Mix well by swirling after each increment of thiosulfate solution.

Make duplicate blank determinations by repeating the entire procedure but omitting the sample; use the same amount of potassium dichromate solution as added in analyzing the sample. Use the average of the blank titrations in calculating the results.

Calculation. Same as in preceding method.

DISCUSSION

During the past 5 years the direct hydrochloric acid decomposition method has been applied successfully to more than a thousand samples of gasoline. The samples tested have been primarily of the saturated type, but they have included all varieties of experimental gasoline blends and motor fuels that have been encountered since the beginning of the present emphasis on aviation gasoline. The method has given satisfactory results on samples of unsaturated gasoline to which have been added several per cent of typical volatile organic compounds, including aldehydes, ethers, amines, esters, and alcohols. Only a comparatively few samples have been analyzed by the iodine decomposition–iodometric method, but satisfactory results have been obtained in all tests, even though all samples were highly unsaturated.

The method is most advantageous in simultaneous analysis of a large number of samples; the various operations (such as evaporation, filtering, titration, etc.) are such that 10 to 15 samples can conveniently be carried along together, each sample requiring a minimum of individual attention. Another attractive feature is the lack of any special apparatus or technique other than that common to general analytical practice. The elapsed time per analysis is generally 3 to 4 hours, depending on the degree of unsaturation of the sample. However, 12 to 15 individual saturated gasoline samples or 8 to 10 highly unsaturated samples can be completed in a normal 8-hour day. Untrained as well as trained analysts generally master the method in several days of steady application. Another desirable feature is that the method is applicable to any gasoline without knowledge of its previous treatment or of possible contaminants present.

The precision and accuracy of the recommended direct decomposition procedures were determined by analysis of synthetic gasoline blends. The blends were made by mixing weighed portions of aviation base stock or unsaturated motor stock with weighed portions of carefully analyzed tetraethyllead fluid in such a manner that the lead content of the mixture was known to

2 parts in a thousand. The tetraethyllead fluid was analyzed by adding the sample to carbon tetrachloride, adding an excess of bromine, carefully evaporating to dryness, and determining the lead gravimetrically as lead sulfate or volumetrically by the excess-dichromate method.

As shown in Table VIII, the precision and accuracy of both methods are within the following maximum amounts:

	Difference of Result from Mean	
	One operator and apparatus	Different operators and apparatus
Gasoline, ml. TEL per gal.	0.01	0.02
TEL fluid, % Pb	0.2	0.4

	Difference of Result from True Value
Gasoline, ml. TEL per gal.	0.02
TEL fluid, % Pb	0.4

The application of these methods by inexperienced analysts has served to emphasize certain technical difficulties. Since these difficulties may be encountered in other laboratories employing this method for the first time, the following suggestions found useful and expedient in analyzing difficult samples are offered:

1. While the methods as written are readily applicable to saturated or unsaturated hydrocarbons, it is suggested that the analyst familiarize himself with the technique by analyzing ordinary saturated aviation gasoline blends before attempting other samples. In such tests, perchloric or sulfuric acid oxidation is not necessary, nitric acid sufficing.
2. For unsaturated samples, for gasolines of unknown composition, and for those containing more than 3 ml. of tetraethyllead per gallon, it is advisable to use 50-ml. samples mixed with 50 ml. of saturated aviation gasoline base stock. This is not to reduce loss of volatile lead but to avoid unnecessary problems in the determination of the lead or in oxidizing extraneous organic material. In some cases, this obviates the necessity of using perchloric or sulfuric acids, repeated oxidation with nitric acid being sufficient.
3. Any mechanical loss during shaking operations must be avoided in application of the hydrochloric acid decomposition method. After the shaking is completed, the stopper must be held firmly in place until all liquid has drained away from the stopper.
4. In the initial evaporation, the flask should be allowed to go completely dry (free from vapor) while standing on the hot plate maintained at the specified temperature; in fact, a short baking period is desirable. Any sublimate in the neck of the flask should be removed by careful heating with the flame of a burner. These conditions greatly expedite the removal of organic matter later and avoid entirely any possibility of "flashing" when the residue is treated with nitric acid.
5. The use of the air-jet evaporator greatly speeds up evaporations, but is not absolutely necessary except to avoid bumping and spattering when analyzing unsaturated gasolines. The use of hot air not only speeds up evaporations but, more important, is more effective in avoiding loss by spattering.
6. If the dry residue is given several thorough preoxidations with nitric acid (taking care to treat any spatterings), there appears to be no danger in the subsequent use of perchloric acid; no instances of explosions are on record in this laboratory. It is important to use the funnel-cover glass spray trap arrangement and to continue the perchloric acid treatment until oxidation is complete.
7. Unless the analyst is experienced in the use of the excess-dichromate lead method, it is advisable each day to make the blank determination specified and to determine the normality of the thiosulfate solution independently, preferably by analyzing a standard lead nitrate solution at the same time that the samples are being analyzed. The volume of thiosulfate solution equivalent to the amount of lead present is given by the difference between the sample titration and blank titration; independent standardization of the thiosulfate and dichromate solutions is unnecessary and increases magnitude of possible errors.

The use of nitric acid and potassium chlorate mixture (*14*) is sometimes advocated as a safe substitute for perchloric acid. Under comparable conditions, this mixture can be just as hazardous as perchloric acid, if not more so. There has been no apparent difficulty in the use of nitric and sulfuric acids for oxidation purposes.

It is known that extraction with aqueous solutions serves to remove certain substances whose presence complicates the oxidation step in these and other methods such as A.S.T.M. D526 (*5*). This preliminary treatment is objectionable because of the possibility of mechanical loss of lead and of loss of lead by solubility in the extracting solution. Serious errors were found in extracting oxidized gasoline, or gasoline exposed to sunlight, with approximately 0.1 *N* acid solution, a loss of 0.15 ml. of tetraethyllead per gallon being found with gasoline (containing 3 ml. of tetraethyllead per gallon) exposed to the sun for only 20 minutes.

The direct hydrochloric acid decomposition procedure has been found useful in the accurate and dependable removal of iron, copper, and other metals from all types of gasoline. The treatment leaves an inorganic residue that is particularly suited for analysis by conventional macro- or micromethods. By suitable changes in apparatus, as much as 500 ml. of sample may be analyzed for traces of metals.

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CORRECTION. In the article entitled "Tentative Procedure for Testing the Variability of Normal and Concentrated Latex" [IND. ENG. CHEM., ANAL. ED., 11, 593 (1939)] the formulas printed at the top of the first column of page 597 should have been written as follows:

$$K_2 = \frac{3h_2Rd}{8L}$$

$$K_3 = \frac{3V}{100\,\pi R^2 g}$$

R. H. GERKE, *Chairman*

Determining the Aromatic Content of Cracked Gasolines by Specific Dispersion

Correction for Olefins

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In determining the aromatic content of cracked gasolines by specific dispersion, correction must be made for the specific dispersion of the olefins. This correction has been calculated from available literature data and found to vary considerably with the class of olefins, with the structure of olefins of the same class, and with the boiling point. An estimate of the reliability of the recommended correction factors is presented; also, as far as possible, the accuracy of the specific dispersion method has been determined experimentally.

THIS method, developed by Grosse and Wackher (*1*), is based on the fact that the specific dispersion of the aromatics is appreciably higher than that of the saturates (naphthenes and paraffins) which is nearly constant. Hence, the aromatic content of a gasoline may be determined by measuring its specific dispersion increment over that of the saturates. (This presupposes that the specific dispersion of the type of aromatics present may be estimated with satisfactory accuracy, which is particularly the case with lower and middle boiling fractions containing single aromatics—viz., benzene or toluene.)

The specific dispersion of the olefins is also higher than that of the saturates, and when present, as in cracked gasoline stock, olefins will cause a specific dispersion increment which, if not corrected for, will give too high a value for the aromatic content.

The aromatic content may be expressed by the following equation:

$$\text{Aromatics, \%w} = \left[\frac{S_s - 98 - f_1 \times \text{Br No.} - f_2 \times M.A.V.}{S_a - 98}\right] 100 + C$$

where

%w = per cent by weight

S_s = specific dispersion, $\frac{n_F - n_C}{d} \times 10^4$ of the sample at 20° C.

S_a = specific dispersion of the aromatics present

98 = estimated average specific dispersion of the saturates. [For greater accuracy, the determined specific dispersion of the saturates as obtained by silica gel treatment is used instead of 98. An adaptation of the silica gel treatment as described by Mair and Forziati (*2*) is used.]

f_1 = factor correcting for the specific dispersion of monoolefins and nonconjugated noncyclic diolefins present

f_2 = factor correcting for the specific dispersion of conjugated diolefins present

C = correction for the deviation from linearity of the relation of specific dispersion to aromatic content

$M.A.V.$ = maleic anhydride value

The bromine number is expressed as grams of bromine consumed by 100 grams of sample, and the maleic anhydride value as milligrams of maleic anhydride consumed by 1 gram of sample.

The necessity of a good evaluation of the olefin correction may be illustrated by the following example: If in calculating the aromatic content of a toluene fraction containing 25% monoölefins, one uses a correction factor which is in error by 15% of its true value, the resulting error in the aromatic content can be shown to amount to 1%, which is already the expected accuracy of the method as applied to olefin-free material. This error increases directly with the olefin content.

Calculation of the correction for the simplest and probably most commonly occurring olefins from available data is outlined below.

CALCULATION OF OLEFIN CORRECTION

The specific dispersion increment due to the presence of olefins is very nearly a linear function of the olefin content; for moderately high olefin contents the deviation may be considered negligible in view of the accuracy of the method. Since the olefin content is measured by the amount of bromine absorbed, the increment is directly proportional to the theoretical bromine number. Hence, the correction factor to be applied equals the specific dispersion increment of olefins per unit bromine number and is obtained by dividing the increment by the theoretical bromine number.

SPECIFIC DISPERSION. Since the interest in specific dispersion of pure hydrocarbons is relatively recent, only a limited amount of data may be found in the literature. The most comprehensive collection is probably contained in Grosse and Wackher's publication (*1*). Therefore, their data have been employed in the present calculations, but augmented and in part supplemented by data accumulated in these laboratories as selected best values from a critical literature review.

The magnitude of the specific dispersion of the main hydrocarbon groups—viz., saturates (naphthenes and paraffins), aro-

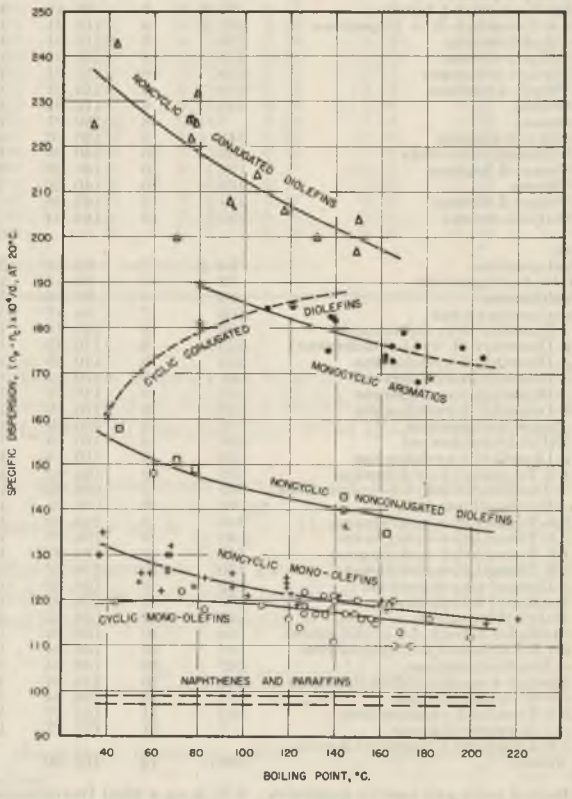

Figure 1. Specific Dispersion of Aromatics, Olefins, and Saturates *vs.* Boiling Point

matics, and olefins—is shown graphically in Figure 1 where they have been plotted against boiling point (data for olefins from Tables I and II).

The average values for naphthenes and for paraffins cannot be distinguished from one another. In general, the specific dispersion values for these two groups lie between 97 and 99, varying somewhat with the extent and mode of branching and with the boiling point. Any trend in this respect has not yet been definitely ascertained, owing to the low accuracy with which most of these measurements have been carried out in the past.

The aromatics in gasoline are almost all monocyclic (benzenes), the specific dispersion trend of which is approximately as indicated in the graph. (Since bicyclic aromatics have much higher specific dispersions, higher boiling fractions containing both mono- and bicyclic aromatics cannot be analyzed by the present method. Though the lowest boiling bicyclic aromatic, naphthalene, boils at 218°, traces have been observed in material boiling as low as 180° C., due to azeotropism. This introduces an error in the analysis of the uppermost fraction of motor gasoline, but this error may be negligible in analysis of the full range gasoline.)

The specific dispersion of the olefins is intermediate between those of the saturates and the aromatics; the conjugated diolefins make an exception, as their specific dispersion is higher than that of the aromatics. There is a distinct difference between the cyclic and noncyclic forms of each class of olefins; the specific dispersion of the former is lower than that of the latter.

On drawing smooth lines through the points, it becomes apparent that the curves, if extrapolated beyond the gasoline range, slowly approach the saturate base line of specific dispersion of about 98. These curves are in general agreement with those obtained by Ward and Kurtz (*3*). The lines cannot be straight, for in that case they would eventually intersect the saturate base line, which is impossible. (The exceptional direction of the curve for cyclic conjugated diolefins may be explained by the combined exalting effect of cyclization and olefinic double bonds.)

THEORETICAL BROMINE NUMBER. By the aid of theoretical bromine number and the boiling points, Figure 2 has been made for the purpose of illustrating the bromine number trend. The shape of the curves will depend on the representation and distribution of olefins of equal carbon number. Although this information is not available, it is probably safe to assume that a smooth curve drawn through the horizontal lines that indicate the boiling range of such olefins represents the loci of the curves fairly well.

SPECIFIC DISPERSION INCREMENT PER UNIT THEORETICAL BROMINE NUMBER. It may be deduced from Figures 1 and 2 that while the specific dispersion increment of the olefins and their bromine number both decrease with rising boiling point, the bromine number decreases faster than the specific dispersion increment. Hence, the increment per unit bromine number, or $i = \frac{S_0 - 98}{\text{Br No.}}$, will increase with the boiling point (S_0 = specific dispersion of olefin). This is shown graphically in Figure 3 obtained from calculated values of i for cyclic and noncyclic monoölefins, noncyclic nonconjugated diolefins, and cyclic and noncyclic conjugated diolefins as listed in Tables I and II.

THE MONOÖLEFIN CORRECTION FACTOR, f_1. The factor f_1 with which to multiply the bromine number of a sample to correct for the specific dispersion increment due to monoölefins is expressed by i. As may be seen from Figure 3, the factors for cyclic and noncyclic monoölefins of the same boiling point differ appreciably. Since the ratio between the contents of these two types of monoolefins cannot be determined, it must be estimated. [An idea of the average ratio between cyclic and

Table I. Specific Dispersion Increment, *i*, per Unit Theoretical Bromine Number of Monoölefins of the Gasoline Range

$$i = \frac{S_0 + 98}{\text{Br No.}}$$

Compound	Boiling Point, ° C.	*C* No.	Theoretical M.W.	Theoretical Br No.	S_0	Reference[a]	*i*
Noncyclic							
2-Pentene (*trans*)	36.2	5	70.13	227.9	130	S.D.	0.140
2-Pentene (*cis*)	37.0	5	70.13	227.9	130	S.D.	0.140
2-Methyl-2-butene	38	5	70.13	227.9	135	G.W.	0.162
4-Methyl-1-pentene	54	6	84.16	189.9	124	G.W.	0.137
4-Methyl-2-pentene (*cis*)	54.7	6	84.16	189.9	126	S.D.	0.147
4-Methyl-2-pentene (*trans*)	58.4	6	84.16	189.9	126	S.D.	0.147
3-Methyl-2-pentene (*cis*)	66	6	84.16	189.9	130	G.W.	0.168
3-Methyl-2-pentene (*trans*)	68	6	84.16	189.9	130	G.W.	0.168
2-Methyl-2-pentene	67	6	84.16	189.9	130	G.W.	0.168
1-Hexene	63.4	6	84.16	189.9	122	S.D.	0.126
3-Hexene (*cis*)	67.0	6	84.16	189.9	126.5	S.D.	0.150
3-Hexene (*trans*)	67.0	6	84.16	189.9	127	S.D.	0.153
2-Hexene	68	6	84.16	189.9	132	G.W.	0.179
2,4-Dimethyl-2-pentene	83	7	98.18	162.8	125	G.W.	0.166
1-Heptene	95	7	98.18	162.8	123	G.W.	0.153
3-Ethyl-2-pentene	95	7	98.18	162.8	126	G.W.	0.172
2,3,3-Trimethyl-1-butene	77.9	7	98.18	162.8	123	S.D.	0.154
2,4,4-Trimethyl-(1 + 2)-pentene	102.0	8	112.21	142.4	121.7	[b]	0.166
3-Ethyl-3-hexene	119	8	112.21	142.4	123	G.W.	0.176
2-Ethyl-1-hexene	121	8	112.21	142.4	121.4	G.W.	0.164
4-Methyl-3-heptene	119	8	112.21	142.4	125	G.W.	0.190
2-Propyl-1-pentene	119	8	112.21	142.4	124	G.W.	0.183
1-Octene	124	8	112.21	142.4	119	G.W.	0.147
2-Octene	?	8	112.21	142.4	121	G.W.	0.162
3-Ethyl-3-heptene	142	9	126.23	126.6	121	G.W.	0.182
2,7-Dimethyl-*x*-octene	160	10	140.26	114.0	119	G.W.	0.184
4-Propyl-3-heptene	161	10	140.26	114.0	120	G.W.	0.193
1-Decene	163	10	140.26	114.0	118	G.W.	0.175
4-Propyl-3-decene	221	13	182.34	84.7	116	G.W.	0.205
5-Butyl-3-nonene	(207)	13	182.34	84.7	115	G.W.	0.194
Cyclic							
Cyclopentene	44.1	5	68.11	234.7	119.0	S.D.	0.089
Methylcyclopentene	73	6	82.14	194.6	122	G.W.	0.123
Cyclohexene	83	6	82.14	194.6	118.1	S.D.	0.103
Ethylcyclopentene	108	7	96.17	166.2	118.7	G.W.	0.124
1,1-Dimethyl-3-cyclohexene	120	8	110.19	145.0	116	G.W.	0.124
1,2-Dimethyl-(3 + 4)-cyclohexene	125	8	110.19	145.0	114	G.W.	0.110
1,3-Dimethyl-3-cyclohexene	125	8	110.19	145.0	119	G.W.	0.145
1,3-Dimethyl-4-cyclohexene	127	8	110.19	145.0	122	G.W.	0.165
1,3-Dimethyl-5-cyclohexene	127	8	110.19	145.0	119	G.W.	0.145
1,4-Dimethyl-1-cyclohexene	127	8	110.19	145.0	117	G.W.	0.131
n-Propylcyclopentene	132	8	110.19	145.0	117	G.W.	0.131
1-Ethyl-1-cyclohexene	136	8	110.19	145.0	117	G.W.	0.131
1,2-Dimethyl-1-cyclohexene	136	8	110.19	145.0	121	G.W.	0.159
1,1,2-Trimethyl-4-cyclohexene	139	9	124.22	128.7	118	G.W.	0.155
1,3-Dimethyl-2-ethyl-1-cyclopentene	140	9	124.22	128.7	120	G.W.	0.171
tert-Butylcyclopentene	140	9	124.22	128.7	110.7	G.W.	0.099
1,3,5-Trimethyl-*x*-cyclohexene	140	9	124.22	128.7	121	G.W.	0.179
1,2,5-Trimethyl-4-cyclohexene	145	9	124.22	128.7	117	G.W.	0.148
1,1,2-Trimethyl-2-cyclohexene	149	9	124.22	128.7	117	G.W.	0.148
1,2,3-Trimethyl-4-cyclohexene	150	9	124.22	128.7	120	G.W.	0.171
1,2-Diethyl-*x*-cyclopentene	152	9	124.22	128.7	116	G.W.	0.140
1-Isopropyl-1-cyclohexene	156	9	124.22	128.7	116	G.W.	0.140
n-Butylcyclopentene	158	9	124.22	128.7	115.2	G.W.	0.134
1-Methyl-2,5-diethyl-1-cyclopentene	164	10	138.24	115.6	119	G.W.	0.182
1,2,4,5-Tetramethyl-1-cyclohexene	166	10	138.24	115.6	120	G.W.	0.190
tert-Amylcyclohexene	167	10	138.24	115.6	110	G.W.	0.104
1-Methyl-4-isopropyl-3-cyclohexene	169	10	138.24	115.6	116	G.W.	0.156
4-*tert*-Butylcyclohexene	174	10	138.24	115.6	110.2	G.W.	0.105
1,2,5-Triethyl-1-cyclopentene	182	11	152.27	105.0	116	G.W.	0.171
4-*tert*-Amylcyclohexene	?	11	152.27	105.0	108.5	G.W.	0.100
1,3,4-Trimethyl-1-isopropyl-3-cyclohexene	(200)	12	166.30	96.1	112	G.W.	0.146

[a] Boiling point and specific dispersion: S.D. from a Shell Development Co. survey of physical properties of hydrocarbons, a critical literature review; G.W. (*1*, pp. 615–22).
[b] Determined in these laboratories.

noncyclic olefin content could conceivably be obtained by determining the change in naphthane-paraffin ratio after hydrogenation, provided this change could be measured with sufficient precision.] Assuming a 50/50 ratio, one obtains an f_1-curve as shown in Figure 4 (the cyclic and noncyclic f_1-curves transferred from Figure 3). This curve, marked 50/50, very nearly coincides with the i-curve for nonconjugated diolefies in Figure 3. Therefore, assuming a 50/50 distribution of cyclic and noncyclic monoölefins, no special correction will be needed for nonconjugated noncyclic diolefins. This is a fortunate coincidence because there is yet no method of determining nonconjugated diolefins in the presence of other olefins.

Conjugated Diolefin Correction Factor, f_2. In contradistinction, the specific dispersion increment per unit bromine number of the conjugated diolefins differs appreciably from that of the monoölefins (Figure 3). In analyzing a cracked gasoline where numerous types of olefins may be present, the portion of the total olefin increment due to conjugated diolefins can be established by a direct determination of the conjugated diolefin content in terms of the amount of maleic anhydride consumed. (There are conjugated dienes which do not react with maleic anhydride and can therefore not be properly accounted for.) This correction procedure follows a similar one employed for some years by Shell Oil Company, Inc., Wood River Research Laboratories.

Table II. Specific Dispersion Increment, i, per Unit Theoretical Bromine Number and Correction Factor, f_2, for Diolefins of the Gasoline Range

$$i = \frac{S_0 - 98}{\text{Br No.}} \qquad f_2 = \frac{S_0 - 98 - f_1 \times 0.6 \times \text{Br No.}^a}{M.A.V.}$$

Compound	Boiling Point, ° C.	C No.	Theoretical M.W.	Theoretical Br No.	S_0	Reference	i	f_2
Noncyclic Nonconjugated								
1,2-Pentadiene	44.7	5	68.11	469.3	158	S.D.	0.128	...
1,5-Hexadiene	59.6	6	82.14	389.2	148	S.D.	0.128	...
1,2-Hexadiene	78	6	82.14	389.2	149	S.D.	0.131	...
4-Methyl-1,2-pentadiene	70	6	82.14	389.2	151	S.D.	0.136	...
2,6-Dimethyl-x,x-heptadiene	144	9	124.22	257.3	140	G.W.	0.163	...
2,6-Dimethyl-1,x-heptadiene	144	9	124.22	257.3	143	G.W.	0.175	...
2,6-Dimethyl-x,x-octadiene	163	10	138.24	231.2	135	G.W.	0.160	...
2,6-Dimethyl-x,x-octadiene	?	10	138.24	231.2	140	G.W.	0.182	...
Noncyclic Conjugated								
2-Methyl-1,3-butadiene (isoprene)	34.1	5	68.11	469.3	225	G.W.	0.270	0.060
1,3-Pentadiene	43	5	68.11	469.3	243	G.W.	0.309	0.072
2,3-Dimethyl-1,3-butadiene	70	6	82.14	389.2	200	G.W.	0.262	0.055
2-Methyl-2,4-pentadiene	76	6	82.14	389.2	226	G.W.	0.329	0.076
2-Methyl-1,3-pentadiene	76	6	82.14	389.2	226	G.W.	0.329	0.076
2,4-Hexadiene (low boiling)	76	6	82.14	389.2	222	G.W.	0.319	0.072
3-Methyl-1,3-pentadiene	78	6	82.14	389.2	225	G.W.	0.326	0.075
2,4-Hexadiene (high boiling)	79	6	82.14	389.2	232	G.W.	0.344	0.081
2,3-Dimethyl-1,3-pentadiene	93	7	96.17	332.4	208	G.W.	0.331	0.075
2-Methyl-2,4-hexadiene	104	7	96.17	332.4	226	G.W.	0.385	0.092
2,4-Heptadiene	105	7	96.17	332.4	214	G.W.	0.349	0.081
2-Methyl-3,5-heptadiene	117	8	110.19	290.1	206	G.W.	0.372	0.088
4-Methyl-2,4-heptadiene	132	8	110.19	290.1	200	G.W.	0.351	0.080
7-Methyl-2,4-octadiene	149	9	124.22	257.3	197	G.W.	0.384	0.090
4-Methyl-3,5-octadiene	150	9	124.22	257.3	204	G.W.	0.411	0.098
Cyclic Conjugated								
Cyclopentadiene	40.4	5	66.10	483.6	161	S.D.	0.130	0.025
Cyclo-1,3-hexadiene	80.3	6	80.12	399.0	181	S.D.	0.208	0.046
Cyclo-1,3-heptadiene	121	7	94.15	339.5	185	G.W.	0.256	0.057

[a] f_1 obtained from curves of Figure 3.

Table III. Olefin Correction Factors for Determination of Aromatic Content of Gasoline Fractions by Specific Dispersion

Mid-boiling Point, ° C.	Monoölefin Factor, f_1: Noncyclic monoolefins, A	Cyclic monoolefins, B	A + B 50/50[a]	Conjugated Diolefin Factor f_2: Conjugated noncyclic diolefins, C	Conjugated cyclic diolefins, D	C + D 50/50
40	0.15	0.09	0.12	0.07	0.02	0.05
50	0.15	0.09	0.12	0.07	0.03	0.05
60	0.15	0.10	0.13	0.07	0.03	0.05
70	0.16	0.11	0.13	0.07	0.04	0.06
80	0.16	0.11	0.14	0.08	0.04	0.06
90	0.16	0.12	0.14	0.08	0.05	0.06
100	0.17	0.12	0.15	0.08	0.05	0.07
110	0.17	0.13	0.15	0.08	0.06	0.07
120	0.17	0.13	0.15	0.09	0.06	0.07
130	0.18	0.14	0.16	0.09	0.06	0.08
140	0.18	0.14	0.16	0.09	0.07	0.08
150	0.18	0.15	0.16	0.09	0.07	0.08
160	0.18	0.15	0.17	0.09	0.07	0.08
170	0.19	0.16	0.17	0.10	0.07	0.08
180	0.19	0.16	0.17	0.10	0.08	0.09
190	0.19	0.16	0.18	0.10	0.08	0.09
200	0.19	0.17	0.18	0.10	0.08	0.09

[a] Also valid for nonconjugated diolefins.

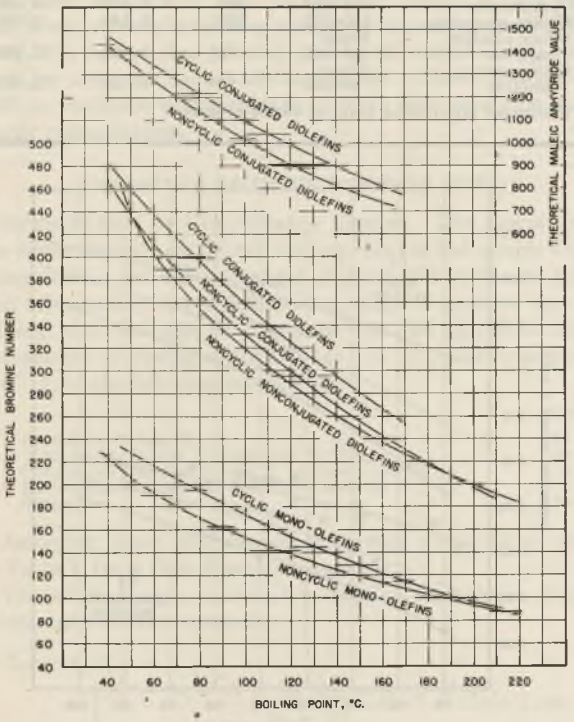

Figure 2. Theoretical Bromine Number and Maleic Anhydride Value of Olefins *vs.* Boiling Point

The specific dispersion increment per unit theoretical maleic anhydride value is $i = \frac{S_0 - 98}{M.A.V.}$. However, since the conjugated diolefins also brominate, this increment has already been partly accounted for as mono-olefin increment, f_1, and the latter, expressed in terms of maleic anhydride value, is

$$f_1 = \frac{\text{Br No. (of conjugated diolefins)}}{M.A.V.}$$

The theoretical correction factor for conjugated diolefins is therefore

$$f_2 \text{ (theoretical)} = \frac{S_0 - 98}{M.A.V.} - f_1 \frac{\text{Br No.}}{M.A.V.}$$

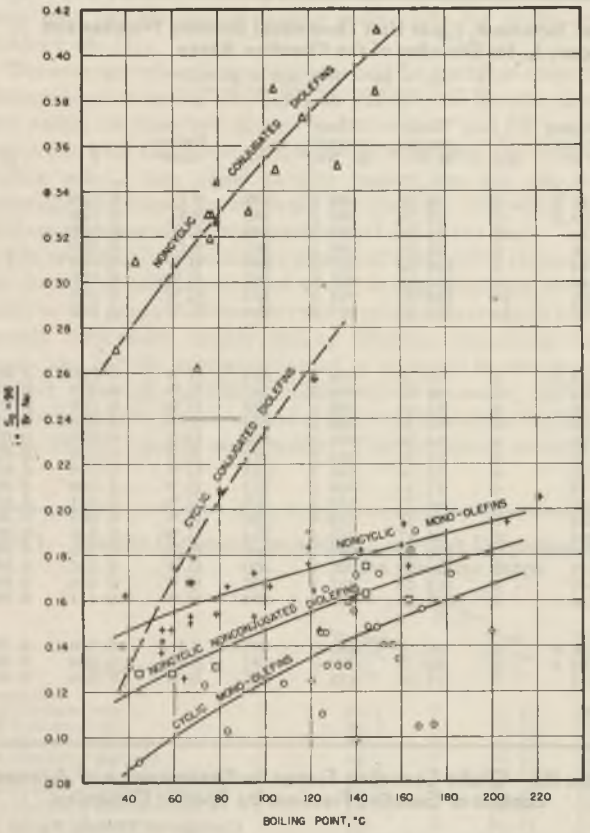

Figure 3. Specific Dispersion Increment per Unit Theoretical Bromine Number of Olefins *vs.* Boiling Point

Actually, conjugated diolefins absorb only some 60% of the theoretical amount of bromine by the methods most reliable for determination of monoölefins (which employ essentially organic media—i.e., acetic acid, carbon tetrachloride). Hence, the factor

$$f_2 = \frac{S_0 - 98 - f_1 \times 0.6 \times \text{Br No.}}{M.A.V.}$$

will be more applicable.

Example. The noncyclic conjugated diolefin 2,4-heptadiene. Boiling point = 105° C., S_0 = 214, theoretical Br No. = 332.4, theoretical $M.A.V.$ = 1019.

The specific dispersion increment per unit maleic anhydride value is $\frac{S_0 - 98}{M.A.V.} = \frac{214 - 98}{1019} = 0.114$. In the presence of the other olefins this increment has been partly corrected for by the noncyclic monoölefin factor f_1, which at 105° C. is 0.170 (see Figure 4), or, expressed in terms of maleic anhydride value, is $f_1 \frac{0.6 \times \text{Br No.}}{M.A.V.} = 0.170 \frac{0.6 \times 332.4}{1019} = 0.033$. Hence, f_2 = 0.114 − 0.033 = 0.081.

Calculated values of f_2 for conjugated diolefins are listed in Table II and shown graphically in Figure 4. As expected, curves similar to the f_1-curves were obtained and the factor of the cyclics is lower than that of the noncyclics. (Although the f_2-curve of the cyclics is based on three points only, it may safely be assumed that its position is approximately as shown.)

Since factor f_2 is a differential correction, it is valid only when a bromine number determination is carried out, which in analyses of cracked gasolines will always be the case.

Recommended Olefin Correction Factors. By aid of the curves in Figure 4, Table III has been made for more convenient use in analyses of any fraction within the gasoline range. Table IV contains the factors applicable to the aromatic fractions, assuming a 50/50 distribution between cyclic and noncyclic olefins, as well as factors applicable to the analysis of full range cracked gasolines (aviation and motor stocks). Since gasolines contain more low-boiling than high-boiling olefins, the mid-boiling point of the olefins will be lower than that of the gasoline; the estimated figures are based on studies of olefin distributions made in these laboratories.

DISCUSSION

It is evident from these calculations that, for the types of olefins investigated, the specific dispersion increment correction factor increases with the boiling point. Thus, over the distilling interval of gasoline it is nearly doubled. Furthermore, the factor for noncyclic olefins is about one and a half times as high as for cyclic olefins of the same class and of the same boiling point. The olefins considered here undoubtedly constitute the overwhelming majority of types present in cracked gasoline stock and therefore the calculated correction factors may be sufficient for practical purposes. Even if the specific dispersion of other olefins were available for calculation of the many individual additional factors, they could not be applied because no method exists for determination of such olefins in the presence of other olefins. (Straight-chain and cyclodiolefins, mono- and diacetylenes, olefin-acetylenes, aromatics and naphthenes with olefinic side chains, cyclomono and diolefins with olefinic side chain, etc.)

These conclusions make it desirable to modify the statement of Grosse and Wackher (*1*, p. 616) regarding the ratio between specific dispersion increment and bromine number for monoölefins and nonconjugated diolefins. Their suggested value of 0.16 for this ratio is derived from Figure 4 (*1*) where the increment of a few olefins was plotted against theoretical bromine number, and a

Table IV. Olefin Correction Factors for Determination of Aromatic Content of Whole Gasoline and of the Aromatic Fractions by Specific Dispersion

(It is assumed that the olefins are 50% cyclic)

Aromatic Fraction	Boiling Range, ° C.	Mid-Boiling Point, ° C.	Mono-olefin Factor, f_1	Conjugated Diolefin Factor, f_2
Benzene	60– 92	76	0.135	0.060
Toluene	92–122	107	0.150	0.070
Xylenes	122–150	136	0.160	0.080
C_9 aromatics	150–180	165	0.170	0.085
Higher aromatics	180–205	192	0.180	0.090
Whole aviation gasoline	About 30–180	75[a]	0.135	0.060
Whole motor gasoline	About 30–205	90[a]	0.140	0.065

[a] Estimated mid-boiling point of olefins present.

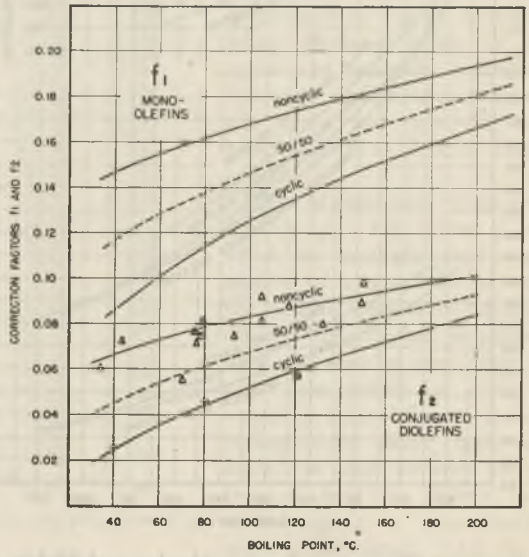

Figure 4. Specific Dispersion Correction Factors for Olefins *vs.* Boiling Point

straight line drawn through the points. By using all of Grosse and Wackher's data (see Figure 5) it becomes apparent that the slope varies appreciably with the bromine number (and hence with the boiling point) as well as with the type of olefin.

Neglecting these variations may lead to a substantial difference in calculated aromatic content of a sample, as illustrated by the following example:

A benzene fraction containing 25% monoölefins (cyclic and noncyclic 50/50), Br No. 47:
Grosse and Wackher's correction factor = 0.16
Correction factor f_1 (from Table IV) = 0.135
With a specific dispersion increment of benzene = 189.3 − 98 = 91.3, difference in aromatic content is $\frac{(0.160 - 0.135) \times 47}{0.913}$ = 1.3% by weight. This discrepancy will increase directly with the olefin content.

Grosse and Wackher's excellent results with known blends containing up to 73% olefin may be explained by the fact that in all their experiments only one olefin was used—namely, 2-ethyl-1-hexene, the correction factor for which (0.164, see Table I) coincides with their factor (0.16).

Reliability of the Factors. The accuracy of the factors per se is independent of the accuracy of the bromine number and maleic anhydride value determinations. However, the factors are subject to the following errors:

1. Error in estimation of the properties of olefins of a given boiling range. It may be assumed that the position of the specific dispersion curve for monoölefins is accurate to ±1 unit, and that of the conjugated diolefins to ±3 units; furthermore, that the position of the bromine number curve is accurate to ±5 units and that of the maleic anhydride value curve to ±20 units. Accumulating these errors for the toluene fraction, the potential error amounts to ±7% of f_1 and ±6% of f_2.
2. Error in estimating the specific dispersion of the saturates may be 1 unit, resulting in a potential error of ±4% of f_1 and ±3% of f_2.
3. Error in estimating the distribution between cyclic and noncyclic olefins. If this be set at 25%, the error in the factors will be ±7% of f_1 and ±11% of f_2.
4. Error in estimating the amount of bromine absorbed by conjugated diolefins. Assuming that the error is 20%, this amounts to ±14% of f_2.

If these errors were noncompensating, which is improbable, they would affect the toluene content to the extent of ±0.5% for each 10% monoölefins present, and to the extent of ±0.3% for each per cent conjugated olefins present. These estimates are well on the conservative side, since there will usually be considerable compensation.

ACCURACY OF THE SPECIFIC DISPERSION METHOD

Enumeration of Conceivable Errors. The magnitude of the errors depends on the mid-boiling point of the sample and its composition. As a hypothetical example will be chosen a fraction representing the middle boiling range of gasoline and containing only one type of aromatics—viz., an untreated toluene concentrate of boiling range 100° to 112° C., mid-boiling point 106° C., of the following composition:

	%w
Aromatics (toluene)	35
Monoölefins (cyclic + noncyclic, 50/50)	30
Conjugated diolefins (cyclic + noncyclic, 50/50)	3
Nonconjugated diolefins (not determinable)	3
Saturates	29

Assuming linear blending, its properties would be as shown in Table V (data from Figures 1, 2, and 4).

The effect of each conceivable error on the toluene content, calculated from the equation

$$\text{Toluene, \%w} = \left[\frac{S_s - 98 - f_1 \times \text{Br No.} - f_2 \times M.A.V.}{S_a - 98}\right] 100 + C$$

will be as shown in Table VI.

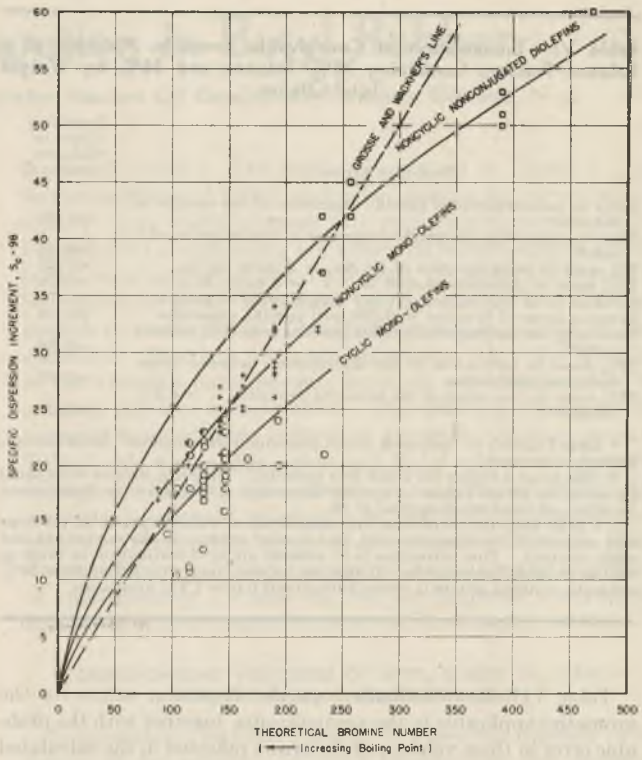

Figure 5. Specific Dispersion Increment *vs.* Theoretical Bromine Number of Monoölefins and Noncyclic Nonconjugated Diolefins

As may be seen from this tabulation, the greatest source of error (±1%) is the uncertainty of the distribution between cyclic and noncyclic olefins. This observation emphasizes the importance of the difference in magnitude of the correction factor for the two types of olefins. The next largest error (±0.7%) is due to uncertainty in the locus of the f_1-curve. However, in calculating this error, the errors in the specific dispersion and in bromine number of the pure olefins were allowed to accumulate.

Table V. Properties

Hydrocarbon Group	Individually			In the Blend				
	S-98	Br No.	*M.-A.V.*	*S*-98	Br No.	*M.-A.V.*	f_1	f_2
Aromatics	86.6	0	0	30.31	0	0	..	..
Mono-olefins	23.0	155	0	6.90	46.50	0	0.15	..
Conjugated diolefins	101.0	334	1025	3.03	6.01	30.75	..	0.07
Nonconjugated diolefins	46.0	310	0	1.38	9.30	0	0.15	..
Saturates	0	0	0	0	0	0	..	..
Total in blend	...	...	..	41.62	61.81	30.75	..	..

To these conceivable errors must be added the error involved in estimating the specific dispersion of the aromatics (S_a). Assuming a fair fractional distillation, as obtained using a 15-plate column, no difficulty should be encountered in the benzene and toluene fractions. In the C_8-aromatic fraction this source of error should also be small, for, although the fraction contains four aromatics, ethylbenzene and *o*-, *m*-, and *p*-xylenes, their distribution ratio is fairly constant—viz., 10 to 20 to 50 to 10, respectively—so that a calculated value of specific dispersion may be used. For higher boiling fractions, however, the error will increase rapidly owing to the ever-increasing number of monocyclic aromatics of undeterminable representation and distribution. (As previously mentioned, in the uppermost fraction the situation may be further complicated by the presence of the bicyclic aromatic naphthalene.)

Table VI. Enumeration of Conceivable Errors in Analysis of a Toluene Fraction Containing 35% Toluene and 36% by Weight Total Olefins

Probable Errors	Resulting Error in Toluene Content, %
Error in measurement of specific dispersion of the sample of 0.3 unit[a]	±0.35
Error in estimation of specific dispersion of the saturates of 1 unit[b]	±0.15
5% error in determination of Br No. = 3 units Br No.	±0.52
10% error in determination of *M.A.V.* = 3 units *M.A.V.*	±0.24
Error in locus of f_1-curve = 0.011 unit specific dispersion	±0.70
Error in locus of f_2-curve = 0.004 unit specific dispersion	±0.14
Assuming the conjugated diolefins brominate to 80% instead of 60%	−0.34
25% error in estimation of the distribution between cyclic and noncyclic olefins	±1.02
25% error in the value of the linearity deviation C = 0.2% toluene[c]	±0.2

[a] Zeiss-Pulfrich or Bausch & Lomb precision refractometer (Abbe not sufficiently accurate).

[b] This error is higher for olefin-free material. The error may be eliminated by using the actual values for specific dispersion of the saturates (as obtained by silica gel treatment) instead of 98.

[c] C is an additive correction, the magnitude of which depends on the type and content of the aromatics and, to a smaller extent, on the naphthene and olefin content. This correction is at present under investigation in these as well as in other laboratories; it reaches its maximum at a little below 50% aromatic content, where it varies from about 0.6 to 2.1% aromatics.

Table VII shows estimated specific dispersion values for the aromatics applicable to the aromatic cuts, together with the probable error in these values and the errors reflected in the calculated aromatic content. Since the magnitude of the latter error depends on the aromatic content, a sample containing 35% by weight of aromatics is again used as an example in order to make a comparison of all conceivable errors possible. The specific dispersion value of full gasoline-range aromatics is difficult to ascertain because the aromatic distribution varies. The figures given in Table VII should therefore be considered as rough estimates only. It is conceivable that a fairly good estimate of the aromatic distribution can be obtained by means of quantitative silica gel adsorption and desorption. It may then develop that for straight-run—viz., olefin-free—gasoline of a given source and for cracked gasoline processed in a given manner, the specific dispersion of the aromatic aggregate may be considered fairly constant. Analyses by fractions should yield more reliable results than full range analyses because all factors applicable to fractions are more nearly correct.

Table VII. Error in Calculated Aromatic Content Due to Uncertainty in Estimation of Specific Dispersion of the Aromatics

Aromatic Fraction	Boiling Range, ° C.	Estimated Specific Dispersion of Aromatics, S_a	Error in Aromatic Content, %w, for 35%w Aromatic Content
Benzene	60– 92	189.3	0
Toluene	92–122	184.6	0
Xylenes	122–150	180.0 ± 0.5	±0.2
C_9 aromatics	150–180	175 ± 2	±0.9
Higher aromatics	180–205	172 ± 3	±1.4
Whole aviation gasoline	About 30–180	180 ± 1	±0.5
Whole motor gasoline	About 30–205	177 ± 2	±0.9

Experimental Evidence of Accuracy. For this demonstration have been chosen gasoline fractions of considerable olefin content, the aromatic content of which can be accurately determined by an independent referee method. Employing the ultraviolet absorption spectrophotometric method, the aromatic content can be determined with an accuracy of ±1% of the aromatic content, provided the sample contains only one type of aromatics. Therefore, the test has been restricted to the analyses of the benzene and the toluene fractions. Results are shown in Table VIII.

It may be noticed from the last line, entitled Δ aromatics, that as a result of accumulation and compensation of the many possible errors, the deviation from the nearly true value increases with the olefin content and may amount to about 2%. Since the greatest source of error is the uncertainty in the estimation of the ratio between cyclic and noncyclic olefins (see Table VI), the aromatic content was also calculated assuming a 25% mistake in the estimation of this ratio. As the resulting set of data shows, this change alone will, in the case of high olefin content, alter the aromatic value by 3%. This discrepancy emphasizes the importance of reliable corrections for the specific dispersion increment due to the presence of olefins.

(Although not required for computation of aromatic content, the olefin content has been included in Table VIII as a matter of orientation. The value of the olefin content likewise depends on the distribution of the two types of olefins, cyclic and noncyclic, because for a given boiling range their difference in molecular weight may amount to as much as ten units. Since the molecular weight of the olefins present in a petroleum fraction cannot be determined, it must be estimated.)

A similar test of the accuracy of the specific dispersion method as applied to the higher boiling polyaromatic fractions and to full range gasolines is not yet feasible because (possibly with the exception of the xylenes fraction) the spectrophotometric method is no longer sufficiently accurate in these ranges to serve as a referee method. It is conceivable, however, that the specific dispersion method could be fully appraised by analyses of known blends composited from hydrocarbon groups that have been isolated by adsorption and desorption on silica gel.

Table VIII. Determination of Aromatics by Specific Dispersion of Unsaturated Gasoline Fractions

Properties	Catalytically Cracked Gasoline				Reformed Untreated Toluene Concentrate		Hydroformate Toluene Concentrate	
	Benzene fraction		Toluene fraction					
Boiling range, ° C.	71–93		93–124		100–112		93–116	
Mid-boiling point, ° C.	82		108		106		104	
Bromine No., grams of Br_2 per 100 grams	126		83		46		10	
(Monoölefins, %w)	(67)		(50)		(25)		(6)	
Maleic anhydride value, mg. M.A. per gram	31		28		34		4	
(Conjugated diolefins, %w)	(2.5)		(2.7)		(3.2)		(0.4)	
Specific dispersion, S_a	124.3		136.8		135.9		130.4	
	[a]	[b]	[a]	[b]	[a]	[b]	[a]	[b]
Monoölefin correction factor, f_1 (Table III)	0.14	0.12	0.15	0.14	0.15	0.14	0.15	0.14
Conjugate l diolefin correction factor, f_2 (Table III)	0.06	0.05	0.07	0.06	0.07	0.06	0.07	0.06
Linearity deviation, C	0.5	0.7	1.0		1.0		1.0	
Specific dispersion of aromatics, S_a (Table VII)	189.3		184.6		184.6		184.6	
Aromatics from specific dispersion, %w	8.0	11.2	29.2	30.5	34.1	35.0	36.4	36.5
Aromatics from ultraviolet spectrophotometry, %w	8.2 ± 0.1		31.5 ± 0.3		35.8 ± 0.4		36.7 ± 0.4	
Δ aromatics, %w	−0.2	+3.0	−2.3	−1.0	−1.7	−0.8	−0.3	−0.2

[a] Assuming cyclic-noncyclic olefins = 50/50.

[b] Assuming cyclic-noncyclic olefins = 75/25.

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Determination of Unsaturation in Butyl Rubber

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A procedure is described for determining the unsaturation in Butyl rubber. The method is based on the reaction of the polymer, in solution, with ozone to give degraded species, the limiting viscosity of which is governed by the original unsaturation. Some relevant information is given on the effects of concentration and molecular weight on the viscosity of the polymer solution, the stability of the ozonized solution, and the effects of certain addition agents. Unsaturation values based on this method and on one involving reaction with iodine chloride are correlated for Butyl rubbers containing various diolefin units.

IN A recent publication (*4*) on unsaturation in Butyl rubber it was pointed out that, of the various methods that had been examined, ozone degradation at the double bonds appears to give the most reliable unsaturation data, the values based on the limiting viscosity of the degraded polymer being in reasonably good agreement with those determined by end group analyses. This paper describes in some detail the analytical method, together with certain modifications that have been introduced with the aim of facilitating routine work.

It was recognized that the earlier procedure had the disadvantage of being too time-consuming, owing mainly to the steps in which the degraded polymer solution is evaporated to dryness, and the residue redissolved in diisobutylene prior to the viscosity measurement. An obvious possible improvement was to develop a procedure whereby the polymer solution could be ozonized and the viscosity of the degraded solution determined directly, thus eliminating evaporation and transfer to a second solvent. It was first necessary, however, to perform the experiments described below; their significance will be explained in the sequel. A further simplification was introduced through the use of carbon tetrachloride of ordinary reagent grade instead of the highly purified solvent. While this substitution usually leads to viscosity-time curves displaying a small negative slope in the region where the purified solvent gives unchanged viscosity values, a simple extrapolation to zero time gives viscosity data that are satisfactory within the limits inherent in the method. In view of the practical requirements of the method and the magnitude of the experimental error, the extra time and effort involved in further purification of the solvent appear to be unwarranted.

DEPENDENCE OF VISCOSITY IN CARBON TETRACHLORIDE ON CONCENTRATION AND MOLECULAR WEIGHT

As it is always necessary to determine the intrinsic viscosity from data obtained at finite polymer concentrations, experiments were carried out to find the effect of concentration and molecular weight on the viscosity. The intrinsic viscosity $[\eta]$ is defined by the relation

$$[\eta] = (2.303 \log \eta_r/c)_{c \to 0} \qquad (1)$$

where η_r is the viscosity of the solution relative to that of the solvent, and c is the solute concentration expressed in grams per 100 cc. of solution. It is apparent that if the function given in Equation 1 varies strongly with concentration, an appreciable error might be introduced in determining $[\eta]$ from a single measurement.

Polyisobutylene samples covering a wide range of molecular weights were dissolved in carbon tetrachloride at various concentrations, and the viscosities of the solutions were measured at 20° C. (It is known, *2*, that the viscosity–molecular weight relationship for polyisobutylene is virtually identical with that for Butyl rubber.) The results are plotted in Figure 1. For polyisobutylenes of higher molecular weights the curves have a negative slope which increases with increasing molecular weight. For polymers of sufficiently low molecular weight, such as are obtained on ozonolysis of Butyl solutions, the slope is observed to be practically zero. With such polymer species it is therefore possible to use Equation 1 directly without imposing the limiting condition. This is a fortunate advantage, since the precision of the viscosity measurements increases considerably with increasing polymer concentration. The practical aspect of this behavior becomes more evident on inspecting the data given in Table I. It is seen that almost any Butyl rubber ordinarily encountered will give, upon ozonolysis, a degraded product the viscosity function of which will be practically independent of polymer concentration in carbon tetrachloride, within the limits shown in Figure 1. GR-I, for example, has an unsaturation value of approximately 1.0 mole %, and the degraded material will have a viscosity-average molecular weight of roughly 10,000.

CORRESPONDING VISCOSITIES OF BUTYL RUBBER IN CARBON TETRACHLORIDE AND DIISOBUTYLENE

In general, the intrinsic viscosity of a polymer depends on the nature of the solvent. In order to determine the molecular weight, and subsequently the original unsaturation, from the intrinsic viscosity of the degraded material in carbon tetrachloride, the corresponding intrinsic viscosity in diisobutylene must be known, since it is only with the latter solvent that the relation-

Table I. Calculated Molecular Weight Data for Butyl Rubber Degraded by Ozone

	Ozonized Polymer	
Unsaturation of Original Rubber	Number-average molecular weight	Viscosity-average molecular weight
Mole %	$\bar{M}_n$	$\bar{M}_v$
0.1	56,100	102,800
0.5	11,200	20,560
1.0	5,610	10,280
2.0	2,805	5,140
3.0	1,870	3,425
4.0	1,403	2,570

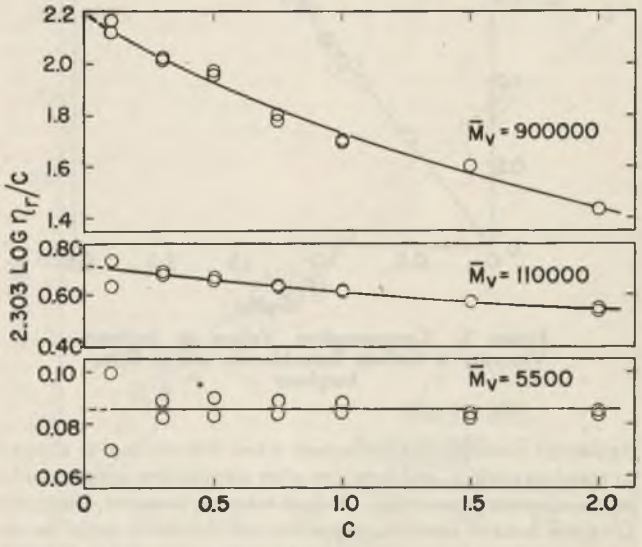

Figure 1. Effects of Concentration and Molecular Weight on Viscosity Function in Carbon Tetrachloride

ship of viscosity to molecular weight has been previously determined (*1*). Corresponding viscosities at 20° in the two solvents were therefore measured for a series of nine Butyl rubbers. The unsaturation values of these samples ranged from 0.6 to 1.7 mole %. Five of the samples were each fractionated into from 6 to 9 fractions; the remaining samples were milled with and without addition of small percentages of zinc stearate and phenyl-β-naphthylamine. A plot of the results is given in Figure 2. It is seen that the intrinsic viscosities in the two solvents are directly proportional, within the limits of error. From the original data it was found that

$$K = [\eta]_{CCl_4}/[\eta]_{\text{Diisobutylene}} = 1.255 \pm 0.005 \qquad (2)$$

The straight line in Figure 2 is obtained from Equation 2.

STABILITY OF OZONIZED BUTYL SOLUTION

It was important to determine whether secondary changes occur when the ozonized solution (which may contain some dissolved ozone) is allowed to stand for any appreciable time, for the analyst may not find it expedient to determine the viscosity until, say, the following day. A solution of a Butyl rubber containing no added antioxidant was prepared in carbon tetrachloride and ozonized for 4 hours. The ozonized oxygen stream was then discontinued, and dry air was passed through the solution in order to sweep out dissolved ozone. Aliquot samples of the solution were removed at known intervals and were allowed to stand in glass-stoppered containers until the following day. The polymers were then recovered by evaporation and their unsaturation values determined from the respective viscosities in diisobutylene. The experiment was repeated with a second sample of the same polymer. The average unsaturation values are given in Table II. It is seen that, within the experimental error, no

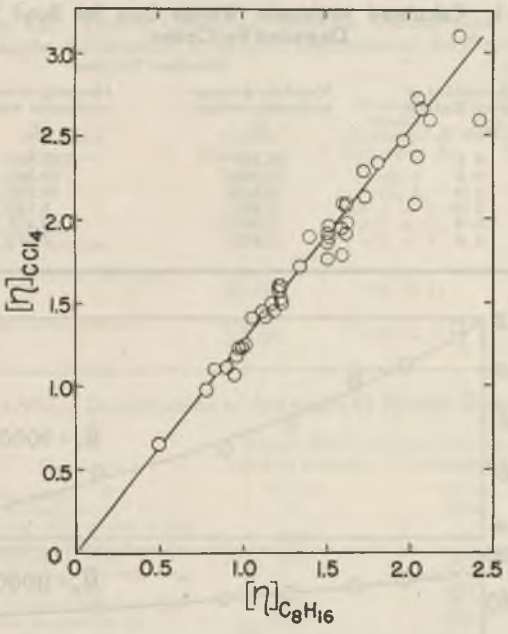

Figure 2. Corresponding Values of Intrinsic Viscosity in Carbon Tetrachloride and in Diisobutylene

significant viscosity change occurs when the solution is allowed to stand overnight, and aeration after ozonization appears to be an unnecessary precaution. It is of interest, however, that even after one hour of aeration, a positive test for ozone could be obtained for the solution (by the potassium iodide test), although no odor of ozone was detected. This result may be due to the development of peroxides or to decomposition products of the solvent.

Table II. Effects of Aeration and Aging on Stability of Ozonized Butyl Solution

Aeration time, minutes	0	30	60
Average unsaturation, mole %	1.4$_0$	1.4$_0$	1.3$_2$

(Unsaturation for unaged solution, 1.4$_1$)

Table III. Effects of Milling and Addition Agents on Unsaturation Values of Butyl Polymers

Polymer and Treatment	Intrinsic Viscosity in Carbon Tetrachloride, by Extrapolation	Corresponding Intrinsic Viscosity in Diisobutylene	Unsaturation, Mole %	Average Unsaturation and Probable Error of Single Observation
I (*a*)	0.20$_2$	0.16$_2$	0.7	
(*b*)	0.24$_0$	0.19$_1$	0.5	
(*c*)	0.19$_7$	0.15$_7$	0.7	0.6 ± 0.06
(*d*)	0.23$_7$	0.18$_9$	0.5	
(*e*)	0.22$_8$	0.17$_8$	0.6	
II (*a*)	0.16$_0$	0.12$_7$	1.0	
(*b*)	0.15$_6$	0.12$_6$	1.0	
(*c*)	0.14$_1$	0.11$_2$	1.2(?)	1.0 ± 0.03
(*d*)	0.15$_6$	0.12$_4$	1.0	
(*e*)	0.14$_9$	0.11$_8$	1.1	
III (*a*)	0.14$_3$	0.11$_4$	1.2	
(*b*)	0.15$_4$	0.12$_2$	1.1	
(*c*)	0.12$_7$	0.10$_1$	1.4	1.3 ± 0.10
(*d*)	0.13$_4$	0.10$_7$	1.3	
(*e*)	0.13$_6$	0.10	1.3	

EFFECTS OF MILLING AND ADDITION AGENTS ON UNSATURATION VALUES

Experiments were carried out to determine whether the presence of zinc stearate and phenyl-β-naphthylamine affects the analytical procedure. Polymers of varying degrees of unsaturation were synthesized in the absence of these substances and were treated in the following ways:

(*a*) Original polymer
(*b*) Milled for 5 minutes at 150° F. with 0.05-cm. (0.02-inch) mill roll setting
(*c*) Milled as in (*b*) with 2.5 parts of zinc stearate
(*d*) Milled as in (*b*) with 0.5 part of phenyl-β-naphthylamine
(*e*) Milled as in (*b*) with 2.5 parts of zinc stearate and 0.5 part of phenyl-β-naphthylamine

The unsaturation values were determined and are given in Table III. Within the limits of error, which is of the order of 0.1 mole %, the addition agents and the milling treatment show no significant influence on the determined values.

APPARATUS AND PROCEDURE

The ozone generator is of the familiar Siemens type and is operated at 15 kilovolts.

In order to obtain higher ozone concentrations, oxygen is used in preference to air. Commercial oxygen is taken from a cylinder through a reduction valve and is passed, in turn, through 30% sodium hydroxide, Drierite, a flowmeter, and into the generator. The ozonized gas passes from the generator through 30% sulfuric acid, anhydrous calcium chloride, and into a glass manifold. A flow rate of 10 to 15 cc. per second is commonly used; this may be varied considerably and is not critical. The ozone concentration depends on the flow rate, as well as on the particular generator. At the above flow rate the apparatus used in this laboratory gives ozone concentrations of 0.14 to 0.18% with air, and 0.39 to 0.56 volume % with oxygen. It was found that at least within these limits, the ozone concentration is not important.

The various glass leads between the oxygen cylinder and the generator are joined with ordinary rubber tubing. Those in the immediate vicinity, and on the delivery side, of the generator are joined with Koroseal tubing. (The authors are indebted to T. L. Gresham of the B. F. Goodrich Company for a supply of this tubing.) Earlier attempts to use all-glass connections led to excessively rigid equipment.

The dry ozonized gas passes into the end of a tubular glass manifold which distributes the gas stream into the ozonizing tubes through three side arms. (The number of side arms and ozonizing tubes can doubtless be increased and thus be adapted to the requirements.) Each connection between the manifold side arms and the ozonizing tubes is made flexible by inserting a piece of Koroseal tubing. The side arms are equipped with stopcocks, so that each tube may be removed without disturbing the operation. The end of the manifold is equipped with a stopcock, so that the ozone stream may be by-passed. The gas streams issuing from the ozonizing tubes are united in an exit manifold similar to that described, and pass into the laboratory hood.

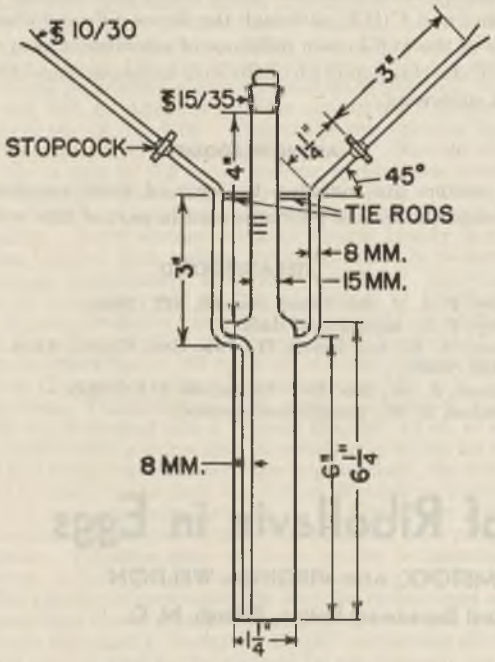

Figure 3. Ozonizing Tube

The ozonizing tubes are held by suitable clamps in an ice-water mixture contained in an insulated rectangular trough equipped with a fitted cover and a drainage valve. The tubes are made of Pyrex, as shown in Figure 3. The dimensions are such that each tube will contain 100 cc. of liquid when filled to a calibration mark near the midpoint of the neck.

During the run some solvent loss through evaporation takes place, and it is necessary to make up the resulting solution to a definite concentration for the subsequent viscosity measurement. This is accomplished by calibrating each tube at 25°, 30°, and 35° with 159.50 grams of carbon tetrachloride (which occupies exactly 100 cc. at 20°, the temperature of the viscosity measurement). The ozonized contents may then be diluted to exact volume at any known room temperature; visual interpolation is sufficiently exact for intermediate temperatures.

In making a run three solutions are prepared for each lot of Butyl rubber; these are ozonized for 1, 2, and 4 hours, respectively. Samples weighing about 1.5 grams are weighed to the nearest milligram and are dissolved in roughly 40-cc. portions of carbon tetrachloride by tumbling for 48 hours in glass-stoppered containers. Each solution is then transferred to an ozonizing tube, the residual solution being washed in with two 10-cc. portions of the solvent. The tubes are clamped into position in the ice-water mixture and ozonized oxygen is passed through the apparatus at a rate of 10 to 15 cc. per second, the rates through the separate ozonizing tubes being occasionally equalized by adjustment of the stopcocks. The rate of cooling of the solution is sufficiently fast to permit ozonization immediately after immersion of the tubes in the cold bath.

After one hour the first tube is removed from the apparatus, ozonization of the others being allowed to continue for the longer periods. A stream of air is passed through the contents for about 15 minutes in order to sweep out excess ozone, and after the contents have been allowed to stand at room temperature for a total time of at least 0.5 hour they are diluted to exact volume with carbon tetrachloride, thoroughly mixed, and the viscosity at 20° determined with a Ubbelohde suspended-level viscometer, proper account being taken of the kinetic energy correction.

TREATMENT OF THE DATA

The ratio of the viscosity of the solution to that of the solvent gives the relative viscosity, η_r; from this and the concentration, c, of the polymer, $[\eta]$ is calculated by Equation 1. Dividing this value by 1.255 (Equation 2), gives the corresponding intrinsic viscosity in diisobutylene. The values thus obtained for the three solutions are plotted against time of ozonization; curves such as are shown in Figure 4 are obtained. The linear portion of the curve, which usually occurs between the 2- and the 4-hour points, but which occasionally includes the 1-hour point, is extrapolated to zero time. From the intercept the viscosity-average molecular weight, $\overline{M}_v$, of the degraded polymer is obtained from the following relation (*1*):

$$\log \overline{M}_v = 5.378 + 1.56 \log [\eta] \quad (3)$$

The number-average molecular weight $\overline{M}_n$ is then given (*4*) by the relation

$$\overline{M}_n = \overline{M}_v/1.832 \quad (4)$$

and the unsaturation, U, of the original polymer, expressed as

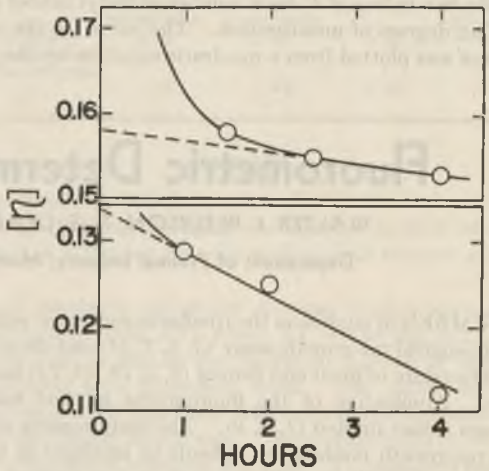

Figure 4. Typical Ozonization Curves

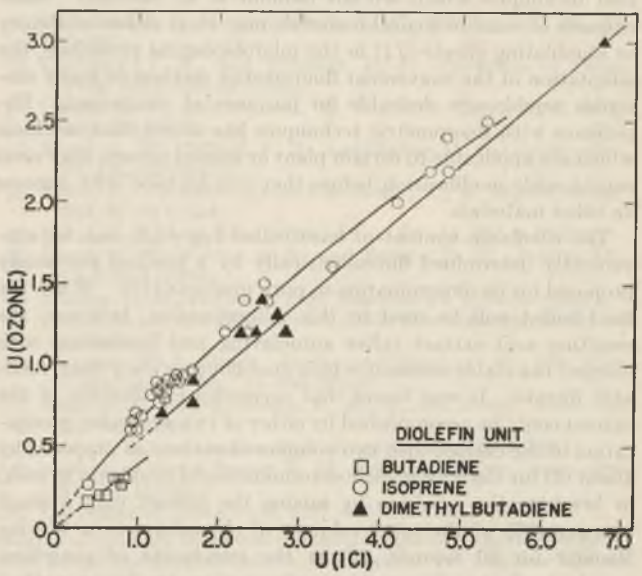

Figure 5. Correlation of Unsaturation Values for Butyl Rubbers Containing Various Diolefin Units

moles of isoprene units per 100 moles of isobutylene units (mole %), follows from

$$U = 5610/\overline{M}_n \quad (5)$$

ALTERNATIVE METHOD OF ESTIMATING *U* FROM IODINE CHLORIDE DATA

For a number of years this laboratory has employed a modification (*5*) of the Kemp-Wijs method for obtaining comparative unsaturation values for Butyl polymers.

In this routine procedure a 0.3-gram sample of the rubber is dissolved in 100 cc. of carbon tetrachloride, 5 cc. of 0.2 *N* Wijs reagent are added, and the mixture is allowed to stand in the dark for 1 hour at room temperature. Twenty cubic centimeters of 15% alcoholic potassium iodide are then added, the mixture is titrated with 0.1 *N* thiosulfate to a canary yellow color, 5 cc. of 0.5% starch solution are added, and the titration is completed. A blank determination is made simultaneously, and from the difference in the titers the unsaturation value *U*(ICl), in mole %, is calculated from the formula

$$U(\mathrm{ICl}) = 0.281 \times (\text{cc. of } 0.1\ N \text{ thiosulfate})/\text{grams of sample} \quad (6)$$

It has been found invariably that these values are approximately twice as large as those obtained by the ozonolysis method, and it has been suggested (*4*) that the difference is due to factors, such as substitution reactions, commonly encountered in iodine chloride determinations. Figure 5 shows the relationship between the two values of *U* for a number of Butyl rubber samples of varying degrees of unsaturation. The curve for the isoprene copolymer was plotted from a quadratic equation by the method of least squares. With the aid of this graph it is possible to obtain at least approximate values of *U* from corresponding values of *U*(ICl) determined by the method described above. More recently Kemp and Peters (*3*) have published an iodine chloride procedure for Butyl rubber in which a different solvent and different reaction conditions are employed. It is likely that their method would be capable of similar use in estimating *U* by this indirect method.

It was considered of interest to include in Figure 5 curves for Butyl polymers containing butadiene and dimethylbutadiene, respectively, as the diolefin units. Over the unsaturation ranges investigated, these materials show linear relationships between *U* (ozone) and *U*(ICl), although the slopes differ by about 20%. In view of the well-known influence of substituent groups on the course of the reaction of an olefin with halogens, these differences are not surprising.

ACKNOWLEDGMENT

The writers are indebted to some of their associates who synthesized the special polymers used in part of this work.

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Fluorometric Determination of Riboflavin in Eggs

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A NUMBER of studies on the riboflavin content of eggs by the biological rat-growth assay (*3*, *5*, *7*, *11*) and the microbiological procedure of Snell and Strong (*2*, *4*, *12*, *13*, *14*) have been reported. Application of the fluorometric method has, however, been rather limited (*1*, *8*, *9*). The early reports of results by the rat-growth method are difficult to interpret in terms of micrograms of riboflavin. The microbiological method, though generally considered to provide reliable results, requires specialized techniques which are not familiar to all chemists. Since extracts of some biological materials may exert either inhibitory or stimulating effects (*14*) in the microbiological procedure, the adaptation of the convenient fluorometric method to many materials would seem desirable for purposes of comparison. Experience with fluorometric techniques has shown that methods which are applicable to certain plant or animal tissues, may need considerable modification before they can be used with success on other materials.

The riboflavin content of hard-boiled egg white can be conveniently determined fluorometrically by a method previously proposed for its determination in pork products (*10*). When the hard-boiled yolk is used in this determination, however, the resulting acid extract (after autoclaving and incubating with clarase) is a stable emulsion which does not provide a clear, readable filtrate. It was found that successful clarification of the extract could be accomplished by either of two methods: precipitation of the extract with two volumes of acetone as proposed by Hand (*6*) for the fluorometric determination of riboflavin in milk, or breaking the emulsion by mixing the extract with a small amount (5% of the total volume) of chloroform in a Waring Blendor for 30 seconds. From the standpoint of analytical speed, simplicity of operation, and accuracy, the latter method was found to be preferable. The acetone precipitation method has the disadvantage of dissolving the yolk carotenols. This necessitates the simultaneous precipitation of an extra aliquot of extract with acetone containing a known amount of pure riboflavin, in order to determine the increment in the fluorometer reading due to the added riboflavin.

In the following comparison of methods, the eggs used were from Rhode Island Red hens which had been receiving a diet containing 6.2 micrograms of riboflavin per gram. In addition to duplicate determinations on individual eggs from different birds, analyses were also made of eggs laid on the second successive day for the same birds. All eggs were analyzed within a few days after being laid.

DESCRIPTION OF METHODS

The eggs are hard-boiled for 6 to 10 minutes, peeled, and the whites and yolks separated, since they are to be determined separately. The first stage in the preparation of the extract is the same for both white and yolk. The entire yolk (weighed) or 10 to 20 grams of white is dropped into 100 cc. of 0.04 *N* sulfuric acid in a Waring Blendor and macerated for 2 minutes at high speed. The creamy mixture obtained is then transferred quantitatively to a 250- or 300-cc. Erlenmeyer flask, using a minimum amount of water from a wash bottle to effect the transfer. The flask is plugged with cotton and autoclaved 15 minutes at 6.8-kg. (15-pounds) pressure. As soon as the flask has cooled, 20 cc. of a 2.5% solution of clarase, freshly prepared in a sodium acetate–acetic acid buffer, are added. [The buffer solution (pH 4.5) is prepared by adding 54.4 cc. of glacial acetic acid to 66.9380 grams of anhydrous sodium acetate together with sufficient distilled water to obtain a solution of the reagents, and then transferred to a 1-liter volumetric flask and made up to volume with distilled water.] The contents of the flask are mixed thoroughly and then incubated at a temperature of 45° C. for 24 hours. The flask is agitated two or three times during the incubation.

Following the incubation period, the extract is brought to a volume of 200 cc. At this point the contents of the flask should

be thoroughly mixed, either by stoppering the flask and shaking thoroughly, or transferring the contents to a Waring Blendor and mixing for 30 seconds.

At this stage, the extract of the egg white may be filtered or clarified by centrifugation and the riboflavin concentration determined as previously described (*10*). The yolk extract, however, is a stable emulsion which cannot be clarified by either filtration or centrifugation. Either of the following treatments will provide clear filtrates.

PRECIPITATION WITH ACETONE. Into each of two test tubes, A and B, are pipetted 5 cc. of the well-mixed yolk extract. Ten cubic centimeters of acetone are added to tube A, and 10 cc. of acetone containing 0.15 microgram of riboflavin per cc. to tube B. Tube B thus contains an increment of 0.1 microgram of riboflavin per cc. over tube A. The solutions containing the precipitated yolk protein are mixed and filtered through No. 597 S. and S. filter paper. Fluorescence readings on the filtrates may be made immediately. With the Coleman electronic photometer (Model 12), the instrument was adjusted each day with a sodium fluorescein solution of such strength that, when its reading was 100, an aqueous solution containing 0.2 microgram of riboflavin per cc. read 70. This range was suitable for most yolk extracts prepared in the above manner. Having obtained readings for A and B, 0.5 cc. of sodium hydrosulfite solution is added to each tube and readings are taken again. The average of these two readings constitutes the reading of the sample blank, or C reading. Since acetone extracts become cloudy in a short time when treated with sodium hydrosulfite, it is necessary to take readings immediately after each addition. Knowing the fluorescence increment due to 0.1 microgram of riboflavin per cc. (B − A), the riboflavin concentration is readily calculated (*10*).

The hydrosulfite solution was prepared by dissolving 5 grams of sodium hydrosulfite in 100 cc. of an ice-cold sodium bicarbonate solution (2 grams of sodium bicarbonate per 100 cc.).

CHLOROFORM TREATMENT. The entire volume of yolk extract (200 cc.) is poured into a Waring Blendor, 10 cc. of chloroform are added from a buret and the solution is mixed for 30 seconds. After being transferred to the original flask, the extract is permitted to stand in the dark for at least 30 minutes, after which it is filtered and approximately 20 cc. of filtrate are collected. The chloroform treatment breaks the emulsion and provides a clear filtrate. Filtrates may be kept under refrigeration for several days without deterioration or change in riboflavin content. The riboflavin concentration may be determined as previously described for its determination in pork products (*10*).

It is important that a "complete blank" containing all the reagents and clarase used in the method be run through the entire procedure, and the resulting concentration subtracted from the value of the sample extract. This applies to both the acetone and chloroform treatments.

Recoveries of riboflavin varied from 97 to 102% when 10, 20, 30, or 40 micrograms of riboflavin were blended with yolk samples and analyzed by the chloroform technique.

Table I. Typical Results of Riboflavin Determinations in Egg Yolks

Bird No.	Acetone Method		$CHCl_3$ Method	
	May 10	May 11	May 10	May 11
	Micrograms/gram		*Micrograms/gram*	
7	3.48	4.07	3.43	3.56
5	3.09	2.83	2.96	2.84
13	3.15	3.43	3.24	3.20
25	4.65	4.74	4.55	4.36
1	4.05	4.10	3.95	4.01
10	4.14	4.26	4.03	4.46

DISCUSSION

Two eggs from each of 18 birds were analyzed. The two eggs were in every case laid on successive days. Table I contains typical results for yolks obtained by the two clarification procedures. If the methods are equally accurate, the variation between eggs by the same bird should be approximately equal, no matter which is used. The standard deviations of eggs from the same bird were 0.36 and 0.26 microgram for the acetone and chloroform techniques, respectively. The difference between these values is not statistically significant but it should be safe to conclude that the chloroform method is at least as accurate as the other. It will be apparent to the analyst that from the standpoint of simplicity of operation and analytical speed, the chloroform clarification procedure is much to be preferred.

Duplicate analyses were made on the yolks of each of 12 eggs by the chloroform technique, using weighed halves of each yolk as duplicates. The standard deviation of duplicates was 0.21 microgram. Comparing this with the standard deviation (0.26 microgram) for eggs from the same bird where each yolk constituted a sample, it is apparent that variation between eggs laid by the same bird on successive days is small.

The standard deviation for duplicate analyses on whites was 0.15 microgram, and the standard deviation of eggs laid on successive days was 0.18 microgram. Again the real day-to-day differences must have been small. In neither case, however, would it be safe to attempt to deduce the range of values likely to be encountered in the eggs of a single bird over a period of several days. There might be cyclic changes; in fact, such changes could account for some of the observed variation from bird to bird.

There were highly significant differences between birds in riboflavin content of yolks, whites, and whole eggs, and in the ratio of riboflavin in yolks to riboflavin in whites. Mean values and standard deviations are listed in Table II. The riboflavin content of whole eggs was calculated from values obtained on yolks and whites.

Table II. Typical Results, Riboflavin Content of Eggs

	Yolk	White	Yolk/White	Whole Egg
	Micrograms/gram	*Micrograms/gram*		*Micrograms/gram*
Mean of 36 eggs	4.09	2.67	1.53	3.18
Standard deviation of eggs from different birds	1.19	0.44	0.48	0.53

SUMMARY

Stable emulsions which interfere in the fluorometric determination of riboflavin in egg yolks can be clarified by mixing the extract in a Waring Blendor with a small amount of chloroform.

Average riboflavin values for all eggs studied were (micrograms per gram): yolk, 4.1; white, 2.7; entire egg, 3.2. Variations from day to day in the riboflavin content of eggs from the same bird were extremely small. The difference in the riboflavin content of eggs from different birds on the same ration was large and highly significant. The average ratio of riboflavin in yolks to riboflavin in whites was 1.53. The standard deviation of the ratio was 0.48.

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Photometric Determination of Fluosilicic Acid in Hydrofluoric Acid

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A method is described which determines 0.005 to 0.3% fluosilicic acid in hydrofluoric acid with an average error of ±0.003%. The fluosilicic acid is converted to silicomolybdic acid, which is determined photometrically. As much as 5 mg. of iron does not interfere.

FLUOSILICIC acid in hydrofluoric acid has been determined by titrating the hydrofluoric acid with standard alkali under conditions that prevent hydrolysis of the fluosilicic acid, subsequently hydrolyzing the fluosilicic acid, and titrating the resulting hydrofluoric acid (*2*, *4*, *5*, *7*). This procedure depends on two end points, the first of which is rather indistinct, and requires large samples (at least 10 grams) for determinations of low fluosilicic acid contents; furthermore, the standard alkali preferably should be free from carbonate and silicate.

Fluosilicic acid, like silicic acid (*3*), reacts with ammonium molybdate to form silicomolybdic acid (*1*).

In the following procedure, this reaction is used for determining fluosilicic acid. Most of the hydrofluoric acid is separated from the fluosilicic acid by evaporation in the presence of sodium chloride (*4*). The small amount remaining as sodium hydrogen fluoride is converted to fluoboric acid, and the fluosilicic acid is converted to silicomolybdic acid, which is determined photometrically. After the blank and the calibration curve have been established, a determination requires about 1 hour.

APPARATUS AND REAGENTS

Fisher AC electrophotometer, with 425-mμ filter and 23-ml. cylindrical cells. Cells put together with optical cement are not suitable.

Sample bomb, 100-ml., preferably of Monel, with 0.6-cm. (0.25-inch) brass Hoke valve and adapter for 0.6-cm. (0.25-inch) tubing.

Bakelite graduate, 25-ml.

Fluosilicic acid, approximately 1 mg. per ml. Mix 0.3 ml. of C.P. 30% fluosilicic acid with 100 ml. of water. Standardize by dissolving in a 25-ml. portion 2 grams of potassium chloride, adding 25 ml. of ethyl alcohol and 5 drops of methyl red indicator, and titrating with standard 0.1 *N* alkali (*6*, *8*). Use as soon as possible after preparation.

Sodium chloride solution, 20 grams per liter.

Boric acid, saturated solution.

Sulfuric acid, 5 *N*.

Ammonium molybdate solution, 10%.

Cylinder of dry nitrogen with pressure regulator.

PROCEDURE

PREPARATION OF CALIBRATION CURVE. Mix, in each of five 50-ml. volumetric flasks, 15 ml. of water, 10 ml. of the sodium chloride solution, a known volume (0.20 to 5.00 ml.) of the standard fluosilicic acid, 10 ml. of the boric acid, 2 ml. of the sulfuric acid, and 5 ml. of the ammonium molybdate solution. In each case dilute to the mark, mix, and allow to stand for 10 minutes. Determine the Scale A reading with the electrophotometer for each solution. Plot milligrams of fluosilicic acid against Scale A readings.

TREATMENT OF SAMPLE. Into the evacuated sample bomb draw a 60- to 80-gram sample, and weigh to the nearest milligram. Pipet 10 ml. of the sodium chloride solution into the Bakelite graduate. Clamp the bomb valve-downward and connect the valve and that of the cylinder of nitrogen to a Saran tee by means of 0.6-cm. (0.25-inch) Saran tubing. To the third opening of the tee connect a piece of Saran tubing that extends almost to the bottom of the graduate, which is fitted with a neoprene stopper bored to fit the tubing loosely enough to permit the escape of effluent nitrogen. Pass nitrogen into the solution at the rate of 1 or 2 bubbles per second. Carefully open the valve on the bomb, and allow 1 to 5 grams of acid to be absorbed in the solution, adjusting the flow rate so that significant quantities of acid mist do not escape. Close the valve on the bomb and continue the flow of nitrogen for about 5 minutes. Transfer the contents of the graduate to a 100-ml. platinum dish and evaporate to dryness at 100° C. on a steam bath. Dissolve the residue in about 15 ml. of water, mix with 10 ml. of the boric acid solution, filter if turbid, and transfer to a 50-ml. volumetric flask. Add 2 ml. of the sulfuric acid and 5 ml. of the ammonium molybdate, dilute to the mark, mix, and allow to stand for 10 minutes. Determine the Scale A reading with the electrophotometer, and determine, from the calibration curve, the weight of fluosilicic acid present.

DETERMINATION OF BLANK. A blank is necessary to compensate for silica in the reagents, which is changed by hydrofluoric acid to fluosilicic acid; no blank is necessary in the calibration, where hydrofluoric acid is substantially absent. Determine the blank by making two determinations, one with a 1-gram sample and the other with a 5- to 7-gram sample of a hydrofluoric acid low in fluosilicic acid, and by extrapolating the results to find the fluosilicic acid corresponding to a 0-gram sample.

Although the foregoing procedure is described for anhydrous hydrofluoric acid, it is easily adaptable for aqueous acid by the use of a platinum sample container (*7*) and omission of the use of nitrogen.

PRECISION AND ACCURACY

Synthetic mixtures were prepared by dissolving known weights of optical quartz, previously acid-washed and ignited, in known weights of anhydrous hydrofluoric acid in a Monel sample bomb. The results of determinations of fluosilicic acid in these samples by the photometric method are given in Table I.

The accuracy and the precision of the photometric method are indicated by an average error of ±0.003% calculated from the data in Table I.

Determinations, in which known amounts of iron were added to hydrofluoric acid samples of known fluosilicic acid content, showed that as much as 5 mg. of iron, which is more than is or-

Table I. Determination of Fluosilicic Acid in Synthetic Mixtures with Hydrofluoric Acid

Weight of Sample	H_2SiF_6 Added[a]	H_2SiF_6 Found[b]	Error
Grams	%	%	%
5.512	0.000	0.009[c]	...
4.469	0.000	0.009[c]	...
2.313	0.187	0.181	−0.006
1.674	0.187	0.184	−0.003
1.188	0.306	0.315	+0.009
1.027	0.306	0.298	−0.008
2.273	0.142	0.145	+0.003
1.885	0.142	0.147	+0.005
3.330	0.101	0.100	−0.001
1.985	0.101	0.106	+0.005
2.430	0.068	0.068	0.000
2.941	0.068	0.065	−0.003
4.479	0.011	0.016	+0.005
2.984	0.011	0.010	−0.001
3.909	0.011	0.011	0.000
4.756	0.006	0.009	+0.003
4.320	0.006	0.007	+0.001
4.274	0.006	0.008	+0.002
1.670	0.048[d]	0.045	−0.003

[a] Added as SiO_2.
[b] Corrected for H_2SiF_6 originally present in HF and for blank.
[c] H_2SiF_6 originally present in HF.
[d] Determined volumetrically (*7*).

dinarily found in a 1- to 5-gram sample of commercial hydrofluoric acid (*5*), does not interfere.

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Determination of Vitamin C in the Presence of Interfering Reducing Substances

Selective Oxidation-Reduction Method

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In a selective oxidation-reduction method for the analysis of true vitamin C, ascorbic acid and interfering substances are catalytically oxidized by the addition of concentrated cucumber juice (ascorbic acid oxidase), followed by the specific reduction of dehydroascorbic acid to ascorbic acid by a suspension of *Escherichia coli* or *Staphylococcus albus*. The ascorbic acid thus formed is then determined by indophenol dye titration. The bacterial suspension reduces dehydroascorbic acid formed by the action of the oxidase on ascorbic acid, in addition to any dehydroascorbic acid present in the original sample, thus permitting a measure of total vitamin C. The method has been successful when applied to milk products, canned fruit and vegetable juices, urine, and blood plasma.

REDUCED ascorbic acid is often estimated by merely acidifying a solution of the material to be analyzed and titrating directly with a standardized solution of 2,6-dichlorophenolindophenol. This procedure is satisfactory for some foods, including raw and pasteurized milk (*16*). However, some products contain other reducing substances which react readily with the indophenol dye solution. In this second group are included milk products and other foods which have been subjected to rather high temperatures—for example, sterilized canned vegetables and evaporated milk.

Various methods have been proposed for estimating vitamin C in the presence of interfering substances (*1*, *2*, *3*, *7*, *9*, *10*, *12*, *13*, *14*, *19*, *20*, *21*) and King (*8*) reviewed the literature in 1941. In many instances interfering substances arise when hydrogen sulfide is used for the reduction of dehydroascorbic acid. The lack of specificity of methods for the determination of ascorbic acid, particularly after hydrogen sulfide reduction of dehydroascorbic acid, as well as a method for minimizing the effect of interfering substances has been discussed by Hochberg, Melnick, and Oser (*7*).

The method described here provides for the determination of both reduced and dehydroascorbic acid as well as interfering reducing substances. It is based on the enzyme-catalyzed oxidation of ascorbic acid and interfering substances by concentrated cucumber juice (ascorbic acid oxidase) with subsequent specific reduction of dehydroascorbic acid to ascorbic acid by a suspension of *Escherichia coli* or *Staphylococcus albus*.

OXIDATION OF ASCORBIC ACID AND REDUCING SUBSTANCES

The oxidation of ascorbic acid to dehydroascorbic acid can be accomplished very rapidly by the addition of ascorbic acid oxidase (*17*, *18*) in the form of concentrated cucumber juice. This oxidation requires exactly one atom of oxygen per molecule of ascorbic acid, and does not proceed beyond the dehydroascorbic acid stage (*6*).

Heating milk to a high temperature produces reducing sub-

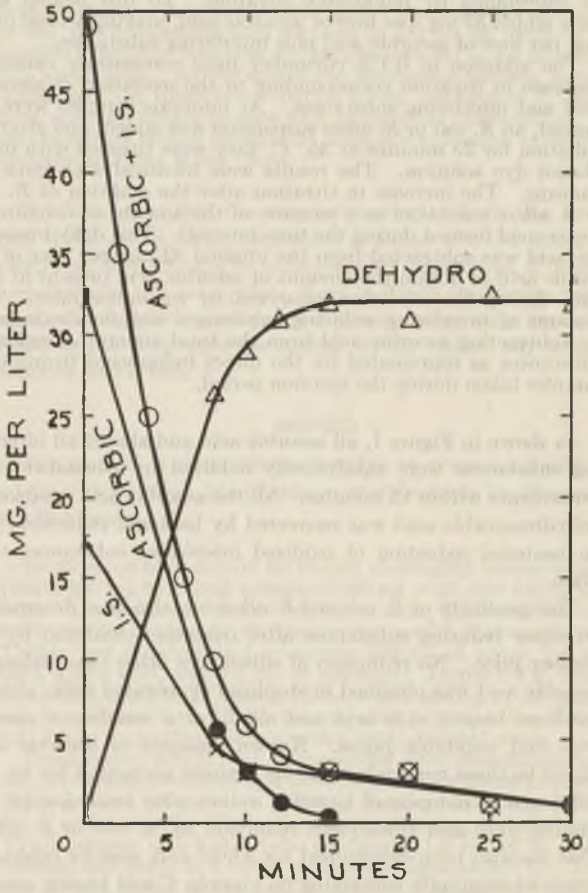

Figure 1. Oxidation of Ascorbic Acid and Interfering Substances Catalyzed by Cucumber Juice

Dehydroascorbic acid determined by reduction with *E. coli* or *S. albus*

stances which are partially or completely oxidized by the addition of concentrated cucumber juice (Figure 1). Canned or bottled fruit and vegetable juices contain interfering reducing substances, whose oxidation is catalyzed by cucumber juice. Thus the basic assumption made by some investigators that ascorbic acid oxidase is specific for catalyzing the oxidation of ascorbic acid does not appear to be true in the above cases. However, the possibility that unpurified cucumber juice may contain additional enzyme systems which bring about this seeming lack of specificity has not been investigated.

REDUCTION OF DEHYDROASCORBIC ACID

The oxidation of ascorbic acid catalyzed by cucumber juice can be reversed by the reduction of dehydroascorbic acid to ascorbic acid by a suspension of *Escherichia coli* or *Staphylococcus albus*. The use of *E. coli* was proposed by Gunsalus and Hand (*5*) for the determination of dehydroascorbic acid in milk, fruit juices, and urine. The bacterial reduction of dehydroascorbic acid in the authors' method is a modification of that given by Gunsalus and Hand which increases its applicability.

The bacterial reduction is very rapid and apparently specific for dehydroascorbic acid. It thus presents a distinct advantage over hydrogen sulfide which has a tendency to reduce other substances and requires a longer time.

The specificity of the bacterial reduction is illustrated in Figure 1. Milk was freed from ascorbic acid by cucumber juice and the resulting dehydroascorbic acid was destroyed by heating. Reducing substances were produced by heating at 100° C. for 30 minutes. The amount produced corresponded to 17 mg. per liter of interfering substances expressed as reduced ascorbic acid as determined by indophenol titration. To this heated milk were added 32 mg. per liter of ascorbic acid, making a total of 49 mg. per liter of ascorbic acid plus interfering substances.

The addition of 0.1% cucumber juice concentrate caused a decrease in titration corresponding to the oxidation of ascorbic acid and interfering substances. At intervals samples were removed, an *E. coli* or *S. albus* suspension was added, and after incubation for 25 minutes at 35° C, they were titrated with indophenol dye solution. The results were identical with both organisms. The increase in titration after the addition of *E. coli* or *S. albus* was taken as a measure of the amount of dehydroascorbic acid formed during the time interval. This dehydroascorbic acid was subtracted from the original 32 mg. per liter of ascorbic acid to obtain the amount of ascorbic acid present at any time during the oxidation catalyzed by cucumber juice. The amount of interfering reducing substances was then calculated by subtracting ascorbic acid from the total amount of reducing substances as represented by the direct indophenol titration of samples taken during the reaction period.

As shown in Figure 1, all ascorbic acid and almost all interfering substances were catalytically oxidized by cucumber juice concentrate within 15 minutes. All the ascorbic acid oxidized to dehydroascorbic acid was recovered by bacterial reduction, but no bacterial reduction of oxidized interfering substances took place.

The specificity of *E. coli* and *S. albus* has also been determined for other reducing substances after oxidation catalyzed by cucumber juice. No reduction of substances other than dehydroascorbic acid was obtained in sterilized evaporated milk, glucose solutions heated with acid and alkali, or a number of canned fruit and vegetable juices. Known amounts of ascorbic acid added to these materials could be entirely accounted for by the difference in indophenol titration values after oxidation by cucumber juice and subsequent reduction by *E. coli* or *S. albus*. This has also been determined for whole milk powder especially prepared from milk containing no vitamin C and known quantities of ascorbic acid added after processing. This specificity is apparently an advantage over the 2,4-dinitrophenylhydrazine method for determining dehydroascorbic acid as proposed by Roe and Oesterling (*15*). The only evidence for nonspecific bacterial reduction was in the case of *d*-isodehydroascorbic acid, which was reduced at about one third the rate of *l*-dehydroascorbic acid. However, the possibility that nonspecific reduction may take place in some materials and under certain conditions cannot be excluded from the above work and this point should be checked for each material being analyzed.

A detailed study has been made concerning certain factors which affect the bacterial reduction of dehydroascorbic acid.

No reduction was obtained unless the living organisms were present. Reducing the number of viable organisms by heating or by freezing and thawing resulted in loss in activity.

A large number of organisms were tested, coliforms and coccus forms being found most likely to reduce dehydroascorbic acid. Of 88 strains of coliform organisms, almost half were able to reduce dehydroascorbic acid.

Complete recovery of dehydroascorbic acid by bacterial reduction was not obtained at temperatures above 40° C. or at pH values above 6.2 because of the rapid destruction of dehydroascorbic acid. A temperature of 35° C. and a pH of 5.9 were found most satisfactory for reducing dehydroascorbic acid within a reasonably short time using a minimum amount of bacterial suspension and without irreversible destruction.

Oxygen determinations have been made in which it was discovered that only after the complete removal of oxygen from solution was there any reduction of dehydroascorbic acid unless sodium cyanide was added. The bacteria themselves remove oxygen from solution and thus establish anaerobic conditions.

Oxygen removal by *E. coli* and *S. albus* is very slow in a phosphate buffer solution, but becomes very rapid when 0.1% cucumber juice concentrate is added. Small amounts of added cucumber juice, in addition to shortening the period required for complete oxygen removal, also increase the rate of actual reduction of dehydroascorbic acid. This factor in cucumber juice is heat-stable.

Gunsalus and Hand (*5*) were able to reduce dehydroascorbic acid in milk, urine, and fruit juices by means of a suspension of *E. coli*, but were unable to obtain any reduction in composite vegetable or sauerkraut juice. They state that in these two juices the *E. coli* acted instead as an oxidation catalyst to decrease the ascorbic acid present.

The authors have investigated this seeming inability of *E. coli* to reduce dehydroascorbic acid under certain conditions. Apparently *E. coli* forms a compound or complex when incubated with vegetable juices, and this can oxidize ascorbic acid in acid solution below a pH of about 3.5.

When *E. coli* is incubated with sauerkraut juice there is rapid and complete reduction of dehydroascorbic acid to ascorbic acid. Subsequent acidification to a pH below 3.5 causes the rapid oxidation of this ascorbic acid and an indophenol dye titration under these conditions is either very small or a blank, depending on how fast the dye is added with respect to the rate of oxidation caused by the compound or complex. Any dye which is reduced by ascorbic acid is then gradually reoxidized and the pink end point tends to increase in color on standing. Ascorbic acid formed from dehydroascorbic acid by *E. coli* in sauerkraut juice can be titrated with indophenol dye at a pH of 4.5 without interference by any oxidation reaction. However, the mixture must be heated or phenol must be added prior to the titration at pH 4.5 to inactivate the *E. coli*, since otherwise the bacteria tend to reduce the dye at this pH and give false values. Heating the mixture before titrating at a pH below 3.5 has no effect, since the compound or complex is apparently heat-stable.

When sauerkraut juice is made 0.001 *M* with respect to sodium cyanide the formation of an oxidizing compound or complex is apparently inhibited, but the dehydroascorbic acid reducing system of *E. coli* is left to function intact. The ascorbic acid formed

under these conditions can be titrated with indophenol dye solution at pH less than 3.5 without interference. Sodium cyanide also enables *E. coli* to reduce dehydroascorbic acid in buffer solution before anaerobic conditions are established.

Vegetable juices in general behave like sauerkraut juice when incubated with a suspension of *E. coli* and then analyzed for ascorbic acid by indophenol dye titration at a pH less than 3.5. The same effect has also been noticed in the case of some samples of urine.

A strain of *Staphylococcus albus* has been found which rapidly reduces dehydroascorbic acid to ascorbic acid in vegetable juices without the production of an interfering compound or complex. This strain of *S. albus* has been used satisfactorily for the analysis of vitamin C in a large number of vegetable juices, urine, and blood plasma. The indophenol dye titrations for ascorbic acid can be made at a pH below 3.5, which eliminates the necessity for heating or adding phenol and titrating at pH 4.5 as in the case of *E. coli*.

REAGENTS

Preparation of Dye Solution. Dissolve 0.135 gram of sodium 2,6-dichlorobenzenoneindophenol in 200 ml. of hot, distilled water. Filter into a 1-liter volumetric flask and add more hot water until the blue colored material has passed through the filter. Adjust to room temperature and make up to volume with distilled water.

Standardization of Dye Solution. Prepare a standard solution of Mohr's salt (*11*) by dissolving 0.500 gram in 1 liter of distilled water containing 10 ml. of concentrated sulfuric acid. Add a 5-ml. portion of the ferrous solution to 10 ml. of 3% metaphosphoric acid and titrate to a stable pink color with the indophenol dye solution. After subtracting a comparable blank, the titration is equivalent to 0.562 mg. of ascorbic acid, or 0.562/dye titration is the dye strength per ml. The ferrous solution is stable for at least a month, but the indophenol dye solution must be standardized daily and a fresh solution prepared every week or 10 days.

Cucumber Juice Concentrate. The authors have found differences in varieties of cucumbers with regard to the ability of their expressed juice to catalyze the oxidation of ascorbic acid. Therefore, several varieties should be tested before selecting one for preparation of the concentrated enzyme. The White Spine variety, about 15 cm. (6 inches) in length, is satisfactory for this purpose. The cucumbers should be frozen (the authors have found it convenient to store the cucumbers in the frozen state for periods as long as one year) and thawed, after which the juice may be drained and concentrated to $^1/_{12}$th the original volume by pervaporation (*17*) and filtered. Such preparations have been kept for several weeks when stored at 10° C. with a trace of toluene as a preservative. The activity should be such that 0.1 ml. in 100 ml. of solution containing 4 mg. of ascorbic acid will catalyze complete oxidation in less than 5 minutes at pH 5.9 and 10° C. Larger amounts of cucumber juice interfere with analysis of vitamin C when *E. coli* is used.

Bacterial Suspensions. The authors have been able to prepare very active suspensions by growth on solid media with subsequent washing into phosphate buffer. A larger number of media have been investigated for the preparation of *E. coli* suspensions before making the final selection. The same medium has been used for growing *S. albus*, although whether or not it is the best for this organism was not determined.

A young culture is more active than one allowed to grow long enough to possess an old population. An incubation temperature of 32° C. and a growth period of 24 hours were found to be most suited for the preparation of an active bacterial suspension.

Of a large number of organisms tested, three were selected as being the most active for reducing dehydroascorbic acid. One of the most active *E. coli* strains was the "Crookes" strain of Esselen and Fuller (*4*) which is listed in the American Type Culture Collection as No. 8739. The authors have isolated a slightly more active coliform which has been listed in the A.T.C.C. as No. 9492. The most satisfactory strain of *S. albus* found has been listed as No. 9491.

Formula and Preparation of Media

Difco proteose tryptone agar	225 grams
Difco veal infusion medium	25 grams
Dextrose	10 grams
Distilled water	5000 ml.

Dissolve the ingredients by heating and distribute 75 ml. to each of 65 flat pint flasks. Wine ovals are well suited for this purpose. Plug the flasks with cotton and autoclave at 7.7 kg. (17 pounds) for 25 minutes. Remove from the autoclave and allow the media to harden with the flasks flat to give a maximum surface area. This medium sets into a firm gel which is not easily broken and supports excellent growth.

Inoculate about 10 ml. of sterile nutrient broth with three or four loops of *E. coli* or *S. albus* from an agar slant. Shake well and immediately inoculate each of three flasks with 2 ml. of the broth culture, making certain that the inoculum is evenly distributed over the agar surface. Incubate at 32° C. for 24 hours. Add 10 ml. of nutrient broth to each of the three flasks and harvest by gently rocking until the growth is removed from the agar surface. Transfer the broth suspension from the three flasks to 100 ml. of nutrient broth, making a total of approximately 130 ml. of broth suspension. Inoculate each of 62 flasks with 2 ml. of this heavy suspension and incubate at 32° C. for 24 hours. Harvest the contents of each flask in 10 ml. of 0.05 *M* sterile phosphate buffer at pH 5.9. One liter of the buffer contains 6.13 grams of KH_2PO_4 and 1.79 grams of $Na_2HPO_4.12H_2O$. Combine the crops and centrifuge the buffer suspension, discard the supernatant liquid, and resuspend in an equal volume of buffer. Repeat for a total of three washings and finally resuspend in a volume of 400 ml. Store in a refrigerator. A suspension of *E. coli* prepared in this manner should contain between 100 and 200 billion, viable count, per ml. Suspensions of *S. albus* tend to have a lower viable count.

Suspensions of *E. coli* have been stored at 8° C. for over 4 months without appreciable loss in activity. Suspensions of *S. albus* are not so stable and usually have to be discarded after about 2 weeks.

Determination of Activity of Bacterial Suspensions. Dissolve 2 to 4 mg. of ascorbic acid in 100 ml. of cold (10° C.) pH 5.9 buffer. Accurately measure 10 ml. of the solution into a test tube containing 1.0 ml. of the bacterial suspension and a drop of cucumber juice concentrate diluted 1 to 10 with water and incubate for about 15 minutes at 35° C. In the meantime, add 0.1 ml. of cucumber juice concentrate to the remaining ascorbic acid solution and hold for 5 minutes, or until all ascorbic acid has been oxidized to dehydroascorbic acid. Immediately add 10 ml. of the dehydroascorbic acid solution to 1.0 ml. of bacterial suspension in another test tube and incubate at 35° C. for 25 minutes in a water bath. At the end of the incubation periods, pour the contents of the tubes into separate 25-ml. portions of 0.1 *N* sulfuric acid and titrate at once with indophenol dye solution. The two titrations should be equal if complete reduction of dehydroascorbic acid has taken place.

It is important that the dehydroascorbic acid solution be kept cold until the bacterial suspension is added and the entire procedure carried out rapidly or irreversible destruction of dehydroascorbic acid will take place. Less bacterial suspension may be used, although it is good practice to use rather large excesses to make certain the reduction occurs rapidly before any dehydroascorbic acid is lost.

METHOD

The method is given here as applied to evaporated milk and whole milk powder. Modifications must be made for the analysis of other foods.

Evaporated milk should be chilled thoroughly before analysis. Dilute 100 ml. to a total volume of 200 ml. with cold 0.07% acetic acid. The final pH should be about 5.9. Reconstitute whole milk powder with cold distilled water and a little acetic acid. This is best accomplished by mixing 230 ml. of water, 1.5 ml. of 10% acetic acid, and 0.5 ml. of detergent (Igepal CTA, General Dyestuff Corp.) in a Waring Blendor and adding 31.25 grams of whole milk powder. If the volume after mixing is less than 250 ml., make up to that volume with a little distilled water.

Immediately add 0.1 ml. of cucumber juice concentrate to 100 ml. of reconstituted milk and allow to stand for about 10 minutes. Pipet a 10-ml. aliquot into a 200-ml. beaker containing 25 ml. of 0.1 *N* sulfuric acid. While the pipet is draining, transfer another 10-ml. aliquot into a test tube containing 1.0 ml. of *E. coli* suspension and place in a water bath maintained at 35° C. Titrate the aliquot added to the sulfuric acid with indophenol dye solution to a pink color which persists for 30 seconds. Incubate the other aliquot with *E. coli* for 25 minutes, add acid, and titrate with indophenol dye. The difference in the two titrations, multiplied by the dye factor, is equivalent to the amount of total

Table I. Reduced Ascorbic Acid, Dehydroascorbic Acid, and Reducing Groups Other Than Ascorbic Acid in Canned Fruit and Vegetable Juices, Dairy Products, Urine, and Blood Plasma

Canned Fruit or Vegetable Juice	Ascorbic Acid	Dehydro-ascorbic Acid	Reducing Groups Other Than Ascorbic Acid
	Mg./100 ml.	*Mg./100 ml.*	*Mg./100 ml.*
Orange, unsweetened	60	0	9
Grapefruit, unsweetened	36	0	6
Pineapple, unsweetened	8	0	2
Apple	3	1	1
Parsley	41	0	53
Lettuce	4	0	7
Sauerkraut No. 1	4	0	8
Sauerkraut No. 2	1	0	1
Dairy Products			
Reconstituted evaporated milk (10 brands)	0.25	0.0	2.3
Reconstituted spray process whole milk powder (100 samples)	1.25	0.0	0.3
Market milk from retail stores in San Francisco–Oakland area (10 brands and 269 samples)	0.37	0.24	0.0
Market milk from homes of consumers in San Francisco–Oakland area (5 brands and 144 samples)	0.35	0.26	0.0
Urine			
1	0.28	0.09	0.47
2	1.14	0.32	2.28
3	0.70	0.52	0.91
4	5.50	0.45	1.85
5	0.65	0.45	1.41
6	0.13	0.26	0.71
7	0.59	0.06	0.25
Blood Plasma			
1	0.80	0.32	0.29
2	0.81	0.33	0.00
3	1.17	0.00	0.00

vitamin C in 10 ml. of reconstituted milk. This may be multiplied by 100 to express the values in the usual terms of mg. per liter.

If the relative amounts of ascorbic acid and dehydroascorbic acid are to be determined, two additional titrations are required: (1) immediately after reconstituting and (2) after incubating with *E. coli* 10 ml. of milk to which no cucumber juice has been added. The difference in titrations is equivalent to the amount of dehydroascorbic acid originally present. This difference may be subtracted from total vitamin C to obtain the amount of ascorbic acid in the sample.

The selective oxidation-reduction method just described has been used in the authors' laboratories for the analysis of several thousand samples of milk products. It has adapted itself well for routine procedure in the hands of laboratory technicians. With a little practice, the analyst can duplicate titrations to within about 0.05 ml., or approximately 0.4 mg. of vitamin C per liter. One person during an ordinary working day can easily analyze 30 samples of whole milk powder or evaporated milk by this method.

A number of canned or bottled fruit and vegetable juices available in retail stores have also been tested for vitamin C by this method as well as for recovery of added ascorbic acid. These juices included grapefruit, orange, pineapple, lemon, peach, papaya, guava, lime, pear, apple, celery, cucumber, parsley, carrot, lettuce, water cress, composite vegetable, rhubarb, and sauerkraut juice. The method was satisfactory when either *E. coli* or *S. albus* was used for the fruit juices and when *S. albus* was used for the reduction in vegetable juices. The juices contained little or no original dehydroascorbic acid, but many, especially vegetable juices, contained rather large amounts of reducing substances other than ascorbic acid which caused no interference with this method.

In general, fruit and vegetable juices were chilled before analysis and were diluted with a cold buffer solution containing a predetermined amount of alkali, so as to make the final concentration of vitamin C less than 5 mg. per 100 ml. and the pH 5.9 to 6.0. A 3% metaphosphoric acid solution was used instead of 0.1 *N* sulfuric acid for the indophenol titration. No attempt has been made to extend the method to highly colored materials which are difficult to titrate visually with indophenol dye. However, no particular difficulty should be found in adapting the method to colorimeter measurements for such materials.

The method has also been applied to urine and blood plasma. *S. albus* was used for these analyses and the general procedure was the same as for fruit and vegetable juices. Urine and blood plasma were adjusted to a satisfactory pH by merely adding an equal amount of 0.25 *M* phosphate buffer of pH 5.9 before adding cucumber juice and the bacterial suspension.

Some typical analyses of canned fruit and vegetable juices, dairy products, urine, and blood plasma are presented in Table I.

Occasionally a solution is so well buffered that the addition of acid prior to indophenol titration is insufficient to cause the inactivation of the bacterial suspension. In such cases the addition of a small amount of phenol has been helpful to prevent the bacterial reduction of the dye itself. It is often necessary to subtract a blank caused by the presence of small amounts of reducing substances in the bacterial suspensions. These substances come from certain lots of media and are difficult to remove completely from the suspensions by washing. The reduction of dye by the bacteria themselves should be carefully checked from time to time in order to avoid serious errors in the determination.

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Electrolytic Determination of Copper and Zinc

In Brass Plating Baths and in Brass Electrodeposits

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IN A PRIOR paper (*1*) reference was made to the present electrolytic method for zinc and copper, in which the copper and zinc are codeposited from a cyanide solution containing ammonium sulfate and ethanolamine, the deposit is dissolved in sulfuric and nitric acids, and the copper is deposited. During the intervening months the procedure has proved more convenient and rapid than was reported, without sacrificing precision and accuracy. It has been successfully used for the determination of copper and zinc in brass plating baths and in electrodeposited brass and has also been adapted to the determination of cadmium, zinc, or copper in plating baths.

Recently, a procedure similar to the present one has been reported by Verdin (*2*). However, his method requires 3 hours for the determination of copper and zinc while the present method requires from 45 to 50 minutes.

APPARATUS

The electrolytic apparatus used consists of one stationary platinum gauze electrode (3.5 cm. in diameter and 4 cm. high) and one central revolving platinum gauze electrode (2 cm. in diameter and 4 cm. high) with a 6-volt rectifier operating from a 110-volt alternating current line. The machine is equipped with an electric hot plate for heating the sample and a reversing switch for changing the polarity of the electrodes. The only other pieces of apparatus required are an ordinary analytical balance and a drying oven.

Table I. Accuracy of Method

Substance (Plus Blank)	Electrodeposit	Error
	Gram	*Gram*
Stock brass-plating solution (blank)	0.2590	.....
Ferrocyanide	0.2594	+0.0004
Silicon (added as silicate)	0.2587	−0.0003
Arsenic (added as arsenite)	0.2665	+0.0075
Lead (added as plumbite)	0.2664	+0.0074
Nickel (added as cyanide complex)	0.2692	+0.0102
Antimony (added as antimonite)	0.2615	+0.0025
Tin (added as stannite)	0.2648	+0.0058

SOLUTIONS

Supporting electrolyte, 200 grams of ammonium sulfate and 40 ml. of Eastman Kodak ethanolamine (practical) per liter. This solution should be filtered before it is used.

Acid mixture, 2 parts of 18 *N* sulfuric acid to 1 part of 6 *N* nitric acid.

Sodium sulfide, 25 grams of sodium sulfide nonahydrate per 50 ml. of solution.

Sodium cyanide, 5% solution.

Brass electrodeposit stripping solution. A solution 8 *N* in ammonium hydroxide and containing 25 grams of ammonium persulfate per liter; 5 drops of 30% hydrogen peroxide are added to each 25-ml. portion of this solution before it is used.

PROCEDURE FOR ANALYSIS OF BRASS-PLATING SOLUTION

Centrifuge a portion of the cyanide brass-plating solution to be analyzed. Pipet 10 ml. of the clear solution into a 180-ml. electrolytic beaker and add 50 ml. of the supporting electrolyte. Heat just to boiling and using a weighed platinum electrode, electrolyze in a covered beaker at 2.5 to 3.0 amperes, maintaining a temperature of 80° C. Wash down the sides of the beaker serveral times during the process of electrolysis. After the sample has run for 15 minutes, test for completeness of copper removal by mixing on a spot plate 1 drop of the solution and 1 drop of concentrated hydrochloric acid, then adding 1 drop of the sodium sulfide test solution. A brown color indicates that the copper has not been completely removed.

When the electrodeposition is complete, wash the electrode with distilled water, dip it in dry acetone, and heat in the oven at 110° C. for 5 minutes. Cool and weigh.

Place the weighed electrode in a 180-ml. electrolytic beaker and add 3 ml. of the prepared acid mixture. Allow a portion of the brass to dissolve in the acid. Without removing the electrode from the beaker, connect the electrode and after adding enough water to cover the brass deposit, strip the brass electrolytically by reversing the original polarity and electrolyzing at 0.5 to 1 ampere till the platinum is bare (about 5 minutes). The polarity is now returned to normal and the copper deposition is begun, first at 0.5 ampere until the electrode is covered (about 1 minute) and then at 1.5 amperes until deposition is complete (about 10 minutes). Wash, dry, cool, and weigh the electrode to determine copper. The weight of copper subtracted from that of brass gives the weight of zinc.

PROCEDURE FOR ANALYSIS OF BRASS PLATE

Using a solution of cellulose acetate in ethyl acetate, mask off 10 sq. inches (65 sq. cm.) of plate area. Deliver the stripping solution from a buret and run it over this area to be stripped until the base metal is clean. Transfer the collected solution of copper and zinc to a 180-ml. electrolytic beaker and boil for 5 to 10 minutes to destroy persulfate. Add the sodium cyanide solution until the blue color of the copper-ammonia complex vanishes and then add 50 ml. of the supporting electrolyte. From this point on, proceed exactly as in the brass-plating solution analysis.

INTERFERENCES

Since the ordinary brass-plating bath contains traces of certain metallic impurities and up to 2 grams of sodium ferrocyanide per liter, the influence of these substances on the accuracy of the method was checked. Ten milligrams of the metal or radical under study were added as a sodium salt to 10 ml. of a stock brass-plating solution and the recommended electrolytic procedure was followed (Table I).

It is clear from Table I that accurate results cannot be expected in the presence of lead, arsenic, antimony, tin, or nickel. When the plating characteristics of the bath point to a possible metallic contamination, a direct chemical analysis for the impurity becomes necessary. Such cases are, however, rare.

DISCUSSION

In the development of the present procedure, the aim was to find a suitable means of freeing copper and zinc in the brass-plating bath from their more stable complexes and converting these metals into complexes permitting rapid yet quantitative deposition of brass. In the search for a suitable electrolyte it was found that ammonium hydroxide speeded the deposition of brass. However, because of the rapidity with which the ammonia was driven out of solution by heating and gas evolution at the electrodes, it was not satisfactory. Consequently experiments were made with other water-soluble amino compounds: 2-amino-1-butanol, hydroxylammonium sulfate, glycine, sulfanilic acid, ethanolamine, diethanolamine, and triethanolamine. Of these compounds, ethanolamine gave the best deposit and the most consistent results. All these compounds gave denser and brighter plates than does ammonia.

A further large increase in the speed of deposition was accomplished through heating to 80° to 90° C., electrolyzing at a high current density, and using a large concentration of ammonium sulfate, the latter salt serving largely to decompose any free sodium cyanide in the solution. With this combination of electrolyte and plating conditions, the deposition of brass can be made extremely rapid. To illustrate the speed of deposition by the present procedure, four 10-ml. samples of a stock brass-plating solution were electrolyzed for 5, 7.5, 10, and 15 minutes, respectively. Of the 0.2387 gram of brass contained in each, the amounts remaining undeposited at the interruption of plating

were, respectively, 0.0263, 0.0138, 0.0007, and 0.0000 gram. Thus without undue haste on the part of the analyst, the copper and zinc content of a brass-plating bath (or the copper-zinc ratio of a brass plate) can be readily determined in 45 minutes.

Perhaps the greatest disadvantage encountered is that some hydrocyanic acid is evolved during the electrolysis. However, experience has shown that if the electrolytic machines are placed in or close to a hood in a well-ventilated room, the method presents no hazard from cyanide fumes.

The precision and accuracy of the method are satisfactory. The average weights of copper and zinc for twenty-five 10-ml. samples of standard brass solution were 0.2210 and 0.0169 gram, respectively, and the average deviation was ±0.0003 gram for both copper and zinc. The solution was standardized by the methods of Miceli and Larson (*1*) and was found to contain 0.2211 gram of copper and 0.0167 gram of zinc per 10 ml.

While the method is hardly an umpire method, it has been found entirely satisfactory for production and experimental control work. The simplicity of the technique involved is also of benefit, especially when the analyses are turned over to a new operator.

ACKNOWLEDGMENTS

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Standards in Vitamin A Assays

U.S.P. Reference Cod Liver Oil *vs.* Beta-Carotene

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Evidence from both biological and spectrophotometric data is presented showing that U.S.P. reference cod liver oil No. 2 contains less than 1700 international units of vitamin A per gram and therefore that the U.S.P. vitamin A unit cannot be considered identical with the international unit for that vitamin. As a result of this discrepancy, assays of vitamin A value performed by bioassay using U.S.P. reference cod liver oil No. 2 as the standard and expressed in terms of international units may be from 30 to 44% higher than the actual value. Data are also presented showing the instability of vitamin A as it occurs in U.S.P. reference cod liver oil No. 2. Pure β-carotene may be used as a standard for the bioassay of vitamin A until a more stable and satisfactory standard is developed.

IN CONNECTION with a study of the utilization of carotene, in which the vitamin A value of a number of samples of carrots was determined by both spectrophotometric and biological methods, evidence has been accumulated indicating that U.S.P. reference cod liver oil No. 2 is lower in vitamin A potency than 1700 international units per gram and thus that the U.S.P. and international vitamin A units are not "identical". As a result of this discrepancy, vitamin A values determined by the bioassay method, using U.S.P. reference oil No. 2 as a standard and expressed in terms of international units, appear higher than they are in reality.

BIOASSAY DATA

The biological assays were conducted in accordance with the method outlined in the U. S. Pharmacopoeia XI, a total of 324 animals being used in the experiments. Both male and female rats from the authors' stock colony were used and the groups of animals to be compared were composed of equal numbers of sex-litter mates. The laboratory in which the animals were kept was maintained at 78° to 82° F. (25.56° to 27.78° C.).

One reference group of animals received U.S.P. reference oil No. 2 in an amount providing 14 units of vitamin A per week, basing calculations on the value assigned to this oil of 1700 units per gram. It was planned that a second reference group should receive β-carotene (pure β-carotene, SMA Corp.) equivalent to the cod liver oil in vitamin A value, considering 0.6 microgram of β-carotene as equivalent to 1 international unit.

As soon as it became apparent that there was a considerable difference between the growth rates of the animals receiving cod liver oil and those receiving β-carotene, the β-carotene content of the solutions used for feeding was determined spectrophotometrically. An aliquot of 0.5 cc. of the β-carotene solution as diluted for feeding was weighed and diluted to 25 cc. with a 1 to 1 mixture of purified Skellysolve, fraction C, and ethyl alcohol. The absorptions at wave lengths 430, 450, and 480 mμ were read by means of a photoelectric spectrophotometer, using a Hilger double monochromator, and absorbencies of an equal amount of cottonseed oil measured at the same wave lengths subtracted from these readings. The average ratios between the absorptions at 430 and 450 mμ and at 480 and 450 mμ were 73.6 and 88.1%, respectively. These ratios indicate that some small amount of impurity was present in the supposedly pure β-carotene and point to the necessity for further purification of even the best commercial preparations.

Calculation of the amount of β-carotene present in each solution was based on the average extinction coefficient for β-carotene of 0.2487. This coefficient has frequently been obtained with these same solvents in the laboratory of the Bureau of Dairy Industry, the highest extinction coefficient for pure β-carotene in petroleum ether–ethyl alcohol obtained in this laboratory being 0.2455. The average value thus obtained for the series of solutions was 25.6 micrograms of β-carotene per gram of solution, which is equivalent to 23.4 micrograms per cc., using 0.915 as the specific gravity of the cottonseed oil at the temperature of the experiment room. This value represents the maximum amount of β-carotene present in the solutions and has been used in calculating the β-carotene intake of the experimental animals (Table I).

The U.S.P. reference oil was diluted prior to feeding each week, using cottonseed oil containing 0.01% hydroquinone as the diluent. No sample of reference oil was used beyond the expiration date marked on the bottle. The concentrated standard solutions of β-carotene in cottonseed oil were diluted at weekly intervals for feeding. The solutions were stored in the dark at a temperature below 0° C. at all times when not actually in use. Supplements, fed thrice weekly from a calibrated syringe, were prepared so that each dose was contained in 0.1 cc. of the cottonseed oil solution. The five assays reported were performed over a period of 2 years; five different lots of U.S.P. reference oil and five different standard solutions of β-carotene were used.

It was found on analysis of the results that in every case the animals receiving β-carotene made greater average gains over the 4-week period of assay than did their litter mates receiving a supposedly somewhat greater amount of vitamin A from the U.S.P. reference oil. As a result of this discrepancy, the vitamin A value of the four samples of carrots under assay in this study appeared to be from 30 to 44% less when β-carotene was used as the standard than when U.S.P. reference oil was the standard on which the calculations were based (see Table I).

Table I. Comparison of U.S.P. Reference Cod Liver Oil No. 2 and β-Carotene as Standards in the Bioassay of Vitamin A Value

Assay	No. of Animals in Each Assay Group[a]	Av. Weight Gain per Week with Standard Error of Animals Receiving: U.S.P. reference cod liver oil, 14 I.U. per week	β-carotene, 7.02γ per week[b]	Vitamin A Value of Carrots: On basis of U.S.P. reference oil	On basis of β-carotene	Difference	
		Grams	*Grams*	*I.U./100 g.*	*I.U./100 g.*	*I.U./100 g.*	%
Carrots, No. 1	10	12.8 ± 1.37	15.8 ± 2.07	6,365	4,180	2,185	34
Carrots, No. 2	15	9.2 ± 1.77	14.0 ± 1.48	17,500	9,750	7,750	44
Carrots, No. 3	8	11.0 ± 0.91	14.6 ± 1.88	6,830	4,775	2,055	30
Carrots, No. 4	15	12.4 ± 2.04	14.4 ± 2.02	13,335	9,360	3,975	30
β-Carotene[c] *vs.* U.S.P. reference oil No. 2	28	10.0 ± 0.683	13.3 ± 0.655	....	...	...	..

[a] Sex-litter mates were assigned to five groups which received U.S.P. reference cod liver oil, β-carotene, supplements of carrots at two levels, and no supplement, respectively.
[b] Determined by spectrophotometric analysis.
[c] Three groups of sex-litter mates were used in this experiment, receiving reference cod liver oil, β-carotene, and no supplement, respectively.

In order to verify these data, 14 male and 14 female sex-litter mate pairs of animals were given the standard doses of reference cod liver oil and β-carotene used in the bioassays, respectively, resulting in similar findings. The rats receiving carotene gained approximately one third more weight during the assay period than did those receiving the U.S.P. reference cod liver oil (Table I).

Baxter and Robeson (*1*) reported a biological potency for vitamin A β-naphthoate of 3,440,000 U.S.P. XI units per gram and compared it with a value of 2,225,000 international units per gram as found by Underhill and Coward (*6*) for the same vitamin A ester. This is a difference of more than 50%, which was attributed by Baxter and Robeson (*1*) to a lack of uniformity in bioassay procedures among different laboratories. However, the discrepancy could well have been due, at least in part, to the fact that U.S.P. reference oil No. 2 was used as a standard by Baxter and Robeson (*1*) while Underhill and Coward employed the international standard β-carotene in their bioassays.

SPECTROPHOTOMETRIC DATA

The unsuitability of the U.S.P. reference cod liver oil No. 2 as a standard in spectrophotometric and colorimetric work is well known and the reasons therefor have been discussed (*2*, *5*), not the least of these being its chemical instability. This instability becomes of increased importance in biological assays, where the use of the sample for a single assay usually extends over a period of at least 1 month. In a series of such assays one sample of the oil might be in use over the full 6-month period, during which it is guaranteed to be safe.

Wiseman and Cary (*7*) of the Bureau of Dairy Industry, Agricultural Research Administration, found $E^{1\%}_{1\ cm.}$ 325 mμ values for the nonsaponifiable fraction varying from 0.764 to 0.88, in a series of U.S.P. reference oils No. 2 picked up from several laboratories in the Washington area and still considered as usable standards by the laboratories. In an attempt to determine the sources of these variations, they analyzed immediately upon delivery two bottles of the U.S.P. reference oil No. 2, which had been packed in dry ice and were in transit less than 24 hours. The absorption of the nonsaponifiable fraction at both 325 mμ and 620 mμ, after treatment with antimony trichloride reagent, was measured by means of a photoelectric spectrophotometer using a Hilger double monochromator. The values for $E^{1\%}_{1\ cm.}$ 325 mμ were 0.870 and 0.873 and the values for $E^{1\ I.U./ml.}_{1\ cm.}$ 620 mμ were 0.1242 and 0.1244, respectively, using the rated potency of the oil in calculating the *E* per I.U.

After aliquots had been removed for analysis, the bottles were resealed under nitrogen, stored at reduced temperatures, and reanalyzed 11 months later. At this time the $E^{1\%}_{1\ cm.}$ 325 mμ value had dropped to 0.675 and the $E^{1\ I.U./ml.}_{1\ cm.}$ 620 mμ value was lowered proportionately to 0.098. About a year after the first samples of reference oil had been received, two additional bottles were obtained, the same precautions being observed in shipping and handling; in this case the values for the freshly opened samples were lower—i.e., the $E^{1\%}_{1\ cm.}$ 325 mμ values were 0.772 and 0.785 and the $E^{1\ I.U./ml.}_{1\ cm.}$ 620 mμ values were 0.1117 and 0.1135, respectively, again calculating the *E* per I.U. as above. This clearly demonstrates that the variability observed in the vitamin A content of the samples of the reference cod liver oil No. 2 is not entirely due to destruction of vitamin A following the opening of the bottle in the laboratory. Three of the samples of the reference oils used in the bioassays described in this report gave $E^{1\%}_{1\ cm.}$ 325 mμ values of 0.728, 0.790, and 0.756, respectively.

DISCUSSION

Oser, Melnick, and Pader (*5*) state that "when parallel tests on the reference oil are used as the basis for arriving at factors for converting *E* values to biological unitage in unknown oils, serious complications result", and that "errors in estimating potency in relation to the U.S.P. reference oil may fall within the limits of error of the bioassay and are difficult to prove".

Hume (*3*) has questioned the assumption that the international and U.S.P. vitamin A units are equivalent, basing this conclusion upon the results of a series of collaborative tests using the U.S.P. reference cod liver oil No. 1; an exhaustive statistical analysis of these results has recently appeared.

In the case of vitamin A bioassays, the limits of error are rather wider than in other types of assay—for instance, as reported by Irwin (*4*) in his statistical examination of the accuracy of vitamin A assays, the limits of error of vitamin A value ($P = 0.99$) obtained from testing several thousand animals lay between 65 and 154%. It is when attempting more precise measurements, such as the establishment of a conversion factor for spectrophotometric values, that the inaccuracies of the bioassay become troublesome and for this reason are considered significant.

However, even from a practical viewpoint, a systematic error of 30 to 44% invariably in the same direction—i.e., increasing the apparent vitamin A value—becomes important, especially when one considers that the only direct way at present of arriving at the vitamin A values of food is by bioassay. Figures of this kind are in widespread use as the basis of calculations for dietary surveys. To the rather wide range of error inherent in the vitamin A bioassay there should not be added a constant error of this magnitude through the use of a standard as unstable as the U.S.P. reference cod liver oil No. 2. At present, pure β-carotene appears to be a more reliable standard for use in vitamin A bioassay than the U.S.P. reference cod liver oil No. 2 and one which could easily be replaced by a vitamin A standard, when a satisfactory preparation is available.

ACKNOWLEDGMENT

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Colorimetric Determination of Nickel with Dimethylglyoxime

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A spectrophotometric study was made of the colorimetric method involving formation of a soluble red complex by treating nickelic ion in ammoniacal solution with dimethylglyoxime. Beer's law applies for concentrations from 0.1 to 5 p.p.m. of nickel, using 1.000-cm. absorption cells. The aqueous system is unstable, but sufficient ethanol makes the color stable for 30 minutes. Of the ions soluble under the conditions used, only auric, cobaltous, and dichromate interfere seriously. Metals which precipitate in ammoniacal solution are removed in the procedure, unless it is possible to prevent precipitation through some suitable reaction.

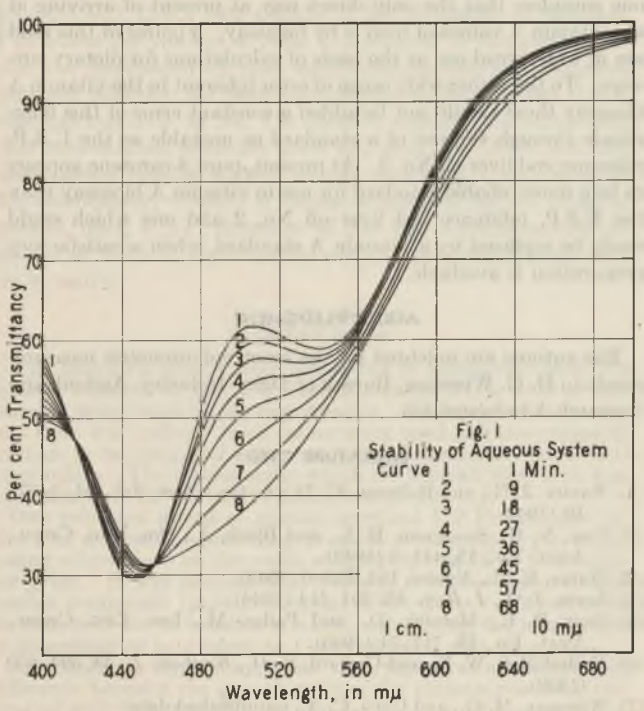
IN 1924 Feigl reported (*5*) that a strong red hue develops when a solution containing nickel is oxidized with lead dioxide, basified with sodium hydroxide, and allowed to react with a solution of dimethylglyoxime. To convert the nickel to the necessary quadrivalent state Rollet (*12*) recommended bromine water as a more convenient oxidant in applying the reaction quantitatively. With this reagent the color develops rapidly without heating. Although Fairhall questioned the general soundness of the method (*4*), its use has been expanding (*2*). Examples of commercial applications are the determination of nickel in aluminum alloys (*6*), copper-base alloys (*6, 14, 16*), iron ore (*1*), silicate rocks (*13*), and steel (*3, 6–10, 15*). Extraction of the colored complex with ether has been suggested (*11*).

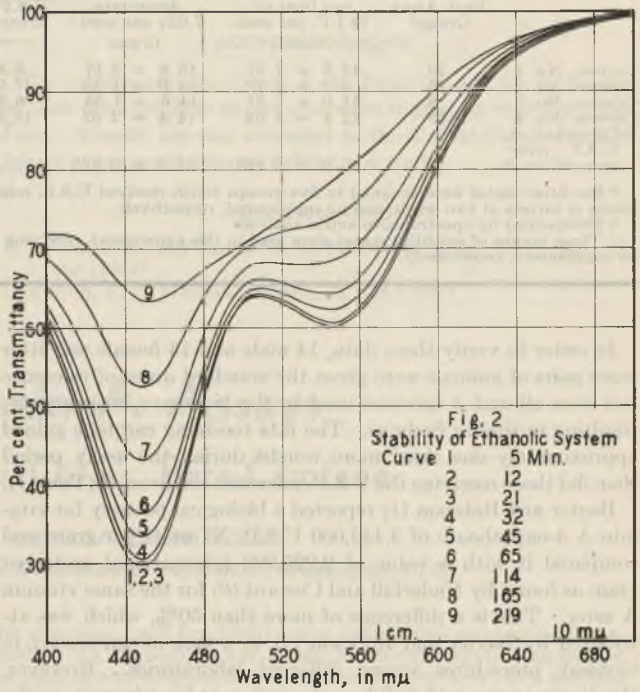
In view of the apparent industrial importance of this method, it seemed worth while to make a spectrophotometric study of the colored system before beginning closely related work. This paper is a summary of the general results obtained.

APPARATUS AND SOLUTIONS

Transmittancy measurements were made in 1.000-cm. cells with a General Electric spectrophotometer adjusted for a spectral band width of 10 mμ.

A stock solution of nickel sulfate, $NiSO_4.6H_2O$, was prepared by suitable dilution of a gravimetrically analyzed solution. In the study of the effect of diverse ions, nitrate, chloride, or sulfate salts were used for cations, and alkali metal salts for anions. The color-forming reagent, dimethylglyoxime, was 0.1% ethanolic solution. The bromine water was a saturated solution. The concentrated ammonia water was reagent quality, and the ethanol was 95% material.





THE COLOR REACTION

The procedure for the colorimetric determination of nickel differs from the gravimetric method chiefly in the oxidation to a higher valence state by bromine water before addition of dimethylglyoxime. In this state the compound formed is a red, soluble complex which, according to Feigl (*5*), has the structure $[HON{=}C(CH_3)C(CH_3){=}NO]_2Ni{=}O$.

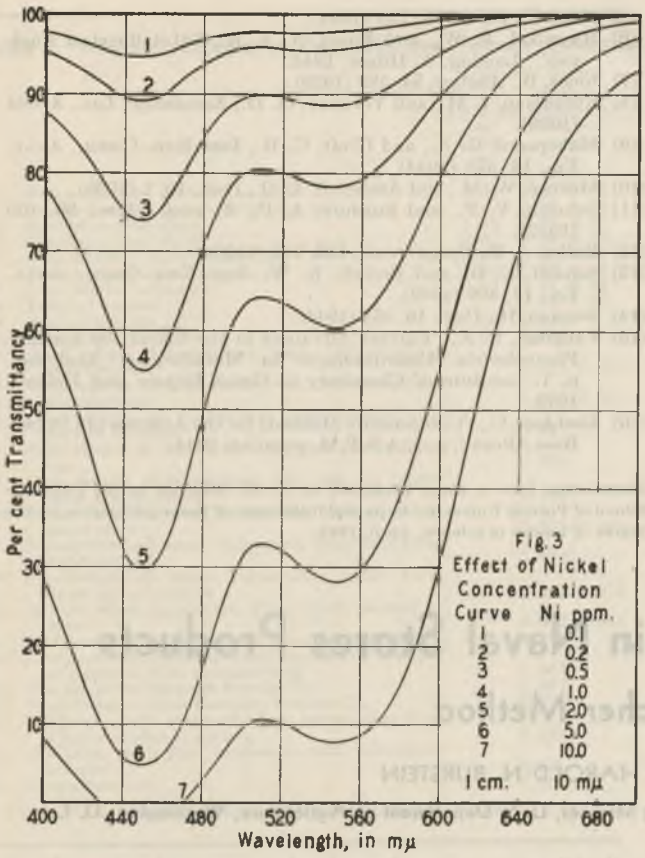
EFFECT OF REAGENT CONCENTRATION. An excess of bromine water, recognized by the yellow color of the solution, is necessary to ensure complete oxidation of the nickel, and therefore complete color development. The bromine water is added first.

Next comes the concentrated ammonia water, its function being to eliminate excess bromine and to adjust the pH value. The reagent is added dropwise until the yellow color from excess bromine disappears. Then 5 ml. more are added, although the color is unaffected by using any volume between 1 and 5 ml. in a total volume of 50 ml. At pH values lower than those thus achieved, the color intensity is less as one goes toward neutrality.

Approximately 1 ml. of 0.1% solution of dimethylglyoxime is required to produce maximum color with 1 p.p.m. of nickel. Excess reagent, at least up to 20 ml., has no further effect.

EFFECT OF SOLVENT. The intensity, stability, and wave length of the transmittancy minima of the colored system depend upon the solvent present.

Water. The solutions whose transmittancy curves are shown in Figure 1 (2 p.p.m. of nickel) contained only the ethanol introduced in 10 ml. of the color-forming reagent. The color did not reach maximum intensity within an hour in the region of the 543 mμ transmittancy minimum. Presumably this indicates a slow shift of some equilibrium reaction. It may be noted that the color has begun to fade at 445 mμ before reaching maximum intensity at 543 mμ. This aqueous solution was too unstable to be satisfactory for analytical determinations. Incidentally, there are two isobestic points in this series of time-study curves, one at 455 mμ and one at 416 mμ.



Ethanol. Difficulties with the aqueous system were avoided by increasing the concentration of ethanol. Both the intensity and the stability of the color were found to be dependent upon the concentration of this solvent. A total of 27.5 ml. of ethanol is the optimum volume for dilution to 50 ml. On using 10 ml. of dimethylglyoxime reagent and 17.5 ml. (±2.5 ml.) of ethanol, the colored system showed the first significant fading after 30 minutes (see Figure 2). The solutions for these curves were identical in composition with those used for Figure 1, except in the concentration of ethanol. If less than 17.5 ml. of ethanol is used, the color shows characteristics approaching those of the aqueous system. Higher concentrations of ethanol increase the rate of fading.

The range of the method, using the ethanol-stabilized system, is 0.1 to 5 p.p.m. with a 1.000-cm. absorption cell (see Figure 3). This system conforms to Beer's law within these concentrations, as determined by measurements at 445 and 543 mμ.

Effect of Diverse Ions. In studying the effect of diverse ions the standard nickel solution was measured with a 2-ml. pipet into a 50-ml. volumetric flask. To this were added 3 ml. of the solution containing the diverse ion and the solution was mixed. After adding an excess of 1 ml. of bromine water, concentrated ammonia water was added dropwise to remove the yellow color and then 5 ml. in excess. Following the successive addition of 10 ml. of dimethylglyoxime solution and 17.5 ml. of ethanol, the solution was diluted to volume with distilled water.

In general, the effect of 600 p.p.m. of diverse ion was determined. If interference was noted, successively smaller amounts were used until the change in transmittancy at 445 mμ amounted to no more than 2% of the nickel, an error considered permissible in such colorimetric measurements. If 600 p.p.m. of the ion did not interfere, it was assumed that the ion would not cause difficulty. Table I summarizes important interferences.

Of the diverse ions studied, the following did not interfere in concentrations 300 times that of the nickel: acetate, arsenate, arsenite, benzoate, borate, bromide, carbonate, chloride, citrate, cyanide, fluoride, formate, iodate, lactate, molybdate, nitrate, nitrite, oxalate, perchlorate, periodate, orthophosphate, pyrophosphate, sulfate, sulfite, tartrate, tungstate, lithium, potassium, and sodium.

Table I. Effect of Diverse Ions

Ion	Added as	Amount *P.p.m.*	Error %	Amount Permissible *P.p.m.*
ClO_3^-	$KClO_3$	600	2	600
$Cr_2O_7^{--}$	$K_2Cr_2O_7$	10	4	5
SeO_4^{--}	Na_2SeO_4	200	2.5	100
NCS^-	KNCS	400	1	400
Au^{+++}	$AuCl_3$	25	2.5	20
Cd^{++}	$Cd(NO_3)_2$	600	0.5	600
Ca^{++}	$Ca(NO_3)_2$	100	1	100
Co^{++}	$Co(NO_3)_2$	5	1	10
Zn^{++}	$Zn(NO_3)_2$	600	0.5	600

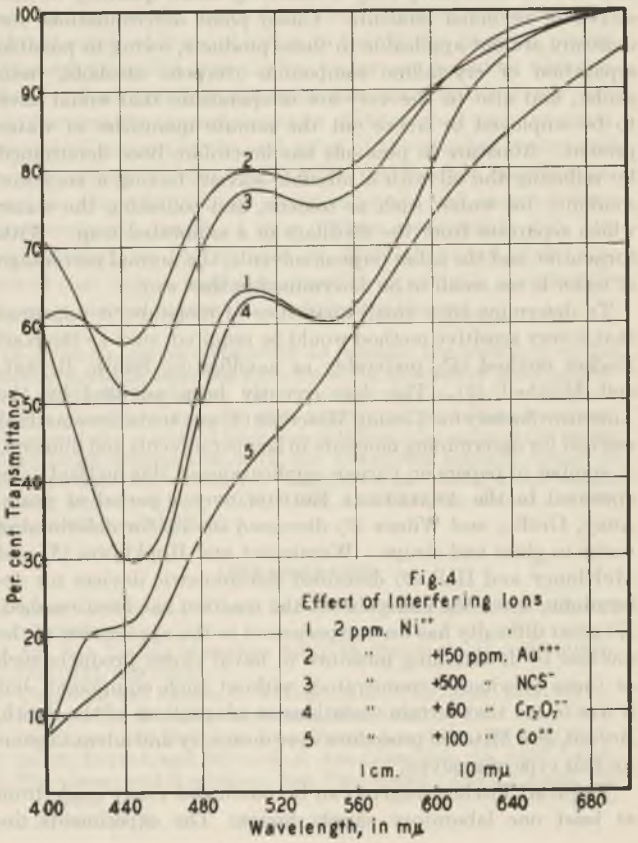
The following ions precipitate and must be removed unless their interference can be prevented by complexation or other applicable reaction: chlorostannous, chlorostannic, iodide, permanganate, silicate, thiosulfate, vanadate, aluminum, antimony, barium, beryllium, bismuth, cerium, chromium, copper, ferrous, ferric, lead, magnesium, manganese, mercuric, mercurous, platinum, silver, strontium, thorium, titanium, uranyl, and zirconium.

Auric, cobaltous, and dichromate ions interfere because of their color. Figure 4 illustrates the nature of the effect. Certain colorless ions interfere when present in large amounts. Examples are the anions chlorate, selenate, and thiocyanate, and the cations cadmium, calcium, and zinc.

From this study of the interference of ions, it is evident that the analysis of various materials, such as alloys, is likely to involve a separation of certain cations before developing the color of the nickel complex. In a specific case it may be possible to eliminate interference by complexation. Thus, tartaric acid functions in this manner for iron in steel analysis (*9*).

RECOMMENDED PROCEDURE

Treatment of Sample. Weigh or measure by volume a quantity of sample containing 0.5 mg. of nickel or less. The proper



treatment of materials depends, of course, on the nature of the sample. When in solution, make the system just acidic by means of hydrochloric acid and/or ammonia water.

SEPARATION AND MEASUREMENT. After bringing the solution to a faint yellow with bromine water, add 2 ml. in excess, then 10 ml. of concentrated ammonia water. If a precipitate forms, stir well and filter, receiving the filtrate in a 100-ml. volumetric flask. If no precipitation occurs, omit the remainder of the separation procedure. Redissolve any precipitate in a minimum amount of hydrochloric acid (1 to 1). Reprecipitate by adding 1 ml. of bromine water and 4 ml. of concentrated ammonia water. Filter, wash, and add the second filtrate to the first. To the combined filtrates add 35 ml. of 95% ethanol and 20 ml. of 0.1% ethanolic dimethylglyoxime reagent. Dilute to volume, mix well, and measure by suitable means. A filter such as Corning No. 440 Signal green is recommended for filter photometers.

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ABSTRACTED from a thesis presented by A. M. Mitchell to the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of master of science, April, 1943.

Determination of Moisture in Naval Stores Products

By the Karl Fischer Method

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DISSOLVED moisture in terpene solvents such as turpentine, pinene, dipentene, and pine oil affects their suitability for certain purposes. For example, turpentine for packaging and distribution in metal cans, pinene for use as a chemical raw material (synthetic camphor), and pine oil for textile preparations and insecticides must carry only a relatively small quantity of dissolved or occluded moisture. Cloud point determinations for moisture are not applicable to these products, owing to possible separation of crystalline compounds (terpene alcohols, resin acids), and also to the very low temperatures that would have to be employed to freeze out the minute quantities of water present. Moisture in pine oils has heretofore been determined by refluxing the oil with a miscible solvent having a very low solubility for water, such as toluene, and collecting the water which separates from the distillate in a calibrated trap. With turpentine and the other terpene solvents, the normal percentage of water is too small to be determined in that way.

To determine such small quantities of moisture, it appeared that a very sensitive method would be required, such as the Karl Fischer method (*5*), preferably as modified by Smith, Bryant, and Mitchell (*7*). This has recently been adopted by the American Society for Testing Materials (*2*) as a tentative standard method for determining moisture in lacquer solvents and diluents. A number of papers on various applications of this method have appeared in the ANALYTICAL EDITION over a period of years. Almy, Griffin, and Wilcox (*1*) discussed its use for determining water in glues and sirups. Wernimont and Hopkinson (*8*) and McKinney and Hall (*6*) described electrometric devices for determining when the end point of the reaction has been reached. No great difficulty has been experienced in the application of the method to determining moisture in naval stores products such as those previously enumerated, without such equipment, but it was found that certain variations or adaptations of the Smith, Bryant, and Mitchell procedure were necessary and advantageous for this type of analysis.

The Karl Fischer reagent can be purchased ready-made from at least one laboratory supply house. The experiments described herein were performed using a reagent prepared and standardized according to the procedure of Smith, Bryant, and Mitchell, except that gaseous sulfur dioxide was used.

MOISTURE IN PINE OIL

The determination of moisture in pine oil by this method proved to be rapid and rather simple, with results which were in very good agreement with those obtained by the method usually used (A.S.T.M. Method D–95 for water in petroleum products, *3*). No additional solvent was required, and excellent checks were obtained on relatively small samples requiring a minimum of reagent. Since the end point with a small sample was sharp, no advantage could be found in using a larger sample of oil requiring from 40 to 50 ml. of reagent. The results obtained on a variety of pine oils and pine oil constituents are given in Table I, along with the results for moisture content as determined by the distillation method.

PROCEDURE FOR PINE OIL (the reversal in the usual order of titration was found to give a closer detection of the end point). Accurately pipet 10 ml. of the Fischer reagent into a 50-ml. Erlenmeyer flask, stopper, and weigh. Immediately run in the pine oil from a 10-ml. microburet, with shaking, until the red-brown iodine color just disappears. At the end point, the change from red-brown to the orange-yellow color of the spent solution is sharp. The weight of the added pine oil is obtained by weighing the flask, or calculating from the specific gravity or density. Duplicate titrations should check to within 0.2 ml.

Calculate the moisture content as follows:

$$\text{Per cent water} = \frac{T \times A}{g} \times 100, \text{ or } \frac{T \times A}{V \times D}$$

where
T = titer or moisture value of 1 ml. of reagent
A = ml. of reagent used
g = weight of sample
D = density (or specific gravity) of sample

The above proportions hold for ordinary pine oil, of up to 0.5% moisture content. For larger moisture content, 20 ml. of reagent

Table I. Moisture Content of Pine Oils and Terpene Solvents

	Sample	Moisture Content A.S.T.M. test D–95 %	Moisture Content Authors' procedure, Fischer reagent %
1.	Steam-distilled amber pine oil	0.45	0.46, 0.46
2.	Steam-distilled water-white pine oil	0.35	0.34, 0.34
3.	Synthetic pine oil, A	1.08	0.87, 0.87
4.	Synthetic pine oil, B	0.51	0.50, 0.52
5.	Destructively distilled pine oil	0.44	0.42, 0.41
6.	Destructively distilled pine oil	1.04	0.86, 0.85
7.	Commercial terpineol	0.43	0.42, 0.44
8.	Fenchyl alcohol	..	0.78, 0.80
9.	Redistilled pine oil	0.00	0.13
10.	Redistilled terpineol	0.00	0.075
11.	Gum spirits of turpentine, fresh, from drum at paint store	..	0.012, 0.015
12.	Gum spirits of turpentine, fresh, from southern storage tank	..	0.020, 0.021
13.	Gum spirits of turpentine from original can packed in South	..	0.032
14.	Gum spirits of turpentine, old, from partly filled can	..	0.074
15.	Gum spirits of turpentine, very old, no longer meet specifications	..	0.380[a]
16.	Steam-distilled wood turpentine from drum at paint store	..	0.022, 0.026
17.	Steam-distilled wood turpentine from can filled at plant, old	..	0.110
18.	Steam-distilled wood turpentine, fresh, from drum at paint store	..	0.017, 0.018
19.	Sulfate wood turpentine, glass bottle packed at plant	..	0.042, 0.049
20.	Sulfate wood turpentine, old, from partly filled can	..	0.28
21.	Destructively distilled wood turpentine, sample as received from manufacturer	..	0.064
22.	Pinane, from Hercules Powder Co.	..	0.031
23.	Para-thinner, from Hercules Powder Co.	..	0.078
24.	α-Pinene, from steam-distilled turpentine, from drum sampled at plant	..	0.081
25.	β-Pinene, from gum turpentine, old, laboratory sample	..	0.13
26.	Dipentene, old	..	0.25

[a] Decomposed reagent at first. Test satisfactory on cooling to 5° C.

are recommended. For anhydrous pine oil, on the other hand, much less than 10 ml. of reagent would be required, so it is preferable to pipet 10 ml. of the sample, weigh, and titrate with the reagent to a red-brown end point.

Only two samples gave results not in agreement with the reflux distillation method. In the case of sample 3, a synthetic pine oil, there is evidence of the presence of polyhydroxy alcohols which are easily decomposed to form water as one of the end products on heating. Sample 6 was a very old (at least 15 years) bottled sample on which no previous data were available. Here again, it is possible that the presence of low-boiling constituents miscible with water, such as wood naphtha, or which decomposed on heating to form water, gave a high water recovery by the ordinary method of determining water in pine oil.

Samples 9 and 10 are included to simulate or represent commercially anhydrous pine oils of very low moisture content. They were prepared by redistilling commercial samples of regular pine oil and of terpineol, with recovery of the material after the initial moisture-containing portion had been rejected. The greater sensitivity of the Karl Fischer method over the ordinary distillation method for such anhydrous pine oils is brought out by the results shown.

TERPENE HYDROCARBON SOLVENTS

Turpentine, pinene, and related terpene hydrocarbons did not lend themselves to direct titration with the Fischer reagent, because of the formation of a precipitate which prevented any observation of the end point. It was possible to overcome this difficulty by the use of excess pyridine which apparently prevents the formation of or dissolves the insoluble compound. As turpentine and methanol are not miscible in all proportions, the reaction mixture forms two layers, the upper one consisting of the turpentine with some methanol and pyridine in solution, the lower one probably a saturated solution of the pyridine salts in methanol. Most of the color developed during the course of the reaction is absorbed by the bottom layer, which gradually takes on the orange-yellow color of the spent reagent. The end point is best observed while the contents of the flask are being vigorously whirled, the change from the orange-yellow to the red-brown being easily discernible. When the layers settle and separate, the bottom layer will be red-brown, the upper layer light yellow or almost colorless.

Procedure for Terpene Solvents. It is advisable to set up three burets, two of 50-ml. capacity for dry pyridine and Fischer reagent, respectively, and one 10-ml. microburet for additional Fischer reagent.

Run 20 ml. of dry pyridine into a 250-ml. Erlenmeyer flask and titrate with the reagent from the larger buret to a red-brown. If the pyridine has not been sufficiently dried, 30 ml. or more of reagent may be required. Then add pyridine dropwise until the red-brown color is just discharged. Stopper and weigh the flask.

Pipet in 100 ml. of the turpentine or terpene solvent under test, stopper, and weigh again. If the turpentine is highly oxidized, a reaction may take place with the spent reagent present, indicated by evolution of heat and decomposition of the spent reagent, with liberation of free iodine. In such case, start over, and place the flask with the pyridine in an ice bath, adding the turpentine slowly with gentle mixing.

Add the reagent from the microburet with vigorous shaking of the flask. Stop occasionally and allow the layers to separate, so the color of the bottom layer can be observed. The change from the orange-yellow to the red-brown color at the end point may require a slight excess of reagent, but this will not affect the results greatly. Most turpentines will require 6 to 10 ml. of reagent. The average of two titrations agreeing within 0.5 ml. can be taken. Calculate the percentage of moisture as with pine oil.

The results obtained on a number of samples of turpentine and related terpene solvents, shown in Table I, cover a rather wide range of condition of sample and moisture content. No comparable results by any other method are available, since no other suitable method has been developed. The Zerewitinoff method (*9*) could not be used, as it measures alcohols as well as water, and Chadwick and Palkin (*4*) showed that small quantities of alcohols were present in turpentine. The results appear to be of logical magnitude and in correlation with the source, identity, appearance, and age of the various samples. Possible sources of error either through addition of iodine to the double bond of pinene, or through liberation of free iodine by terpene peroxides were considered. Such reactions generally take place rather slowly, and would occur after the main reaction of the reagent with the water. Free iodine present at the end point of the reaction is absorbed by the terpene solvents only after the reaction mixture has stood for about 30 minutes, as an end point will endure for approximately this length of time. These observations were also substantiated by the findings of McKinney and Hall, who showed that only a negligible quantity of iodine is absorbed by pinene or pine oil, under the conditions of the test.

In order to test as completely as possible the accuracy of this procedure, check analyses were made on samples of gum turpentine, dried by refluxing over calcium oxide and redistilled. Known quantities of water were introduced by adding measured volumes of the standard moisture solution, the amounts of water added being comparable with the quantities actually found in authentic samples. Results: calculated for water, from weight of water added: 0.020, 0.034, 0.062, and 0.089%; found: 0.020, 0.034, 0.062, and 0.090%.

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Estimation of Riboflavin, Thiamine, and N^1-Methylnicotinamide

Rapid Field Methods

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Rapid field methods for estimating riboflavin, thiamine, and N^1-methylnicotinamide in small volumes of urine are described. They employ simple, rugged apparatus and visual fluorometry, and can be used under primitive field conditions.

THE stimulus of repeated surveys has led to the development of a field nutrition laboratory for use even under primitive conditions (*4*). The primary requirements for field chemical methods are that they should demand small samples, should require the transportation of the least possible bulk of reagents, should allow ease and rapidity of manipulation, and should employ simple apparatus that is rugged and light in weight. The methods described below satisfy these requirements and in addition have two features not common to orthodox fluorometric methods. (1) Visual fluorometers allow readings to be made with as little as 0.5 ml. of solution, and in contrast to the usual types of delicate photoelectric fluorometers are rugged and do not demand stable sources of power. (2) All reactions are carried out in a single glass vessel to avoid the necessity of transferring, filtering, fractionating, or centrifuging and to make the methods faster than the usual chemical estimations of riboflavin, thiamine, and N^1-methylnicotinamide.

RIBOFLAVIN

PRINCIPLE. The riboflavin in urine is extracted into isobutyl alcohol after preliminary treatment with potassium permanganate followed by hydrogen peroxide in the presence of pyridine and acetic acid (*5*).

APPARATUS. A source of ultraviolet light consists of a General Electric Type H-4 mercury vapor bulb enclosed in a box with louvers for air cooling and a Wood filter for absorbing visible light. A General Electric autotransformer Type 59G18 is placed in series between the bulb and any suitable source of 110-volt alternating current. In the field simple motor generators without line stabilization can be used.

Fluorometric comparator consisting of a thin wooden rack containing 10 Corning glass-stoppered thin-walled 2-ml. test tubes, outside diameter 10 mm. and length 75 mm. Holes are bored so that these tubes which contain standard solutions are flanked by empty holes to hold 10 × 75 mm. thin-walled test tubes containing the unknown solution. The rack is constructed to slide conveniently in a direction perpendicular to the ultraviolet ray and 8 cm. from the Wood filter. On the side of the rack nearest the eye a suitable yellow filter is fixed. It should have maximal transmission at 560 mμ.

A suitable darkened room for reading unknown solutions. This need not be completely light-tight.

Glass-stoppered test tubes of 10-ml. capacity are conveniently made from standard 10-ml. glass-stoppered footed mixing cylinders by removing the foot with a sharp tap of a tack hammer and rounding the bottom in the blast lamp.

Suitable beakers, delivery pipets, and other usual appurtenances, including a large number of 10 × 75 mm. thin-walled test tubes. One special pipet that is of great assistance is an automatic syringe pipet calibrated to deliver 1.5 ml.

REAGENTS. Oxalic acid, dry, powdered. A mixture of 1 part pyridine and 1 part glacial acetic acid. Aqueous 4% potassium permanganate. Aqueous 3% hydrogen peroxide.

Isobutyl alcohol of low blank. *n*-Butyl alcohol is equally satisfactory. Each fresh batch of alcohol should be examined before use before the ultraviolet light and redistilled if there is perceptible fluorescence.

Anhydrous sodium sulfate.

PROCEDURE. Freshly voided urine collected in glass vessels or unwaxed paper cups of low blank is stored in amber glass bottles with the addition of about 100 mg. of oxalic acid for every 25 ml. of urine. Ponting (*8*) has emphasized the suitability of oxalic acid for stabilizing ascorbic acid, and in addition riboflavin, thiamine, and N^1-methylnicotinamide are maximally stable at the acidity obtained by the above step.

All manipulations should be carried out in diffuse light. In the experience of this laboratory only direct sunlight destroys riboflavin rapidly enough to make extreme precautions necessary against light.

One-half milliliter of urine is delivered into a 10-ml. glass-stoppered test tube, and 0.5 ml. of the pyridine–glacial acetic acid mixture is added. One drop of potassium permanganate is added and mixing is effected by gentle shaking of the rack. The time of standing may be anywhere from 0.5 to 5 minutes at this stage without affecting the results. Two drops of hydrogen peroxide are added and mixed by gentle shaking of the rack. The permanganate should be destroyed within a few seconds. If it is not, another drop of hydrogen peroxide may be necessary. In temperatures near freezing the tube may be gently warmed in a water bath at 70° F.

Isobutyl alcohol (1.5 ml.) is added by means of either an ordinary pipet or preferably an automatic syringe pipet, which avoids getting unpleasant vapors in the mouth. The glass stopper is inserted and the tube is shaken vigorously by hand with an up and down motion 25 times. The distribution coefficient of riboflavin between the aqueous phase and the alcohol-pyridine–acetic acid phase is such that more shaking is unnecessary. The test tube is allowed to stand in the rack until the aqueous phase is nearly separated. This usually takes from 1 to 5 minutes, being faster the higher the temperature. The glass stopper is removed, a small wide-mouthed funnel is placed in the neck of the tube, and a small spatula of sodium sulfate is added to the tube. A gentle rotary motion imparted to the tube assists clearing of the alcohol layer. The tube is allowed to stand in the rack until it is water-clear, which usually takes 1 to 2 minutes; a few turns on a hand centrifuge will materially hasten this step.

Approximately 1 ml. of the alcohol layer is transferred to a standard thin-walled Pyrex test tube of dimensions 10 × 75 mm. This is best achieved with a small syringe fitted with a lumbar puncture needle, but any pipet can be used. Two precautions are necessary: The needle must be washed immediately after the completion of a run to avoid corrosion of the needle after prolonged action of pyridine and acetic acid. The pipet or syringe need be rinsed with isobutyl alcohol between deliveries only when one expects wide variations in vitamin concentrations between samples. Reading is conducted before the ultraviolet lamp. The alcohol solutions remain stable for at least 2 hours even at high temperatures. There is invariably a silvery blue fluorescence, which is absorbed by a suitable yellow filter, and is not destroyed by ultraviolet light. After the initial reading if one wishes to obtain true riboflavin it is necessary to expose the solution to strong ultraviolet light for an hour or more. If this step is omitted satisfactory comparative results are still obtained, since it has been found in 90 random specimens of urine from 90 different subjects that the amount of fluorescence not destroyed by ultraviolet light is remarkably constant, and amounts to about one half of the initial reading. In specimens collected after loading tests, the concentration of riboflavin is usually so high that the ultraviolet stable fluorescence amounts at the most to one fifth of the initial reading.

STANDARD SOLUTIONS. Standards are prepared by running appropriate dilutions of riboflavin with the reagents and technique described above. Solutions are conveniently prepared from vitamin tablets supplied by reputable drug houses, provided each batch is assayed before use. A convenient range of riboflavin standards consists of samples equivalent to 0, 25, 50, 75, 100, 125, 150, 200, 250, and 300 micrograms per 100 ml. of original solutions. These must be checked daily or oftener with a fresh

standard, and renewed when the exposure to ultraviolet light has significantly changed them.

THIAMINE AND N^1-METHYLNICOTINAMIDE

These two substances are estimated by methods so similar that they are best discussed together except in the last steps. The method of Hennessy and Cerecedo (*3*) and the modifications of Egaña and Meiklejohn (*2*) have been incorporated in the present analysis of thiamine and the method of Najjar and Wood (*7*) has been followed for estimating N^1-methylnicotinamide. A considerable saving in time and manipulation has been achieved by adsorbing both substances on activated zeolite and eluting with potassium chloride not in a base-exchange column but in the vessels where thiamine is converted to thiochrome and where factor F_2 is formed by addition of alkali. Both vitamin derivatives are shaken into isobutyl alcohol and are estimated by visual fluorometry.

Apparatus. All equipment is the same as for riboflavin, except that no light filter is used in the fluorometric comparator.

Reagents. Oxalic acid.

Activated zeolite. Considerable time, patience, and sometimes experimentation are required to get a satisfactory product which will settle out rapidly and also adsorb thiamine well. Commercial zeolite is crushed in a stone crusher or suitable mortar. Permutit and Decalso are as good as zeolite. That portion of the product which will pass a 100-mesh sieve is saved and suspended in 1% acetic acid in distilled water in large cylinders. The granules which settle to the bottom rapidly—that is, in about 2 minutes—are separated from the lighter particles by decanting and are boiled 3 times with 1% acetic acid with settling and decanting between fresh additions of acetic acid. The product is washed with distilled water and dried in the oven at 110° C. It should be a white homogeneous powder and when about 200 mg. are shaken with water in a 10-ml. mixing cylinder all the particles should settle rapidly to the bottom in not more than 2 minutes. There may be great variability from batch to batch. When test runs with synthetic thiamine are made, recovery is sometimes poor and the product may have to be reactivated by the technique of Najjar and Wood (*7*). If addition of ferricyanide results in the formation of Prussian blue, the product has to be washed with warm fairly strong solutions of hydrochloric or nitric acid. When a satisfactory product is finally obtained, its activity remains constant for months if it is kept dry.

Aqueous potassium chloride, approximately 25%. At temperatures near freezing the solubility properties necessitate lower concentrations.

Aqueous 15% sodium hydroxide.

Aqueous 0.25% potassium ferricyanide. This should be made up fresh every day, most conveniently from weighed samples stored dry in small stoppered test tubes.

Isobutyl alcohol. This must have a blank of essentially zero and each new batch has to be tested before use. It can be recovered by redistillation of the residue after analysis. *n*-Butyl alcohol can be used instead of isobutyl.

Dilute acetic acid, approximately 1% in distilled water.

Procedure. Urine is collected and stored as under riboflavin. N^1-Methylnicotinamide is stable for days and thiamine for weeks even at high environmental temperatures. The initial steps are identical for N^1-methylnicotinamide and thiamine, and it is therefore convenient to run both at the same time but with separate aliquots until the final stages, which are differentiated below.

Two milliliters of urine are pipetted into a glass-stoppered test tube. If the urine is likely to be high, 0.5 ml. is used. About 200 mg. of zeolite are delivered from a small spatula into the tube, most easily through a small funnel, and mixed by about 10 rapid vigorous shakes of the rack.

(1) In the case of urine from normal men neither preliminary treatment with isobutyl alcohol to remove interfering substances in the estimation of thiamine nor preliminary treatment with Norite to remove substances interfering with the estimation of N^1-methylnicotinamide has any significant effect in the present method and both can be omitted with safety. (2) The adsorption of thiamine and N^1-methylnicotinamide on the zeolite is not critically affected by acidity in the present method. So long as the urine is between pH 3 and 6 recovery of added thiamine is essentially the same. Oxalic acid added to the urine as specified provides this acidity. (3) The time of standing at this stage is not critical.

Adsorption is complete after 10 shakes. Approximately 8 ml. of acidified water are added to the tube, the top is closed with a clean finger, and the tube is mixed by inversion 10 times. After a few seconds' standing in the rack until the particles of zeolite have begun to settle, the few particles adherent to the top and sides are washed down by closing the top with a clean finger and administering a single sharp upward jerk. The tube is replaced in the rack until the zeolite particles have settled to the bottom. In urine containing oxalic acid there is usually slight permanent turbidity which is disregarded in deciding when the zeolite has settled. The supernatant fluid is withdrawn by means of suction through a long needle of stainless steel and is discarded. A syringe or vacuum pump may be used.

Approximately 8 ml. of acid are added and the processes of standing, inversion, and removal of the supernatant fluid are repeated. This washing step is critical in the estimation of both N^1-methylnicotinamide and thiamine but for different reasons. In the case of thiamine, if washing is not effective, the fluorescence obtained in the final step is off-color with a silvery blue admixture to the true thiochrome mauve and reading is wholly unsatisfactory. Tap water can usually be used instead of distilled water but it may be necessary to add more acetic acid and sometimes even to wash three times before the thiochrome color is satisfactory. Thiamine is firmly bound to the zeolite and repeated washings remove the interfering substances without affecting the thiamine. Experimentation determines how many washings with the available water supply are required in order to get satisfactory results for thiamine. In the case of N^1-methylnicotinamide in contrast to thiamine each washing diminishes the final fluorescence. Apparently N^1-methylnicotinamide is relatively easily washed off the zeolite, whereas thiamine is firmly bound. In making routine surveys it is therefore essential to adopt and follow scrupulously a fixed technique of washing in order to be able to compare values for N^1-methylnicotinamide from one place to another.

Potassium chloride (0.5 ml.) is added to the tube and the rack is shaken gently, so as to mix the zeolite with the potassium chloride but not to streak the zeolite far up the sides. The time necessary for elution does not exceed 30 seconds. From this point on the estimations of N^1-methylnicotinamide and thiamine are different.

Final Steps for Thiamine. Potassium ferricyanide (0.1 ml.) is added and mixed by a few gentle shakes of the rack; 0.25 ml. of sodium hydroxide is added and mixed with a gentle shake of the rack. The time necessary for completing the oxidation from thiamine to thiochrome is only a few seconds. Once formed in this mixture it is stable for at least 0.5 hour. (Although thiochrome in alkaline aqueous solutions is destroyed in a relatively short time, a considerable body of experimental data has been obtained, demonstrating that in the presence of zeolite it is stable for periods up to 0.5 hour.) Isobutyl alcohol (2 ml.) is added, the glass stopper is inserted, and the tube is shaken vigorously 25 times up and down its long axis. This number of shakes ensures complete distribution of thiochrome between the alcohol and aqueous phases; more shaking is superfluous. The tube is allowed to stand in the rack until separation of the phases is almost complete or is centrifuged a few seconds in a hand centrifuge if separation is unsatisfactory, as it sometimes is in cold places. About 1 ml. of the supernatant fluid is transferred to a small test tube by means of a pipet or a syringe and needle, and the fluorescence is matched in the visual comparator against standards prepared by running solutions of thiamine hydrochloride through the method, thus compensating for variability in the reagents. Convenient standards are 0, 5, 10, 15, 20, 30, 50, 75, 100, 150, and 200

Table I. Reproducibility of Results with Aqueous Solutions and Recovery from Normal Urine

Substance	Amount Added	Amount Recovered: Water	Amount Recovered: Normal urine
	Micrograms per 100 ml.		
Riboflavin	25	25	25
	50	52	47
	100	102	110
	150	145	150
	200	190	210
Thiamine hydrochloride	5	6	5
	10	11	10
	20	20	20
	50	52	52
	100	100	102
N^1-Methylnicotinamide chloride	250	250	250
	500	500	600
	1000	1100	1100
	2000	1900	2100
	4000	3950	4100

Table II. Comparison of Field Methods with Usual Laboratory Methods

Substance	Method	Concentration of Vitamin Sample 1	Sample 2	Sample 3
		Micrograms per 100 ml.		
Riboflavin	Present field	40	60	70
Riboflavin	Najjar (*5*)	40	56	75
Thiamine	Present field	13	20	50
Thiamine	Egaña and Meiklejohn (*2*)	10	18	52
N^1-Methylnicotinamide	Present field	250	250	500
N^1-Methylnicotinamide	Najjar and Wood (*7*)	300	300	550

micrograms of thiamine hydrochloride per 100 ml. The fluorescence of the isobutyl layer is stable for several days even in high temperatures.

In the urine of normal young men, not receiving drugs and not deficient, no correction for F_1 or F_2 need be made unless the ratio of N^1-methylnicotinamide to thiamine is high. In the present method, such an occurrence is detected at once because the thiochrome color is then distinctly tinged with a greenish component, and blank correction can be made. Such specimens have been rare in any of the surveys so far conducted by this laboratory. Najjar and Ketron (*6*) have recently demonstrated that in the usual case the F_2 correction is small in magnitude and becomes important only in samples low in thiamine.

Final Steps for N^1-Methylnicotinamide. Two milliliters of isobutyl alcohol are delivered into the tube (no mixing is necessary at this point), 0.25 ml. of sodium hydroxide is added, and the tube is stoppered with its glass stopper and shaken 25 times as under thiamine. The addition of sodium hydroxide should be followed by shaking at once, because factor F_2 in aqueous alkaline solution breaks down to nonfluorescent end products within a very few minutes but is stable in the isobutyl layer for hours. The tube is replaced in the rack until separation of the phases is complete, or it is centrifuged for a few seconds in a hand centrifuge. One milliliter of the supernatant layer is transferred to a small test tube as under thiamine.

Maximal F_2 fluorescence is developed relatively slowly, so that reading must be made not sooner than 5 minutes after the shaking with sodium hydroxide. Fluorescence is matched in the comparator against standard solutions of F_2 in isobutyl alcohol, prepared by running a series of appropriate aqueous solutions of N^1-methylnicotinamide chloride through all stages of the method. A convenient series of aqueous standards is 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.5, 3.75, 5.0, 7.5, and 10.0 mg. per 100 ml. (In the field it is sometimes convenient to use aqueous solutions of quinine sulfate dissolved in approximately 0.1 *N* sulfuric acid. The relation between the two types of standard is linear, as is shown by an experiment in which standards of F_2 representing aqueous solutions of N^1-methylnicotinamide chloride in the concentrations 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.5, 3.75, 5.0, and 7.5 mg. per 100 ml. of matched quinine standards which read 0, 5, 10, 15, 20, 30, 50, 75, 100, and 150 micrograms of quinine sulfate per 100 ml. The quinine color is not a perfect match against F_2 but is satisfactory.) No blank correction is made unless the apparent concentration of N^1-methylnicotinamide is over 1.5 mg. per 100 ml., the blank correction being insignificant for normal urine of low concentrations.

RESULTS AND DISCUSSION

The present methods yield reproducible results with aqueous solutions of riboflavin, thiamine hydrochloride, and N^1-methylnicotinamide chloride, and recovery is satisfactory when these substances are added to urine (Table I). The method for N^1-methylnicotinamide compares favorably with that of Coulson, Ellinger, and Holden (*1*). (Reference standards should be obtained from E. Fullerton Cook, Chairman, U. S. Pharmacopoeial Revision Committee, 43rd St. and Woodland Ave., Philadelphia 4, Pa. Synthetic N^1-methylnicotinamide chloride is obtainable from W. A. Taylor Co., Baltimore, Md.)

With the urine of well-fed young men who are not receiving drugs substantially the same results are obtained by the present methods and by the methods from which they are derived. Typical results for samples of low, intermediate, and high concentrations are presented in Table II. With some kinds of abnormal urine, preliminary treatment of the urine is necessary before reliable results can be obtained. For instance, when men are receiving atabrine or quinine, thiamine can be estimated accurately after the urine has been treated with isobutyl alcohol and a very small amount of Norite A; and accurate estimations of N^1-methylnicotinamide are possible after the urine has been shaken with a relatively large amount of Norite A.

Means and ranges are given in Table III for field surveys conducted by this laboratory under a variety of climatic conditions. Such data show the type of results that may be expected in populations of supposedly well-fed healthy young men. They were all living on field rations, some of which had been supplemented with yeast or synthetic vitamins. This accounts for the high values sometimes observed.

The present field methods are so rapid that four technicians can perform duplicate estimations of riboflavin, thiamine, and N^1-methylnicotinamide in 100 specimens of urine in 2 working days without undue haste. As described above, they are applicable to urine from healthy, well-fed subjects, and should not be applied to urine from other types of subjects without careful comparison with standard laboratory methods. Systematic work is now in progress on modifications in procedure that may be necessary for burned patients, for patients receiving drugs, and for frankly deficient patients. The specificity of the present methods for testing urines from normal subjects is that of the standard procedures from which they were derived. The most reliable results are perhaps for vitamin tolerance tests, in which any increment in urinary excretion must be due to the test dose.

Table III. Urinary Excretion of Vitamins[a]

Thiamine Mean	Thiamine Range	N^1-Methylnicotinamide Mean	N^1-Methylnicotinamide Range	Riboflavin Mean	Riboflavin Range
Micrograms per hour		*Mg. per hour*		*Micrograms per hour*	
149 Men in the Mojave Desert					
2	0– 7	0.15	0–1.00	40	2–157
85 Men in Subarctic Conditions (6 Observations Each)					
11	1–66	0.30	0–1.30	50	13–320
35 Men in New England Spring and Autumn (3 Observations Each)					
4	1– 9	0.30	0.05–0.85	35	12–120
595 Men in the Rocky Mountains, Summer (2 Observations Each)					
13	1–65	0.45	0.05–2.10	41	3–246

[a] Specimens collected in morning after arising but before breakfast.

ACKNOWLEDGMENTS

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CORRECTION. In the article on "Colorimetric Estimation of Aluminum in Aluminum Steel" [IND. ENG. CHEM., ANAL. ED., **17**, 206 (1945)], an error occurred under the heading "Solutions Required". Both the first and second paragraphs should read "add 1 ml. of 10% benzoic acid in methanol".

C. HOWARD CRAFT
G. R. MAKEPEACE

Vacuum Drying Apparatus for Unstable Polymeric Materials

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DURING the course of research conducted in these laboratories on unsaturated high molecular weight polymers like natural rubber, GR-S, etc., there developed a need for an efficient drying apparatus capable of rapidly and completely removing from these materials volatile substances such as water, acetone, benzene, while ensuring protection against oxidation. The regular laboratory drying methods, such as the use of a high static vacuum in a vacuum desiccator or a constantly changing inert atmosphere at ordinary temperature, were found slow and inefficient, thus being the source of considerable delay in the

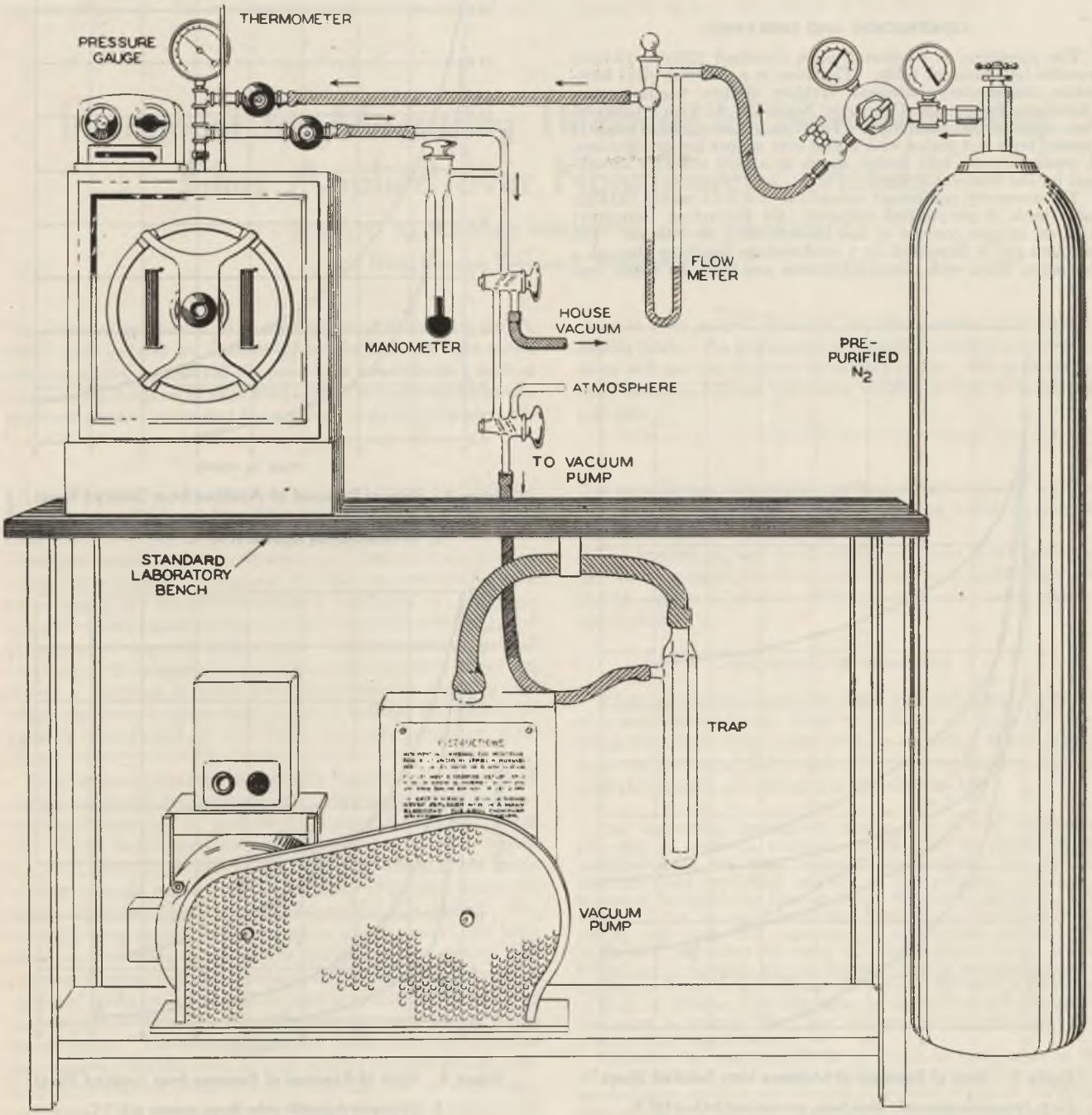

Figure 1. Diagram of Apparatus

prosecution of the research work. Because of the susceptibility of the above materials to oxidation the use of open electrically heated ovens is inadvisable.

Experience has shown that the rate of drying of rubber and rubberlike materials is dependent upon temperature, the amount of exposed surface area per unit weight of the material, the type and amount of the sorbed matter, and the concentration of the volatile matter in the atmosphere (humidity) surrounding the surface of the rubber. By making use of presently available laboratory equipment the authors have constructed a highly efficient drying apparatus at moderate cost which employs both elevated temperatures and a constantly changing inert atmosphere to accelerate the drying process and at the same time protect the material against oxidation. A sketch of the apparatus is shown in Figure 1. The apparatus is operated at low pressures for the purpose of obtaining as much as possible an oxygen-free atmosphere and for the most economical use of the inert gas.

CONSTRUCTION AND OPERATION

The apparatus is supported on a standard 120-cm. (4-foot) wooden-top chemical table. The oven is a stainless steel automatic temperature-controlled Webber electric vacuum oven (American Instrument Company, No. 4-158 A) with a temperature range of 20° to 150° C. The vacuum chamber is made of pressed steel and plated with nickel over copper for air-tightness. A small-diameter lead gasket serves as a very effective airtight seal for the removable door.

The accessory equipment consists of a 6.3-cu. meter (224-cu. foot) tank of prepurified nitrogen (Air Reduction Company) with an oxygen content of less than 0.002% by volume. The nitrogen gas is dispensed by a double-stage regulator through a flowmeter filled with dibutylphthalate and into the upper connection to the vacuum chamber. The exit valve of the vacuum chamber is connected to a mercury manometer and to a series of two three-way stopcocks with leads attached to the house vacuum system, to the atmosphere, and to a Duo-Seal vacuum pump. A glass trap is included in the line to remove condensable vapors during the operation of the system, solid carbon dioxide in acetone being used as the coolant. Vibrations are held to a minimum by mounting the pump on a rubber mat 0.6 cm. (0.25 inch) in thickness. All connections are made with heavy-walled rubber tubing. For airtightness all rubber-to-glass and rubber-to-metal joints are cemented with shellac. The flowmeter and manometer are supported by metal rods (not shown in sketch) attached to the wooden table top.

The apparatus is operated by placing the samples on stainless steel wire-mesh trays in the vacuum chamber and securing the door tightly in place by means of the screw-clamp handle.

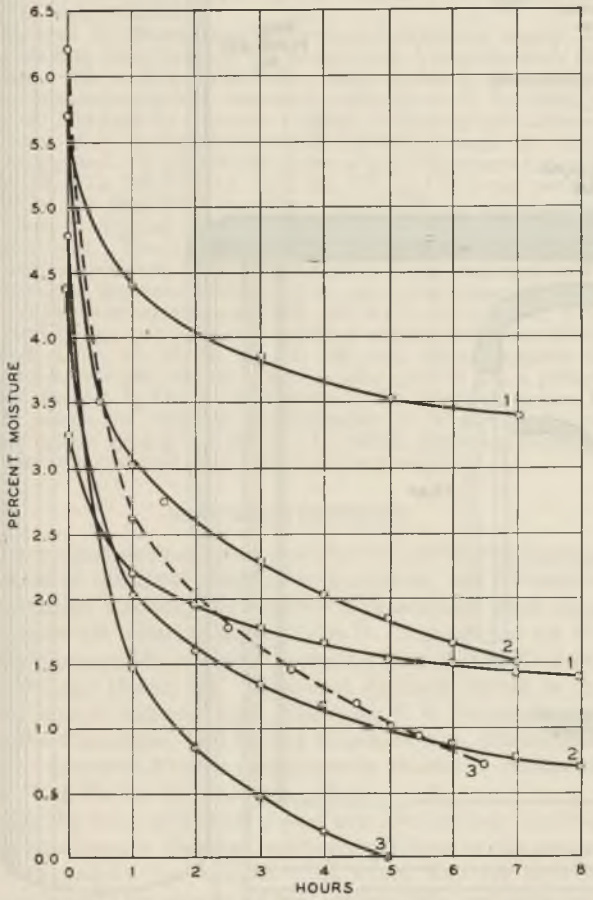

Figure 2. Rate of Removal of Moisture from Smoked Sheet

1. In vacuum desiccator under 3-mm. pressure over P_2O_5 at 25° C.
2. In vacuum-drying apparatus at 55° C.
3. In vacuum-drying apparatus at 80° C.

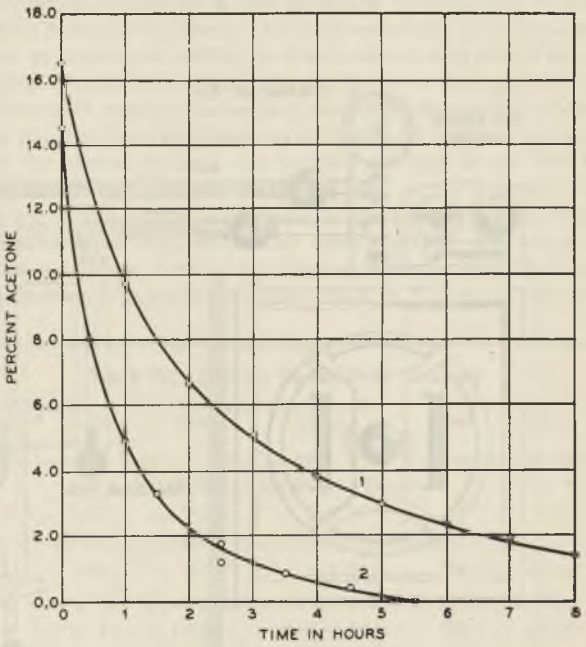

Figure 3. Rate of Removal of Acetone from Smoked Sheet

1. In vacuum desiccator under 3-mm. pressure at 25° C.
2. In vacuum-drying apparatus at 50° C.

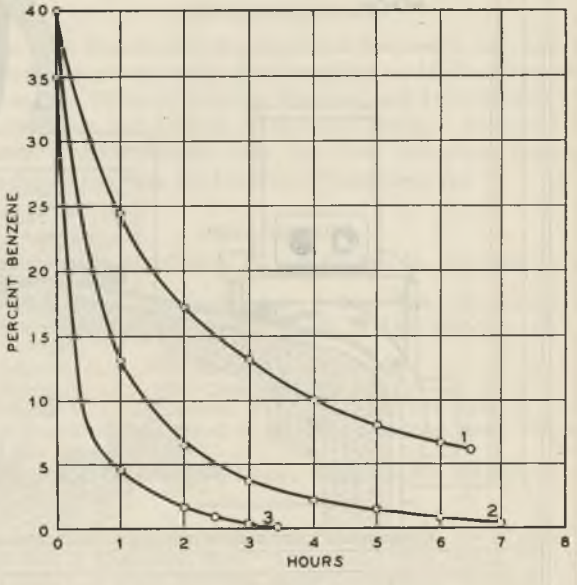

Figure 4. Rate of Removal of Benzene from Smoked Sheet

1. In vacuum desiccator under 3-mm. pressure at 27° C.
2. In vacuum-drying apparatus at 55° C.
3. In vacuum-drying apparatus at 80° C.

The vacuum chamber is evacuated and recharged to atmospheric pressure with the prepurified nitrogen. This is repeated twice. When the mercury manometer becomes steady the valve attached to the gas regulator is opened slightly to where the difference in the two levels of the dibutylphthalate in the flowmeter is 3 mm. with an orifice of about 2.0 mm. This corresponds to a gas flow of 78 liters of expanded gas per hour by the flowmeter which was calibrated at atmospheric pressure with a wet-test gas meter. The samples are removed by shutting off the vacuum and filling the vacuum chamber with nitrogen to slightly above atmospheric pressure. The stopcock arrangement provides for the immediate release of the vacuum outside the pump as a safeguard against drawing the pump oil into the system. After cooling to room temperature, the materials are weighed and the operation is repeated until a constant weight is obtained. The house vacuum system serves well for overnight drying or when a considerable amount of volatile matter is to be removed.

FACTORS INFLUENCING DRYING RATE OF RUBBER

The rate of drying of rubberlike materials depends upon a number of factors. Elevated temperatures, a large exposed surface, and a rapidly changing low "humidity" atmosphere favor rapid drying. Figures 2, 3, and 4 show, for example, the effect of temperature on the rate of removal of water, acetone, and benzene from a 3.75-cm. (1.5-inch) square 0.3-cm. (0.125-inch) thick piece of Grade A smoked sheet rubber. Curves designated as 1 represent the slow rate of drying under ordinary laboratory conditions, as compared with curves 2 and 3 which show a much more rapid rate when carried out in the drying apparatus at elevated temperatures. By increasing the temperature to 110° C. it was found that it required about 0.5 hour to remove 5% of moisture from the smoked sheet. The same drying rate was found in cases where the drying was carried out at atmospheric pressure instead of under vacuum. This indicates that the drying process depends principally upon diffusion rather than evaporation from the liquid phase.

In drying delicate biological substances there exists a need for safe and efficient drying procedures and it is hoped that the present work may assist others in this connection.

Instrument for Measuring Thickness of Nonconducting Films Applied over Nonmagnetic Metals

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The extensive application of camouflage paint to military aircraft, whose paint surfaces are constructed largely from the light metals and their alloys, emphasized the need for a nondestructive method for measuring thickness of paint films applied to nonmagnetic metals. In aircraft painting, control of film thickness is doubly important because of strict weight allowance and the necessity of providing a durable finish. An instrument is described for making such measurements and data are presented illustrating its use. The gage satisfactorily measures coatings containing metallic as well as nonmetallic pigments.

IN THE study of the properties of paint films, methods for determining thickness have been helpful in making more uniform measurements, since some properties of paint films are dependent largely upon the thickness at which they are applied to any given surface. In the past, the most common instruments for measuring dry film thicknesses have been based on a magnetic principle which results in their use for the study of films applied to surfaces of magnetic metals only. Lack of a similar method for studying films applied to the light metals and their alloys has proved a handicap to paint technicians studying finishing materials for aircraft, whose painted metal surfaces are almost invariably constructed of aluminum and magnesium or their alloys.

Paint technologists are familiar with the ordinary type of gage making use of the magnetic principle, illustrated by the Aminco-Brenner Magnegage (*1*) and the G.E. enamel thickness meter (*3*). These instruments have proved most valuable in aiding the paint technologist to hold film thickness within given limits in order to study closely various properties of the film which are largely dependent upon the thickness at which it is applied. For studying films applied to nonmagnetic metals, resort has been made to the measurement of film thickness by means of dial gages or micrometers which involve either the measurement of the panel thickness before paint is applied or the destruction of at least a portion of the film usually somewhere near the edge of a sample panel. The method discussed herein describes a means for measuring the thickness of films applied to nonmagnetic conducting substances without destroying the film at any point, regardless of the area involved. The outstanding limitation of the instrument in its present stage of development is the fact that measurements cannot be made on curved surfaces, and surfaces not providing a level area as much as 1 inch in diameter cannot be measured accurately.

The instrument has been successfully used over aluminum, various aluminum alloys, copper, brass, and magnesium, and should perform adequately over any conducting metal which is nonmagnetic.

DESCRIPTION OF APPARATUS

When an alternating current flows in a coil near the surface of a nonmagnetic metal, eddy currents are set up in the metal which will affect the inductance of the coil when placed upon or near the surface of the metal. The instrument was designed with the thought of utilizing this phenomenon (*2*).

The measuring instrument makes use of the heterodyne principle in order to compare the variable frequency of one oscillator with the fixed frequency of another. The LC (inductance and capacity) product of the variable oscillator is adjusted to equal the fixed value of the fixed oscillator. The value of L for the variable oscillator depends on the distance of a pickup coil from the nonmagnetic conducting surface. The value of C necessary to make LC, the required value, is controlled by a variable air condenser. This is the usual type of condenser found in an ordinary radio set. By using a frequency of 50 kc. or greater, the inductance, as the pickup coil approaches the metal, is independent of the thickness of the metal, providing it is not less than 0.02 inch.

Consider two oscillators, one of which is fixed whereas the other is variable. The two oscillators beat against each other, producing an audible note having a frequency equal to the difference of the two oscillator frequencies. By adjusting the capacitance in the variable oscillator circuit by means of a condenser,

this tone can be eliminated. The extent of adjustment depends upon the exact distance of the pickup coil from the base metal, which in turn depends upon the thickness of the insulating layer. The audible note can be heard by means of ear phones which are preferred on a portable model or better, perhaps, by means of an amplifier and a loud speaker which are preferred in the laboratory instrument. In each case a buzzing sound is produced, characteristic of poor radio reception, as a result of considerable interference.

Figure 1 is a diagrammatic sketch of the portable instrument which is powered by dry cells and can be conveniently carried into the field for making measurements on equipment in use. Figure 2 illustrates this instrument fully assembled. Figure 3 is a diagrammatic sketch of the laboratory instrument, whose sensitivity is somewhat higher and whose power is derived from a regular 115-volt alternating current supply. This apparatus is shown fully assembled in Figure 4.

The pickup unit, Figure 5, consists of a lattice-wound coil conveniently mounted in a Bakelite holder. The sensitivity of the apparatus has been shown to be independent of the value of the inductance of this coil for a given value of the LC product—i.e., for a given frequency. Referring again to Figure 5, the outer shell of Bakelite, 1, is cemented to the bottom covering, 4, and the entire inside painted with Aquadag to provide electrostatic shielding. The 16-millihenry coil, B, is connected to the coil holder, 2, which in turn is fastened to a brass weight, 3. The center conductor, A, is soldered to one end of the coil, B. The shield of the cable is grounded to the chassis of the apparatus and to the brass weight. This is also connected to the other end of the coil. A cable clamp, C, removes all strain from internal wire connections. The brass weight, 3, incorporated in the pickup unit ensures that the same pressure is always applied to any given surface under measurement.

Except in cases where the film being measured is exceptionally soft, this pressure is not sufficient to deform or deface the film to any degree. The area of the conducting surface of the pickup unit is so large relative to its weight that the pressure of the unit area is small indeed. A denser weight over the same area might prove even more effective in obtaining reproducible results. The pickup unit should always be placed lightly on the surface of the film to be measured and no artificial pressure should be applied.

USE OF THE INSTRUMENT

Although it is possible to calibrate dial numbers to read directly in mils, readings may be readily referred to calibration curves, which permits the use of standard dials. Calibration curves were prepared by measuring the thickness of uniform sheets of insulating material of various thicknesses at several points by means of a micrometer. Sheets of cellophane or Pliofilm are suitable for this purpose. The same sheets were then placed over a bare piece of nonmagnetic metal and additional measurements made by means of the thickness gage. The pickup unit was placed over the insulating material, its weight being sufficient to press the film tightly against the metal to obtain easily reproducible dial readings. In this manner, a number of known points were obtained for the calibration curves by selecting film sheets whose thicknesses occurred in the range of interest. The use of this method is also convenient for checking the accuracy of the instrument from time to time during operation.

The technique was followed in preparing two sets of curves for the laboratory instrument which operates in two separate ranges of thickness. The left-hand dial (Figure 4) is used for measuring thicknesses at 0 to 10 mils. The right-hand dial is used for measuring thicknesses at 0 to 25 mils. The curve obtained for the 0 to 10 range is practically a straight line except for those points where the film is extremely thin. All thicknesses above 0.5 mil are easily determined, but smaller measurements are rather difficult, inasmuch as this is below the practical limitation of calibration for the instrument. Over the 0- to 10-mil range there are about 20 dial divisions for each mil thickness. It is possible, therefore, to adjust the dial to one division, which describes an increment of 0.05 mil. Since the dial can be read to about 0.25 division, it is apparent that the sensitivity of the instrument is limited only by the areas involved in placing the pickup unit uniformly on portions of the film to be measured.

When the thickness of the coating exceeds 10 mils, the right-hand dial is used. However, the sensitivity is decreased somewhat, owing to the fact that the 25 mils are spread over the same dial range as are the 10 mils on the first scale. In addition, the

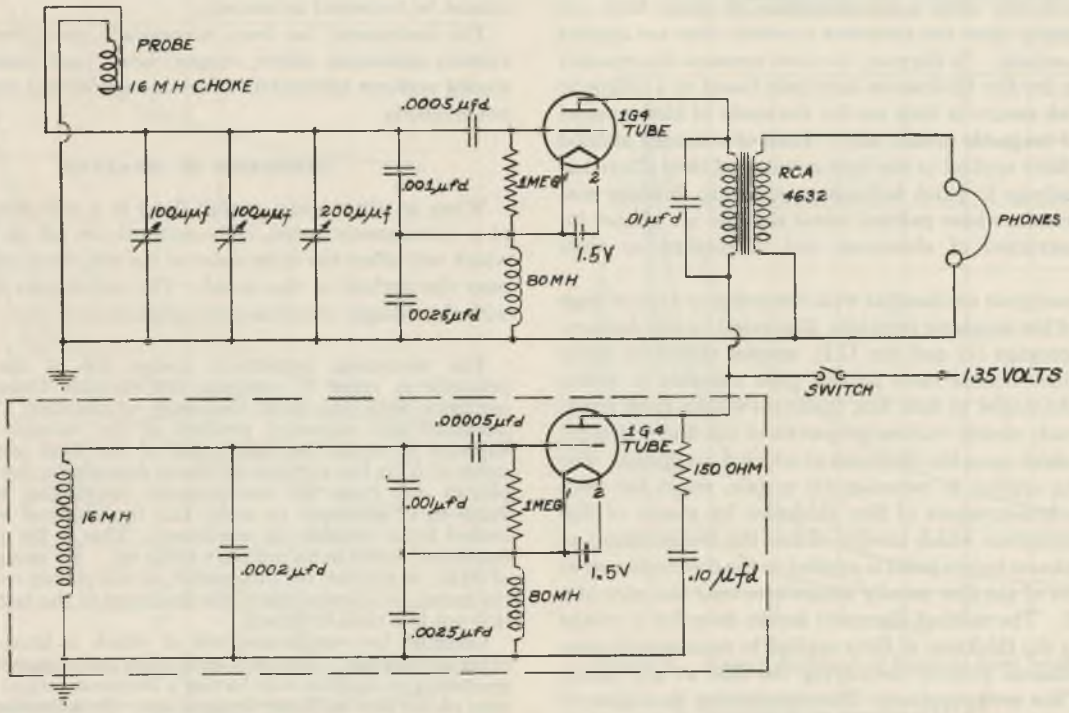

Figure 1. Portable Battery-Operated Film-Thickness Gage

Figure 2. Assembled Portable Instrument

approximate linear relationship existing in the first case does not hold over the entire range.

The actual operation of the instrument is simple and rapid. After the power is applied, the instrument should be allowed to warm up for several minutes. If it is to be used intermittently during the day, it should be left on continuously. To adjust the zero setting, the pickup coil is placed upon a sheet of bare metal of the type over which the film is applied. In making measurements on laboratory panels, the uncoated back of the finished panel serves well. When the contact surface of the pickup unit is placed on the bare metal, both dials are set at zero, in which case a buzzing noise is produced. The frequency of this noise is gradually diminished by turning the right-hand dial until the noise ceases, the left-hand dial remaining at zero. The pickup is next placed upon the film to be measured and a similar buzzing noise is produced. By turning the left-hand dial carefully until the noise is eliminated, a dial reading is obtained which can be referred to the calibration curve and the thickness read in mils. This arrangement is used for measurements of the 0- to 10-mil range. In order to measure films on the 0- to 25-mil range, the zero setting is obtained by the left-hand dial and the measurement made by adjusting the right-hand dial until the noise is eliminated. The dial reading is converted to mils by reference to a calibration curve.

The dials must always be turned counterclockwise from the low reading toward the high one. There is a considerable range on the dial scale over which no sound is heard, owing to the fact that the two oscillators couple. If the approach is made in a clockwise direction, the result obtained will be meaningless. It is possible to redesign the instrument to eliminate this possibility but only at a considerable sacrifice in the simplicity of the circuit.

Until the circuits have had an opportunity to warm up, the zero setting has a tendency to drift somewhat over a narrow range; therefore, when the instrument is used after a short warm-up period, it should be checked at frequent intervals.

TYPICAL MEASUREMENTS

In order to illustrate the wide adaptability of the instrument to the measurement of several types of coatings applied to different alloys with several surface treatments, a number of experiments were made.

Panels were prepared from 24ST Alclad aluminum, 24ST aluminum, 17ST aluminum, and 75ST aluminum as well as zinc-

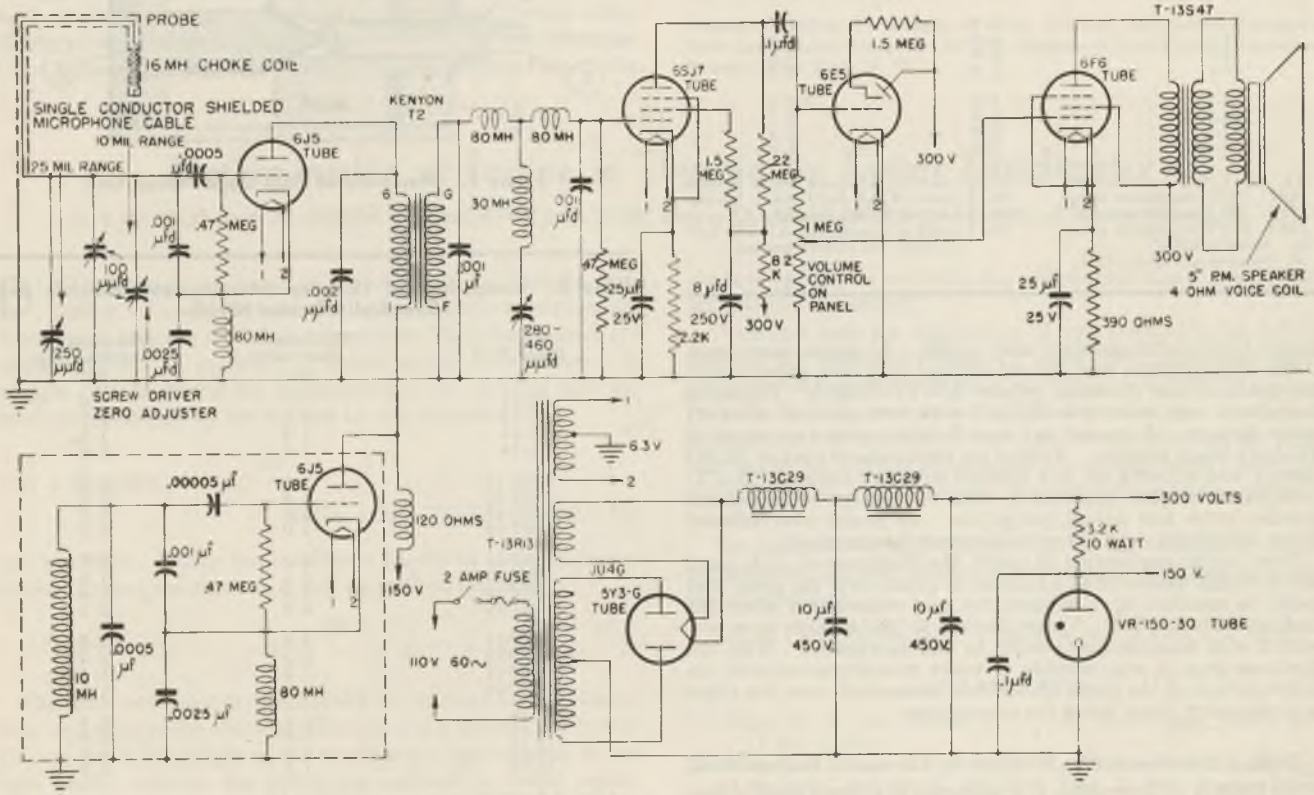

Figure 3. Laboratory Alternating Current Film-Thickness Gage

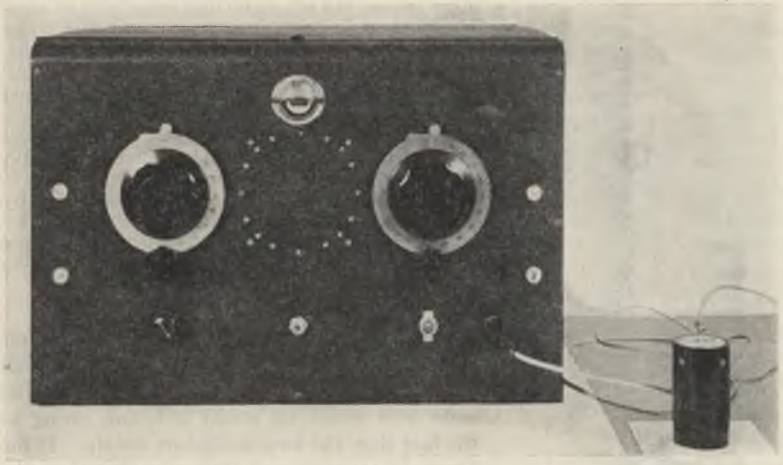

Figure 4. Assembled Laboratory Instrument

Table I. Comparison of Thickness Measurement Methods over Chemically Cleaned Metals

Panel No.[a]	Thickness by Micrometer *Mils*	Thickness by Electrical Gage *Mils*
AL-01	1.5	1.6
AL-02	1.4	1.1
AL-03	1.0	1.0
AL-04	1.4	1.4
24ST-01	1.8	1.6
24ST-02	2.1	2.5
24ST-03	0.9	1.0
24ST-04	1.5	1.7
17ST-01	1.6	1.9
17ST-02	1.8	2.6
17ST-03	1.0	1.0
17ST-04	1.4	1.7
75ST-01	2.7	1.6
75ST-02	2.0	2.2
75ST-03	1.1	0.9
75ST-04	1.5	1.6
B-1	1.8	1.7
B-2	2.0	2.2
B-3	0.9	0.3
B-4	1.5	1.5
M-1	1.6	1.5
M-2	1.9	2.1
M-3	0.7	0.4
M-4	1.2	1.7

[a] AL. 25ST Alclad aluminum
24ST. 24ST aluminum alloy
17ST. 17ST aluminum alloy
75ST. 75ST aluminum alloy
B. Zinc-Cu brass
M. Magnesium alloy (J1H)

01. 2 coats of AN-L-21 white lacquer
02. 2 coats of AN-L-21 black lacquer
03. 1 coat of M-543 enamel
04. 2 coats of aluminized AN-TT-L-51 lacquer (12 ounces per gal.)

copper brass and magnesium alloy (J1H). In one instance, each of the above metals was solvent-cleaned and then primed with two coats of zinc chromate primer AN-TT-P-656A. Following the primer, one series was finished with two coats of AN-L-21 white lacquer. A second set was finished with two coats of AN-L-21 black lacquer. A third set received one coat of M-543 enamel, and a fourth set was finished with two coats of AN-TT-L-51 clear lacquer pigmented with 12 ounces of aluminum powder (extra fine lining) per gallon. All panels were allowed to dry for several days before measurements were made.

Prior to the application of paint, the thickness of each panel was carefully measured at a number of points over the areas that could be reached by a micrometer and remeasured after the coatings were applied. Values derived in this manner were correlated with measurements made by the instrument. With the thickness gage it was possible to make measurements over the entire surface of the panel rather than somewhere near the edges as is necessary when using the micrometer.

Table I lists the results obtained by the use of each method. From a study of these data, it is apparent that some small differences exist in results obtained by the two methods. Because the pickup unit of the film gage has a diameter of about 1.5 inches and the micrometer approximately 0.25 inch, some variation is to be expected. For example, a pigment particle could tilt the pickup coil enough to account for some of the differences, while the chance of covering a single particle with the micrometer is much smaller. Such discrepancies can largely be overcome by making several measurements and selecting an average.

In the data listed in Tables I through III, at least four micrometer measurements were made on the bare panel. The average thicknesses of the panel measurements were used against each of the individual measurements obtained on the finished panel to obtain a number of readings. The average of four readings is listed in the tables of data. Similarly, a minimum of four measurements was made with the film thickness gage and the average reported in the listed data.

Table II lists the results obtained when the paint systems described above were applied to aluminum and magnesium alloy which had been surface-treated before painting.

The aluminum alloys were solvent-cleaned and then given a chromic acid anodization, whereas the magnesium alloy panels were solvent-cleaned, immersed in 15 to 20% hydrofluoric acid at room temperature for several minutes, rinsed in cold water, and then placed in a boiling 10 to 15% solution of potassium dichromate. They were finally rinsed with hot water and allowed to dry in the air for a few minutes before application of the first prime coat.

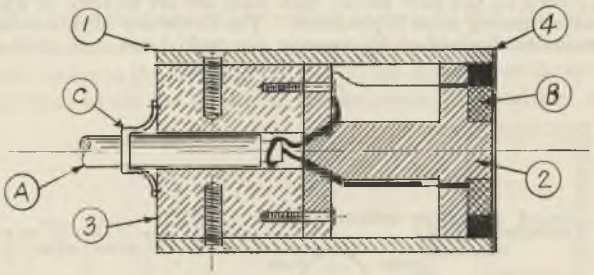

Figure 5. Assembly of Paint Gage Pickup Unit

Table II. Comparison of Thickness Measurement Methods over Chemically Treated Metals

Panel No.[a]	Thickness by Micrometer *Mils*	Thickness by Electrical Gage *Mils*
AL-11	2.1	2.0
AL-12	2.0	2.2
AL-13	1.4	1.2
AL-14	1.8	2.0
24ST-11	2.1	2.0
24ST-12	1.8	2.3
24ST-13	1.1	1.5
24ST-14	1.9	2.2
17ST-11	2.2	3.0
17ST-12	2.1	1.3
17ST-13	1.3	1.8
17ST-14	2.0	2.5
75ST-11	2.3	2.5
75ST-12	2.0	2.5
75ST-13	1.3	1.4
75ST-14	2.0	1.8
M-11	2.1	2.4
M-12	1.8	2.6
M-13	0.9	1.5
M-14	1.5	2.8

[a] Panel designations same as in Table I.

Finally a set of data was obtained (Table III) in which the panels were merely cleaned and given finishing coats without prior application of primer.

Table III. Comparison of Thickness Measurement Methods over Solvent Cleaned Metals without Primer

Panel No.[a]	Thickness by Micrometer	Thickness by Electrical Gage
	Mils	*Mils*
AL-21	2.6	3.0
AL-22	2.2	2.5
AL-23	1.6	1.5
AL-24	1.0	1.2
24ST-21	2.8	2.8
24ST-22	2.1	2.2
24ST-23	1.8	2.1
24ST-24	1.4	1.5
17ST-21	2.5	2.8
17ST-22	2.1	2.3
17ST-23	1.8	1.8
17ST-24	1.2	1.5
B-21	2.3	2.9
B-22	2.3	3.0
B-23	1.8	1.7
B-24	1.3	1.5
M-21	2.3	2.5
M-22	2.1	2.3
M-23	1.5	1.5
M-24	1.1	1.1

[a] Panel designations same as in Table I.

The first instrument was designed to measure the thickness of an insulating layer over a nonmagnetic conductor. It seemed to be of interest to determine the effect of a slightly conducting coat applied to a nonmagnetic conductor. Some clear lacquer was pigmented to produce a film displaying conductivity. The thickness of the film was first determined with the micrometer and finally by the electrical gage. In every instance the results agreed as closely as those presented in the tables for nonconducting films. This appeared surprising at first but, when it is considered that the paint is an extremely poor conductor compared to the metal underneath, the result is somewhat understandable. However, a point can be reached where the film whose thickness to be measured displays a conductivity approaching that of the metal, in which case a situation is reached where the electrical gage will no longer function.

DISCUSSION AND SUMMARY

From an examination of the data it is evident that the average readings made by the electrical gage are on the average slightly higher than those obtained by the micrometer. Reasons for this differential have been pointed out. In a very few instances micrometer readings are higher. More uniform agreement was obtained on unprimed panels (Table III), which may be explained by the fact that the primer pigment was not so well dispersed as were the top coat pigments; this resulted in a slight unevenness in the primer coat and was accentuated as the film was built up by additional coats. In one or two instances where the film was thin, wider discrepancies exist, but these readings fall below the 0.5-mil range where calibration of the instrument is much less accurate. As film thickness increases, the accuracy of the measurement is greater.

Among the advantages of the gage is the fact that measurements can be made rapidly and with reproducible accuracy. No limit of its usefulness is imposed by the type of metal over which the film is applied except that it be nonmagnetic. In this respect it complements the magnetic instruments (*1*, *3*) so widely used throughout the coatings industry. Accuracy is limited more by the nature of the film surface on which the pickup coil must rest during operation than by the sensitivity of the instrument itself.

Films applied to curved surfaces cannot be measured accurately and a flat surface as much as 1 inch in diameter is necessary. If the edge of the pickup coil is placed nearer than 0.5 inch to the edge of the panel the inductance of the coil is affected. Finally, the zero point must be adjusted by contact with a sample of the unpainted metal.

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PRESENTED before the Division of Paint, Varnish, and Plastics Chemistry, Paint and Varnish Group, at the 108th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y.

Determination of Iodine in Thyroid by Cerate Oxidimetry

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DURING the examination of a number of methods for estimating iodine in thyroid and other organic materials, attention was given to a method proposed by Hilty and Wilson (*1*) which involved an oxidation of iodide ion by ceric sulfate. It became evident during the examination of the method that the reaction postulated by the authors for the oxidation

$$NaI + 6Ce(SO_4)_2 + 3H_2O \xrightarrow{HCl} NaIO_3 + 3Ce_2(SO_4)_3 + 3H_2SO_4 \quad (1)$$

was incorrect. Under the conditions employed it could be assumed (*2*) that the reaction would proceed as follows:

$$2I^- + 4Ce^{++++} + 2Cl^- \xrightarrow{HCl} 4Ce^{+++} + 2ICl \quad (2)$$

This was confirmed experimentally by the addition of chloroform and observance of the appearance of the iodine color in this layer in the initial stages of the reaction and later change to the light brown color as the iodine was oxidized to iodine monochloride. This method of detection of the end point was checked agains tthe one using the *o*-phenanthroline indicator with satisfactory agreement.

Upon this basis the equivalence of each atom of iodine is 2 instead of 6. Hilty and Wilson (*1*) state that "each cubic centimeter of 0.005 *N* ceric sulfate is equivalent to 0.0003178 gram of iodine in thyroid combination". This is correct upon the basis of Equation 2, but not of 1. Hence, the results reported are properly evaluated and the conclusions drawn from them are not invalidated, even if they are not in accordance with the assumed reaction.

The formulation of the expression for the calculation of the normality of the ceric sulfate is misleading. It should be represented simply as

$$\text{Ml. of } Ce(SO_4)_2 \text{ required} \times \text{its } N = \text{ml. of } FeSO_4 \times \text{its } N$$

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Determining the Hydroxyl Content of Certain Organic Compounds

Macro- and Semimicromethods

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A modification of macroprocedures for determining the hydroxyl content of hydroxylated fatty acids and alcohols is described, in which an internal indicator is used. For colored solutions a potentiometric method has been developed. A semimicroprocedure using an internal indicator is also presented. An acetylating solution of 1 volume of acetic anhydride in 3 volumes of pyridine and a hot-water hydrolysis are used.

THE West, Hoagland, and Curtis (*14*) modification of the Verley and Bölsing (*13*) procedure for determining hydroxyl specifies the acetylation of fats and fatlike material with a solution of 1 volume of acetic anhydride in 7 volumes of pyridine, followed by hydrolysis with hot water and titration of the acid formed with alcoholic alkali solution. Peterson and West (*10*) recommended an acetylating solution of 1 volume of acetic anhydride in 2 volumes of pyridine, and Marks and Morrell (*6*) adopted the use of a solution of 1 volume of acetic anhydride in 3 volumes of pyridine. In the last two procedures, the excess acetic anhydride is hydrolyzed by adding ice water to the reaction mixture. Malm, Genung, and Williams (*5*) studied the effects of time, temperature, and concentration of the acetic anhydride in the anhydride-pyridine solution on the acetylation of cellulose derivatives. They found that an acetylation period of 24 hours was required to obtain calculated free hydroxyl values when they used a 0.5 molar acetic anhydride reagent (1 part of acetic anhydride to 19 parts of pyridine). Titrations were conducted electrometrically in an open beaker.

The methods of West, Hoagland, and Curtis and of Marks and Morrell gave low results with hydroxylated higher fatty aids, certain oxidation products of higher fatty acids, and long-chain alcohols analyzed in this laboratory. However, by combining the acetic anhydride acetylating solution of Marks and Morrell with the hot-water hydrolysis and the homogenization with *n*-butanol of West, Hoagland, and Curtis, good results were obtained on the types of material mentioned. Since a concentration of 1 part of acetic anhydride in 7 parts of pyridine gave incomplete acetylation, the more dilute solution of Malm, Genung, and Williams was not investigated. Other work (*1–4*, *8*, *9*, *11*, *12*) has been described in which both acetic anhydride and acetyl chloride were used for determining the hydroxyl content of organic compounds.

In each of the methods, except that of Malm *et al.*, an internal indicator is employed, which limits the accuracy of analysis of samples producing dark solutions. For these materials a procedure using potentiometric titrations was developed. When the reaction was carried out in the usual manner in an iodine flask, it was necessary to transfer the reaction mixture to a beaker to make the potentiometric titration. During this transfer, small amounts of acetic acid were inevitably lost, introducing relatively large errors. To eliminate these errors, a modified iodine flask was designed that would permit the electrometric titration to be made in the reaction vessel when a Beckman pH meter equipped with extension electrodes is used.

This flask (Figure 1) was made by sealing side arms containing No. 16 standard taper stoppers on opposite sides of a 250-ml. flask. The side arms allow the tips of the electrodes to be immersed in the solution without touching the bottom of the flask.

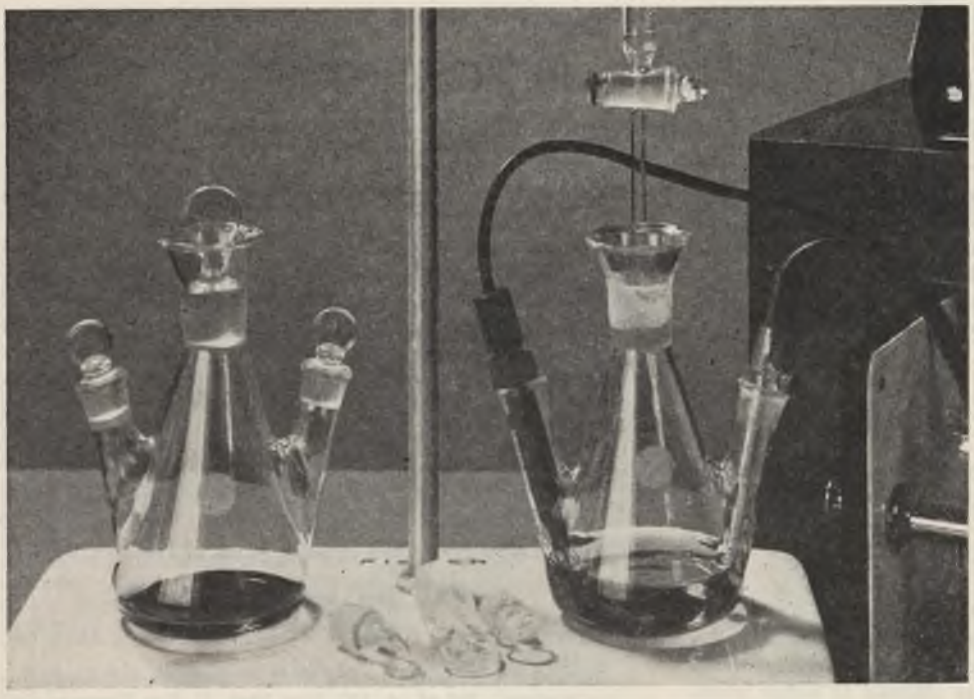

Figure 1. Modified Iodine Flask for Potentiometric Titrations

A semimicromethod using the same reagents employed for the macrodetermination was developed for analyzing samples too small for the macroprocedure. A glass-stoppered, pear-shaped flask (Figure 2) was designed so that the small volumes of the sample and of the reagent would be held in the conical tip, ensuring complete mixing. A 50-ml. flask provides sufficient volume for the addition of the titrating solution. Four tenths milliliter of the acetic anhydride–pyridine solution (1 to 3) is used, since this quantity is sufficient for complete acetylation and also requires less than 25 ml. of 0.1 *N* alkali for neutralization. This volume of reagent permits the use of a 25-ml. buret (calibrated), which delivers a measured volume with the necessary accuracy.

The acetylating reagent is measured from an S-shaped capillary buret of 1-mm. bore (Figure 2) having a mercury

column in contact with the solution. The buret is filled and the reagent expelled by releasing and applying pressure to the rubber tubing which serves as the mercury well of the buret. This is done by manipulating two screw clamps, *A* and *B*, in contact with the rubber tubing. Since a fixed volume of the acetic anhydride–pyridine reagent is to be delivered, two hair lines are placed on the buret at the points that would allow the delivery of approximately 0.4 ml.

REAGENTS

Acetylating reagent. One volume of A.C.S. grade acetic anhydride mixed with 3 volumes of reagent grade pyridine.

n-Butanol (Eastman Kodak, practical).

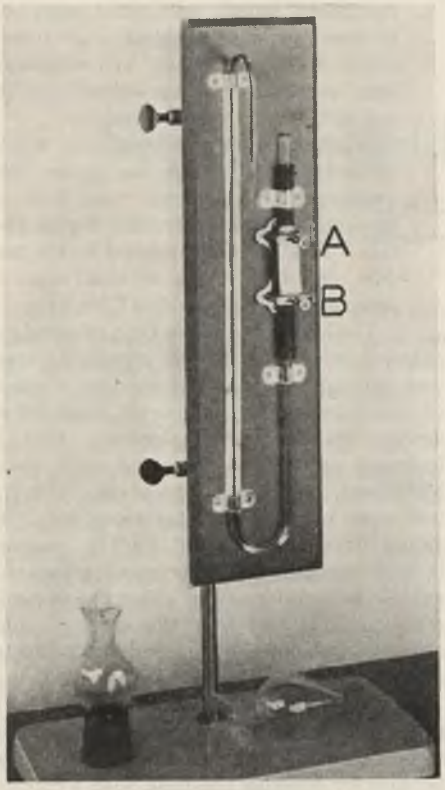

Figure 2. Capillary Buret and Semimicro Reaction Flasks for Semimicromethod

Mixed indicator solution (*4*). One part of 0.1% aqueous solution of cresol red neutralized with sodium hydroxide and 3 parts of 0.1% thymol blue neutralized with sodium hydroxide.

Standard alcoholic sodium hydroxide solutions. Macroprocedure, approximately 0.5 *N*. Semimicroprocedure, approximately 0.1 *N*.

Prepare the solutions from saturated aqueous soldium hydroxide solution (approximately 18 *N*) and aldehyde-free ethanol made by alkaline aluminum reduction. Standardize the alcoholic solutions to ±2 parts per 1000 against either potassium acid phthalate or a standard acid of approximately the same normality, using the mixed indicator.

MACROPROCEDURE

Place a weighed sample containing from 1 to 2.5 milliequivalents of hydroxyl in the modified iodine flask and add exactly 3.00 ml. of the acetic anhydride–pyridine solution (1 to 3) from a reservoir-type 5-ml. microburet with a Drierite protecting tube in the reservoir. Moisten all stoppers of the flask with pyridine and place the two stoppers firmly in the electrode arms. The center, or main stopper, should be loosely seated. Place the flask on a steam bath. After heating for 45 minutes, add 5 to 6 ml. of water to the cup of the flask and loosen the stopper in such a manner as to rinse the stopper and inside walls of the flask. Continue heating for 2 minutes and then cool under the tap with the main stopper partly removed. With 10-ml. of *n*-butanol rinse the three stoppers and inside walls of both the flask and side arms. Insert the glass and calomel electrodes in the side arms and titrate with 0.5 *N* alcoholic alkali to pH 9.8 (volume *A*). Make a blank determination (volume *B*) on 3.00 ml. of the acetic anhydride–pyridine solution.

Determine the free acid of the sample by repeating the procedure described above, using pyridine instead of acetic anhydride–pyridine solution and adding 5 ml. of neutral ethanol just prior to the titration to make the solution homogeneous. Shake well and titrate with 0.5 *N* alcoholic alkali. Calculate the volume of alkali required to neutralize the acidity of 1 gram of the sample (volume *C*).

CALCULATION.

$$\frac{[B - (A - C \times \text{wt. of sample for —OH determination})] \times N \times \frac{\text{OH}}{1000} \times 100}{\text{weight of sample for —OH determination}} = \% \text{ OH}$$

Because of the ease of manipulation, it is recommended that for colorless solutions ordinary iodine flasks and the mixed indicator be used with this procedure instead of the special flasks and the potentiometric titration.

SEMIMICROPROCEDURE

Weigh a sample having a hydroxyl content equivalent to approximately 2 ml. of 0.1 *N* alkali directly into the dry, pear-shaped reaction flask. Draw the acetic anhydride–pyridine reagent into the S-shaped buret to a point below the lower hairline. Wipe the tip of the buret with a towel and then with the fingertips to deposit a slightly oily film, which ensures a more uniform removal of the reagent from the tip. Hold the inside wall of a beaker against the buret tip and bring the mercury meniscus to the lower mark by carefully closing the screw clamp, *A*. Replace the beaker with the reaction flask held so that the buret tip touches the inner wall, and rotate the flask as the reagent is slowly discharged from the buret by tightening *A* and then *B* until the meniscus is even with the upper mark. Immediately connect the flask to a water-cooled condenser and seal the glass joint with a few drops of pyridine. Place the flask and the condenser on a steam bath with the tip of the flask extending approximately 1.25 cm. (0.5 inch) through a tightly fitting ring. Acetylate for 30 minutes, add 3 ml. of distilled water through the condenser, and hydrolyze by heating for 2 minutes longer if no carboxyl groups are present or 30 minutes longer if the sample contains organic acids.

Add 1 ml. of pyridine to the cup and disconnect the flask in such a manner that the pyridine rinses the condenser tip. Loosely insert a stopper and immediately cool the flask to room temperature under running water. Add 3 ml. of *n*-butanol to the cup and loosen the stopper so that the stopper and the walls of the flask are rinsed. Add 3 drops of the mixed indicator and cover the flask with a rubber dam, held in place by a rubber band, to prevent the contents from absorbing carbon dioxide from the air near the end of the titration. Insert the buret tip through a pinhole in the dam and titrate the excess of acetic acid and any acid in the sample with 0.1 *N* alcoholic sodium hydroxide until the solution changes to gray (volume *A*).

Make a blank determination on the acetic anhydride–pyridine solution (volume *B*). Determine any free acid as described in the macroprocedure or by dissolving the sample in ethanol which has

Table I. Effect of Strength and Age of Acetic Anhydride–Pyridine Mixture on Macrodetermination of Hydroxyl in Dihydroxystearic Acid and Oleyl Alcohol

Sample	Acetylating Reagent: Ratio of anhydride to pyridine	Volume, ml.	Age	% Hydroxyl Found		Average
Dihydroxystearic acid[a]	1:7	3	Fresh	10.51	10.29	10.39
	1:7	6	Fresh	10.54	10.48	10.51
	1:7	3	4 days	10.38	10.12	10.25
	1:3	3	Fresh	10.83	10.78	10.81
	1:3	3	4 days	10.88	10.74	10.81
Oleyl alcohol 1[b]	1:7	3	Fresh	5.20	5.46	5.33
	1:7	6	Fresh	6.25	6.24	6.25
	1:7	3	4 days	3.97	3.49	3.73
	1:3	3	Fresh	6.30	6.31	6.31
Oleyl alcohol 2[b]	1:7	3	Fresh	5.60	5.34	5.47
	1:7	3	4 days	4.59	4.10	4.35
	1:3	3	Fresh	6.34	6.39	6.37
	1:3	3	4 days	6.35	6.40	6.38

[a] Theoretical % OH = 10.75; weight of sample = 0.2 gram.
[b] Theoretical % OH = 6.34; weight of sample = 0.8 gram.

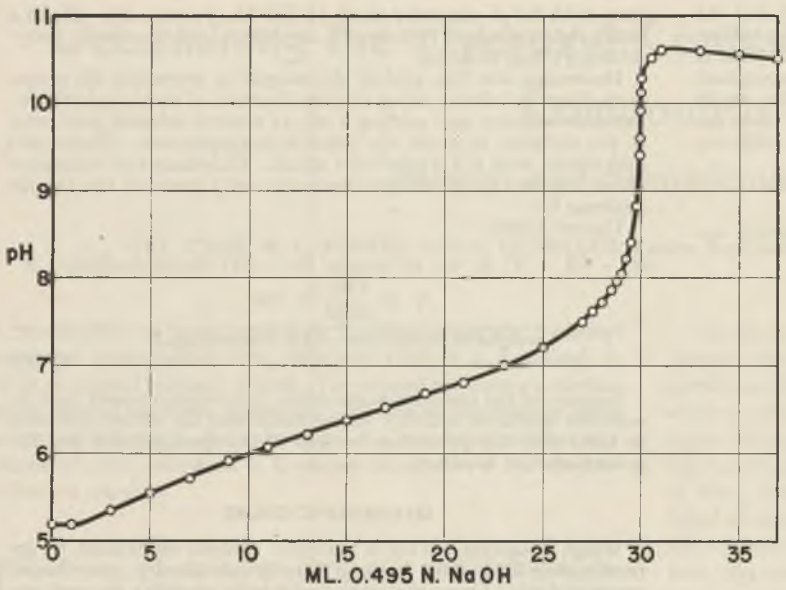

Figure 3. Titration Curve for Reaction Mixture

Table II. Macrodetermination of Hydroxyl

Sample	Indicator Method 1	Indicator Method 2	Indicator Method Av.	Potentiometric Method 1	Potentiometric Method 2	Potentiometric Method Av.	Theoretical
	%	%	%	%	%	%	%
Dihydroxystearic acid	10.78	10.73	10.76	10.72	10.81	10.77	10.75
Monohydroxystearic acid	5.60	5.58	5.59	5.70	5.62	5.66	5.69
Oleyl alcohol 1	6.30	6.31	6.31	...	...	...	6.34
Oleyl alcohol 2	6.34	6.39	6.37	...	...	...	6.34
Methyl ricinoleate	5.47	5.62	5.55	...	...	...	5.44
Cyclohexanol[a] (Eastman Kodak White Label)	16.45	16.55	16.50	16.58	16.58	16.58	16.81
Benzyl alcohol[a] (Eastman Kodak White Label)	15.36	15.35	15.36	15.35	15.37	15.36	15.73
No. 4588 (dark colored)[b]	2.40	2.76	2.58	2.50	...	2.50	...
No. 5565 (dark colored)[c]	...	...	...	2.05	1.96	2.01	...
No. 5687 (dark colored)[b]	...	...	...	1.61	1.51	1.56	...

[a] Not further purified.
[b] Residues left in pot after distillation of methyl esters of fatty acids.
[c] Residue left in pot after distillation of an oxidized oleic acid polymer.

been previously neutralized (mixed indicator), and titrating with 0.1 *N* alcoholic sodium hydroxide, using a rubber dam. From this titration calculate the volume of 0.1 *N* sodium hydroxide equivalent to the free acid in a 1-gram sample (volume *C*). The calculations are the same as for the macroprocedure.

DISCUSSION

The titration curve for alcoholic alkali versus acetic acid in a pyridine-water–*n*-butanol–ethanol solution is shown in Figure 3. The point of color change for the mixed indicator in this solution was at pH 9.8. Since the vertical portion of the curve extends between 9.2 and 10.3, a pH of 9.8 was selected for the potentiometric end point to make the potentiometric and indicator procedures interchangeable.

As indicated in Table I, both the age and the strength of the acetic anhydride–pyridine acetylating solution are important. The reagent made by mixing 1 volume of acetic anhydride with 3 volumes of pyridine gave theoretical results and remained effective for at least 4 days. A 1 to 7 acetic anhydride–pyridine solution was so dilute that it resulted in incomplete acetylation, as shown by the data for dihydroxystearic acid and oleyl alcohol. As this reagent aged, it became less effective as an acetylating agent, giving still lower results. The 1 to 3 acetic anhydride–pyridine solution permits the use of reagent grade pyridine without its further purification, since a sufficient excess of the acetic anhydride is assured for complete acetylation of the sample, even though some has been consumed by moisture or other impurities in the pyridine.

For colorless solutions, identical values were obtained by the indicator method and the potentiometric method, as shown in Table II. Good precision was obtained with both methods. The cyclohexanol and benzyl alcohol, Eastman Kodak Company White Label reagents, were not further purified. The compounds for which theoretical values are cited were established as pure by such physical and chemical constants as iodine value, neutralization equivalent, saponification equivalent, melting point, and carbon and hydrogen analysis.

In the semimicroprocedure, it was necessary to carry out the reaction under water-cooled condensers to prevent the loss of small amounts of acetic anhydride during the acetylation. This loss also occurred in the macroprocedure, but here it was so small that it was not detected by titration with 0.5 *N* alkali.

The longer hydrolysis time required in the semimicroprocedure for samples containing organic acids was probably due to the formation of small amounts of mixed anhydrides, which are more difficult to hydrolyze than is acetic anhydride. High semimicro hydroxyl values were obtained with the 2-minute hydrolyses, probably owing to the failure to hydrolyze these mixed anhydrides completely. Since high values were not obtained by the macroprocedure, it was assumed that only small amounts of mixed anhydride were formed in either the semimicro or the macromethods and that the amount unhydrolyzed after the 2-minute heating period was not significant in the macroprocedure.

To obtain accurate hydroxyl values in the semimicroprocedure, all solutions must be protected from carbon dioxide during the titrations. This can best be done by covering the flask with a rubber dam and inserting the tip of the buret through a pinhole in the dam.

Table III presents a comparison of the values obtained by the semmicro- and macroprocedures.

Groups such as primary and secondary amines, and sulfhydryl, which contain active hydrogen and form acetylated products not hydrolyzed by hot water, interfere in the analysis. Comparison of the method of Mitchell, Hawkins, and Smith (*7*) for determining primary and secondary amines with the one herein described indicates that it may be possible to adapt this hydroxyl method to the determination of these amines and other interfering substances of the type noted above. Any compound which undergoes condensation to produce hydroxyl groups, such as aldehydes, interferes in the procedure described.

Table III. Determination of Hydroxyl by Semimicromethod

Sample	Semimicro Values 1	Semimicro Values 2	Semimicro Values 3	Average	Macro Values	Theory
	%	%	%	%	%	%
Dihydroxystearic acid	10.72	10.79	10.73	10.75	10.76	10.75
Oleyl alcohol 1	6.30	6.38	6.30	6.33	6.31	6.34
Partheniol	7.61	7.65	...	7.63	...	7.64
Cyclohexanol (Eastman Kodak White Label)	16.61	16.58	16.43	16.54	16.50	16.81
Benzyl alcohol (Eastman Kodak White Label)	15.33	15.29	15.46	15.36	15.36	15.73

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Semimicro-Kjeldahl Nitrogen Determination

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IN THE course of an investigation involving the preparation of derivatives of α-aminopyridine, a convenient and accurate method for analyzing these compounds was needed. At the time this project was initiated, the Kjeldahl nitrogen method of Pepkowitz and Shive (*3*), recently published, seemed to fill this need. These authors claimed that their method, in which perchloric acid is used to hasten digestion of the sample, is applicable to the semimicroanalysis of organic compounds and reported some results bearing out this contention.

Several nitrogen heterocyclic compounds, prepared in this laboratory, were analyzed by their procedure, but in every case the result obtained was much lower than the calculated value. The method was then used in the analysis of organic compounds which had been purified by repeated recrystallizations and then thoroughly dried. Table I shows that low values were obtained in every analysis.

When 2 to 10 mg. of nitrogen, in the form of ammonium sulfate, were carried through the method, no loss of nitrogen was observed, even with long periods of digestion. It seems, therefore, that loss of nitrogen occurred before the nitrogen in the organic compound was converted to ammonium hydrogen sulfate.

The Kjeldahl nitrogen method described by Clark (*1*) was selected next and used in the analysis of several well-known pure organic compounds and research preparations. Excellent results were obtained in all but one case (see Table I). Nitrogen heterocyclic compounds and nitro compounds (polynitro compounds, too, were accurately analyzed) as well as amines and amides yielded their nitrogen quantitatively in this method of digestion. Clark (*1*) found that the method has its limitations in the cases of certain semicarbazones. To these must be added aminopyrine which gave low results on analysis.

Several slight changes were made in the method in order to adapt it to the facilities at the authors' disposal. These modifications, it is believed, make the method easier to use in the average organic laboratory, where the Kjeldahl nitrogen method is not a frequent analysis, and increase its accuracy.

The sample is weighed by difference on aluminum foil rather than on cigaret paper. Less time is consumed in the weighing and digestion of the sample (since it is no longer necessary to digest the paper in addition to the sample) and a smaller blank is obtained (the cigaret paper contains an appreciable amount of nitrogen).

Ordinary test tubes, 175 mm. long with an internal diameter of 20 mm., are substituted for the digestion flasks. (These test tubes can be heated in an ordinary digestion rack with microburners rather than with the more elaborate electric heaters.) Less wash water is needed in transferring the digested sample quantitatively to the distilling apparatus. The resulting smaller volume of liquid to be steam-distilled lessens the likelihood of the alkaline reaction mixture bumping over into the distillate.

The mercuric oxide and potassium sulfate used in the digestion are more conveniently used in the form of an intimate mixture which can be added with a spatula made from a 190-mm. length of glass tubing 6 mm. in inside diameter, sealed near one end in

Table I. Determination of Nitrogen

Name or Type of Compound	Molecular Formula	Nitrogen Found: Method of Pepkowitz and Shive	Nitrogen Found: Modified method of Clark		Nitrogen Calculated
		%	%		%
3-Nitro-4-amino-phenetole	$C_8H_{10}N_2O_3$	13.41 13.40	...		15.38
3-Nitro-2-amino-toluene	$C_7H_8N_2O_2$	10.21 10.65	...		18.42
o-Dinitrobenzene	$C_6H_4N_2O_4$	12.40 12.48	...		16.67
Sulfanilamide	$C_6H_8N_2O_2S$	15.59 15.77	16.28 16.26		16.27
Sulfathiazole	$C_9H_9N_3O_2S$	15.85 15.78	16.47 16.60 16.57		16.46
Phenobarbital	$C_{12}H_{12}O_3N_2$	11.87 11.82	12.11 12.30	12.09 11.95	12.05
Aminopyrine	$C_{13}H_{17}ON_3$	11.93 11.87	14.73 14.57		18.18
Theobromine	$C_7H_8O_2N_4$	30.67 29.96	31.44 31.56		31.11
Acetophenetidin	$C_{10}H_{13}O_2N$	7.23 7.18	7.82 7.78 7.86		7.82
2-Aminopyridine	$C_5H_6N_2$	28.90 28.99	29.73		29.77
K-88A[a]	$C_8H_{24}ON_2Cl_2$	10.06 9.89	11.45		11.33
W-105[b]	$C_8H_9O_2NS$	6.80 6.89	7.73 7.73		7.65
W-106[b]	$C_8H_9O_2NS$	...	7.65 7.72		7.65
W-145[b]	$C_8H_9O_2NS$	...	7.68		7.65
W-143A[b]	$C_9H_{11}O_3N_2S$	...	12.44 12.41	12.58 12.36	12.39
W-143B[b]	$C_9H_{11}O_3N_2S$	...	12.46		12.39
W-143C[b]	$C_9H_{11}O_3N_2$	...	12.40		12.39
K-157[c,d]	$C_{14}H_{13}N_3O_4$	...	14.52 14.63	14.59	14.63
K-150[c,d,e]	$C_{22}H_{20}O_{11}N_6$	...	15.31		15.47
K-170[c,d,e]	$C_{24}H_{24}O_{11}N_6$	...	14.70 14.56	14.74	14.68
K-174[c]	$C_{16}H_{19}O_3N_2$	...	14.73 14.77		14.73
K-175[c]	$C_{18}H_{23}O_2N_3$	... ...	13.39 13.37		13.41
K-137[c,e]	$C_{22}H_{21}O_9N_5$	...	14.04 13.94	14.10	14.03
K-143[c,e]	$C_{24}H_{25}O_8N_5$	... ...	13.15 13.24		13.28
Thebaine	$C_{19}H_{21}O_3N$	...	4.48		4.50
Dihydrothebaine	$C_{19}H_{23}O_3N$	...	4.51		4.47
Dihydrocodeinone	$C_{18}H_{21}O_3N$	...	4.59		4.68

[a] Substituted diamine.
[b] Derivative of aminophenol containing thio or thiol group.
[c] Derivative of α-aminopyridine.
[d] Compound contains nitro group.
[e] Salt of picric acid.

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a sharp flame and bent at right angles to the remainder of the tubing so as to form a cup 19 mm. in height. The cup delivers about 500 mg. of the mixture.

The mixed indicator of Ma and Zuaga (*2*) gives a sharper end point in the final titration than the methyl red indicator recommended by Clark. The end point is reached when the solution is almost colorless, the sharp interval between the red color of the acid solution and the green color of the alkaline solution. Two milliliters of 2% boric acid solution are used instead of the same volume of the 4% solution.

A more precise end point is obtained by titrating the distillate with 0.3 *N* hydrochloric acid until the indicator turns red and then back-titrating with approximately 0.003 *N* sodium hydroxide, standardized before use, until the solution becomes almost colorless.

If an ordinary laboratory balance, damped or undamped, rather than a semimicro- or microbalance, is the only means of weighing the sample, as is the case in this laboratory, samples of 20 ± 3 mg. should be used in the analysis. The factor of sensitivity of the balance should be adjusted to 0.10 to 0.12 in order to weigh a sample of this magnitude with the accuracy demanded by the method. It is advisable, also, to use calibrated weights. The volume of distillate collected in the steam-distillation is extremely important and depends upon the amount of nitrogen in the sample. For samples containing up to 5 mg. of nitrogen, Clark recommends collecting a volume of 8 ml. with the tip of the condenser under the surface of the boric acid solution and 1 ml. more with the end of the condenser above the surface of the boric acid. For the amounts of nitrogen that may be found in samples of about 20 mg. it has been found safer to collect 13 ml. of distillate followed by 2 ml. more for washing down the sides of the receiver (a 50-ml. Erlenmeyer flask).

With these changes the Kjeldahl nitrogen determination can be carried out in a relatively short period of time, as contrasted with such time-consuming procedures as the carbon and hydrogen or Dumas determinations, on small amounts of sample and with the analytical equipment usually found in the average organic research laboratory. After a few runs, results within 1% of theory, with an average error of ±0.5%, can be obtained.

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Microdetermination of Copper with the Polarograph

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COPPER was first determined by amperometric titration by precipitation with salicylaldoxime by Neuberger (*4*). However, it was thought feasible to determine copper by measuring the amounts of salicylaldoxime removed by cupric ions under the right conditions rather than by measuring the diffusion current of copper.

APPARATUS AND REAGENTS

Heyrovský polarograph, Model XI (E. H. Sargent & Co.).

Phosphate buffer mixtures, pH 5.4, 0.067 *M* and 0.083 *M* with respect to both disodium phosphate and monopotassium phosphate.

Standard copper solution made approximately 0.06 *N* from copper sulfate and the copper content determined iodometrically by the method of Foote and Vance (*3*).

Salicylaldoxime (Eastman), the oxime content of which was determined by adding a weighed sample of the oxime in 95% alcohol to an excess (calculated) of the diluted standard copper solution, and the copper salt of the salicylaldoxime precipitated by the procedure of Biefeld and Howe (*1*). Representative samples of the lot used throughout this work contained 99.34% salicylaldoxime.

The stock solutions of salicylaldoxime were kept in the ice box until ready for use. A solution of 3.61×10^{-4} *M* oxime as precipitant was prepared as follows: To 20 ml. of the alcoholic stock solution (1000 mg. of oxime dissolved in 100 ml. of 95% alcohol) in a 200-ml. volumetric flask were added 180 ml. of 0.067 *M* phosphate buffer, the resulting mixture being 0.059 *M* in phosphate buffer and 7.22×10^{-3} *M* in salicylaldoxime. Then 5 ml. of this solution were made up to 100 ml. in a volumetric flask with 0.067 *M* buffer to give 3.61×10^{-4} *M* oxime in the buffer mixture of about 0.065 *M*.

EXPERIMENTAL

EFFECT OF pH UPON CURRENT-VOLTAGE CURVES OF SALICYLALDOXIME. The influence of pH was studied to ascertain at what pH (necessarily above that at which copper is quantitatively precipitated, *1*) the best defined current-voltage curves could be obtained. The results are shown in Table I; maximum height of the wave occurred at pH 3.7 and 5.4.

The calibration data (Table II) were obtained by dissolving 1000 mg. (993.4 mg. of oxime) of salicylaldoxime in 100 ml. of 95% alcohol to make the stock solution. By diluting portions of this alcoholic solution with 0.067 *M* phosphate buffer, 1.4 to 7.2×10^{-4} *M* solutions were made. Finally portions of the latter were diluted with the buffer solution to make 0.578 to 4.309×10^{-4} *M* solutions of salicylaldoxime, all of which had practically the same low ethanol content.

The diffusion currents were measured (*4*) and the polarograms made after placing the salicylaldoxime solutions in shell vials and then removing any oxygen with a stream of nitrogen bubbles. The rate of flow of mercury for the capillary in milligrams per second, *m*, and the drop time in seconds (*5*) were measured at a potential of −1.2 volts (in the supporting electrolyte) with respect to the saturated calomel electrode. For the 0.575 to 4.309×10^{-4} *M* salicylaldoxime solutions i_d/C is constant (Table II) and either the relationship $i_d/C = K$ or a calibration curve can be used for the analysis of copper.

PROCEDURE FOR STANDARD COPPER SOLUTIONS

The copper sulfate standard stock solution was diluted with distilled water to make various concentrations of cupric ions. Various dilutions of other standard copper sulfate stock solution were prepared and samples not exceeding 10 ml. were delivered from recalibrated pipets into 50-ml. volumetric flasks. Samples of less than 10 ml. were made up to 10 ml. by the addition of distilled water. Then 40 ml. of 0.083 *M* phosphate buffer containing salicylaldoxime were added, so that the final molarity was 0.067 *M*. A blank without cupric ions but containing 10 ml. of distilled water was diluted to the mark with the salicylaldoxime–phosphate buffer solution. This blank gave the amount of salicylaldoxime originally present. Blank and standards were allowed to stand overnight to ensure complete precipitation of the copper salt in the standards. After determining the amount of oxime left in solution from the calibration curve after precipitation of the copper salt, the amount of copper recovered was easily calculated. The recovery of copper from 0.1516 to 0.6029 mg. is shown in Table III, the error being from 1 to 3%. Molarities of the salicylaldoxime solutions used for precipitation are also shown.

Sample Calculation. The blank containing distilled water and salicylaldoxime in 50 ml. gave a diffusion current of 1.49 microamperes (corrected) which corresponded to 1.86×10^{-4} *M* salicylaldoxime from the calibration curve. A sample containing 0.1904 mg. of copper in 50 ml. had a corrected diffusion current of 0.50 microampere which was equivalent to 0.62×10^{-4} *M* salicylaldoxime. Then $1.86 \times 10^{-4} - 0.62 \times 10^{-4}$ gave 1.24×10^{-4} *M* salicylaldoxime removed by cupric ions. In milligrams of salicylaldoxime this amounted to $1.24 \times 10^{-4} \times 137.4$ or 0.017 gram or 1.7 mg. per liter, or 0.085 mg. in 50 ml. Since 1 mg. of salicylaldoxime will precipitate 0.2314 mg. of copper, the copper recovered was 0.085×0.2314 or 0.1966 mg.

Solutions of such salicylaldoxime concentration should be used that the difference between the diffusion current of the

blank and of the lowest concentration of copper is sufficient for accurate measurement.

For the determination of small quantities of copper either the molarity of the salicylaldoxime has to be decreased, or the precipitation reaction must be carried out in a smaller volume, so that the difference in the diffusion current between the blank and the standard will be large enough for accurate measurement.

For 3.8 to 22.8 micrograms of copper about 1×10^{-5} *M* salicylaldoxime in 50 ml. would give a sufficient difference in the measurement of i_d but it was found that the oxime was not sufficiently concentrated to precipitate the copper. Then the standards were delivered to 5- and 10-ml. volumetric flasks and diluted with 4 or 8 ml., respectively, of 0.083 *M* buffer containing about 1.15×10^{-4} *M* salicylaldoxime. Precipitation occurred immediately and since a few micrograms of copper were sufficient to lower the oxime molarity below that of the blank, an accurate measurement of i_d was possible. The results on the recovery of 3.8 to 74 micrograms of copper are shown in Table III; 3.8 to 15.2 micrograms could be determined with an error of about 3%. As with the larger amounts of copper, the volumetric flasks were allowed to stand overnight to ensure complete precipitation of the copper salt of salicylaldoxime.

INFLUENCE OF OTHER IONS

Since this method was devised primarily for the determination of copper in animal tissue, the effect of sodium, potassium, calcium, magnesium, and iron upon the recovery of small amounts of copper was investigated. In the majority of animal tissues iron is the only element found in sufficient amount that might interfere. Since the author has already completed the mineral analysis of normal and hyperplastic epidermis (*2*), it was possible to calculate the amount of each mineral found in the epidermis per mouse and to ascertain their effect upon the procedure.

Assuming that the hyperplastic epidermis from 20 mice would be used for an analysis, and calculating the amount of minerals that would be present in 5 ml. of buffer-salicylaldoxime solution, the latter was made up to contain the following metals as chlorides:

1.7×10^{-2} *M* in K; 1.4×10^{-4} *M* in Na; 1.5×10^{-3} *M* in Mg; 2.0×10^{-4} *M* in Ca; 1.33×10^{-3} *M* in Fe (ferric)

The amount of iron added was in the ratio of 20 Fe to 1 Cu, a ratio exceeding that found in normal and hyperplastic epidermis according to preliminary work. When the mineral mixture was added to the phosphate buffer, ferric phosphate precipitated immediately, but the solution was used without filtering to determine its effect upon the recovery of small amounts of copper.

Table I. Wave-Height Change for Approximately 1×10^{-4} M Salicylaldoxime with pH of Supporting Electrolyte

Supporting Electrolyte	pH	Diffusion Current[a] (Uncorrected) *Microamperes*
Potassium acid phthalate	3.7	1.54
Phosphate buffer	5.4	1.47
Phosphate buffer	6.7	1.00
Phosphate buffer	7.8	0.27
NH_4Cl–NH_4OH	9.1	No wave

[a] Constants for dropping mercury electrode: $m = 1.657$ mg. sec.$^{-1}$; $t = 2.99$ sec.; $h = 53$ cm. Diffusion current measured at -1.2 volts with respect to saturated calomel electrode.

Table II. Calibration Data for Salicylaldoxime

(Various concentrations of salicylaldoxime in 0.067 *M* phosphate buffer of pH 5.4 at 25° C. Air removed with nitrogen. $h = 64$ cm. $t = 2.74$ sec. $m = 1.015$ mg. sec.$^{-1}$. Diffusion currents measured at -1.2 volts with respect to saturated calomel electrode)

Salicylaldoxime $M \times 10^{-4}$	Observed *Microamperes*	Corrected *Microamperes*	i_d/C *Microamp./mmole/l.*
4.309	3.51	3.42	7.94
3.600	2.95	2.86	7.94
2.884	2.37	2.28	7.90
2.156	1.79	1.70	7.88
1.800	1.54	1.45	8.05
1.437	1.25	1.16	8.07
0.575	0.56	0.47	8.17
0.000	0.09[a]	..	..

[a] Residual current of supporting electrolyte alone.

Table III. Assay of Known Copper Sulfate Solutions

Molarity of Salicylaldoxime $M \times 10^{-4}$	Copper Added *Mg.*	Copper Recovered *Mg.*	Difference *Mg.*
6.3	0.6029	0.6096	−0.0067
3.4	0.4556	0.4506	−0.0050
3.4	0.3799	0.3729	−0.0070
3.4	0.3048	0.3015	−0.0033
1.8	0.1904	0.1966	+0.0062
1.8	0.1516	0.1490	−0.0026
0.90 to 1.17	0.0759	0.0761	+0.0002
	0.0570	0.0571	+0.0001
	0.0381	0.0373	−0.0008
	0.0228	0.0232	+0.0004
	0.0152	0.0155	+0.0003
	0.0076	0.0078	+0.0002
	0.0038	0.0039	+0.0001

Table IV. Assay of Known Copper Sulfate Solutions

[In presence of Na, K, Mg, Ca, and Fe (ferric) chlorides]

Molarity of Salicylaldoxime $M \times 10^{-4}$	Copper Added *Micrograms*	Copper Recovered *Micrograms*	Difference *Micrograms*
0.94 to 0.96	38.0	38.9	−0.9
	30.4	31.3	+1.1
	22.8	23.8	+1.0
	7.6	7.3	−0.3
	3.8	3.6	−0.2

Since 3.8 to 30.4 micrograms could be recovered with an error of 4 to 5%, the effect of the minerals was not appreciable (Table IV). Under the conditions in these experiments ferric phosphate does not react with the salicylaldoxime nor does ferric iron produce a diffusion current. However, the residual current of the mineral phosphate buffer mixture was 0.14 microampere.

The importance of having the same composition of supporting electrolyte in the calibration data and in the determination of unknowns is exemplified by the fact that i_r was increased by 0.05 microampere by the presence of the minerals added. Although this was a small increase, it affected i_d considerably when the concentration of salicylaldoxime was low.

Zinc interferes in the determination of copper, since the current-voltage curve of this metal, which is not precipitated as a salicylaldoxime salt under the conditions of this experiment, starts at about -1.0 volt with respect to the saturated calomel electrode and thus makes impossible the measurement not only of i_r (in the presence of material containing zinc) but also of i_d of salicylaldoxime since both waves plateau at about the same applied potential. Zinc interferes also at pH 3.7 and 6.7.

SUMMARY

Salicylaldoxime was investigated as a reagent for the polarographic determination of small amounts of copper.

It was found that the diffusion current of this reagent in a phosphate buffer mixture of pH 5.4 is proportional to its concentration, and that the wave height decreased from pH 3.7 to 7.8, disappearing at pH 9.1. The half-wave potential of salicylaldoxime at pH 5.4 is around -0.98 volt with respect to the saturated calomel electrode. From 0.1516 to 0.6029 mg. of copper can be determined with an error of about 1 to 3%, and 3.8 to 15.2 micrograms with an error of about 3%.

Sodium, potassium, calcium, magnesium, and ferric iron do not interfere, whereas zinc interferes strongly.

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Spectrophotometric Method for Determining Formaldehyde[1]

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ALTHOUGH there have been many publications dealing with the determination of formaldehyde and higher aliphatic aldehydes, no reference has been found to a specific spectrophotometric method for determining formaldehyde.

Denigès (*3*) suggested a method for detecting formaldehyde in the presence of higher aliphatic aldehydes by making use of the fact that the color produced by acetaldehyde and higher aldehydes with Schiff's reagent disappears on standing a few hours, whereas that given by formaldehyde increases in intensity. Blaedel and Blacet (*1*) have made this method semiquantitative by using a colorimeter to measure the magenta color after the solution has stood 6 hours.

This method, involving Schiff's reagent, leaves much to be desired: Because of the instability of the fuchsin reagent, it is necessary to make up fresh standards every day. For rapid control work, 6 hours is too long a period to wait for a formaldehyde determination. On standing 6 hours, a slight magenta color is sometimes obtained from the reagent alone.

More recently, Hoffpauir, Buckaloo, and Guthrie (*6*) have suggested a method for determining combined formaldehyde in organic compounds involving a hydrolysis in 12 *N* sulfuric acid followed by a formaldehyde determination according to the method of Blaedel and Blacet (*1*). Because this method gave unsatisfactory results when applied to certain formals and a rapid and sensitive method was needed for determining formaldehyde in the presence of higher aliphatic aldehydes, a search was made for a reaction which would lead to a specific test for formaldehyde.

Eegriwe (*5*) proposed chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) as a spot reagent for detecting formaldehyde. He recommended heating a drop of the solution to be tested for formaldehyde with a small amount of chromotropic acid in 72% sulfuric acid for 10 minutes at 60° C. Formaldehyde gave a purple color, whereas acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, isovaleraldehyde, crotonaldehyde, chloral hydrate, glyoxal, benzaldehyde, salicylaldehyde, phthalic aldehyde, vanillin, and cinnamic aldehyde gave no reaction. Glyceric aldehyde gave a yellow color and furfural gave a brownish color. Acetic acid, formic acid, oxalic acid, acetone, glycerol, glucose, and mannose also gave no reaction in the spot test.

Boyd and Logan (*2*) used chromotropic acid for the colorimetric determination of formaldehyde which was liberated from serine by periodate oxidation and then distilled from the reaction mixture. These authors used a Duboscq colorimeter for their measurements, and state that for an accuracy of 2% the unknown should have a formaldehyde concentration within 50% of the standard.

It seemed likely, therefore, that chromotropic acid was specific for formaldehyde and if proper conditions could be found for developing the purple color, a spectrophotometric method could be obtained.

RECOMMENDED PROCEDURE

Prepare a solution of chromotropic acid by dissolving 2.5 grams of the dry powder in 25 ml. of water, and filter if there is any insoluble material. Although this solution gradually changes color on standing, it is perfectly stable as far as color development is concerned for 2 weeks. However, since the color of the reagent does change on standing, it is desirable to run a reagent blank each day and make all readings for the day against this blank. The calibration curve remains the same if this precaution is observed.

The sample to be analyzed should not contain over 100 micrograms of formaldehyde and should have a volume of 0.4 to 0.9 ml. If a dry sample is to be analyzed, take a suitable weight of sample in a test tube (18 × 150 mm., preferably glass-stoppered) and add 0.5 ml. of water to it. After 0.5 ml. of the chromotropic acid solution is added, slowly pour 5 ml. of concentrated sulfuric acid into the test tube with continuous shaking. Then stopper the test tube and place it in a beaker of boiling water for 30 minutes. Cool the test tube and after diluting the contents of the test tube and cooling again, dilute the solution to approximately 50 ml. in a volumetric flask. When the diluted solution has reached room temperature, adjust the volume accurately to 50 ml. and read the transmission of the colored solution against a reagent blank at 570 mμ. Then, after the extinction (extinction = log $1/T$) for the solution is computed, the amount of formaldehyde equivalent to this extinction can be evaluated from the calibration curve. The percentage of formaldehyde in the sample is calculated by dividing the weight of formaldehyde found by weight of the original sample and multiplying by 100.

EXPERIMENTAL

In the preliminary experiments, a small amount of formaldehyde in 1 ml. of solution in a test tube was treated with 60 mg. of chromotropic acid and 5 ml. of 72% sulfuric acid and then heated at 60° C. ± 1° for 1 hour in a constant-temperature bath. After this solution was diluted to 50 ml., a transmission curve (using a spectral band width of 2 to 4 mμ) was run. A minimum in the transmission curve was found at 570 mμ. However, when an attempt was made to set up a calibration curve by plotting the extinction versus the concentration of formaldehyde, a concave curve instead of a straight line was obtained.

The conditions which affect the development of the purple color were then critically investigated. Technical chromotropic acid as obtained from one source was lumpy and was found to contain about 40% sodium chloride. When this technical reagent was used, erratic results were obtained. Before any conclusive work could be done, it was necessary to have some fairly pure chromotropic acid.

This technical reagent can be purified by dissolving 10 grams in 100 ml. of water, filtering off the insoluble material, evaporating the filtrate to 8 to 10 ml., and then adding about 250 ml. of ethanol. A nearly white crystalline material separates which can be filtered and dried. It was found later that chromotropic acid obtained from the Eastman Kodak Company, although sold as a practical grade, contains little or no chloride and can be used without purification. Chromotropic acid for spot tests (ST-13) from Paragon Testing Laboratories was equivalent to the purified material. Eastman Kodak reagent, spot test reagent, and the purified chromotropic acid gave identical results.

In the preliminary experiments, the conditions for color formation had to be carefully duplicated in order to ensure reproducible results. In this connection, it was found that the intensity of color increased slowly when the reaction mixture was heated for longer than 1 hour at 60° C. Therefore, it seemed likely that a higher temperature for heating the mixture and a higher sulfuric acid concentration would give more sensitive and possibly more uniform results.

Accordingly, 50 mg. of chromotropic acid in 0.5 ml. of water were added to 50 micrograms of formaldehyde in 0.5 ml. of water in test tubes. These solutions were treated with various amounts of concentrated sulfuric acid. The solutions were heated for 30

Table I. Effect of Sulfuric Acid Concentration

Sulfuric Acid Added *Ml.*	Transmission at 570 mμ %	Extinction (−log T)
1	69.0	0.160
2	33.8	0.470
3	32.5	0.489
4	33.0	0.481
5	32.5	0.487
6	33.3	0.477
7	32.9	0.483

[1] Since the galley proof of this paper was received, an article by D. A. MacFayden, *J. Biol. Chem.*, 158, 107 (1945), has appeared in which chromotropic acid is tested under different conditions, but which substantiates the results of this paper.

Table II. Effect of Bath Temperature

Temperature of Heating, ° C.	Extinction
60	0.493
70	0.489
80	0.492
90	0.480
100	0.487
120	0.485

Table III. Effect of Heating Time at 100° C.

Time of Heating, Min.	Extinction
0	0.328
5	0.683
7	0.765
10	0.850
15	0.895
30	0.906
45	0.898
60	0.890

minutes in a boiling water bath and then diluted to 50 ml. in volumetric flasks. The results in Table I show that the color developed is constant regardless of the acid concentration as long as at least 3 ml. of concentrated sulfuric acid are used for each ml. of water.

To determine the optimum temperature for developing the purple color, six samples, each containing 50 micrograms of formaldehyde and 50 mg. of chromotropic acid in 1 ml. of water, were treated with 5 ml. of concentrated sulfuric acid. These solutions were heated at various temperatures for 30 minutes and then diluted to 50 ml. (Table II).

From Table II, it is obvious that the temperature at which the color is developed is not too critical. However, for convenience and for obtaining complete hydrolysis of various formals, all solutions were heated in a boiling water bath for 30 minutes.

In order to determine the most favorable time of heating, 5 ml. of concentrated sulfuric acid were added to test tubes containing 0.10 mg. of formaldehyde and 50 mg. of chromotropic acid in 1 ml. of water. These solutions were heated for various lengths of time in a boiling water bath. The results are tabulated in Table III. It can be seen that if a solution is heated for at least 30 minutes at 100° C., complete color development is obtained.

Experiments were carried out according to the established procedure, with 0.06 mg. of formaldehyde, to determine the optimum concentration of reagent. The results shown in Table IV indicate that a 500 to 1 ratio of reagent to formaldehyde is necessary. In another series of experiments, it was found that 0.1 mg. is the maximum amount of formaldehyde that can be conveniently determined by this method. Therefore, 50 mg. of reagent should be used for each determination.

The next uncertainty was the stability of the purple color. The color from 20 and 80 micrograms of formaldehyde was developed according to the recommended procedure. The extinctions of these solutions were read as soon as they were diluted to 50 ml. and cooled to room temperature, and then at various intervals. The results are tabulated in Table V.

Obviously, the color is stable for at least 48 hours and in all probability for much longer. However, it is necessary to allow the solutions to reach room temperature before taking the spectrophotometric readings. The extinction of the purple solution is directly proportional to the temperature of the solution. If this precaution is not observed, the stability of the color may not appear to be so good as shown in Table V.

Table IV. Effect of Chromotropic Acid Concentration

Weight Ratio of Reagent to Formaldehyde	Extinction at 570 mμ
10:1	0.235
50:1	0.532
100:1	0.620
500:1	0.630

The recommended procedure was used to obtain transmission curves on a reagent blank and three different concentrations of formaldehyde. Figure 1 shows that the minimum of the colored solution is at 570 mμ and that this minimum is independent of the concentration of formaldehyde. It is apparent that the blank is only 97% transmitting at 570 mμ. Therefore, it is necessary to make all calibration readings against a reagent blank.

A calibration curve, using various amounts of formaldehyde, was made. This curve is practically linear up to an extinction of 0.750 but beyond this value the absorption starts to deviate slightly from Beer's law. Since the absorption is so reproducible, it is possible to determine as much as 0.10 mg. of formaldehyde by referring to the calibration curve.

INTERFERENCES

Using the recommended procedure, formaldehyde is the only aldehyde that has been found which will react with chromotropic acid to give a purple color. This is in agreement with the original reference to this reagent (*5*). However, any compound that will give formaldehyde on hydrolysis with sulfuric acid will give a purple color.

Table V. Stability of Color

Amount of Formaldehyde, γ	Extinction		
	Immediately	After 12 hours	After 48 hours
20	0.205	0.205	0.208
80	0.751	0.752	0.750

Methanol and ethanol do not react with the reagent and therefore, by using 0.5-gram samples, it is possible to determine as little as 2 p.p.m. of formaldehyde in these alcohols.

Higher aliphatic alcohols seem to hinder the formation of the formaldehyde color. The effect of *n*-propyl alcohol is much less than that of *n*-amyl (Table VI).

Acetaldehyde, acrolein, and β-hydroxypropionaldehyde give a yellowish brown color with chromotropic acid and in addition interfere markedly with the formaldehyde test. However, it is possible to determine 0.04 mg. of formaldehyde in the presence of 4 mg. of acetaldehyde by using 300 mg. instead of the customary 50 mg. of reagent. So far, a method to eliminate the interference of acrolein and β-hydroxypropionaldehyde has not been found.

Benzaldehyde alone does not react with the reagent, nor does it inhibit the formation of the formaldehyde color. Therefore,

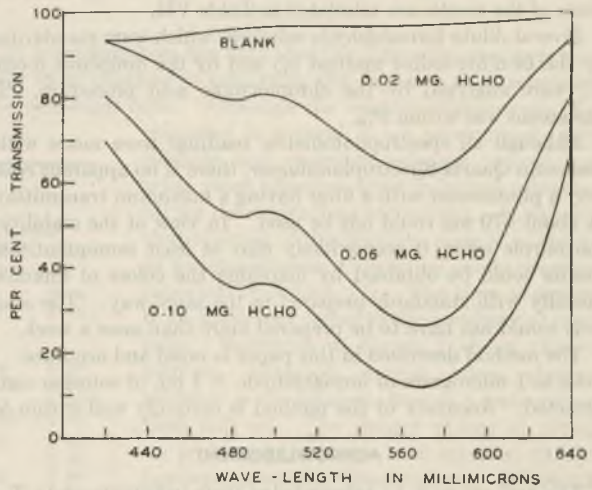

Figure 1. Transmission Curves

Table VI. Effect of *n*-Propyl and *n*-Amyl Alcohols

Weight Ratio, Propyl Alcohol to Formaldehyde	Formaldehyde Added	Formaldehyde Found
	Mg.	*Mg.*
5:1	0.035	0.035
10:1	0.035	0.033
15:1	0.035	0.033
Amyl Alcohol to Formaldehyde		
2:1	0.044	0.042
5:1	0.044	0.037
10:1	0.044	0.030
100:1	0.044	0.021

Table VII. Determination of Formaldehyde in Formals

Material	Formaldehyde Theory	Formaldehyde Found[a]
	%	%
Methylal (7.3% methanol)	36.4	36.2
Pentaerythritol diformal	37.50	37.5
Pentaerythritol monoformal	20.27	20.2
Piperonal	20.00	21.2
n-Propyl formal[b]	22.6	19.7
Trioxane	100.0	102

[a] No free formaldehyde found in any of these samples.
[b] Purity not known. By the 2,4-dinitrophenylhydrazine procedure, 19.9% formaldehyde was found.

very small amounts (0.02%) of formaldehyde can be determined in the presence of this aromatic aldehyde and probably in the presence of other aromatic aldehydes.

Acetone, while it does not give a color with chromotropic acid, causes the purple color due to formaldehyde to fade when the solution is diluted with water. However, this interference can be eliminated by diluting the solution to 50 ml. with 18 *N* sulfuric acid instead of water. As little as 1 part of formaldehyde in 5000 parts of acetone can be accurately determined by this modification.

Diacetone alcohol and methyl ethyl ketone interfere very markedly with the development of the formaldehyde color. In the presence of 200 mg. of each of these materials, 0.04 mg. of formaldehyde could not be detected.

The mechanism of the reaction between formaldehyde and chromotropic acid has not been investigated. For this reason, no explanation can be offered to account for the various interferences mentioned.

RESULTS AND APPLICATIONS

The method described in this paper can be used to determine free formaldehyde or combined formaldehyde which is liberated by acid hydrolysis. Various formals which were subjected to the recommended procedure were quantitatively hydrolyzed. Some of the results are tabulated in Table VII.

Several dilute formaldehyde solutions which were standardized by the bisulfite-iodine method (*4*) and by the dimedone method (*7*) were analyzed by the chromotropic acid procedure. The agreement was within 3%.

Although all spectrophotometric readings were made with a Beckman Quartz Spectrophotometer, there is no apparent reason why a photometer with a filter having a maximum transmittance at about 570 mμ could not be used. In view of the stability of the purple color, it seems likely that at least semiquantitative results could be obtained by matching the colors of unknowns visually with standards prepared in the same way. The standards would not have to be prepared more than once a week.

The method described in this paper is rapid and accurate. As little as 1 microgram of formaldehyde in 1 ml. of solution can be detected. Accuracy of the method is certainly well within 5%.

ACKNOWLEDGMENT

The authors wish to acknowledge their indebtedness to T. R. Aalto for his encouragement and criticism during the development of this method. Marie Corse and Robert Yale deserve much credit for their faithfulness in applying and proving the value of this method for determining formaldehyde.

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PRESENTED before the Division of Analytical and Micro Chemistry at the 108th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y.

BOOK REVIEW

The Amino Acid Composition of Proteins and Foods. *R. J. Block* and *Diana Bolling.* 396 pages. Charles C. Thomas, Springfield, Ill., 1945. Price, $6.50.

The recent pronounced increase in amount of research attention paid to proteins and amino acids, particularly with respect to the roles they play in nutrition, makes this book timely. No previous text or reference book contains in such detail the published amino acid analyses of proteins and foods. In addition, the authors have rendered a distinct service by outlining and discussing in detail the methods used for the determination of amino acids.

The first eight chapters—Diamino Acids, Aromatic Amino Acids, Sulfur-Containing Amino Acids, β-Hydroxy Amino Acids, "Leucines", Dicarboxylic Amino Acids, Glycine and Alanine, and Proline and Hydroxyproline—contain the available information on methods of estimation and occurrence. In many cases sufficient detail is included, so that the determinations can be made without reference to the original papers. The authors suggest which methods are most reliable and comment pertinently on the various alternate procedures. The tables are extensive and inclusive and contain many hitherto unpublished analyses. Although many of the data are entirely unreliable and of historical interest only, they should make extensive early literature searches unnecessary for future workers in this field. The authors indicate which of the analytical results they consider to be the closest approximations to true values.

It is unfortunate, although doubtless unavoidable, that the references are complete only through part of 1943. Inasmuch as a considerable number of important papers dealing with amino acid methods and analyses have appeared since then, the most acceptable results for the more difficultly determinable amino acids in many cases are now no longer the same as those included in the tables. In particular, data obtained by microbiological methods have required some revision of previous data on the amino acid composition of proteins and foods.

Chapter IX describes general methods for the hydrolysis of proteins, the separation and determination of amino acids, and tests for carbohydrates.

Chapter X includes a series of 14 tables of amino acid compositions of proteins. Here the entire amino acid moiety is represented, in contrast to the tables in the previous chapters in which the amino acids are presented individually or in groups of two or three.

The three tables in the final chapter on the essential amino acid requirement of man include an estimated annual average per capita consumption of essential amino acids, the daily essential amino acid requirements, and the percentage of optimal daily requirements supplied by 100 grams of proteins from several of the commonest foods.

The bibliography of more than 700 references and the author and subject indexes have been carefully prepared and help to make the book a handy manual for ready reference. Protein chemists and those interested in the protein phases of nutrition will use it as such.

HAROLD S. OLCOTT

Catalytic Activity of Selenates in the Kjeldahl Method for Determination of Nitrogen

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Although selenates have been found to be more effective catalysts in the Kjeldahl digestion for protein nitrogen than elemental selenium, prolonged digestion gives low results.

IN 1931, Lauro (*4*) discovered that small amounts of selenium were effective in catalyzing the decomposition of proteins in Kjeldahl digestions. Although the action of selenium, selenium oxychloride, and certain selenites in catalyzing these decompositions has been fairly extensively investigated since that time, there appears to be no study of the effect of selenates on digestion time.

Sreenivasan and Sadasivan (*7*) employed copper selenate in a study of the mechanism of selenium catalysis, but did not report the effect of the salt on digestion time. They proposed a mechanism for the catalysis in which selenium is alternately oxidized to selenious acid and reduced to elemental selenium. The selenium, after complete oxidation of protein, is present as selenious acid. However, in the presence of mercuric oxide, all the selenium is presumably oxidized to selenic acid. Osborn and Krasnitz (*5*) report that a combination of mercuric oxide and selenium acts much more effectively than either mercuric oxide or selenium alone in catalyzing the decomposition of proteins. In view of this fact and in the event that selenic acid does play a role in selenium catalysis in the presence of mercuric oxide, possibly a selenate would be more effective as a catalyst than either selenium or a selenite.

The purpose of the present investigation was to determine the relative catalytic action of several selenates in the Kjeldahl determination and compare their effectiveness to elemental selenium in this respect.

EXPERIMENTAL

The determinations were carried out with the conventional Kjeldahl apparatus in the usual manner, using a gas flame as a source of heat. Elemental selenium used was a preparation of Eimer and Amend. The selenates were prepared by treating analytical reagent carbonates of the metals with selenic acid, the preparation of which has been described (*3*). The salts were twice recrystallized from water. The selenates in hydrated form were weighed out in amounts such that the selenium content of each sample was 0.10 to 0.15 gram. This was added directly to the weighed sample of protein. Bradstreet (*2*) reports that more than 0.25 gram of selenium gives low results. The digestions were carried out for varying lengths of time in the presence of the four catalysts: selenium, copper selenate, calcium selenate, and cadmium selenate. All analyses represent the average of at least two determinations. The results of duplicate determinations were in error by no more than 0.2%.

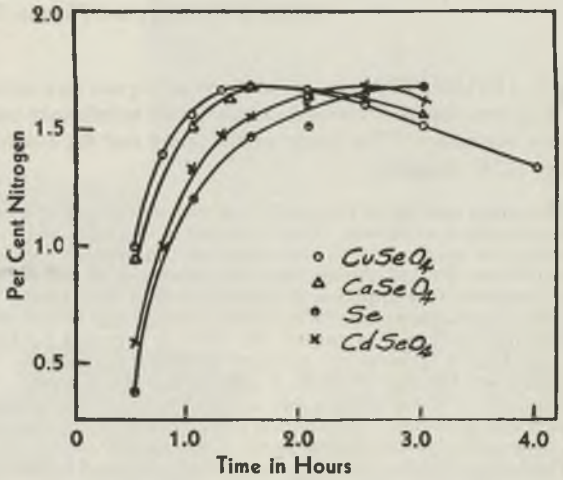

Figure 1. Effect of Selenates on Digestion Time

While the data recorded in Table I are only a portion of those obtained in the study, they are fairly representative. Sample I was pea meal, very difficult to decompose completely. Six hours were required for the decomposition using metallic selenium as a catalyst; with copper selenate the time was cut to 2.5 hours; with calcium selenate to 3 hours; and with cadmium selenate to 3.5 hours. Nitrogen obtained by the official method (Kjeldahl-Gunning-Arnold) is somewhat lower than the maximum obtained using selenium or selenates as catalysts. However, in three other samples, data for one only of which are included here, the nitrogen content by the official method agreed well with the maximum values obtained with the selenium or selenate catalysts. Data for sample No. II are shown graphically in Figure 1.

Table I. Effect of Selenium Catalysts

Protein Sample No.	$HgO + K_2SO_4$ Time	$HgO + K_2SO_4$ N	Se Time	Se N	$CuSeO_4$ Time	$CuSeO_4$ N	$CaSeO_4$ Time	$CaSeO_4$ N	$CdSeO_4$ Time	$CdSeO_4$ N
	Hours	%	*Hours*	%	*Hours*	%	*Hours*	%	*Hours*	%
I	1	2.59	6	2.63	2	2.59	2	2.56	2	2.49
					2.5	2.63	3	2.61	3	2.61
					3	2.60	4	2.51		
					6	2.30				
II	1	1.67	0.5	0.37	0.5	0.98	0.5	0.94	0.5	0.57
			0.75	0.84	0.75	1.37	0.75	1.39	0.75	0.97
			1	1.20	1	1.55	1	1.59	1	1.32
			1.25	1.44	1.25	1.66	1.33	1.62	1.25	1.47
			1.5	1.46	1.5	1.67	1.5	1.67	1.5	1.55
			2	1.51	2	1.66	2	1.64	2	1.60
			2.5	1.63	2.5	1.59	2.5	1.60	2.5	1.66
			3	1.67	3	1.50	3	1.57	3	1.61
					4	1.36				

DISCUSSION

It is evident from the data that the three selenates were markedly more effective in catalyzing the digestion than selenium in the elemental form. Although the relative effectiveness of the selenates in general is not pronounced, copper selenate and cadmium selenate proved somewhat more effective than cadmium selenate in all the determinations carried out. It was noted that clearing of the digestion mass is no criterion as to completeness of digestion, a fact previously reported by Ashton (*1*). The time of digestion is an important factor in the accuracy of the results. Reference to Figure 1 shows that the nitrogen obtained, except when elemental selenium is the catalyst, rises to a maximum and then falls off. The danger of loss of nitrogen on prolonged digestions with selenium catalysts has been reported by Sandstedt (*6*). It would not appear practicable, therefore, to employ selenates as catalysts, since the digestion time for maximum

yield of nitrogen would have to be rather accurately determined and controlled for each type of protein sample.

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An Automatic Gas Circulating Pump

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AN AUTOMATIC gas circulating pump for use on a vacuum system has been devised and used with satisfactory results in this laboratory. The design of the pump and the controls is shown in the diagram.

The pump consists of two small one-way valves and two 50-cc. bulbs arranged as shown. Gas is drawn in through valve *A* by lowering the mercury in *C*, thus reducing the pressure inside the pump below that of the system; the lowering of the mercury is accomplished by applying a vacuum on bulb *D*. In this step *B* acts as a check valve. When the mercury has been lowered sufficiently so that *C* is empty, the cycle is reversed by letting air into *D* and allowing the mercury to flow back into *C*. Gas is forced out through *B* while *A* acts as the check valve. The pump used by the authors made a complete cycle once a minute at 200-mm. pressure. This period could be varied by changes in the dimensions of the various parts of the apparatus.

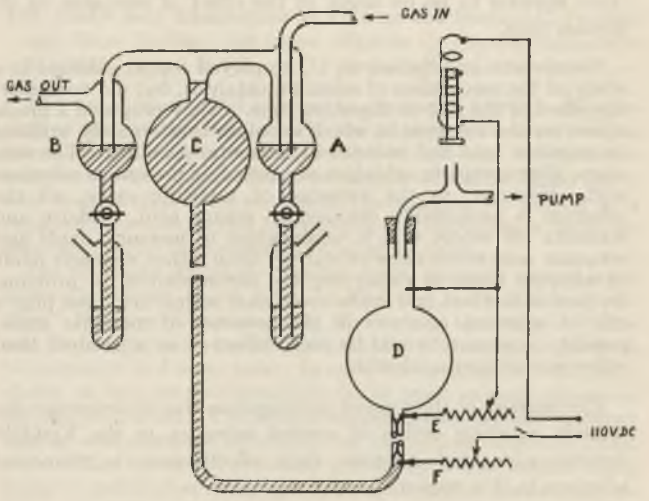
The levels of the mercury in *A* and *B* are adjusted by means of the reservoirs below them. The pump can be made to operate at pressures as low as 3 to 4 mm. by suitably adjusting these levels. This is the pressure required to overcome the resistance of the mercury check valves. If other check valves were used this minimum might be reduced. Residual gas in the pump can be let out into the system by drawing the mercury down into the reservoirs.

The pump was designed to operate with a continually varying pressure inside the system. This is accomplished by constructing the right arm of the U-tube of small-bore tubing; pressure variations register on this arm while the maximum height in *C* remains practically unchanged. The height between the bottom of this small-bore tubing and the top of *C* should exceed the mercury equivalent of one atmosphere pressure for safe operation at pressures down to a few millimeters.

The minimum operating pressure in millimeters of mercury is equal to or greater than the height of the bottom of *C* above the upper contact on *D*, plus the resistance of one check valve, and the lowest pressure reached by the operating vacuum pump. The maximum operating pressure is equal to or less than the atmospheric pressure, minus the resistance of one check valve, minus the height of the top of *C* above the lower contact on *D*. The operating range of pressures for any set of fixed dimensions or level of bulb *D* is the difference between the maximum and minimum operating pressures and is equal to atmospheric pressure minus the sum of the distance between the top and bottom of *C*, the distance between the top and bottom contacts on *D*, the lowest pressure of the operating pump, and twice the resistance of one check valve. For safety of operation, so that mercury from *C* does not enter the valve chambers, the minimum pressure encountered in millimeters of mercury plus the height of the top of *C* above the rest level of the mercury in the small-bore tubing should be equal to or greater than atmospheric pressure.

For operating at pressures down to a few millimeters the upper contact on *D* must be below the bottom of *C* by at least the lowest pressure of the operating pump plus the resistance of one check valve. The maximum operating pressure under these conditions is equal to atmospheric pressure minus the height between the lower contact on *D* and the top of *C*. Of course, the lower *D* is the shorter the period of operation but the lower the maximum pressure. For operating at pressures above this maximum *D* must be raised relative to *C*. In this case the minimum pressure at which the pump will operate will increase by the amount that the upper contact of *D* is raised above the highest level that it could have for operating down to a few millimeters. The height of *D* can be made adjustable by connecting it to the glass system by a flexible rubber tubing.

The operation of the pump is made automatic by an electrical device based on the fact that it requires less force to hold an iron core in a solenoid than to lift it into this position against the force of gravity, particularly as the vacuum below the air leak provides an additional resistance to be overcome in lifting the solenoid core.

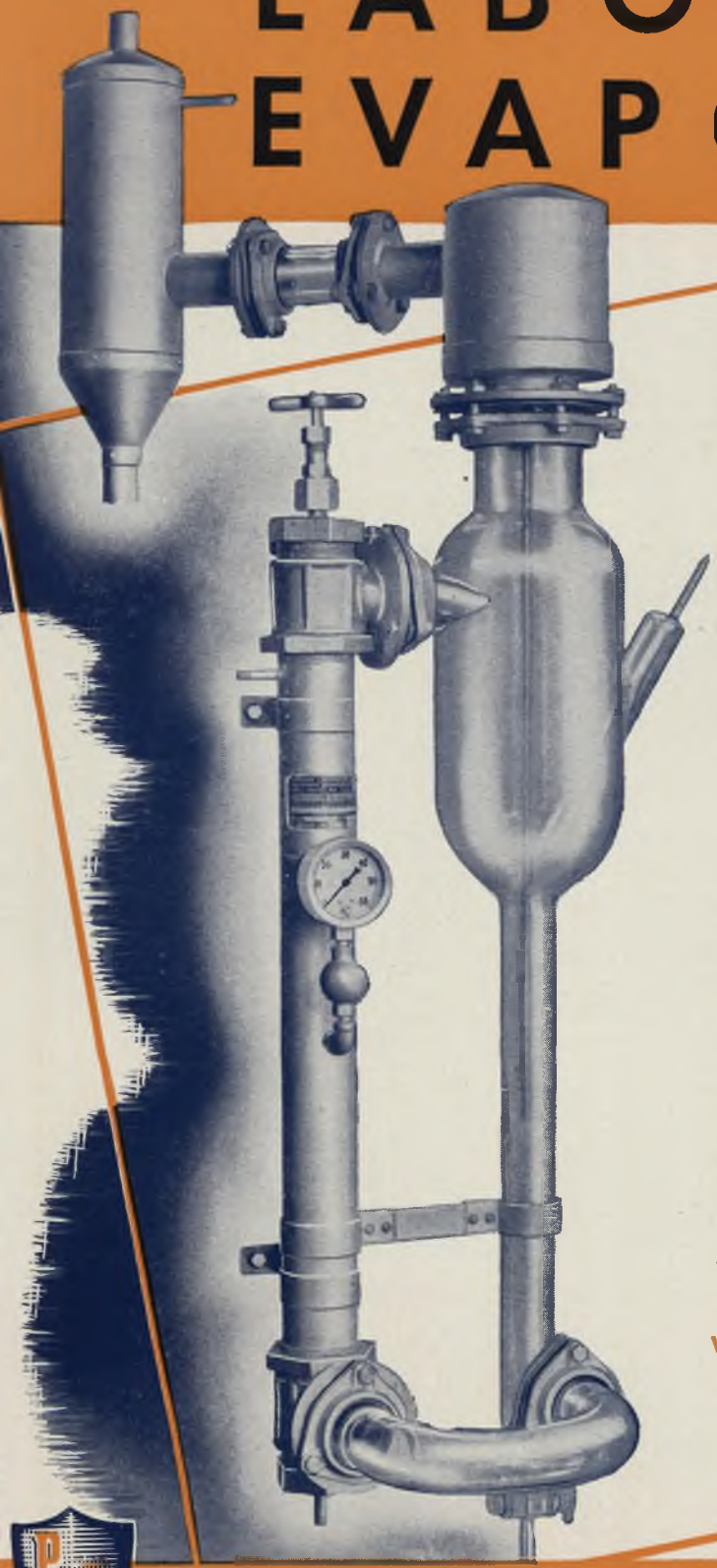


When the mercury starts rising in the right arm of the U-tube, it completes a circuit through the sealed-in contact at the bottom of bulb *D*. The current through the solenoid is sufficient to support the weight of the rod, but not sufficient to lift the rod and overcome the vacuum force, so the air leak remains closed. The mercury continues to rise until it makes contact with the lead at the top of *D*. A larger current then flows through the solenoid, and the rod is pulled off the air leak. This contact is immediately broken, since air rushes into *D* and the mercury starts to fall. The air leak does not close, since the current through the lower contact is sufficient to hold the rod in the solenoid. The mercury continues to fall until the lower contact is broken. At this point no current flows through the solenoid, the rod falls, closing the air leak, and the cycle is repeated.

In the original apparatus an ordinary aspirator supplied the vacuum and an air leak of 7-mm. tubing was used. The stop on the air leak was made of 0.6-cm. (0.25-inch) iron rod and weighed 15 grams. The open end of the glass tube was ground flat. A cork was fitted to the lower end of the plunger rod by means of a centrally bored hole. The rod entered this hole partway through the cork. The hole was enlarged at the lower end, and a rubber disk cemented to the bottom face. An air cushion was thus provided above the rubber disk. A direct current solenoid of approximately 400 turns was used, which required a current of 2.3 amperes to overcome both forces on the rod and 1.0 ampere to maintain the weight of the rod. Resistances *E* and *F* were approximately 50 and 30 ohms.

Alternating current could be used by using an alternating current solenoid; the design could be further modified by using relays to cut down the current through the mercury.

"PRECISION"
LABORATORY EVAPORATOR



The evaporation of solutions for the purpose of reducing volumes and concentrating solids is one of the more frequently encountered operations common to many production and research problems. However, many laboratories still carry on with the outmoded steam cones and round bottom flasks as basic equipment.

Developed in cooperation with the Clinton Company, Clinton, Iowa, this evaporator is constructed on the same general principles as large industrial units, for the evaporation of aqueous solutions to recover dissolved solids. It will duplicate closely results obtained with commercial-sized tube evaporators. Bumping is eliminated and protein or other food product solutions, almost impossible to handle in flasks because of excessive foaming, can be evaporated with little or no difficulty. Evaporation is rapid and easily carried on. Ideally suited for experimental study on agricultural residues.

PERFORMANCE

On tests using water, the model shown will evaporate 12 liters per hour (26.4 lbs. per hour), with an evaporator having a surface area of .5 sq. ft., or the equivalent of 53 lbs. per sq. ft. per hour. On tests conducted on corn steep liquor, the evaporation was equivalent to 45 lbs. per sq. ft. per hour.

FEATURES

Made entirely of Pyrex glass and stainless steel to eliminate corrosion. Model shown has a maximum capacity of 3,000 cc. of liquid and will handle batches as small as 500 cc. Heating tube—18-8 stainless steel, with a surface area of .5 sq. ft. Built-in dual vapor condensate traps and drawoff cock. Steam inlet connection, steam valve and pressure gauge.

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