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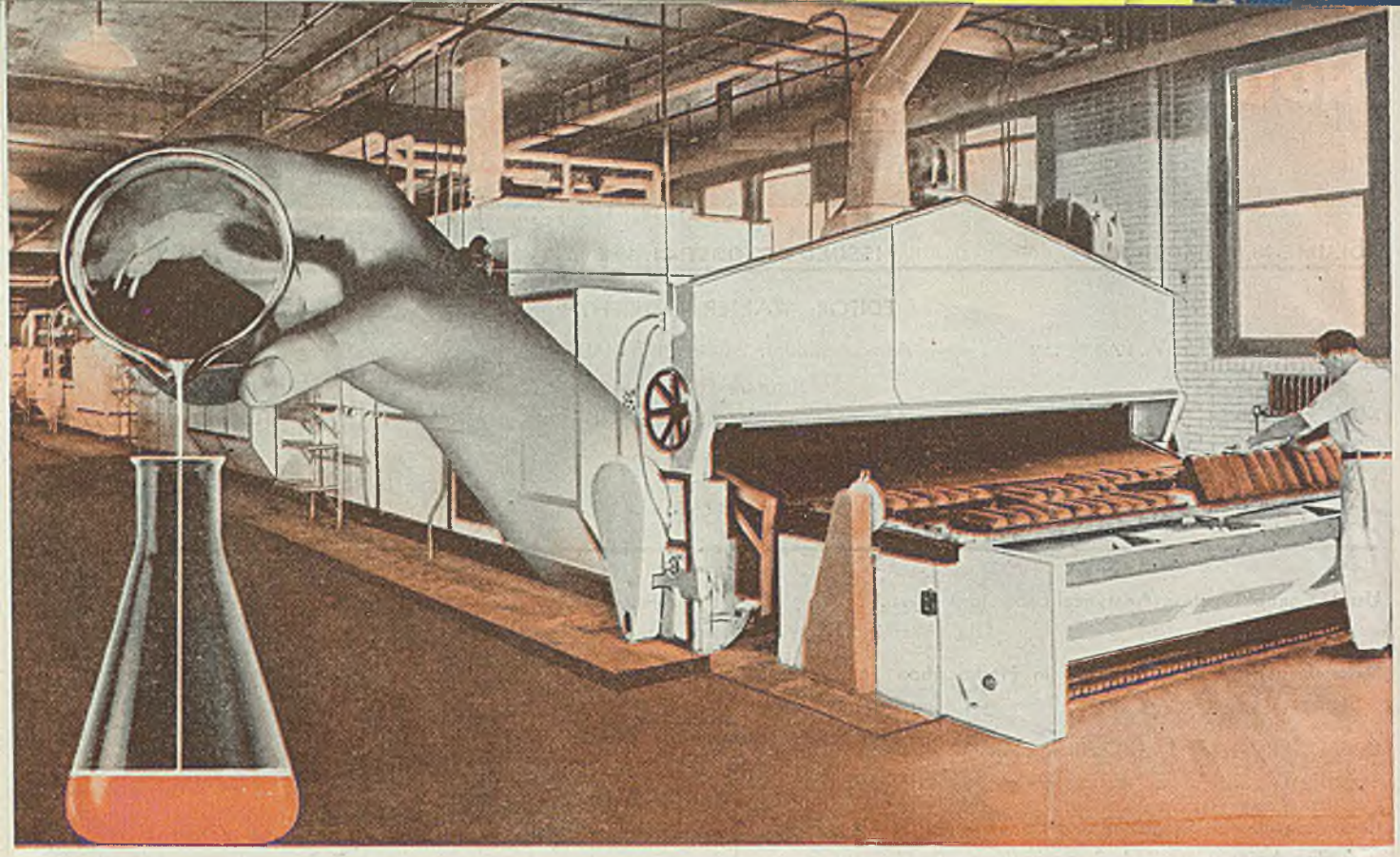

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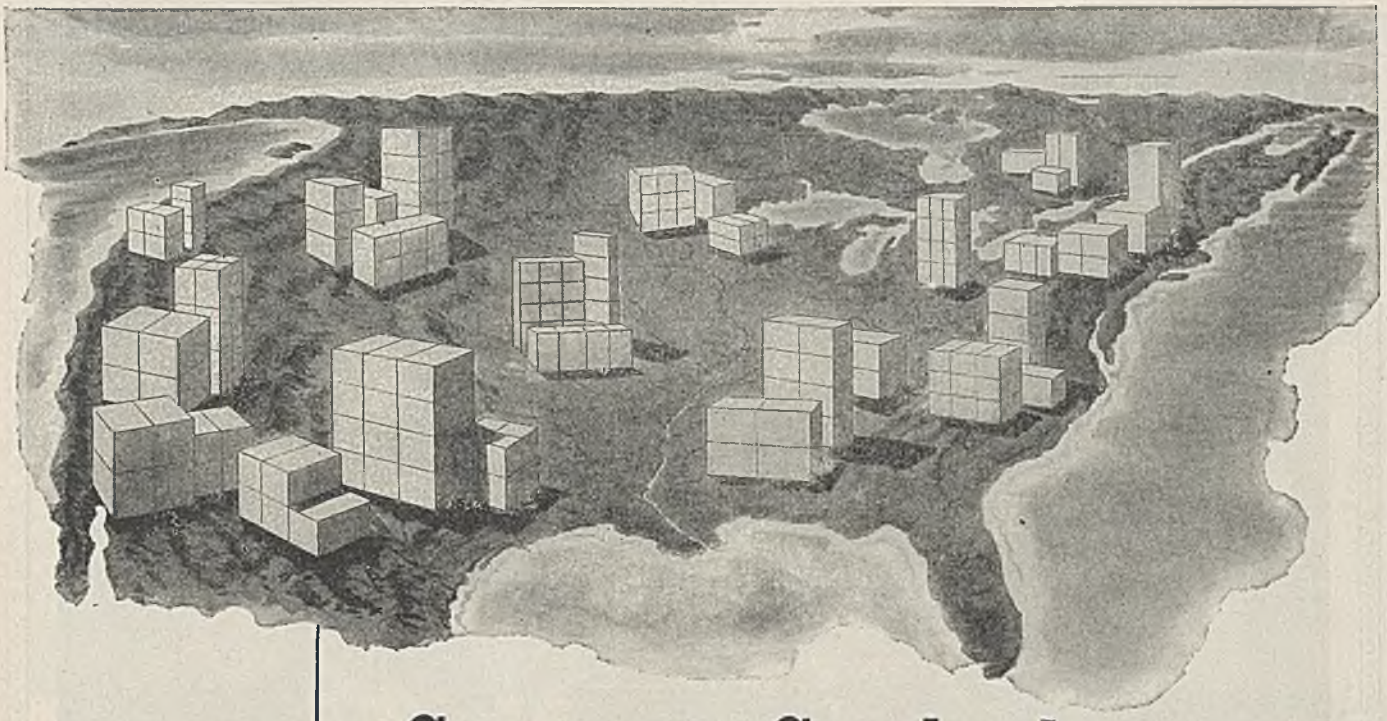

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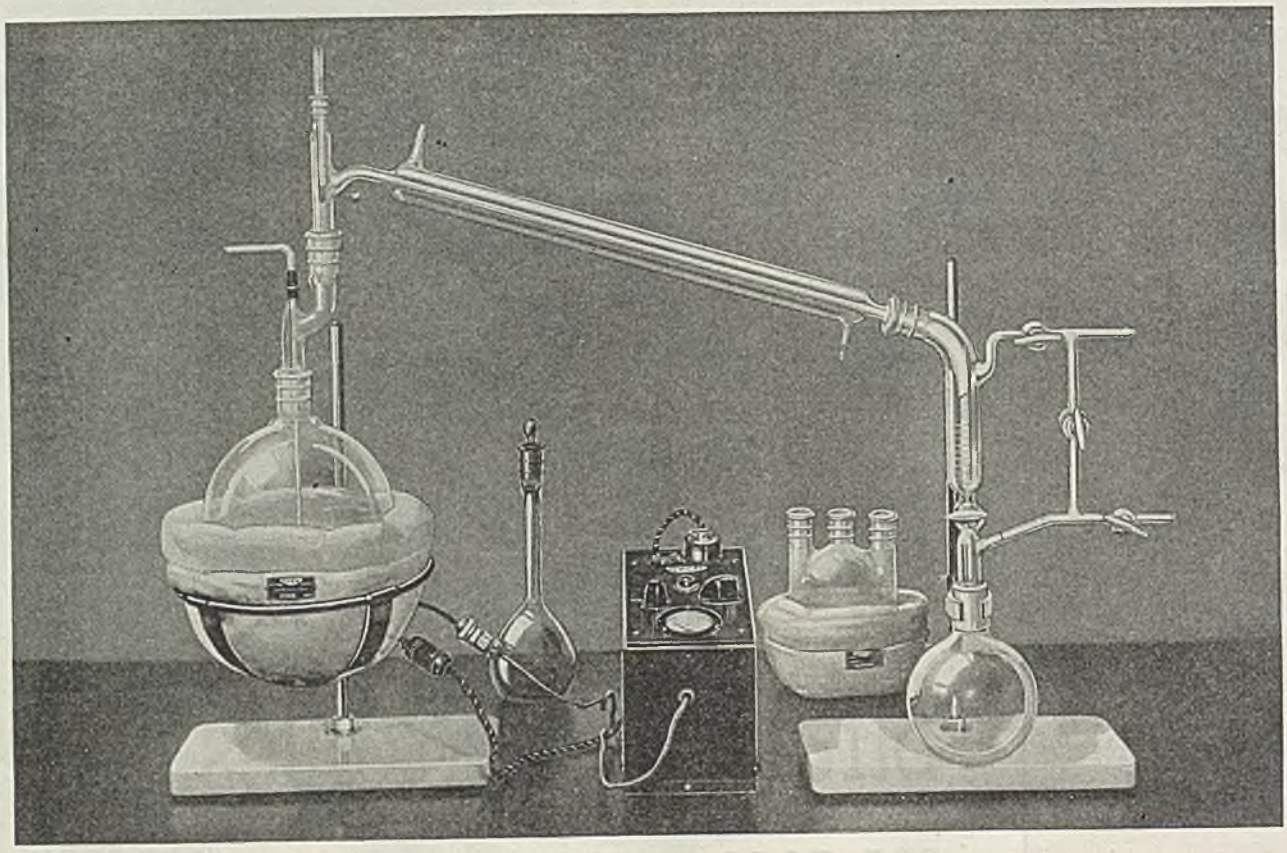

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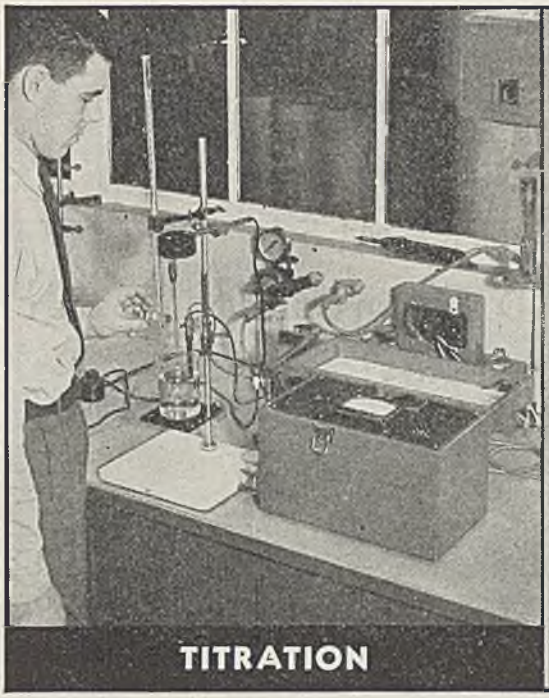

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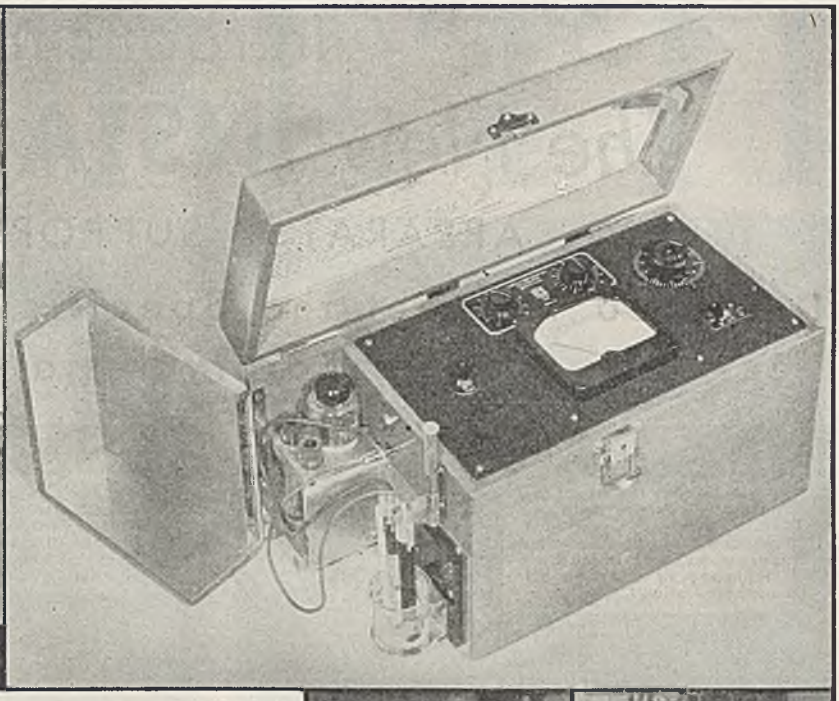

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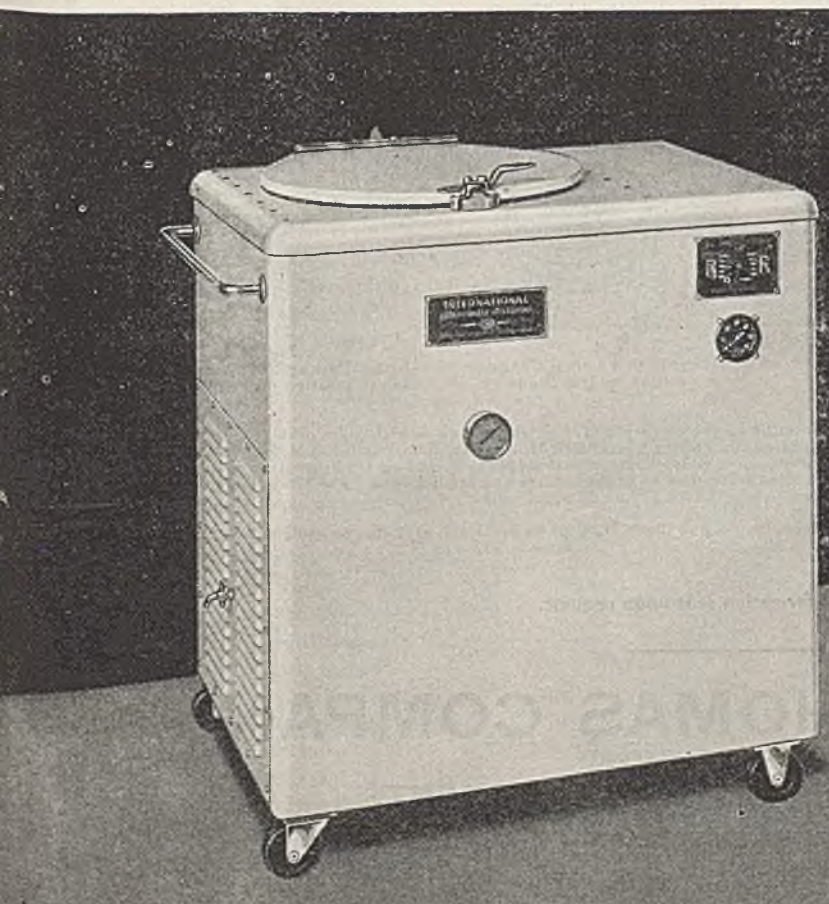

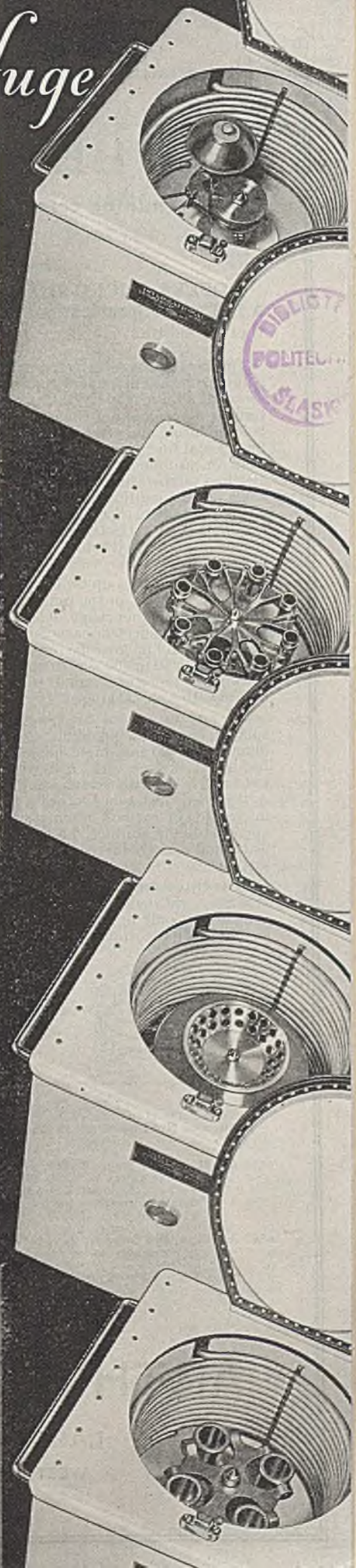

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Use of Control Charts in the Analytical Laboratory

GRANT WERNIMONT, Industrial Laboratory, Eastman Kodak Company, Rochester 4, N. Y.

Control charts are useful in the testing laboratory for comparing the over-all variability of test data with the average variability of small groups of the data, and are simpler to understand than complicated statistical methods of analysis of variance. Operational meaning of control charts depends on the manner in which arbitrary small groups are chosen.

STATISTICAL methods have been used for a long time to present the average of test method results. However, as Fisher has pointed out (*5*), the variation among test results usually has not been an object of study, but has been recognized rather as a troublesome circumstance which detracted from the value of the average.

The estimates of test method precision which analysts do present are often not correct because they are made on the basis of a small amount of data covering only a short period of time. In many cases, a great deal of effort has been taken to eliminate all assignable causes of variation while the precision of the test method is being studied, even though it would not be desirable or even possible to do so when the test method is used for routine control purposes. W. S. Gosset, who published under the pseudonym of "Student", stated all this aptly when he observed (*17*) that an analyst who wishes to impress his clients will arrange to do repetition analysis as nearly as possible at the same time, but if he wishes to diminish his real error he will separate them by as wide an interval as possible.

The application of statistical methods to industrial manufacturing problems has been pioneered in the United States by Shewhart (*14*). During the war, the War Production Board sponsored some thirty-three short courses on the industrial application of Shewhart's methods in which almost two thousand men were trained. With the coming of these "quality control" methods into chemical-process manufacturing, it will be increasingly important for analysts to be able to determine how much of any observed process variation is to be ascribed to the test procedure itself. The control chart method of Shewhart is probably the most practical method of studying the precision and accuracy of routine test methods, and several authors have already mentioned its application to the study of chemical test procedures (*4, 7, 8, 10, 20*).

No attempt will be made here to develop the mathematics upon which control charts are based; and only a brief reference will be made to the simple arithmetical calculations involved because this part has been adequately presented in many publications (*1, 2, 6, 12–16*). An attempt will be made to show the operational meaning which can be put into control charts when applied to problems in the analytical laboratory.

MAKING A CONTROL CHART

A control chart may be described as a graphic presentation of test data in such a manner that the variability of all the results is compared with the average variability within (arbitrary) small groups of the test results. The chart is said to show evidence of "control" when there is no more variation throughout the entire set of results than corresponds, statistically, to the average variation within the (arbitrary) small groups.

In order to illustrate the preparation of a control chart, use can be made of a comprehensive experiment made by Shewhart (*14*), in drawing marked chips from a bowl. In his experiment, 998 chips were marked as shown in Figure 1, and 1000 groups of four chips were drawn with replacement from the well-mixed bowl. The averages and ranges (difference between highest and lowest) of the first fifty drawings are plotted in Figure 2. The grand average was −0.08 and the average range of four was 2.03. It can be seen that this graph of random drawings is similar to a graph for routine test results, in that both the averages and ranges fluctuate and the question immediately arises as to whether any limits for these variations can be set up.

Table I can be used to set up these limits as follows: The upper

Table I. Factors for Computing 3-Sigma Control Limits

No. of Observations, n	Chart for Individuals, Factors for Control Limits: Av. standard deviation, I_1	Chart for Individuals, Factors for Control Limits: Av. range, I_2	Chart for Averages, Factors for Control Limits: Av. standard deviation, A_1	Chart for Averages, Factors for Control Limits: Av. range, A_2	Chart for Standard Deviations: Factor for central line, c_2	Chart for Standard Deviations: Factors for control limits, B_1	Chart for Standard Deviations: Factors for control limits, B_4	Chart for Ranges: Factor for central line, d_2	Chart for Ranges: Factors for control limits, D_3	Chart for Ranges: Factors for control limits, D_4
2	5.32	2.66	3.76	1.88	0.5642	0	3.66	1.128	0	3.27
3	4.15	1.77	2.39	1.02	0.7236	0	2.69	1.693	0	2.57
4	3.76	1.46	1.88	0.73	0.7979	0	2.33	2.059	0	2.28
5	3.57	1.29	1.60	0.58	0.8407	0	2.13	2.326	0	2.11
6	3.45	1.18	1.41	0.48	0.8686	0	2.00	2.534	0	2.00
7	3.38	1.11	1.28	0.42	0.8882	0.10	1.90	2.704	0.08	1.92
8	3.32	1.05	1.18	0.37	0.9027	0.17	1.83	2.847	0.14	1.86
9	3.28	1.01	1.09	0.34	0.9139	0.23	1.77	2.970	0.18	1.82
10	3.25	0.97	1.03	0.31	0.9227	0.27	1.73	3.078	0.22	1.78

Control limits for individual observations $= \bar{\bar{X}} \pm \frac{3\bar{\sigma}}{c_2} = \bar{\bar{X}} \pm I_1\bar{\sigma} = \bar{\bar{X}} \pm \frac{3\bar{R}}{d_2} = \bar{\bar{X}} \pm I_2\bar{R}$

Control limits for averages of groups of $n = \bar{\bar{X}} \pm \frac{3\bar{\sigma}}{\sqrt{n}c_2} = \bar{\bar{X}} \pm A_1\bar{\sigma} = \bar{\bar{X}} \pm \frac{3\bar{R}}{\sqrt{n}d_2} = \bar{\bar{X}} \pm A_2\bar{R}$

Control limits for standard deviations $= \left(1 \pm \frac{3}{\sqrt{2n}\,c_2}\right)\bar{\sigma} = B_3\bar{\sigma}$ to $B_4\bar{\sigma}$

Control limits for ranges $= \bar{R} \pm 3\sigma_R = D_3\bar{R}$ to $D_4\bar{R}$

$\bar{\bar{X}}$ = grand average; $\bar{\sigma}$ = average standard deviation; $\bar{R}$ = average range

With the exception of factors in columns for I_1 and I_2, this table is taken from (*1*) Table I, page 50; the formulas are explained on pages 52 and 53.

and lower limits for range variation are equal to the average range, 2.03, multiplied by the factors 0 and 2.28 (which are found in columns D_3 and D_4 corresponding to a group size of four under chart for ranges). This gives a lower limit of 0 and an upper limit of 4.6 for the variation of ranges of four drawings. Ranges greater than 4.6 (and less than 6.0) are possible but they should be observed only one or two times per thousand drawings by chance alone.

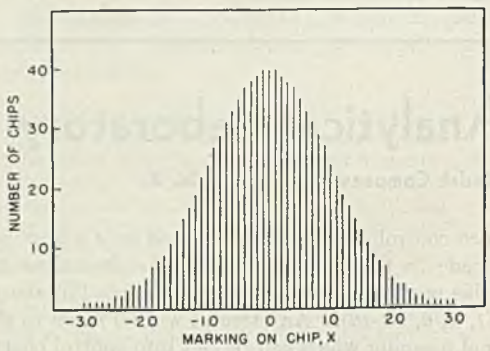

Figure 1. Distribution of 998 Chips in Shewhart's Sampling Experiment

Average, 0.0
Standard deviation, 1.007

In a similar manner, the limits of variation for the averages of four about the grand average are equal to the average range, 2.03, multiplied by the factor 0.73 (which is found in column A_2, corresponding to a group size of four under chart for averages). This gives the limits -0.08 ± 1.48 for the variation of averages of four drawings. A summary of these calculations is given in Table II.

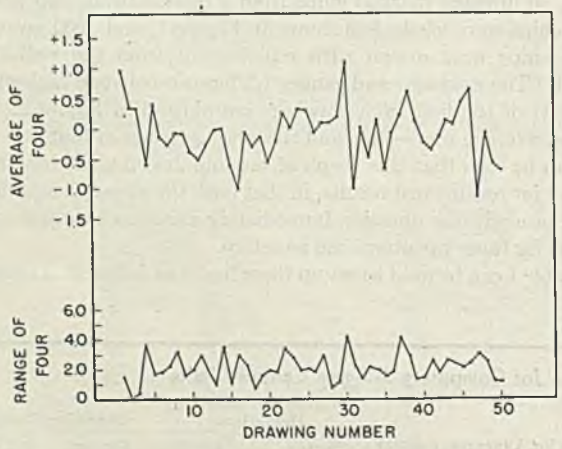

Figure 2. Two Hundred Drawings from Shewhart's Bowl of Chips in Groups of Fours

These so-called "control limits" are drawn in as shown in Figure 3, and it can be seen that the ranges and averages are all within their control limits, as of course they should be, if Shewhart's experiment was carried out properly. The control chart tells us graphically that there was no more variation among the fifty averaged values than corresponds, statistically, to the average range of four individual chips drawn at one time. If an infinite number of drawings were tabulated and summed up, the results would make a distribution curve like that shown at the right of Figure 3; the control limits correspond, approximately, to the points on either side of the grand average where this distribution curve approaches the zero base line.

The table of factors can be used to estimate control limits for

Table II. Summary of Calculation of Control Limits for Shewhart's Drawing Experiment

Drawing No.	X_1	X_2	X_3	X_4	$\bar{X}$	R
1	1.7	0.2	1.4	0.5	0.950	1.5
2	0.3	0.3	0.4	0.4	0.350	0.1
3	0.4	0.4	0.1	0.4	0.325	0.3
4	−1.8	−0.9	−0.6	1.7	−0.400	3.5
5	0.5	−0.7	0.8	1.0	0.400	1.7
..	...	...	...	...	...	...
..	...	...	...	...	...	...
..	...	...	...	...	...	...
46	0.6	2.2	0	−0.2	0.650	2.4
47	0.5	−0.7	−1.9	−2.5	−1.150	3.0
48	0.3	−1.5	1.0	−0.1	−0.75	2.5
49	−1.2	0	−0.8	−0.5	−0.625	1.2
50	−0.7	0	−1.0	−1.1	−0.700	1.1
			Sum		−4.00	101.4
			Av.		−0.08	2.03

Control limits for $R = (0 \times 2.03)$ to $(2.28 \times 2.03) = 0$ to 4.6
Control limits for $\bar{X} = -0.08 \pm (0.73 \times 2.03) = -1.56$ to $+1.40$
For complete data see (*14*) Table A, p. 442.

ranges, standard deviations, and averages of small groups of test results as well as for the individual results themselves. When these limits are drawn into the graph, the resulting control chart shows at a glance whether there is more variation among the groups (or among all the individual results) than corresponds, statistically, to the average variation within the groups. If excessive variation is found to be present, the control chart will often help to find its cause.

CHECKING PERFORMANCE OF ROUTINE TEST METHODS

Many laboratories maintain a "controlled sample" which is used at regular intervals to check the performance of a routine test method. The control chart in Figure 4 shows such test results for a simple viscometer. The controlled sample is run every day just before the production samples are tested. The five weekly results for the controlled sample are grouped in order to estimate control limits. The lower graph for weekly ranges indicates no variations greater than are to be expected by chance alone, and the upper graph shows that there is no more variation among the individual results from week to week than corresponds, statistically, to the average variation of any one week. These limits of test variation are satisfactory to the production department.

This kind of chart is invaluable when new test operators are being trained or when it becomes necessary to change the controlled sample. The chart also stops effectively most of the arguments between the testing laboratory and the production department over the validity of production test results which are not as they should be.

Sometimes it is possible to keep a satisfactory check on a rou-

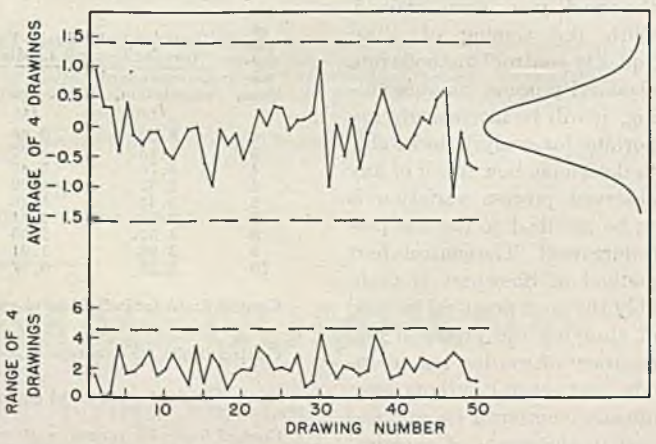

Figure 3. Control Chart for Averages of Four Drawings from Shewhart's Bowl of Chips

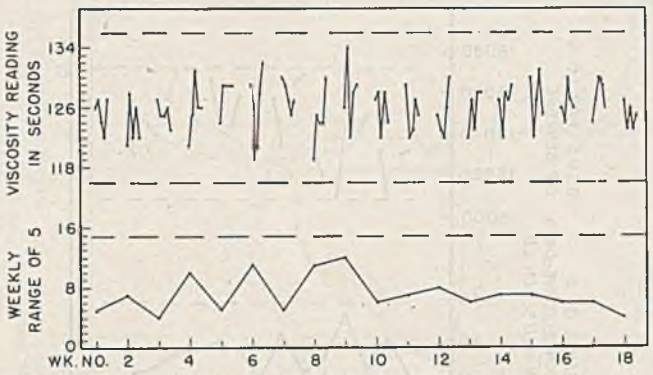

Figure 4. Control Chart for Viscosity of a Standard Sample

tine test method without the use of a controlled sample. Figure 5 shows a control chart for the moisture content of production distillation lots of an organic solvent. The lower graph shows that the differences between two consecutive lots of the solvent do not fluctuate more than corresponds to chance alone. The upper graph for individual lots indicates that there is no more variation from lot to lot than corresponds, statistically, to the average difference between two consecutive lots. This control chart combines the variations of the production department and the testing laboratory, but the chart still serves a useful purpose in the laboratory as long as all results are within their own control limits and the control limits are within the specification limits (in this case 0 to 0.6% moisture).

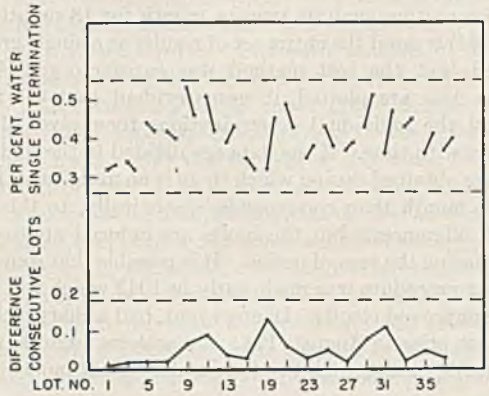

Figure 5. Control Chart for Routine Moisture Determinations on Production Lots of an Organic Solvent

When the routine test results show lack of control, the analyst checks his reagents and then verifies his results on replicate samples from the suspected lot. The use of such a control chart sometimes makes it possible to reduce the amount of routine testing when a production process is not giving trouble. As soon as lack of process control is indicated, the amount of routine testing can be increased immediately.

COMPARING MERITS OF ALTERNATIVE TEST PROCEDURES

It is often desirable to compare the merits of alternative test procedures, and a control chart will usually help to do this in a manner which is both objective and quantitative. Figure 6 shows the results of a comparison between the so-called method of single swings and the more commonly used multiple-swing method for weighing on the analytical balance.

Four individual comparisons of two 1-gram and two 100-gram weights were made on ten different days using the two methods. With one exception, the daily variation (standard deviation) does not fluctuate significantly greater than it should by chance alone and, with four exceptions, the individual weighings are all within their control limits.

No assignable causes were found which would account for the occasional lack of control for individual weighings, but they may be the result of air drafts or faulty manipulation in releasing the beam rests. A control chart for daily averages would indicate some evidence of more variability in the weighings from one day to another than corresponds to the average daily variability. However, there is no evidence that either of the two weighing procedures is superior to the other for weighing loads up to the full capacity of the balance. One important thing to note in favor of the method of single swings is the fact that it requires about one quarter as much time as the multiple-swing method.

Mention has been made of control charts for individual test results and for averages of test results. It is always better, from a statistical point of view, to chart averages rather than the individuals themselves. However, there are many occasions in the analytical laboratory where single observations rather than averages are used or reported. Thus, only rarely would weighings be made in replicate and the average weight used or reported. In such cases the individuals can be charted, although they must be grouped arbitrarily in order to estimate control limits. It is often desirable to chart averages as well as individuals because the chart for averages gives valuable information about the general pattern of a set of test results, even though it is only the individual results which are reported or used.

CONTROLLING ERRORS OF CALIBRATION OF TESTING EQUIPMENT

The calibration of testing equipment is often plagued with the errors of the calibration method itself, and control charts will offer help in such cases. Figure 7 shows the results of calibrating nine burets by weighing the water delivered at a known temperature to the 39-, 40-, and 41-ml. marks. The lowest graph shows the range of three consecutive individual tests made for each mark, and it can be seen that there are no variations from range to range greater than correspond to chance alone, although the upper limit of 0.03 ml. seems high. These range variations were not the result of insufficient drainage time, and they seem to represent real differences in the amount of water held up on the walls of the buret.

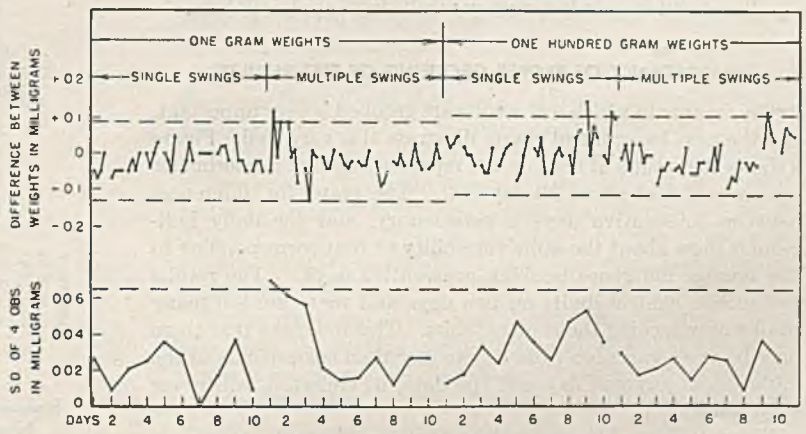

Figure 6. Control Chart for Comparing Weighing Methods

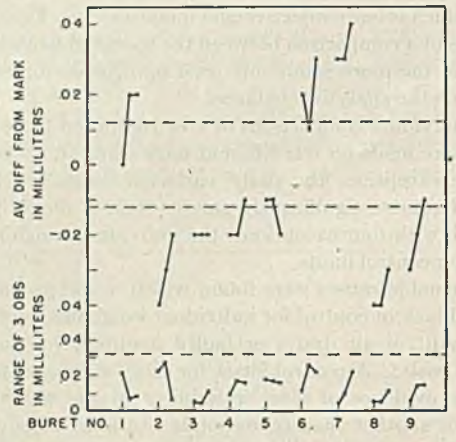

Figure 7. Control Chart for Buret Calibrations

Each buret checked at 39-, 40-, and 41-ml. marks

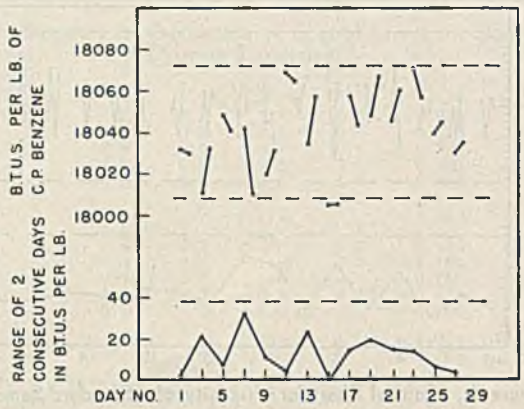

Figure 8. Control Chart for Heat of Combustion of C.P. Benzene

Data of Dean, Williams, and Fisher (3)

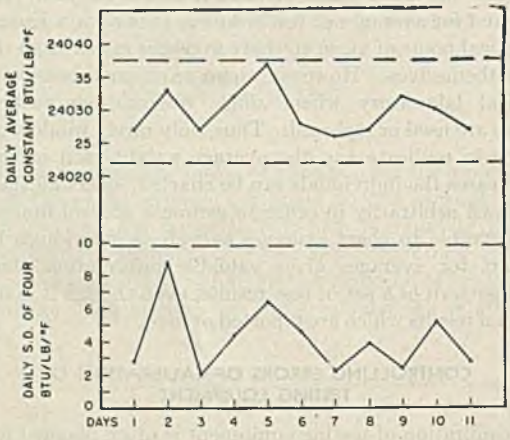

Figure 9. Control Chart for a Calorimeter Constant

The control limits for averaged corrections have been drawn about zero as the mean, in the upper graph, because all corrections would be zero if the burets were perfectly marked. There is more variation among the corrections than corresponds, statistically, to the average range of three consecutive individual calibrations. Therefore, the corrections are applied if they are greater than ±0.01 ml. but no correction is justified if it is ±0.01 ml. or less. In other words, corrections which fall within the control limits based on the variability of the method of calibration are not significant and no gain in accuracy results from applying them.

IMPORTANCE OF PROPER GROUPING OF TEST RESULTS

The manner in which test results are grouped is very important, and the next two control charts illustrate this very well. Figure 8 shows the results of tests on the reproducibility of a calorimeter over a period of about 30 days (*3*). The graph for differences between consecutive days is satisfactory, and the daily individuals show about the same variability as that corresponding to the average difference between consecutive days. The results are outside control limits on two days and there are too many results approaching the control limits. This indicates that there may be more variation from day to day than corresponds to the variation in any one day and the data, as collected, will never show it up.

Figure 9 shows a chart for the calibration of a fuel calorimeter, in which replicate calibrations were made on the same day. Daily standard deviations and averages are again all within their control limits, which, in this case, means that there is no more variation from day to day than corresponds, statistically, to the average variation on any one day. The two charts shown in Figures 8 and 9 illustrate how the same type of experimental work can be made to have different operational meanings merely by carefully choosing the method of grouping the tests.

Another illustration of the importance of picking the proper arbitrary small group is shown in Figure 10. A controlled sample of sodium hydroxide in potassium hydroxide was submitted for routine analysis twice a month for 18 months. The authors (*21*) treated the entire set of results as a single group and concluded that the test method was satisfactory. However, when the data are plotted, it seems evident that the monthly range and the individual determinations themselves show less variability with time. If the data are divided in the middle, two periods are obtained during which there is no more variation from month to month than corresponds, statistically, to the average monthly differences; but the limits are reduced approximately one half during the second period. It is possible that some change in the test procedure was made early in 1942 which will account for these improved results. In any event, had a chart such as this been in use prior to August, 1942, the authors might have found an assignable cause for the two low results of that month.

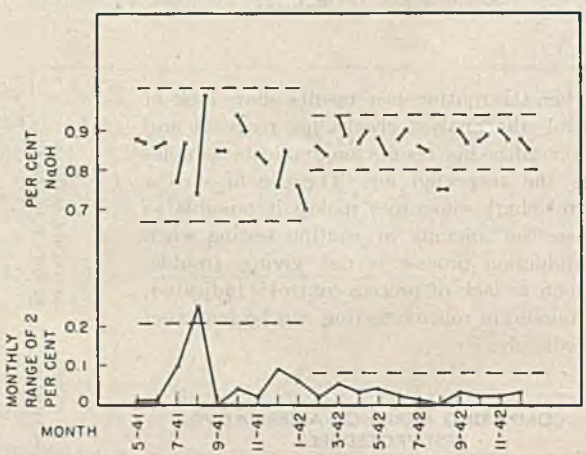

Figure 10. Control Chart for Determination of Sodium Hydroxide in a Potassium Hydroxide Control Sample

Data of Williams and Haines (*21*)

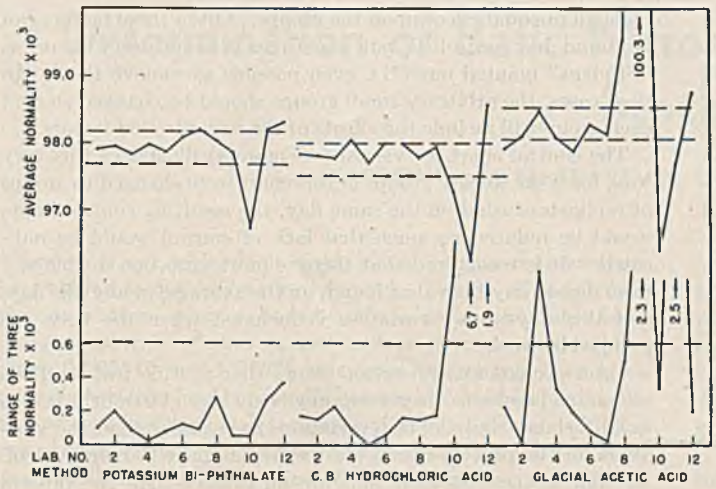

Figure 11. Control Chart for Sodium Hydroxide Standardizations

The application of control chart methods to data already collected often shows that the same amount of experimental work would have yielded a great deal more useful information if it had been planned in a slightly different way.

COMPARISON OF COLLABORATIVE STUDIES OF TEST METHODS

An obvious application of control charts is to the comparison of the results of the collaborative study of test methods, and several examples will be presented to show this. Figure 11 shows the results of such a study of the standardization of 0.1 *N* solutions of sodium hydroxide (*11*). Unknown samples of the solution were standardized in twelve different laboratories by means of three primary standards. The lower graph shows the range of three individual standardizations made at the same time, and the upper graph shows the average of the three. This control chart indicates that there is some uncontrolled factor which affects the ranges of some of the analysts more than corresponds to chance alone, and there is also more variation among the analysts than corresponds, statistically, to the average range of the individual standardizations made by each analyst. Three laboratories are consistently out of control and one laboratory gave bad results when using one of the standards. The cause of these abnormal variations might be in the standardizing procedure, in the controlled sample of sodium hydroxide, in the standardizing materials, or in the analysts, and the control chart should help materially in locating the cause. No conclusions about the relative merits of the three primary standards can be safely drawn until the cause of this lack of control is removed.

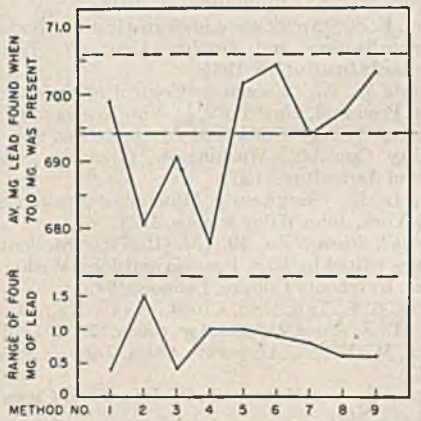

Figure 12. Control Chart for Comparing Various Test Methods for Lead

Data of Lykken (*9*)

The control chart in Figure 12 summarizes data for nine methods of determining lead (*9*). Known solutions containing exactly 70.0 mg. of lead were tested by each method with four replicates at the same time in each test. The methods were all different, five gravimetric and four volumetric. The lower graph shows that the range fluctuations are no greater than correspond to chance alone, although the authors expressed the opinion, based on other considerations, that method 5 showed excessive variation. The control limits for averages were drawn about 70.0 mg. rather than the grand average of all the results because the controlled samples all contained 70.0 mg. of lead. Three of the methods gave excessively low results, based on the average range of the nine tests.

The control chart in Figure 13 summarizes the results of a collaborative study of gravimetric and volumetric methods for determining sulfur in coal (*18*). The sulfur was determined as barium sulfate in both gravimetric methods, but in one case calorimeter bomb washings were used while in the other separate samples of coal were carried through the Eschka method. Bomb washings were used for both volumetric methods but different indicators served to detect the end point of the final sulfate titration with barium chloride.

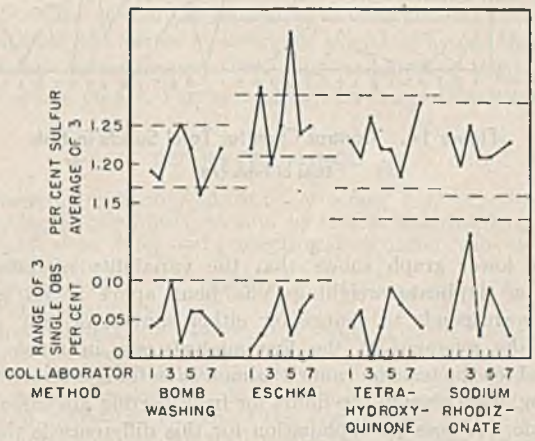

Figure 13. Control Chart for Methods of Determining Sulfur in Coal

Data of Tomkins (*18*)

The lower graph for range of each collaborator's individual determinations shows that the gravimetric methods are appreciably less variable than the volumetric methods and that in both cases the ranges do not show more variation than corresponds to chance alone. One might conclude from this that the volumetric methods are less satisfactory than the gravimetric methods. This is not the case, however, because the upper graph for averages shows that the collaborators cannot agree as well using the gravimetric methods as they do using the volumetric methods.

Unless the cause of this lack of agreement between collaborators using the gravimetric methods can be found and removed it would be better to use the volumetric methods even though they are somewhat less precise. Nothing can be said about the accuracy of the methods because the sulfur content of the controlled samples of coal was not known.

The control chart in Figure 14 shows the results of a collaborative study of three methods for determining the total solids in fish (*19*). Two controlled samples of fish (canned salmon and fresh herring) were tested by five analysts, using a vacuum oven method, a double oven method, in which the samples were heated first to 70° C. and then to 130° C., and a vacuum desiccator method. The weighed samples were dried for arbitrary lengths of time and weighed. The samples were then dried for one additional hour and weighed again.

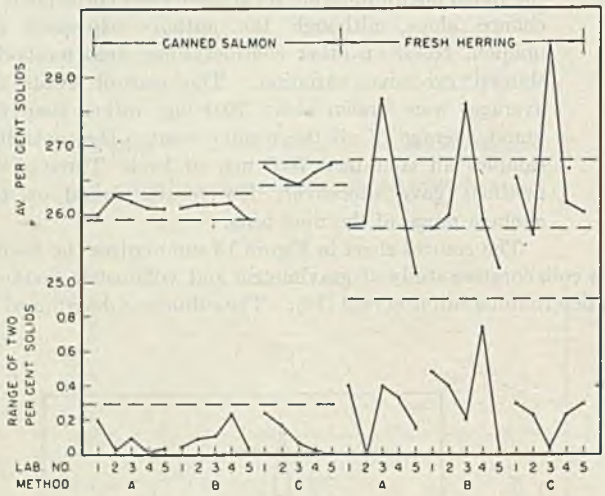

Figure 14. Control Chart for Total Solids in Fish

Data of Tubis (*19*)

The lower graph shows that the variability of differences between duplicate weighings one hour apart is no greater than corresponds to chance for either sample of fish. However, the averages of the five analysts are in much better control for the tests on canned salmon than for the tests on fresh herring, even though the limits for fresh herring are three times as wide. A possible explanation for this difference is that the cooked fish has been broken down so that it requires less drying time than the fresh fish. It seems possible that the results for fresh fish might show satisfactory control if the drying treatment were extended before the first weighing was made.

CONCLUSIONS

Control charts have been found useful in the testing laboratory whenever it is desirable to compare the over-all variability of test data with the average variability of small groups of the data, and they are simpler to understand than the more complicated statistical methods of analysis of variance. Operational meaning can be put into the charts, depending entirely on the manner in which the arbitrary small groups are chosen.

When the small groups consist of replicate tests, made at the same time on the same sample, the resulting control chart subjects the data to the most rigid test that is possible, because most of the uncontrolled factors, which contribute to variations in any test method, affect the groups more than they affect the individual tests within each group.

Test methods which show evidence of control, based on this kind of grouping, may still lack satisfactory precision. In such cases, it is obviously necessary to control more of the factors which contribute to individual test variations within the small groups.

Test methods which show lack of control, based on this kind of grouping, are being influenced by uncontrolled factors which have an unequal effect upon the groups. Often these factors can be found and controlled, but sometimes it is not desirable or, as "Student" pointed out (*17*), even possible to remove them. In such cases, the arbitrary small groups should be changed so that each group will include the effects of the uncontrolled factors.

The control chart for viscosity (Figure 4) illustrates this very well, for if the weekly groups in this chart were changed to groups of replicates tested on the same day, the resulting control limits would be reduced so much that lack of control would be indicated. It is recognized that there is more variation in this test from day to day than that found, on the average, in any one day, and this day-to-day variation is included when the tests are grouped by weeks.

Control chart analysis often shows that routine test methods are not so precise as they were previously thought to be. It also usually shows that the test variations are smaller than the variations in the process which the test is designed to help control. As long as the test variations are no greater than the process variation, replicate testing of single samples may sometimes be uneconomical because the additional evidence of the replicates does not materially help in controlling the process. In such cases, it may be better to replicate the samples in such a manner that more control of the process results, and then test each sample only once.

Only a few of the many applications of control charts have been presented here, but it is safe to say that their use in the analytical laboratory is limited only by the ingenuity of the analyst.

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Determination of Basic Nitrogen in Hydrocarbon Feed Stocks

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Ammonia is not retained when very dilute gaseous concentrations are bubbled through dilute sulfuric acid. Storage of such dilute gaseous mixtures in iron cylinders results in loss of appreciable basic nitrogen content. Ammonia and amines in concentrations between 0.000015 and 0.008% calculated as ammonia have been successfully determined in normally gaseous hydrocarbon feed stocks by a new method that involves passage of a cooled (about −60° C.) hydrocarbon sample into a substantially nonaqueous standard solution of sulfuric acid, evaporation of the hydrocarbon, and titration of the excess acid with standard alkali. About 4 hours are required for a 400-gram sample.

TRACE impurities in hydrocarbon gases have a serious and harmful effect upon certain catalytic processes. The elimination of such impurities and control of their concentration are dependent upon suitable analytical methods.

Methods for determining minute quantities of water (*1*) and oxygen (*2*) in such gases have been described and the present paper describes a suitable method for ammonia or other basic nitrogen compounds.

In the investigation of methods for determining basic nitrogen, two general types of procedure were considered for transferring the basic compounds from the hydrocarbon to the aqueous media in which they subsequently could be determined by conventional methods. Both methods involved aqueous acid extraction; in one the sample was treated in the gaseous phase and in the other, in naphtha solution.

Where the sample was treated in the gaseous phase, fritted-glass scrubbers containing dilute sulfuric acid failed to retain small concentrations of ammonia. In a run on 70 grams of hydrocarbon sample, in which six such scrubbers in series were used, the first showed 0.00005% of ammonia and the sixth 0.00004%. In almost every test the second scrubber collected practically as much base as the first in the series.

Treatment of a sample in naphtha solution involved diluting a liquefied sample with naphtha, from which basic compounds were extracted by dilute sulfuric acid using ordinary separatory funnels. Good results were obtained on C_4 hydrocarbon samples but when appreciable quantities of C_3 or lower boiling hydrocarbons were present the manipulations were hazardous and impractical because of the pressures involved.

The procedure which proved satisfactory involves condensation of the hydrocarbon sample into a substantially nonaqueous standard solution of sulfuric acid in acetone, evaporation of the hydrocarbon, and titration of the excess acid to methyl red indicator. The condensation is effected by a dry ice–acetone bath (at about −60° C.); constituents not retained at this temperature are measured on emergence from the condensation flask. Though the lower boiling hydrocarbons are not completely condensed, their basic constituents are quantitatively retained in the nonaqueous acid through which they pass. Sample size is determined from the sum of the condensed and uncondensed portions of the sample, the former by weighing, the latter by gas metering and gravity determination. The time required to complete a determination on a 400-gram sample is not more than 4 hours.

DETAILS OF METHOD

REAGENTS. Sodium hydroxide, aqueous, 0.02 *N*. Prepare by diluting clarified 50% sodium hydroxide solution with carbon dioxide–free water and protecting from carbon dioxide with a soda-lime tube.

Sulfuric acid, 0.02 *N*. Prepare by diluting aqueous 4 *N* sulfuric acid with acetone.

Methyl red indicator, a saturated solution of the free acid in 0.1 *N* aqueous sodium hydroxide.

PROCEDURE. Half fill the condensing bath with acetone and in it carefully immerse a perforated can containing small pieces of dry ice. Acetone will enter the can through its perforated bottom and rapidly dissipate the dry ice. Remove the empty can, refill with dry ice, and again immerse in the acetone bath, repeating the operations until the effervescence, which is violent at first, practically ceases. Then add several pieces of dry ice directly to the bath to maintain its temperature at about −60° C.

Pipet 20 ml. of 0.02 *N* solution of sulfuric acid in acetone into the dry condensing flask and a like portion into a 250-ml. Erlenmeyer flask containing 50 ml. of water; the latter serving as blank is reserved until the sample is titrated, when it also is titrated.

Stopper the condensing flask with the one-hole glass plug and carefully dip it into the cold acetone bath until all the coils of the flask are immersed. Remove the flask from the bath, quickly wipe the bottom, set upon a cork ring on a pan balance, weigh rapidly within 1 gram, and record as tare weight.

Reimmerse the flask in the cold bath and assemble the apparatus as shown in Figure 1. The assembly may be mounted on a suitable rack for convenience in carrying to and from sampling points.

Completely fill the gas-sampling pipet with water, as well as the lower arm of the tee to which it is attached. This may be conveniently accomplished by upward flow. Close stopcock S_4 and leave stopcock S_5 open to the water reservoir.

Introduce the sample through the Ascarite scrubber, and by manipulating stopcock S_2 bypass the condensing flask for a few moments to flush the tubing through stopcock S_3. A rate of

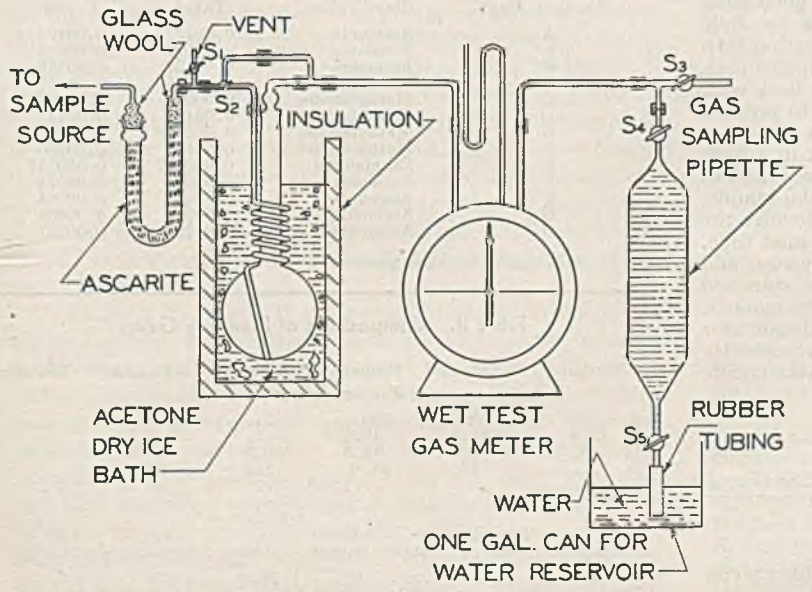

Figure 1. Sample Collection Unit

passage as high as 170 liters (6 cubic feet) per hour as indicated by the gas meter has been found to yield good results. Open vent S_1, and record the initial reading of the meter, its temperature, and the barometric pressure. Readjust stopcock S_2, close S_1, and start introduction of the sample into the flask. Add pieces of dry ice as required to the condensing bath to maintain its temperature at about −60° C. If the sample is completely retained in the condensing flask, the meter will, of course, indicate no gas passage. If uncondensed gases are present, take a sample for gravity determination after about 0.2 cu. foot (5663 cc.) have by-passed the sampling pipet and assured purging of air, etc. For a truly representative sample, small portions should be drawn over the entire collecting period after purging, but for most purposes the sample may all be collected at one time. Regulate stopcocks S_3 and S_4, so that substantially atmospheric pressure is indicated on the manometer with which the gas meter is equipped. When approximately 1 liter of gas has been collected, detach sampling pipet and determine the specific gravity of the gas against air.

When sufficient sample has been taken, stop its flow, detach the condensing flask, and weigh rapidly as before. In most cases a 400-gram sample is adequate, but when extremely low concentrations are involved, the sample should be increased. Record the new weight and meter reading.

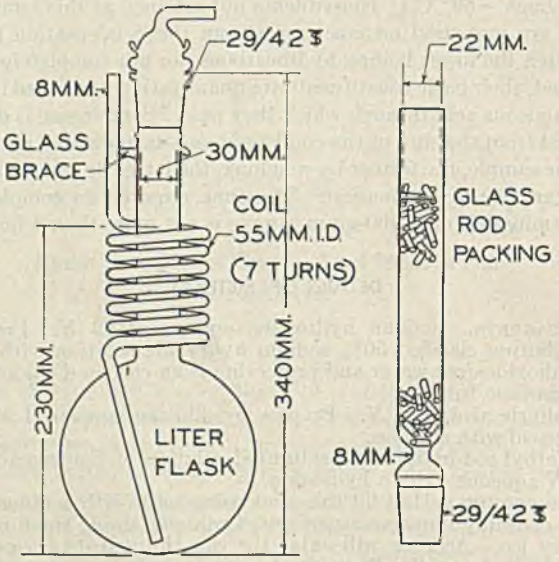

Figure 2. Glass Condensing Flask-Evaporation Trap

Gently swirl the flask and set it on a cork ring in a good hood for evaporation of its hydrocarbons. Remove the one-hole plug from its neck. Pour water through the evaporation trap shown in Figure 2, drain a few moments, and insert in the neck of the flask. Close the inlet tube of the condensing flask with a short piece of rubber tubing fitted with a glass plug to prevent ejecting liquid during the subsequent evaporation step.

To hasten evaporation carefully immerse the flask in a bath of water at about 60° C. Less than 45 minutes are required to evaporate the hydrocarbons. Upon evaporation of the sample, add 50 ml. of water to the flask through the trap. Remove the trap as well as the rubber tubing and plug from the inlet tube. Rinse down the coil or inlet tube of the flask with water, add methyl red indicator to the flask, and titrate with standard 0.02 N sodium hydroxide. Titrate the blank in a like manner.

CALCULATIONS. *Noncondensed Gas.* (The calculation for noncondensed gas is generally sufficient. However, to be strictly correct, the volume of noncondensed gas, V, and its specific gravity, G, should be calculated to a dry basis.)

$$\text{Noncondensed gas (grams)} = \frac{V \times 28.32 \times G \times 1.223 \times 288.6 \times P}{T \times 760}$$

where V = cubic feet measured at meter
G = specific gravity of noncondensed gas (specific gravity of air = 1.0000)
T = average meter temperature, degrees absolute
P = barometric pressure, mm. of mercury
28.32 = liters per cubic foot
1.223 = weight in grams of 1 liter of air at 15.6° C. and 760 mm. of mercury

This may be simplified to:

$$\text{Noncondensed gas (grams)} = \frac{13.15 \times V \times G \times P}{T}$$

Per Cent Basic Nitrogen.

$$\%\ \text{basic nitrogen (calcd. as } NH_3) = \frac{(A - B) \times N \times 1.7}{\text{grams of sample}}$$

where A = ml. of sodium hydroxide required to titrate the blank
B = ml. of sodium hydroxide required to titrate the sample
N = normality of sodium hydroxide
Grams of sample = grams of noncondensed gas plus grams of condensed gas

DISCUSSION

The accuracy of this method is indicated by the typical values shown in Table I. Concentrations of ammonia or amine as low as 0.000015% and as high as 0.008%, calculated as ammonia, have been successfully determined. It is apparent that as the concentration decreases to a minimum, a small titration error will contribute to a large percentage error.

Ammonia, methylamine, dimethylamine, trimethylamine, ethylamine, isopropylamine, and methylethylamine, ranging in boiling points from −33° to +35° C., were all used to prepare known samples for analysis. The quality of the results was unaffected by the nature of the base employed.

In preparing the samples, ammonia and methylamine were measured gasometrically in small sample pipets under controlled conditions of temperature and pressure, and then flushed out of the measuring pipets with the hydrocarbon gas concerned.

Samples containing the other nitrogen bases were prepared by adding standard alcoholic or pentane solutions of the base to liquefied hydrocarbons.

Acetone is used to dilute the aqueous 4 N sulfuric acid. The initial dilution is made with water because it is expected that

Table I. Typical Data Obtained with This Method

Blending Gas[a]	Base Taken	Base Calculated as Ammonia, % Taken	Found
A	Ammonia	0.00123	0.00123
B	Ammonia	0.00022	0.00022
B	Ammonia	0.00127	0.00129
C	Ammonia	0.00125	0.00127
C	Methylamine	0.00097	0.00097
D	Methylamine	0.00147	0.00147
E	Methylamine	0.000016	0.000024
E	Methylamine	0.00120	0.00120
E	Ethylamine	0.000137	0.000137
F	Ammonia	0.00073	0.00074
F	Ammonia	0.00004	0.00004
G	Ammonia	0.00003	0.00004
G	Ammonia	0.00223	0.00227

[a] Composition of blending gases shown in Table II.

Table II. Composition of Blending Gases

	Methane	Ethane	Propane	Iso-butane	*n*-Butane	Heavier
			Per cent by volume			
A	...	...	100	...	...	...
B	12.4	37.1	49.0	...	1.5	...
C	...	8.4	44.5	0.5	45.8	0.8
D	...	14.6	41.0	1.9	42.5	...
E	...	...	...	...	100	...

	Ethylene	Propylene	*n*-Butane	Iso-butane	1-Butylene Iso-butylene	Iso-pentane	1,3-Butadiene
F	1.6	3.3	...	69.7	22.9	2.5	...
G	...	...	53.5	...	...	...	46.5

neutralization reactions will proceed with greatest ease in the presence of water. Acetone is used, because it is a solvent for both liquefied hydrocarbons and water, apparently has no effect upon the standard acid, and lowers the freezing point of the solution to below −60° C. The titer of 0.02 *N* sulfuric acid in acetone dropped less than 1% in 144 hours. Other solvents, ketones and alcohols, were not so satisfactory for one or more reasons involving high freezing point, poor miscibility, or instability of the titer of the standard acid prepared with them.

It was found that samples cannot be kept or weighed in iron containers, though free of brass valves and fittings, since reaction or adsorption occurs, reducing the base content of the hydrocarbon. Solutions of ammonia in *n*-butane, kept in iron cylinders, changed from an initially determined value of 0.00081% to lower and lower values until after 16 hours' storage only 0.0001% remained. Solutions of methylamine in *n*-butane, similarly stored, changed from 0.00149 to 0.00103% in 16 hours. Similar solutions prepared with these bases, as well as with bases up to and including isopropylamine, but in pressure glass tubing, retained their initial concentrations after storage, demonstrating that loss of base was not due to reaction between it and the hydrocarbon solvent. The Florence flask, equipped with condensing coil, eliminates the need for metal sample containers.

ACKNOWLEDGMENT

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Use of High-Frequency Oscillators in Titrations and Analyses

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Energy absorption from high-frequency fields forms the basis for a new method of conductometric analyses. Distinct advantages are that this method eliminates the use of electrodes, and that values may be read directly on a meter. It is very flexible and highly sensitive. Any changes in ionic or dipole content in ionized or un-ionized solutions may be followed. The electrical circuit is described and a variety of types of titrations are presented.

ELECTRICAL methods for titrations and analyses are generally limited in their scope and application by the necessity of using electrodes of various types. Galvanic methods usually are restricted to ionized solutions and ordinarily are not usable in organic liquids, particularly if the organic liquid is a very poor ionizing medium. Conductometric methods have a wide field of application but frequent readjustments are usually required. By using the field of a high-frequency oscillator it is possible to produce ionic or dipole motion without the introduction of electrodes. The energy required to produce this motion causes a change in the oscillator current which is easily measured. Thus when an oscillator is loaded by the introduction of a liquid or a solution into its tank circuit, its characteristics are altered. Chiefly three factors govern the magnitude of the loading: the type and circuit constants of the oscillator, the volume of the solution and its location within the tank circuit, and the conductance of the solution due to its ionic or dipole concentration. The changes in conductance during a reaction cause variations in loading, whereby the course of the reaction can be followed.

Oscillators differ widely in the manner in which they load. In Figure 1 are shown types of loading curves which can be obtained from various oscillators or even from the same oscillator by changing its frequency or its circuit constants. Conditions which produce linear curves of steep slope are desirable, since oscillators producing such curves show high and uniform sensitivity throughout their loading range. However, oscillators with such high sensitivity also load out of oscillation readily. Therefore provision for restoring oscillation is highly essential. A study of the characteristics of various types of oscillators (*5*) indicated that the tuned-plate tuned-grid oscillator was the most adaptable to analytical work, since it could be made highly sensitive and yet maintain reasonable stability, and could be readily brought back into oscillation when necessary.

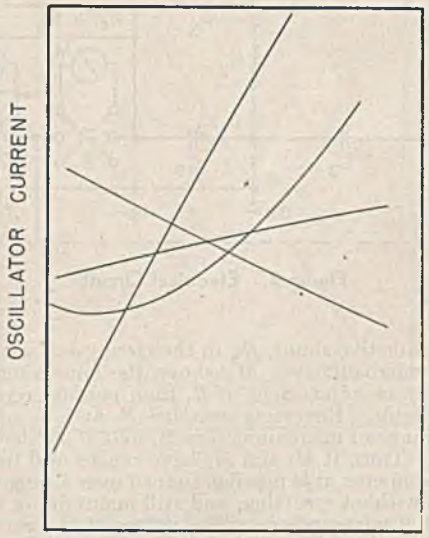

Figure 1. Typical Loading Curves

ELECTRICAL CIRCUIT

In Figure 2 is shown the electrical circuit of a tuned-plate tuned-grid oscillator with a sensitive metering system in the positive power-supply lead. A regulated power source furnishes high voltage. Oscillations occur when the two tank circuits, L_1C_1 and L_2C_2, are resonant at approximately the same frequency. The condenser, C_1, is the primary factor in determining the frequency of oscillation, while condenser C_2 controls the excitation to the grid of tube *A*. Any triode vacuum tube having good oscillating characteristics may be used. Current flowing through resistance R_1 produces the grid-bias voltage. The trimmer condenser, C_3, by-passes high-frequency energy around R_1 and is adjusted to give maximum sensitivity to loading in the plate tank circuit. Introduction of the glass tube, *T*, containing the solution into coil L_1 accomplishes the loading. Condensers C_4 to C_9, inclusive, and choke coil L_3 reduce stray high-frequency

currents and eliminate body-capacity effects, thus stabilizing the circuit.

The oscillator current is read on milliammeter M_3, which also assists in determining the manner in which the circuit goes in or out of oscillation. By adjustment of resistance R_4 in the series R_3-R_4-R_5, the voltage across resistance R_2 can be balanced by an equal and opposite voltage across R_3 and a portion of R_4.

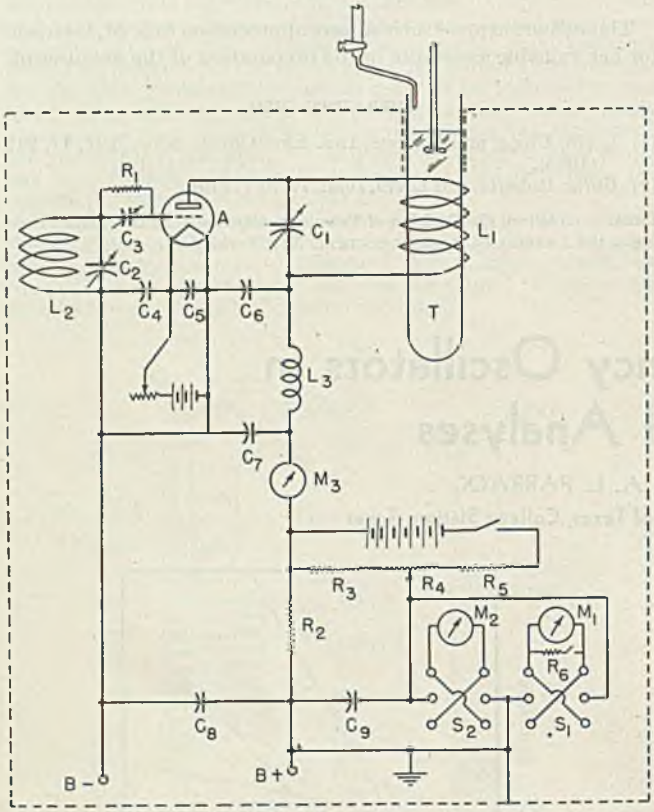

Figure 2. Electrical Circuit

With the protective shunt, R_6, in the circuit and with switch S_1 closed, the microammeter, M_1, shows the approximate state of balance. Closer adjustment of R_4 then permits removal of R_6 from the circuit. Reversing switches S_1 and S_2 serves to increase the range of microammeters M_1 and M_2 without reducing sensitivity. Thus, if M_1 and M_2 have ranges of 0 to 200 and 0 to 50 microamperes, it is possible to read over a range of 400 microamperes without resetting, and still maintain an accuracy in reading of 0.2 microampere. The values of the various circuit constants are not highly critical. The following will give good results.

R_1. Resistance, 40,000 ohms
R_2. Resistance, 1,000 ohms
R_3, R_5. Resistance, 10,000 ohms
R_4. Wire-wound potentiometer, 10,000 ohms
C_1, C_2. Variable condenser, 100 micromicrofarads
C_3. Adjustable mica condenser, 100 micromicrofarads
C_4–C_9. Mica condenser, 0.01 microfarad
S_1, S_2. Double-pole double-throw anticapacity switch
M_1. Microammeter, 0 to 200 microamperes
M_2. Microammeter, 0 to 50 microamperes
M_3. Milliammeter, 0 to 150 milliamperes
L_1, L_2. Coil, 4 turns of No. 6 copper wire, spaced 1.5 inches; coil diameter, 2.25 inches
L_3. Radio frequency choke, 2.5 millihenries
A. Vacuum tube, type S01

High sensitivity is obtainable by increasing the size of R_2 up to certain limits, or by adjusting C_2 and C_3 to such values that tuning with C_1 causes the circuit to go in or out of oscillation with extreme rapidity. As sensitivity is increased a slow drift in the meter reading frequently becomes apparent. Good shielding and grounding are essential. The shield around T fits snugly

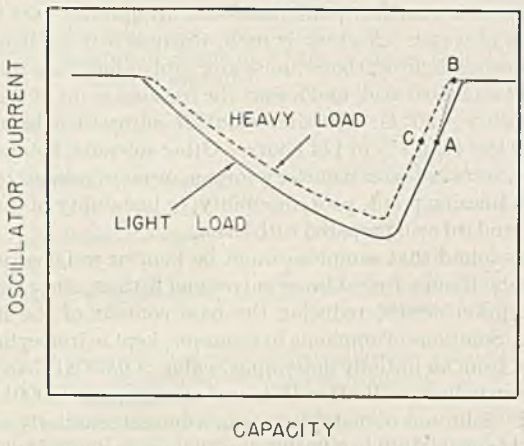

Figure 3. Variation of Oscillator Current with Tuning of C_1

and extends well below the liquid level inside the tube. The length of this shield is determined experimentally and is adjusted so that changes in the liquid volume during analysis will have no effect upon the loading.

The choice of frequency is not critical, except that an oscillator of a given design may have maximum sensitivity at a certain frequency. Frequencies in the range of 15 to 20 megacycles give satisfactory results. At higher frequencies the control of stray currents is more difficult, while coils and condensers tend to become excessively large at lower frequencies. Figure 3 illustrates the manner in which a tuned-plate tuned-grid oscillator responds to tuning by C_1. The circuit is in oscillation only when the two tank circuits are resonant at approximately the same frequency. Increase in loading during titration causes a change in the impedance and in the resonant frequency of the plate tank circuit. However, its original resonant frequency can be restored by changing the capacitance in the plate tank circuit. Thus, with C_1 set to give good loading characteristics as at A, a sufficient

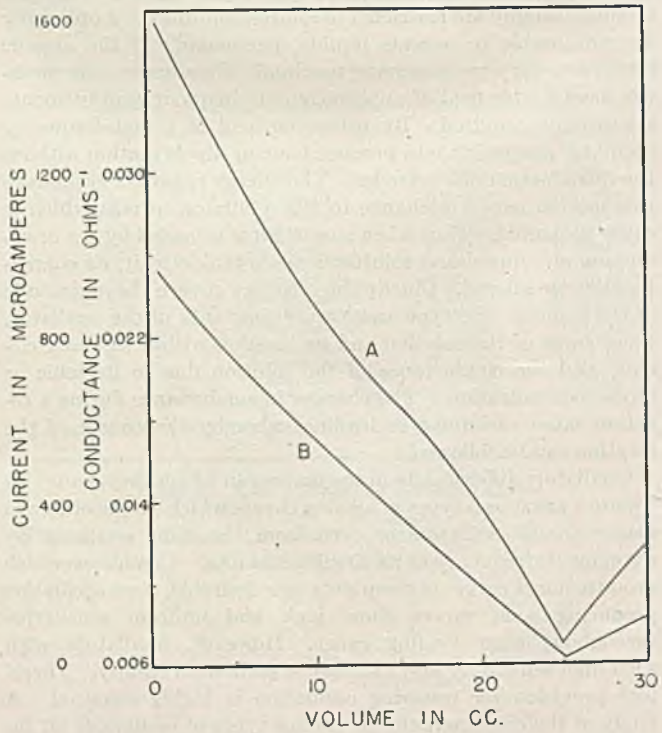

Figure 4. Titration of Hydrochloric Acid with Sodium Hydroxide

A. High-frequency method. *B*. Conductance method

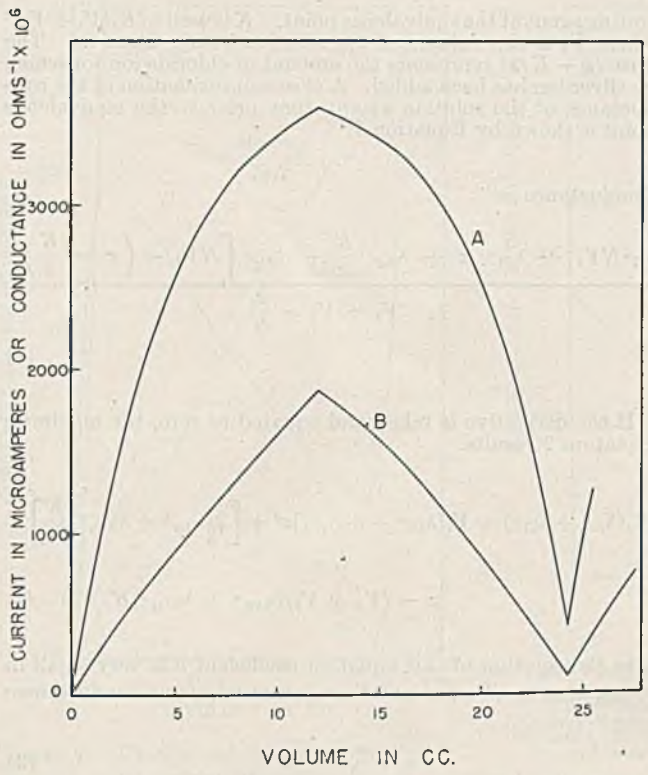

Figure 5. Titration of o-Phthalic Acid in Acetone with Sodium Methylate in Methanol

A. High-frequency method. *B.* Conductance method

increase in loading during titration may cause the circuit to go out of oscillation as at *B*. Then by simply retuning with C_1, the circuit can readily be brought back into oscillation. Since the two loading curves shown on Figure 3 are nearly parallel up to the point where the circuit begins to go out of oscillation, the slopes of the titration curves are not materially changed by retuning with C_1 in this manner, particularly if the retuning is done before the circuit goes out of oscillation. In actual practice C_1 can be very conveniently used as a means of resetting when the current increases or decreases beyond the range of the microammeters. Minor adjustments can be made with R_4. With this technique no observable changes in the slopes of the titration curves are produced by resetting.

TYPICAL ANALYSES

In carrying out a titration the solution in tube *T* is suspended within coil L_1 and the motor stirrer is started. By means of C_1, and also C_3 if necessary, the circuit is adjusted so that it goes into and out of oscillation with the desired sensitivity as observed on M_3. Balance on meters M_1 and M_2 is obtained by adjustment of R_4. If the temperature of the solution differs from that of the interior of the system, a slow drift of the meter needle is apparent until thermal equilibrium is reached. Ordinarily this effect can be neglected in titration.

In the various types of titrations, illustrated in Figures 4 to 9, 25 cc. of solution in the order of 0.1 *N* were diluted with 100 cc. of water and were titrated with solutions also in the order of 0.1 *N*. End points appear as changes in the slopes of the curves. If the disappearing ion has a higher conductance than the ion replacing it, the excess of the titrating agent beyond the end point produces a reversal in the curve. Comparison is made between this method and the usual conductance method on Figures 4 and 5. The scale for the microampere ordinate is greatly reduced for comparison purposes. In cases such as the titration of acidified ferrous ammonium sulfate solution with potassium permanganate solution, addition of the titrating agent beyond the end point actually dilutes the solution and lowers its conductance, since the solution contains a relatively high concentration of sulfuric acid. Thus as seen from curve *A* on Figure 8, the end point appears but no reversal takes place. If, however, the conductance of the potassium permanganate solution is increased by the addition of inert potassium sulfate, the typical reversal occurs at the end point as shown by curve *B*. Jander and his co-workers (*1*, *3*, *4*), in their work on conductometric titrations in solutions of high foreign salt content, add the concentrated titrating agent from a specially constructed microburet in order to avoid large volume changes. However, the sensitivity of the high-frequency method is sufficiently great, so that adding relatively large amounts of inert salt with the titrating agent does not destroy its accuracy.

Titrations in organic solvents or mixtures of organic solvents frequently present interesting problems. Since the degree of ionization is usually low, loading due to rotation of dipoles may constitute an appreciable part of the total effect. Furthermore, if the titration involves more than one solvent, changes in the

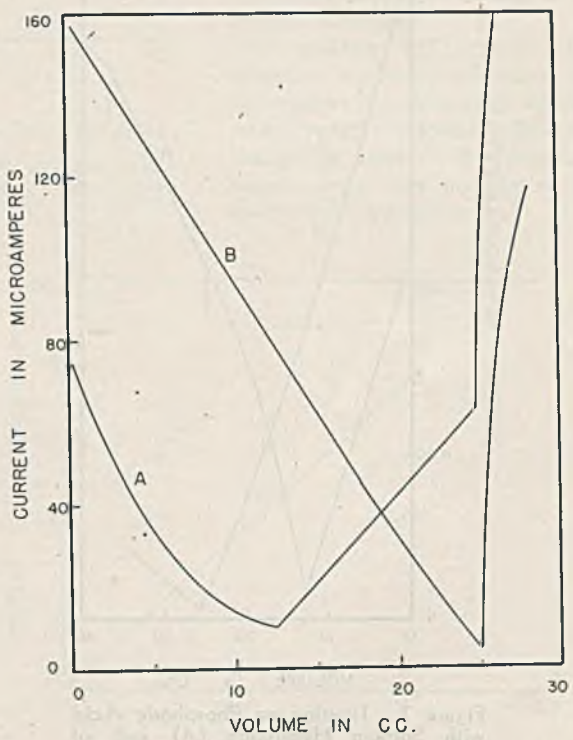

Figure 6. Titration of Sodium Carbonate with Hydrochloric Acid (*A*) and of Potassium Chloride with Silver Nitrate (*B*)

degree of ionization and in the dipole content during titration materially affect the slope of the curve. The end point on curve *B*, Figure 9, is completely masked, owing to the increase in the number of methanol molecules which are more polar than the acetone molecules, and the increase in the solubility and the degree of ionization of the sodium benzoate as methanol progressively enters the solution. When the sodium methylate is made up in benzene with only sufficient methanol to maintain solubility, a sharp end point is obtained as seen by curve *C*. Initially, conductance increases until the limit of solubility of the sodium benzoate is reached, and thereafter decreases because of the removal of benzoic acid as insoluble sodium benzoate. This suggests a possible method for the determination of solubilities.

Results of these titrations are listed in Table I. The known end point values are those obtained by standard volumetric methods or calculated from the known strengths of the solutions. The observed end point values are obtained by use of the oscillator.

Table I. Comparison of Known and Observed Values

Substance Titrated	Titrating Agent	Known End Point, Cc.	Observed End Point, Cc.
H_3PO_4	NaOH	25.25	12.66 25.20 37.87
Na_2CO_3	HCl	12.20	12.18 24.40
$FeSO_4(NH_4)_2SO_4$	$KMnO_4$	25.00	24.98
$FeSO_4(NH_4)_2SO_4$	$KMnO_4$–K_2SO_4	25.04	25.03
$Na_2C_2O_4$	$KMnO_4$–K_2SO_4	25.13	25.10
KCl	$AgNO_3$	24.92	24.90
C_6H_5COOH in acetone	CH_3ONa in benzene	23.98	24.00
HCl	Na_2CO_3	25.50	25.50
$C_6H_4(COOH)_2$ in acetone	CH_3ONa in MeOH	24.00	12.00 24.00

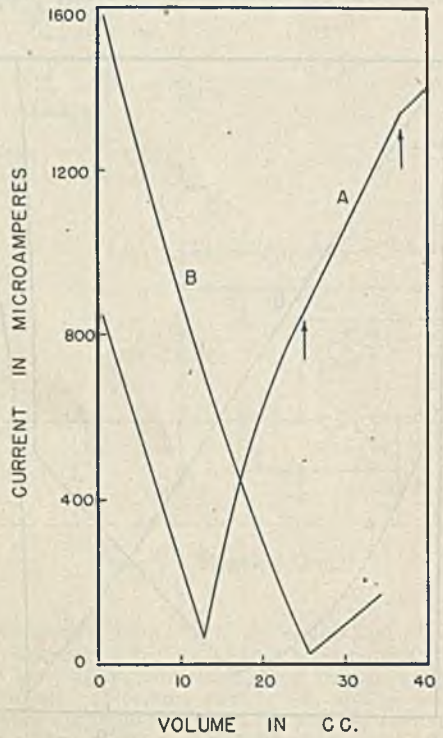

Figure 7. Titration on Phosphoric Acid with Sodium Hydroxide (*A*) and of Hydrochloric Acid with Sodium Carbonate (*B*)

DILUTE SOLUTIONS

Adjustment of C_2 and C_3 and tuning with C_1 can cause the circuit to go in or out of oscillation with such extreme rapidity that even slight changes in conductance produce relatively large changes in the oscillator current. Curve *A*, Figure 10, shows the titration of 25.00 cc. of 0.01000 *N* potassium chloride solution, diluted to 300 cc., with 0.01000 *N* silver nitrate solution. The minimum conductance point at 23.90 cc. does not coincide with the true equivalence point of 25.00 cc. for several reasons. The silver chloride becomes more soluble as the equivalence point is approached and dilution of ions takes place as titration proceeds. However, a simple approximate correction can be made in the following manner:

If x is the number of gram-ions of chloride ion present at any time, then K/x, NV_1, and $NV_1 - (x - K/x)$ are the amounts of silver, potassium, and nitrate ions, respectively, where N is the normality of the titrating agent, and V_1 is the volume of the titrating agent at the equivalence point. K is used as $K_s(V_1 + V_2)^2$ where V_2 is the volume of the solution before titration. The term $(x - K/x)$ represents the amount of chloride ion for which no silver ion has been added. A close approximation of the conductance of the solution at any time prior to the equivalence point is shown by Equation 1.

$$\text{Conductance} = \frac{\lambda_{K^+}NV_1 + \lambda_{Cl^-}\,x + \lambda_{Ag^+}\frac{K}{x} + \lambda_{NO_3^-}\left[NV_1 - \left(x - \frac{K}{x}\right)\right]}{V_1 + V_2 - \frac{x}{N}} \quad (1)$$

If the derivative is taken and equated to zero, the quadratic Equation 2 results.

$$[V_1(\lambda_{K^+} + \lambda_{Cl^-}) + V_2(\lambda_{Cl^-} - \lambda_{NO_3^-})]x^2 + \left[2(\lambda_{Ag^+} + \lambda_{NO_3^-})\frac{K}{N}\right] \times$$

$$x - (V_1 + V_2)(\lambda_{Ag^+} + \lambda_{NO_3^-})K = 0 \quad (2)$$

In the solution of this equation coefficient b is very small in comparison to $\sqrt{-4ac}$ and therefore at minimum conductance:

$$x = \sqrt{-c/a} \quad (3)$$

or

$$x = \sqrt{\frac{(V_1 + V_2)^3(\lambda_{Ag^+} + \lambda_{NO_3^-})K_s}{V_1(\lambda_{K^+} + \lambda_{Cl^-}) + V_2(\lambda_{Cl^-} - \lambda_{NO_3^-})}} \quad (4)$$

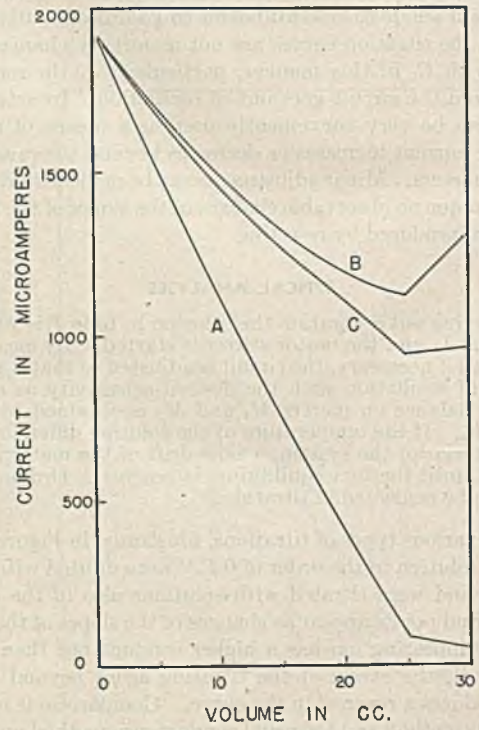

Figure 8. Effect of High Ion Concentration

A. Titration of 0.1 *N* ferrous ammonium sulfate with 0.1 *N* potassium permanganate
B. Titration of 0.1 *N* ferrous ammonium sulfate with 0.1 *N* potassium permanganate and 0.4 *N* potassium sulfate
C. Titration of 0.1 *N* sodium oxalate with 0.1 *N* potassium permanganate and 0.5 *N* potassium sulfate

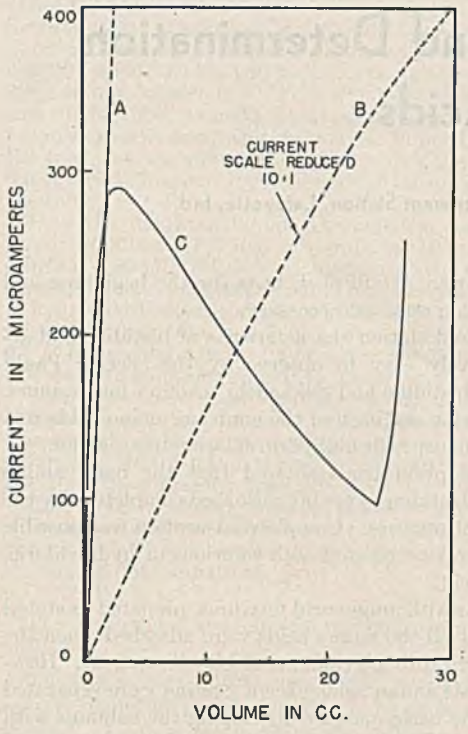

Figure 9. Titration of Benzoic Acid in Acetone

A, B. With sodium methylate in methanol
C. With sodium methylate in benzene

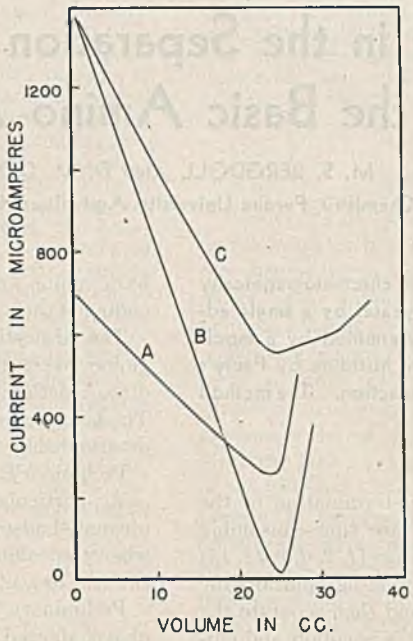

Figure 10. Titrations in Dilute Solutions

A. 0.01 *N* potassium chloride with silver nitrate
B. 0.01 *N* potassium chloride with silver acetate
C. 0.001 *N* hydrochloric acid with sodium hydroxide

Figure 11 illustrates another type of analysis which may be performed readily by this method. The curve shows the variation in oscillator current as the concentration of hydrogen chloride in dry benzene is changed. Since a change in hydrogen chloride concentration from 0 to 0.275% by weight produces a change of 100 microamperes, it is possible to read a change in hydrogen chloride content in the order of 0.0006% by weight.

To some extent temperature changes affect the oscillator current. With an increase in temperature the ionic conductances become greater, causing an increase in the current. The temperature effect on the dielectric constant causes a change in the opposite direction.

In addition to titrations, this simplified conductance method can be applied for following reaction rates, control work, etc. Wherever changes in ionic or dipole conductances occur, they may be readily observed by this means.

MacInnes, Shedlovsky, and Longsworth (*6*) give the ionic conductances at 25° C. of potassium, chloride, silver, and nitrate ions at 73.50, 76.32, 61.90, and 71.42 reciprocal ohms, respectively. The value of K_s for silver chloride is calculated as 1.7×10^{-10} from its solubility (*2*) at 25° C. Using these values in Equation 4 and replacing V_1 by its approximate equivalent of 0.0239 liter (the volume at minimum conductance), the amount of chloride ion at minimum conductance is 1.24×10^{-5} gram-ion. K/x is 0.15×10^{-5} gram-ion and $(x - K/x)$ is 1.09×10^{-5} gram-ion. This is equivalent to 1.09 cc. of 0.01000 *N* silver nitrate solution. Addition of this correction to the 23.90 cc. gives an end point of 24.99 cc.

In Equation 4 the term $(\lambda_{Cl^-} - \lambda_{NO_3^-})$ appearing in the denominator represents the difference in the ionic conductances between the disappearing ion and the ion replacing it. If instead of the nitrate ion, the acetate ion, which has a conductance 40.87 reciprocal ohms (*6*), is used, this difference becomes greater, thereby decreasing the value of x. On curve *B*, Figure 10, the minimum conductance point is much sharper and appears at 24.68 cc. when silver acetate is used. Calculating the correction from Equation 4 but replacing the ionic conductance of the nitrate ion with that of the acetate ion, the end point is found as 25.05 cc.

It can be seen in Equation 4 that small values of K_s, as well as large differences in conductance between the disappearing ion and the ion replacing it, will produce small corrections. In acid-base titrations the hydrogen ion with a conductance of 349.72 reciprocal ohms (*6*) disappears and is replaced by a positive ion of much lower conductance. Also, since the ion product constant is 1.0×10^{-14}, the corrections will become very small or negligible. As shown on curve *C*, Figure 10, the titration of 25.00 cc. of 0.000996 *N* hydrochloric acid solution in 300 cc. of redistilled water with 0.001003 *N* sodium hydroxide solution produces a minimum conductance point at 24.80 cc. The known end point is 24.83 cc. The correction of 0.003 cc. is negligible. The change in the slope of the curve at 32 cc. is due to titration of carbon dioxide which has dissolved during titration. Its position varies with the time used for titration.

Cases may occur in which the disappearing ion has a lower conductance than the replacing ion. Then, since the curve has a positive slope throughout, no minimum point is produced and Equation 4 does not apply. However, the excess of titrating agent entering the solution as the end point is passed causes a break in the curve and no correction is necessary.

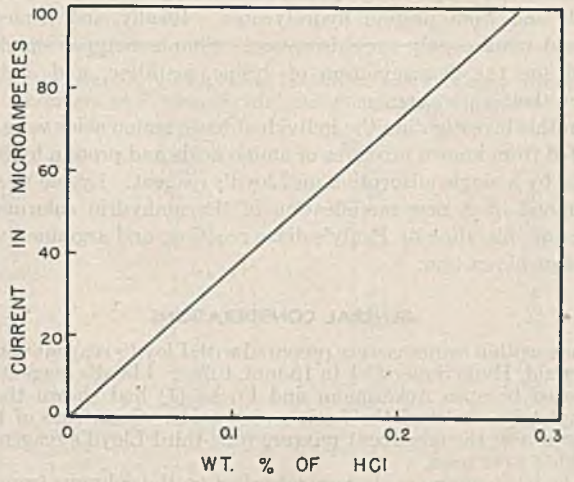

Figure 11. Determination of Hydrogen Chloride in Benzene

ACKNOWLEDGMENT

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Chromatography in the Separation and Determination of the Basic Amino Acids

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The individual basic amino acids were separated chromatographically from amino acid mixtures and protein hydrolyzates by a single adsorption on Lloyd's reagent. Lysine was determined by a modification of the ninhydrin colorimetric reaction, histidine by Pauly's diazo reaction, and arginine by the Sakaguchi reaction. The method is simple, rapid, and reliable.

THE present chemical methods for the determination of the basic amino acids, particularly lysine, are time-consuming and not entirely specific. Previous investigators (*1, 2, 6, 7, 12, 15*) have shown that it is possible to separate chromatographically the basic amino acids from the nonbasic ones and then separate the individual basic amino acids by subsequent adsorption and elution procedures. Very little work was done with protein hydrolyzates, but the results indicated that chromatography could be applied successfully to them.

The purpose of this investigation was to find, if possible, a chromatographic method for complete separation of the basic amino acids, particularly lysine, from known mixtures of amino acids and from protein hydrolyzates. Ideally such a method should require only one adsorption. Simple methods should be used for the determination of lysine, histidine, and arginine after their separation.

In this investigation the individual basic amino acids were separated from known mixtures of amino acids and protein hydrolyzates by a single adsorption on Lloyd's reagent. Lysine was determined by a new modification of the ninhydrin colorimetric reaction, histidine by Pauly's diazo reaction, and arginine by the Sakaguchi reaction.

GENERAL CONSIDERATIONS

Adsorption columns were prepared with Lloyd's reagent and the filter aid, Hyflo Super-Cel, in 19-mm. tubes. Lloyd's reagent was selected because Ackermann and Fuchs (*1*) had shown that it completely adsorbed the basic amino acids. Quantities of 12 to 20 grams of the adsorbent mixture (one-third Lloyd's reagent by weight) were used.

The basic amino acids were adsorbed on the columns from water or from 1% hydrochloric acid solutions of their hydrochlorides. The interchanging of water and 1% hydrochloric acid as solvents had little effect on the adsorption characteristics of the basic amino acids if the amount used for each adsorption were less than 20 ml. Samples of 1 to 8 mg. of lysine and histidine and 2 to 16 mg. of arginine were used for each adsorption. The larger amounts of arginine were used because many proteins contain larger quantities of arginine than of lysine and histidine.

The amino acid mixtures were adsorbed from 1% hydrochloric acid solutions. A representative mixture was: 20 mg. of *dl*-alanine, 25 mg. of *dl*-aspartic acid, 20 mg. of *l* (−)-cystine, 25 mg. of *l* (+)-glutamic acid, 30 mg. of glycine, 20 mg. of *dl*-isoleucine, 20 mg. of *dl*-leucine, 10 mg. of *dl*-methionine, 20 mg. of *dl*-norleucine, 20 mg. of *dl*-phenylalanine, 20 mg. of *dl*-threonine, 20 mg. of *l* (−)-tryptophane, 50 mg. of *l* (−)-tryosine, 20 mg. of *dl*-valine, 8 mg. of *l* (+)-arginine, 4 mg. of *l* (−)-histidine, 4 mg. of *l* (+)-lysine, and 100 mg. of ammonium chloride, per 10 ml. of solution.

QUALITATIVE STUDIES ON AMINO ACID ADSORPTION

Although the adsorption and elution characteristics of many compounds are affected by the presence of similar compounds, the basic amino acids behaved the same under identical conditions of adsorption and elution either alone or in amino acid mixtures. Thus, when conditions for a separation method for the basic amino acids were established, tests for the beginning and ending of their elution were not necessary.

The adsorption and elution characteristics of histidine and arginine were relatively easy to observe by the specific Pauly diazo reaction for histidine and Sakaguchi reaction for arginine. The behavior of lysine and each of the nonbasic amino acids was determined by the nonspecific ninhydrin colorimetric reaction.

Preliminary adsorption trials showed that the basic amino acids, particularly histidine, were not adsorbed completely by the untreated adsorbent mixture. Complete adsorption was possible when the column was pretreated with solutions of hydrochloric, sulfuric, or acetic acid.

Preliminary trials with amino acid mixtures, prepared as stated above, showed that all the amino acids were adsorbed when the mixtures were passed through columns of Lloyd's reagent. However, all the nonbasic amino acids except cystine were separated completely from the basic ones by developing the columns with hydrochloric acid. Cystine was completely separated from the basic amino acids when it was reduced to cysteine before chromatographing, by addition of zinc dust to the amino acid solution acidified with hydrochloric acid. The adsorption and elution characteristics of the nonbasic amino acids were determined by chromatographing each of the nonbasic amino acids with lysine.

Many possible eluants were tested for their effect on the elution characteristics of lysine, histidine, and arginine. The only solvents that were tried in this investigation were water and solvents completely miscible with water. The eluants tested may be classified as follows: strong and weak bases, strong and weak acids, salts, and buffer solutions.

The stronger basic compounds such as sodium hydroxide could not be used with Lloyd's reagent because their passage through the column was too slow.

Sodium bicarbonate proved the most useful of the weaker basic compounds, as it facilitated complete separation of histidine from arginine and removed most of the histidine before elution of lysine began.

Of the acids tested, hydrochloric acid gave the only useful separation. It eluted lysine completely before any histidine was removed and removed much of the histidine before elution of arginine began. However, complete separation of histidine and arginine was not possible.

Salts such as sodium chloride eluted in the same order as did hydrochloric acid, but the elutions were not complete nor the separations clear cut.

The elution powers of pyridine were so general that it was useful only when a single amino acid was on the column or when group elution was desirable.

Of the solvents miscible with water, methanol gave an unexpected elution. Methanol solutions of hydrochloric acid eluted arginine before lysine and histidine, but the separation was not clear-cut.

A study of the elution properties of the various compounds tested suggested a logical separation procedure. Since hydrochloric acid gave the greatest spread among lysine, histidine, and arginine, and removed lysine completely before removing any histidine or arginine, it was used as the first eluant. Sodium bicarbonate was used as the second eluant because it completely separated histidine from arginine. Arginine was removed with pyridine.

PROCEDURE FOR QUANTITATIVE SEPARATION AND DETERMINATION OF BASIC AMINO ACIDS

Make the amino acid solution, 10 ml. of which contains 1 to 6 mg. of lysine, 1 to 4 mg. of histidine, and 1 to 8 mg. of arginine,

about 0.3 *N* with hydrochloric acid, add 30 to 50 mg. of zinc dust for each 10 ml. of solution, heat to 80° C., and cool.

Insert a 400 by 19 mm. chromatographic tube into the top of a bell jar and attach the bell jar to a vacuum line with a pressure of 30 to 40 mm. of mercury. Push a small wad of cotton to the bottom of the tube, pour 20 grams of absorbent mixture (one-third Lloyd's reagent and two-thirds Hyflo Super-Cel by weight) into the tube, tap the side of the tube to pack the column, and place 0.6 cm. (0.25 inch) of Hyflo Super-Cel on top of the absorbent in the tube.

Pass the following liquids through the column in the order given: 50 ml. of 1.7 *N* hydrochloric acid, 10 ml. of the amino acid solution, 180 ml. of 0.5 *N* hydrochloric acid (use first 10 ml. to rinse the amino acid solution onto the absorbent), 200 ml. of 1.0 *N* hydrochloric acid, 150 ml. of 0.125 *M* sodium bicarbonate, 100 ml. of 10% pyridine in 0.7 *N* hydrochloric acid, and 40 ml. of 0.5 *N* hydrochloric acid. Add a new solution just as the last of the previous solution is about to disappear onto the absorbent.

Cuts were made when 80, 275, 480, 625, and 730 ml. of eluant had passed into the absorbent. The first fraction was discarded. The second fraction contained the nonbasic amino acids and ammonia, the third contained lysine, the fourth contained histidine, and the fifth contained arginine.

Run a blank column, in which 0.3 *N* hydrochloric acid treated with zinc replaces the amino acid solution, for each set of determinations. This serves as a reagent blank for the individual amino acid determinations.

The absorption and elution procedure requires from 4 to 5 hours.

Since each basic amino acid occurs singly in a separate fraction, it should be possible to determine each one by any reliable method. In this investigation the following procedures, which have been applied routinely in this laboratory, were used.

Neutralize the histidine fraction to litmus with hydrochloric acid and make to volume. Determine the histidine quantitatively by a modification of Pauly's diazo reaction (*9*).

Neutralize the arginine fraction to litmus with sodium hydroxide and make to volume. Determine the arginine quantitatively by a modification of Sakaguchi's reaction (*4*) (use one 25-ml. flask in place of the three 10-ml. flasks called for).

Make the lysine fraction just acid to phenolphthalein with sodium hydroxide. Add 15 ml. of a pH 7.4 buffer solution (50 ml. of 0.4 *M* potassium dihydrogen phosphate and 4.1 ml. of 1.0 *N* sodium hydroxide diluted to 100 ml.) and make the solution to volume. Determine lysine quantitatively by the authors' modification of the ninhydrin colorimetric method: Pipet an aliquot containing 0.02 to 0.10 mg. of lysine into a 25-ml. flask and make to 3 ml. with the blank solution. Add 1 ml. of the ninhydrin solution (5 mg. of triketohydrindene hydrate, 0.4 mg. of sodium hydroxide, and 263 mg. of sodium chloride per milliliter) and 5 ml. of glycerol (added with a large-bore pipet), mix the solution thoroughly, and place in a boiling water bath. After 30 minutes remove the solution, cool, and make to volume with 95% ethanol. Within 15 minutes determine the transmission value of the colored solution in a photoelectric colorimeter and evaluate the amount of lysine by reading this value from a standard curve prepared by using known amounts of lysine in the procedure outlined above.

The transmission values of the colored solutions can be determined in any photoelectric colorimeter with which a spectral region with a maximum transmission at 5400 Å. can be isolated. In this investigation a KWSZ photoelectric photometer was used with Corning filters 978 and 351, 1-cm. adsorption cells, 5% copper sulfate in the cooling cells, and 67.5 volts on the photocell circuit.

The lysine in the lysine fractions from some of the protein hydrolyzates was also determined by the total amino nitrogen method (*13*), by the α-amino nitrogen method (*14*), and by the method involving the difference between the total and α-amino nitrogen (*14*).

The proteins used in this investigation were hydrolyzed with 20 ml. of 20% hydrochloric acid for 18 hours. The hydrolyzates were filtered, concentrated under vacuum, and reconcentrated three times after addition of water to remove the hydrochloric acid. One milliliter of concentrated hydrochloric acid was added and the solutions were made to 50 ml. When decolorizations were made, acid- and alkali-washed Norit was used. The nucleic acid used in the experimental work was treated in the same manner as the proteins.

RESULTS AND DISCUSSION

Recoveries of lysine, histidine, and arginine adsorbed from amino acid mixtures were excellent (Table I). The slightly high recovery of lysine may have been due either to lysine impurities in the other amino acid preparations or to traces of nonbasic amino acids that escaped removal before elution of lysine began.

The amounts of the basic amino acids found in several proteins compare fairly well in most cases with those which Block (*3*) considers "best values" (Table II), especially since different preparations of the same protein may vary somewhat in their analysis. Since the lysine values were higher than most reported values, it is possible that other ninhydrin-color-producing substances were present in the lysine fraction. To check this possibility the amounts of lysine present in the lysine fractions of hydrolyzates from several proteins were determined by the ninhydrin colorimetric method and by amino nitrogen determinations (Table III). Since the errors in the amino nitrogen determinations may be high when small amounts of lysine are determined, the comparisons are considered good. The results indicate that only traces of interfering substances could have been present in the lysine fraction.

Stanley (*10*) reported no lysine present in tobacco mosaic virus. Knight (*8*) found 1.35% lysine in the virus, but decided his findings were inconclusive, since he could not isolate any lysine by

Table I. Recovery of Lysine, Histidine, and Arginine from a Mixture of Amino Acids[a] and Ammonium Chloride

Amino Acid	Amount in Solution	Amount in Eluate	Recovery
	Mg.	*Mg.*	%
Lysine	4.00	4.04	101.0
	4.00	4.21	105.3
	4.00	4.13	103.3
	1.00	1.01	100.3
Histidine	4.00	3.97	99.3
	4.00	4.01	100.3
	4.00	4.04	101.0
	1.00	0.99	99.0
Arginine	8.00	7.76	97.0
	8.00	8.08	101.0
	8.00	7.90	98.8
	2.00	1.96	98.0

[a] Tyrosine, tryptophane, methionine, glutamic acid, aspartic acid, cystine, glycine, alanine, valine, norleucine, isoleucine, leucine, phenylalanine, threonine, histidine, arginine, lysine.

Table II. Basic Amino Acid Content of Proteins[a]

(16% nitrogen basis)[b]

Proteins	Nitrogen	Lysine		Histidine		Arginine	
		Adsorption value[c]	"Best value"[d]	Adsorption value[c]	"Best value"[d]	Adsorption value[c]	"Best value"[d]
	%	%	%	%	%	%	%
Casein (Pfanstiehl)	13.6	8.6	7.5	3.3	2.5	3.7	4.2
Fibrin (c.p., City Chemical Corp.)	13.2	9.1	7.5	2.7	2.4	6.9	7.8
Lactalbumin (Labco, Borden)	13.6	11.2	9.6	2.2	2.0	3.4	3.9
Egg albumin (c.p., City Chemical Corp.)	12.3	8.0	5.0	2.7	2.0	6.0	5.8
Blood albumin (c.p., City Chemical Corp.)	11.3	9.7	10.0	3.2	2.8	5.7	5.5
Gelatin (Sargent)	16.1	4.5	4.5	0.75	0.8	8.0	8.0
Tobacco mosaic virus (Stanley)	?	1.42	0.0[e]	0.0	0.0[e]	9.3	9.0[e]

[a] Protein hydrolyzates not treated with Norit.
[b] Values calculated to 16% nitrogen basis for comparison only.
[c] Values did not vary more than ±3%.
[d] Block and Bolling (*3*).
[e] Stanley (*10*).

Table III. Comparison of Methods for Determining Lysine in Lysine Fraction from Protein Hydrolyzates (Norit-Treated)

Protein	Lysine by Ninhydrin Colorimetric Method	Lysine by Total Amino Nitrogen Method	Lysine by α-Amino Nitrogen Method	Lysine by Total Amino Nitrogen Minus α-Amino Nitrogen Method
	%	%	%	%
Casein	7.3	7.2	7.5	7.0
Fibrin	7.5	7.8	7.6	8.0
Lactalbumin	9.3	9.5	9.4	9.7
Egg albumin	5.9	6.1	6.1	6.2
Blood albumin	6.5	6.5	6.5	6.6
Gelatin	4.5	4.6	4.9	4.4
Corn 1	0.23	0.23	0.23	0.23
Corn 2	0.26	0.26	0.29	0.22
Corn 3	0.25	0.28	0.26	0.29
Corn 4	0.24	0.23	0.27	0.19
Corn average	0.25	0.25	0.26	0.23

precipitation methods. Stokes *et al.* (*11*) by use of microbiological techniques found 1.36% lysine in the virus. The 1.42% found by the adsorption method is in good agreement with Knight's and Stokes's findings. All the above determinations were made on tobacco mosaic virus prepared by Stanley.

The histidine values might also be questionable, since there are other naturally occurring basic compounds that give a color with Pauly's diazo reaction. The compounds most likely to be present in quantities sufficient to cause interferences are the purine and pyrimidine bases occurring in nucleic acids. The values for histidine, arginine, and lysine were not affected when hydrolyzed yeast nucleic acid, in amounts several times higher than would occur in proteins, was added to the amino acid solutions before chromatographing (Table IV).

Decolorization of the acid hydrolyzates affected slightly the basic amino acid values, particularly histidine and arginine values (Table V). However, it was found that this preparation of Norit adsorbed slight amounts of the basic amino acids. The variation of values from the treated samples were greater in some cases than the values from the untreated samples. Even though there was some color removed during the elution of histidine, this did not account for the differences obtained in the histidine values. It is recommended that the protein hydrolyzates be chromatographed without decolorization.

The values for arginine and histidine found in some corn grain hydrolyzates are compared to values obtained from the same samples by Doty *et al.* (*5*) by use of phosphotungstic acid to separate the basic amino acids before determination of histidine and arginine (Table VI). There were no corrections made for the solubility of the phosphotungstates, which accounts, at least in part, for the lower values obtained by the phosphotungstic acid precipitation method.

Table IV. Effect of Nucleic Acid on Basic Amino Acid Values[a]

Protein	Lysine		Histidine		Arginine	
	No nucleic acid	100 mg. of nucleic acid	No nucleic acid	100 mg. of nucleic acid	No nucleic acid	100 mg. of nucleic acid
	%	%	%	%	%	%
Lactalbumin	9.4	9.3	1.8	1.8	2.8	2.8
Egg albumin	6.2	6.3	2.1	2.1	4.6	4.7
Blood albumin	7.1	7.1	2.2	2.2	3.8	3.8

[a] Protein hydrolyzates treated with Norit.

Table V. Effect of Decolorization of Protein Hydrolyzates with Norit on Basic Amino Acid Values

Protein	Lysine		Histidine		Arginine	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
	%	%	%	%	%	%
Casein	7.4	7.6	2.8	2.8	3.2	3.2
Fibrin	7.7	7.7	2.3	2.2	5.6	5.6
Lactalbumin	9.7	9.4	1.9	1.8	2.9	2.8
Egg albumin	6.3	6.2	2.2	2.1	4.9	4.6
Blood albumin	7.0	7.1	2.3	2.2	4.1	3.8
Gelatin	4.6	4.5	0.78	0.64	8.1	7.5

CONCLUSIONS AND SUMMARY

The individual basic amino acids have been separated chromatographically from amino acid mixtures and protein hydrolyzates by use of Lloyd's reagent. This is the first time that a single adsorption has been used successfully for the separation of the basic amino acids. The recoveries of the basic amino acids when they were adsorbed from amino acid mixtures were 103 ± 2% for lysine, 100 ± 1% for histidine, and 99 ± 2% for arginine. The method is simple, rapid, and reliable. Arginine was determined by a modification of the Sakaguchi reaction and histidine by a modification of Pauly's diazo reaction. Lysine was determined by a modification of the ninhydrin colorimetric reaction which was standardized for quantitative determination. Evidence indicates that substances from protein hydrolyzates that may interfere with this reaction were absent. Accurate determination of lysine in protein hydrolyzates was possible even when the percentage of this amino acid was small. Nucleic acid did not interfere with the adsorption or subsequent determination of the basic amino acids.

Table VI. Basic Amino Acids in Corn

Sample	Nitrogen	Histidine		Arginine		Lysine
		Adsorption value	PTA value	Adsorption value	PTA value	Adsorption Value
	%	*Mg./g.*		*Mg./g.*		*Mg./g.*
1	1.50	2.9	2.0	3.9	3.6	2.3
2	1.59	2.7	2.2	4.0	4.1	2.6
3	1.45	2.5	2.0	3.7	3.8	2.5
4	1.45	2.6	2.3	4.0	3.9	2.4

Values of the basic amino acids determined in seven protein hydrolyzates were compared to literature values. The lysine and histidine values obtained by the adsorption method were generally higher than the average of the reported values, but the adsorption values checked more closely with the most recent literature values. The adsorption values for arginine compared satisfactorily with the average literature values.

The basic amino acids were determined in four corn grain hydrolyzates. The values for arginine and histidine were compared to values obtained when the basic amino acids were separated from hydrolyzates from the same corn grain samples by phosphotungstic acid precipitation. The arginine values checked very closely, but the adsorption values for histidine were appreciably higher than those obtained after phosphotungstic acid precipitation.

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Aids to Computation in Spectrophotometric Analysis of Binary Mixtures

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Several methods are described for facilitating the computation of concentrations of binary mixtures from the observed spectrophotometer data. The methods are especially useful for cases of Beer's law failure. One is a graphical method in which the observed optical densities of a mixture determine the location of a point inside a coordinate network reading directly in concentrations. The other method utilizes a specially constructed slide rule on which concentrations are read when the position is adjusted according to the densities. Illustrative data for benzene-toluene mixtures are shown. The saving in time over numerical calculation can amount to more than a factor of 20. The graphical method can be extended to three-component mixtures.

THE spectrophotometric analysis of mixtures of absorbers requires measurement of the optical density at selected wave lengths and solution of the resulting simultaneous equations. These equations are nonlinear in cases in which there is an apparent failure of Beer's law and are consequently tedious to solve directly. A method of solution based on successive approximations has been described (*1*). The present paper describes several devices which have been used for some time to shorten the labor of computation for binary mixtures.

An example which has been of considerable practical importance in the production of nitration grade toluene is the analysis of benzene-toluene mixtures in a transparent solvent such as isooctane.

For this case, using a particular set of experimental conditions (constants must be determined for every optical system used) and assuming no Beer's law failure, the following equations apply:

$$D^{268.9} = 1.01\ C_b + 20.8\ C_t \quad (1)$$

$$D^{260.9} = 16.3\ C_b + 19.4\ C_t \quad (2)$$

where the optical densities, D, are measured at characteristic absorption peaks at the indicated wave lengths expressed in millimicrons (the peaks for the two compounds happen almost to coincide in wave lengths but not in intensities and for the author's work were found most sensitive to relative composition) and the concentrations, C, are given in volume per cent. Equations 1 and 2 may be transformed to the more convenient form:

$$C_b = 0.0651 D^{260.9} - 0.0607\ D^{268.9} \quad (3)$$

$$C_t = -0.0032\ D^{260.9} + 0.0510\ D^{268.9} \quad (4)$$

GRAPHICAL SOLUTION

These equations may be thought of as defining a transformation from D axes to C axes, as illustrated in Figure 1. Here the densities at 268.9 mμ for various concentrations of the pure compounds in a transparent solvent are plotted as a function of the densities at 260.9 mμ, the toluene axis having approximately unit slope because the peaks are about of equal intensity, while the slope for the benzene axis is much lower because the 268.9 peak is much weaker than that at 260.9 for this compound. The densities of a mixture are represented by a point somewhere inside these two lines. It will be seen that, speaking in the language of vectors, the components along the concentration axes of a point defined by components along the density axes can be obtained graphically by projecting back parallel to the concentration axes.

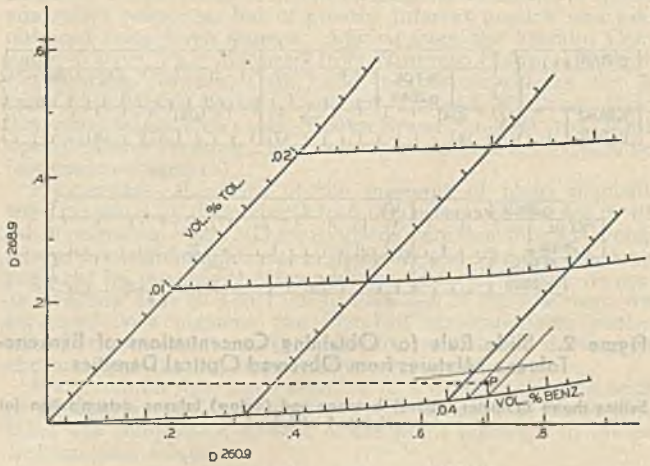

Figure 1. Graphical Method of Obtaining Concentrations of Benzene-Toluene Mixtures from Observed Optical Densities

Example shown at *P* represents mixture 1 of Table I

The network consisting of the concentration axes repeated at regular intervals of benzene and toluene concentration, as indicated in Figure 1, is drawn in merely for convenience. Concentrations can be obtained in this way in a much shorter time than by numerical solution of Equations 3 and 4. The chief advantage, however, is found in the case in which the densities are not linear functions of the concentrations. This situation arises when benzene-toluene mixtures are measured on a Beckman quartz spectrophotometer, which requires an effective slit width (0.30 mm. corresponding to about 0.8 mμ) comparable with the width of the absorption bands. In this case the data can be represented by a curvilinear coordinate system for the concentration axes which is a distortion of the previous case, the concentrations being obtained by following a curvilinear network. Figure 1 actually rep-

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resents Beckman spectrophotometer data. It will be seen that the curvature of the axes is not great but cannot be ignored for accurate work. Linear interpolation between any two successive 0.01% intervals on the concentration scales is satisfactory.

SLIDE RULE SOLUTION

Another device which has been found convenient in practice is a slide rule, Figure 2, with appropriate scales reading directly in concentrations. These scales are drawn on strips of paper which are pasted on a conventional slide rule and covered with label varnish. The action of the rule depends on the fact that each concentration in Equations 3 and 4 is expressed as the difference between two linear terms, so that if the density scales on the rule are each drawn with units adjusted in length according to the corresponding coefficients the subtractions can be performed mechanically. The operation is thus analogous to the operation of division on a conventional slide rule and requires the same length of time to obtain each concentration as a simple division. The rule has one density scale for 268.9 mμ but a separate density scale for 260.9 mμ is used for each compound. Based on the data of Equations 3 and 4 and assuming no Beer's law failure, unit length on the $D^{260.9}$ scale used for the toluene determination would be 0.0032/0.0510 the unit length on the $D^{268.9}$ scale, while unit length on the $D^{260.9}$ scale used for the benzene determination would be 0.0651/0.0607 that of the $D^{268.9}$ scale. The concentration scales are not in equivalent positions on the rule because the algebraic signs of the coefficients in Equations 3 and 4 are interchanged. In operation the movable hair line is set at the observed value on one of the $D^{260.9}$ scales, the $D^{268.9}$ scale moved to read the observed value for this wave length at the hair line, and the concentration in volume per cent read opposite the index arrow.

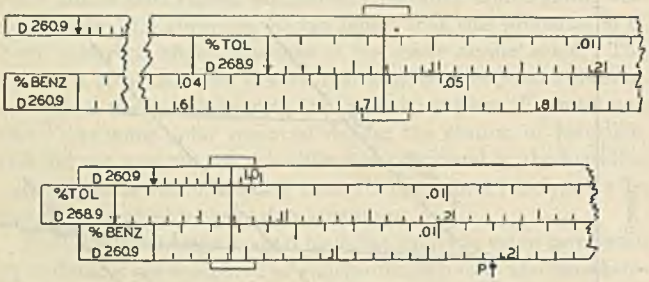

Figure 2. Slide Rule for Obtaining Concentrations of Benzene-Toluene Mixtures from Observed Optical Densities

Setting shown illustrates (above) benzene and (below) toluene determination for mixture 1 of Table I

The construction of the slide rule for the nonlinear case which is actually represented in Figure 2 is not quite so simple and is best carried out with the aid of the corresponding graph—i.e., Figure 1. The following procedure has been found to yield exact results, although other choices could doubtless be made. (It is possible that this method of attack might not be valid in all cases.)

The $D^{260.9}$ scale used for the determination of benzene is laid out linearly as before. With the zero of the movable scale in coincidence with that of the fixed scale, the $D^{268.9}$ scale is laid out from the readings of the toluene axis of the graph—i.e., suitable increments of the $D^{268.9}$ scale are drawn in opposite the associated values of the $D^{260.9}$ scale. For example, 0.2 on the $D^{268.9}$ scale would be placed opposite point P corresponding to the associated density on the $D^{260.9}$ scale. The movable hair line and the movable scale are then adjusted to correspond to associated densities for various concentrations of pure benzene, as obtained from the benzene axis, and the values for the C_b (% benzene) scale drawn in opposite the index arrow for each setting. The same procedure is now followed for the toluene scales, using the $D^{268.9}$ scale already obtained, drawing in another $D^{260.9}$ scale from data from the benzene axis with the zero points in coincidence, and then laying out the C_t (% toluene) scale from associated densities obtained from the toluene axis.

Table I. Comparison of Different Procedures for Converting Observed Densities of Benzene-Toluene Mixtures to Concentrations

Mixture No.	Constituent	Volume Per Cent Known	Shell	Graph	Rule
1	Benzene	0.0424	0.0421	0.0423	0.0423
	Toluene	0.0012	0.0014	0.0012	0.0012
	Total	0.0436	0.0435	0.0435	0.0435
2	Benzene	0.0290	0.0288	0.0288	0.0289
	Toluene	0.0060	0.0060	0.0059	0.0060
	Total	0.0350	0.0348	0.0347	0.0349
3	Benzene	0.0122	0.0121	0.0121	0.0121
	Toluene	0.0121	0.0122	0.0122	0.0122
	Total	0.0243	0.0243	0.0243	0.0243
4	Benzene	0.0116	0.0114	0.0114	0.0113
	Toluene	0.0194	0.0195	0.0196	0.0194
	Total	0.0310	0.0309	0.0310	0.0307
5	Benzene	0.0081	0.0078	0.0076	0.0075
	Toluene	0.0421	0.0424	0.0427	0.0424
	Total	0.0502	0.0502	0.0503	0.0499
6	Benzene	0.0033	0.0033	0.0033	0.0032
	Toluene	0.0168	0.0168	0.0168	0.0168
	Total	0.0201	0.0201	0.0201	0.0200
	Benzene	0.0018	0.0019	0.0020	0.0018
	Toluene	0.0388	0.0386	0.0386	0.0387
	Total	0.0406	0.0405	0.0406	0.0405
	Benzene	0.0007	0.0007	0.0007	0.0006
	Toluene	0.0155	0.0154	0.0155	0.0155
	Total	0.0162	0.0161	0.0162	0.0161

The resulting slide rule is similar to that used in the case of no Beer's law failure, except that only one scale is linear, the others converging or diverging. Linear interpolation on these scales between any two successive 0.01% concentration or 0.1 density points is convenient and satisfactory.

COMPARISON OF METHODS

A comparison of the different methods of treating the same data is shown in Table I. The Shell method refers to the successive approximation procedure described in (*1*). The results reported under graph were obtained with a curvilinear graph 50 × 50 cm., while a 50-cm. (20-inch) slide rule was used for those listed under rule. The average time required for obtaining both the benzene and toluene concentrations from the observed densities for a given mixture is approximately 5 to 8, 0.5, and 0.3 minute for the respective methods. The construction of the graph requires 3 or 4 hours, and the slide rule scales require about 2 working days.

THREE-COMPONENT MIXTURES

Linear equations for three-component mixtures may be solved with a two-dimensional graph by using as one of the equations the condition that the sum of the concentrations is known. The optical densities at any one wave length may be indicated on a conventional triangular composition diagram as a contour map. It will be found that lines of constant density for different compositions are straight parallel lines. For another wave length the constant density lines will have a different slope and different spacing for the same density interval. The composition is determined by the point of intersection of the measured density lines at the two wave lengths. The method is more rapid than numerical solution. It has not been tested for cases of Beer's law failure but should also apply if the deviations are not so large as to introduce contour curvatures with more than one point of intersection.

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Conditions Affecting the Sequence of Organic Compounds in Tswett Adsorption Columns

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TSWETT'S columnar or chromatographic adsorption method is particularly useful for the resolution of mixtures of organic compounds and for the detection and estimation of the individual components. Under given conditions, compounds adsorbed in the columns form a series of bands or zones in definite sequence. As a result, organic substances are frequently identified and named with respect to their relative positions in the adsorption columns (*13*, *15*). In several instances, prognostications regarding chemical structures of organic compounds have been based upon their adsorbability relative to that of substances of known constitution (*9*).

This sequence or adsorption order of the bands is, however, not the same under all circumstances; it varies with the conditions in the columns, with the solvent and the adsorbent, and with the kinds of substances adsorbed (*9*, *10*). Variations of the adsorption sequence have been observed most frequently when mixtures of dissimilar compounds are adsorbed under different conditions (*1*, *6*, *9*). For example, mixtures of the green chlorophylls and the yellow xanthophylls isolated from the green parts of plants often yield different adsorption sequences (*10*). Variations of the adsorption sequence have also been observed when mixtures of similar substances, such as the xanthophylls themselves, are resolved by adsorption (*13*).

Further investigation of the relative adsorbability of organic compounds has now revealed many additional examples of variation of the adsorption sequence. These variations have resulted from alteration of one or more factors such as the solvent, the adsorbent, the temperature, the concentration, the presence of an impurity, or the concentration of hydrogen ion. As experience is gained, additional conditions which influence the adsorption sequence will undoubtedly be found.

The number of possible adsorption sequences is related to the number of components in the mixtures that are adsorbed. With a binary mixture, two sequences of the bands are possible. Under certain conditions, one solute will form the upper band; under other conditions, this solute may form the lower band. As in a series of permutations, increase in the number of components of a mixture multiplies the number of possible sequences of the adsorption bands. For 3 components there are 6 possible adsorption sequences; with 4 components there are 24 possible sequences; with 5 components there are 120 sequences; and for 10 components there are 3,628,800 possible sequences. Only with binary mixtures have all the theoretically possible sequences been obtained.

Alteration of the adsorption sequence may be utilized to increase the sensitivity and to broaden the applicability of the chromatographic adsorption method. Variation of the adsorption sequence with conditions in the columns illustrates precautions to be observed in new applications of the columnar adsorption technique.

MATERIALS AND METHODS

Effects of various conditions upon the adsorption sequence were determined by use of colored solutes, which are readily visible in adsorption columns. These mixtures were made of chlorophylls, of chlorophylls and xanthophylls, of xanthophylls, or of common water-soluble dyes.

PIGMENTS AND THEIR SOURCES. Because of their lability, chlorophylls and xanthophylls were prepared in small quantities as needed. Chlorophylls *a* and *b* were isolated by adsorption of the freshly prepared extracts of green leaves (*11*). The labile isomer of each of these pigments, chlorophyll *a'* and chlorophyll *b'*, was obtained by adsorption of extracts of heated leaves (*12*). Chlorophyll *d* was prepared from red algae (*Rhodophyceae*) (*7*).

Xanthophylls, the alcohol-soluble oxygen derivatives of polyene hydrocarbons, were extracted from a variety of plant sources and were isolated by adsorption. Lutein and neoxanthin were obtained from leaves, fucoxanthin from the kelps or brown seaweeds (*Pheophyceae*), diadinoxanthin and peridinin (sulcatoxanthin) from the symbiotic alga of a sea anemone, diatoxanthin from diatoms, violaxanthin from pansies, and taraxanthin and tareoxanthin from dandelions (*13*). Zeaxanthin was isolated from the fruit pods of the Chinese lantern (*Physalis*) (*9*). On the Pacific Coast, sources of many of these pigments are available throughout the year.

SOLVENTS AND ADSORBENTS. Solvents were purified by distillation in an all-glass apparatus. Commercial adsorbents were employed, usually without further treatment.

Heat-treated siliceous earths, Hyflo Super-Cel and Celite 501, 503, 535, and 545, from Johns-Manville, were all weakly adsorptive (comparable in adsorptive capacity to powdered sugar). All these products are manufactured by the same alkali flux calcination process and differ principally in particle size.

Powdered sugar (confectioners' powdered sugar, grade XXXXXX, containing 3% starch to prevent caking), which was obtained from several sources, exhibited rather uniform adsorptive properties. Cellulose was prepared for use in columns by disintegration of finely cut filter paper in water in a Waring Blendor. Preparations of activated magnesium silicate of similar adsorptive properties but of greatly different particle size were obtained from three sources: Florisil from the Floridin Company, Warren, Pa.; Magnesol from Westvaco Chlorine Products Corporation, Newark, Calif., and magnesium silicate No. 34 from Philadelphia Quartz Company Limited, Berkeley, Calif. Activated magnesium oxide, Micron Brand No. 2641 from Westvaco Chlorine Products Corporation, was mixed with Celite before use in columns (*8*).

PROCEDURE. Solutions of the mixtures of plant pigments were prepared by dissolving 1 to 5 mg. of each substance in 100 ml. of petroleum ether. These solutions were then filtered through adsorption columns (1.5 cm. in diameter and 12 to 20 cm. long) prepared by pressing the dry adsorbent into the tube. As soon as a narrow zone (0.3 to 1 cm.) at the top of the adsorbent was saturated with pigment, the adsorbed materials were washed with fresh solvent (or solvent mixture), in order to develop the chromatogram and to reveal the adsorption sequence.

Water-soluble dyes were dissolved directly in water or in acidic or alkaline solution. Concentration of the dyes in these solutions was comparable to that of the plant pigments in the petroleum ether solutions.

CONDITIONS INFLUENCING ADSORPTION SEQUENCE OF COMPONENTS OF BINARY MIXTURES

ADSORPTION FROM DIFFERENT SOLVENTS. In columns of a given adsorbent, adsorption sequences are determined, in part, by the properties of the solvent. There are many examples of a chlorophyll and a xanthophyll adsorbed in two sequences when different solvents are used.

With petroleum ether or benzene as solvent and with Celite as adsorbent, chlorophyll *b* forms a yellow-green band above the yellow band of neoxanthin. Addition of about 25% of acetone to the petroleum ether or benzene causes the chlorophyll *b* to be adsorbed below the neoxanthin.

With petroleum ether, benzene, or 1,2-dichloroethane as solvent, chlorophyll *d* is adsorbed above fucoxanthin in columns of powdered sugar. If 0.5 to about 3% of an alcohol is added to these solvents, chlorophyll *d* is adsorbed below fucoxanthin.

When petroleum ether containing about 5% acetone is used as solvent, chlorophyll *a* is adsorbed above lutein in columns of

Celite. The presence of about 0.75% ethanol in the petroleum ether plus acetone causes the chlorophyll *a* to be adsorbed below the lutein.

Many similar alterations of the adsorption sequence have been produced by adsorption of binary mixtures of chlorophyll *a* and a xanthophyll. When petroleum ether is the solvent and powdered sugar is the adsorbent, chlorophyll *a* is adsorbed above each of the following xanthophylls: violaxanthin, fucoxanthin, diadinoxanthin, tareoxanthin, taraxanthin, diatoxanthin, zeaxanthin, and lutein. With petroleum ether containing 0.5 to 1% propanol as solvent, chlorophyll *a* is adsorbed below each of these xanthophylls.

Substances of the same type, such as the xanthophylls themselves, are often adsorbed in different sequences when different solvents are used with columns of a given adsorbent. With chloroform as solvent, zeaxanthin is adsorbed below violaxanthin in columns of magnesia plus Celite (1 to 1). With petroleum ether plus about 25% of ketones (such as acetone, ethyl methyl ketone, or methyl nonyl ketone) as solvent, zeaxanthin is adsorbed above violaxanthin.

With 1,2-dichloroethane as solvent, fucoxanthin is adsorbed above violaxanthin in columns of sugar. But with petroleum ether plus about 0.5% of an alcohol such as methanol, ethanol, propanol, or butanol as the solvent, fucoxanthin is adsorbed below violaxanthin.

Variations of the proportions of a mixture of solvents may also suffice to alter the sequence of substances adsorbed on a given adsorbent. When petroleum ether plus about 5% acetone and 0.5% ethanol is used as solvent, fucoxanthin is adsorbed above chlorophyll *a* in columns of Celite. Increase of the alcohol concentration to about 2% causes the fucoxanthin to be adsorbed below the chlorophyll *a*. At one intermediate concentration of alcohol, fucoxanthin and chlorophyll *a* are difficultly separable in the columns of Celite.

Impurities in Solvents. Very small quantities of alcohols which may occur as impurities in a solvent often alter the sequence of adsorbed chlorophylls and xanthophylls. In columns of sugar, where fucoxanthin is normally adsorbed below chlorophyll *a* when petroleum ether is used as solvent, as little as 0.05% amyl alcohol in the petroleum ether causes the fucoxanthin to be adsorbed above the chlorophyll *a*. A similar effect is produced by as little as 0.1% cholesterol or ergosterol dissolved in the petroleum ether. Unsaturated hydrocarbons, chlorinated hydrocarbons, ethers, esters, organic acids, and amides of organic acids added to petroleum ether accelerate the movement of fucoxanthin and chlorophyll *a* through columns of sugar, but the adsorption order is identical with that produced by petroleum ether alone.

Addition of very small quantities of alcoholic substances to a solution of fucoxanthin and chlorophyll *a* in petroleum ether before adsorption in a column of sugar causes the chlorophyll to form the lower band. But as the column is washed with fresh, unadulterated portions of petroleum ether, the fucoxanthin gradually becomes adsorbed below the chlorophyll *a*.

Alcohols are common in plants, and it seems probable that they may cause the reversal of the adsorption order of chlorophylls and xanthophylls which occurs when extracts of plants are adsorbed. When a petroleum ether extract of the brown seaweeds is filtered through a column of powdered sugar, chlorophyll *a* is adsorbed below fucoxanthin, the principal xanthophyll. As the chromatogram is developed with fresh petroleum ether, fucoxanthin slowly appears below the chlorophyll *a*. In analogous fashion, a petroleum ether extract of the leaves of higher plants yields chlorophyll *a* adsorbed below the principal xanthophyll lutein. Development of the chromatogram with petroleum ether carries the lutein below the chlorophyll *a*.

Use of Different Solvents for Development of One Chromatogram. As indicated by the adsorption of pigments from solutions containing impurities, bands of two substances separated in one sequence by adsorption from one solvent may have their relative positions reversed by further development of the chromatogram with another solvent. Because the resolution effected by the first solvent must be overcome by the second solvent in the lower unused portions of the adsorbent, long adsorption columns are usually required.

If a mixture of chlorophyll *a* and zeaxanthin in solution in dichloroethane is adsorbed on a column of sugar, zeaxanthin forms the lower band. Further development of the chromatogram with petroleum ether containing about 0.5% ethanol causes the chlorophyll *a* to overtake and to pass through the zeaxanthin band, thus reversing the adsorption sequence. Through the alternate use of these two different solvents in a long column of sugar, the adsorption sequence of chlorophyll *a* and zeaxanthin may be reversed several times.

Mixtures of either chlorophyll *a*, *b*, or *d* with one xanthophyll have also been separated in first one sequence and then in the other by the successive use of different solvents for development of the chromatogram. Mixtures of two xanthophylls have likewise been separated in one sequence and then in the other by development of the chromatogram with two different solvents. This may be illustrated by the adsorption of taraxanthin and lutein in columns of a mixture of magnesia and Celite. With dichloroethane as solvent, lutein forms a band below that of taraxanthin. When the column is subsequently washed with petroleum ether containing about 25% acetone, taraxanthin advances through the band of lutein. Conversely, lutein first separated below taraxanthin by adsorption on magnesia from solution in petroleum ether plus acetone can be made to form the upper band by washing the column with 1,2-dichloroethane.

Effect of Hydrogen Ion. Different concentrations of hydrogen ion in aqueous solution may cause mixtures of water-soluble substances to separate in different sequences. As an example, on columns of cellulose bromothymol blue (dibromothymol sulfonephthalein) is adsorbed below fluorescein when the solutions are alkaline (ammoniacal). From neutral, buffered solutions or acidic solutions, however, bromothymol blue is adsorbed above fluorescein.

Concentration of Solutes. At higher concentrations, a solute usually migrates through an adsorption column faster than at lower concentrations. For this reason, increase in the concentration of a more adsorbed constituent of a binary mixture may cause the band of this more adsorbed component to advance beyond the band of the less adsorbed component. From dilute ammoniacal solution, fluorescein is slightly more adsorbed than bromothymol blue in columns of cellulose (and in strips of filter paper) when about equal quantities of the pigment are adsorbed. If the ratio of fluorescein to bromothymol blue is increased to about 50 to 1, fluorescein appears ahead of the bromothymol blue. But the advancing band of fluorescein has a dilute, diffuse, trailing boundary that moves slower than the band of bromothymol blue.

Table I. Adsorption Sequences of Chlorophyll *b*, Chlorophyll *a*, and Fucoxanthin

	Columns of Magnesia and of Celite		
Adsorbents	Magnesia + Celite (1 to 1)	Celite	Celite
Solvents	Petroleum ether + 25% acetone	Petroleum ether + 0.75% ethanol	Petroleum ether + 0.75% ethanol + 5% acetone
Adsorption sequences	Chlorophyll *b* Chlorophyll *a* Fucoxanthin	Fucoxanthin Chlorophyll *b* Chlorophyll *a*	Chlorophyll *b* Fucoxanthin Chlorophyll *a*
	Columns of Sugar		
Adsorbents	Sugar	Sugar	Sugar
Solvents	Petroleum ether + 5% acetone	Petroleum ether + 0.75% ethanol	Petroleum ether + 5% acetone + 0.25% ethanol
Adsorption sequences	Chlorophyll *b* Chlorophyll *a* Fucoxanthin	Fucoxanthin Chlorophyll *b* Chlorophyll *a*	Chlorophyll *b* Fucoxanthin Chlorophyll *a*

As a result, the band of bromothymol blue appears as a blue zone in the middle of a rapidly widening yellow band of fluorescein. If the concentration of the fluorescein is not too great, the original band is not too wide, and the column is long enough, the band of fluorescein widens; its rate of movement decreases; and it gradually falls behind the band of bromothymol blue.

Different Adsorbents. The sequence of two substances adsorbed from a given solvent varies from adsorbent to adsorbent. In columns of Celite, chlorophyll *a* is adsorbed below lutein when petroleum ether plus 0.5% ethanol plus 5% acetone is used as solvent; but in columns of sugar, chlorophyll *a* is adsorbed above lutein. In columns of magnesia, with petroleum ether plus 5% acetone as solvent, chlorophyll *a* is adsorbed above lutein, but in columns of sugar these pigments are adsorbed in the inverse order.

Relative adsorbability of the xanthophylls also varies with the adsorbent. When magnesia plus Celite is the adsorbent and dichloroethane is the solvent, zeaxanthin is adsorbed above fucoxanthin; but in columns of sugar, zeaxanthin is adsorbed below fucoxanthin. From solution in petroleum ether plus about 10% acetone, lutein is adsorbed below taraxanthin in columns of Celite or of powdered sugar; but in columns of magnesia or of magnesia plus Celite lutein is adsorbed above taraxanthin. In this latter example, the resolving power of the magnesia tending to separate the pigments in one sequence is greater than the resolving power of the Celite tending to separate the pigments in the other sequence.

In columns of different adsorbents, water-soluble substances also separate in different sequences. From ammoniacal solution bromothymol blue is adsorbed above fluorescein in columns of Celite or of magnesium silicate. In columns of magnesia plus Celite, in columns of cellulose, or in strips of filter paper (by capillary analysis) bromothymol blue is less adsorbed than fluorescein.

Alteration of the Adsorbent. Treatment of an adsorbent in different ways may serve to invert the sequence of adsorbed substances. In columns of Celite, fucoxanthin is adsorbed above chlorophyll *a* when petroleum ether plus about 0.75% ethanol is employed as solvent. If, however, the Celite is moistened uniformly with water (about 5 to 10% by weight) before it is packed into the adsorption tube, the fucoxanthin is weakly adsorbed, forming a band just below the chlorophyll *a*. By contrast, chlorophyll *a* is adsorbed above fucoxanthin in columns of dry or of moist Celite when petroleum ether plus 5% acetone is used as solvent.

Impurities on an adsorbent sometimes cause substances to be adsorbed in different sequences. Sugar used for adsorption of extracts of plant material and recovered by drying often retains impurities in sufficient quantities to cause chlorophyll *a* to be adsorbed below xanthophylls such as fucoxanthin, zeaxanthin, and lutein (with petroleum ether as solvent), whereas in columns of fresh sugar chlorophyll *a* is adsorbed above these xanthophylls. A similar effect is obtained when sugar that has been washed with alcohol is completely dried before re-use in columns.

Layers of Adsorbents. Layers of different adsorbents in a single adsorption column may cause the bands of adsorbed compounds to alter their relative positions as they are carried through the adsorbent. In a column composed of a deep lower layer of magnesia and a shallower upper layer of sugar, chlorophyll *a* is adsorbed below zeaxanthin in the sugar layer but gradually becomes adsorbed above zeaxanthin in the magnesia layer when petroleum ether plus 0.5% ethanol are employed as the solvent.

Adsorption at Different Temperatures. Variation of the temperature may sometimes change the adsorption sequence. At 95° C., lutein dissolved in decalin plus 0.5% propanol is adsorbed below chlorophyll *a* in columns of powdered sugar. Under similar conditions but at 20° C., lutein is adsorbed above chlorophyll *a*. At the higher temperature, spontaneous isomerization of chlorophyll *a* causes formation of a wide green band which does not separate into distinct bands of chlorophyll *a* and chlorophyll *a'* (*12*).

At 95° C. bromothymol blue and fluorescein, in dilute ammoniacal solution, pass through adsorption columns of magnesium silicate slightly faster than at 20° C. At each temperature, fluorescein forms the lower band.

Table II. Adsorption Sequences

Chlorophyll *b*, Fucoxanthin, and Zeaxanthin			
Adsorbents	Sugar	Sugar	Magnesia + Celite (1 to 1)
Solvents	1,2-Dichloroethane	Petroleum ether + 0.5% ethanol	Petroleum ether + 25% acetone
Adsorption sequences	Chlorophyll *b* Fucoxanthin Zeaxanthin	Fucoxanthin Chlorophyll *b* Zeaxanthin	Chlorophyll *b* Zeaxanthin Fucoxanthin
Chlorophyll *a*, Fucoxanthin, and Zeaxanthin			
Adsorbents	Magnesia + Celite (1 to 1)	Magnesia + Celite (1 to 1)	Sugar	Sugar
Solvents	Petroleum ether + 3% ethanol	Petroleum ether + 25% acetone	Petroleum ether + 0.5% ethanol	Petroleum ether + 5% acetone + 0.25% ethanol
Adsorption sequences	Chlorophyll *a* Fucoxanthin Zeaxanthin	Chlorophyll *a* Zeaxanthin Fucoxanthin	Fucoxanthin Zeaxanthin Chlorophyll *a*	Fucoxanthin Chlorophyll *a* Zeaxanthin
Chlorophyll *a*, Violaxanthin, and Lutein			
Adsorbents	Sugar	Magnesia + Celite (1 to 1)	Sugar	Sugar
Solvents	1,2-Dichloroethane	Petroleum ether + 25% acetone	Petroleum ether + 0.5% ethanol	Petroleum ether + 5% acetone + 0.25% ethanol
Adsorption sequences	Chlorophyll *a* Violaxanthin Lutein	Chlorophyll *a* Lutein Violaxanthin	Violaxanthin Lutein Chlorophyll *a*	Violaxanthin Chlorophyll *a* Lutein
Fucoxanthin, Violaxanthin, and Zeaxanthin			
Adsorbents	Celite	Sugar	Magnesia + Celite (1 to 1)
Solvents	Petroleum ether + 5% acetone	Petroleum ether + 0.5% ethanol	Petroleum ether + 25% acetone
Adsorption sequences	Fucoxanthin Violaxanthin Zeaxanthin	Violaxanthin Fucoxanthin Zeaxanthin	Zeaxanthin Fucoxanthin Violaxanthin

MIXTURES WITH SEVERAL COMPONENTS

Some mixtures of three components have now been separated in three of the six possible sequences. A few mixtures of three components have been resolved in four of the six possible sequences.

Examples of three sequences obtained by adsorption of two chlorophylls and one xanthophyll are illustrated in Table I. These three sequences resulted from variation of both solvent and adsorbent. Similar sequences were observed in columns of a single adsorbent by variation of the solvent alone. Thus far it has not been possible to alter the relative adsorbabilities of chlorophylls *a*, *b*, and *d*, which are adsorbed in the order *b*, *d*, and *a*, with *a* being least adsorbed (*7*).

Examples of three adsorption sequences obtained by adsorption of chlorophyll *b* and the two xanthophylls, fucoxanthin and zeaxanthin, are reported in Table II. Mixtures of chlorophyll *a* and these two xanthophylls were resolved in four of the six possible sequences.

With mixtures of chlorophyll *a*, violaxanthin, and lutein, four adsorption sequences were likewise obtained.

Mixtures of the three xanthophylls, fucoxanthin, violaxanthin, and zeaxanthin, were readily resolvable, yielding three sequences.

Adsorption of a mixture of two chlorophylls and two xanthophylls has yielded as many as 6 of the 24 possible sequences, as illustrated in Table III. Some of the sequences reported there were altered by very slight changes in the concentration of alcohol in the solvent. In fact, different preparations of powdered sugar

Table III. Adsorption Sequences of Chlorophyll b, Chlorophyll a, Fucoxanthin, and Zeaxanthin

Adsorbents	Sugar	Sugar	Magnesia + Celite (1 to 1)	Magnesia + Celite (1 to 1)	Sugar	Celite
Solvents	Petroleum ether + 1% ethanol	Petroleum ether + 5% acetone + ca. 0.75% ethanol	Petroleum ether + 3% ethanol	Petroleum ether + 25% acetone	Petroleum ether + 5% acetone + ca. 0.5% ethanol	Petroleum ether + 5% acetone + ca. 0.5% ethanol
Adsorption sequences	Fucoxanthin Chlorophyll *b* Zeaxanthin Chlorophyll *a*	Fucoxanthin Chlorophyll *b* Chlorophyll *a* Zeaxanthin	Chlorophyll *b* Chlorophyll *a* Fucoxanthin Zeaxanthin	Chlorophyll *b* Chlorophyll *a* Zeaxanthin Fucoxanthin	Chlorophyll *b* Fucoxanthin Chlorophyll *a* Zeaxanthin	Chlorophyll *b* Fucoxanthin Zeaxanthin Chlorophyll *a*

required very careful adjustment of the alcohol concentration in order to provide the adsorption sequences shown in the table.

By variation of the amounts of alcohol and of acetone added to petroleum ether, fucoxanthin has been made to occupy four different positions relative to the bands of chlorophylls *b*, *d*, and *a* in columns of sugar. With petroleum ether containing about 1% alcohol, fucoxanthin is adsorbed above the most adsorbed green pigment, chlorophyll *b;* with petroleum ether containing about 5% acetone, fucoxanthin is adsorbed below the least adsorbed green pigment, chlorophyll *a*. At intermediate concentrations of alcohol and of acetone, fucoxanthin occupies positions between chlorophylls *b* and *d* and between *d* and *a*. These four components thus yield four sequences.

In columns of sugar and with petroleum ether containing different amounts of alcohol and acetone, fucoxanthin has been made to occupy positions above and below each of the following chlorophylls: *b*, *b'*, *a*, and *a'*. In this way five components have been made to yield five sequences.

In all the experiments leading to the results summarized in this paper, relatively few adsorbents and solvents were employed. The ease with which chlorophylls and carotenoids are decomposed by many adsorptive solids necessitated rapid work with some adsorbents and precluded the use of many others. Further investigation of the adsorption of these plant pigments will undoubtedly reveal additional adsorption sequences.

DISCUSSION

Variation of the adsorption sequence with changes of conditions in the adsorption columns demonstrates the great sensitiveness of the chromatographic adsorption method. Substances inseparable under one set of conditions may be readily separable under slightly different conditions. If substances are to be identified and named in relation to their relative adsorbabilities (*13*, *15*), unusual care must be exercised in the purification of solvents and in the preparation and description of adsorbents.

In order to be regarded as "chromatographically homogeneous" (*9*) substances should be adsorbed on a variety of adsorbents from dilute solution in different solvents and solvent mixtures. Determination of the identity or nonidentity of two substances by adsorption of a mixture of them upon Tswett columns will be most conclusive when different solvents and adsorbents are employed.

Reversibility of the adsorption sequence often provides a convenient and rapid method for preparation of substances in a high state of purity, for the purity of adsorbed substances is related to the sequence. When a mixture of substances is separated in a Tswett column, each adsorbed compound may be contaminated with small, trailing portions of each less adsorbed substance (*4*). By contrast, each adsorbed substance is entirely free of the compounds which are adsorbed above it in the column. Consequently if a substance which forms an upper band can be readsorbed under conditions such that it then forms the least adsorbed band it will be free of the contaminants.

Substances adsorbed on a given adsorbent in one sequence from one solvent and in another sequence from another solvent can be made to occupy various intermediate positions by adsorption from mixtures of the two solvents. Analogously, substances adsorbed from a given solvent in one sequence in columns of one adsorbent and in another sequence in columns of a second adsorbent should be adsorbable in various intermediate positions in columns composed of mixtures of the two adsorbents.

Variation of the adsorption sequence especially among compounds of the same class indicates that the relationship between adsorbability and chemical structure is much more complex than was at first supposed. Experience with the chlorophylls and xanthophylls suggests that the relative adsorbabilities depend upon competition of solvent, solute, and adsorbent for one another. Adsorbability of some molecules is especially sensitive to the attraction between solvent and adsorbent. Adsorbability of other molecules appears to be particularly dependent upon the attractive forces between solvent and solute. With some molecules, such as indicators, change of solvent may alter the molecular structure and the reactivity of functional groups, thus affecting the adsorbability. In static systems, the summation of these effects has usually been expressed by the adsorption isotherm, but the relation between the adsorption isotherm and the chemical structure of the solute has been difficult to interpret (*1*, *2*).

Recent theoretical and experimental work has shown that the rate of migration of a solute through an adsorption column is a function of the adsorption isotherm (*1*, *2*, *4*, *5*, *6*, *8*, *14*). It follows, therefore, that the adsorption sequence is also a function of the adsorption isotherms of the several solutes. Variation of the adsorption sequence with different conditions in the columns must result from disproportionate variation of the adsorption isotherms.

As a rule the slope of the adsorption isotherm is more nearly constant at low concentrations of solutes than at higher concentrations (*2*). Consequently, the adsorption sequences should be subject to less variation and the separation of the bands from one another should be more complete when mixtures of solutes are adsorbed from dilute solution than when adsorbed from more concentrated solution.

As solutes pass through an adsorption column the bands widen, so that the concentration of each solute decreases constantly. The final relative positions of the bands in a long column will, therefore, be determined by the slope of the adsorption isotherms at low concentration. Theoretically, the trailing boundaries of adsorption bands provide a better basis for estimation of the adsorption sequences than do the leading boundaries (*4*, *5*), but the diffuse trailing boundaries are usually more difficult to locate than the better defined leading boundaries, and they may be distorted because of the slowness with which the last traces of solute diffuse from porous particles of the adsorbent.

The resolving power of an adsorption column depends upon the properties of the adsorbent, the solvent, and the solutes. It depends in part upon the relative concentration of the solutes, upon the amount of solution added to the column, and presumably also upon the temperature. The interrelationship of these properties and conditions is so complex and the tendency of many adsorbents to combine irreversibly with the solutes is so common that the selection of solvents and adsorbents for the resolution of unknown mixtures remains largely a matter of trial and error.

Conditions that fix the sequence of solutes in columns should determine the relative adsorbabilities of these solutes in other adsorption procedures such as the fractional adsorption technique, described by Craig and co-workers (*3*), in which equal portions of the adsorbent are exposed in succession to one portion of the solution and then in turn to successive portions of the

solvent. Variation of the adsorbability may also be utilized to enhance the sensitivity and to broaden the applicability of most analytical methods based upon the phenomenon of adsorption.

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Determination of Chlorine in 2,2′-Dihydroxy-5,5′-dichlorodiphenylmethane

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Rapid determination of 2,2′-dihydroxy-5,5′-dichlorodiphenylmethane is accomplished by a modification of the U.S.P. XII method for assay of iodophthalein sodium. The compound is decomposed by alkaline permanganate solution and the chloride formed is determined by the Volhard titration or by a photometric turbidity method. The application of the method for analysis of powders, emulsions, and fabrics is of particular interest.

IN CONNECTION with work on the compound 2,2′-dihydroxy-5,5′-dichlorodiphenylmethane, an efficient mildew-proofing agent (*5*) known also as DDM, or as Compound G-4 to the textile trade, it became necessary to devise a rapid method of analysis suitable for control operations. The procedure developed depends upon the oxidation of DDM with alkaline potassium permanganate and, after acidification, reduction of excess permanganate with sodium nitrite. The liberated chloride is then determined by the Volhard (*9*) method or by the photometric measurement of the turbidity of silver chloride suspensions.

The method generally used for determination of this compound is based upon that of Willard and Thompson (*10*) and is unsatisfactory here because it is a procedure for total chlorine. Furthermore, it involves a number of steps which prolong the procedure beyond the time available for control analysis. The authors have avoided these difficulties, and have developed a method applicable to technical or pure DDM powders, solutions, or emulsions, and impregnated fabrics. The behavior of organo-iodine compounds with a method (*2*) similar to the proposed indicates that organo-halogen compounds which are alkali-soluble and easily oxidized are included in the reaction; hence the procedure is not a specific test but is admirable for control purposes.

The method here presented is similar to that of Leclercq (*4*), later modified by Butler and Burdett (*1*). The latter determined iodine in sodium tetraiodophenolphthalein by oxidation of the organic matter with alkaline potassium permanganate, and, after acidification, reduced the excess permanganate with sodium bisulfite. The iodide was then titrated with silver nitrate and the end point detected with an adsorption indicator. Excellent results were obtained which were checked by the Pregl (*6*) combustion method on a semimicro scale. The Butler-Burdett method is now one of the official U.S.P. XII (*8*) procedures for the determination of iodine in organic pharmaceuticals.

The determination of iodine in organic compounds by an acid permanganate oxidation is well known. Seeker and Mathewson (*7*) used an acid permanganate oxidation to determine the iodine content of erythrosine. Of more recent date is the work of Clark and Jones (*2*) who employed both an acid and alkaline permanganate method for the determination of iodine in organic compounds soluble in acids or bases. To the knowledge of the authors, the alkaline permanganate oxidation method has not yet been applied to the determination of chlorine in organic compounds. No claim is made of its general applicability to organic compounds containing chlorine, but it has been successfully applied to the estimation of DDM.

Table I. Analysis of Purified DDM

Method	Chlorine	Deviation from Theory
	%	%
Proposed	20.12	−0.24
Pregl	26.46	+0.10

PROCEDURE FOR DDM POWDER

Place approximately 0.2 gram of DDM powder, accurately weighed, into a 500-ml. Erlenmeyer flask and dissolve in 15 ml. of 10% sodium hydroxide. Add 35 ml. of saturated potassium permanganate; the characteristic purple color of the permanganate should persist. If the mixture is green, add more saturated potassium permanganate. Add a few glass beads to control bumping and boil the mixture gently for 10 minutes. Cool under the tap to room temperature and add 75 ml. of distilled water followed by 10 ml. of concentrated nitric acid. Reduce the excess permanganate immediately with 10% sodium nitrite, preferably added from a buret. Add 25 ml. of 0.1 *N* silver nitrate and titrate the excess with 0.1 *N* ammonium thiocyanate, using 2 ml. of ferric alum as the indicator.

Perform a blank determination with the same quantities of the same reagents and in the same manner.

DISCUSSION OF METHOD

The proposed method was checked by analysis of both technical and pure DDM. Purified DDM, prepared from the technical grade by three crystallizations from ethylene dichloride followed by a single crystallization from distilled water, was analyzed by the proposed method and checked by a Pregl microcombustion analysis (Table I). From the good agreement obtained with these independent methods, it is apparent that oxidation of the DDM is complete with alkaline permanganate, and that the method is more than sufficiently accurate for control procedures.

Table II. Analysis of Technical DDM

Sample	Per Cent DDM Proposed method	Pregl method
A	94.2	99.6
B	93.9	96.4

Table III. Analysis of DDM on Fabric

Sample No.	Per Cent of DDM on Fabric Gottlieb-Marsh method	Silver chloride turbidity method
1	0.89	0.84
		0.80
2	1.09	1.04
		1.05
3	1.51	1.56
		1.55
4	1.72	1.68
		1.64

The proposed method has been applied to the analyses of technical DDM samples. An inspection of the results in Table II shows that analysis of technical DDM by total halogen determination may lead to erroneous conclusions as to the DDM content. The lower DDM values obtained by the proposed method compared with those obtained by Pregl microcombustion analysis would indicate the presence in technical DDM of organic halogen compounds which were not oxidized by the alkaline permanganate method. A check of these technical DDM samples revealed a negligible amount of inorganic halogen present.

ANALYSIS OF EMULSIONS

The analysis of emulsions containing DDM, frequently used to impart water-repellency as well as mildew-proofness to fabrics, requires little change in the procedure described previously for DDM powder. For the purpose of choosing a sample weight or aliquot volume, 0.1 gram of DDM requires 7.43 ml. of 0.1 *N* silver nitrate. The wax used in these emulsions presents no difficulty, since it readily separates to the surface during oxidation and does not interfere with the final titration.

ANALYSIS OF FABRICS

Because of the small quantity of DDM generally applied to fabrics, the chloride content of the oxidized extract does not permit convenient titration. However, the liberated chloride is readily determined by photometric methods. The acidity of the solution following addition of sodium nitrite is adjusted so that the silver chloride is maintained in suspension. The turbidity of this suspension is then measured with a suitable photometer—a Fisher electrophotometer was employed in the work presented here—and the DDM content determined by reference to a standard calibration curve.

Solutions. Nitric acid, 1 to 4. Dilute 50 ml. of concentrated nitric acid, sp. gr. 1.42, with 150 ml. of distilled water.

Sodium carbonate, 0.025%. Dissolve 0.5 gram of anhydrous sodium carbonate in 2 liters of distilled water.

Sodium hydroxide, 2.5%. Dissolve 5 grams of sodium hydroxide in 195 ml. of distilled water.

Phenolphthalein indicator, 0.5%. Dissolve 0.5 gram of phenolphthalein in 100 ml. of 95% ethyl alcohol.

Standard Curve. Since the logarithm of the transmittance of silver chloride suspensions is an inverse and very nearly linear function of the turbidity, four determinations are adequate to establish the curve. To illustrate this point, 2, 4, 6, 10, and 15 mg. of DDM in a final volume of 200 ml. gave respective photometric logarithmic readings (corrected for reagent blank) of 9.8, 18.0, 28.1, 48.1, and 74. Establish the curve by first dissolving 500 mg. of DDM in sufficient 2.5% sodium hydroxide to make 100 ml. of solution. Use suitable aliquots of this solution, containing 5 mg. of DDM per ml., to cover the range of 2 to 15 mg. The procedure followed is exactly as described below for the fabric extract.

Fabric Analysis. Weigh a 2-gram sample of the very finely cut fabric to the nearest milligram and place in a 150-ml. beaker. Extract three times with successive 50-ml. portions of 0.025% sodium carbonate by boiling gently for 5 minutes and decanting the hot solution into a 200-ml. Pyrex volumetric flask. Wash the extracted fabric twice with 15-ml. portions of 0.025% sodium carbonate and combine with the extract solution. Dilute to the mark with 0.025% sodium carbonate after the flask has cooled to room temperature. Filter about 50 ml. through a dry filter. Then follow the oxidation procedure for analysis of DDM powder with these exceptions: Use a 25-ml. aliquot of the filtrate; reduce the volume of saturated potassium permanganate to 25 ml.; omit the dilution with water after oxidation. Following reduction of the excess permanganate with 10% sodium nitrite, add a few drops of phenolphthalein indicator and then sufficient 10% sodium hydroxide to produce a faint pink color. Acidify with 10 ml. of 1 to 4 nitric acid and transfer to a 200-ml. volumetric flask. Cool to room temperature, add 4 ml. of 0.1 *N* silver nitrate, dilute to the mark with distilled water, and invert the flask several times. Let stand 10 minutes and read with the Fisher electrophotometer, using a 525 filter.

With the above procedure it is necessary to establish two blank values—extract blank and reagent blank. For the extract blank, pipet a 25-ml. aliquot of the filtrate into a 200-ml. volumetric flask, add 125 ml. of 0.025% sodium carbonate, 10 ml. of 1 to 4 nitric acid, and 4 ml. of 0.1 *N* silver nitrate, and dilute to the mark. Invert the flask several times and read after 10 minutes. This extract blank, representing inorganic chloride, is frequently negligible. For the reagent blank, follow the procedure described above, omitting the 25-ml. extract aliquot. This reagent blank value need be determined only once for a set of reagents.

The extract procedure above described is essentially the same as that developed by Gottlieb and Marsh (*3*) for the determination of DDM by the color reaction with 4-aminoantipyrine in the presence of potassium ferricyanide and dilute sodium carbonate. Table III gives the comparative results obtained from analysis of four samples of fabric by the Gottlieb-Marsh colorimetric and silver chloride turbidity methods.

CONCLUSIONS

The alkaline permanganate procedure used successfully for the analysis of certain organic iodine compounds has been adapted for the routine analysis of DDM. Analysis of purified DDM indicates the reaction is complete, as substantiated by a Pregl microcombustion analysis. The alkaline permanganate method appears to be of particular value in the analysis of technical DDM which has organic chlorine-containing impurities. Analysis of such technical material indicates that these impurities are not as susceptible to oxidation by the proposed method as by the Pregl microcombustion total halogen method. Of further particular interest is the applicability of the described method for analysis of powders, emulsions, and fabrics containing DDM.

ACKNOWLEDGMENT

The authors are indebted to Ralph O. Clark of the staff of the Gulf Research and Development Company for the Pregl microcombustion halogen analyses. Thanks are also due to F. W. Adams of the Spool Cotton Company for many helpful suggestions.

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Viscosities of Pure Hydrocarbons

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THE purpose of this paper is to record the viscosities of pure hydrocarbons.

The viscosities were determined in several viscometers of the type shown as Figure 1, which is but a slight modification of the type previously recommended for nonviscous materials (*4*).

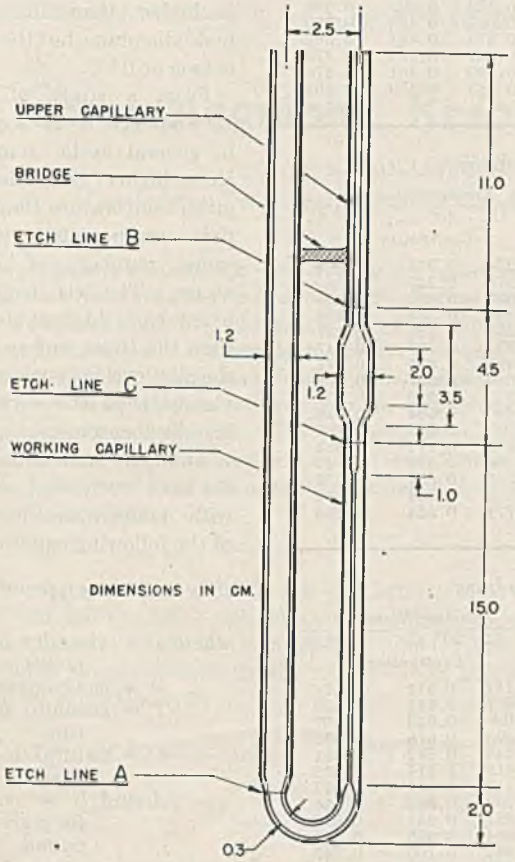

Figure 1. Type C Viscometer

After this work was finished it was found that the viscometer shown as Figure 2 is faster to use and just as accurate, and it is being used in extending this work. For viscous liquids above 800 centipoises it is more accurate because the larger diameter and lower reservoir reduces the effect of drainage on the driving fluid head. It is used in the manner described previously (*4*). For the low viscosity data reported here, this drainage effect is below 0.05%.

The most troublesome source of error in the viscosity range below 2 centipoises is the so-called "kinetic energy" error. This was kept small by so designing the viscometers that velocities were kept low enough to prevent this correction from exceeding 0.2%. As the probable error in making this correction is of the order of ±20%, a net error of 0.04% may occur from this source. Other sources of error in viscosity measurements have been discussed in detail (*4*).

The type C viscometer shown as Figure 1 is loaded as previously described (*4*). A slight excess of 0.2 or 0.3 cc. is then added to the instrument which is placed in an accurately controlled constant-temperature bath. The 0° C. bath was controlled to within ± 0.05° C. while the 20° C. and 40° C. baths were controlled to within ±0.01° C. When bath temperature is attained, slight pressure is applied to the wide arm of the viscometer and the bottom of the meniscus is brought to etched line *A*. The slight excess liquid is then wiped from the capillary arm. The excess so removed will be somewhat different from the excess charged, because the volume of liquid will change in going from room temperature to the test temperature of the bath. This simple technique allows the same viscometer constant to be used over a wide temperature range, although other methods of making the necessary slight corrections in the viscometer constant for each temperature are satisfactory. The viscometers were calibrated with distilled water at 20° C. and checked by hydrocarbons which had been standardized in master viscometers (*3*). The viscosity of the test sample is obtained by multiplying the efflux time in seconds (the efflux volume timed is that between lines *B* and *C*) by the viscometer constant. This yields kinematic viscosity in centistokes, which in turn must be multiplied by density in order to obtain absolute viscosity in centipoises.

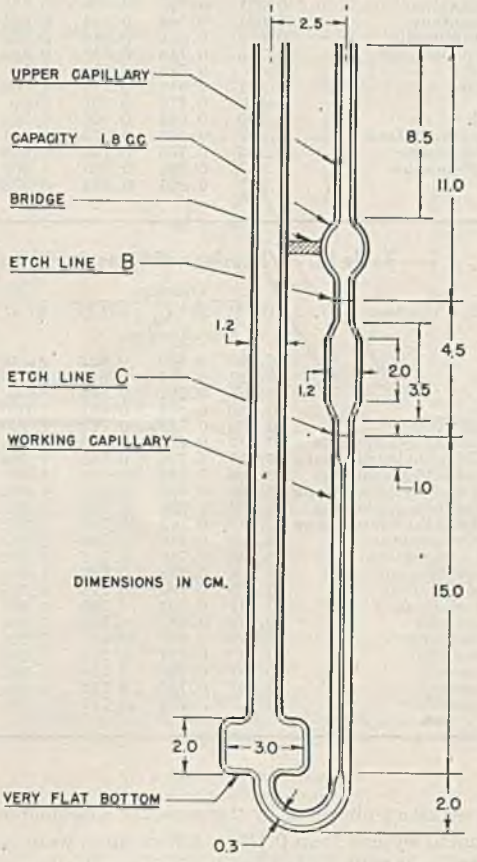

Figure 2. Type E Viscometer

All samples were tested with two separate viscometers and stop watches. The stop watches were spring-wound and tested against a large electric clock operated by constant-frequency constant-voltage power. The spring-wound stop watches were found more reliable than several types of electric stop clocks which operate by a magnetic clutch engaging and disengaging a constant-speed rotor. In these the clutch frequently slips and sometimes fails to make a sharp disengagement, so that the clock drifts past the true stopping point. The correction necessary on the spring-wound stop watches employed never exceeded 0.05%.

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Table I. Viscosities of Octanes

(Listed in decreasing value at 20° C.)

Hydrocarbon	Density			Kinematic Viscosity			Absolute Viscosity		
	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.
	Gram per cc.			*Centistokes, $cm^2 \times sec.^{-1} \times 10^{-2}$*			*Centipoise, $g. \times cm.^{-1} \times sec.^{-1} \times 10^{-2}$*		
2,2,3,3-Tetramethylbutane	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid
2,3,3-Trimethylpentane	0.741	0.726	0.711	1.227	0.933	0.739	0.909	0.677	0.525
2,2,3-Trimethylpentane	0.731	0.715	0.699	1.074	0.836	0.674	0.785	0.598	0.471
3-Methyl-3-ethylpentane	0.742	0.727	0.712	1.034	0.799	0.642	0.768	0.581	0.457
2,3,4-Trimethylpentane	0.734	0.719	0.703	0.969	0.764	0.624	0.712	0.549	0.438
n-Octane	0.718	0.712	0.686	0.985	0.771	0.626	0.707	0.542	0.429
2,2-Dimethylhexane	0.711	0.695	0.679	0.975	0.758	0.611	0.694	0.527	0.414
2,2,4-Trimethylpentane	0.707	0.691	0.675	0.917	0.738	0.598	0.649	0.504	0.403
2-Methylheptane	0.713	0.698	0.681	0.909	0.717	0.586	0.648	0.500	0.399
3,3-Dimethylhexane	0.726	0.710	0.694	0.890	0.703	0.575	0.645	0.499	0.399
2,3-Dimethylhexane	0.729	0.713	0.697	0.881	0.698	0.571	0.643	0.497	0.398
3,4-Dimethylhexane	0.734	0.718	0.703	0.862	0.686	0.564	0.633	0.493	0.396
3-Methylheptane	0.721	0.705	0.689	0.886	0.697	0.570	0.639	0.492	0.393
2-Methyl-3-ethylpentane	0.735	0.719	0.702	0.856	0.678	0.556	0.628	0.488	0.390
2,5-Dimethylhexane	0.710	0.693	0.677	0.883	0.698	0.571	0.626	0.484	0.386
4-Methylheptane	0.720	0.704	0.689	0.829	0.659	0.542	0.597	0.464	0.373
2,4-Dimethylhexane	0.716	0.700	0.683	0.824	0.658	0.542	0.590	0.461	0.370
3-Ethylhexane	0.729	0.714	0.697	0.799	0.635	0.523	0.583	0.453	0.364

Table II. Viscosities of Some 5-, 6-, and 7-Carbon Hydrocarbons

Hydrocarbon	Density			Kinematic Viscosity			Absolute Viscosity		
	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.
	Gram per cc.			*Centistokes*			*Centipoises*		
n-Pentane	0.644	0.626	Vapor	0.426	0.362	Vapor	0.274	0.227	Vapor
Isopentane	0.638	0.619	Vapor	0.435	0.363	Vapor	0.278	9.225	Vapor
Cyclopentane	0.764	0.745	0.726	0.728	0.590	0.489	0.555	0.440	0.355
n-Hexane	0.677	0.659	0.641	0.564	0.468	0.397	0.382	0.308	0.254
2-Methylpentane	0.671	0.653	0.635	0.523	0.437	0.372	0.351	0.286	0.237
3-Methylpentane	0.681	0.664	0.646	0.544	0.451	0.382	0.371	0.299	0.247
2,3-Dimethylbutane	0.679	0.661	0.642	0.626	0.508	0.422	0.425	0.336	0.271
Methylcyclopentane	0.766	0.748	0.730	0.848	0.677	0.555	0.650	0.507	0.405
Cyclohexane	Solid	0.778	0.759	Solid	1.259	0.926	Solid	0.980	0.703
Cyclohexene	0.831	0.813	0.794	1.066	0.816	0.653	0.886	0.664	0.518
Benzene	Solid	0.879	0.857	Solid	0.739	0.573	Solid	0.649	0.492
n-Heptane	0.700	0.683	0.666	0.745	0.602	0.499	0.521	0.411	0.333
2,2,3-Trimethylbutane	0.707	0.690	0.673	1.141	0.853	0.668	0.806	0.589	0.449
Ethylcyclopentane	0.783	0.766	0.749	0.928	0.740	0.609	0.726	0.567	0.456
Methylcyclohexane	0.787	0.769	0.753	1.263	0.954	0.750	0.993	0.734	0.564
Toluene	0.883	0.865	0.848	0.878	0.676	0.549	0.774	0.585	0.465

Table III. Viscosities of Some 8-, 9-, and 10-Carbon Hydrocarbons

Hydrocarbon	Density			Kinematic Viscosity			Absolute Viscosity		
	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.
	Grams per cc.			*Centistokes*			*Centipoises*		
o-Xylene	0.897	0.880	0.862	1.240	0.922	0.723	1.111	0.811	0.624
m-Xylene	0.881	0.864	0.847	0.916	0.714	0.581	0.807	0.617	0.492
p-Xylene	Solid	0.861	0.843	Solid	0.748	0.602	Solid	0.643	0.507
Ethylbenzene	0.886	0.868	0.851	1.010	0.781	0.631	0.895	0.678	0.536
Ethylcyclohexane	0.804	0.788	0.772	1.420	1.068	0.842	1.142	0.842	0.651
cis-1,2-Dimethylcyclohexane	0.812	0.796	0.780	1.988	1.399	1.048	1.615	1.114	0.818
trans-1,2-Dimethylcyclohexane	0.791	0.776	0.760	1.404	1.053	0.825	1.111	0.817	0.627
cis-1,3-Dimethylcyclohexane	0.802	0.785	0.769	1.501	1.103	0.850	1.203	0.866	0.654
trans-1,3-Dimethylcyclohexane	0.782	0.766	0.750	1.063	0.825	0.667	0.831	0.631	0.500
cis-1,4-Dimethylcyclohexane	0.799	0.783	0.766	1.531	1.119	0.861	1.224	0.875	0.659
trans-1,4-Dimethylcyclohexane	0.779	0.762	0.746	1.222	0.926	0.733	0.951	0.706	0.547
n-Propylcyclopentane	0.792	0.776	0.756	1.123	0.877	0.711	0.889	0.681	0.540
Isopropylcyclopentane	0.792	0.776	0.761	1.162	0.909	0.735	0.920	0.706	0.559
1,1,3-Trimethylcyclohexane	0.794	0.778	0.763	1.510	1.120	0.871	1.199	0.873	0.664
n-Propylcyclohexane	0.809	0.793	0.778	1.744	1.268	0.976	1.410	1.005	0.759
Isopropylcyclohexane	0.817	0.803	0.786	1.880	1.369	1.049	1.535	1.097	0.824
n-Propylbenzene	0.878	0.862	0.845	1.348	0.995	0.778	1.183	0.857	0.657
Isopropylbenzene	0.879	0.862	0.845	1.226	0.917	0.725	1.077	0.790	0.613
2-Methylnonane	0.742	0.727	0.712	1.644	1.199	0.925	1.220	0.872	0.659
3-Methylnonane	0.747	0.733	0.718	1.582	1.159	0.898	1.182	0.849	0.645
4-Methylnonane	0.747	0.732	0.716	1.498	1.100	0.854	1.118	0.804	0.612
5-Methylnonane	0.747	0.732	0.717	1.526	1.116	0.866	1.140	0.816	0.620

The viscosities obtained in the separate viscometers were in disagreement by less than 0.1%. Efflux times were maintained above 200 seconds in order to make accurate time measurements possible as well as keep the kinetic energy error low. The accuracy of the viscosity data is dependent upon the accuracy to which the viscosity of water at 20° C. is known, since it was employed as the primary calibrating liquid. A value of 1.007 centistokes was used, as recommended by the American Society of Testing Materials (*1*). Because of the great difficulties in determining absolute viscosities, the error inherent in the 1.007 value may be of the order of ±0.5%.

However, the precision of the data recorded here is ±0.1%. Therefore, while absolute viscosities may be in error by as much as 0.5%, the comparative viscosities are in error by only 0.1% and one compound may be compared with another to that degree of precision.

The viscosities of the eighteen isomeric octanes are listed in Table I according to decreasing absolute viscosity at 20° C. Many of the compounds have widely different viscosity temperature coefficients; thus at 20° C. the absolute viscosity of 3,4-dimethylhexane is higher than that of 3-methylheptane, but the reverse is true at 0° C.

From a study of Tables II and III it is seen that in general cyclic compounds have higher viscosities at a given temperature than paraffinic compounds with the same number of carbon atoms. The cis compounds listed have higher viscosities than the trans, and in general the alkylcyclohexanes are more viscous than the corresponding alkylbenzenes.

Andrade and others (*2*, *6*) have correlated viscosity with temperature by means of the following equation:

$$\eta = A(e)^{B/RT}$$

where η = viscosity in centipoises
R = gas constant
T = absolute temperature
e = natural logarithm base
A and B = constants for a given compound

With the data listed in Table I the values of A and B were determined for 2,2,4-trimethylpentane, and viscosities at other temperatures were then calculated. Evans (*5*) measured the viscosities of this compound at several temperatures; hence it is possible to compare the calculated values with measured values (Table IV).

Table IV. Viscosity of 2,2,4-Trimethylpentane

Temperature	Observed Viscosity, Evans	Calculated Viscosity, Andrade Equation
° C.	*Centipoise*	*Centipoise*
5	0.608	0.607
15	0.534	0.535
30	0.447	0.449
45	0.380	0.383
60	0.327	0.331
75	0.284	0.290
90	0.248	0.257

ACKNOWLEDGMENT

The samples of A.P.I.-N.B.S. hydrocarbons were made available by the American Petroleum Institute and the National Bureau of Standards through A.P.I. Research Project 44 on the Collection, Analysis, and Calculation of Data on the Properties of Hydrocarbons. The samples were purified at the National Bureau of Standards by A.P.I. Research Project 6 on the Analysis, Purification, and Properties of Hydrocarbons, under the supervision of Frederick D. Rossini, from material supplied by the following laboratories: *o*-xylene, Standard Oil Development Company, Elizabeth, N. J., through the courtesy of William J. Sweeney; 2,2-dimethylbutane, A.P.I. Research Project 6 at the National Bureau of Standards; the four methylnonanes by the Ethyl Corporation through the courtesy of George Calingaert; all others by A.P.I. Research Project 45 on the Synthesis and Properties of Hydrocarbons of Low Molecular Weight, at the Ohio State University under the supervision of Cecil E. Boord.

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PART of a thesis submitted by J. M. Geist in partial fulfillment of the requirements for the degree of master of science in chemical engineering at The Pennsylvania State College.

Chromatographic Resolution of the Quinone Oximes

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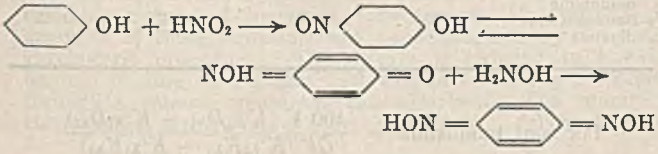
A chromatographic procedure is described for the separation of *p*-benzoquinone monoxime from *p*-benzoquinone dioxime and products of side reactions which take place during the nitrosation of phenol and oximation. The sample is dissolved in acetone and adsorbed on a column of activated alumina. The monoxime forms a brilliant green band which is removed from the column and eluted with aqueous sodium hydroxide. The concentration is determined by spectrophotometric measurements at 363 and 399 mμ. The chromatographic resolution and optical properties of the ortho isomers are also discussed.

BOTH *p*-benzoquinone dioxime and its derivatives have been used for the nonsulfur vulcanization of rubber (*8*), and are of current interest for the vulcanization of GR-I. The parent compound is usually formed by the nitrosation of phenol (*17*), followed by oximation (*12*).



During the course of work on the preparation of this material it was of interest to follow the rate of oximation, and determine the concentration of unreacted monoxime in the final product.

As this substance is tautomeric with *p*-nitrosophenol, a method described by Clauser (*5*) for the determination of the nitroso group by reaction with phenylhydrazine and measurement of evolved nitrogen was investigated. However, under these conditions a large volume of gas is obtained from the dioxime. Both compounds are reduced by potassium iodide in hydrochloric acid solution (*6*), but the reduction of the monoxime proceeds more rapidly. This suggested the possibility of a polarographic analysis. At a pH of 7.0 in a phosphate buffer, the half-wave potential of the monoxime was found to be 0.15 volt more positive than that of the dioxime, and on solutions of the pure compounds, the diffusion currents are well defined. However, an unresolved wave is usually obtained on the crude product, owing to an unfavorable concentration ratio and the presence of other electroreducible materials in the mixture.

p-Benzoquinone monoxime is readily soluble in dilute sodium hydroxide with the formation of the sodium salt, which is believed to exist largely in the ionized quinone oxime form (*1*, *10*). The principal absorption maximum of the ion is at 399 mμ (Figure 1), while that of the neutral molecule in acid solution is at 301.5 mμ. Anderson and Yanke (*1*) point out that the absorption spectrum of the system is sensitive to changes in hydrogen-ion concentration between pH 3 and 7 and is independent of this factor in more strongly acid or alkaline solutions. In order to ensure reproducibility of the extinction coefficients, and provide a

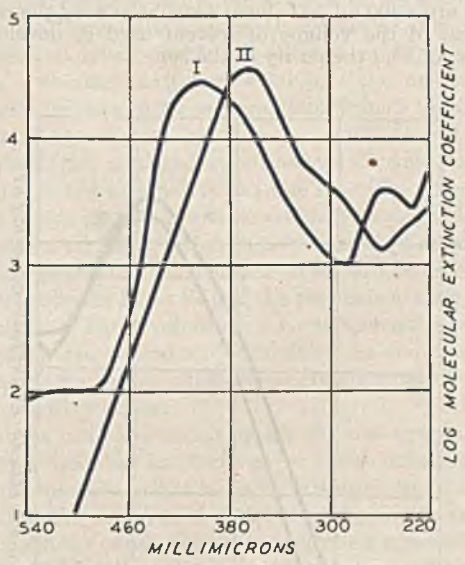


Figure 1. Absorption Spectra of Quinone Oximes in 0.1 *N* Aqueous Sodium Hydroxide Solution

I. *p*-Benzoquinone monoxime
II. *p*-Benzoquinone dioxime

medium in which the product is sufficiently soluble, the quantitative measurements described in this paper were made in 0.1 *N* sodium hydroxide solution. Quinone dioxime behaves in an analogous manner. The principal maximum attributable to the free ion is at 363 mμ (Figure 1), while that of the neutral molecule occurs at 317 mμ (Figure 2). As both compounds obey the Beer-Lambert law within experimental error, a direct calculation of the composition of a binary mixture is possible from optical readings made at 363 and 399 mμ on solutions of the sodium salts, providing no interfering substances are present and the concentration ratio is favorable. As these conditions cannot be predicted, and in general are not satisfied, it is necessary to remove the products

of side reactions which take place during the nitrosation of phenol and concentrate the monoxime at the expense of the dioxime without loss of the former.

When a solution of the crude oximation product in acetone is percolated through a column containing activated alumina, the constituents of the mixture adsorb in easily distinguishable zones which can be further resolved by washing the column with a more polar solvent. The monoxime forms a green zone, while the dioxime forms a brilliant yellow zone. The schematic diagram (Figure 3) illustrates the separations normally obtained. Band I is dark brown in color, and contains an amorphous material of indefinite composition produced by resinification of the monoxime (*17*). Band II contains *p*-benzoquinone monoxime, and Band III, *p*-benzoquinone dioxime. In the lower section of the column, narrow blue, red, and yellow bands appear which are completely removed from the column during development with solvent. The initial fraction of the percolate is orange yellow in color, and contains substances not readily adsorbed from acetone by alumina. On evaporation of the solvent and readsorption from petroleum ether, these materials can be further resolved.

The green zone containing quinone monoxime initially forms directly below the brown resinous material and is not readily separated from it by continued washing. Complete resolution is achieved by treatment of the column with a dilute solution of acetic acid in acetone. The acid is adsorbed less strongly than the resinous material, and more strongly than the monoxime which results in the formation of a colorless zone between the two materials. The development of the column is completed by washing with 300 ml. of a 5% solution of methanol in acetone. The effect of this solvent is to wash the lower zones and part of the dioxime into the percolate, and improve the separation of the oximes. At the end of this treatment the green zone contains all of the monoxime but is contaminated with 20 to 35% dioxime which is not readily removed by further washing. As a spectrophotometric correction can be made for residual dioxime, a complete separation is unnecessary. The analytical values in Table I show that after 300 ml. of solvent have been used the results are independent of the volume of solvent used in developing the chromatogram and the purity of the zone.

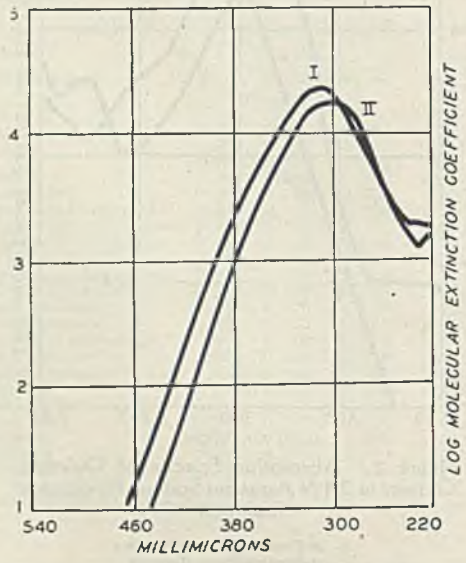


Figure 2. Absorption Spectra of Quinone Oximes in 0.1 *N* Aqueous Sulfuric Acid Solutions

I. *p*-Benzoquinone monoxime
II. *p*-Benzoquinone dioxime

The analysis is completed by separating the adsorbent containing monoxime from the rest of the column and eluting it with aqueous sodium hydroxide. A yellow-brown solution is obtained on which the monoxime content is determined by absorption measurements at 399 and 363 mμ. In making the analysis as outlined, low results are obtained owing to incomplete elution of the material from the adsorbent. Experiments made on the pure compound indicate that the average recovery is 95.0 ± 0.8%. When this factor is incorporated into the calculations, results close to theory are obtained.

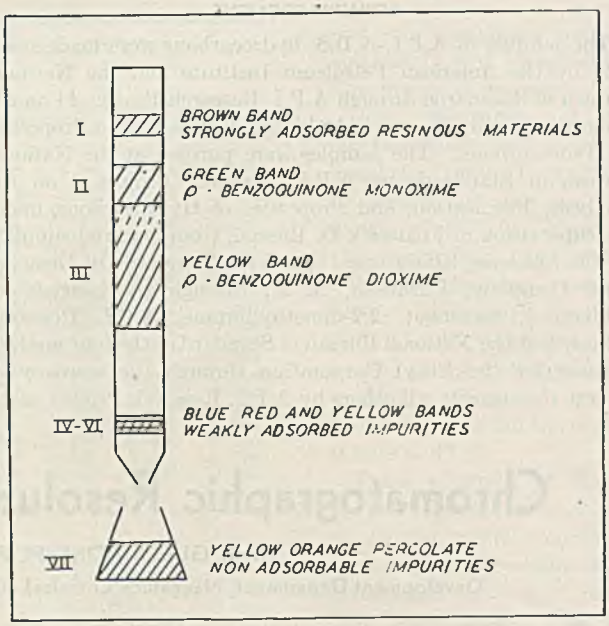


Figure 3. Chromatographic Resolution of Crude *p*-Benzoquinone Dioxime

Table I. Effect of Volume of Solvent Used in Development of Chromatogram on Results of Monoxime Analysis and Purity of Zone

Solvent	Monoxime Found	Dioxime in Monoxime Zone
Ml.	%	%
0	4.9	72
100	5.5	28
200	5.4	34
300	5.6	23
500	5.6	25
800	5.6	35
1000	5.6	20

Table II. Analytical Constants

Compound	*K* at 399 mμ	Slit Setting *Mm.*	*K* at 363 mμ	Slit Setting *Mm.*
p-Benzoquinone monoxime	224	0.10	109	0.15
p-Benzoquinone dioxime	283	0.10	65.7	0.15

$$\text{Per cent monoxime} = \frac{100\ V\ (K'_{\lambda_2}D_{\lambda_1} - K'_{\lambda_1}D_{\lambda_2})}{GF\ (K'_{\lambda_2}K_{\lambda_1} - K'_{\lambda_1}K_{\lambda_2})}$$

where D_{λ_1} and D_{λ_2} are the measured optical densities at 399 and 363 mμ, G is the sample weight, V is the volume in liters to which the solution is diluted, K'_{λ_1} and K_{λ_2} are the specific extinction coefficients of the monoxime at 399 and 363 mμ, K'_{λ_1} and K'_{λ_2} are the specific extinction coefficients of the dioxime at 399 and 363 mμ, and F is the decimal per cent monoxime recoverable after adsorption on alumina.

This equation is a modification of the type generally used in the spectrophotometric analysis of binary mixtures where the absorption bands overlap. Details concerning the derivation are given by Ashley (*3*).

Spectrophotometric constants determined on solutions of the pure compounds in 0.1 *N* sodium hydroxide are shown in Table II. The optical measurements were made with a Beckman Model DU spectrophotometer in 1-cm. quartz cells, using a tungsten lamp and a red purple filter. Both compounds obey the Beer-Lambert law within experimental error at concentrations corresponding to a density range of 0.4 to 1.0.

APPARATUS

Adsorption columns are conveniently made from 35-cm. lengths of 18-mm. outside diameter Pyrex tubing constricted to an internal diameter of about 0.5 cm. about 10 cm. from the end.

Table III. Analyses of Synthetic Mixtures

Monoxime Added %	Monoxime Found %	Deviation %
5.5	5.7	+0.2
4.3	3.9	−0.4
4.5	4.3	−0.2
4.6	4.4	−0.2
6.5	6.7	+0.2
4.9	5.5	+0.6
5.8	5.5	−0.3
6.0	6.2	+0.2
7.8	8.0	+0.2
4.8	5.0	+0.2
4.8	4.8	0.0
7.0	6.9	−0.1
Av. 5.54	5.58	±0.23

Table IV. Analysis of Crude Reaction Products

Monoxime, %	4.9	5.6	3.5	4.8	4.4
Dioxime, %	90.7	84.5	79.4	77.8	91.3
Resinous material, %	2.6	4.5	14.6	10.9	3.1
Inorganic, %	0.8	2.9	1.6	1.1	0.1
Total	99.0	97.5	99.1	94.6	98.9

The shorter end is connected to a suction flask and the constriction plugged with glass wool. The column is filled to a height of 15 cm. with a mixture of 90 parts by weight of grade F-1 — 80-mesh activated alumina (Aluminum Company of America) and 10 parts of Hyflo Super-Cel (Johns Manville Company) which is included to increase the percolation rate. The adsorbent is added to the column in 2- to 3-cm. portions and tamped firmly into place with a wooden dowel. Solvent is delivered continuously to the head of the column from a 500-ml. separatory funnel.

REAGENTS

Anhydrous analytical reagent grade solvents are used for the preparation of the chromatogram. The column is developed with a 1% by volume solution of acetic acid in acetone, and a 5% solution of anhydrous methanol in acetone. The adsorbent is eluted with aqueous *N* sodium hydroxide.

PROCEDURE

A 1-gram sample is dissolved in 100 ml. of acetone under reflux and filtered through a tared Selas crucible to remove acetone-insoluble resin and inorganic constituents. If desired, the amounts of these materials can be determined by the usual gravimetric procedures. The filtrate is reduced to half its original volume on a steam bath and after cooling percolated through a column containing the adsorbent. The column is then washed with 10 ml. of acetic acid solution, followed by 300 ml. of methanol in acetone. The top brown layer is removed with a spatula and discarded. The green segment is transferred quantitatively to a 250-ml. beaker and eluted with successive portions of sodium hydroxide. A total volume of about 100 ml. is required. The separation of the yellow zone need not be complete, as a correction for residual dioxime is made in the calculations. After filtration, the elutriate is diluted to 1 liter with distilled water and then rediluted to a concentration at which optimum optical density readings can be obtained. The measurements are made at 399 and 363 mμ with a Beckman spectrophotometer.

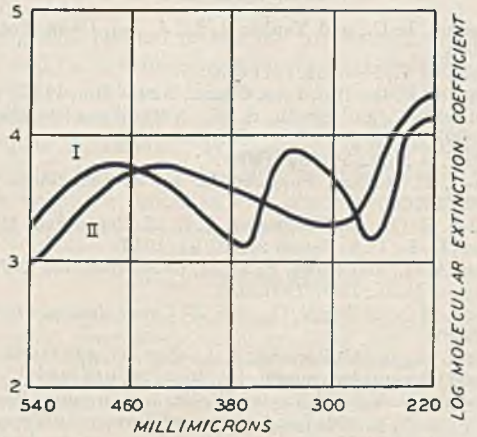


Figure 4. Absorption Spectra of Quinone Oximes in 0.1 *N* Aqueous Sodium Hydroxide Solution

I. o-Benzoquinone monoxime
II. o-Benzoquinone dioxime

PRECISION AND ACCURACY

The appearance of the adsorption column is subject to variation, depending on the composition of the sample and differences in the techniques used in preparing and developing the chromatograms. Impurities in the solvents and changes in the adsorptive capacity of the alumina may alter the widths of the bands formed from equal amounts of monoxime. Although a measurement of the band width will not give a quantitative estimate of the concentration, the appearance of a green zone provides direct visual evidence of the presence of the monoxime. A band width of 2 to 6 mm. is obtained on samples containing 10 mg. of this compound. Thus, the method is sufficiently sensitive to establish the presence of this substance in mixtures in which it occurs to the extent of less than 1%.

In order to evaluate the accuracy of the method under the conditions normally encountered in the analysis of crude reaction products, weighted quantities of the pure monoxime were added to a sample on which a preliminary analysis of 5.6% was obtained. This value was then subtracted from the analytically determined results. This information is shown in Table III. The average deviation is ±0.23%, while the average of the analytically determined values is 0.04% higher than theory. The precision of the method as determined by the analysis of 64 samples in duplicate by a routine analyst is ±3.6% of the material present. This series included samples containing from 2 to 55% quinone monoxime.

The use of this method for the analysis of crude reaction products is illustrated by the data in Table IV. The quinone dioxime content of the materials was determined by a gravimetric procedure based on its oxidation to polymeric "*p*-dinitrosobenzene" (*13*) with potassium ferricyanide in a carbonate buffer. The materials in bands IV to VI and the percolate are not included in the analyses. These values are not representative of the yields obtained on nitrosation and oximation, as the determinations were made on samples which were partly purified by separation from the mother liquor.

While the results obtained by the chromatographic procedure are not comparable in precision to those obtained by direct spectrophotometric methods under favorable conditions, in this application the removal of interfering impurities and the concentration of the minor constituent permits a reasonably accurate analysis which would otherwise be impossible to obtain.

DISCUSSION

The para isomer is the principal reaction product formed in the nitrosation of phenol. However, Veibel (*17*) has shown that as much as 10% of the *o*-nitrosophenol may be formed under certain conditions. As this compound is tautomeric, oximation would result in the presence of *o*-benzoquinone dioxime in the crude product. In order to investigate the possibility of interference from this source, the chromatographic behavior and optical properties of the ortho isomers were investigated. The experimental data are outlined in Table V.

Both ortho isomers adsorb above *p*-benzoquinone dioxime, and if present, can be mechanically removed from the column before elution. In the reaction products examined, these bands were not evident, probably owing to loss by solubility of the *o*-monoxime in the mother liquor.

Table V. Chromatographic and Spectrophotometric Properties of Quinone Oximes

	o-Monoxime	o-Dioxime	p-Monoxime	p-Dioxime
Position of band	I	II	III	IV
Color of band	Red	Orange	Green	Yellow
$\lambda_{max.}$ (ion) (mμ)	470	433.5	399	363
$\epsilon_{max.}$ (ion)	6,130	5,600	27,570	39,010
$\lambda_{max.}$ (molecule) (mμ)	400	398	301.5	317
$\epsilon_{max.}$ (molecule)	1,110	5,720	16,820	23,300
Dipole moment ($\mu \times 10^{18}$)	...	3.84	4.72	2.37

The isomeric dioximes are adsorbed in the order of their dipole moments, the ortho isomer with a moment of 3.84 *D* (*11*) forming the upper orange band, and the para isomer with a moment of 2.37 *D* forming the lower yellow band. This behavior is analogous to that of the position isomers of benzene, and to the cis-trans isomers of azobenzene in which the compound with the higher dipole moment is usually most strongly adsorbed (*2*, *15*). The moment of *p*-benzoquinone monoxime in dioxane solution has been reported to be 4.72 *D* (*7*). This compound is adsorbed between the *o*- and *p*-dioximes. Data on the electric moment of the *o*-monoxime are not available. However, a direct correlation between dipole moment and adsorbability should not necessarily be expected in this case, owing to possible shifts in the tautomeric equilibria. The *o*-monoxime is adsorbed as a red band at the top of the column, while the para isomer appears as a green band. This suggests that the ortho compound is adsorbed in the quinoid form, while the para isomer is adsorbed as *p*-nitrosophenol, for the green color is similar to that of compounds containing the free nitroso group (*14*). The proximity of the functional groups in the ortho compound, on the other hand, might lead to chelation with the adsorbent. This would tend to shift the equilibrium to the quinoid modification.

A more direct correlation is observed between the order of adsorption and the wave lengths of the spectrophotometric maxima in alkaline solution. In this case the compounds which absorb light of longer wave length are bound more tightly. It is evident that the compounds are polarized by contact with the alumina, for the free acids are pale yellow to almost colorless. While the formation of colored bands is not requisite to a good separation, it is of great practical convenience in locating and distinguishing between the adsorbed compounds.

Owing to restricted rotation about the C=N bond, the existence of cis-trans isomers of the benzoquinone dioximes was considered possible. However, under the experimental conditions described, the bands appear homogeneous and no differences in properties can be detected between materials eluted from the upper and lower portions of the columns.

The choice of the solvent, adsorbent, and eluant was guided by a consideration of the physical properties of the compounds. Acetone is the only solvent readily available in which they are easily soluble and from which they can be adsorbed by alumina. However, it is difficult to free from traces of water and alcohol and undergoes a condensation reaction in contact with the adsorbent which results in the presence of a high-boiling liquid in the percolate (*18*). These factors may cause variations in the widths of the bands, and complicate recovery of the impurities found in the percolate, but otherwise do not interfere with the analysis. Alumina, magnesia, talc, silica gel, and a number of other materials were tested as adsorbents, but, aside from alumina, magnesia was found to be the only one of potential value. Of the possible eluants, aqueous sodium hydroxide is the most effective. The compounds are readily removed from the adsorbent and optical measurements can be made directly on the filtered and diluted solutions without further adjustments.

The chromatographic adsorption method of Tswett (*16*) has found extensive use in the purification and estimation of naturally occurring substances. However, applications in the field of industrial organic analysis have been comparatively few. This may in part be ascribed to the relatively poor precision obtained when compared to standard gravimetric and volumetric methods, and to the small quantities of materials which can be isolated on columns of convenient size. However, when used in conjunction with absorption spectrophotometry, the usefulness of both methods is greatly extended, for the small amounts of materials recovered from the column can often be measured conveniently, while frequently compounds can be separated which would otherwise produce unresolved absorption spectra. While optical methods have been proposed in which a number of constituents are determined from a series of measurements made on a complex reaction mixture, it is evident that they are completely valid only in cases where the qualitative composition of the mixture can be predicted with certainty. If the properties of the materials permit, a preliminary separation will frequently augment the reliability of the analysis.

EXPERIMENTAL

p-Benzoquinone monoxime was prepared by the nitrosation of phenol, and purified by repeated recrystallizations from water until a pale yellow product was obtained with a melting point of 126° C.

o-Benzoquinone monoxime was prepared by the oxidation of phenol with hydrogen peroxide in the presence of cupric acetate and hydroxylamine hydrochloride (*4*). The reaction mixture was acidified and extracted with petroleum ether, and the monoxime concentration determined iodometrically (*6*). Spectrophotometric measurements were made on a solution obtained by extracting an aliquot of the petroleum ether with aqueous sodium hydroxide.

p-Benzoquinone dioxime was prepared by oximation of the monoxime with hydroxylamine acid sulfate. The crude product was adsorbed on alumina, the zones containing impurities removed and discarded, and the dioxime displaced by treatment with a solution of acetic acid in acetone. After evaporation of the solvent, the product was further purified by precipitation from acetone solution by the addition of petroleum ether. A pale yellow product was obtained with a decomposition point of 239° C.

o-Benzoquinone dioxime was prepared by the oxidation of *o*-nitraniline with sodium hypochlorite (*9*), followed by reduction of the benzfurazan oxide with sodium hydrosulfide. The crude product was dissolved in aqueous ammonia, filtered, and precipitated by the addition of acetic acid. Yellow needles with a melting point of 145° C. were obtained.

ACKNOWLEDGMENTS

The methods for the gravimetric determination and purification of *p*-benzoquinone dioxime were devised by H. P. C. Burrell. Many of the spectrophotometric measurements were made by Virginia S. Martin.

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Determination of Free Fatty Acids in Dried Egg Powders

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A method for the determination of free fatty acids in dried egg powders is described. The egg powders are extracted with acetone and the traces of cephalin are removed with magnesium chloride before titration. The removal of the cephalin, previously shown to be responsible for as much as 70% of the total acidity of ether extracts of egg powders, facilitates accurate measurement of small amounts of free fatty acid acidity. The acidity of extracts of egg powders from which cephalin has been removed by the acetone–magnesium chloride treatment is not appreciably influenced by variations in the moisture content of the powder (at least within the range 0.3 to 6.5%). Errors caused by the formation of a fatty acid–protein complex have been studied. Fatty acids are bound by basic proteins in a nonextractable form when the egg is in the liquid state or when dried egg powder is reconstituted. The fatty acids thus bound can be recovered completely only if the egg emulsion is adjusted to pH 4 before drying and extracting. Fatty acids which develop in the dry egg powder during storage are not bound and can be completely extracted by the proposed method.

A RECENT paper (*9*) was concerned with the differences between the total acidity of the ether extract of dried egg powders as determined by the A.O.A.C. method (*4*) and the free fatty acid acidity of the powders. In some cases over 70% of the acidity of the ether extract of unstored egg powders was found to be due to cephalin. The amount of cephalin appearing in the ether extract, in contrast to the free fatty acid content, was influenced by the moisture content of the powder, so that it was necessary that the powders be uniformly dried, as specified in the A.O.A.C. procedure, in order to obtain reliable total acidity values.

When recovery experiments were carried out (*9*), it was found that oleic acid, that had been added to liquid egg mixtures, was apparently bound in some way that prevented its extraction from the corresponding dried egg powders unless the liquid egg was adjusted to about pH 4 before drying. Thus it appeared that reliable free fatty acid values for the dried egg powders could be obtained only by reconstituting the dried egg, acidifying to pH 4, and redrying prior to extraction. In addition, a correction for the cephalin content of the extract was necessary.

This paper describes a more satisfactory method of determining the true free fatty acid content of dried egg powders and presents additional information on the combination of free fatty acids in the liquid whole egg.

The proposed method makes use of the differential solvent power of cold (room temperature) acetone. Glycerides and fatty acids are fully soluble in cold acetone, whereas the phospholipides (phosphatides) are only slightly soluble. Traces of dissolved phospholipide are removed by treatment with alcoholic magnesium chloride (*8*). The phospholipide concentration is thus reduced to negligible amounts, the phosphorus content of the treated oil is usually less than 0.01%.

ANALYTICAL PROCEDURE

A 2-gram sample of the dried egg powder was transferred to a 40-ml. conical, graduated centrifuge tube, and 15 ml. of C.P. acetone were added. The sample was centrifuged after it had stood in the acetone for 0.5 hour. The acetone extraction was repeated four times, using 10-ml. portions of acetone and only 5- to 10-minute extraction times. The acetone suspension of the egg powder was frequently stirred during each of the extraction periods. All the extracts from a given sample were combined in a single centrifuge bottle. The final volume of acetone extract was kept below 60 ml.

One milliliter of a saturated solution of magnesium chloride hexahydrate in absolute ethanol was then added with rapid swirling to the combined extracts. The treated acetone solution was centrifuged and the supernatant liquid transferred to a 500-ml. separatory funnel (with stem cut to 0.5 inch, 1.25 cm.). The magnesium chloride–phospholipide precipitate was washed twice by suspending in 5 ml. of acetone and centrifuging. The washings were combined with the original supernatant in the separatory funnel.

Thirty milliliters of petroleum ether (Skellysolve B) were added to the acetone solution in the separatory funnel and thoroughly mixed. Two hundred to 250 ml. of distilled water were then added and the contents of the funnel were agitated gently to prevent emulsion formation. The volume of acetone in the separatory funnel should be maintained as small as possible (60 to 80 ml.) because the acetone concentration in the washing procedure must lie between 20 and 30% in order to prevent losses of fatty acids in the washing step. This means that 200 to 250 ml. of water must be added if the initial acetone volume is 70 to 80 ml.

After separation of the phases, the aqueous acetone was discarded. The aqueous phase was turbid, owing to traces of magnesium chloride and phospholipide. The petroleum ether was washed with two 25-ml. portions of distilled water. The washings gave a negative chloride test. The petroleum ether solution was then transferred quantitatively to a 125-ml. glass-stoppered Erlenmeyer flask.

Fifty milliliters of isopropanol and 10 drops of 1% ethanolic phenophthalein were added, and a stream of carbon dioxide–free air was bubbled through the solution for 10 minutes. The solution was titrated with 0.025 *N* sodium ethylate with the stream of carbon dioxide–free air passing through the solution. Oleic acid has been reported to constitute approximately 50% of the egg fatty acids (*10*). Therefore, as a matter of convenience, the results were calculated as oleic acid.

RESULTS AND DISCUSSION

RECOVERY OF OLEIC ACID ADDED TO DRY EGG POWDERS. Recovery of oleic acid was determined by adding known amounts of oleic acid in acetone solution to samples of a spray-dried egg powder suspended in acetone. These samples and controls were carried through the procedure as outlined above. The data in Table I show satisfactory recovery of the added acid.

ANALYSIS OF STORED EGG POWDERS. Samples of spray-dried egg powders, containing only the free fatty acids developed during storage in the dry state, were analyzed by the proposed method. Samples of the same egg powders were also exhaustively extracted with diethyl ether in a Soxhlet apparatus, the ether removed from the Soxhlet extracts under a stream of nitrogen, and the residual oil taken up in acetone and subjected to the magnesium chloride treatment and washing as outlined above. Thus only the effect of different types of extraction was studied. The results in Table II indicate that the shorter acetone extraction procedure yields results from 2 to 7.5% lower, but an error of this magnitude is probably permissible for many purposes. In some cases, the more time-consuming ether Soxhlet extraction may be desirable.

The phospholipide content of all extracts treated with magnesium chloride was negligible, as evidenced by phosphorus contents

Table I. Recovery of Oleic Acid Added to Spray-Dried Egg Powder

Acid Initially Present as Oleic Acid	Oleic Acid Added[a]	Acid Recovered as Oleic Acid	Recovery
Mg.	*Mg.*	*Mg.*	%
10.0, 9.8, 10.1, 10.1	5.0	15.1, 15.1, 15.1, 15.1	101.5
10.0, 9.8, 10.1, 10.1	25.1	34.0, 34.4, 34.3, 34.6	96.4
10.0, 9.8, 10.1, 10.1	50.2	59.0, 59.4, 57.6, 58.5	96.6

[a] Added in acetone solution to acetone suspension of egg powder sample in first step of procedure.

Table II. Free Fatty Acid Values of Dried Egg Powders as Influenced by Extraction Method and Pretreatment

Sample	Treatment	Oleic Acid	Average	Standard Deviation
		Mg. per gram of dry powder		
A	Soxhlet extraction with ether	20.7, 21.2, 21.0 20.8, 20.2, 20.5, 20.8	20.8	0.3
A	Proposed method	20.6, 19.6, 19.9 18.5, 18.6, 19.0, 18.6	19.3	0.8
A	Sample adjusted to pH 4.0 Soxhlet extraction with ether	19.4, 19.6, 19.5, 19.8 19.7, 19.9, 19.6, 20.0	19.7	0.2
A	Sample adjusted to pH 4.0 Proposed method	18.5, 19.1, 19.4, 19.4 19.3, 19.0, 19.2, 18.8	19.1	0.3
C	Soxhlet extraction with ether	3.82, 3.94	3.88	....
C	Proposed method	3.50, 3.52, 3.73, 3.63, 3.52, 3.65 3.49, 3.55, 3.55, 3.54, 3.72, 3.65	3.59	0.08
C	Sample adjusted to pH 4.0 Soxhlet extraction with ether	3.47, 3.57, 3.52, 3.54	3.52	0.03
C	Sample adjusted to pH 4.0 Proposed method	3.37, 3.47, 3.47	3.44	....

(method of Allen, *3*) of 0.001 to 0.01%. Cephalin, therefore, could make only a very small contribution to the acidity values of these extracts.

Effect of Moisture Content of Powders on Free Fatty Acid Values. The proposed method does not require preliminary drying of the egg sample prior to extraction. Average free fatty acid values, calculated to the moisture-free basis, were 17.1, 16.8, 16.9, and 16.6 mg. of oleic acid per gram of powder at moisture levels of 6.5, 4.9, 1.8, and 0.3%, respectively. This relative constancy of results over the moisture range was expected, since the cephalin content of the extracts was negligible as the result of the magnesium chloride treatment. However, the total acidity of ether extracts that are not treated with acetone and magnesium chloride may vary as much as 30% over comparable moisture ranges. This variation has been shown (*9*) to be due to the different amounts of cephalin extracted by ether at the different moisture levels. This, of course, is the reason for the preliminary drying of the samples in the A.O.A.C. procedure for the acidity of the ether extract.

Errors Resulting from Fatty Acid–Protein Complex Formation. Another possible source of error in the free fatty acid determination in egg powders was suggested by the observation that oleic acid, which had been added to whole liquid egg mixtures, was incompletely recovered in the ether extract of the subsequently dried powder unless the pH of the egg mixture was adjusted to 4 prior to drying (*9*).

A similar situation exists for acetone extraction. Only 50 to 55% of the oleic acid added to liquid egg mixtures was extracted by neutral acetone. Therefore, a modified procedure based on the use of acidified acetone (0.088 *N* with respect to hydrochloric acid) was tried on samples to which oleic acid was added before drying. The acidified acetone was used in the first two extractions and neutral acetone in the last three extractions. Acidified acetone was not used in the last three extractions, since high results due to the extraction of phosphorus compounds that were not removed by the magnesium chloride treatment were obtained. Acidified acetone in the first two extractions did not extract phosphorus compounds that could not be removed by magnesium chloride.

Although the pH of the suspension of the residues from the acid acetone extraction was near 4.0, only 80 to 90% recovery could be attained by this technique. Therefore, the acid acetone extraction is not recommended as a means of obtaining complete extraction of fatty acids from egg samples which may contain bound fatty acids. Rather, the procedure involving reconstitution, adjusting to pH 4, and lyophilizing is recommended in these cases. The fatty acids in the dried, adjusted powder may then be determined by the proposed method.

Although the recovery of oleic acid added to or present in liquid egg mixtures prior to drying is not complete unless the pH of the liquid mixture is adjusted to pH 4.0, extraction is complete from egg powders which have developed free fatty acid acidity while stored in the dry state. Tables II and III show that the recovery of fatty acids, by the proposed method, is not increased by the adjustment of the acidity of samples of either spray-dried or lyophilized egg powders to pH 4.

If, on the other hand, samples of egg powders, in which acidity had developed, are reconstituted and allowed to stand in the liquid state at its natural pH for a time before being lyophilized, then part of the fatty acids are no longer extractable. For example, a suspension of reconstituted egg powder had a pH of 6.7; after redrying by lyophilizing, only 86% of the fatty acids were recovered. Recovery of free fatty acids from a similar sample which had been adjusted to pH 4 was complete.

These results may be explained by assuming that liberation of free fatty acids in the dry egg powder occurs in a substantially fatty medium—i.e., the fatty acids are, in a microscopic sense, in or near the egg lipides—and, for this reason or because the medium is essentially nonaqueous, the free fatty acids have little or no opportunity to combine with bases present in the egg. When fatty acids are present in the liquid egg mixture, they are free to combine with bases to an extent determined by the pH of the mixture.

It might be expected that egg white would bind fatty acids more strongly than the egg yolk, since the proteins of the white contain many basic groups that may combine with fatty acids. Lysozyme, in particular, is an unusually basic protein constituent of egg white (*2*) which has been shown to form salts with strong acids (*1*). The higher fatty acids would doubtless also form salts with lysozyme and would hardly be expected to be removed from the lysozyme in the pH region of 6 to 8 which is midway between the isoelectric point of lysozyme and the pK of the acids. Support for this assumption is also obtained from reports (*5*, *6*) that the lower fatty acids and serum albumin combine in solution to give complexes that are more heat-stable than serum albumin dissolved in solutions containing only inorganic ions. These workers have also presented evidence, based on electrophoretic measurements, for anion-albumin association which increases with chain length of the anion.

An experiment was performed to determine whether there was a difference in the fatty acid binding capacity of egg white and egg yolk. Known amounts of oleic acid were added to fresh mixed yolks and the fresh mixed whites. Portions of each sample were adjusted to pH 8 and also to pH 4 before drying by lyophilization. At pH 8, only 4% of the added oleic acid was recovered from the whites, while 81% was recovered from the yolk. At pH 4, 84% of the oleic acid was recovered from the whites and 96% from the yolks.

The formation of the nonextractable fatty acid–protein complexes is of analytical significance in the case of samples of unknown history or in those cases where opportunity for contact

Table III. Free Fatty Acid Content of Stored Lyophilized Eggs as a Function of pH[a]

Sample	pH	Oleic Acid	
		Mg./g.	
1	7.7	10.9	10.6
	4.0	11.0	10.4
2	7.5	11.5	11.4
	3.8	11.2	11.4
3	7.4	10.0	10.0
	3.7	10.0	9.9
4	7.1	11.4	11.1
	4.1	11.2	11.2

[a] pH of suspension of 1 part egg powder and 3 parts distilled water.

between fatty acids and proteins in aqueous media has been known to exist. Thus the free fatty acids in shell eggs which have undergone some deterioration would have an opportunity to combine with protein and the pH should be adjusted to 4 prior to drying the sample.

The lowest moisture content at which interaction of the fatty acids and proteins occurs is not known. However, egg powders containing as much as 8% moisture gave the same free fatty acid values before and after adjustment of the pH. Since few dried egg samples are prepared at higher moisture levels, no adjustment of the pH of dried powders that have been prepared from good shell eggs seems necessary.

INTERFERENCES AND LIMITATIONS

The production of lactic acid in certain types of egg deterioration has been reported (*7*). Lactic acid is, of course, easily soluble in water and is not determined in the method outlined above. Amounts of lactic acid corresponding to 14 to 50 mg. per gram of egg powder were added to samples of the powder and were shown to make no contribution to the acidity of the samples.

The method would not be applicable to samples of materials likely to contain fatty acids that are water-soluble, since they would probably be incompletely partitioned between the aqueous acetone and the petroleum ether in the washing process, thus leading to low results. Since the lower fatty acids have been shown to be absent from egg fat (*10*), such losses do not occur in the determination of egg fatty acids arising from the usual type of glyceride hydrolysis.

Moderate excesses of acids, added to lower the pH of the sample, do not interfere, since they are removed during the washing procedure.

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Iodometric Method for the Assay of Penicillin Preparations

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TWO methods for the chemical estimation of penicillin, recently proposed (*4, 5*), depend on the liberation of an acidic group upon inactivation of penicillin with penicillinase or with alkali. The simple, rapid inactivation by alkali was being utilized in this laboratory in conjunction with the development of an iodometric assay method which was suggested by observations (*3*) on the behavior towards iodine of penicillin and certain of its inactivation products. Penicillin is inert to iodine in neutral aqueous solution, while penicilloic acid (*1*), the inactivation product formed from penicillin by the action of penicillinase or of alkali, consumes from 6 to 9 equivalents of iodine, depending on the conditions used. The difference in the iodine consumption under standard conditions before and after inactivation by alkali might therefore be expected to be approximately proportional to its penicillin content as ascertained by biological assay. This was found to be the case, with certain limitations.

EXPERIMENTAL

Iodine Equivalent of Pure Sodium Penicillin G Inactivated with Alkali. The iodine consumption of a neutral solution of alkali-inactivated sodium penicillin G is a function of time, and under the conditions given below becomes constant after 25 to 30 minutes.

A weighed sample (3 to 5 mg.) of crystalline sodium penicillin G is place in a glass-stoppered flask, dissolved in 5 to 10 cc. of water, and treated with 0.5 cc. of 1 *N* sodium hydroxide. The alkaline solution is allowed to stand for 15 minutes and then neutralized with 0.5 cc. of 1 *N* hydrochloric acid. A measured excess of 0.01 *N* iodine solution (about 10 cc.) is added. After 30 minutes the unconsumed iodine is titrated with 0.01 *N* thiosulfate. It was found that 1 mg. of sodium penicillin G when inactivated will consume under these conditions 2.52 cc. of 0.01 *N* iodine solution, corresponding to 8.97 equivalents of iodine per mole, which is in reasonably good agreement with the range given (*3*) for penicilloates (8.5 to 8.9). This value is well reproducible and independent within fairly wide limits of the penicillin concentrations and the excess iodine used.

Sodium penicillin F (crystalline, anhydrous) after inactivation treated in this manner consumed 2.64 cc. of 0.01 *N* iodine solution per milligram, or 8.8 equivalents per mole, showing that the double bond in this compound is inert to iodine under these conditions. This was confirmed by the blank titration (without prior alkali treatment) which showed an uptake of only 0.07 cc. per milligram. However, it is probable that penicillin X might give abnormally high values in this procedure.

Assay of Unknowns. In the assay of unknowns an amount of material corresponding to approximately 1000 to 5000 units is inactivated with alkali, and the neutralized solution is treated with iodine and back-titrated after 30 minutes as described above for pure sodium penicillin G. A blank titration in which the treatment with alkali is omitted is carried out on a separate sample of the same magnitude. The difference between both titrations can be used, as indicated below, to calculate either international units or micromoles of penicillin.

When relatively pure preparations (800 to 1000 micrograms per mg.) were assayed in this manner, the figures agreed well with the bioassay values regardless of the time allowed for contact of the blank sample with excess iodine (30 minutes as in inactivated sample, or immediate back-titration), the small amounts of iodine-consuming impurities present in such preparations having little influence on the final result. However, with preparations of lower potency containing proportionately greater amounts of iodine-consuming impurities, the iodometric results were considerably lower than the bioassay figures when the 30-minute interval between addition of iodine and back-titration was maintained also in the blank determination. A possible explanation for this is that products formed from these impurities by reaction with iodine catalyze the slow hydrolysis of penicillin to penicilloic acid normally occurring even in neutral reaction, giving rise to an erroneously high blank value, since the latter would include the penicilloic acid thus formed from penicillin. This assumption is supported by the finding that the addition of crystalline penicillin to an impure sample increases the blank titration (after 30 minutes' contact with excess iodine) over the blank value (also determined after 30 minutes standing) given by the impure sample alone.

It was found empirically that the discrepancy between iodometric and bioassay values in the case of low-grade preparations was minimized when the blank value arrived at by immediate back-titration was used in computing the results. In the final

Table I. Correlation between Iodometric and Bioassay Values

Sample No.	Iodometric Assay γ/mg.	Cup Test γ/mg.
890	790	800
	816	
	800	
1080	942	968
	964	
	947	
A69-71	120	100
	107	
	126	
D041	495	502
	492	
	468	
D042F-1	345	336
	330	
	340	
1088 u.u.f.	None	None
DE43	530	480
	507	

Table II. Comparison of Biological and Chemical Assay Methods on Solutions of Commercial Penicillin Sodium Salts

Na Salt Solution	Biological		Chemical	
	2-cc. test (3)	Cup test	Iodometric	Alkalimetric[a]
	Micrograms per cc.			
3419	90,900	71,400	88,000	90,000
3422	87,100	85,000	92,500	91,600
3423	90,400	81,000	88,800	81,000
3420	107,300	90,300	112,500	90,400
3424	87,000	70,700	88,500	
3425	89,000	77,600	91,500	89,300
3426	70,000	68,700	83,600	66,300
3427	86,000	79,000	84,000	92,000
3505	101,603	94,500	98,800	99,200
3506	113,700	108,700	105,500	106,000
3428	96,300	89,100	100,200	81,200
3429	96,500	89,300	101,000	90,700
3430	97,900	88,500	94,500	94,500
3431	85,000	80,300	88,000	87,000
3507	110,000	106,800	114,400	111,500
3428-2	90,900	73,700	83,600	92,300
3432	72,089	74,000	76,500	86,100
3433	93,280	92,900	98,500	107,500
3434	96,400	93,000	98,000	107,600
3435	103,600	105,200	99,300	95,200
3436	116,900	114,000	112,500	114,200
3437	70,900	69,400	68,000	68,800

[a] Method to be published.

procedure adopted for the assay of high- as well as low-grade preparations, the blank sample is therefore back-titrated immediately after the addition of the iodine solution, whereas with the alkali-inactivated sample the 30-minute interval is maintained.

Example. A sample of 6.386 mg. of a commercial penicillin salt (bioassay 800 micrograms per mg.) was dissolved in water and the volume was made up to 10.0 cc. A 5-cc. aliquot was pipetted into a 125-cc. glass-stoppered flask, and 10.0 cc. of a 0.01 *N* iodine solution were added. Immediate back-titration with 10.0 *N* thiosulfate showed that 0.92 cc. of 0.01 *N* iodine had been consumed. When the back-titration was delayed for 30 minutes, practically the same result (0.95 cc.) was obtained.

The remainder of the solution (together with the pipet washings) was made alkaline with 0.50 cc. of 1 *N* sodium hydroxide. After standing for 15 minutes, the solution was neutralized with 0.50 cc. of 1 *N* hydrochloric acid, treated with 10 cc. of 0.01 *N* iodine solution, and back-titrated after 30 minutes. The iodine uptake corresponded to 4.86 cc.

Calculation. Potency (units per mg.) =

$$\frac{(4.86 - 0.92) \times 661}{3.193} = 816$$

The factor 661 represents the ratio of the potency of sodium penicillin G (1667) to the number of cubic centimeters of 0.01 *N* iodine consumed by 1 mg. of alkali-treated sodium penicillin G (2.52).

With the use of this procedure good correlation between the iodometric and bioassay values was obtained on a variety of carefully bioassayed solid sodium salt preparations of widely varying potency (Table I). Table II gives a comparison of the values obtained by the iodometric, the alkalimetric (*5*), and two standard bioassay methods This survey was conducted in the Squibb Biological laboratories on solutions of commercial sodium salt preparations ranging in potency from 900 to 1150 units per mg. The figures show that the agreement between the two chemical methods, and between the latter and the 2-cc. test is in most cases satisfactory enough for practical purposes. It is possible that the iodometric method can be used for rapid control work in the intermediate stages of the plant process, but no data on such materials are as yet available.

The iodometric procedure has certain advantageous features not possessed by the alkalimetric method. It requires less material than the latter, and therefore can be applied to more dilute solutions; it also can be adapted to the assay of buffered solutions, which is valuable particularly in chromatographic work.

Finally, the comparison of bioassay figures with the iodometric values, or for that matter with the results obtained by any assay method which measures the number of penicillin molecules, is predicated on the condition that the penicillin in the unknown sample be of the same type as in the pure standard preparation used in both kinds of assay. Since the various penicillin species (F, G, X, K, etc.) differ somewhat in molecular size, the iodine consumed after alkaline inactivation will be inversely proportional to the molecular weight. Consequently, when the composition of the unknown sample deviates materially from that of the standard in regard to the type or types of penicillin present, the iodometric value for the unknown will not correspond exactly to its penicillin content if the latter is expressed in terms of milligrams. However, the errors caused by this circumstance are comparatively small and may be disregarded in practice.

A more serious complication is encountered when the iodometrically measured penicillin content of an unknown sample is expressed in terms of international penicillin units and then compared with the unitage ascertained by biological assay. It is well known that the various penicillin species differ widely in their bacteriostatic potency towards the test organism commonly used, *Staph. aureus*. Thus the potencies of the sodium salts of penicillins F, G, and K determined by standard assay methods employing this test organism are 1400, 1667, and 2350 units per mg., respectively. It is obvious that when the unitage of an unknown preparation is computed from the iodometrically determined penicillin content by means of the conversion factor 1 mg. = 1667 units for sodium penicillin G (the International Standard), marked discrepancies from the values ascertained by bioassay are to be expected if penicillins other than G are present as major components. Since quantitative information on the latter point is hardly ever available, the applicability of this as of any chemical method is necessarily restricted to the comparative assay of products similar in respect to derivation and mode of preparation. Therefore, in cases in which the measurements reveal a consistent discrepancy between the chemical and the bioassay data, it is preferable to base the computation of unitage from the chemical assay on a conversion factor empirically determined with an impure standard preparation of the same type rather than on the factor for pure sodium penicillin G given above.

ACKNOWLEDGMENT

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Spectrophotometric Changes during Oxidation of Vitamin A Oils

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Both the quality of the carrier oil and the quantity of vitamin A are important for the evaluation of fish liver oils containing vitamin A. The former develops maxima at 235 and 280 mμ during oxidation. These can be used advantageously for estimating the quality of fish liver oils by measuring the ratio $E^{1\%}_{1cm.}$ 280/328, and from the percentage by which the absorption of the unsaponifiable fraction is lower than that of the whole oil at 280 mμ. The quantity of the vitamin A in oils of high potency can be estimated from the unsaponifiable fraction absorption at 325 mμ since the unsaponifiable fraction does not change appreciably during the early stages of oxidation.

FISH liver oils containing vitamin A are essentially solutions of the esters of that vitamin in fatty oil (carrier oil). It may be divided into two fractions, the saponifiable and the unsaponifiable carriers—i.e., the portions respectively nonextractable and extractable with ether after saponification of the oil. Knowledge of the quality of the carrier oil is of utmost importance for the over-all evaluation of vitamin A oils, since carrier oils containing degradation products due to oxidation have considerable influence on the extinction coefficient at 328 mμ and the whole vitamin A curve. While the vitamin A ester itself has a sharp maximum at 328 mμ, biologically inactive substances, which may accompany it, do not show this sharp peak, but have maxima at wave lengths sufficiently close to 328 mμ to produce considerable absorption at this point, thus causing an apparent broadening of the vitamin A absorption band and an erroneously high value at this point. Oxidized oils can also interfere with the vitamin A absorption in vivo (*7*, *12*, *13*, *16*) and so inhibit its action.

The method of Oser *et al.* (*18*) of plotting absorption curves of vitamin A oils in terms of absorption ratios ($E^{1\%}_{1cm.}$ λ328) could be useful in detecting anomalous or oxidized oils. However, the method for measuring the ratio $E^{1\%}_{1cm.}$ 300/328 with the limit of 0.72 for acceptable oils (*11*, *19*) did not prove satisfactory. Work in this laboratory had disclosed the fact that an oil could be considerably oxidized and still show a satisfactory $E^{1\%}_{1cm.}$ 300/328 ratio and Oser *et al.* (*19*) showed that oils with an acceptable $E^{1\%}_{1cm.}$ 300/328 ratio did not always show the biological potency corresponding to their $E^{1\%}_{1cm.}$ value at 328 mμ.

It therefore seemed desirable to study spectrophotometric curves of both components of the carrier oil, apart from vitamin A, during accelerated oxidation, in an effort to establish their influence upon the absorption curve of vitamin A oils. Such a study could also be helpful in developing better criteria for the evaluation of vitamin A oils.

EXPERIMENTAL

Outline of Procedure. Freshly prepared oils were oxidized at 75° C., and the absorption curves measured in the region from 220 to 400 mμ on both the whole oil and the unsaponifiable fraction, the difference between the two representing the absorption of the saponifiable carrier. The absorption of a part of oxidized vitamin A which is washed out during the saponification procedure (*18*) is also included in the subtracted curve. In order to obtain the curve of the unsaponifiable carrier, the unsaponifiable fraction was destructively irradiated. On the assumption that only vitamin A is being destroyed by irradiation, the absorption curve obtained after irradiation would represent the absorption of the unsaponifiable carrier. By subtracting the absorption values of the unsaponifiable carrier from those of the unsaponifiable fraction a vitamin A curve can be obtained representing essentially the pure vitamin A absorption. The values so obtained are referred to as the modified values (*17*).

Preparation of Oils. Two test oils were used: a grayfish (*Squalus suckleyi*) liver oil and a ling cod (*Ophiodon elongatus*) liver oil. The grayfish liver oil was prepared by steaming minced fresh livers with live steam for 10 minutes, and then centrifuging; the ling cod liver oil by similarly steaming minced fresh livers for 10 minutes, extracting the steamed livers with a mixture of methylene chloride and ethylene dichloride, and distilling off the solvent in vacuum. The oils showed a very high stability. The grayfish oil especially had a stability far in excess of that observed on commercially prepared grayfish liver oils.

Apparatus, Reagents, and Procedures. The oxidation of the oils was carried out at 75° C. in a constant-temperature oil bath, using the following technique: From 0.33 to 0.36 gram of the oil was introduced into vials of 23-mm. outside diameter and 25 mm. high. These were then placed in a frame bored with holes which, when lined with felt, were of a suitable size to hold the vials firmly upright. The frame was kept immersed in an oil bath maintained at a constant temperature of 75° C. For each oxidation 4 to 10 vials were used. The oils were heated for varying lengths of time in order to obtain oils at different stages of oxidation. The oxidized oils from these vials were afterwards combined and kept for analyses in full ampoules at −20° C.

The spectrophotometric analyses were carried out in duplicate with a Beckman quartz spectrophotometer, using an ultraviolet phototube and, as light source, a hydrogen discharge tube below 320 mμ and a tungsten lamp above 320 mμ. All determinations were made in quartz cuvettes with isopropanol as solvent. The latter was purified by refluxing for one hour with zinc dust and sodium hydroxide, and then distilling.

The saponifications were carried out essentially according to the method of Oser *et al.* (*18*). Blank saponifications made on the reagents were used as blanks for the spectrophotometric determinations. The irradiation of the saponified samples was carried out with a Uviarc lamp UA 32A6, and a Corning filter No. 597. The quartz cuvette containing the material to be irradiated was placed in a water-cooled holder at a distance of about 20 cm. from the lamp and irradiated for 1 hour. Corning filter 597 does not give such a sharp maximum as the arrangement used by Little (*17*) but has a flat maximum at about 350 mμ and cuts out all wave lengths below 300 mμ.

DISCUSSION OF RESULTS

In Figures 1 to 10 are plotted the absorption curves of both test oils; the numbers on the individual curves refer to hours heated at 75° C. In Table I are collected those characteristic values which were considered important for the present investigation.

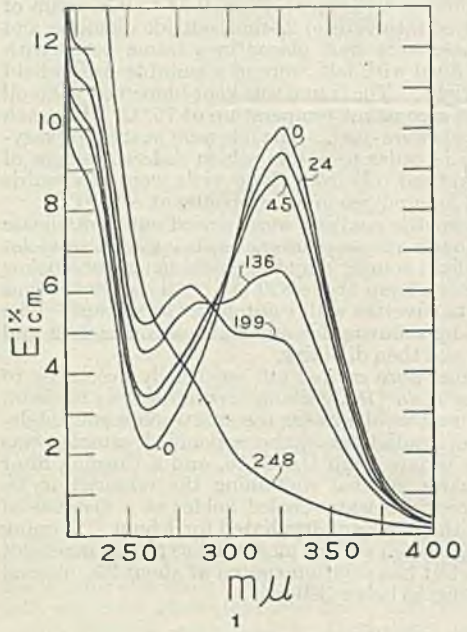
Saponifiable Carrier. In Figures 4 and 9, representing the absorption of the saponifiable carrier, it can be seen that during oxidation new maxima at 235, 275 to 280, and 335 to 345 mμ are formed. The latter increases at first but decreases in the later stages of oxidation, indicating instability of the substance which causes this maximum. Formation of the maximum at 335 to 345 mμ could be due to oxidation of highly unsaturated acids which are known to be present in fish liver oils.

The shape of the curves of the oxidized saponifiable carrier of both test oils resembles the curves of rancid and oxidized fats. Holman *et al.* (*14*, *15*) found the formation of the 235 and 275 to 280 mμ maxima during oxidation of dienoic and trienoic acids and of lard. Bradley (*2*) made a similar observation on heat-bodied linseed oil, Edisbury *et al.* (*8*) on ling oil, and Castle *et al.* (*4*) found that vitamin A carefully oxidized with chromic acid absorbs at 275 to 280 mμ.

Table I. Characteristics of Test Oils during

Time of heating, hours	Whole Oil: Vitamin A estimate[c]	Decrease in vitamin A estimate, %	Maximum at mμ	F.F.A., %	Peroxide value	$E^{1\%}_{1\ cm.}$ 260/328	280/328	300/328	350/328	Unsaponifiable: Vitamin A estimate[c]	Decrease in vitamin A estimate, %	Maximum at mμ	$E^{1\%}_{1\ cm.}$ 280/328	300/328	350/328
Grayfish Liver oil (Unsaponifiable 21.0%)															
Original	19,930	0	327	0.46	0.2	0.22	0.37	0.67	0.57	19,620	0	325	0.34	0.68	0.50
24	18,600	6.7	326	0.64	1.9	0.33	0.47	0.71	0.58	17,660	10	325	0.36	0.68	0.51
45	17,490	12.2	325	0.71	3.1	0.39	0.54	0.74	0.57	16,200	17.4	325	0.38	0.70	0.51
136	12,780	35.9	323	0.91	5.1	0.71	0.87	0.90	0.58	10,380	47.1	325	0.40	0.71	0.53
199	9,300	53.3	281	1.21	5.6	1.13	1.32	1.09	0.57	6,900	64.8	325	0.58	0.81	0.52
248	2,080	89.6	...	5.79	62.4	5.53	3.88	2.12	0.56	316	98.4	280	3.68	2.84	0.60
Ling Cod Liver Oil (Unsaponifiable 10.5%)															
Original	68,820	0	328	2.3	0.9	0.14	0.28	0.58	0.62	65,040	0	325	0.26	0.59	0.54
6	67,740	1.6	328	2.6	2.3	0.16	0.30	0.59	0.62	61,640	5.2	325	0.28	0.61	0.54
18	64,340	6.5	328	2.6	4.8	0.22	0.36	0.63	0.61	55,420	14.8	325	0.29	0.61	0.54
24	55,160	19.8	328	2.6	5.8	0.29	0.43	0.67	0.62	44,560	31.5	325	0.33	0.63	0.55
32	33,440	51.4	327	2.8	11.7	0.63	0.78	0.84	0.58	26,800	58.7	325	0.40	0.70	0.54
40	20,800	69.8	280	2.9	16.4	1.19	1.35	1.20	0.59	15,580	76.0	325	0.56	0.77	0.53
72	8,960	87.0	275	3.7	33.0	3.56	3.50	2.20	0.61	1,540	97.6	...	2.70	1.88	0.55

[a] Unsaponifiable minus irradiated unsaponifiable.
[b] Per cent of absorption of whole oil.
[c] Determined spectrophotometrically.



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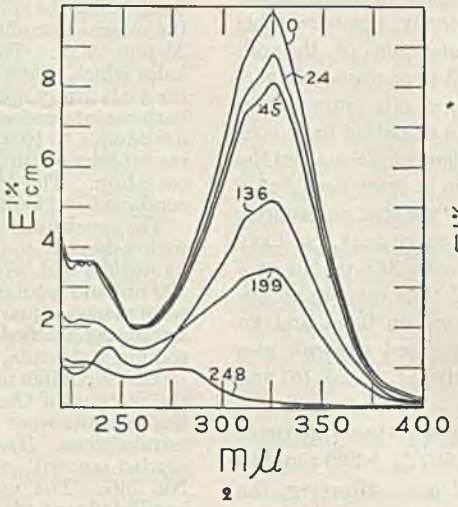

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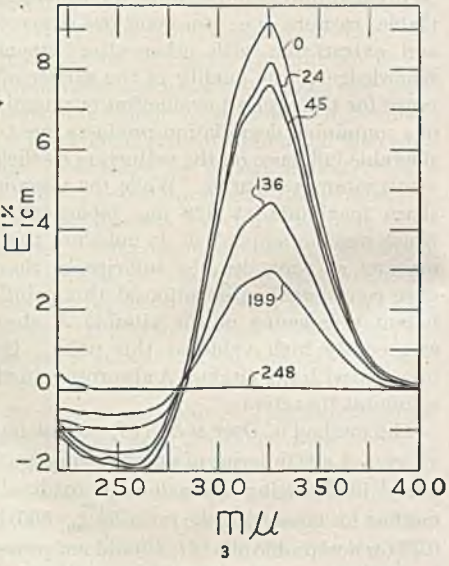

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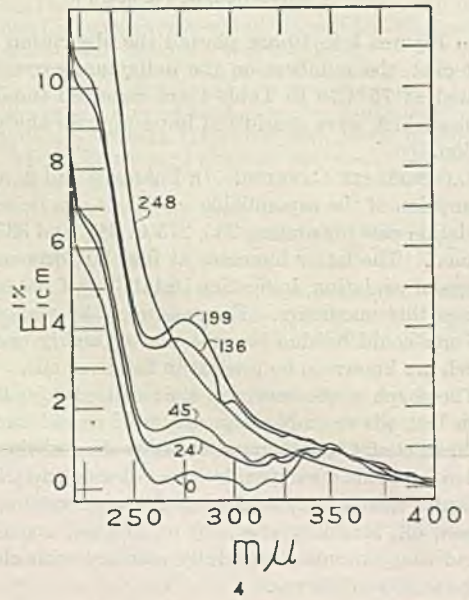

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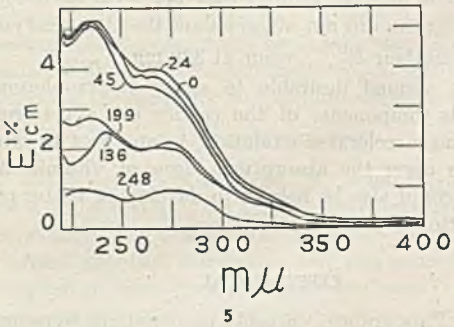

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Figures 1 to 5. Absorption Curves of Fractions of Grayfish Liver Oil Samples Oxidized at 75° C.

1. Whole oil
2. Unsaponifiable fraction
3. Modified values (unsaponifiable minus irradiated unsaponifiable)
4. Whole oil minus unsaponifiable (saponifiable carrier)
5. Irradiated unsaponifiable (unsaponifiable carrier)

Accelerated Oxidation at 75° C.

Vitamin A estimate[c,d]	Modified Values[a] Decrease in vitamin A estimate, %	Maximum at mμ	$E^{1\%}_{1\text{ cm.}}$ 300/328	$E^{1\%}_{1\text{ cm.}}$ 350/328	Per Cent Extraneous Absorption[b] Saponifiable Carrier At 328 mμ	At 280 mμ	At 260 mμ	Unsaponifiable carrier at 328 mμ	Total at 328 mμ
Grayfish Liver Oil (Unsaponifiable 21.0%)									
18,060	0	325	0.57	0.51	1.6	20	19.2	7.8	9.4
16,100	10.9	325	0.53	0.51	5.0	28.4	34.8	8.4	13.4
14,980	17.0	325	0.56	0.52	7.3	41.0	41.7	7.1	14.4
9,220	49.0	325	0.55	0.54	18.7	63.0	77.7	9.2	27.9
5,900	67.4	325	0.58	0.54	25.8	67.9	71.4	10.8	36.6
140	99.2	...	0.82	0.69	84.8	75.5	85.9	8.5	93.3
Ling Cod Liver Oil (Unsaponifiable 10.5%)									
61,280	0	325	0.48	0.55	5.5	16.1	12.9	5.5	11.0
58,280	4.9	325	0.50	0.54	9.0	17.9	23.5	5.0	14.0
49,280	19.6	326	0.49	0.56	13.9	34.4	41.5	9.5[d]	23.4[d]
40,400	34.1	326	0.56	0.57	19.2	39.0	44.1	7.6	26.8
23,980	60.9	325	0.55	0.55	19.6	54.6	60.0	8.7	28.3
13,400	78.1	325	0.52	0.55	25.1	69.4	73.5	10.5	35.6
940	98.5	315	1.00	0.58	82.8	87.3	86.6	7.7	89.5

[d] Values too low, owing to absorption of irradiated vitamin A.
[e] Value too high, owing to insufficient irradiation of sample.

The appearance of the new maxima during oxidation is generally attributed to the formation of different conjugated systems, produced by isomerization in the case of unconjugated fats and by the destruction of a part of the four conjugated double bonds in the case of vitamin A. Both components of the oxidized saponifiable carrier (fatty acid and oxidized vitamin A) can therefore be responsible in various degrees for the formation of the 275 mμ band. The extinction coefficients of conjugated fatty acids are of such a high order (*3*) that their formation even in small quantities can markedly affect the shape of the vitamin A curve.

It can be seen in Figures 1 and 6 that the whole left side of the vitamin A curve is affected by the formation of the three new maxima. The minimum of the vitamin A curve at 260 mμ steadily increases during oxidation despite loss in potency. This causes a broadening of the curve, an increase of the $E^{1\%}_{1\text{ cm.}}$ 300/328 ratio, and a shifting of the 328 mμ peak to a lower wave length in the later stages of oxidation. By studying the curves of the saponifiable carrier (Figures 4 and 9) it can be seen that the point at 260 mμ (minimum of vitamin A curve) or at about 280 mμ (maximum of the saponifiable carrier) is more indicative for taking the ratio than the 300 mμ point. At these points the $E^{1\%}_{1\text{ cm.}}$ values increase during oxidation, while at the same time $E^{1\%}_{1\text{ cm.}}$ values at 328 mμ decrease (Figures 1 and 6). At 300 mμ the $E^{1\%}_{1\text{ cm.}}$ values remain rather constant while those at 328 mμ decrease. The point at 300 mμ was apparently empirically selected not for any significance in the detection of extraneous absorption but for the convenience of taking readings at a wave length where the use of quartz cuvettes and of a hydrogen discharge lamp is unnecessary. In Figure 11 are plotted the ratios of $E^{1\%}_{1\text{ cm.}}$ 300/328, 280/328, and 260/328 of the oxidized test oils. The steeper curves of the 260 and 280 ratios are the graphical results of the considerations mentioned above.

The extraneous absorption (expressed in per cent of the absorption of the whole oil) of the saponifiable carrier steadily increases during oxidation and could also be used as a criterion for the evaluation of the quality of vitamin A oils. At 328 mμ the extraneous absorption of the saponifiable carrier increases only slightly during oxidation, the increase being dependent mostly on formation of the maxima at 275 to 280 mμ and at 335 to 345 mμ. The increase in per cent extraneous absorption is therefore mainly due to the decrease of vitamin A. Such an increase of per cent extraneous absorption at 328 mμ was also observed by Oser *et al.* (*18*) on different high-potency oils and by Coy *et al.* (*5*, *6*) on cod liver oils. It can be seen, however, in Figure 12, that the first stages of oxidation, which are important from the practical standpoint, result in only a slight increase and are possibly of little value as criteria. At 260 and 280 mμ the per cent of extraneous absorption increases significantly during the first stages of oxidation. It is most pronounced at 260 mμ because here the influence of the 235 mμ maximum is greater than at 280 mμ. Both points, however, could be used as criteria. A certain advantage can be given to the 260 mμ point, at which wave length the determination can be carried out more accurately on the flat of the curve.

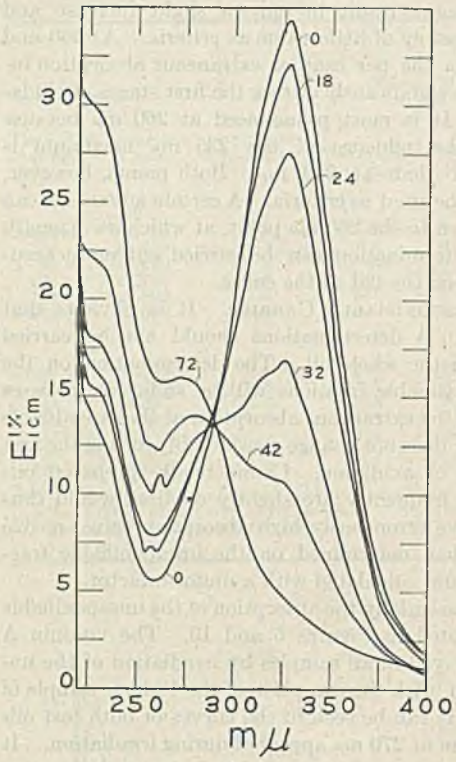
Unsaponifiable Carrier. It is obvious that vitamin A determinations should not be carried out on the whole oil. The determination on the unsaponifiable fractions will be sufficient in cases where the extraneous absorption of the saponifiable carrier does not change considerably during the first stages of oxidation. Commercially prepared oils which frequently are slightly oxidized would thus not give erroneously high absorption values at 325 mμ when determined on the unsaponifiable fractions and calculated with a uniform factor.

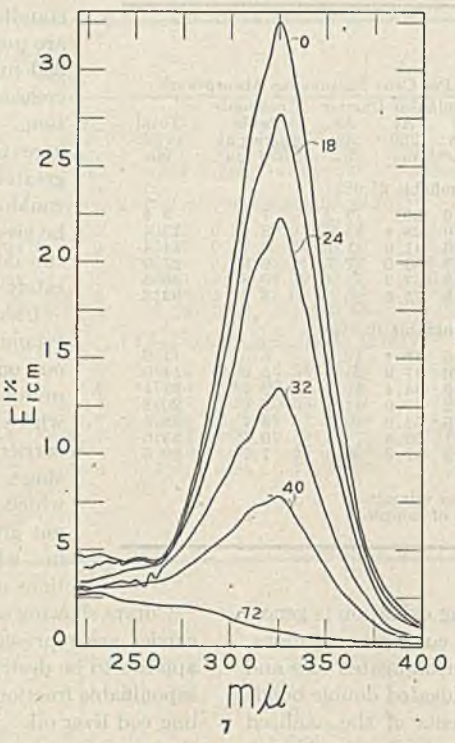
Curves showing essentially the absorption of the unsaponifiable carrier are represented in Figures 5 and 10. The vitamin A appears to be destroyed in all samples by irradiation of the unsaponifiable fraction, with the exception of the 18-hour sample of ling cod liver oil. It can be seen in the curves of both test oils that a new maximum at 270 mμ appeared during irradiation. It represents a substance formed from vitamin A during destructive irradiation. The formation of a similar maximum at 275 mμ during irradiation of vitamin A acetate was observed by Sobotka *et al.* (*21*). The absorption band of the irradiated unsaponifiable fraction includes, therefore, not only the absorption of substances originally present in the unsaponifiable fraction, but also that of the irradiation product of vitamin A.

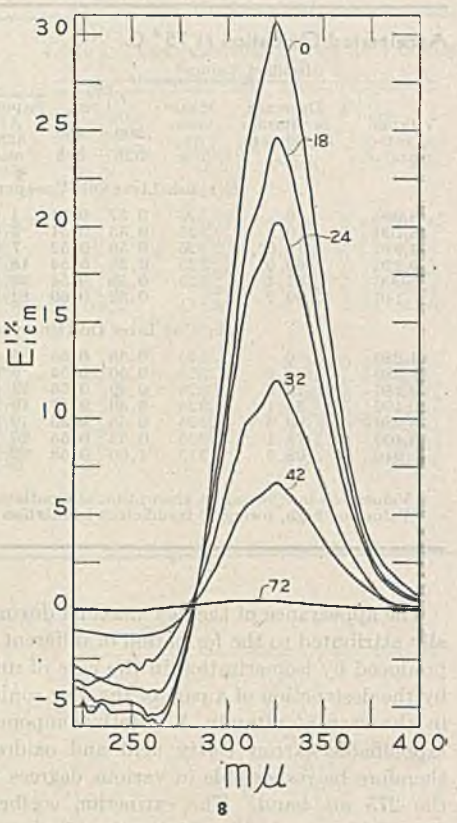
The additional absorption of the irradiation product of vitamin A affects considerably the curves of the modified values (Figures 3 and 8) which, as mentioned above, are obtained by subtraction. The entire left side of the modified curves appears steeper and results in negative values below 280 mμ. Part of the absorption at 325 mμ can be caused by the absorption of the irradiation product which seems to extend beyond 325 mμ, with the result that the modified vitamin A value appears to be too low.

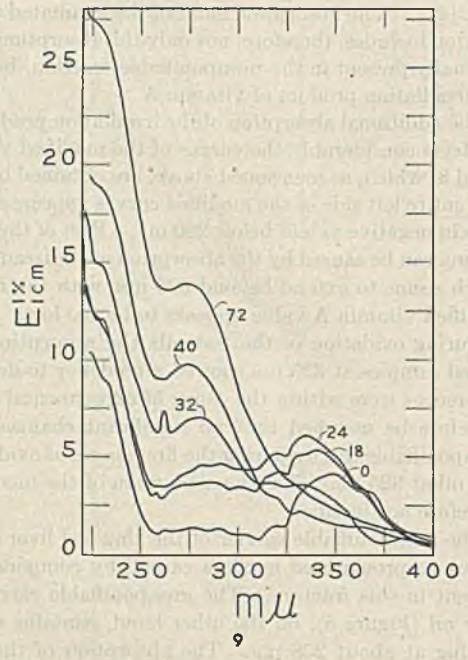
During oxidation of the test oils the absorption of the irradiated samples at 325 mμ showed a tendency to decrease but the differences were within the range of experimental error. It can therefore be assumed that no significant changes occur in the unsaponifiable carrier during the first stages of oxidation of either test oil at 325 mμ. The determination of the modified values is therefore not justified.

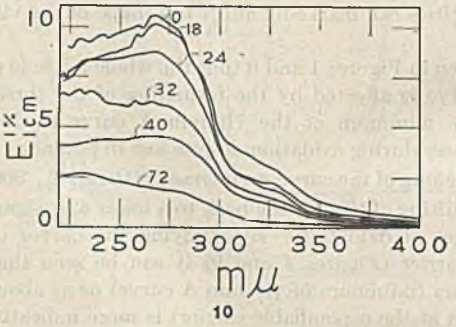
The unsaponifiable carrier of the ling cod liver oil (Figure 10) shows no pronounced maxima caused by components originally present in this fraction. The unsaponifiable carrier of grayfish liver oil (Figure 5), on the other hand, contains substances absorbing at about 238 mμ. The absorption of these substances partly masks the absorption of irradiated vitamin A at 270 mμ. The absorption at 238 mμ, however, does not affect the 325 mμ point. It remains constant in the first stages of oxidation, but decreases considerably later. In order to identify the substances, the unsaponifiable fraction of a grayfish liver oil was chromatographed, using Swain's method (*22*) of the flowing chromatogram. The absorption curves of three fractions obtained by elution with different solvents are presented in Figure 13. The amount of absorption of the separate fractions is expressed in $E^{1\%}_{1\text{ cm.}}$ values based on the weight of the total unsaponifiable fraction. The absorption curve for the vitamin A eluate is omitted in the figure. It can be seen that the main substance absorbing at 238 mμ belongs to the glyceryl ether fraction, prob-











Figures 6 to 10. Absorption Curves of Fractions of Ling Cod Liver Oil Samples Oxidized at 75° C.

6. Whole oil
7. Unsaponifiable fraction
8. Modified values (unsaponifiable minus irradiated unsaponifiable)
9. Whole oil minus unsaponifiable (saponifiable carrier)
10. Irradiated unsaponifiable (unsaponifiable carrier)

ably being a higher unsaturated homolog of selachyl alcohol (*22*). Another substance absorbing very strongly at 238 mμ was discovered in the methanol eluate. This substance of unknown constitution is present in only very small quantities.

Although the unsaponifiable carrier of the two test oils did not show oxidizable substances which would affect considerably the absorption curve of vitamin A, some fish liver oils can contain substances which carry through to the unsaponifiable fraction and produce an anomalous vitamin A curve. In presence of such substances as kitol (*10*), vitamin A_2 (*9*), anhydro vitamin A (*20*), and others, an accurate estimate of vitamin A by means mentioned above is impossible. The commercial conversion factor of 2000 allows a certain amount of extraneous absorption in all oils because the crystalline vitamin A alcohol was shown to have a conversion factor 2460 (*1*). In cases where the substances which produce excessive extraneous absorption in the unsaponifiable fraction do not interfere with the Carr-Price reaction, the colorimetric estimate can be used advantageously (*19*).

CONCLUSIONS

The ratio $E^{1\%}_{1\,cm.}$ 280/328 was found more indicative of the quality of fish liver oils containing vitamin A than the ratio $E^{1\%}_{1\,cm.}$ 300/328 since, while the 300 mμ point was arbitrarily selected,

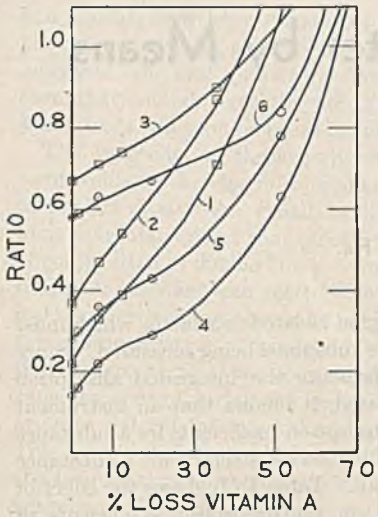


Figure 11. Changes in Absorption Ratios of Test Oil Samples Oxidized at 75° C.

1. Grayfish liver oil, ratio 260/328
2. Grayfish liver oil, ratio 280/328
3. Grayfish liver oil, ratio 300/328
4. Ling cod liver oil, ratio 260/328
5. Ling cod liver oil, ratio 280/328
6. Ling cod liver oil, ratio 300/328

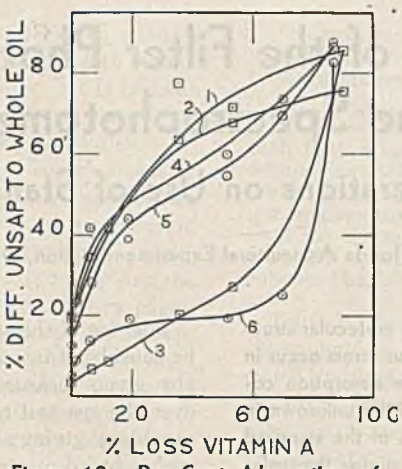


Figure 12. Per Cent Absorption of Saponifiable Carrier

$$\frac{\text{Whole oil, minus unsaponifiable}}{\text{whole oil}} \times 100$$

1. Grayfish liver oil at 260 mμ
2. Grayfish liver oil at 280 mμ
3. Grayfish liver oil at 328 mμ
4. Ling cod liver oil at 260 mμ
5. Ling cod liver oil at 280 mμ
6. Ling cod liver oil at 328 mμ

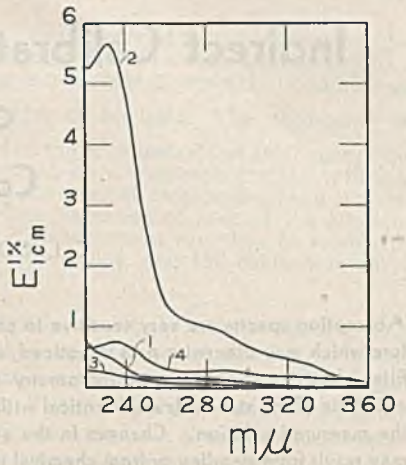


Figure 13. Absorption Curves of Fractions of Unsaponifiable Grayfish Liver Oil Obtained by Flowing Chromatography

$E^{1\%}_{1\,cm.}$ values calculated on basis of total unsaponifiable

1. Petroleum ether, eluate, 1.4% (hydrocarbons)
2. Ether eluate, 81.4% (glyceryl ethers)
3. Chimyl alcohol, m.p. 59.5-50°, crystallized from ether eluate, 20.8%
4. Methanol eluate, 2.6%

Not shown, benzene eluate (vitamin A and sterols), 11.2%

the saponifiable carrier develops at 280 mμ a definite maximum during oxidation. The formation of this maximum is responsible for the increase in the ratios on the left slope of the vitamin A curve in oxidized fish liver oils. The 280 mμ point shows therefore a proportionately greater increase in its absorption value at the same degree of oxidation than the 300 mμ point. Alternatively, the ratio $E^{1\%}_{1\,cm.}$ 260/328 could be used because at 260 mμ, the minimum in the vitamin A curve, the determination can be carried out more accurately than on the slope. The 260 mμ point is not only affected by the maximum at 280 mμ, but also by the increased absorption at wave lengths lower than 260 mμ caused by oxidation of the saponifiable carrier.

Considering the great difference in the ratios of both original test oils, it seems that the establishment of a definite limit for its magnitude for evaluating the quality of fish liver oils is not satisfactory. A rigorous limitation for good oils and a range for doubtful ones would be more satisfactory. It is suggested that in doubtful cases the percentage of extraneous absorption of the saponifiable carrier $\frac{E^{1\%}_{1\,cm.}\text{ whole oil} - E^{1\%}_{1\,cm.}\text{ unsaponifiable}}{E^{1\%}_{1\,cm.}\text{ whole oil}} \times 100$ at 280 or 260 mμ might be helpful in the estimation of the quality of fish liver oils.

From the present work and from other experience in this laboratory it appears that the value of 0.72 for the $E^{1\%}_{1\,cm.}$ 300/328 indicates that oxidation has taken place, in the case of high-potency oils at least. Further work, particularly biological, will be necessary to establish proper limitations of the values to be used as criteria.

The irradiation technique for determining vitamin A was not found suitable for high-potency fish liver oils because the irradiated vitamin A which has a maximum at about 275 mμ shows a certain absorption at 325 mμ. This results in the modified absorption values at 325 mμ being too low.

In both test oils the absorption of the unsaponifiable carrier at 325 mμ was found to remain stable during oxidation. Taking into account the fact that the unsaponifiable fraction of grayfish liver oil is composed mostly of glyceryl ethers and that of ling cod liver oil of cholesterol, it appears that a fairly accurate estimation of the quantity of vitamin A can be made in high-potency fish liver oils on the unsaponifiable fraction, even when the oils are oxidized.

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Indirect Calibration of the Filter Photometer by Means of the Spectrophotometer

Considerations on Use of Standards

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Absorption spectra are very sensitive to changes in molecular structure which may otherwise pass unnoticed, and serious errors occur in filter photometry and spectrophotometry unless the absorption coefficients of the standards are identical with those of the unknown in the measured solution. Changes in the absorption of the standard may result from standing or from chemical treatment during the isolation procedure. This situation occurs frequently, since standards are usually subject to a rigorous purification process whereas the analytical determinations are usually made on simple extracts in which the substance measured has had little opportunity to undergo degradation. This type of error can be avoided and the method simplified by use of appropriate calibration procedures which may involve interchangeability of spectrophotometric data.

COLORIMETRIC and spectrophotometric analytical methods may be broadly classified into two groups: those in which the substance or element is treated with a reagent to form a system having suitable colorimetric properties and those in which the substance itself has absorption characteristics suitable for the analytical measurement. By far the greater number of colorimetric methods fall into the first category, and adequate reviews have been published emphasizing the limitations and the precautions to be observed with this type of method (*7*, *8*). While comparatively few systems are found in the second group, many of the biologically important compounds, especially the complex substances of high molecular weight, have absorption spectra which allow measurement without the need for chemical transformations.

If the analytical results are to have any meaning, it is essential that the absorption spectrum of the standard be identical with that of the compound under consideration as measured in the unknown solution. This paper emphasizes the precautions which should be observed where absorption spectra of purified substances are used for analytical purposes, and more particularly where purified substances are used as calibration standards in filter photometry. Advantages of an indirect method, which involves calibration of the filter photometer by means of the spectrophotometer and established absorption coefficients, are presented. Although this work has been developed employing photoelectric colorimeters, the method should be applicable to visual instruments as well.

SPECTROPHOTOMETRIC METHODS

Within the past few years the availability, at reasonable cost, of commercial spectrophotometers capable of absolute absorption measurements has placed this facility at the disposal of a great many laboratories throughout the country. While the use of published absorption coefficients must always be approached with caution, it seems evident that if the physical and instrumental factors are satisfactorily taken into account, significant errors should not be introduced by using coefficients on instruments other than the one on which they were established (see *4* for definition of absorption coefficient). Methods are available by which the worker can determine for himself that his individual spectrophotometer is giving linear response, is in proper wavelength adjustment, and that significant amounts of scattered radiation are absent (*6*, *9*).

The size of the spectral region isolated is a factor which must be considered in terms of the substance being measured. Since any given measurement represents the integrated absorption over the spectral region isolated, it follows that an instrument capable of giving absolute absorption coefficients for a substance with a broad maximum may not be satisfactory for a substance with a narrow absorption peak. Table I (*2*) shows the effect of the spectral region isolated on the absorption coefficients of chlorophyll *A* in diethyl ether solution; these measurements were made with a photoelectric spectrophotometer at Purdue University similar to that described by Hogness, Zscheile, and Sidwell (*6*) but containing a Müller-Hilger double monochromator. It is clear that for absolute measurements on this particular system the spectral region isolated should not exceed 31 to 36 Å. at 6600 Å., or about 60 Å. at 4290 Å.; at the broad minimum near 4720 Å. significant errors are not introduced even when the region isolated approaches 155 Å. Zscheile *et al.* (*15*) have reported similar information for β-carotene. These data illustrate how this particular instrumental requirement for absolute measurements on a given system may be determined. The spectral region isolated for given slit settings on an instrument may usually be obtained from the manufacturer's specifications but should be checked by established methods (*6*).

It is axiomatic that a worker should determine absorption coefficients on his own instrument whenever possible. In the case of many of the biologically important substances, however, it is very difficult to obtain the isolated highly purified material with its absorption properties unaltered; in other words, differences between laboratories may more often be due to variations in the samples measured than to instrumental differences. It is in such instances that interchangeability of spectrophotometric data becomes of definite advantage. Zscheile, Comar, and Mackinney (*14*) exchanged samples and solutions of purified chlorophylls and inorganic salts (Weigert's solution) and made comparative measurements on the Purdue spectrophotometer mentioned above and another instrument described by Smith (*11*). There were no instrumental differences and it was evident that interchangeability of data on the systems studied was feasible. Comar (*3*) showed that absolute absorption coefficients for the chlorophyll components as determined on the Purdue spectrophotometer could be used with a Cenco-Sheard spectrophotelometer to give accurate analytical results. Vandenbelt, Forsyth,

Table I. Effect of Spectral Region Isolated on Absorption Coefficients of Chlorophyll *A* in Diethyl Ether Solution

Wave Length 6600 Å. (Maximum)		Wave Length 4290 Å. (Maximum)		Wave Length 4720 Å. (Minimum)	
Region isolated (Å.)	$\text{Log}_{10}\frac{I_0}{I}$	Region isolated (Å.)	$\text{Log}_{10}\frac{I_0}{I}$	Region isolated (Å.)	$\text{Log}_{10}\frac{I_0}{I}$
10	0.442	10	0.582	9	0.484
15	0.445	13	0.581	11	0.486
20	0.444	16	9.581	13	0.484
25	0.442	21	0.582	18	0.487
31	0.442	26	0.583	22	0.486
36	0.437	33	0.581	33	0.485
41	0.430	41	0.581	44	0.486
51	0.424	49	0.576	55	0.487
61	0.416	66	0.574	66	0.487
71	0.409	82	0.568	89	0.487
81	0.402	99	0.563	110	0.486
102	0.389	114	0.556	155	0.489
153	0.359	164	0.536	177	0.492

and Garrett (*12*) have demonstrated that a Beckman spectrophotometer can give absolute absorption coefficients with certain inorganic salts and the natural Vitamin A ester. It is obvious then that interchangeability of spectrophotometric data on a sound basis will soon be possible among many laboratories.

The sensitivity of the absorption spectra of complex organic compounds to changes in molecular structure is a disadvantage in analytical studies. Small modifications of the structure of high molecular weight compounds, such as changes in the degree of conjugation of double bonds or in the elements comprising the conjugated system, can scarcely be detected by ordinary chemical methods and yet they may profoundly influence the absorption characteristics. Therefore it should always be ascertained that the spectrum of the compound in question has not undergone any change during the isolation and purification procedure prior to determination of the absolute absorption coefficients. This can be done critically and conveniently by employing the absolute absorption coefficients at several wave lengths for the analysis of the simple extract. As an illustration, consider the chlorophyll components; it was necessary to demonstrate that the absorption of the chlorophylls after rigorous purification was identical with that of the pigments after immediate extraction of the leaf material with solvent. Comar and Zscheile (*5*) obtained analytical agreement between measurements at several wave lengths when simple plant extracts were analyzed spectrophotometrically using the established absorption coefficients, which was considered sufficient evidence for the validity of the original absorption data. The analytical results are extremely sensitive to changes in the absorption and satisfactory values are not obtained if there are significant discrepancies in the absorption data. This procedure may also be used to determine the adequacy of a spectrophotometer for a given compound when a purified preparation is unobtainable. For instance, if a simple plant extract were analyzed satisfactorily for the chlorophyll components at several wave lengths using established absorption coefficients, this would indicate that the particular spectrophotometer was adequate for absolute measurements on this system.

FILTER PHOTOMETRY

Whenever possible it is desirable that a spectrophotometric method be adapted for use with the photoelectric colorimeter. Practically every laboratory has a colorimeter available and this instrument is less complicated and more easily used than the spectrophotometer. Absolute absorption measurements are not possible with today's photoelectric colorimeter and each instrument must have its own calibration curve for each substance. Interchangeability of calibration and absorption data is not to be recommended even between instruments of the same make and design.

The filter photometer is usually calibrated by using weighed amounts of the purified compound and making up solutions of known concentration. Some function of the absorption of each solution is plotted against the concentration to give the calibration curve. In the case of labile substances, this method may produce errors which pass unnoticed and are therefore all the more serious. This situation arises because the substance used for the calibration standard has usually been exposed to conditions during isolation, purification, and storage which may cause changes in the absorption, whereas the analytical measurements are customarily made on simple extracts in which absorption changes are at a minimum. This type of error is illustrated below.

A practical solution to this problem, where absorption coefficients have been established, involves calibration of the photoelectric colorimeter by means of spectrophotometric measurements on the simple extract. This method has proved entirely satisfactory for chlorophyll analysis (*1, 4*). The general procedure is as follows:

The worker prepares a simple extract in the manner decided upon for the colorimetric analysis. From the original extract a series of known dilutions is made which covers the optimum $\log_{10} \frac{I_0}{I}$ range of the colorimeter to be used. The absorbency of each dilution is measured on the colorimeter, and an aliquot of the original extract is measured on a spectrophotometer which is known to be satisfactory for absolute measurements on the system under study. The concentration of each of the dilutions used for the colorimetric measurement can then be calculated from the spectrophotometric value, and the calibration curve constructed.

Table II (see *4* for details) shows the results obtained with chlorophyll by this method and also points out the deviations which resulted when various purified preparations were used as calibration standards. The spectrophotometric as well as the colorimetric measurements were made on aliquots from the same leaf extract. These data support the viewpoint that colorimetric results must be viewed with suspicion where the calibration standard has not been studied spectrophotometrically.

Table III shows how this indirect method of calibration may be employed for the determination of total carotene.

In this study a Fisher Electrophotometer was used with 23-ml. cylindrical cells, and the 425 filter supplied with the instrument. The data for the direct calibration were obtained in the usual way by using weighed amounts of crystalline carotene (90% β, 10% α, from S.M.A. Corporation) in hexane. This sample of carotene had been stored under vacuum, in the dark, at refrigerator temperature until used. For the indirect calibration a plant extract was prepared from fresh carrots according to the method

Table II. Per Cent Deviation from Spectrophotometric Value for Total Chlorophyll

Plant Leaf Material	Photoelectric Colorimeter Calibrated with Chlorophyll in plant extract	Chlorophyll sample Z.-C.[a]	Chlorophyll sample 1[b]	Chlorophyll sample 2[c]
Norway maple	3.5	22.6	35.2	75.9
Muskmelon	1.0	21.1	37.7	82.9
Alfalfa	−3.9	14.7	30.0	70.4
Crimean linden	1.8	23.0	35.0	76.7
Tomato	0	20.1	37.3	78.1
Lima bean	−3.7	13.3	32.1	69.7
Watermelon	9.8	30.8	49.0	94.4
Peanut	−4.8	14.8	29.2	72.2
Bush string bean	−1.6	12.0	39.1	67.4
Pepper	−1.0	12.8	36.0	67.0
Oats	−3.5	16.3	32.2	71.3
Wheat	−2.0	18.3	33.7	74.8
Broccoli	−6.2	13.8	27.5	69.2
Spinach	−4.0	16.0	32.0	70.4
Beets	−2.7	19.1	41.8	72.7
Carrot	−3.3	16.7	32.5	73.2
Blue grass	−0.8	18.5	34.3	74.6
Sunflower	−4.8	14.3	31.2	72.5
Rhubarb	0	20.3	36.7	77.2
Black locust	−7.3	13.3	27.6	67.3
Hollyhock	−4.2	15.7	31.5	72.7
Peach	10.0	32.4	50.7	99.1
Clover	−2.2	16.4	30.3	69.3
Burdock	−4.3	14.7	32.2	73.9

[a] Prepared by method of Zscheile and Comar (*13*), absorption coefficient at 6600 Å. in diethyl ether 83 liters per gram cm.
[b] Research grade commercial chlorophyll, absorption coefficient at 6600 Å. in diethyl ether 44 liters per gram cm.
[c] Research grade commercial chlorophyll, absorption coefficient at 6600 Å. in diethyl ether 33 liters per gram cm.

Table III. Data for Calibration of Photoelectric Colorimeter for Carotene Analyses

(Direct calibration using crystalline carotene compared with indirect method using spectrophotometric determination on plant extract)

Direct Calibration with Crystalline Carotene		Indirect Calibration with Plant Extract	
Carotene concentration	$\log_{10} \frac{I_0}{I}$	Carotene concentration	$\log_{10} \frac{I_0}{I}$
Mg./l.		*Mg./l.*	
4.73	0.802	4.53	0.796
3.15	0.695	3.02	0.675
2.10	0.552	2.01	0.529
1.40	0.417	1.34	0.412
0.93	0.312	0.90	0.296
0.62	0.207	0.60	0.205
0.41	0.140	0.40	0.143

of Moore (*10*). Aliquots of this extract were measured on the colorimeter and the carotene concentrations were determined by measurements at 4520 Å. on a Beckman spectrophotometer, using the absorption coefficients as reported for pure carotenes in hexane by Zscheile *et al.* (*15*). The slit width was 0.03 mm. and 1-cm. cells were employed.

These data, when plotted, give essentially the same calibration curve. This indicates that when a good preparation of carotene is used, the analytical results will be the same with either the direct or indirect method of calibration. Any discrepancy in a comparison of this type will probably be due to deterioration of the crystalline carotene standard, so that the indirect calibration curve is considered the more reliable.

ADVANTAGES OF INDIRECT CALIBRATION

The fundamental advantage of the indirect method of calibration as outlined here is the reliability of the results obtained, which will lead to dependable interlaboratory agreements where this situation is now far from satisfactory in many cases. Another advantage lies in the simplicity of the indirect method. After the details have been worked out, the spectrophotometric determination on an aliquot of the extract is usually a simple matter of making measurements on a solution at a few given wave lengths. It can usually be arranged to have the solutions shipped to another laboratory for the spectrophotometric measurement, if necessary. This practice eliminates the need for obtaining the purified material either by purchase or preparation and for its carefully controlled storage, and eliminates the operation of weighing out the standard and the necessity for spectrophotometric study of the standard prior to each use.

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Determination of Conjugated Diolefins with Chloromaleic Anhydride

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A new method of determining conjugated diolefins in hydrocarbon mixtures utilizes chloromaleic anhydride as the dienophilic reagent. This method is based on the quantitative addition of chloromaleic anhydride to conjugated dienes to form an adduct containing a highly reactive tertiary chlorine atom which can be determined by the Volhard method after refluxing with aqueous silver nitrate; the vinyl chlorine of the reagent is completely unreactive under the same conditions.

DURING the early phases of the synthetic rubber program, an investigation of the preparation of conjugated dienoid monomers by the cracking of terpenes was undertaken in this laboratory. It soon developed that the determination of conjugated dienes in the pyrolysis products was one of the more important phases of the problem. Since no satisfactory procedure was available for the analysis of such mixtures, a number of physical and chemical methods were considered.

It was recognized that several physical methods including ultraviolet absorption, infrared absorption, and mass spectrometry give excellent results on mixtures of known constituents. However, to obtain reliable results by the absorption methods it is generally necessary to know not only the functional groups but also the molecular species present. Moreover, a simple chemical method requiring no special equipment appeared desirable for the routine determination of conjugated dienes in the crude products from cracking experiments. Consequently, a number of reagents were considered which included bromine (*4, 7*), sulfur dioxide (*10, 12*), aromatic diazonium coumpounds (*11*), cuprous chloride (*9*), maleic anhydride (*3, 12*), and chloromaleic anhydride. All these reagents except the last two either proved nonspecific for conjugated dienes or did not react quantitatively.

The first procedure developed involved fractionation of the samples and determination of the conjugated diene content of the cuts by a refined gas volumetric procedure utilizing maleic anhydride as the diene absorbent. However, the need for a more rapid procedure—particularly one that would not involve preliminary fractionation of the sample—resulted in the development of a second method using the same reagent. In this procedure the conjugated diene content was obtained by reacting the sample with a known excess of maleic anhydride and determining the unreacted reagent polarographically. Both procedures were time-consuming and provided only an indirect determination of the conjugated dienes.

The belief that chloromaleic anhydride would also react quantitatively with conjugated dienes and, in addition, would provide a direct and more rapid determination led to the development of the present method.

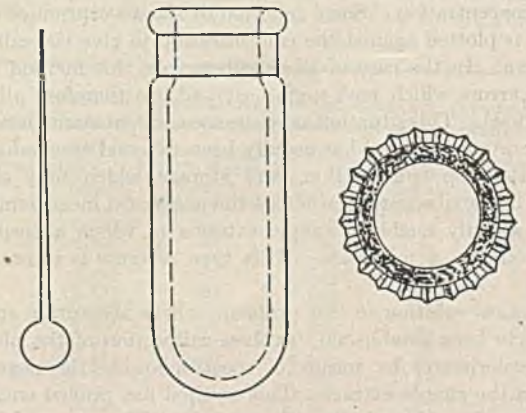


Figure 1. Sample Bulb, Pressure Bottle, and Bottle Cap

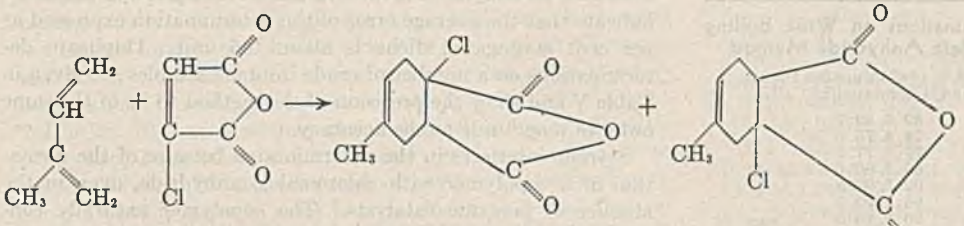
Table I. Determination of Chlorine in Isoprene–Chloromaleic Anhydride Adduct[a]

Compounds Added	Conditions of Pretreatment: Temp., ° C.	Conditions of Pretreatment: Time, hours	Chlorine in Adduct: Theoretical, %	Chlorine in Adduct: Found, %
None	...	...	17.68	17.7
None	...	...	...	17.6
None	...	...	...	17.6
Chloromaleic anhydride, 1 gram	55	2	...	17.9
Chloromaleic anhydride, 1 gram	50–55	2.5	...	17.8
Mixed Amylenes, 0.2 ml.	50–55	2.5	...	17.7
Chloromaleic anhydride, 1 gram	100	2	...	17.7
	100	2	...	17.7
Inhibitor solution, 2 drops	75–80	1	...	17.8
	75–80	1	...	17.8

[a] Sample weights varied from 0.2 to 0.5 gram.

DEVELOPMENT OF CHLOROMALEIC ANHYDRIDE METHOD

Chloromaleic anhydride reacts with conjugated diolefins in the same manner as maleic anhydride to form a Diels-Alder adduct. Thus, isoprene (I) reacts with chloromaleic anhydride (II) to give 1-chloro-4-methyl-4-cyclohexene-1,2-dicarboxylic anhydride (III) and 2-chloro-4-methyl-4-cyclohexene-1,2-dicarboxylic anhydride (IV).



I II III IV

The difference in reactivity between the highly active tertiary chlorine of the adduct and the unreactive vinyl chlorine of the reagent permits a quantitative determination of the adduct in the presence of excess reagent. Refluxing with aqueous silver nitrate removes the chlorine completely from the adduct, leaving the chlorine of the chloromaleic anhydride untouched.

It is believed that the utilization of the high reactivity of tertiary halogens as a basis of organic analysis is a principle that has considerable possibilities and has been exploited to only a limited extent. This same principle has been applied recently in the authors' laboratories to the determination of α,*p*-dimethylstyrene in styrene mixtures (*5*).

The practicality of the new method was demonstrated by substituting chloromaleic anhydride for maleic anhydride in the gasometric absorption procedure, and also by synthesizing pure isoprene–chloromaleic anhydride adduct (melting point 35–36° C.) and showing that the chlorine of the adduct could be determined by the Volhard method after refluxing with aqueous silver nitrate. As the results in Table I show, the agreement with theoretical is equally good when the determination is made in the presence of compounds added to simulate conditions of an actual analysis.

Samples containing conjugated dienes were then heated with excess chloromaleic anhydride in specially designed pressure bottles (*6*) (Figure 1), and the amount of adduct formed was determined as outlined above. It was soon apparent, as is shown by the data in Table II, that it was necessary to purify the commercial-grade chloromaleic anhydride to obtain quantitative adduct formation. Chloromaleic acid proved to be one of the most troublesome impurities, as it is readily formed by hydrolysis of the anhydride. The low and erratic results obtained in the presence of appreciable amounts of this acid, shown in Table III, may be due to the acid catalyzing the dimerization and polymerization of conjugated dienes, thus rendering them unavailable for adduct formation. The formation of chloromaleic acid can, however, be prevented by proper care in handling the anhydride.

Even with extensive purification it was difficult to obtain chloromaleic anhydride that gave theoretical adduct formation. Consequently, it was found desirable to use chloromaleic anhydride that gave results between 95 and 100% of theory and apply an empirical correction factor determined by analyzing a sample of known conjugated diene content. However, it is believed that if absolutely pure chloromaleic anhydride were available, it would be possible to eliminate the correction factor, since in one instance it has been reduced to 1.005.

Low and variable results were also obtained in the presence of peroxides which catalyze the copolymerization of conjugated dienes with chloromaleic anhydride. Apparently the copolymer contains more than a 1 to 1 ratio of conjugated diene to chloromaleic anhydride, and furthermore, it is so insoluble in aqueous silver nitrate that the analysis is unsatisfactory. In view of this adverse effect of natural peroxides, a small amount of inhibitor, such as *p-tert*-butylcatechol, was added as a precaution in all subsequent analyses.

A study of the optimum time and temperature for adduct formation showed that heating at 55° C. for 2 hours was sufficient for samples containing over 25% isoprene or for cyclopentadiene. Samples containing smaller amounts of isoprene should be heated at higher temperatures and/or for longer periods of time—for example, samples containing 5 to 10% should be heated for 4 hours at 75° C. Butadiene reacts more slowly than isoprene and it is necessary to heat the samples at 55° C. for 12 hours to obtain satisfactory results. Isoprene-butadiene mixtures containing 5 to 10% butadiene require 6 to 8 hours at 55° C. and mixtures containing 20 to 25% butadiene require 8 to 12 hours at 55° C. The method should be applicable to samples containing less than 5% diene, but the optimum reaction conditions have not been established experimentally. The accuracy in this range might be improved by increasing the sample weight.

CHLOROMALEIC ANHYDRIDE REAGENT

Commercial chloromaleic anhydride is purified by filtering the crystals from the oily mother liquor and washing them with dry hexane. (Commercial chloromaleic anhydride may be pur-

Table II. Effect of Purity of Chloromaleic Anhydride on Determination of Conjugated Dienes

Chloromaleic Anhydride: Sample purification	Chloromaleic Anhydride: Melting point, ° C.	Conjugated Diene, Analysis Results, % of Theory
Commercial	27 (ca.)	62.2 ± 5
One recrystallization	31.5–32.5	91.6 ± 2
Two recrystallizations	32–34	93.1 ± 1
Three recrystallizations	32.5–34	95.4 ± 1
Two recrystallizations, one distillation	...	99.0 ± 1
Theoretical	33 (*1*) 34.5 (*8*)	100.0

Table III. Effect of Chloromaleic Acid on Determination of Conjugated Dienes with Chloromaleic Anhydride

Chloromaleic Acid Added, Weight % of Total Reagents	Weight % of Isoprene Found (Av.)
0	93.9
1	90.6
2	92.0
3	91.5
10	85.3
50	82.2

Table IV. Analysis of Standard Conjugated Diene Samples with Chloromaleic Anhydride

Composition of Sample	Conjugated Dienes, Weight %: Present	Found[a]	Deviation
Isoprene	100.0	99.7	−0.3
	100.0	99.7	−0.3
	100.0	99.7	−0.3
	100.0	100.0	±0.0
	100.0	100.1	±0.1
Isoprene-amylene mixture	75.5	75.2	−0.3
	75.5	75.1	−0.4
	60.8	60.6	−0.2
	60.8	61.2	+0.4
	60.8	59.6	−1.2
	60.8	61.8	+1.0
	60.8	59.7	−1.1
	25.7	25.2	−0.5
	25.7	26.2	+0.5
	25.7	25.4	−0.3
Isoprene-pentane mixture	9.9	9.5	−0.4
	9.9	9.9	±0.0
	9.9	10.2	+0.3
	9.9	9.7	−0.2
	5.0	4.6	−0.4
	5.0	4.8	−0.2
Cyclopentadiene	98.9	97.5	−1.4
	98.9	98.5	−0.4
Butadiene[b]	98.4	97.8	−0.6
	98.4	97.9	−0.5

[a] Results corrected by a factor of 1.03.
[b] Butadiene samples heated for 12 hours at 55° C. instead of 2 hours.

Table V. Precision of Typical Determinations on Wide Boiling Range Isoprene Samples by Chloromaleic Anhydride Method

Sample Designation	Weight % of Conjugated Dienes Found, Calculated as C_5H_8
A	89.6, 89.7
B	74.4, 75.0
C	77.7, 77.5
D	85.3, 86.0
E	92.5, 92.8
F	95.4, 95.3
G	95.9, 96.0
H	91.6, 91.0
I	28.2, 27.3
J	93.8, 94.0

chased from the National Aniline Division, Allied Chemical and Dye Corporation, 40 Rector St., New York, N. Y. By special request one sample of chloromaleic anhydride was obtained which was of satisfactory analytical grade as received. Should such a reagent become generally available, the purification procedure would be unnecessary.) The crystalline chloromaleic anhydride is dissolved in the minimum amount of dry benzene and then hexane is added to incipient turbidity at room temperature. The mixture is seeded and cooled in an ice bath or a refrigerated room (+3° C.). The recrystallized product is filtered as before and then distilled in vacuo (water pump with drying tube in the line) under dry carbon dioxide or nitrogen. The fraction boiling at 110° C. at 44 mm. of mercury is collected and sealed in small glass ampoules under nitrogen or carbon dioxide until ready for use. The purified product melts at 32° to 34° C. and is completely soluble in dry benzene (insoluble material is usually chloromaleic acid). The correction factor for the reagent is determined by analyzing a sample of isoprene of known purity. If it is greater than 1.05, further purification is recommended.

ANALYTICAL PROCEDURE

For the analysis of volatile samples weigh a small glass bulb (Figure 1) similar to those used in acid analysis to the nearest 0.1 mg. Warm the weighed bulb slightly with a small flame and invert the stem into the sample, using a lead washer for support. Cool the bulb with a snugly fitting piece of dry ice until the desired amount of sample (0.1 to 0.2 gram) is drawn into it and then quickly seal the stem in a hot flame. To prevent carbonization and fractionation during sealing, keep the bulb well cooled by holding a small piece of dry ice just below the tip of the stem. Reweigh the bulb after it reaches room temperature and place it in a pressure bottle (Figure 1) with 1.0 ± 0.1 gram of chloromaleic anhydride. [These small pressure bottles, for use with commercial bottle caps, are available from the Ace Glass Co., Vineland, New Jersey. Bottle caps with cellophane liners ("spots") are recommended.] Add one drop (approximately 0.02 ml.) of a 10% solution of *p-tert*-butylcatechol in nitrobenzene, cap the pressure bottle using a household bottle capper, and break the sample bulb by striking the bottle sharply against a moderately hard object like the heel of one's shoe.

Heat for 2 hours at 55° C., cool, open the bottle, and transfer the reaction products to a 250-ml. Erlenmeyer flask through a funnel with 10 ml. of acetone and distilled water (50 to 75 ml.). (These conditions are for samples containing over 25% isoprene. See the preceding discussion regarding the conditions to use for other samples.) Use 2 ml. of the acetone to rinse the bottle cap. Add 20 ml. of 0.2 *N* aqueous silver nitrate solution and reflux the mixture for 1 hour. After cooling, filter, wash the precipitate thoroughly with distilled water, add 5 ml. of 1 to 1 nitric acid and 1 ml. of ferric alum indicator to the filtrate, and titrate the excess silver nitrate with 0.1 *N* potassium thiocyanate solution.

Nonvolatile samples may be weighed directly into the pressure bottle from a small weight buret. From this point the procedure is the same.

Calculate the results as follows (for unknown samples an approximate molecular weight may be assumed or the results may be expressed as diene numbers):

$$\% \text{ conjugated diene} = [(\text{ml. of } AgNO_3 \times N\ AgNO_3) - (\text{ml. of KSCN} \times N\ \text{KSCN})] \times \left[\frac{\text{mol. wt. of diene} \times \text{correction factor}}{\text{wt. of sample} \times 10}\right]$$

DISCUSSION OF RESULTS

The conjugated diene content of a number of standard samples of isoprene, isoprene-amylene mixtures, isoprene-pentane mixtures, cyclopentadiene, and butadiene was determined by the chloromaleic anhydride method. The results given in Table IV indicate that the average error of the determination expressed as per cent conjugated diene is about 0.5 unit. Duplicate determinations on a number of crude isoprene samples are given in Table V and show the precision of the method to be of the same order of magnitude as the accuracy.

Styrene interferes in the determination because of the formation of a copolymer with chloromaleic anhydride, even in the absence of peroxide catalysts. The copolymer naturally contains tertiary chlorine which is determined along with the chlorine of the conjugated diene-chloromaleic adduct.

Although the method has been used most extensively for the determination of isoprene, it has been applied successfully to the determination of butadiene, cyclopentadiene, and other conjugated dienes. Preliminary experiments indicate that *trans*-piperylene and 2-methyl-1,3-pentadiene can be determined with chloromaleic anhydride, although 4-methyl-1,3-pentadiene cannot. The behavior of the latter compound is not surprising, since it has been found (*2*) that it will not form a Diels-Alder adduct with maleic anhydride. It is probable that the method could be applied to other conjugated dienes by varying the conditions of adduct formation to obtain a quantitative reaction.

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Indicator Properties of Derivatives of 4′-Nitrophenylazo-1-naphthol

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Three new indicators of the 2-(4′-nitrophenylazo)-1-naphthol class are described and indicator properties given.

CONGO red indicator test paper, in common use in the dyestuff industry, possesses the practical advantage of changing from the normally red neutral form through a series of grays and blue-blacks to the blue acid form. With experience, changes in pH of 0.2 may be detected. During some experiments on the properties of 1-naphthol derivatives, the author observed that the monoazo dye, resulting from coupling *p*-nitrophenyl diazonium chloride to 1-naphthol-4,8-disulfonic acid, had indicator properties similar to Congo red but was acid- instead of alkali-sensitive. Analogous compounds prepared from derivatives of 1-naphthol and *p*-nitroaniline had similar properties, changing from the normal red neutral form to the blue alkaline form over various pH ranges and hence were useful in testing common alkaline mixtures—e.g., sodium bicarbonate and carbonate.

Hewitt (*3*) has described the indicator 4-(4′-nitro-3′-sulfophenylazo)-1-naphthol which changes from yellow (acid) to purple (alkali). Wenker (*6*) examined various 2,4-dinitrophenylazo-1-naphthol sulfonic acids but concluded that the dyes resulting from ortho coupling were lacking in sensitivity to pH changes. This paper describes the indicator properties of three typical dyes made by coupling 4-nitrophenyl diazonium chloride (or the 2-methoxy derivative) to 1-naphthol-3,8- or 4,8-disulfonic acids which permit of only ortho coupling.

EXPERIMENTAL

I. Disodium Salt of 2-(4′-Nitrophenylazo)-1-naphthol-4,8-disulfonic Acid. Alpha blue. A mixture of 138 grams (1 mole) of *p*-nitraniline and 72 grams of technical (95%) sodium nitrite in 1 liter of water was slowly added to an agitated mixture of 1000 grams of ice, 250 ml. of commercial 20° Bé. hydrochloric acid, and 2 grams of sodium nitrite. After 2 hours the resultant diazonium chloride solution was filtered to remove sludge and slowly added to a solution of 1 mole of 1-naphthol-4,8-disulfonic acid (*4*), 370 grams of sodium carbonate, and 1000 grams of ice in 1.5 liters of water. The combination was allowed to agitate overnight and was then filtered, reslurried in 3 liters of 10% sodium chloride solution, filtered, and dried at 60° C. in an atmospheric oven.

Pure coal-tar dye (as disodium salt) by titration with titanous chloride, 67.1%.

II. Disodium Salt of 2-(4′-Nitrophenylazo)-1-naphthol-3,8-disulfonic Acid. Epsilon blue. One mole of *p*-nitraniline was diazotized as above and then slowly added to a solution of 1 mole of 1-naphthol-3,8-disulfonic acid (*2*), 336 grams of sodium carbonate, and 1000 grams of ice in 2 liters of water. The resultant dye was isolated and dried as above.

Pure coal-tar dye (as disodium salt) by titration with titanous chloride, 93.7%.

III. Disodium Salt of 2-(2′-Methoxy-4′-nitrophenylazo)-1-naphthol-4,8-disulfonic Acid. Nitroanisole blue. A solution of 74 grams of 95% sodium nitrite in 150 ml. of water was added slowly to a mixture of 168 grams (1 mole) of 5-nitro-2-aminoanisole (E. I. du Pont de Nemours & Co.), 250 ml. of 20° Bé. hydrochloric acid, and 1000 grams of ice. After 2 hours' agitation, the diazonium chloride solution was filtered and

Table I. Wave Lengths and Indicator Exponents

	I	II	III
Wave length $\left(\frac{\Delta \log D}{\Delta \text{pH}} = \text{max.}\right)$, mμ	610	640	630
pK	9.6	12.0	10.0

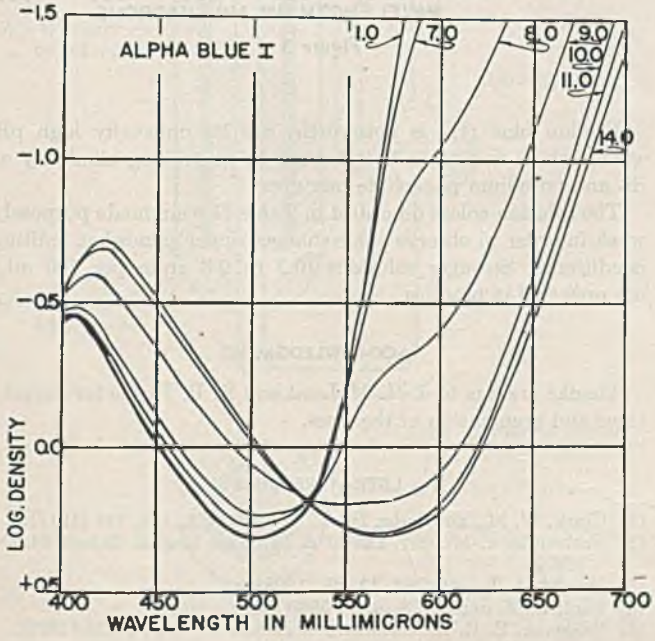

Figure 1

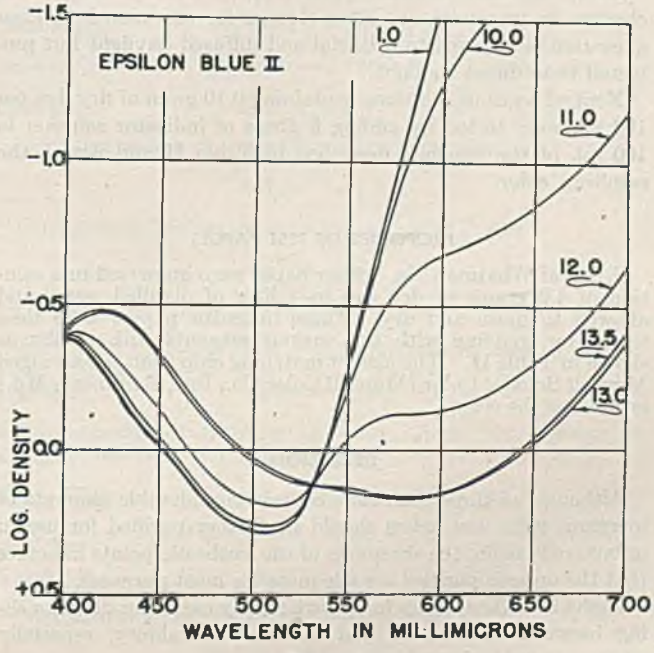

Figure 2

coupled to 1-naphthol-4,8-disulfonic acid in the same manner as for I.

Pure coal-tar dye (as disodium salt) by titration with titanous chloride, 74.1%.

INDICATOR PROPERTIES

LIGHT ABSORPTION. Spectral transmission curves (Figures 1, 2, and 3) of buffer solutions containing 10 p.p.m. of dry dye were obtained by means of the General Electric recording spectrophotometer, using a path length of 50 mm., band width of 10 mμ, temperature of 30° C., and distilled water as reference solvent. Buffer solutions for pH from 1 to 8 were essentially as described by Clark and Lubs (*1*); for pH 9, 10, 11, and 12, mixtures of sodium carbonate and bicarbonate were prepared; and for pH 13 and 14 solutions of sodium and potassium hydroxides were employed. All buffer solutions were checked by a Beckman pH meter just prior to determining the transmission curves. The presence of boric acid in Clark and Lubs buffers pH 9 and 10 was found to interfere with the dissolved dye.

Isosbestic points (*5*) in the visible region were at 531 mμ for I, 532 mμ for II, and 551 mμ for III.

INDICATOR EXPONENTS

Indicator exponents (pK) were determined graphically by observing the inflection points of the curves obtained by plotting optical density *vs.* pH. The density, D, was determined from the spectral transmission curves (Figures 1, 2, and 3) at the wave lengths of maximum difference of log density per unit change in pH—i.e., when $\frac{\Delta \log D}{\Delta \text{pH}} = \text{max.}$

Selected wave lengths and resultant indicator exponents are given in Table I.

PROPERTIES OF SOLUTIONS

Dilute aqueous solutions (10 p.p.m.) showed only small changes in transmittance after storage for one month in clear glass bottles exposed to artificial and diffused daylight but protected from direct sunlight.

Neutral aqueous solutions containing 0.10 gram of dry dye per 100 ml. were tested by adding 3 drops of indicator solution to 100 ml. of the reagents described in Table II and noting the resulting color.

PROPERTIES OF TEST PAPERS

Strips of Whatman No. 1 filter paper were immersed in a solution of 4.0 grams of dry dye in 1 liter of distilled water and allowed to drain and dry. These indicator papers were then tested by spotting with the various reagents with results as shown in Table II. The closest matching chip from the abridged Munsell Book of Color (Munsell Color Co., Inc., Baltimore, Md.) is given as the color.

DISCUSSION

Although all three indicators contain considerable amounts of inorganic salts and hence should be further purified for use in unbuffered media, the sharpness of the isosbestic points indicates that the organic purities are adequate for most purposes.

Alpha blue (I) appears to be particularly useful for distinguishing between bicarbonate and carbonate alkalinity, especially when used in the form of test paper.

Table II. Properties of Solutions and Test Papers

Reagent	Color of Solutions			Color of Test Paper		
	I	II	III	I	II	III
Distilled water	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
Alcohol	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
1 *N* HCl	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
0.1 *N* HCl	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
5% acetic acid	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
5% sodium bicarbonate	Pink	Pink	Pink	5R 6/10	5R 5/12	10RP 5/10
5% $Na_2HPO_4.12H_2O$	Pink	Pink	Pink	10RP 4/10	5R 5/12	5R 5/12
5% sodium carbonate	Light purple	Purplish pink	Light purple	10PB 3/8	10R 4/8	10PB 3/8
5% $Na_3PO_4.12H_2O$	Light purple	Pale bluish purple	Light purple	10PB 3/10	5R 3/4	10PB 3/10
0.1 *N* NaOH	Light purple	Pale bluish purple	Light purple	10PB 3/8	10PB 3/8	10PB 3/8
Buffer, pH						
8	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
9	Purplish pink	Pink	Pink	2.5RP 3/4	5R 5/12	10RP 4/10
10	Light purple	Pink	Purplish pink	5P 3/4	5R 5/12	2.5RP 4/6
11	Light purple	Pink	Light purple	10PB 3/8	5R 5/12	10PB 3/8
12	Light purple	Purplish pink	Light purple	10PB 3/8	10R 4/8	10PB 3/8
13	Light purple	Light purple	Light purple	10PB 4/10	10PB 3/8	10PB 4/8

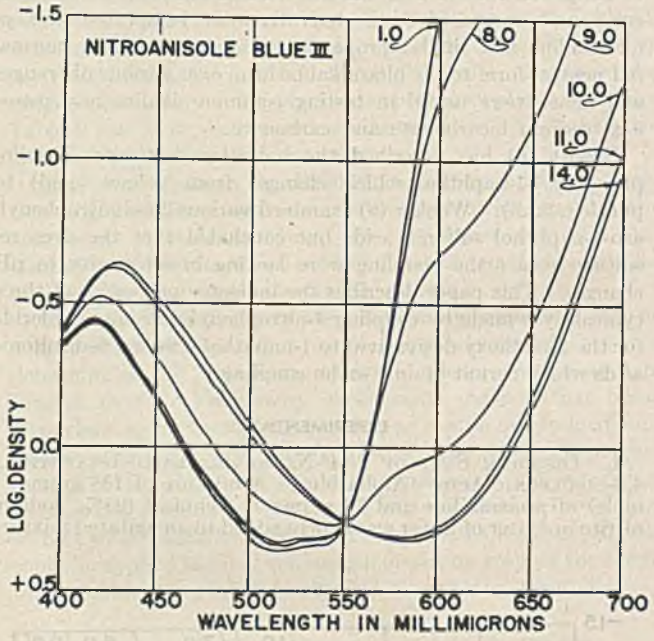

Figure 3

Epsilon blue (II) is noteworthy for its unusually high pK value, which recommends it for use in controlling alkalinity of di- and trisodium phosphate mixtures.

The solution colors described in Table II were made purposely weak in order to observe color changes under somewhat limiting conditions. Stronger solutions (0.2 to 0.3 gram per 100 ml.) are preferred in practice.

ACKNOWLEDGMENT

Thanks are due to J. H. McLeod and R. B. Payne for suggestions and preparation of the dyes.

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Theory and Operation of a Cartesian Diver Type of Manostat

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THE idea of using a float, consisting of an inverted tube with entrapped air, for vastly increasing the sensitivity of pressure measurements dates as far back as 1704, when Caswell published the details of a baroscope embodying this principle (*1*), which is generally known today as the Cartesian diver. More recently, the idea was modified to yield the low-pressure Dubrovin gage (*2*), the theory of which is described in a recent paper (*3*). An adaptation of the Cartesian diver to control the pressure in a closed system has been available for purchase for the past few years from the Emil Greiner Co., New York, N. Y., where the manostat was developed. This device has proved useful in adequately maintaining constant pressures in vapor-liquid equilibrium measurements and vacuum distillations ranging from a moderate vacuum of a few millimeters of mercury to atmospheric pressure.

OPERATION

Mercury is introduced into the container of Figure 1 until the disk of the float just makes contact with the orifice, when the pressure is equalized inside and outside the float. The device is connected to the vacuum pump, and to the system by way of a large reservoir and a suitable manometer. With the stopcock open, the pressure in the system is reduced by way of a by-pass between the pump and system until the desired value as read on the manometer is reached, then both the stopcock and by-pass are closed; the device will automatically maintain the desired pressure within limits determined by the dimensions of the constituent parts of the device. If the system is vacuum-tight, the pressure will maintain itself; however, a slight leak, which may be introduced intentionally, will cause the pressure to rise slightly. This will produce a displacement of the mercury level downward outside the float and a corresponding displacement upward inside the float; the buoyant force on the float is consequently diminished and when the reduction in buoyancy becomes sufficient to overcome the suction force at the orifice due to the pressure differential, the disk will break away from the orifice and permit the pump to evacuate sufficient gas from the system to restore the original pressure. When the original pressure is restored, the disk will return to its former position and seal off the orifice. The cycle is repeated indefinitely, if the size of the leak in the system does not exceed the capacity of gas flow that is possible through the orifice, and the pump is of sufficient rating to carry the load.

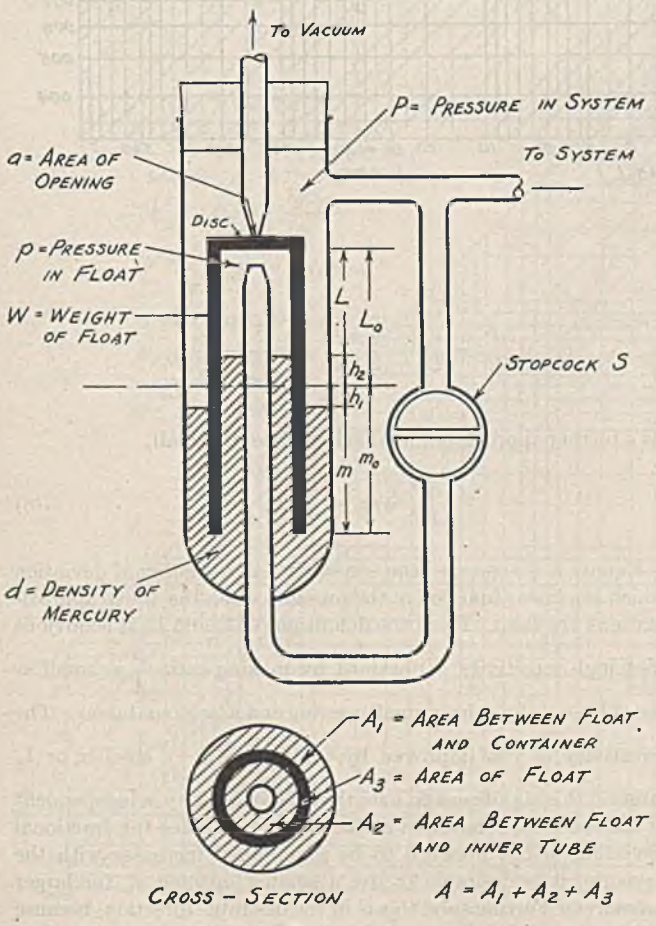

Figure 1

THEORY

In designing a Cartesian diver manostat, it is desirable to know the relationship between its sensitivity and physical dimensions, so that the particular requirements for its use may be met. Such a relationship is readily derived using the symbols of Figure 1.

By means of the stopcock, S, and the by-pass, the system is brought to the desirable pressure, P_0, as described above. The initial pressure inside the float, p_0, will be equal to that initially in the system, P_0, and the level of mercury inside and outside the float will be the same—i.e., h_1 and h_2 are initially 0. As the pressure increases in the system, owing to slight leaks, the liquid level is depressed outside the float and is correspondingly elevated inside the float, so that the buoyant force on the float is decreased. When the pressure in the system reaches a sufficiently high value, P, so that the buoyant force is diminished enough to counteract the force at the opening, a, due to the pressure differential, the float breaks away from the opening and the vacuum can restore the initial pressure. As soon as the initial pressure, P_0, is restored, the float cuts off the vacuum and the cycle is repeated indefinitely. The maximum variation in pressure, $P - P_0$, is a reciprocal measure of the sensitivity of the instrument and can be expressed quantitatively by setting up the following equations:

Pressure Equalities. $P = p + h_1 + h_2$ and $P_0 = p_0$ (pressure expressed as a height of mercury)

Combining, $$P - P_0 = p - p_0 + h_1 + h_2$$

and letting $$\Delta P = P - P_0 \text{ and } \Delta p = p - p_0$$

Therefore, $$\Delta P = \Delta p + h_1 + h_2 \qquad (1)$$

Equality of Volumes of Displaced Mercury.

$$h_1 A_1 = h_2 A_2 \qquad (2)$$

Isothermal Compression of Ideal Gas in Float.

$$pL = p_0 L_0$$

or $$pL - p_0 L = p_0 L_0 - p_0 L = p_0(L_0 - L) = p_0 h_2$$

and $$L\,\Delta p = (L_0 - h_2)\,\Delta p = P_0 h_2 \text{ (remembering that } P_0 = p_0)$$

Therefore, $$L_0 \Delta p = h_2(\Delta p + P_0) \qquad (3)$$

Equilibrium of Buoyant Forces. Assuming pressure in vacuum line is zero and neglecting buoyant forces due to gas,

$W + A_2(m_0 + h_2)d = (A_2 + A_3)md + aPd$, and $W = A_3 m_0 d$

Combining, $A_3 m_0 d + A_2 m_0 d + A_2 h_2 d = A_2 md + A_3 md + aPd$

Simplifying, $$h_1(A_2 + A_3) + h_2 A_2 = aP$$

Substituting Equation 2, and making use of $\Delta P = P - P_0$

$$h_1 A = a(\Delta P + P_0) \qquad (4)$$

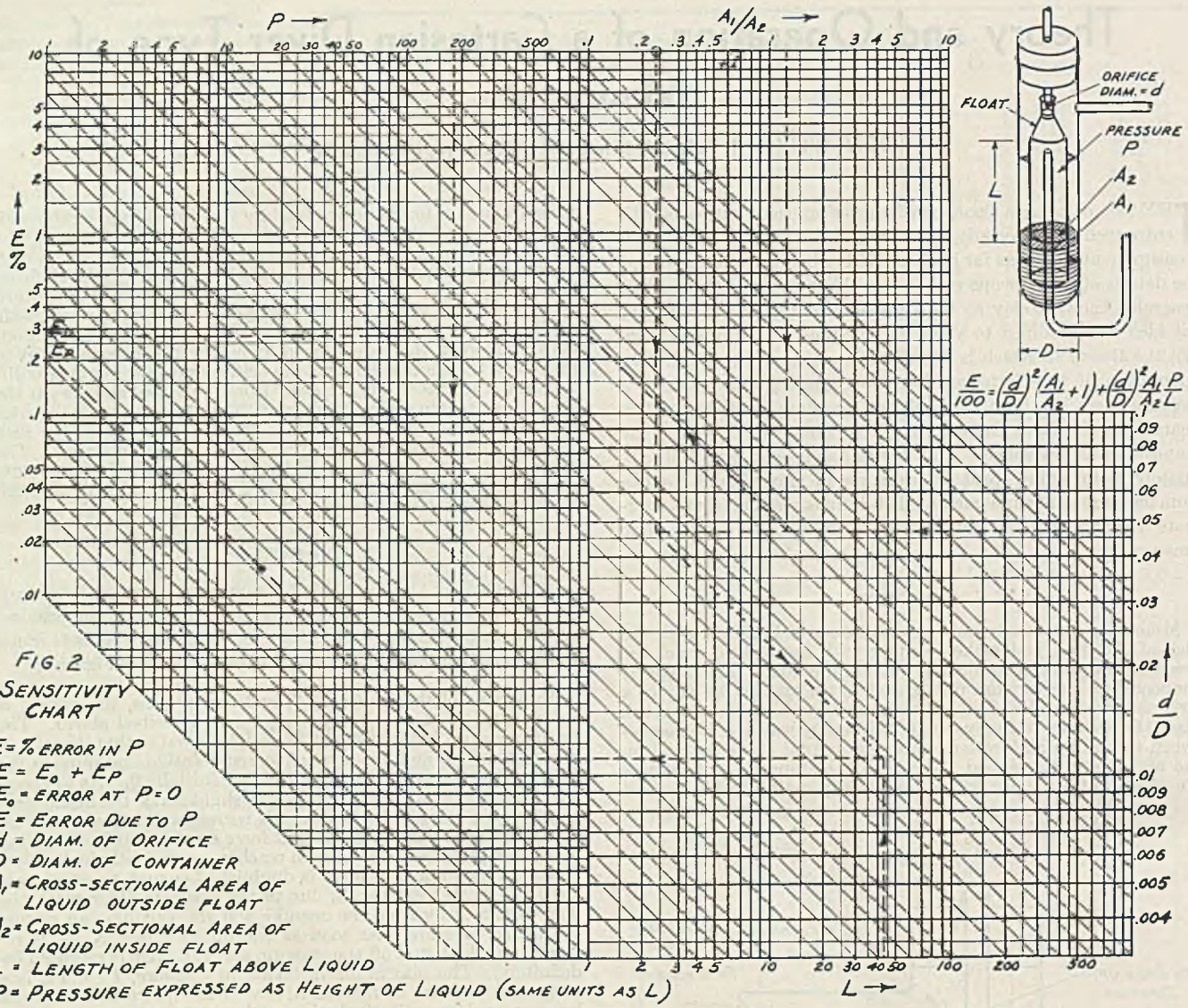

FIG. 2
SENSITIVITY CHART

E = % ERROR IN P
E = E_0 + E_P
E_0 = ERROR AT P = 0
E_P = ERROR DUE TO P
d = DIAM. OF ORIFICE
D = DIAM. OF CONTAINER
A_1 = CROSS-SECTIONAL AREA OF LIQUID OUTSIDE FLOAT
A_2 = CROSS-SECTIONAL AREA OF LIQUID INSIDE FLOAT
L = LENGTH OF FLOAT ABOVE LIQUID
P = PRESSURE EXPRESSED AS HEIGHT OF LIQUID (SAME UNITS AS L)

Equations 1 to 4 may be solved simultaneously for ΔP in terms of P_0, L_0, a, A, A_1, and A_2 by eliminating h_1, h_2, and Δp:

Combining 2 and 4 to eliminate h_1 and letting

$$k_1 = \frac{a}{A}\,\frac{A_1}{A_2}$$

gives
$$h_2 = k_1(\Delta P + P_0) \tag{5}$$

Combining 3 and 5 to eliminate h_2 and simplifying,

$$\Delta p(L_0 - k_1\Delta P - k_1P_0) = k_1P_0(\Delta P + P_0) \tag{6}$$

Combining 1, 2, and 4 so as to eliminate h_1 and h_2 and letting

$$k_2 = \frac{a}{A}\left(\frac{A_1}{A_2} + 1\right)$$

gives
$$\Delta P(1 - k_2) - k_2P_0 = \Delta p \tag{7}$$

Combining 6 and 7 to eliminate Δp and simplifying,

$$(\Delta P)^2 - \left(\frac{L_0}{k_1} - 2\,P_0\right)\Delta P + \frac{k_2P_0L_0}{k_1(1 - k_2)} + P_0^2 = 0$$

Now k_1 and k_2 should be made very small to give high sensitivity, so that the square term may be neglected and $(1 - k_2)$ may be taken as unity; therefore as a close approximation,

$$\frac{\Delta P}{P_0} = \frac{k_2L_0 + k_1P_0}{L_0 - 2\,k_1P_0} = \frac{k_2 + \dfrac{k_1}{L_0}P_0}{1 - 2\,k_1\dfrac{P_0}{L_0}}$$

As a further approximation, if k_1 is sufficiently small,

$$\frac{\Delta P}{P_0} = k_2 + \frac{k_1}{L_0}P_0 \tag{8}$$

Equation 8 expresses the sensitivity as a fractional deviation which is a linear function of the pressure when the above approximations are valid. From the definitions of k_1 and k_2, it is obvious that high sensitivity is obtained by making ratio $\frac{a}{A}$ as small as possible—i.e., by using a small opening and a large container. The sensitivity is also improved by making ratio $\frac{A_1}{A_2}$ smaller or L_0 larger. It is significant to note that the sensitivity is independent of the cross-sectional area of the float, A_3. Since the fractional deviation in the pressure to be maintained increases with the pressure, it is desirable to use a smaller opening, a, for larger pressures. Fortunately, this is in the desirable direction, because at higher pressures leakage is smaller and a higher pressure differ-

ential exists across the opening, so that more rapid evacuation is possible. To maintain very low pressures, a larger opening is required to take care of leaks and to give more rapid evacuation; since the last term in Equation 8 becomes negligible, the fractional deviation may then be conveniently approximated by:

$$\frac{\Delta P}{P_0} = k_2 = \frac{a}{A}\left(\frac{A_1}{A_2} + 1\right) \qquad (9)$$

To enable one rapidly to compute the sensitivity as given by Equation 8, the chart in Figure 2 was constructed. In this chart the symbols have the same meaning as before with the following addition:

$$E = \%\text{ error in } P = \frac{\Delta P}{P_0} \times 100$$

$$\left(\frac{d}{D}\right)^2 = \frac{a}{A}$$

The per cent error in P is divided into two terms—namely, one which is independent of pressure, E_0, and one proportional to the pressure, E_p. The values of d/D, A_1/A_2, and L are determined by the dimensions of the device—e.g., 0.047, 0.23, and 43 mm., respectively—and P is arbitrarily chosen—e.g., 175 mm. of mercury. On the chart, the initial point of this representative example is marked by O on the A_1/A_2 axis; this is continued vertically downward to meet the value of d/D projected across horizontally. The point of intersection determines one of the 45° lines, which is intersected by the value of L projected upward vertically. From this second point of intersection a horizontal line is projected to meet the vertical line projected down from the P axis corresponding to the desired pressure. The intersection of these lines determines a 45° line which is projected to the E axis and determines the value of E_p in per cent. The above path is shown by the dotted line. To determine E_0, the path indicated by the short and long dashed line is obtained as follows: From the point marked O on the A_1/A_2 axis, the path proceeds horizontally to the value of $(A_1/A_2) + 1$ by adding unity to the value of A_1/A_2, and then vertically to the value of d/D, then at 45° to the axis corresponding to $L = 1$, and finally horizontally to give the value of E_0. The total per cent error in the pressure is the sum of E_0 and E_p:

$$E = E_0 + E_p = 0.28 + 0.22 = 0.50\%$$

Hence the pressure of 175 mm. of mercury will then fluctuate

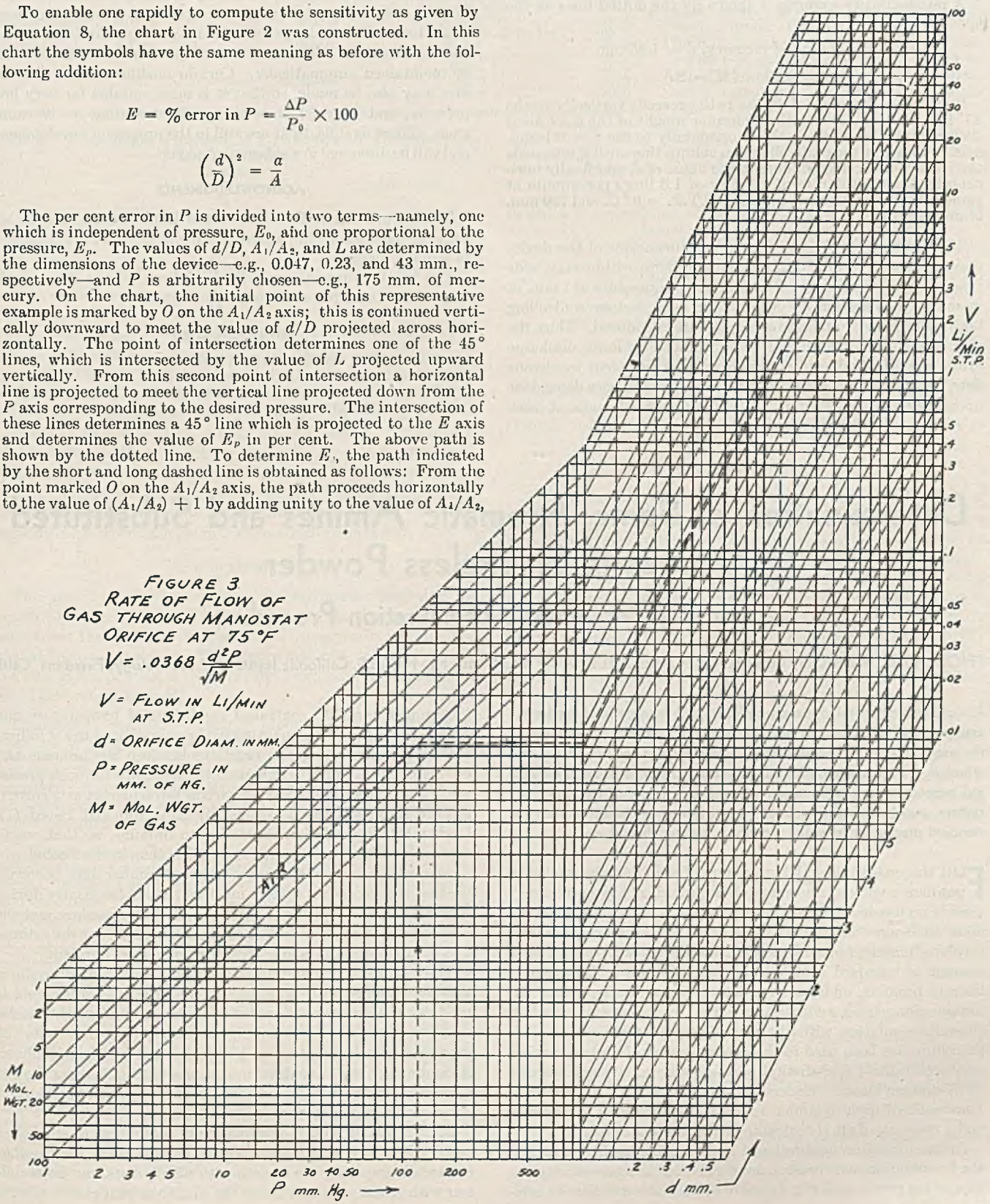

FIGURE 3
RATE OF FLOW OF GAS THROUGH MANOSTAT ORIFICE AT 75°F

$V = .0368 \frac{d^2P}{\sqrt{M}}$

V = FLOW IN LI/MIN AT S.T.P.

d = ORIFICE DIAM. IN MM.

P = PRESSURE IN MM. OF HG.

M = MOL. WGT. OF GAS

by 0.9 mm. of mercury. Higher sensitivity is possible by decreasing the ratio d/D, say, by decreasing the size of the orifice. However, the size of the orifice limits the capacity of gas removal from the system, so that increased sensitivity by decreasing the size of the orifice is obtained at the expense of decreased capacity.

In Figure 3, the rate of gas flow is plotted from a suitable orifice flow equation (Fliegner's equation) modified to include the molecular weight of the gas, although otherwise based on air flow.

A representative example is shown by the dotted lines on the chart for:

$$P = 120 \text{ mm. of mercury, } d = 1.25 \text{ mm.}$$

$$\text{for air flow } (M = 29)$$

Starting at the value of P, the path proceeds vertically to the 45° line corresponding to the molecular weight of the gas (air is shown by the heavy line), then horizontally to the d = 0.1-mm. axis. From here the path follows an oblique line until it intersects the vertical line corresponding to the value of d, and finally horizontally to give the rate of gas flow of 1.3 liters per minute at standard temperature and pressure (S.T.P. = 0° C. and 760 mm. of mercury pressure).

Thus, by means of these charts, the dimensions of the device may be chosen to give desired characteristics within very wide ranges. These charts do not extend below a pressure of 1 mm. of mercury, because for lower pressures new factors controlling both the sensitivity and rate of gas flow are introduced. Thus, the rate of gas flow equation has to be modified by lower discharge orifice coefficients due to change-over from turbulent to viscous flow. Moreover, the sensitivity of the device becomes dependent upon the relative motion of the float, which is adequate at pressures above about 1 mm. of mercury; below this pressure, modifications in design are needed to magnify the displacement of the float.

CONCLUSIONS

The simplicity of this type of manostat and the ease with which it may be designed to meet a wide range of requirements make it ideal for practically all laboratory needs in place of complicated electrical hookups. Built of heavy glass or metal, the unit may be easily adapted for superatmospheric pressures. Furthermore, by suitable design, the device may be used for large industrial applications whenever constant-pressure conditions must be maintained automatically. Certain modifications of the device may also be made, so that it is more suitable for very low pressure, and in which it is also a direct-reading semivacuum gage. These modifications are still in the process of development and will be discussed in a subsequent paper.

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Determination of Some Aromatic Amines and Substituted Ureas in Smokeless Powder

Improved Volumetric Bromination Procedure

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The volumetric bromination procedure which is in general use for the estimation of stabilizers in smokeless powder has been modified by the use of glacial acetic acid as a solvent for the sample to be brominated. The modified procedure is more convenient than the standard procedure in which carbon tetrachloride is used in a two-phase system, and less dependent on conditions of bromination than the standard procedure in which alcohol is used as the solvent.

FOR the quantitative determination of stabilizers in smokeless powders a volumetric bromination procedure has been widely used in preference to gravimetric procedures, which are generally more time-consuming. The volumetric bromination procedure involves treatment of a solution of a powder extract with a known amount of standard bromate-bromide solution, acidification to liberate bromine, addition of iodide in excess at the end of the bromination period, and titration of the free iodine with standard thiosulfate solution with the use of starch as indicator. This procedure has been used in the determination of diphenylamine, ethyl centralite (N,N'-diethyl-N,N'-diphenylurea), and acardite (N,N-diphenylurea). Under the conditions of the procedure 1 molecule of diphenylamine reacts with 4 molecules of bromine, and 1 molecule of ethyl centralite or acardite reacts with 2.

In the procedure as developed for centralite by Levenson (*2*), the bromination and titration are carried out in a one-phase solution in the presence of ethyl alcohol to keep the stabilizer in solution, under carefully controlled conditions of temperature and time in order to obtain quantitative bromination of the stabilizer without accompanying side reactions between the bromine and the alcohol. In order to eliminate the necessity for such precise control a procedure employing carbon tetrachloride as a solvent for the stabilizer was developed by Ellington and Beard (*1*). Carbon tetrachloride is nonreactive to bromine, so that conditions of bromination are much less critical than in the alcohol procedure. The manipulations are somewhat cumbersome, however, because the two-phase system must be shaken frequently during bromination and titration. Both the alcohol procedure and the carbon tetrachloride procedure are in common use for the estimation of diphenylamine and centralite in smokeless powders.

The authors have found that the advantages of both procedures may be obtained in a procedure in which glacial acetic acid is used as the solvent for the substances to be brominated. Acetic acid is nonreactive to bromine; moreover, its use permits the bromination and titration to be carried out in a single phase. Accordingly, they have developed a procedure involving the use of acetic acid and have tested this procedure by determinations of ethyl centralite, diphenylamine, and acardite.

Most procedures for the estimation of stabilizers in smokeless powder involve extracting the powder sample directly with ether or else decomposing the sample with alkali, distilling the stabilizer with steam, and extracting the distillate with ether; the re-

Table I. Analysis of Pure Samples of Stabilizers

Stabilizer	Method	Time of Bromination, Minutes	Temperature of Bromination, ° C.	Per Cent of Sample Detected: Individual determinations	Average
Ethyl centralite	HOAc	5	Room	101.5, 101.0, 102.0, 102.8	101.8
		1		100.2, 99.9, 99.8, 100.0	100.0
		45 sec.		99.8	99.8
		30 sec.		99.5	99.5
		15 sec.		99.3	99.3
	Alcohol	45 sec.	15–20	99.3, 99.6, 99.7, 99.2	99.5
	CCl_4	5	Room	99.8, 99.8, 98.3, 100.7, 99.9, 99.6, 100.8, 99.3, 99.6	99.8
Diphenylamine	HOAc	1	Room	99.9, 99.0	99.4
	CCl_4	5	Room	99.6, 99.8, 99.2, 99.9	99.6
Acardite	HOAc	10	Room	99.0	99.0
		5		99.1, 98.3	98.7
		1		84.5, 82.8	83.6
	CCl_4	3 hours	Room	81.3	81.3
		5		30.9	30.9

Table II. Comparative Analyses of a Nitroglycerin Propellant for Ethyl Centralite

	Carbon Tetrachloride %	Acetic Acid %
Centralite	0.986	0.971
	0.997	0.973
		0.978
		0.981
Av.	0.992	0.976

sulting ether solution is evaporated, and the residue from the evaporation is analyzed for stabilizer. The authors have found that the use of these procedures on samples that were subsequently analyzed for ethyl centralite or diphenylamine by the carbon tetrachloride bromination procedure frequently led to results which were low by as much as 10% of the stabilizer present. Subsequent studies indicated that the discrepancies were due to the presence of difficultly volatile peroxides in the ether. The replacement of ether by methylene chloride as an extracting solvent eliminated the discrepancy entirely and led to satisfactory results. Accordingly its use is recommended in the procedure given below.

PROCEDURE

This procedure may be used when diphenylamine, centralite, or acardite are present in a smokeless powder in the absence of other substances that react with bromine. Nitroglycerin and diethyl phthalate do not interfere. When more than one stabilizer is present in a given powder, a more involved procedure than the one given below must be used.

Reagents. Standard 0.1000 *N* bromate-bromide solution is prepared by dissolving 2.784 grams of recrystallized potassium bromate (dried to constant weight at 110° C.) and 15 grams of potassium bromide in distilled water and diluting to 1 liter in a volumetric flask. Standard 0.05 *N* sodium thiosulfate solution, 0.5% starch indicator solution, and a 15% solution of potassium iodide are prepared according to standard iodometric practice (*3*).

The methylene chloride used for extraction is distilled from technical grade material; adequate head and tail fractions are discarded. Reagent grade glacial acetic acid is used in the bromination procedure.

Extraction and Bromination. A sample of powder containing not more than 0.075 gram of ethyl centralite, 0.02 gram of diphenylamine, or 0.06 gram of acardite is finely divided and introduced into a Soxhlet extraction apparatus, which is attached to a 250-ml. glass-stoppered iodination flask containing 100 ml. of methylene chloride. The sample is extracted for 2 hours or more, depending on its state of subdivision. The flask is then detached and the methylene chloride is completely evaporated by means of a stream of dry air, with suitable precautions to minimize the hazard due to possible detonation of the nitroglycerin in the extract. The residue is dissolved by the addition of 60 ml. of glacial acetic acid, and 25.00 ml. of 0.1000 *N* bromate-bromide solution are added with a pipet. Five milliliters of concentrated hydrochloric acid are added, the flask is stoppered, and the contents are mixed by swirling. The bromination is allowed to proceed for 1 ± 0.25 minutes from the time the solution was acidified if centralite or diphenylamine are being determined, or at least 5 minutes in the case of acardite.

Titration. At the end of this time 10 ml. of 15% potassium iodide solution are added, the flask is swirled, and the gutter and walls are washed down with distilled water from a wash bottle. The solution is titrated immediately with 0.05 *N* sodium thiosulfate solution. Five milliliters of 0.5% starch solution are added when the solution has assumed a light yellow color, and the titration is continued to the disappearance of the blue color. A blank determination is made, under the same conditions of bromination and titration, on a 60-ml. portion of glacial acetic acid. The percentage of stabilizer in the powder sample is calculated by means of the equation

$$\text{Percentage of stabilizer} = \frac{(1 - A/B)\,NVC}{W}$$

in which A = volume of thiosulfate consumed in titration of sample
B = volume in milliliters of thiosulfate solution consumed in titration of blank
N = normality of standard bromate-bromide solution
V = volume of pipet in milliliters
W = weight of powder sample in grams
C = one tenth of equivalent weight of stabilizer; 6.709 for centralite, 2.115 for diphenylamine, and 5.306 for acardite

The nitroglycerin remaining in the solution at the end of the titration should be destroyed by boiling with an excess of ferrous chloride or by some other appropriate procedure.

RESULTS

The accuracy and precision of the new method and the effect of time of bromination were determined by analysis of purified samples of ethyl centralite, acardite, and diphenylamine, as well as powder samples containing ethyl centralite; comparison analyses were also made by one or both of the standard procedures mentioned above. The results of these experiments are presented in Tables I and II. Additional experiments, the results of which are not given in the tables, demonstrated that acetic acid is virtually inert toward bromination under conditions of the procedure and that the presence of either nitroglycerin or diethyl phthalate does not introduce serious error into the estimation of centralite.

The data in Tables I and II demonstrate that this volumetric procedure involving bromination in acetic acid solution is satisfactorily accurate and precise, and that the results are not critically dependent on the time allowed for bromination. As it is more convenient to carry out than the carbon tetrachloride and alcohol procedures, it is recommended for the estimation of stabilizers in smokeless powders.

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Analysis of Organoselenium Compounds

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A procedure developed for the determination of selenium in organic compounds involves combustion of the substance in a hydrogen-oxygen flame, followed by collection and titration of the selenium dioxide formed. Iodometric methods for the determination of the equivalent weights of certain types of organoselenium compounds reduced by potassium iodide and of diaryl diselenides are also described. The methods are all rapid and accurate.

INVESTIGATION of the known methods for the analysis of organic compounds of selenium showed these procedures to be either tedious and time-consuming or inaccurate. In testing the available methods, best results were obtained by use of a modification of the combustion method of Niederl and Niederl (*4*) which is based on the methods of Alber and Harand (*1*) and Umezawa (*5*) and involves combustion of the substance in oxygen gas on a glowing platinum surface. However, it was found more expedient and fully as accurate to determine the selenium dioxide formed by an improved iodometric method based on that of van der Meulen (*3*). The titration as described by van der Meulen is erratic, particularly where hydrochloric acid is used or where chlorine is present in the substance being analyzed. This erratic behavior is due to oxidation of iodide by oxygen of the air which is apparently catalyzed by something present in the solution. This difficulty was overcome by modifying the method as described in the procedure below. In spite of this and other improvements, the method was still lengthy and required constant attention and skill during the combustion. It gives good results, however, in the hands of a skilled operator.

The new analytical methods described in the present paper are of two types: (1) the flame combustion of the organoselenium compound which leads directly to the determination of the selenium content and (2) volumetric methods which yield the equivalent weights of the compounds being analyzed.

FLAME COMBUSTION METHOD

A method for the determination of halogens in organic compounds by flame combustion has been described by Winter (*6*). The simplicity and accuracy of this procedure led the present workers to investigate its possibilities as a method for selenium analysis. Preliminary tests showed that selenium was oxidized completely to the tetravalent state when the organoselenium compound was burned in a flame supplied with an ample quantity of oxygen. In an ordinary air-supplied gas flame, part of the selenium came through as the element and formed a red deposit in the chimney.

APPARATUS AND PROCEDURE. The apparatus is shown in Figure 1. The burner, *ABCD*, is made of transparent quartz tubing. Although the dimensions do not seem to be critical, the inside diameters of the tubing actually used are 6 mm. for arm *AB*, 3 mm. for side arm *D* and the inner jet of burner *C*, and 10 mm. for the outer tube of the burner. The chimney and absorption tube, *EFGH*, are of Pyrex, the inside diameters being 30 mm. at *E*, 6 mm. at *F*, and 15 mm. at *H* with a 10/30 ⅀ joint at *G*. Other dimensions are shown roughly to scale in the figure. The absorption tube, *H*, is tightly packed with Pyrex glass-wool which had been soaked in chromic acid cleaning solution and thoroughly rinsed with water.

In use, the burner, thermometer, and lower part of the chimney are placed inside an electric furnace, *L*, contained in a 10 × 20 cm. Pyrex electrolytic beaker, *K*. The heater consists of 5.4 meters (18 feet) of 22-gage Chromel-A wire (1.0 ohm per foot) wound on a Transite frame and is energized through a variable transformer.

The apparatus is assembled into three independently movable units consisting of the quartz burner and thermometer, the furnace, and the chimney and absorption apparatus.

Although only solid samples have been analyzed in this laboratory, there is no apparent reason why liquid and gaseous samples could not be handled if one modified the apparatus as described by Winter for halogen analysis. The sample size was adjusted to contain 20 to 80 mg. of selenium, the governing factors being the normality of the sodium thiosulfate, the size of the buret, and whether or not a method of aliquots is employed in the titration. The sample may be compressed into a pellet or weighed directly into the small platinum-foil boat, *B*.

The weighed sample is introduced at *A* and shaken down to *B*. Hydrogen gas is then introduced at *D* at a rate of about 200 ml. per minute and after the original air has been swept out, the gas is lit at *C*. The flame should be about 1 cm. high. The oxygen is then slowly turned on until a flow rate of about 100 ml. per minute is reached. Once started, the flame is very stable and does not go out during an analysis. The furnace and chimney assemblies are then brought into place and suction applied at *J* sufficient to give an air flow through the chimney of about 1 liter per minute, which is rapid enough to sweep all products of combustion into the absorber. Although successful analyses have been performed without the use of flowmeters, it is recommended that some kind of gage be placed in each of the three lines mentioned.

The current to the heater is then started and the voltage raised to the point where a suitable rate of volatilization of the sample is obtained. Experience alone will tell the most satisfactory temperature for the vaporization of a given substance, but the operator soon becomes skilled in this regard. The otherwise colorless flame assumes a blue color, the intensity of which is proportional to the rate at which the selenium compound is being burned. This color serves as a good indication of the onset of vaporization as well as of the completion of the analysis. Most of the selenium dioxide formed in the reaction deposits in the tube at *F*, but a significant quantity makes its way to the glass-wool in the absorber, *H*.

A large proportion of the compounds analyzed vaporized cleanly without decomposition or residue. However, in some cases decomposition accompanies volatilization, so that a dark, nonvolatile, selenium-containing residue remains. When this is the case, the thermometer is removed and the temperature is gradually raised to the point where the compound slowly burns in the oxygen stream. This is the advantage of introducing the sample into the oxygen rather than into the hydrogen. In some cases the decomposition can be prevented by vaporizing at a higher temperature, the rate of vaporization being increased more than the rate of decomposition as the temperature rises. Increasing the rate of oxygen flow also helps in these cases.

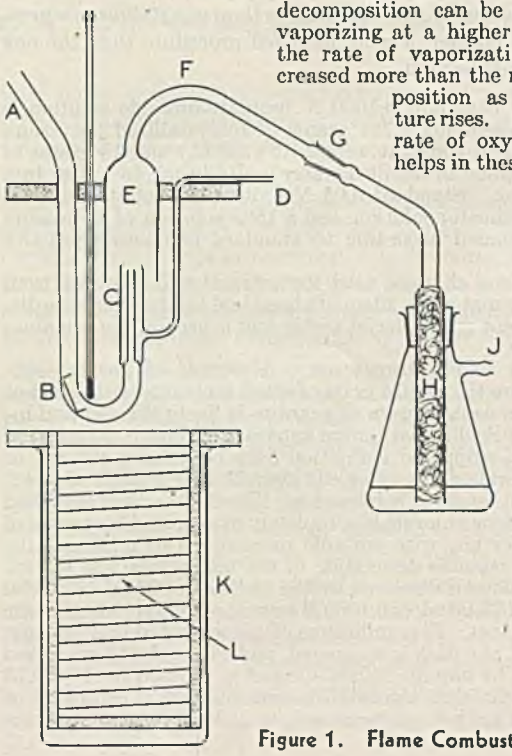

Figure 1. Flame Combustion Apparatus

Table I. Analysis of Organoselenium Compounds by the Flame Combustion Method

Compound	Formula	Selenium Theory %	Selenium Analysis %	Deviation %
Di-*p*-tolylselenium	$C_{14}H_{14}Se$	30.23	30.22	−0.03
Dibenzyl diselenide	$C_{14}H_{14}Se_2$	46.42	46.53	0.24
Diphenyl diselenide	$C_{12}H_{10}Se_2$	50.60	50.51	−0.18
Bis (*p* - methoxyphenyl) selenium	$C_{14}H_{14}O_2Se$	26.93	26.83	−0.37
Bis (*o* - chlorophenyl) selenium	$C_{12}H_8Cl_2Se$	26.14	26.14	0.00
p-Tolyl selenocyanate	C_8H_7NSe	40.27	40.37	0.25
p-Nitrophenyl selenocyanate	$C_7H_4N_2O_2Se$	34.77	34.60	−0.49
Phenylseleninic acid	$C_6H_6O_2Se$	41.76	41.70	−0.14
α - (*o* - Biphenylylseleno) propionic acid	$C_{15}H_{14}O_2Se$	25.87	25.77	−0.39

When combustion is complete, which requires 15 to 45 minutes, the hydrogen and oxygen are stopped and the furnace is shut off and lowered to permit the apparatus to cool. The suction is left on during the cooling process. When cool, the chimney is removed from the absorber and the selenium dioxide rinsed into a 250- or 500-ml. volumetric flask with water. By alternately applying suction to *G* and *J*, the glass-wool is rinsed with three or four 25-ml. portions of water, each portion being drawn up and down slowly several times, then added to the volumetric flask. This rinsing must be carefully done in order to remove all the selenious acid. The final rinse water should not show a color when added to an acidified solution of starch and potassium iodide. After rinsing is complete, the volumetric flask is filled to the mark and aliquots are titrated by the procedure below.

Titration of Selenious Acid. The solution containing the selenious acid is boiled gently for a few minutes to remove dissolved oxygen, then cooled to room temperature in an ice-water mixture. Air is subsequently kept out of the flask by addition of small pieces of dry ice from time to time or by passage of carbon dioxide or nitrogen gas into the flask. Although this latter precaution is not absolutely necessary, more reliable results have been obtained in this way. To the solution are then added 15 ml. of 2% starch solution, 10 ml. of 1.5 *M* potassium iodide, and 10 ml. of 6 *N* sulfuric acid in the order named, mixing thoroughly after each addition. The solution is then titrated at once with approximately 0.05 *N* sodium thiosulfate. The end point is sharp and is marked by a change from a turbid brown to a transparent red color. 1.000 ml. of 0.05000 *N* $Na_2S_2O_3$ = 0.9870 mg. of Se.

Results and Discussion. The accuracy of the flame combustion method is indicated by the results given in Table I for the analysis of a number of carefully purified substances.

The above examples show that the method yields excellent results for compounds of widely differing types containing selenium together with carbon, hydrogen, oxygen, nitrogen, and chlorine. The method in its present form does not give good results when bromine, iodine, or sulfur is present, since these elements yield substances which reduce tetravalent selenium in the absorber. However, bromine and iodine also interfere with other combustion methods, although sulfur does not because of its oxidation to sulfur trioxide (rather than dioxide) on the platinum contacts. It is this interference by sulfur in the flame combustion method which makes it advisable to use hydrogen as a fuel rather than commercial gas which is apt to contain sulfur. The flame combustion method has a distinct advantage over other methods in being more rapid and requiring less skill and attention on the part of the analyst.

VOLUMETRIC METHODS

A number of types of organoselenium compounds which contain halogen or oxygen directly bonded to selenium lend themselves to iodometric procedures for determination of equivalent weight. These compounds react with aqueous potassium iodide to form triiodide, which may then be titrated with sodium thiosulfate. The titration of diarylselenium dihalides has been described in a previous communication (*2*). The reduction products and the equivalents per mole for the various types of compounds are indicated in Table II.

Table II. Reduction Products and Equivalents per Mole for Various Types of Organoselenium Compounds

Type of Compound	Reduction Product	Equivalents per Mole
R_2SeX_2	R_2Se	2
R_2SeO	R_2Se	2
$RSeX_3$	R_2Se_2	3
$RSeX$	R_2Se_2	1
$RSeO_2H$	R_2Se_2	3

Table III. Equivalent Weights of Some Selenium Compounds Reduced by Potassium Iodide

Compound	Formula	Equivalent Weight Theory	Equivalent Weight Experimental	Deviation, %
Diphenylselenium dichloride	$(C_6H_5)_2SeCl_2$	152.1	151.9	−0.13
Diphenylselenium dibromide	$(C_6H_5)_2SeBr_2$	196.5	196.6	0.05
Di-*p*-tolylselenium dibromide	$(C_7H_7)_2SeBr_2$	210.5	210.3	−0.10
Dibenzoselenophene dibromide	$C_{12}H_8SeBr_2$	195.5	195.4	−0.05
4 - Methoxydiphenylselenium dichloride	$C_{13}H_{13}OSeCl_2$	167.1	167.7	0.36
4 - Bromodiphenylselenium dibromide	$C_{12}H_9BrSeBr_2$	236.0	235.1	−0.38
4 - Methyldiphenylselenium dibromide	$C_{13}H_{12}SeBr_2$	203.5	203.6	0.05
4,4′ - Diethoxydiphenyl selenoxide	$(C_8H_9O)_2SeO$	168.6	169.0	0.24
Phenylselenium tribromide	$C_6H_5SeBr_3$	131.9	131.4	−0.30
Phenylselenium monobromide	C_6H_5SeBr	236.0	236.3	0.13
Phenylseleninic acid	$C_6H_5SeO_2H$	63.0	63.2	0.32

Table IV. Iodometric Titration of Diaryl Diselenides

Compound	Formula	Equivalent Weight Theory	Equivalent Weight Experimental	Deviation, %
Diphenyl diselenide	$(C_6H_5)_2Se_2$	52.01	51.97	−0.1
			51.53	−0.9
Di-*p*-tolyl diselenide	$(C_7H_7)_2Se_2$	56.70	56.98	0.5
Di(*o*-biphenylyl) diselenide	$(C_{12}H_9)_2Se_2$	77.38	76.84	−0.7

Procedure for Compounds Reducible by Potassium Iodide. The weighed sample (0.1 to 0.5 gram) is placed in a glass-stoppered flask containing 5 ml. of carbon tetrachloride, 25 ml. of 0.3 *M* potassium iodide, and 2 ml. of 6 *M* sulfuric acid. After shaking, the mixture is titrated with standard sodium thiosulfate. Starch solution is added just before the end point.

Titration of Aryl Diselenides. Aryl diselenides may be titrated iodometrically to the iodine monochloride end point if the hydrochloric acid concentration is kept at 5.0 to 5.5. *F* at the end point. Because of a small amount of side reaction, possibly oxidation of the diselenide to selenonic acid, the results are sometimes low by 0.5 to 1.0%. Although the reactions are probably more complex than indicated, the stoichiometry is represented by the equations:

$$R_2Se_2 + 6\ ICl = 2\ RSeCl_3 + 3\ I_2 \quad (1)$$

$$2\ I_2 + IO_3^- + 6H^+ + 5\ Cl^- = 3\ H_2O + 5\ ICl \quad (2)$$

Procedure for Aryl Diselenides. A solution of iodine monochloride is prepared by titration of 1 ml. of 1.5 *M* potassium iodide in 50 ml. of 12 *M* hydrochloric acid, using 5 ml. of carbon tetrachloride as an indicator. When the carbon tetrachloride is just colorless, 25 ml. of 12 *N* hydrochloric acid and the weighed sample of diselenide are added. After shaking, the mixture is titrated with standard potassium iodate solution.

Tables III and IV show results from volumetric determination of equivalent weights of a number of different compounds.

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Oxide Films Formed on Alloys at Moderate Temperatures

Electron Diffraction and Electron Microscope Study

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Supplementing previous papers, electron microscope and electron diffraction data are presented concerning the structure of electrochemically and chemically stripped films from a series of 16 alloys consisting principally of iron, cobalt, nickel, and chromium. Both reflection and transmission methods of electron diffraction were used.

THE nature of the protective properties of metals and alloys is a question of great importance in modern technology. Many theories have been proposed, some of which are based on the role of the oxide film in preventing further reaction. A complete knowledge of the chemical and physical structure of the oxide crystals in the surface film may aid in determining the necessary conditions for protection, but unfortunately, a complete knowledge is impossible for very thin films with the present state of instrumentation. In previous papers (*8*, *10*, *15*) the use and limitations of the electron diffraction and electron microscope techniques were discussed. This paper presents electron microscope and electron diffraction data concerning the structure of electrochemically and chemically stripped films from a series of 16 alloys consisting principally of iron, cobalt, nickel, and chromium.

SURVEY OF LITERATURE

The literature on the removal of oxide films from iron and other metals by chemical and electrochemical methods developed largely by Evans and co-workers (*4*) was reviewed in a previous paper (*15*).

The electrochemical method has been applied to the stripping of oxide films from alloys of iron and chromium by Evans and Stockdale (*5*). A very thin film of oxide was stripped from 13% chromium steel by a long anodic treatment. The film, which carried striae of the original metal surface, also contained opaque flakes. These workers also obtained from 18-8 stainless steel a thin skin which contained large amounts of residual metal. The film was opaque to light.

The chemical method has been applied with success to the removal of the surface film on 18-8 stainless steel by Vernon, Wormwell, and Nurse (*17*). The metals present in the film were determined by chemical analyses and the contents of the corresponding oxides computed. The thickness of the oxide film increased with the degree of polish. The effect of polishing was to enrich the chromium in the film as compared with the underlying steel. The surface film of brightly polished specimens contained 90% chromic oxide. Nickel, on the other hand, was not found to be enriched in the surface film. The authors have suggested that the enrichment of chromium is associated with surface flow.

In the macroscopic film thickness range considerable information is available on the enrichment and concentration of the several metals making up the oxide film. Pfiel (*14*) has made chemical analyses on films of the order of 0.25 cm. (0.1 inch) or more in thickness. These films formed at temperatures of the order of 1000° C. existed in the form of three layers. In a study of eight alloys the two outer layers contained only very small amounts of the alloying elements compared with the original steel except in the case of manganese steel. With a few exceptions nonferrous elements present in the alloy before oxidation were concentrated in the innermost of the three layers of scale. Pfiel (*14*) considers the oxidation process as one involving iron atoms diffusing outward through the oxide film.

Recently Kornilov and Sidorishin (*11*) investigated the oxide films formed on iron-chromium-aluminum solid solution alloys by electron diffraction and chemical analysis in the temperature range 400° to 1000° C. At moderate temperatures an isomorphous mixture of oxides of the spinel type was found. With increasing temperature the lattice constant of the solid solution of the oxides decreased and approached that of pure γ-alumina. The oxide film which formed on the surface crumbled readily and had no protective properties.

Table I. Analysis and Preparation of Alloy Specimens

Alloy	Analysis	Heat Treatment, Hours at 1000° C., Furnace Cooled	Polishing
Mild steel[a]	0.18 C, 0.028 S, 0.030 P	15 in dry H_2	Emery papers through 2/0, uniwax wheel, 320 abrasive, uniwax wheel 600 aloxite, No. 3 alumina
13 CrFe	13 Cr	10 in dry H_2	Emery papers through 1/0, wax wheel, 320 abrasive, chrome rouge, Nos. 1 and 3 alumina
18-8 SS	18 Cr 8 Ni	15 in Ammogas	Emery papers through 2/0, chrome rouge, Nos. 1 and 3 alumina
K42B[a]	41.7 Ni, 22.5 Co, 20.0 Cr, 12.0 Fe, 1.89 Ti, 0.21 Al, 0.57 Mn, 1.21 Si	10 in wet H_2	Emery papers through 2/0, chrome rouge, Nos. 1 and 3 alumina
Inconel (*3*)	79.5 Ni, 13 Cr, 6.5 Fe, 0.25 Mn, 0.25 Si, 0.08 C, 0.20 Cu	15 in Ammogas	Emery papers through 1/0, 320 abrasive wheel, chrome rouge, Nos. 1 and 3 alumina
Nichrome V	80 Ni, 20 Cr	14 in Ammogas	Emery papers through 1/0, 320 abrasive wheel, chrome rouge, Nos. 1 and 3 alumina
30 CoFe[a]	30.4 Co, 0.22 C	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
Hipernik	49 Ni, 49 Fe, 2 Mn	10 in dry H_2	Emery papers through 1/0, wax wheel, 320 abrasive, chrome rouge, Nos. 1 and 3 alumina
5 CrFe[a]	4.63 Cr, 0.044 C	10 in dry H_2	Emery papers through 1/0, wax wheel, 320 abrasive, chrome rouge, Nos. 1 and 3 alumina
5 NiFe[a]	4.89 Ni, 0.015 C	10 in dry H_2	Emery papers through 1/0, wax wheel, 320 abrasive, chrome rouge, Nos. 1 and 3 alumina
5 CoFe	.....	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
5 MnFe[a]	4.85 Mn, 0.035 C	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
5 SiFe	.....	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
3 VFe	.....	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
Kovar	54 Fe, 18 Co, 28 Ni	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
4 WFe[a]	4.10 W, 0.028 C	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina

[a] Analyzed at research laboratories.

APPARATUS AND METHOD

The details of the apparatus and methods used in this study have been described (*8*, *10*).

The specimens of the alloys are heat-treated and given a metallographic polish according to the procedures tabulated in Table I. The samples are then mounted in the electron diffraction camera furnace and oxidized under carefully controlled conditions of time, temperature, and oxygen pressure. The surface lattice structure is studied in situ by the electron diffraction reflection method. Three photographs are taken: (1) the sample in vacuum and at the temperature of the experiment; (2) after the oxidation and while the specimen is at the elevated temperature; (3) after the specimen is cooled under vacuum conditions to room temperature.

The sample is now removed and cut into two pieces. Light micrographs are made of the surface of one half of the specimen by reflected light at 100 and 1000×. The other half is subjected to the electrolytic or chemical stripping techniques (*15*). After the film is loosened, it is washed and then manipulated carefully onto a small stainless steel specimen screen. The screen and specimen are vacuum-dried before being placed in the microscope for study. Several electron micrographs are taken at 6800× of typical portions of the stripped oxide film. The film is also studied by the electron diffraction transmission method, using the electron diffraction adapter of the electron microscope.

CHOICE AND PREPARATION OF SPECIMENS

The alloys in this study may be divided into four general classes, although a given alloy may belong to two or more classes—for example, both Inconel and K42B are refractory alloys but they also have good protective qualities. The following classification is used in the discussion of results:

1. Protective. 13 CrFe, 18-8 stainless steel
2. Refractory. K42B, Inconel, Nichrome V
3. Magnetic. Hipernik, 30 CoFe
4. Sealing. Kovar

In addition to commercial alloys, certain experimental alloys were made to determine whether the lattice type of the alloying element would have any effect on the oxidation products. These ferrous alloys contain alloying metals of the body-centered cubic, face-centered cubic, and other structural types. In each the percentage of the alloying metal does not exceed 5%.

The specimens are machined from bars of the alloys to cylinders of 0.94 cm. (0.375-inch) diameter and 0.94 cm. (0.375-inch) length. After cleaning, they are heat-treated at elevated temperatures in dissociated ammonia or wet or dry hydrogen. The specimens are next given a fine metallographic polish. Details of the heat-treatment and polishing procedures are given in Table I. The specimens are stored in a desiccator over anhydrous calcium chloride until used.

The alloy specimen is placed in the electron diffraction camera furnace and heated to the desired temperature. Oxygen to a pressure of 0.1 atmosphere is admitted and the specimen is oxidized for a predetermined time. The time and temperature conditions of the oxidation are estimated from rate measurements where these are available. For most of the alloys no rate measurements are available, so that intelligent guesses as to oxidation conditions are necessary in order that the oxide film thickness shall lie within the optimum range for investigation with the electron microscope.

INTERPRETATION OF DATA

Electron Diffraction. The electron diffraction reflection method has been discussed in previous papers (*8*, *10*, *15*) and the interpretation of data by the transmission technique has been discussed in a recent work on metals (*15*). The transmission method yields information on the structure of the whole film, while the reflection method may indicate only the structure of the outer surface. This is important, since the surface structure may not be the same as the structure of the body of the film. The oxide layer which forms initially may consist of various oxides present on the surface in the same mole ratio as the metals in the alloy. As the oxidation proceeds a stratification of layers of the several oxides may occur, since the factors influencing their formation may be different. Thus, in a binary alloy, an oxide of one of the metals may concentrate in the surface layer while an oxide of the other metal may concentrate in contact with the metallic substrate.

Table II. Lattice Parameters of Metals and Metallic Oxides

Substance	*a*	*b*	*c*	*α*	Structural Type
Fe	2.86	...	...	.....	Body-centered cubic
W	3.16	...	...	.....	Body-centered cubic
Cr	2.88	...	...	.....	Body-centered cubic
V	3.03	...	...	.....	Body-centered cubic
Ni	3.52	...	...	.....	Face-centered cubic
Co	2.51	...	4.07	.....	Hexagonal close-packed
Mn	8.89	...	...	.....	Cubic
Si	5.42	...	...	.....	Cubic (diamond)
FeO	4.28	...	...	.....	Face-centered cubic
CoO	4.25	...	...	.....	Face-centered cubic
NiO	4.17	...	...	.....	Face-centered cubic
WO_2	4.86	...	2.77	.....	Tetragonal
Cr_2O_3	5.35	...	...	54°58′	Rhombohedral
α-Fe_2O_3	5.42	...	...	55°17′	Rhombohedral
W_2O_3	...	...	...	.....	Rhombohedral
WO_3	7.28	7.48	3.82	.....	Monoclinic
V_2O_3	5.43	...	...	53°53′	Rhombohedral
V_2O_4	11.48	4.36	3.55	.....	Orthorhombic
SiO_2	4.90	...	5.39	.....	Hexagonal
MnO	4.44	...	...	.....	Face-centered cubic
MnO_2	4.44	...	2.89	.....	Tetragonal
Mn_3O_4	5.75	...	9.42	.....	Tetragonal
Fe_3O_4	8.40	...	...	.....	Spinel (cubic)
Co_3O_4	8.11	...	...	.....	Spinel (cubic)
γ-Fe_2O_3	8.32	...	...	.....	Cubic
$MnO.Cr_2O_3$	8.42	...	...	.....	Spinel (cubic)
$NiO.Cr_2O_3$	8.31	...	...	.....	Spinel (cubic)
$CoO.Cr_2O_3$	8.32	...	...	.....	Spinel (cubic)
$FeO.Cr_2O_3$	8.35	...	...	.....	Spinel (cubic)
$MnO.Fe_2O_3$	8.51	...	...	.....	Spinel (cubic)
$NiO.Fe_2O_3$	8.34	...	...	.....	Spinel (cubic)
$CoO.Fe_2O_3$	8.39	...	...	.....	Spinel (cubic)

The interpretation of the information obtained by the electron diffraction method is more difficult in the case of alloys than of metals. Because of the similarity of the lattice parameters of many of the oxides, there is difficulty in distinguishing α-Fe_2O_3 from Cr_2O_3, $NiO.Fe_2O_3$ from Fe_3O_4, etc. Table II shows the lattice parameters of the various oxides of interest in this study. Other complicating factors are the phenomena of various types of solid solution of the several oxides in each other. For example, on an iron-chromium alloy where both α-Fe_2O_3 and Cr_2O_3 may be present it is difficult by the electron diffraction technique to determine whether α-Fe_2O_3 or Cr_2O_3 is present or a solid solution of one in the other. There may also occur a random replacement of ferric ions by chromic ions in the Fe_3O_4 (spinel type) lattice. The occurrence of such solid solution phenomena may have effects on the lattice parameters and thus complicate the identification of the oxidation products.

An interesting comparison can be made of the reflection and transmission electron diffraction patterns. In general, one should expect to find certain oxidation products by reflection and additional products by transmission. However, the oxides in the outer layer as determined by reflection may comprise such a small fraction of the complete oxide film that one or more of them may not give good diffraction patterns in the transmission investigations.

Electron Microscope. The two methods of studying surfaces of opaque bodies by means of the electron microscope have been compared in a recent work on metals (*15*). Since we are interested primarily in the details of crystals making up the body of the oxide film, the stripped film technique is used exclusively in this study.

Information Recorded. Electron micrographs of the stripped oxide film are taken at 6800× and enlarged optically to 34,000×. The following information is recorded from the electron micrographs of the stripped film: (1) particle size, (2) particle size distribution, (3) particle shape, (4) uniformity in film thickness, and (5) type of micrograph. The particle size is obtained by averaging measurements on a number of crystals, while the particle size distribution indicates the variation in particle size. The particle shape is determined from an examination

Table III. Electron Diffraction Data

(Oxide films formed on alloys at 0.1 atmosphere of O_2 at various times and temperatures)

Alloy	Temp. °C.	Time of Oxidation Min.	Diffraction Patterns: Preoxidation by R	Oxidized by R	Oxidized at 25° C. by R	Stripped by T
Protective						
13 CrFe	600	5	None	Cr_2O_3* MO	Cr_2O_3, M O	Cr_2O_3, Fe_3O_4*, 8.41, S
18-8 SS	600	5	α-Fe_2O_3, D	Fe_3O_4* 8.44, α-Fe_2O_3, S	Fe_3O_4*, 8.44, α-Fe_2O_3, S	Cr_2O_3, S
18-8 SS	600	40	Fe_3O_4, 8.45, α-Fe_2O_3*, D	Fe_3O_4* 8.45, α-Fe_2O_3*, S	Fe_3O_4, 8.45, α-Fe_2O_3, S	Cr_2O_3, S
Refractory						
K42B	600	5	α-Fe_2O_3, D	Fe_3O_4, 8.43, S	Fe_3O_4, 8.43, S	Cr_2O_3, Fe_3O_4, 8.36, S
K42B	600	30	α-Fe_2O_3, D	Fe_3O_4, 8.44, α-Fe_2O_3, S	Fe_3O_4, 8.44, α-Fe_2O_3, S	Cr_2O_3, Fe_3O_4*, S
Inconel	600	5	Fe_3O_4, VD	Fe_3O_4, 8.43, S	Fe_3O_4, 8.43, S	Spinel, 8.32, Cr_2O_3, Ni, OD
Inconel	600	30	Fe_3O_4, VD	Fe_3O_4, 8.43, S	Fe_3O_4, 8.43, S	Cr_2O_3, NiO, VDO
Nichrome V	600	5	Cr_2O_3, VD	Cr_2O_3, S	Cr_2O_3, S	Cr_2O_3, S
Nichrome V	600	30	Cr_2O_3, VD	Cr_2O_3, S	Cr_2O_3, S	Cr_2O_3, M
Magnetic						
Mild steel	250	5	None	Fe_3O_4, 8.43, DO	Fe_3O_4, 8.43, DO	Fe_3O_4, 8.36, M
Mild steel	250	30	None	Fe_3O_4, 8.42, M	Fe_3O_4, 8.42, M	Fe_3O_4, 8.39, VS
Mild steel	300	5	Fe_3O_4, 8.43, α-Fe_2O_3, DO	Fe_3O_4, 8.43, α-Fe_2O_3, MO	Fe_3O_4, 8.43, α-Fe_2O_3, MO	Fe_3O_4, 8.41, SO
30 CoFe	300	30	Fe_3O_4, VD	Fe_3O_4, 8.43, M	Fe_3O_4, 8.43, M	Fe_3O_4, 8.36, M
Hipernik	300	5	Fe_3O_4, MO	Fe_3O_4, 8.42, α-Fe_2O_3, S	Fe_3O_4, 8.42, α-Fe_2O_3, S	Spinel, 8.34, α-Fe_2O_3, NiO, SO
Sealing						
Kovar	400	5	Fe_3O_4, 8.43, M	Fe_3O_4, 8.43, SO	Fe_3O_4, 8.43, SO	Fe_3O_4, 8.35, S
Miscellaneous						
5 CrFe	400	5	Fe_3O_4, DO	Fe_3O_4, 8.43, Cr_2O_3, *S	Fe_3O_4, 8.43, S	Fe_3O_4, or FeO.Cr_2O_3, 8.34, DO
5 NiFe	300	5	None	Fe_3O_4, 8.43, α-Fe_2O_3, M	Fe_3O_4, 8.43, α-Fe_2O_3, M	Fe_3O_4, 8.34, M
5 CoFe	300	5	Fe_3O_4, 8.43, M	Fe_3O_4, 8.43, M	Fe_3O_4, 8.43, S	Fe_3O_4, 8.35, SO
5 MnFe	300	10	α-Fe_2O_3, VD	Fe_3O_4, 8.43, S	Fe_3O_4, 8.43, S	Fe_3O_4, 8.32, S
5 SiFe	300	5	None	Fe_3O_4, 8.43, D O	Fe_3O_4, 8.43, DO	α-Fe_2O_3, M
3 VFe	300	5	Fe_3O_4, 8.42, D	Fe_3O_4, 8.42, D	Fe_3O_4, 8.42, M	Fe_3O_4, 8.33, DO
4 WFe	300	5	Fe_3O_4, VDO	α-Fe_2O_3, S	α-Fe_2O_3, S	α-Fe_2O_3, M

R, reflection. T, transmission. S, sharp. M, medium. D, diffuse. O, oriented. V, very. *, trace.

of the more typical shapes in the pattern. Uniformity in film thickness refers to the presence of thick and thin portions of the film. The type of pattern refers to a number of features, including: (1) the sharpness of the crystal edges, (2) the presence of overlapping crystals, and (3) the presence of extraneous material. These features are of importance in classifying a micrograph but may not be easily interpreted.

Light Microscope. Light micrographs of the oxidized surface are taken at 100× and 1000× by the use of reflected light. The oxidation process acts like a chemical etching solution in revealing the grain boundaries of the grains in the metal or alloy surface. The light micrographs also reveal the presence of inclusions and the roughness of the surface layer.

Spectroscopic Analysis. Whenever possible, sections of the stripped films were analyzed spectroscopically. In some cases these analyses showed the presence of metals not found in the alloys but present in the oxides used in the polishing procedures.

Table IV. Lattice Parameter Deviations of Oxides

Alloy	Temp. °C.	Time of Oxidation Min.	Reflection Patterns: Composition and parameter	Deviation %	Transmission Patterns: Composition and parameter	Deviation %
Protective						
13 CrFe	600	5	Cr_2O_3	+0.30	Cr_2O_3	+0.04
	600	5	....	....	Fe_3O_4, 8.41	+0.12
18-8 SS	600	5	α-Fe_2O_3	+0.52	....	....
	600	5	Fe_3O_4, 8.44	+0.48	....	....
	600	5	....	....	Cr_2O_3	−0.02
18-8 SS	600	40	α-Fe_2O_3	+0.30	....	....
	600	40	Fe_3O_4, 8.45	+0.60	....	....
	600	40	....	....	Cr_2O_3	00.0
Refractory						
K42B	600	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.36	−0.48
	600	5	....	....	Cr_2O_3	+0.07
K42B	600	30	Fe_3O_4, 8.44	+0.48	....	....
	600	30	α-Fe_2O_3	+0.32	....	....
	600	30	....	....	Cr_2O_3	+0.07
Inconel	600	5	Fe_3O_4, 8.43	+0.36	Spinel, 8.32	....
	600	5	....	....	Cr_2O_3	−0.11
	600	5	....	....	NiO	Diffuse
Inconel	600	30	Fe_3O_4, 8.43	+0.36	....	....
	600	30	....	....	Cr_2O_3	Too diffuse
	600	30	....	....	NiO	Too diffuse
Nichrome V	600	5	Cr_2O_3	+0.25	Cr_2O_3	−0.04
	600	30	Cr_2O_3	+0.30	Cr_2O_3	+0.04
Magnetic						
Mild steel	250	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.36	−0.48
Mild steel	250	30	Fe_3O_4, 8.42	+0.24	Fe_3O_4, 8.39	−0.12
Mild steel	300	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.41	+0.12
	300	5	α-Fe_2O_3	+0.25	....	....
30 CoFe	300	30	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.36	−0.48
Hipernik	300	5	Fe_3O_4, 8.42	+0.24	Spinel, 8.34	−0.72
	300	5	α-Fe_2O_3	+0.10	α-Fe_2O_3	−0.03
	300	5	....	....	NiO	0.00
Sealing						
Kovar	400	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.35	−0.60
Miscellaneous						
5 CrFe	400	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.34	−0.72
5 NiFe	300	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.34	−0.72
	300	5	α-Fe_2O_3	+0.25	....	....
5 CoFe	300	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.35	−0.60
5 MnFe	300	10	Fe_3O_4, 8.43	+0.36	Spinel, 8.32	−0.96
5 SiFe	300	5	Fe_3O_4, 8.43	+0.36	....	....
5 SiFe	300	5	....	....	α-Fe_2O_3	0.00
3 VFe	300	5	Fe_3O_4, 8.42	+0.24	Spinel, 8.33	−0.84
4 WFe	300	5	α-Fe_2O_3	+0.16	α-Fe_2O_3	+0.08

RESULTS

Electron Diffraction. The results of the electron diffraction study are shown in Table III. Three reflection and one transmission patterns are taken of the oxide film formed on the surface of the alloy. The first reflection pattern of the metal is taken in the vacuum of the camera before oxidation and at the temperature of the experiment. The second is taken after the oxidation and at the temperature of the experiment. The third is taken after cooling the oxidized sample to 25° C. in a vacuum. The transmission pattern of the stripped oxide film is also included in order to compare the body structure of the film with its surface structure. Table III shows the conditions of oxidation, the chemical structure of the surface oxide, the unit cell size (A_0) where readily calculable, and the type of diffraction pattern obtained.

Let us consider the oxide film formed on mild steel at 250° C. and 0.1 atmosphere of oxygen with an oxidation time of 5 minutes. A diffuse and oriented pattern of Fe_3O_4 is found by reflection. The unit cell size is calculated to be 8.43 Å. After

Table V. Summary of Electron Diffraction and Spectrographic Data

Alloy	Oxidizing Conditions Temp. °C.	Time Min.	Composition and Parameter: Transmission	Reflection	X-ray data (literature)	Spectrographic data
Protective						
13 CrFe	600	5	Fe_3O_4*, 8.41, Cr_2O_3	Cr_2O_3	Fe_3O_4, 8.40	Cr, Fe, Ag*
18-8 SS	600	5	Cr_2O_3	Fe_3O_4*, 8.44, α-Fe_2O_3	Fe_3O_4, 8.40	.....
18-8 SS	600	40	Cr_2O_3	Fe_3O_4, 8.45, α-Fe_2O_3	Fe_3O_4, 8.40	.....
Refractory						
K42B	600	5	Fe_3O_4, 8.36, Cr_2O_3	Fe_3O_4, 8.43	Fe_3O_4, 8.40	Cr, Fe* (Mg)
K42B	600	30	Fe_3O_4*, Cr_2O_3	Fe_3O_4, 8.44, α-Fe_2O_3	Fe_3O_4, 8.40	.....
Inconel	600	5	Spinel, 8.32, Cr_2O_3, NiO	Fe_3O_4, 8.43	Fe_3O_4, 8.40, $NiO.Cr_2O_3$, 8.31	Cr, Ni, Fe (Al, Si, Co)
Inconel	600	30	Cr_2O_3, NiO	Fe_3O_4, 8.43	Fe_3O_4, 84.0	
Nichrome V	600	5	Cr_2O_3	Cr_2O_3	....	Cr, Fe* (No Ni)
Nichrome V	600	30	Cr_2O_3	Cr_2O_3	....	Cr, Fe* (No Ni)
Magnetic						
Mild steel	250	5	Fe_3O_4, 8.36	Fe_3O_4, 8.43	Fe_3O_4, 8.40	.....
Mild steel	250	30	Fe_3O_4, 8.39	Fe_3O_4, 8.42	Fe_3O_4, 8.40	.....
Mild steel	300	5	Fe_3O_4, 8.41	Fe_3O_4, 8.43, α-Fe_2O_3	Fe_3O_4, 8.40	.....
Hipernik	400	5	Spinel, 8.34, α-Fe_2O_3, NiO	Fe_3O_4, 8.42, α-Fe_2O_3	Fe_3O_4, 8.40, $NiO.Fe_2O_3$, 8.34	Mn, Ni, Fe*
30 CoFe	300	30	Spinel, 8.36	Fe_3O_4, 8.43	Fe_3O_4, 8.40, $CoO.Fe_2O_3$, 8.39	Fe, Co, (Cr*)
Sealing						
Kovar	400	5	Fe_3O_4, 8.35	Fe_3O_4, 8.43	Fe_3O_4, 8.40	.....
Miscellaneous						
5 CrFe	400	5	Spinel, 8.34	Fe_3O_4, 8.43	Fe_3O_4, 8.40, $FeO.Cr_2O_3$, 8.35	Cr, Fe*
5 NiFe	300	5	Spinel, 8.34	Fe_3O_4, 8.43	Fe_3O_4, 8.40, $NiO.Fe_2O_3$, 8.34	.....
5 CoFe	300	5	Spinel, 8.35	Fe_3O_4, 8.43	Fe_3O_4, 8.40, $CoO.Fe_2O_3$, 8.39	Fe, Cu, (Al*), Ni, Co
5 MnFe	300	10	Spinel, 8.32	Fe_3O_4, 8.43	Fe_3O_4, 8.40, $MnO.Fe_2O_3$, 8.51	Fe, Mn, (Cr, Al*)
5 SiFe	300	5	α-Fe_2O_3	Fe_3O_4, 8.43	Fe_3O_4, 8.40	.....
3 VFe	300	5	Spinel, 8.33	Fe_3O_4, 8.42	Fe_3O_4, 8.40	.....
4 WFe	300	5	α-Fe_2O_3	α-Fe_2O_3		

* Trace.

deviations of the oxides formed in each oxidation experiment are shown in Table IV. This table shows the conditions of oxidation, the composition, and unit cell size where readily calculable and the deviation in per cent from the accepted x-ray values given in Table II. Both the reflection and transmission patterns are tabulated. The deviations are calculated from the unit cell size as given in Table III or from the d/N values for those oxides which do not obey the cubic lattice.

A summary of the electron diffraction and spectrographic data obtained on the 16 alloys is shown in Table V. Both the transmission and reflection data are given, together with the x-ray data obtained from the literature.

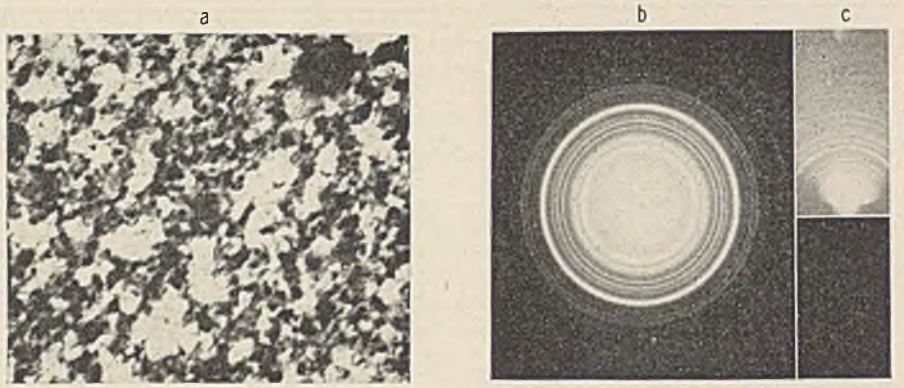
Electron Microscope. Figures 1 to 11 show the light micrographs, electron micrographs, electron diffraction transmission, and electron diffraction reflection patterns for the stripped oxide films from the alloy specimens. The lengths of 1, 10, or 100 microns are shown on the photographs. The light micrographs of the unstripped oxide film are taken at 100 and 1000×, while the electron micrographs of the stripped oxide film are taken at 6800× and enlarged optically to 34,000×. These micrographs are reduced subsequently in the printing process. The actual magnifications can be readily calculated from the fact that each centimeter of the length of the micron line shown in the micrograph equals 10,000×.

cooling the sample to room temperature, the oxide film is stripped from the metal. A medium pattern of Fe_3O_4 is found using the transmission method on the stripped film. The unit cell size is calculated to be 8.36 Å. Figure 10, *b* and *c*, shows the electron diffraction photographs for this oxidation experiment. In a similar manner the results of the other experiments on mild steel and the fifteen other alloys are shown in Table III and Figures 1 to 11.

In general the lattice parameters obtained in this study deviate from the accepted x-ray diffraction values. The lattice parameter

Table VI. Electron Microscope Analyses of Stripped Oxide Films of Alloys

Alloy	Oxidizing Conditions 0.1 Atmosphere of O_2 Min.	°C.	Film Color on Metal	Figure	Particle Size, Å.	Size Distribution, Å.	Shape	Uniformity	Type of Micrograph
Protective									
13 CrFe	5	600	Blue	1	450	200 to 700	Irregular	Nonuniform	Medium, overlapping crystals
18-8 SS	5	600	Blue and brown	2	300	100 to 600	Irregular	Uniform	Medium
	40	600	Yellow and blue	3	700	300 to 1500	Irregular	Nonuniform	Sharp, overlapping crystals
Refractory									
K42B	5	600	Yellow and mauve	4	350	200 to 750	Irregular	Nonuniform	Medium, clusters of crystals
	30	600	Reddish blue	..	450	300 to 800	Irregular	Nonuniform	Sharp, clusters of crystals
Inconel	5	600	Light blue	5	400	300 to 800	Irregular	Nonuniform	Medium, overlapping crystals, grain boundaries show
Inconel	30	600	Red	..	450	300 to 900	Irregular	Nonuniform	Medium, overlapping crystals
Nichrome V	5	600	Light blue	6	250	100 to 450	Irregular	Fairly uniform	Medium, overlapping crystals
	30	600	Yellow	..	350	200 to 550	Irregular	Fairly uniform	Medium, overlapping crystals
Magnetic									
Mild steel	5	250	Dark blue	7	700	300 to 1600	Irregular	Nonuniform	Medium overlapping crystals
	30	250	Dark blue	..	800	400 to 1200	Irregular	Nonuniform	Medium, overlapping crystals, striations
	5	300	Light blue, hiatus	..	750	250 to 900	Irregular	Nonuniform	Medium, overlapping crystals
30 CoFe	30	300	Light mauve	8	700	300 to 1200	Irregular, angular	Nonuniform	Sharp, overlapping crystals
Hipernik	5	400	Blue	..	300	200 to 500	Irregular	Uniform, thicker at grain boundaries	Medium, overlapping crystals, grain boundaries show
Kovar	5	400	Pink and light blue	..	400	250 to 750	Irregular	Nonuniform	Medium, clusters of small crystals
Miscellaneous									
5 CrFe	5	400	Light blue	9	250	100 to 400	Irregular	Nonuniform	Medium, chains of clusters of crystals
5 NiFe	5	300	Yellow and mauve	..	600	250 to 1000	Irregular	Nonuniform	Medium, overlapping crystals
5 CoFe	5	300	Pink and blue	..	400	250 to 750	Irregular	Nonuniform	Diffuse, overlapping crystals, clusters of crystals
5 MnFe	10	300	Light blue, hiatus	10	350	250 to 600	Irregular	Uniform	Sharp, overlapping crystals
5 SiFe	5	300	Reddish-blue	11	500	250 to 750	Irregular	Uniform	Sharp, overlapping crystals
3 VFe	5	300	Mauve and blue	..	250	200 to 300	Irregular	Uniform	Medium, large clusters of crystals on fine grained matrix
4 WFe	5	300	Mauve	..	300	200 to 400	Irregular, indistinct	Fairly uniform	Diffuse, network of thin strips



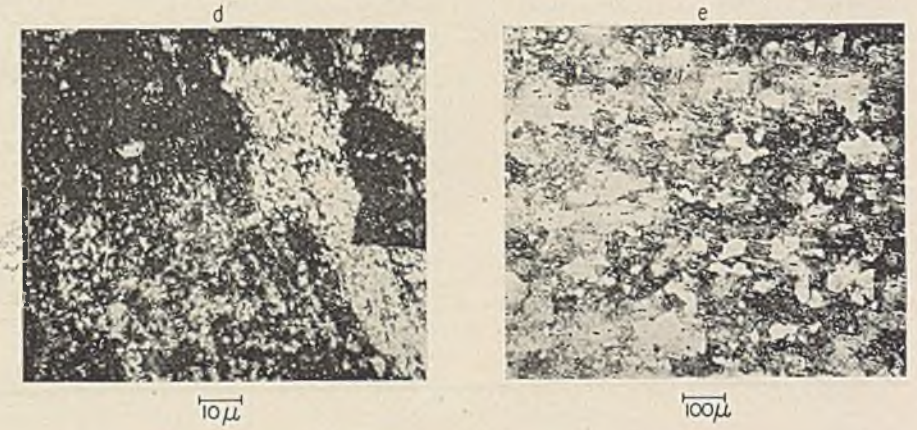


Figure 1. Oxide Film of 13% Chrome-Iron, 13 Cr 5–600

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, *e*. Light micrographs, film on metal

Table VI summarizes the information recorded from the electron microscope: (1) color of the oxide film on the metal, (2) particle size in Ångströms, (3) particle size distribution, (4) particle shape, (5) film uniformity, and (6) type of micrograph.

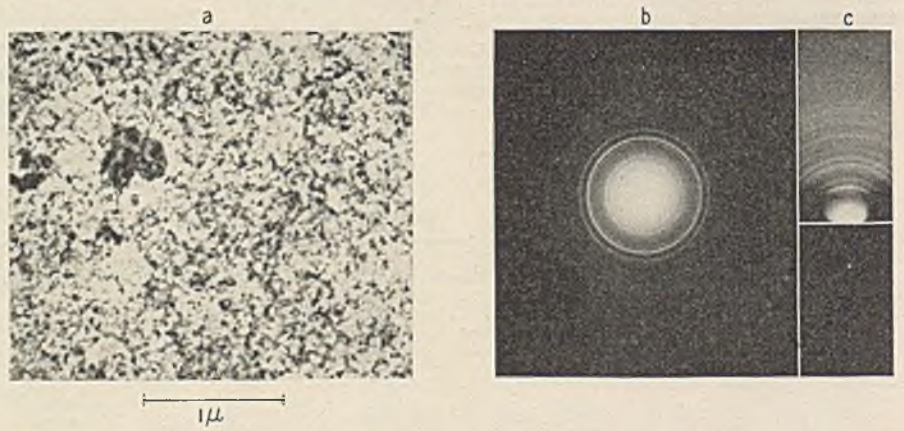
DISCUSSION

The factors which determine the chemical and physical structure of the oxide film on the surface of an alloy have been discussed in a previous paper (*10*):

1. Rates of formation and diffusion of the various metal ions and electrons through the oxide lattice
2. The rate of diffusion of the oxygen molecule, ion or atom through the oxide lattice
3. Thermodynamic stabilities of the oxides formed
4. Lattice type and its resemblance to the original metal or alloy lattice
5. Chemical reactions occurring in the oxide film between the several oxides or between metal ions or oxygen atoms and the several oxides
6. Preoxidation treatment given to the alloy, such as annealing, polishing, and cleaning
7. Rate of nucleation and growth of the oxide crystal

Factors 3 and 4 are known from thermodynamic and lattice structure data, while factor 5 can be estimated from thermodynamic data. Factors 1, 2, and 7 are unknown from both theory and experiment. Some evidence is available experimentally on factor 6. In a previous paper (*8*) the authors studied the effect of abrasion treatment on the surface oxide films formed on iron at high temperatures. Vernon, Wormwell, and Nurse (*17*) have studied the effect of polishing procedures on the oxide films formed on 18-8 stainless steel.

An analysis of the factors listed above would show that on alloys one might expect to obtain, after oxidation, reaction products which concentrate one or more of the oxides at the expense of the other components in the alloy. This has been shown in a previous paper (*10*). Thus NiO is never observed on the surface in the oxidation of its alloys containing up to 80% nickel. Cr_2O_3 and Fe_3O_4 or γ-Fe_2O_3, however, are observed on alloys where chromium and iron may be present in amounts less than 5%.



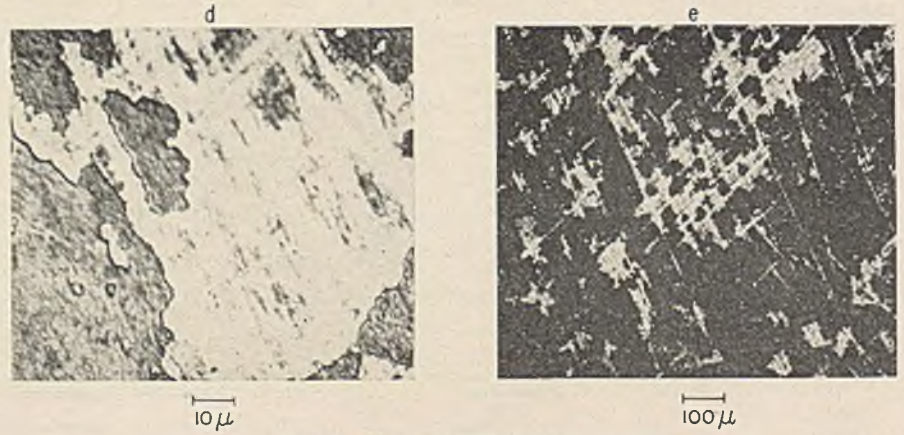


Figure 2. Oxide Film of 18-8 Stainless Steel, SS5–600

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, *e*. Light micrographs, film on metal

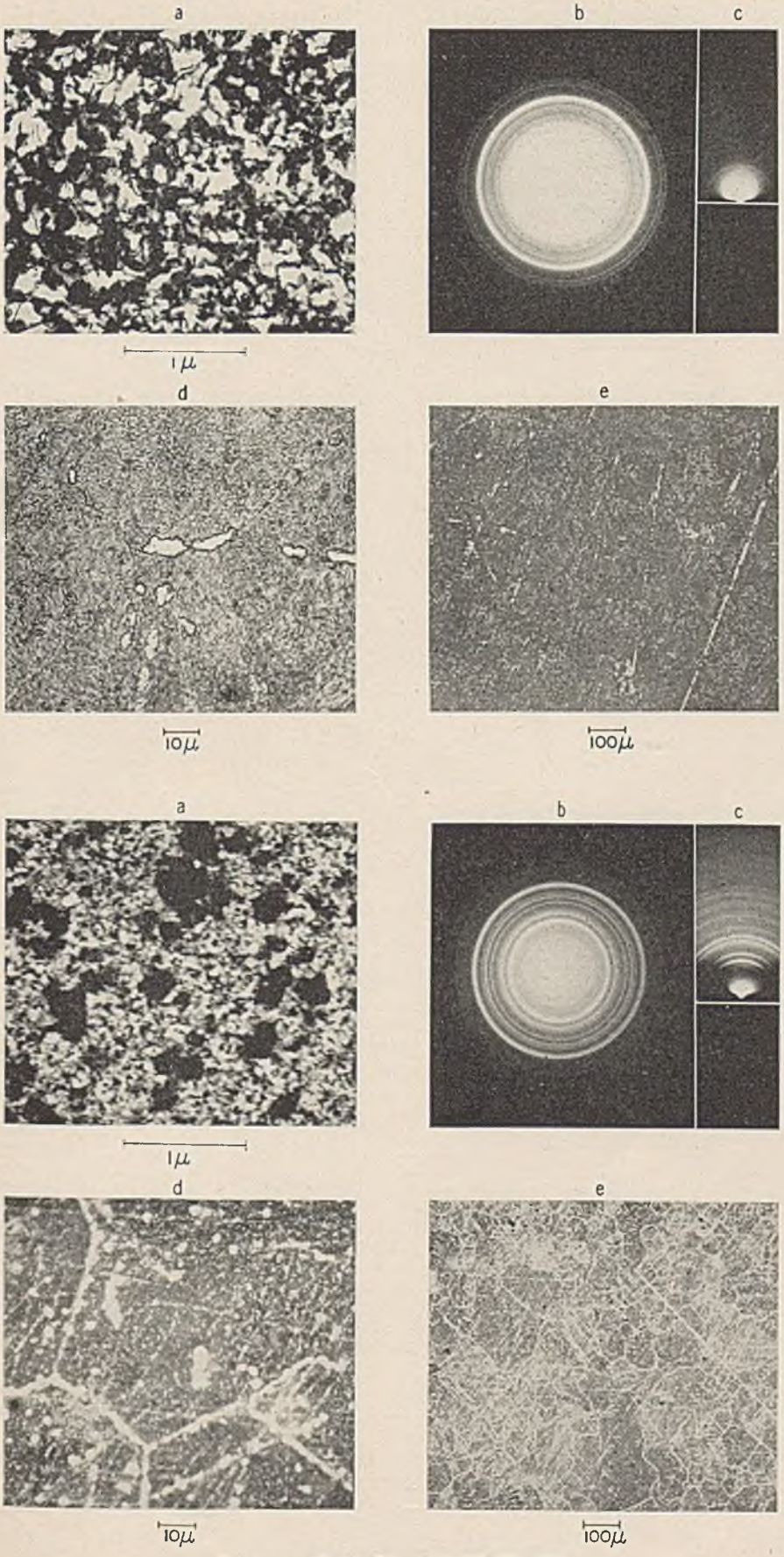


Figure 4. Oxide Film of K42B, K5–600

a. Electron micrograph, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, *e*. Light micrographs, film on metal

Figure 3. Oxide Film of 18-8 Stainless Steel, SS40–600

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, *e*. Light micrographs, film on metal

The use of the stripping technique to remove the oxide film and a study of the stripped oxide film by the transmission method should show further evidence, when compared with reflection measurements, of the concentration of the several components in the oxide film.

A previous electron microscope study (*15*) of the stripped oxide films formed on iron, cobalt, nickel, tungsten, chromium, molybdenum, columbium, aluminum, and copper has shown that the films consist of small oxide crystals of 100 to 2500 Å. in size. The films consist largely of crystals of irregular shapes, although a few films show definite crystal outlines. The oxide crystals are of the order of 10^{-3} to 10^{-5} of the linear dimension of the metal crystal or grain and 10^{-6} to 10^{-10} of the area.

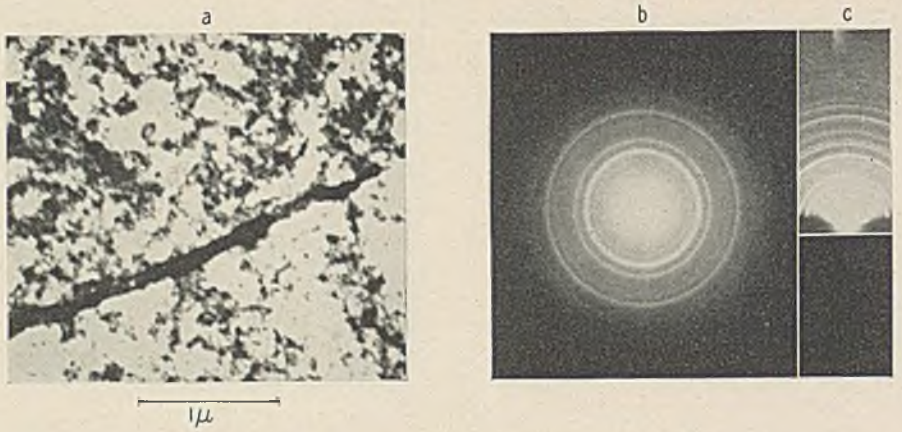
A systematic study of stripped oxide films on metals is difficult using electron microscope techniques. This is due to several factors: (1) The limitation imposed on the sample by the electron microscope—i.e., the thickness range of the specimen is limited to samples of about 100 to 500 Å. (2) The limitation in the resolving power of the electron microscope; with a resolving power of 40 Å. it is difficult to determine the shapes and the nature of the boundaries of crystals 100 to 200 Å. in size. (3) The scattering and diffraction of the electrons by the sample. If diffraction effects occur at grain boundaries, the nature of the boundary zone may not be determined.

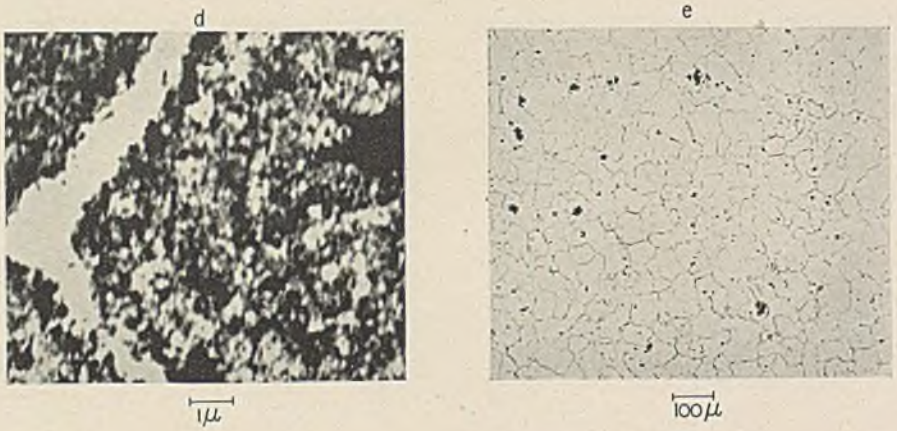
The study on alloys is further complicated in some cases by the presence of several oxides in the film. An examination of Table V shows that mixtures of oxides occur on 13 CrFe, K42B, Inconel, and Hipernik.

In the previous study (*15*) it was proposed that nucleation and growth of crystals had resulted in the formation of a mosaic structure completely covering the surface. This was noticed even for films less than 75 Å. thick. It would appear that at no time is the metal or alloy surface exposed to the gas atmosphere after the formation of the first chemi-adsorbed layer of oxygen atoms. The transformation from the first chemi-adsorbed layer of oxygen atoms to the continuous layer of small crystals is a continuous process of nucleation

Figure 5. Oxide Film of Inconel, 5–600

a, *d*. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
e. Light micrographs, film on metal





and growth. This continuous layer of small crystals is shown for the oxide film formed on silicon iron in Figure 11.

It is of interest to contrast this type of thin film with that formed by the evaporation of metals. Here the metal condenses into a discontinuous group of crystals. No evidence of a mosaic structure is noticed.

The continuous film of oxide crystals covering the surface of the metal or alloy may be the mechanism by which the property of protection is given to the metal or alloy. Since the chemi-adsorbed layer transforms readily to a continuous layer of crystals, a mechanical break in the film is readily repaired.

Protective Alloys. 13 CrFe and 18-8 stainless steel are classified as protective alloys, since they react only very slightly to oxygen atmospheres at moderate temperatures. Here a more quantitative concept is used in defining the term protective—i.e., a metal or alloy which obeys a parabolic oxidation rate law at a given temperature and pressure is considered to be protective. All the 16 alloys considered here are studied under conditions where the alloy follows the parabolic rate law.

13 CrFe. This alloy consists of a solid solution of chromium in the body-centered cubic lattice of α-iron. The oxide film formed at 600° C. in 0.1 atmosphere of oxygen for 5 minutes gives a pattern of Cr_2O_3 and in addition a trace of Fe_3O_4 using the transmission technique. Spectrographic analysis indicates the presence of chromium and iron as shown in Table V.

The electron microscope study of the stripped oxide film shown in Figure 1 and Table VI indicates a nonuniform film of irregularly shaped particles with a size distribution of 200 to 700 Å. while the average crystal size is about 450 Å.

Mott's theory of oxidation (*13*) would predict the occurrence of oxides of iron on the surface and Cr_2O_3 in contact with the substrate.

18-8 Stainless Steel. This alloy consists of a solid solution of chromium and nickel in the face-centered cubic lattice of γ-iron. Two oxidation experiments are made at 600° C., the first for 5 minutes and the second for 40 minutes. A mixture of Fe_3O_4 and α-Fe_2O_3 is found by the reflection study, while Cr_2O_3 is the only oxide found in the stripped film using the transmission technique. No spectrographic analyses were made. The evidence indicates that oxides of iron are con-

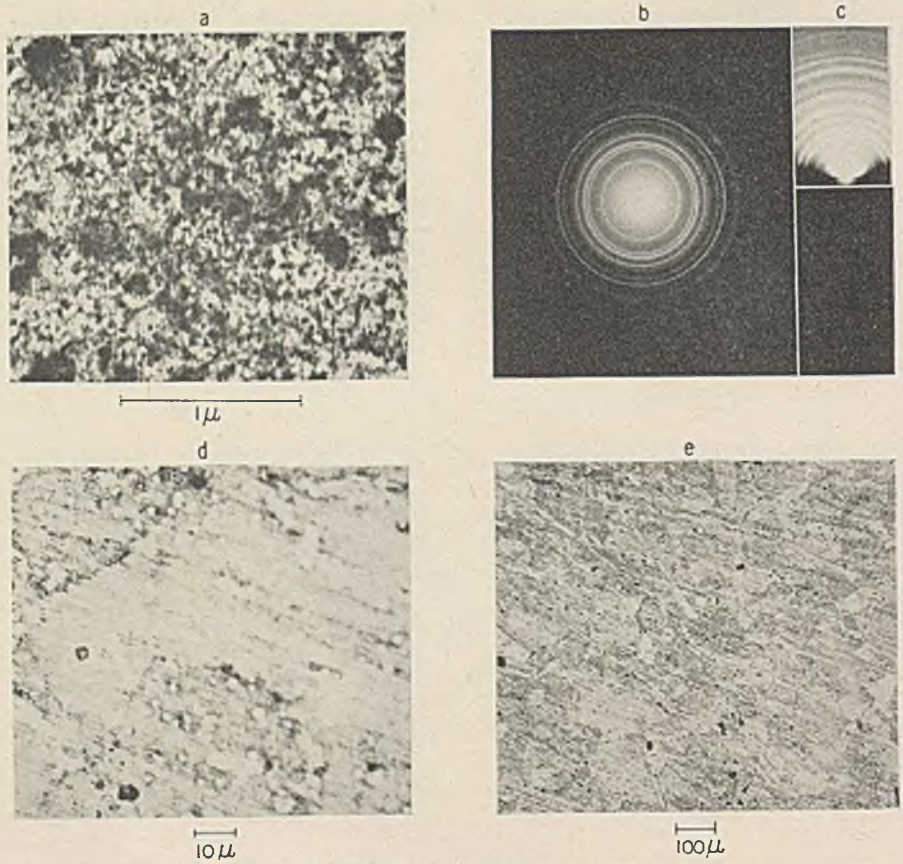
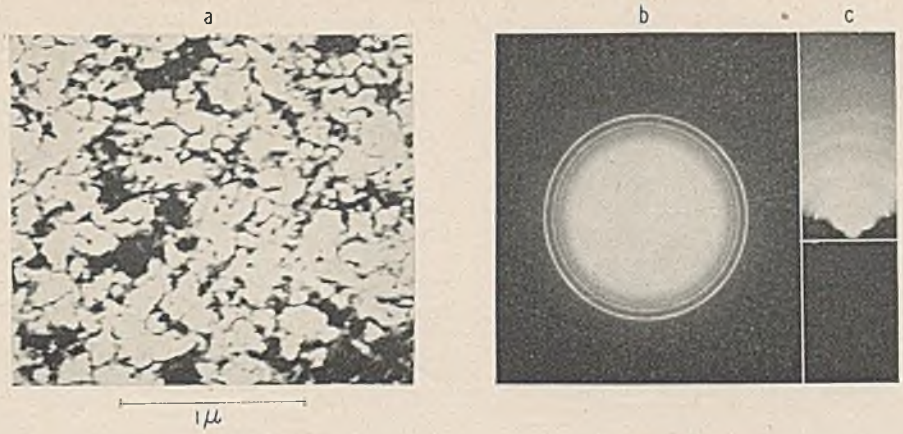


Figure 6. Oxide Film of Nichrome V, NC5–600

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, *e*. Light micrographs, film on metal



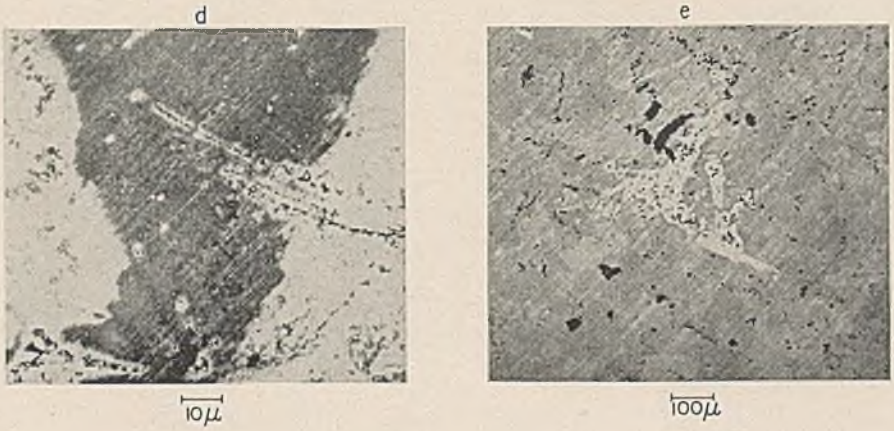
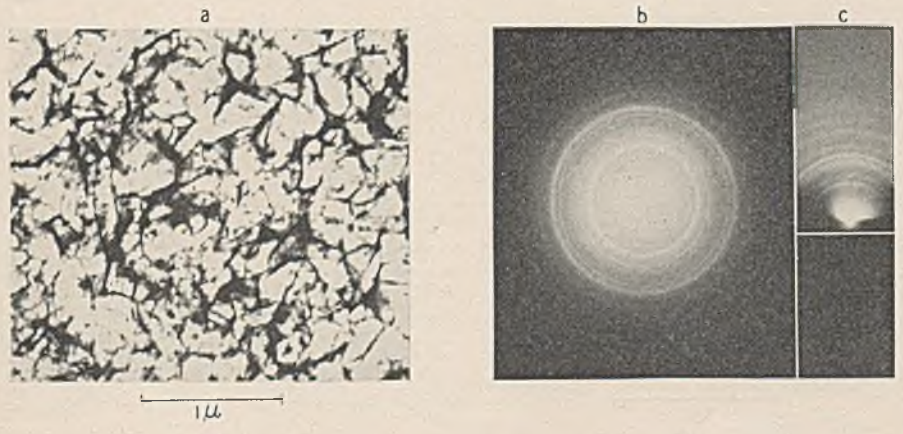


Figure 7. Oxide Film of Mild Steel, MS5–250

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, *e*. Light micrographs, film on metal



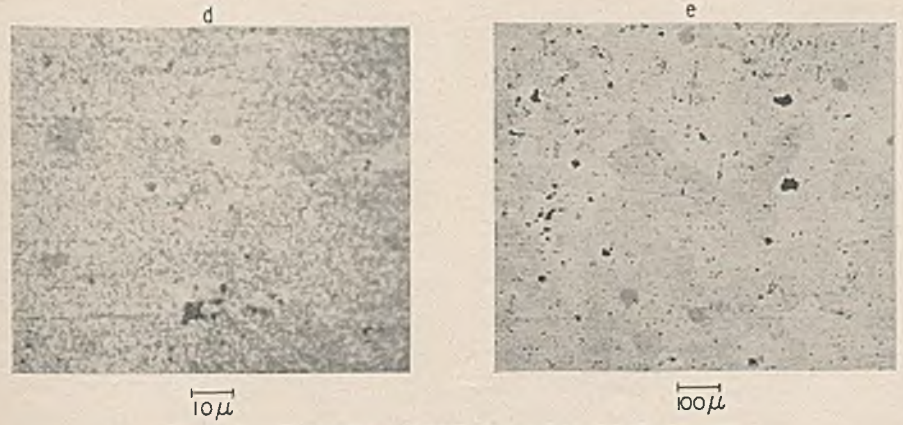


Figure 8. Oxide Film of 30% Cobalt-Iron, 30 Co 30–300

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, *e*. Light micrographs, film on metal

centrated in the outer part of the film, while Cr_2O_3 is the main component of the film. These results, when compared with those obtained on 13 CrFe as shown in Table VI, indicate that the presence of nickel in the lattice has had an effect on the relative rates with which chromium and iron get to the surface.

The electron microscope data shown in Table VI and Figures 2 and 3 indicate that small, irregular crystals of 100 to 600 Å. are formed for the 5-minute oxidation while somewhat larger, irregular crystals of 300 to 1500 Å. are formed in the 40-minute oxidation. The average size increases with time of oxidation from 300 to 700 Å. as shown by the two experiments. The 5-minute oxidation shows a uniform film while the 40-minute oxidation shows a nonuniform film.

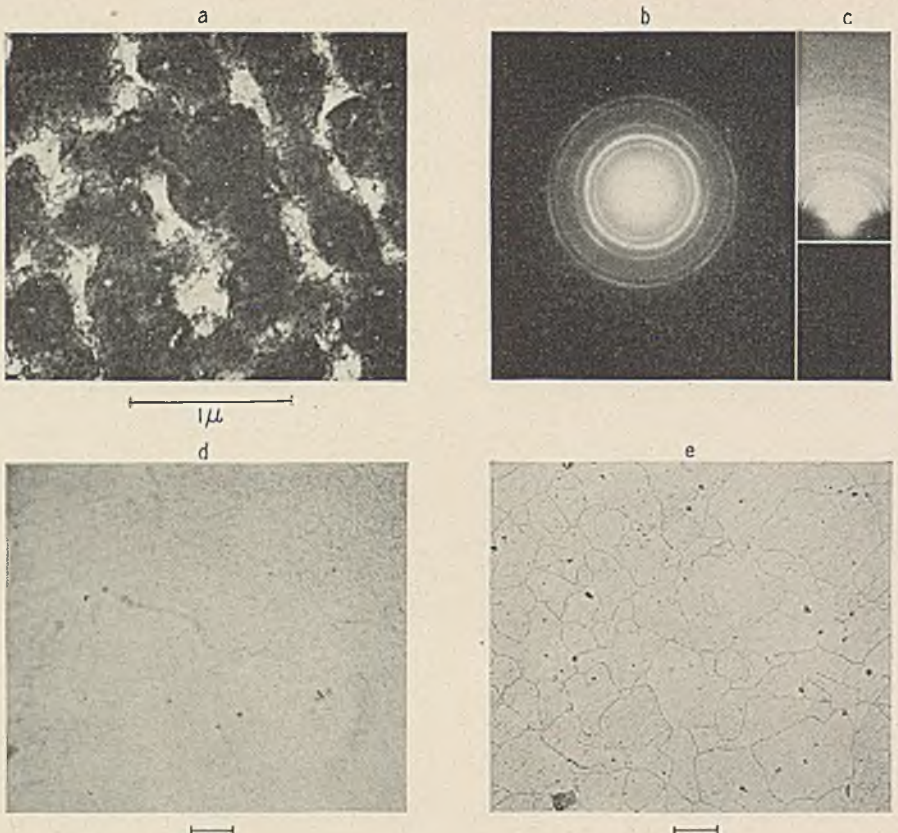
Refractory Alloys. These alloys are not only protective but exhibit great strength at high temperatures. They are usually complex chemically and in many cases are not structurally homogeneous.

K42B. K42B is a complex alloy and consists largely of a solid solution of the several metals in a face-centered cubic lattice. Two experiments are made with K42B at 600° C. The first oxidation is for 5 minutes, while the second is for 30 minutes. The reflection electron diffraction data shown in Tables III and V indicate a spinel structure on the surface which the authors have assigned the formula Fe_3O_4, because of its lattice parameter. The 30-minute oxidation study indicates the presence of α-Fe_2O_3 in addition to Fe_3O_4 on the surface. This is to be expected from previous observations on iron by the authors (*8*) where α-Fe_2O_3 is formed under conditions of an unlimited oxygen supply and long oxidation time. The transmission diffraction patterns show the presence of Cr_2O_3 and a spinel, while spectrographic analysis shows both chromium and iron with chromium making up the bulk of the film. It is of interest that stratification occurs even in films 100 to 300 Å. in thickness. Since the 30-minute oxidation shows only a trace of Fe_3O_4 by transmission while the reflection data indicate that the surface consists of oxides of iron, Cr_2O_3 appears to be in contact with the substrate. This is in agreement with results for stainless steel and in contrast with those for 13 CrFe.

The electron microscope data are summarized in Table VI and shown in Figure 4. Nonuniform films of irregular crystals are formed in both experiments. Crystals from 200 to 750 Å. in size, with an average size of 350 Å., are found for

Figure 9. Oxide Film of 5% Chrome-Iron, 5 Cr 5–400

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, e. Light micrographs, film on metal



the 5-minute oxidation, while the size distribution is 300 to 800 Å. and the average size 450 Å. for the 30-minute experiment. The average crystal size increases with an increase in the time of oxidation.

Inconel. This alloy is probably a solid solution type of alloy with a face-centered cubic lattice. Two experiments are made at 600° C.; the first oxidation is for 5 minutes, while the second is for 30 minutes.

The reflection studies indicate the presence of Fe_3O_4 for both experiments. A transmission study of the stripped film shows the presence of a spinel, Cr_2O_3, and NiO for the 5-minute oxidation, and Cr_2O_3 and NiO for the 30-minute oxidation. Spectrographic analysis shows the presence of chromium, nickel, and iron in the film.

An analysis of these results indicates that a stratification of the oxides occurs in the film formed on Inconel with a spinel, probably Fe_3O_4, on the surface and a spinel, probably $NiO.Cr_2O_3$, and Cr_2O_3 and NiO making up the body of the film. The data available do not permit a determination of the relative positions of the Cr_2O_3 and NiO in the film. It is interesting to note that NiO and Cr_2O_3 can exist together in the film without forming completely the spinel $NiO.Cr_2O_3$. An x-ray diffraction study of the equilibrium system Cr_2O_3-NiO by Thomassen (*16*) is shown in Figure 12. No part of the chart shows the existence of NiO and Cr_2O_3 except as the spinel $NiO.Cr_2O_3$.

The electron microscope data are summarized in Table VI and the micrograph is shown in Figure 5. Nonuniform oxide films are obtained with irregularly shaped crystals. The crystals vary in size from 300 to 900 Å. with an average size of 400 to 450 Å. An increase in the time of oxidation does not result in an appreciable increase in average crystal size.

Nichrome V. Nichrome V is an alloy used in making electrical resistance heaters. It consists largely of a solid solution of chromium in nickel with a face-centered cubic lattice.

Two oxidations are made, the first for 5 and the second for 30 minutes at 600° C. The electron diffraction data as summarized in Tables III and V show that Cr_2O_3 is found by both the reflection and transmission methods. This is in agreement with a previous study (*9*) on the existence diagram for Nichrome V. Mott's theory (*13*) would, however, predict the occurrence of NiO on the surface and Cr_2O_3 in contact with the substrate. Spectrographic analysis shows the presence of chromium and a trace of

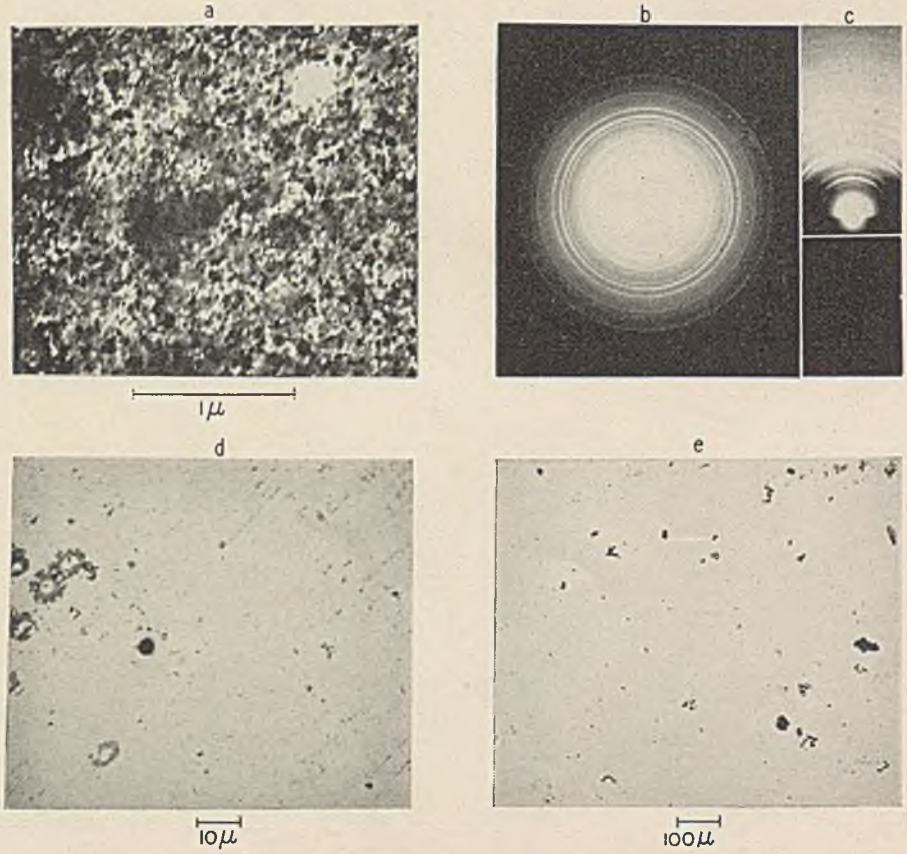


Figure 10. Oxide Film of 5% Manganese Iron, 5 Mn 10–300

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, e. Light micrographs, film on metal

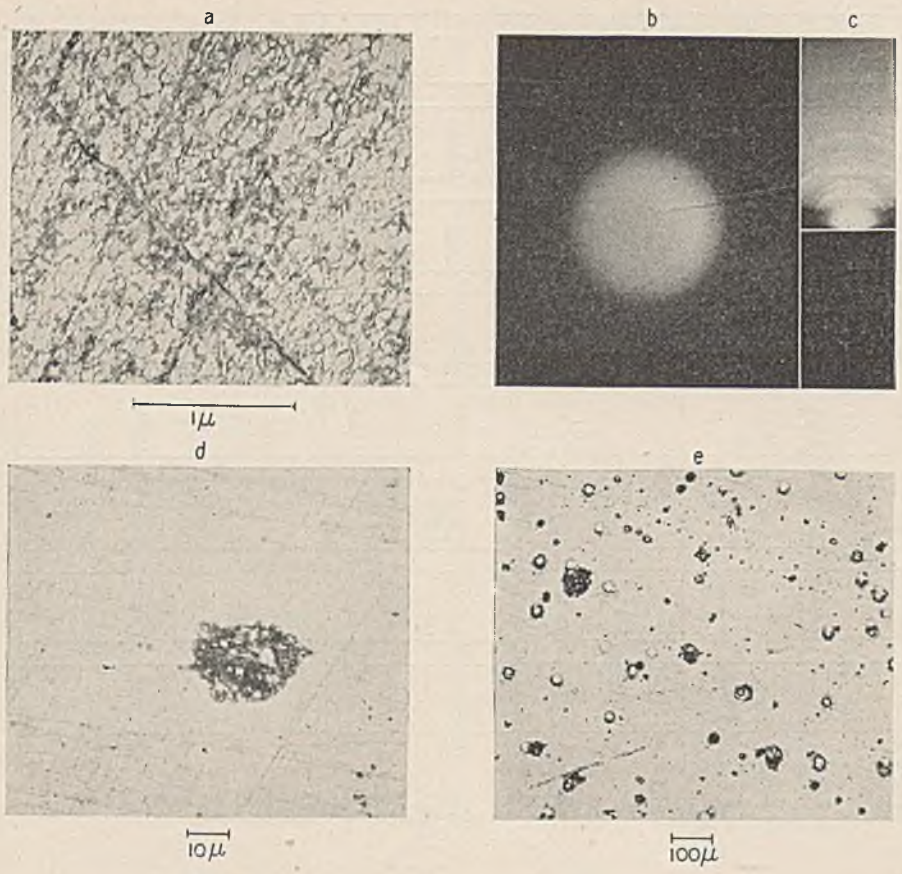


Figure 11. Oxide Film of 5% Silicon Iron, 5 Si 5–300

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, e. Light micrographs, film on metal

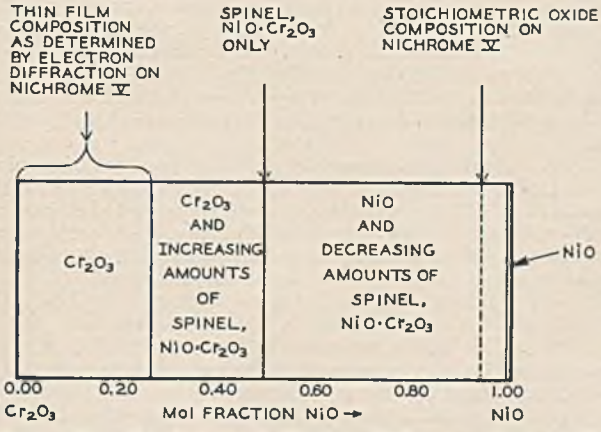


Figure 12. Correlation of Electron Diffraction Study with Equilibrium Diagram Study of System Cr_2O_3–NiO (15)

iron but no nickel. The authors' data also differ from the results of Chalmers and Quarrell (*2*) who report that the oxide formed on nickel-chromium alloys has a spinel structure and probably a composition corresponding to $NiO.Cr_2O_3$.

It is of interest to compare results for Nichrome V with the results of the equilibrium diagram for the Cr_2O_3–NiO system as determined by Thomassen (*16*) and shown in Figure 12. If the alloy were completely converted to the oxides, the diagram shows that one should obtain NiO and the spinel $NiO.Cr_2O_3$. The authors obtain from electron diffraction data only Cr_2O_3. Referring again to Figure 12 one can see that Cr_2O_3 would be the equilibrium oxide forming up to NiO mole fractions of 0.27, the NiO being present in solid solution in the Cr_2O_3. This example illustrates the difficulty of applying equilibrium data to a thin film reaction product forming on alloys.

The lattice parameters for the Cr_2O_3 lattice found in this study are shown in Figure 13, together with the other possible lattices which might be formed. The agreement with the Cr_2O_3 lattice is good.

Electron microscope data summarized in Table VI and the electron micrograph shown in Figure 6 indicate the presence of fairly uniform oxide films made up of irregularly shaped crystals. The crystals have a size distribution of 100 to 550 Å. with an average size of 250 Å. for the 5-minute oxidation and 350 Å. for the 30-minute oxidation. The time effect of size increase is small but can be noticed.

Magnetic Alloys. *Hipernik.* This alloy is of the solid solution type and has a face-centered cubic lattice. It is best known for its magnetic properties. It is an interesting alloy, since it consists of 49% iron, 49% nickel, and 2% manganese. In terms of the atomic concentration of the metals on the surface of the alloy, oxides of both iron and nickel should occur.

The oxide film formed at 400° C. in 0.1 atmosphere of oxygen for 5 minutes shows the presence of Fe_3O_4 and α-Fe_2O_3 on the surface by the reflection technique. The transmission data show the presence of a spinel with a lattice parameter of 8.34 Å. as well as α-Fe_2O_3 and NiO. The lattice parameter of the spinel does not permit a positive identification, since Fe_3O_4 has a parameter of 8.40 Å. and $NiO.Fe_2O_3$ has a parameter of 8.34 Å. Spectrographic analysis shows the presence of manganese, nickel, and a trace of iron.

The electron microscope data summarized in Table III indicate the presence of a uniform film with thicker sections at the grain boundaries. The crystals are irregular in shape and show evidence of overlapping. The crystal size varies from 200 to 500 Å. with an average size of 300 Å.

30 CoFe. This alloy is of the solid solution type and has a body-centered cubic structure. It is known for its magnetic rather than for its protective properties.

The oxide film formed at 300° C. in 0.1 atmosphere of oxygen for 30 minutes shows the presence of Fe_3O_4 (8.43 Å.) on the surface by reflection and a spinel (8.36 Å.) by transmission. The two spinels which are possible are Fe_3O_4 (8.40 Å.) and $CoO.Fe_2O_3$ (8.39 Å.). The body of the film may be either of these or a mixture of the two. Spectrographic analysis shows iron, cobalt, and a trace of chromium.

The electron microscope data given in Table III and the electron micrograph shown in Figure 8 indicate the presence of a non-uniform film composed of irregular, angular crystals with a size distribution of 300 to 1200 Å. and an average crystal size of 700 Å. Considerable overlapping of crystals is noted.

Mild Steel. Mild steel may be classified as a magnetic alloy with poor protective quality. It has a body-centered cubic structure.

Oxidations with 0.1 atmosphere of oxygen at 250° C. for 5 and 30 minutes show the presence of Fe_3O_4 by both techniques. Oxidation at 300° C. for 5 minutes shows Fe_3O_4 and α-Fe_2O_3 by reflection from the surface and Fe_3O_4 by transmission

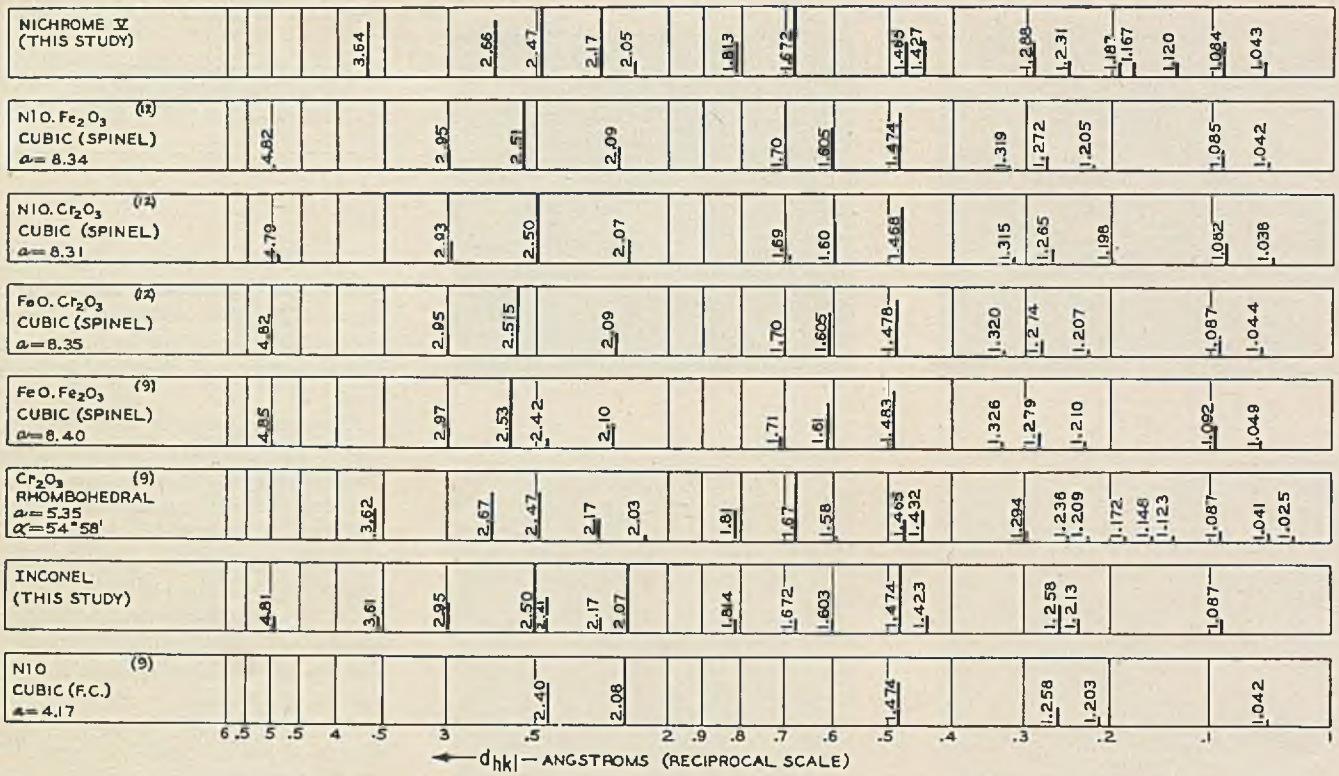


Figure 13. Experimental Diffraction Data for Oxides Which May Occur on Nichrome V and Inconel

The electron micrograph shown in Figure 7 indicates the presence of nonuniform film consisting of overlapping crystals with a size distribution of 250 to 1600 Å. and an average size of 750 Å. for the three oxidations. The size of the crystals does not appear to be affected by temperature.

Sealing Alloy. Kovar is a solid solution type of alloy having a face-centered cubic structure. It is used primarily in making glass to metal seals, and is not considered to be a protective alloy. However, the nature of the oxide film is very important in preparing good seals.

Oxidation with 0.1 atmosphere of oxygen at 400° C. for 5 minutes shows the presence of Fe_3O_4 by reflection and a spinel with lattice parameter of 8.35 Å by transmission. Since the parameter of $NiO.Fe_2O_3$ is 8.34 Å. while that of Fe_3O_4 is 8.40 Å. and $CoO.Fe_2O_3$ is 8.39 Å., it would appear that the nickel spinel may be formed. Spectrographic tests were not made on this alloy and therefore a positive identification is impossible.

The electron micrograph indicates the presence of a nonuniform film consisting of clusters of small irregular crystals with a size distribution of 250 to 750 Å. and an average size of 400 Å.

Miscellaneous Alloys. These binary alloys are composed of iron and metals of the body-centered cubic, face-centered cubic, and other structural types in solid solution in the α-iron lattice. In all cases the alloying metal is not present in excess of 5%. The alloying metals tungsten, chromium, and vanadium have body-centered cubic structures; Nickel is face-centered cubic; cobalt is hexagonal close packed; Manganese has a complex cubic structure; and silicon has a cubic structure of the diamond type. Previous work by the authors (*10*) on abraded samples of these alloys indicated that oxides of the alloying metal were not found on the surface. This study has been undertaken to determine whether polishing may increase the concentration of the alloying metal to such an extent that its oxides may occur on the surface and to determine whether oxides of the alloying metal occur in the body of the film.

5 CrFe. Transmission data show the presence of a spinel with a lattice parameter of 8.34 Å. in the body of the film. Spectrographic analysis shows the presence of chromium and a trace of iron. This would indicate that $FeO.Cr_2O_3$ (8.35 Å.) may be the oxide in the body of the film with Fe_3O_4 on the surface. It may also be possible that γ-Fe_2O_3 (8.32 Å.) is present in the body of the film.

3 VFe. The transmission data show the presence of a spinel with a lattice parameter of 8.33 Å. Although a spinel with the formula $FeO.V_2O_3$ may exist, no x-ray data appear to be available for this substance. The body of the film may, therefore, be composed of this spinel or it may be γ-Fe_2O_3 (8.32 Å.). No spectrographic analysis is available to check these predictions.

5 NiFe. Although the surface spinel appears to be Fe_3O_4, the formula of the spinel occurring in the body of the film may be $NiO.Fe_2O_3$. The lattice parameter by transmission (8.34 Å.) is in agreement with the assignment of the formula $NiO.Fe_2O_3$ (8.34 Å.). No spectrographic analysis is available.

5 CoFe. The spinel occurring in the body of the oxide film cannot be determined uniquely by electron diffraction, since there are two possible spinels, Fe_3O_4 and $CoO.Fe_2O_3$, having almost identical lattice parameters. However, the spectrographic analysis shows the presence of iron, copper, and a trace of aluminum but no cobalt. This would seem to indicate that γ-Fe_2O_3 (8.32 Å.) may be the oxide in the body of the film.

5 MnFe. Transmission data show the presence of a spinel with a lattice parameter of 8.32 Å. Since three spinels are possible Fe_3O_4 (8.40 Å.), γ-Fe_2O_3 (8.32 Å.), and $MnO.Fe_2O_3$, it would appear that γ-Fe_2O_3 is the oxide present in the body of the film. Spectrographic analysis shows the presence of iron, manganese, and traces of chromium and aluminum. Manganese may occur in solid solution in the γ-Fe_2O_3 lattice.

The two remaining alloys, 5 SiFe and 4 WFe, show only the presence of α-Fe_2O_3 by transmission.

The surface oxide on 5 SiFe as determined by the reflection technique appears to be Fe_3O_4. This case is rather unusual, since α-Fe_2O_3 is in contact with the substrate and Fe_3O_4 on the surface. Usually α-Fe_2O_3 is formed only when there is an unrestricted supply of oxygen and equilibrium conditions are being approached.

The surface oxide on 4 WFe appears to be α-Fe_2O_3. This is the only case in this series of alloys where α-Fe_2O_3 is found on the surface.

In summation, the structure of the alloying metal does not seem to have an appreciable effect on the occurrence of a given oxide on the surface. Polishing may have an effect in concentrating the alloying metals in the surface layer of the oxide but this increase in concentration, if it occurs, does not appear to result in the formation of an oxide of the alloying metal on the surface of the film. The data are not conclusive enough to decide whether the alloying metal may or may not be present in the various spinels which occur in the body of the oxide films.

The electron micrographs for these alloys are shown in Figures 9 to 11. The interpretations of these micrographs are given in Table III.

The micrographs of the oxide layers on 5 MnFe, 5 SiFe, 3 VFe, and 4 WFe show the presence of films which are uniform and composed of irregularly shaped crystals with average crystal sizes of 350, 500, 250, and 300 Å., respectively.

The micrographs on 5 CrFe, 5 NiFe, and 5 CoFe show the presence of films which are nonuniform and composed of crystals of irregular shapes with average crystal sizes of 250, 600, and 400 Å., respectively.

There does not appear to be any close correspondence existing between the structure of the alloying metal and the size and shape of the oxide crystals which form when the alloy is oxidized. With the exception of 5 CrFe, all oxidations were carried out under identical conditions. Since the oxidation rates of these alloys are probably different, there may be some differences in the thicknesses of the oxide films.

DEVIATION OF CALCULATED LATTICE PARAMETERS FROM X-RAY VALUES

A previous work by the authors (*8*) indicated that the electron diffraction reflection method gives lattice parameters for the oxide films formed on abraded samples of iron, cobalt, nickel, chromium, and copper which are slightly larger than the x-ray values. These parameters showed negligible time and temperature effects.

Transmission data obtained by the authors (*15*) on the same metals show small negative deviations. These samples had been submitted to metallographic polishing prior to oxidation. On the polished metals reflection data yield lattice parameters which are on the average 0.7% high, while the transmission method gives values which are on the average of 0.2% low.

In a recent paper (*10*) on alloys the lattice parameters of the oxides formed on abraded samples show in general that positive deviations occur. The average deviation was calculated to be 0.3%. The lattice parameter deviations of the oxides found in this work are shown in Table V.

The reflection method yields results which are, on the average, 0.4% high, while the transmission method gives values which are, on the average, 0.3% low. The positive deviations are greatest for Fe_3O_4 and least for α-Fe_2O_3 and Cr_2O_3. The negative deviations result almost entirely for Fe_3O_4 and are negligible for α-Fe_2O_3 and Cr_2O_3.

In the calculation of the deviations from the transmission data, Fe_3O_4 was used as the unknown spinel in all cases. Since other spinels may be present, as discussed earlier, the deviations may actually be smaller than those listed.

The positive deviations for Fe_3O_4 have a range of +0.24 to +0.60% and an average of 0.40%. The α-Fe_2O_3 lattice has an average deviation of +0.35% while the one observation of the Cr_2O_3 lattice gives a deviation of +0.30%. Goldschmidt (*6*) has shown that a variation in the stoichiometric ratio of iron to oxygen atoms in the lattice may give deviations of the order of magnitude observed here. Bernard (*1*) also reports that an increase in the lattice parameter of FeO from 4.28 to 4.298 Å. is caused by the solid solution of iron in the oxide lattice.

The differences observed in the lattice parameters as found by the reflection and transmission methods thus may be due to one or more of the following reasons:

The presence of strains in the oxide film on the alloy and subsequent relief of these strains when the film is stripped.

Transformation of Fe_3O_4 to γ-Fe_2O_3 by the stripping process.

Variations in the stoichiometric ratio of iron to oxygen atoms in the lattice.

Stratification of the several components as a function of the film thickness.

CONCLUSION

Electron Diffraction. The results obtained by the electron diffraction reflection technique show that the oxides found on the outer surface of the oxide layer may not be directly correlated with the composition of the alloy. In some cases oxides are found on the surface by reflection and are not observed after the film is stripped and examined by transmission. The cause of this difference may be a chemical change in the film as a result of the stripping technique or the presence of the outer oxide layer as such a small fraction of the whole film that the transmission technique is not sensitive enough to show its presence.

The difference in composition of the outer layer and the body of the oxide film may result from the following experimental facts observed on the alloys in addition to any possible effects which may result from the stripping technique:

Iron and chromium ions appear to diffuse more readily to the surface than other metal ions.

NiO is never observed on the surface, although it does occur in the body of the film on certain alloys; this may indicate a low diffusion rate for the ion.

CoO is not observed on the surface or in the body of an oxide even when the percentage of cobalt is high as in 30% CoFe; there may, however, be a solid phase reaction between CoO and Fe_2O_3 to form the spinel, $CoO.Fe_2O_3$.

The protective and refractory alloys, such as 13 CrFe, 18-8 stainless steel, K42B, Inconel, and Nichrome V, yield Cr_2O_3 as one of the oxides in the body of the film, although Cr_2O_3 may not be present on the surface of the oxide film. In two of these cases, 13 CrFe and Nichrome V, Cr_2O_3 is found both on the surface and in the body of the oxide film. None of the alloys shows the presence of Cr_2O_3 on the surface if it is not present in the body of the film. This would seem to indicate that in many alloys containing both iron and chromium, Cr_2O_3 is in contact with the metallic substrate while oxides of iron occur in the outer surface of the oxide film. An examination of those alloys shown in Table V which contain both chromium and iron indicates that iron in most cases has a greater tendency than chromium to get to the surface.

All of the magnetic, sealing, and miscellaneous alloys are oxidized in the temperature range 250° to 400° C. On Hipernik the presence of NiO, α-Fe_2O_3, and a spinel with a lattice parameter of 8.34 Å. suggests that a solid phase reaction may have occurred between NiO and α-Fe_2O_3 to form $NiO.Fe_2O_3$ with a lattice parameter of 8.34 Å. On Kovar the spinel in the body of the oxide film may be $NiO.Fe_2O_3$, $CoO.Fe_2O_3$, Fe_3O_4, or a mixture of any two or all three of these, although the lattice parameter of 8.35 Å. suggests that $NiO.Fe_2O_3$ may be predominant. Unfortunately, no spectrographic analysis is available to confirm this. The spinels in the body of the oxide films formed on 5 CrFe, 5 NiFe, and 5 CoFe may be $FeO.Cr_2O_3$, $NiO.Fe_2O_3$, and $CoO.Fe_2O_3$, respectively. Further work on the analysis of these oxide coatings is required in order that more positive identifications may be made.

It is of interest to compare the oxides found on polished samples in this study with those found on abraded samples recently reported by the authors (*10*). These comparisons shown in Table VII are made at the same temperature and time of oxidation although the oxygen pressure was 1 mm. with the abraded and 0.1 atmosphere with the polished samples.

There is a 1 to 1 correspondence of oxides obtained in the two methods of surface preparation. This indicates that polishing appears to have little or no effect or the oxides occurring in the

outer layer of the oxide surface. The oxygen pressure effect also appears to be negligible.

The lattice parameters of the oxides observed on polished samples of the alloys are in general agreement with x-ray values although slightly larger by the reflection technique and slightly smaller by the transmission technique. In those cases where more than one time of oxidation is used, no variation of lattice parameter with film thickness is noted.

Table VII. Comparison of Oxide Film Structures on Abraded and Polished Samples by Reflection Method

Alloy	Abraded Samples	Polished Samples
13 CrFe	α-Fe_2O_3 or Cr_2O_3	Cr_2O
18-8 SS	α-Fe_2O_3 + Fe_3O_4	α-Fe_2O_3 + Fe_3O_4
K42B	Fe_3O_4	Fe_3O_4
Inconel	Fe_3O_4	Fe_3O_4
Nichrome V	Cr_2O_3	Cr_2O_3

The authors' recent study (*10*) indicated that no unique oxide lattice could be correlated with the protective quality of an alloy. Neither could any statement be made relative to the position of the protective part of the oxide film. This study indicates that those chromium alloys, which are protective or refractory, always show the presence of Cr_2O_3 in the body of the oxide layer and may also show it in the surface film. This would seem to indicate that Cr_2O_3 may be functioning as a protective oxide layer and that it is actually in contact with the substrate. Further work is needed where the surface of the oxide in contact with the substrate is examined by the reflection diffraction technique in order to determine if this inner oxide surface actually is Cr_2O_3.

Electron Microscope. The selection of the various temperatures and times of oxidation is made such that the oxide films obtained are of comparable thickness. It is estimated that the thickness range lies between 100 and 300 Å. Within this thickness range all the alloys may be considered to be protective and they are probably obeying a parabolic rate law of oxidation.

The refractory and protective alloys included in this study are all oxidized at 600° C. In cases where two experiments at different times of oxidation are performed, the average crystal size increases with an increase in the time of oxidation. This increase is the most pronounced for stainless steel with relatively slight increases observed for K42B, Inconel, and Nichrome V. Of these four alloys Nichrome V is probably the most protective while stainless steel may be the least protective. The increase in crystal size is greatest for stainless steel and least for Nichrome V. There may, therefore, exist a correlation between the size of the oxide crystals formed and the protective quality of the alloy. Further work involving shorter and longer times of oxidation and higher temperatures is required in order to check this possibility. All the oxide films on these alloys consist of irregular crystals with considerable overlapping. Most of the films are nonuniform.

The magnetic, sealing, and miscellaneous alloys are oxidized in the temperature range 250° to 400° C. All the oxide films are composed of crystals of irregular shapes. The manganese, silicon, vanadium, and tungsten alloys are characterized by uniform films, while the cobalt, chromium, and nickel alloys form nonuniform films. The average crystal size varies more widely on these alloys than it does on the protective or refractory alloys ranging from 250 Å. on 3 VFe or 5 CrFe to 600 Å. on 5 NiFe.

The occurrence of nonuniform oxide films on alloys is not unexpected, since the initial layer may consist of the several oxides which have different rates of growth. In addition, each oxide possesses certain preferred directions of growth. A third factor may be concerned with the ease with which a metallic ion gets to the surface and is oxidized. All these factors would favor the formation of nonuniform films. One might expect, therefore, to find thicker and thinner sections of oxide film existing on the surface. In a previous paper on metals (*15*) the authors have explained overlapping of crystals as the result of the physical overlap of two crystals, and the contact zone between crystals occurring at an angle to the electron beam. This overlapping phenomenon is observed in many of the films on the alloys.

SUMMARY

The thin film oxidation process occurring on alloys composed principally of iron, cobalt, nickel, and chromium is studied by electron diffraction and the electron microscope. Both the reflection and transmission methods of electron diffraction are used. The film stripping techniques developed by Evans and co-workers are used for preparing the film for the transmission studies. Typical commercial protective, refractory, magnetic, and sealing alloys are studied. In addition a miscellaneous group of experimental alloys is included.

The following electron diffraction results are obtained: (1) Iron and chromium ions diffuse more readily to the surface of the oxide film than the other metal ions. (2) Stratification of the oxides occurs even for films of 100 to 300 Å. thick. (3) Cr_2O_3 is always observed in the oxide film on those alloys in this study which are classified as protective or refractory. In those cases where stratification occurs, Cr_2O_3 appears to be in contact with the substrate. (4) NiO is never observed on the surface of the oxide film, although it does occur in certain cases in the body of the film. This fact may indicate a low diffusion rate for the nickel ion. (5) Solid phase reactions may occur between two simple oxides to form the spinel type of oxide structure. (6) With the exception of iron and chromium, metals which constitute no more than 5% of the alloy do not occur as simple oxides on the outer surface of the film. These oxides do appear to form spinels in the body of the film. (7) Simple oxides of cobalt, manganese, silicon, vanadium, and tungsten are not observed in this study.

The following electron microscope results are obtained: (1) The thin oxide films consist of a continuous film of oxide crystals of 100 to 1600 Å. in size. (2) The oxide films are not of uniform density and the crystals have irregular shapes. (3) The effect of longer oxidation periods is to increase the average size of the crystals. (4) The crystals found on the nonprotective alloys such as mild steel at 250° C. are of the same size as the crystals found on the protective and refractory alloys at 600° C. (5) At a given temperature a correlation may exist between the size of the oxide crystals and the protective quality of the oxide film. (6) The thickness of the boundary zone between crystals in the mosaic structure is of the order of 50 Å. or within the resolving power of the microscope.

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Preparation of Powdered Materials for Electron Microscopy

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A discussion is given of the basic problems involved in the preparation of powdered materials for electron microscopy, with special reference to effecting and maintaining an adequate dispersion of ultimate particles. Various tested methods are described for forming thin films of clear resins and for mounting dispersed powders on these films. Procedures are also given for dispersing and mounting powders suspended in resin solutions. The properties and application of various resins and solvents are described, and electron micrographs of several types of mounts are shown. These procedures for mounting particulate materials should be helpful for extension and refinement of the techniques of sample preparation for the electron microscope.

FINELY divided substances were among the first objects studied by the electron microscope, and the literature contains accounts of numerous investigations of specific materials (*9*). In many cases descriptions are given of the methods used to mount the specimens for electron microscopic study, but a general survey of mounting methods for particulate materials has heretofore been lacking. The traditional mounting methods of light microscopy are not in general suitable for the electron microscope, because of the low penetrating power of electrons and the consequent necessity for operating in a high vacuum, which make impossible the use of standard glass slides and immersion liquids. It has therefore been necessary to develop special mounting techniques for electron microscopy. The aim of this paper is to discuss the problems involved in dispersing and mounting powdered materials for electron microscopy, and, since there is no single universally successful method of preparation, to describe various specific procedures found useful in this laboratory.

GENERAL CONSIDERATIONS

DISPERSION. When the purpose of microscopic study is to examine the characteristic size and shape of a few isolated particles, the preparation of a mount can be extremely quick and simple, involving no more than dusting a small quantity of the powder on the thin nitrocellulose films generally used as specimen supports. One purpose of pigment microscopy is to produce micrographs that can be studied statistically to furnish particle-size distribution data (*6*, *12*), from which can be calculated specific surface, covering power of pigments, and other properties. Such a statistical study is almost without significance if the particles are clumped together so that few particle diameters can be measured; therefore, the microscopic study must be preceded by adequate dispersion (Figure 1).

An ideally dispersed mount consists of a field of particles crowded enough for many to be studied on one plate, yet with each particle sufficiently separated from its neighbors to permit measuring. The dispersion of a powdered material involves the breaking of aggregates without comminution of the ultimate particles, and the prevention of flocculation during the subsequent preparation of the mount. Some form of work—mechanical, thermal, electrical (*10*), magnetic, or chemical—must be applied in order to break these aggregates down to the ultimate working unit, which is a single crystal, fragment of crystal, or small hard aggregate that functions as an ultimate particle (*4*).

FLOCCULATION. Flocculation is generally considered to occur when a powder is "poorly wetted" by the mounting medium, and if it occurs, the selection of another medium will often bring about a deflocculated mount. If the powder is known to be hydrophobic or hydrophilic, the mounting medium should be chosen accordingly. Wetting agents are sometimes helpful, but there is some evidence that they may make the image of a particle fuzzy by concentrating at the particle-medium interface; therefore, it is preferable to rely on the wetting properties of a properly chosen mounting medium, and to avoid the use of an additional wetting agent.

FILMS AND SCREENS. Electron microscope preparations are usually mounted on very thin (100 to 300 Å.) resin films. The fragile films are themselves supported on 0.3-cm. (0.125-inch) disks of perforated metal (nickel-plated copper), or of calendered screening of stainless steel, phosphor bronze, or copper. The size of hole varies from 20 microns for the electrolytic screens to over 100 microns for the woven screens. The small holes afford more support for films, but the large ones give a greater field of view. It is convenient to have punches of $^1/_{64}$, $^1/_{32}$, and $^1/_{16}$ inch with which to make a large hole in the center of a screen to facilitate the location of large objects in the electron microscope. Such punched screens are also used for electron diffraction work to eliminate all diffracted electrons coming from the surface of the screen wires. The film over such a large hole must be heavier than normally used, but for moderately low magnifications will not interfere with the quality of the image.

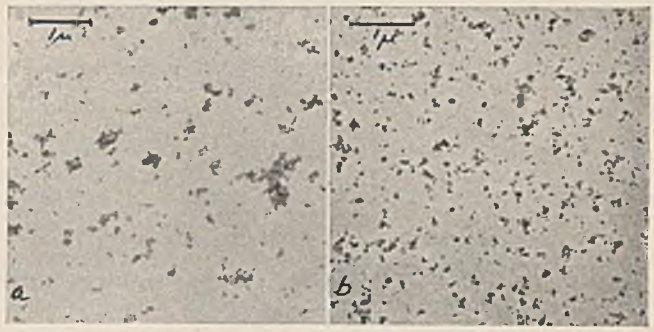

Figure 1. Dispersion

a. Iron blue in nitrocellulose, stirred
b. Iron blue in nitrocellulose, dispersed mechanically

USE OF LIGHT MICROSCOPE. It is strongly recommended that all stages of the preparation of mounts for the electron microscope be followed by observation in the light microscope. Experience in comparative observations with both instruments enables the microscopist to save much time by discarding unsatisfactory mounts before they are ready for the electron microscope.

MOUNTING ON A CLEAR RESIN FILM

FILMS. The resin film should be as nearly transparent to electrons as possible, should show no structure of its own, and should contain no suspended impurities. One exception to this generalization is in the case of stereoscopic studies of large particles, where a little fine dirt on the supporting film supplies a reference plane that is psychologically helpful in studying the stereoscopic micrographs. The thinner the film, the more transparent it will be. An ideally thin film is one which breaks under the full intensity of the electron beam, but which holds together under intensities sufficient to give a clearly visible image on the fluorescent screen of the microscope. A fragile film can be toughened by subjecting it first to very low beam intensities, and gradually building up to the desired brightness, both by bringing the source into focus at the specimen plane and by increasing the beam current. Usually larger particles will require thicker films for proper support.

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Resins. The most generally used resin is a purified nitrocellulose called Parlodion which is soluble in the usual nitrocellulose solvents. Greater toughness and heat resistance are exhibited by a polyvinyl formal polymer, Formvar 15/95, Grade E; this resin dissolves in a relatively limited number of solvents. All the resins listed in Table I, and doubtless many others, may be used to form films, but the two mentioned above have been adequate for a large part of the work in this laboratory.

Spread Films. A common method of film formation is to drop a small amount of resin solution on water, let it spread and dry, place screens on it, and pick up the screens with a special annular tool or with a glass microscope slide. The thickness of the film is controlled by the concentration of the original solution, by the volatility of the solvent (the more volatile solvents evaporate faster, and the film area is consequently smaller than with a less volatile solvent), and by the spreading coefficient of the solution (*1*). The spreading coefficient is a function of the surface tension of the water, the surface tension of the solution, and the interfacial tension of the two liquids; the coefficient must be positive if spreading is to occur. In the absence of a surface-active agent, the spreading coefficient of the solvent controls the spreading of the solution. Thus, Parlodion in methyl, ethyl, amyl, or Cellosolve acetates (all with positive coefficients) spreads well; whereas Formvar in ethylene dichloride or dibromide (negative coefficients) will not spread, but forms lenses on the water instead. Formvar (5%) in dichloroethylene or propylene dichloride will spread fairly well, since the unsymmetrical nature of those molecules enables them to spread. Formvar in ethylene dichloride will spread if it contains a sufficient amount of wetting agent, such as Aerosol OT (suggested by A. Y. Mottlau, Standard Oil Development Co., Elizabeth, N. J.) or oleic acid, although the films obtained with the former contain many holes and are lacy in appearance. This lacy structure may be desirable as a support for weak or exceptionally thin films, and can also be achieved by dispersing camphor in an equal or slightly larger volume of 10% Parlodion in amyl acetate and spreading the mixture on water.

The water on which the films are spread is contained in a rectangular dish of Pyrex whose edges are ground flat, and whose inner surface and edges are lightly coated with paraffin. When the dish is filled to the brim with distilled water, the meniscus rises above the edge of the dish and can be swept clean with paraffined glass rods or chromium-plated brass bars. The water is swept twice in this manner before every operation. The distilled water must be changed daily, since any impurity tending to lower the surface tension of the water will concentrate at the water-air interface and seriously reduce the spreading of solutions on the water. Any bacteria growing in the water will be observed on the preparation; therefore the dish must be cleaned regularly.

Mercury may also be used as a substrate on which to spread films. Its high surface tension makes it theoretically excellent for this purpose, but the practical difficulties of cleaning and handling mercury are considerable.

Cast Films. Resin films can be formed by casting onto glass. Dilute solutions of the resin (0.2 to 0.4% by weight) in very volatile solvents are taken up in a micropipet formed by drawing out soft glass tubing in a flame (*2*). A slight bend in the tubing will prevent the solution from running back into the rubber bulb and becoming contaminated. In forming the film, the long narrow shank of the pipet is held close to and parallel with a clean glass slide; the liquid is gently expelled and is drawn between pipet and slide by capillarity, then quickly spread out across the slide with a sideways motion of the pipet. To eliminate streaking, the slide is held vertically in an atmosphere of the solvent for the resin, preferably in a large jar or beaker, where it is allowed to drain until dry. The film thickness depends on the concentration of the solution, the volatility of the solvent, the length of time for draining, and to some extent on the manner in which the solution is spread. The film can be formed more simply by dipping the slide into a jar of the solution, but the pipet method is preferred for two reasons: the large surface of the dipping jar exposes the solution to dust contamination, an important consideration in many laboratories, and the tendency of ethylene dichloride (the usual Formvar solvent) to become acid on exposure to light and air is aggravated. All ethylene dichloride solutions should be stored in brown bottles, since a markedly acid solution forms weak films that are difficult or impossible to strip.

Manipulation of Cast Films. After casting and drying, the film is scored around the edge with a needle and usually cut into 0.25-inch squares for easy manipulation of the screens. The slide is lowered into the water at a low angle, film side uppermost, and the edge of the film is teased loose from the slide with a needle. This is easier if the rim is thickened with one or two extra coats of resin. The film slowly peels off the slide and floats on the surface of the water with little help from the needle; breathing on the slide before and during peeling will facilitate the operation. If the squares of film show any interference colors when examined by reflected light, they are too thick. A pale, first-order gray is the color of the heaviest film that can be employed; the best films are nearly invisible on the water surface.

The glass slides should be clean and free from scratches, because scratches are reproduced in the film. Dirt will contaminate the film and sometimes prevent it from peeling off the slide. To minimize these difficulties, new microscope slides of good quality are employed; they are cleaned in soap and water, rinsed, and dried. Just before use, a slide is removed from the water, drained, then polished with fine magnesium oxide on a small wad of moist lens paper, and wiped dry with a clean soft cloth. If the slides are used only for this purpose, they may be cleaned and reused several times before becoming too badly scratched.

The film may be transferred directly from the slide to the screen by a method described by Schaefer and Harker (*13*). The screen is placed on a film-covered slide, both are covered with condensed moisture by breathing on the slide, and Scotch tape is quickly pressed down over screen and slide. When the tape is gently peeled off the slide, the screen comes up with the film on it.

Dry Mounting. The specimen may be placed on the film in a variety of ways. The simplest is to dust the dried powder onto either a film-covered screen or a film-covered slide. In the latter case, a suitable area is selected in the light microscope, and a screen is placed over that area. A small drop of rubber cement on one edge of the screen will keep it from slipping off the selected area as film and screen are peeled off together.

Mounting Powders Suspended in a Liquid. Any powder that can be dispersed in a volatile liquid which does not dissolve the film on the screen, can be easily mounted by placing a drop of the suspension on a film-covered screen and allowing it to dry; however, the particles tend to clump together as they go to dryness. It is possible to keep the particles dispersed to the last moment by applying some form of vibration, as described below. It is also possible to immobilize a deflocculated suspension by quick-freezing in liquid air or a dry ice–alcohol mixture, and then to sublime the ice under high vacuum. Particles can also be directly centrifuged onto the film-covered screen (*3*).

Removing Soluble Impurities. Soluble impurities in a suspension are often troublesome, since they crystallize on drying and are visible in the electron microscope. The mounting film can be used as a dialyzing membrane to remove water-soluble substances: a drop of the suspension is placed on the film as it lies on the water and water is allowed to dialyze through the film. The drop swells as water passes through the film by osmosis. A screen is placed on the drop, and the excess water withdrawn by

Table I. Resins and Solvents

Resin	Recommended Solvents: For dispersion[a]	For mounts cast on glass[b]	For mounts spread on water[c]	For mounts drawn down on glass[c]
Nitrocellulose (Parlodion)	Cellosolve acetate	Methyl acetate Ethyl acetate	Methyl acetate Ethyl acetate Amyl acetate Cellosolve acetate Octyl acetate	Ethyl acetate Amyl acetate
Formvar 15/95	Dioxane plus a few drops of dimethyl dioxane Nitroethane	Ethylene dichloride Nitroethane	Dichloroethylene Propylene dichloride Ethylene dichloride plus 0.1% oleic acid Nitroethane 1,1-Dichloro-2-nitroethane	Dioxane Nitroethane
Polystyrene	Xylene	Ethylene dichloride Toluene	Benzene Toluene Xylene	Benzene Toluene
Methyl methacrylate or cellulose acetate	Methyl amyl ketone Methyl ethyl ketone plus 10% isophorone	Acetone	Methyl ethyl ketone Methyl amyl ketone Isophorone	Methyl ethyl ketone

[a] 10 to 20% solutions, approximately.
[b] 0.1 to 0.5% solutions, approximately.
[c] No fixed concentration; suspension allowed to dry to suitable consistency.

a micropipet, dropping the screen on the film. Film and screen are removed and dried in the usual manner.

DISPERSION OF POWDERS

Turpentine Dispersion. The manner in which work is applied to obtain a dispersion may vary greatly. A standard pigment-dispersion method used in light microscopy may be adapted for electron microscopy (*5*).

The pigment is rubbed out on a microscope slide in a drop of fresh triple-distilled turpentine, using a back and forth circular motion of a glass rod held parallel to and touching the slide horizontally. As the last of the turpentine evaporates, the particles dry in wedges. In some areas of these wedges, the particle density will be an optimum, neither too crowded nor too sparse. Such a dispersion may be flooded with a dilute resin solution, drained, dried, and stripped onto water, as in the formation of a cast resin film; or it may be stripped by the method of Schaefer and Harker. The film partly surrounds the pigment particles and lifts them off the slide.

This method works fairly well with powders of large and fairly uniform particle size (0.5 micron and greater). The method may also be reversed—that is, the turpentine dispersion may be made directly on a film-covered slide. The film is unavoidably torn by the largest particles under the rod, and it may be awkward to peel onto water; however, a continuous scrap of film $^1/_{16}$ inch square is ample for electron microscopy.

Dispersion of Soft Substances. Many substances are too soft to be rubbed out mechanically without deformation. These materials can be placed in a mortar with solid carbon dioxide and ground until just before the dry ice has all sublimed. The low temperature makes most soft substances sufficiently brittle to shatter. The particles can later be mounted in any convenient manner. Since commercial dry ice contains appreciable amounts of oil, the particles should be washed by decantation with an oil solvent.

Dispersion with Vibrators. Air flocculates may sometimes be broken apart using a simple mechanical vibrator. The dry sample is placed on a film-covered microscope slide fastened to the vibrator. Materials of very large particle sizes have a tendency to separate into different size ranges as they are vibrated. Sonic vibration in the upper audio range (15,000 cycles per second) was also tried. An arm rigidly attached to the vibrator of a loudspeaker strikes the bottom of a glass cell made of a ground-glass ring cemented to a cover slip. The cell contains water on which is floated a film-covered screen, which in turn supports the drop of liquid containing the sample in suspension. Supersonic vibration (550 kilocycles per second) was used in a similar manner (*7*). A watch glass containing water is placed directly on an electrode of the supersonic vibrator and a screen floated on the water as before. The latter two methods were tried only with carbon black suspended in water. Although the results were not entirely satisfactory, the method as described might be useful in special problems, upon proper selection of suspending medium and adjustment of particle concentration.

Dispersion with a Spark. Dispersions may also be made using a high-frequency spark generated by a Tesla coil. The dry sample is placed in a small pile on one end of a film-covered microscope slide, and the slide is clamped on a wooden support with the sample-bearing end projecting. A metal plate is supported about 0.25 inch above the slide. A spark from the coil is passed several times across the bottom of the slide. The powder scatters radially outward and usable areas may be found around the edges of the slide. The spark cannot be applied directly to the sample, since it alters the film, making it difficult to strip onto water. This method gives fair results, but is unsuitable for easily fused materials.

Dispersions with Mills. Mechanical dispersions may be made in ball, colloid, rubber, or roller mills, or any others, but these commercial mills require a relatively large sample, consume considerable time, and in some cases contaminate the sample severely. It should be remembered that nitrocellulose mixtures may degrade or explode on milling. Mortar and pestle, muller and plate, or an all-glass motor-driven micromill (*11*) are cleaner and use a smaller sample, but they have not been found to give the most satisfactory dispersions. An agate mortar and pestle are indispensable for very refractory materials.

Dispersion with a Spatula. The authors have found that the best dispersions for electron microscopy are made with the edge of a stiff spatula on a flat white glass table with a fine-grain "suede" finish. The table is cleaned and re-surfaced with No. 600 Carborundum, followed by a good cleansing powder, and liberal rinsing. Plate glass surfaced in the same manner is satisfactory. The best spatulas are of the straight palette knife type, with a fairly stiff 4-inch blade; the edge should be dull, smooth, and straight, and the blade kept polished brightly with fine emery paper. The use of stainless steel is advantageous, as some of the solvents may corrode an ordinary steel blade. Only the largest aggregates are comminuted by being pressed directly against the table by the knife, since fine particles are usually smaller by many orders of magnitude than the average distance between the relatively rough dispersing surfaces. Dispersion is largely accomplished by creating shearing forces in the liquid in which the particles are suspended; the greater the viscosity of that liquid, the greater will be the shearing force that can be produced. This desirable condition is attained by mixing the powder with a solution of the mounting resin in a solvent of moderate volatility. As the solvent slowly evaporates, the mixture becomes more and more viscous. Just before going to dryness, it has the consistency of tar and can be smeared out on the dispersion table.

The knife is held with the edge parallel to the table and the blade tilted slightly in the direction of smearing, which is normal to the edge of the knife. The mixture is rubbed out over an area of a few square inches, scraped up, and resmeared until it is too stiff to work without breaking the spatula. The mixture may be wetted with more of the same solvent and reworked, if the dispersion is not complete. The solvent should have sufficiently low volatility to give adequate rubbing time, usually about half an hour for 0.5 cc. of mixture. After the initial mixing, it is not necessary to rub the sample continuously, since the most effective work is accomplished in the last 10 minutes, and several dispersions can thus be in progress at the same time.

Particle Density. The proportion of dry pigment to resin solution that will give a mount of proper particle concentration varies with the average particle size of the pigment. The finer the pigment, the smaller is the proportion of pigment volume to resin-solution volume, since, of course, a given weight of a fine pigment has a greater projected area than an equal weight of coarser pigment, and consequently will produce a more crowded field. Thus, 1 or 2 mg. of a fine carbon black will be adequate for 0.5 cc. of 10% resin solution, while a bulk of a coarse lead white equal to or greater than that of the resin solution will be needed to produce a sufficiently concentrated mount.

Mounts for Electron Diffraction. Pigment mounts for electron diffraction must be more dense than for microscopic studies, in order to provide a sufficient number of surfaces for diffraction, so that continuous rings rather than separate spots will be formed on the photographic plate. It is possible to obtain fairly satisfactory micrographs and diffraction patterns from the same preparation, by making a compromise in the particle density between optimum density for diffraction and optimum density for micrography.

MOUNTING DISPERSED POWDERS

After a powder has been dispersed in a resin, there are several methods for forming it into a film suitable for electron microscopy. Each method has its advantages and disadvantages; conse-

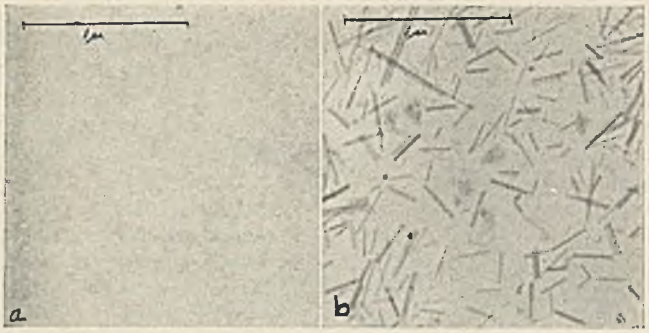

Figure 2. Mounts Cast on Glass

a. Phthalocyanine green in Formvar
b. Attapulgus clay in Formvar

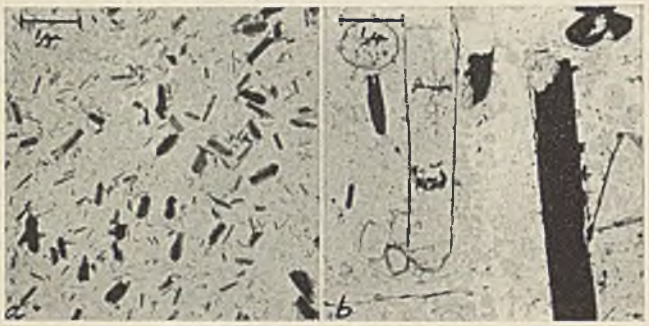

Figure 3. Mounts Spread on Water

a. Iron oxide in nitrocellulose
b. Antimony oxide in nitrocellulose; although this material volatilizes in the electron beam, the particle size and shape are clearly outlined by the nitrocellulose

quently, the choice of a mounting method must be made with reference to the particular substance to be studied.

Cast Mounts. Materials of colloidal dimensions dispersed in a resin may be resuspended in a fairly volatile solvent and cast onto a clean glass slide as described above. The dry smear on the dispersion table is taken up with solvent and transferred to a test tube. A small amount of this is placed in another test tube and diluted with the same solvent. From this suspension a film is cast, and if it is not thin enough, the dilution is repeated and another film formed. This procedure is continued until the film becomes too thin to remain coherent when peeled onto the water; the dilution just preceding this one is used to make the final film.

While this method is tedious, it produces films of exceptional thinness and clarity, and is the only method suited to the finest carbon blacks and organic pigments. Formvar diluted with ethylene dichloride is usually employed because of its superior toughness. Materials that flocculate while being cast cannot be mounted in this way, but another combination of resin and solvent may sometimes avoid flocculation. Materials that have too large a particle size to stay in suspension for several minutes cannot be mounted by casting, since the larger sizes will settle out in the test tube, and the mount will not properly represent the original sample. Figure 2 shows mounts typical of the casting method and Table I suggests various resin and solvent combinations.

Spread Mounts. A method modified from one originally suggested in the RCA electron microscope instruction book is useful for medium-sized particles (0.2 to 2 microns). For this method the dispersed mixture is taken up in a sol ent of positive spreading coefficient, allowed to evaporate until it reaches the consistency of thin cream, and then spread on water by drawing a drop on the end of a spatula rapidly across the surface. Screens are placed on thin, carefully chosen areas of the spread film, removed, and dried as usual (Figure 3).

This method is quick and convenient, but has the disadvantage that the films often contain many holes (Figure 4, *a*), for reasons not clearly understood. While electrostatic charges in the electron microscope have been blamed for this condition, the holes are often visible in the light microscope when the film is still moist and before it is placed in the electron microscope. Resin solutions containing surface-active agents may form lacy films, suggesting surface activity as one cause of hole formation. There is probably also some connection between hole formation and water-solubility, since holes are readily formed in nitrocellulose films when using the highly water-soluble esters, methyl formate and methyl acetate, and also with the highly insoluble octyl acetate. Solvents of moderate water solubility seem best for spreading nitrocellulose, as, for example, amyl acetate and Cellosolve acetate. In the case of resins such as Formvar and polystyrene, which dissolve only in almost totally water-insoluble solvents, the relative water solubility of the solvents seems to have no influence on hold formation. The holes in the film are sometimes found concentrated around the edges of the particles (Figure 4, *a*), indicating poor wetting of the particle by the solvent, but more frequently the holes have no apparent connection with the presence of particles.

There are other variables affecting the quality of spread films, such as the concentration ratio of pigment to resin to solvent, the cleanliness and temperature of the water, the speed with which the knife carrying the suspension is drawn across the surface of the water, and most important, the choice of area on which to place a screen, since spread films are very nonhomogeneous. The procedure is highly empirical, but with a little practice consistently good results can be obtained with any powder to which the method is suited.

"Draw-Down" Mounts. The most widely applicable method of mounting a dispersed powder is to make a draw-down on a clean glass slide, strip the dried film onto water, and place screens on the thinnest areas (Figure 5, *a*, *b*, *c*, *d*). In this method the dispersed mixture is suspended in a solvent of volatility equal to or greater than that used for spreading films on water; it is then stirred and allowed to evaporate to the consistency of heavy cream; the mixture is finally drawn down on a clean glass slide in a series of short strokes, using either the edge or the tip of the spatula. After drying, the film is cut into squares and floated off onto water.

Such a film exhibits a great variation in thickness, and the screens are placed on the thinnest areas. Even the thinnest areas consist of microscopic stripes of different thicknesses resulting from the irregularities of the knife edge. When such a film is examined in the electron microscope, some screen holes are seen to have no film, and others will be only partially covered, but many will be completely covered with film. The irregularities of the mount assure a large range of particle density on any one specimen screen, so that a field of any desired density may be found upon sufficient scanning. This type of mount should be made with a somewhat higher pigment-resin ratio than the other methods. Samples mounted in this manner exhibit fewer holes and less flocculation than other methods of mounting. If there is some tendency to flocculation, it may be minimized by employing a more volatile solvent and making the draw-down more rapidly. By this means, the particles are mechanically deflocculated and fixed in position by drying before they have time to reflocculate. This method is excellent for particles of all sizes except the extremes of fine and coarse.

Draw-Downs in Liquids. Powders of the largest particle size range for which the electron microscope is useful (1 to 10 microns) are mounted by a variation on the draw-down procedure. The powder is wetted with a minimum amount of a high-boiling hydrocarbon (such as ink oil No. 896, Gulf Refining Co., boiling point, 270–315° C.; this oil must be redistilled under vacuum and stored in a brown bottle) or dibutyl phthalate and dispersed on the table. It is then drawn down on a film-covered slide with

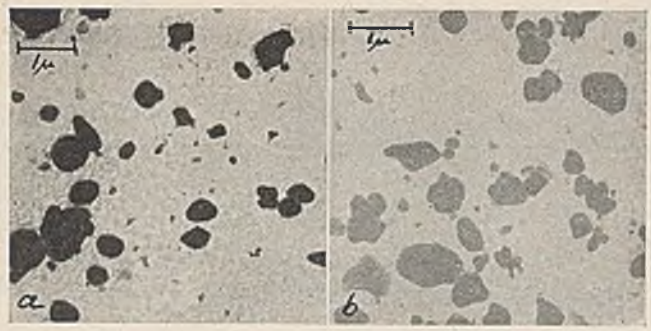

Figure 4. Holes in Films Near Particles

a. Chromium oxide in nitrocellulose, spread on water
b. Chromium oxide in nitrocellulose, drawn down on glass

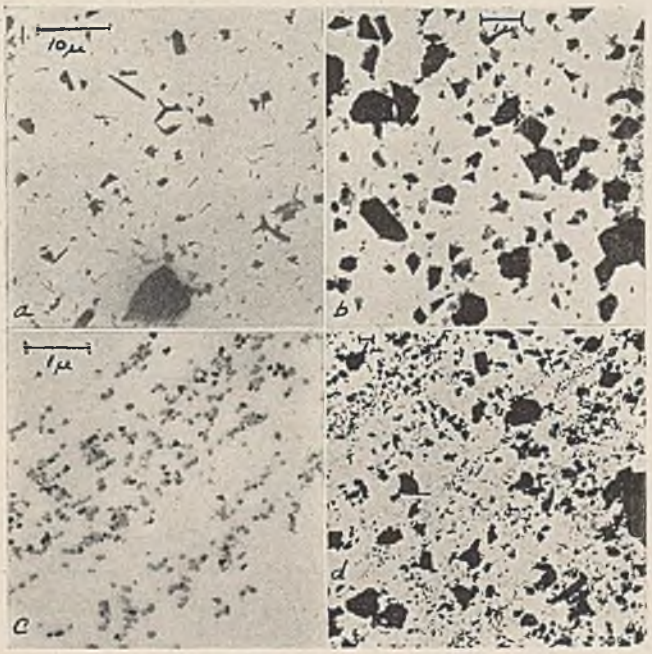

Figure 5. Mounts Drawn Down on Glass

a. Zinc oxide in Formvar
b. Ultramarine in nitrocellulose
c. Iron blue in castor oil
d. Calcium carbonate in a high boiling hydrocarbon

short strokes as in the resin draw-down method. The film is floated onto water and mounted on screens with large holes. The hydrocarbon and dibutyl phthalate are practically nonvolatile at atmospheric pressures and room temperatures, but disappear completely in the high vacuum of the electron microscope. The dispersions made by this method are never so complete as in the resin draw-down method, since the lower viscosity of the hydrocarbon limits the shearing action that breaks the aggregates. It is, however, possible to study large particles stereoscopically to aid in the differentiation between an aggregate and a single particle. Since the particles are not incorporated in the film, they seldom rupture it under electron bombardment (Figure 5, *d*).

Suspensions of solids in semivolatile and nonvolatile media, such as paints or printing inks, may be mounted for electron microscopy by smearing them onto film-covered slides (*8*). The nonvolatile residues naturally cause the electron beam to scatter, with the result that the images of the particles are rather fuzzy (Figure 5, *c*). There is, however, an obvious advantage in studying such material in a condition as near as possible to its original state.

The various methods of mounting powders can be modified and combined to suit different materials. For instance, a powder in a slurry may be drawn down on a glass slide, dried, and thereafter handled in the manner of the turpentine dispersions. Different conditions will suggest different modifications of mounting methods, with consequent flexibility of application of the electron microscope to the field of powdered substances.

ACKNOWLEDGMENT

The authors express their thanks to Interchemical Corporation for permission to publish this paper; to Henry Green for his valuable advice; and to Vincent Salines, Olive Hodgson, and Gertrude Pfeifer for their able assistance

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Calculation of Specific Dispersion of Pure Hydrocarbons and Petroleum Fractions—Correction

Above is Figure 1 as it should have appeared in connection with the article by M. R. Lipkin and C. C. Martin entitled "Calculation of Specific Dispersion of Pure Hydrocarbons and

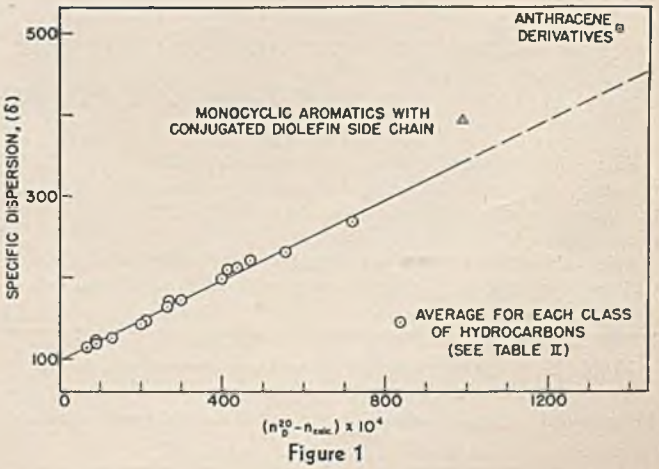

Figure 1

Petroleum Fractions" [Ind. Eng. Chem., Anal. Ed., 18, 433 (1946)]. As will be seen, the point for anthracene derivatives on the graph agrees with the numerical data for this point as given in Table I, page 433, of the article. This was not true in the figure published with the article, owing to an unintentional alteration in the process of preparing the engraving.

The Editors

NOTE ON ANALYTICAL PROCEDURES

Analysis of Boron Trifluoride in Organic Liquids (Ethers)

S. L. WALTERS AND R. R. MILLER

Naval Research Laboratory, Office of Research and Inventions, Anacostia Station, Washington, D. C.

CONSIDERABLE difficulty is encountered in analyzing boron trifluoride in organic liquids by conventional methods for boron and fluorine. This is due in part to the fact that the hydrolysis of boron trifluoride gives not only a mixture of boric and hydrofluoric acids, but some fluoboric acid, HBF_4, in addition. Several methods of decomposition have been used. Pflaum and Wenzke (*2*) first made a fusion in a Parr sulfur bomb and determined boron and fluorine in the products of fusion. Bowlus and Nieuwland (*1*) heated with fuming nitric acid in a closed tube to decompose the sample. Vaughn and Nieuwland (*3*) determined fluorine in organic compounds by the use of liquid ammonia and sodium, but did not report any results for boron.

A problem in this laboratory required the routine determination of boron trifluoride dissolved in various low-boiling ethers. These solutions contained no nonvolatile matter. A convenient method of analysis was developed based on the reaction of boron trifluoride with sodium fluoride to form sodium fluoborate. This compound is stable and can be heated to 200° to 300° C. with no appreciable decomposition; therefore, the method should apply to organic solvents with rather high boiling points.

PROCEDURE

About 5 grams of anhydrous sodium fluoride were placed in a 100-ml. wide-mouthed extraction flask, and the flask was stoppered and weighed. Approximately 2 grams of the sample were placed in a tared 15 × 40 mm. weighing bottle and weighed. Samples were conveniently transferred with a small hypodermic syringe and in a dry-box, since boron trifluoride compounds are very hygroscopic.

The weighing bottle and sample were placed in the flask with tweezers, the stopper was removed, and the flask was attached to a reflux condenser. After refluxing for about 30 minutes, the flask was removed and the liquid evaporated on a hot plate. The flask was stoppered, cooled, and weighed. The gain in weight minus the tare of the weighing bottle gave the weight of boron trifluoride in the sample.

Analysis by this method was found to be reliable and accurate within 0.5%.

Three analyses on a single sample of boron trifluoride ethyl etherate gave 48.3, 48.1, and 48.5% boron trifluoride; the theoretical boron trifluoride content was 47.8%. Additional work on mixed butyl and ethyl etherates indicated satisfactory results with this method.

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CORRESPONDENCE

Effect of Acetic Acid on the Spectrophotometric Estimation of Gossypol in Aqueous Alcoholic Solution

SIR: Since publication of an improved rapid method for the determination of gossypol (*1*) it has been found with different lots of alcohol that the intensity of the color and the precision of the analyses depend upon the acidity of the alcohol used.

Very satisfactory results are obtained when sufficient acetic acid (0.1 to 0.2 ml. per liter) is present to give the 95% alcohol an acidity of 0.002 to 0.004 *N*. The upper limit of acidity is not critical, since as much as 6 ml. of glacial acetic acid per liter may be used.

Table I. Effect of Acetic Acid on Development of Color of Dianilino Gossypol in Aqueous Alcohol

[As measured by per cent transmittance[a] at 445 mμ (6 determinations each concentration)]

	Per Cent Transmittance					
	0.025 Mg. of Gossypol per 25 Ml.		0.100 Mg. of Gossypol per 25 Ml.		0.175 Mg. of Gossypol per 25 Ml.	
	No AcOH	0.2 Ml. of AcOH per liter	No AcOH	0.2 Ml. of AcOH per liter	No AcOH	0.2 Ml. of AcOH per liter
Lowest	77.4	74.0	35.7	30.4	15.6	12.4
Highest	92.0	76.4	47.5	31.6	24.5	13.0
Mean	82.38	74.96	41.03	30.93	22.32	12.96

[a] Coleman double monochrometer spectrophotometer.

Table I shows that the addition of only 0.2 ml. of glacial acetic acid per liter of alcohol increases the intensity of the color and greatly reduces the variation between replicate determinations. The acid added increased the normality from 0.0003 to 0.0037. The equation for the concentration–log transmittance curve for all data with acidified alcohol is: mg. of gossypol in 25 ml. $= \frac{2 - \log T}{5.017}$. This equation agrees with that previously found and published.

It is recommended that in the determination of gossypol by the previously published method (*1*) the alcohol used for the extraction be adjusted to an acidity between 0.002 and 0.004 *N* with acetic acid.

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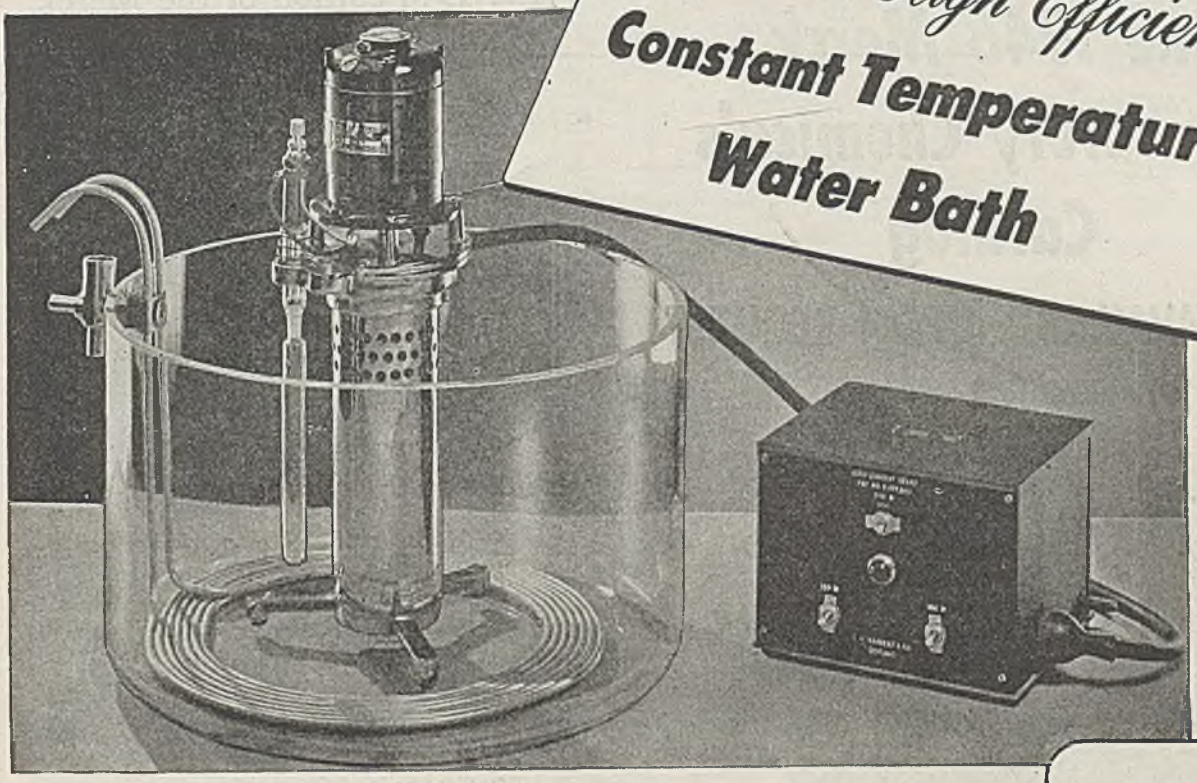
F. H. SMITH

Department of Animal Industry
North Carolina Agricultural Experiment Station
Raleigh, N. C.

Sargent High Efficiency Constant Temperature Water Bath



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Of the total surface area of the bath which is approximately 190 square inches, only 15 square inches is occupied by control equipment. All remaining space is entirely free for insertion of vessels.

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A cooling coil is supplied and should be used when operating temperatures are less than 3°C above average room temperature. A compact constant water level device attached to the wall of the container permits rapid overflow and replacement of water displaced by immersed apparatus.

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SCIENTIFIC LABORATORY SUPPLIES

Current Developments in

INSTRUMENTATION IN ANALYSIS

Discussed by Ralph H. Müller

SEPTEMBER 16 to 20 represents an important landmark in American instrumentation. The first National Instrument Conference and Exhibit was held in Pittsburgh with the theme "Instrumentation for Tomorrow", a slogan which was characteristic of many of the papers and particularly prophetic in the choice of exhibits. The Instrument Society of America, under whose auspices this meeting was held, accomplished many important things, among the most commendable of which was the election of C. Owen Fairchild as president. The principal events of this meeting are described in *Chemical and Engineering News* (Oct. 10, 1946), and the complete program, abstracts of papers, and descriptive list of the exhibits are to be found in *Instruments* (Vol. 19, No. 9, Program Supplement).

Although the efforts of many individuals were responsible for the success of this meeting, it is evident to anyone who has followed the slow and steady development of instrumentation in this country, that its principal protagonist has been the editor of *Instruments*, Major M. F. Behár. It is relatively easy for one to view the passing scene, to comment on points of interest, and to trace the development of any subject in its proper chronological order. For nearly a quarter of a century, Behár has unerringly caught the significance of each major development and sensed the implications, beyond mere technical details. He has witnessed the acceptance of many of his earlier predictions and pleas and has graciously chosen to forget the sneers or catcalls which accompanied their proposal. He has long insisted that instrumentation is a profession. This meeting has demonstrated that all the factors essential for a true profession are in evidence: a common interest, a well defined set of principles and practices, a wide assortment of special skills, and a well educated and trained body of experts completely dedicated to this field.

One of the most interesting contributions to this meeting was the address of H. B. Cronshaw of London, England, who discussed "Instrument Developments in Britain". It was apparent from his remarks that America may take great pride in her supremacy in industrial instrumentation but that the attention given to the more academic and scientific aspects of instrumentation is relatively inadequate. Instrumentation in Britain is more closely related to the university, and the active research of the scientists and important developments have usually been associated with these groups rather than with industry. It is therefore not surprising that most controls and regulators to be found in that country are of American manufacture, where they are regarded with high esteem. Dr. Cronshaw seemed surprised that no equivalent of the writer's proposed Institute for Instrument Research has been established in America, whereas essentially that has been effective in England for 28 years.

Although the conference was concerned with all phases of instrumentation, there were many papers and numerous exhibits of direct interest for the analyst. For example, H. D. Middle of the General Electric Co. described "Four New Methods of Gas Analysis" which were concerned with an acoustic analyzer, a dew point recorder, a mass spectrometer, and an x-ray photometer. These developments illustrate an important trend in modern instrumentation. They involve no fundamentally new principles; yet they embody all the means for automatic compensation and recording of the results. The x-ray photometer should prove particularly useful, inasmuch as the absorption is primarily an atomic function and very slightly dependent upon the state of combination of the respective atoms. A commercially available model of the photometer was shown in the General Electric exhibit.

Computers

An important and instructive session on computing was held, at which two papers served to illustrate the more important types of computing devices. C. E. Berry of the Consolidated Engineering Corp. provided an excellent summary of electrical analog computers. An analog computer as distinguished from digital computers, of which the adding machine office computer is a familiar example, utilizes physical quantities which are introduced in magnitude proportional to the numbers which they are to represent. Thus the common slide rule uses length, as inscribed on a scale, and by relative motion along other scales provides means for a variety of mathematical operations. It was shown how addition can be carried out both by series and parallel electrical networks; multiplication by means of pairs of voltage dividers, one feeding the other; and integration and differentiation with combinations of reactive elements in conjunction with vacuum tube circuits. Numerous examples were given, illustrating intercombinations of these devices, including the computer for solving linear simultaneous equations. This instrument is familiar to many analysts. It has been particularly useful in routine mass spectrographic and infrared analyses. The possibilities of electrical analog computers are almost unlimited and the analytical instruments of the future may be expected to make extensive use of these devices.

Continuous computing mechanisms were discussed by Macon Fry of the W. L. Maxson Corp. This very complete summary of mechanical computers was largely illustrative of the complex devices used in fire-control instruments. It was shown how all the useful mathematical functions can be accommodated by differential gears, cams, Scotch yokes, and linkages and their intercombinations. These mechanical systems are characterized by very high precision and reliability, and numerous ingenious compensating schemes have been developed to supplement systems which furnish first-order approximations to the desired function. In some cases, electrical and electronic components are introduced to supplement the mechanical units, but as a rule the latter provide ultimate reference values in the computation.

A few generalizations are possible in comparing the two types of computers. The electrical are somewhat more flexible, and in some cases, particularly where the function is handled electronically, there are no speed limits. In general the cost is lower. The mechanical systems have some speed limitations, the possibility of wear, and although costs are higher, these are usually attributable to design and initial tooling costs.

Atomic Energy

An interesting symposium on "Instrumentation in Atomic Energy Research" was held jointly with the Physical Society of

Instrumentation

Pittsburgh at which the controls on a chain-reacting pile and the remote control of chemical laboratories dealing with intense radiations were discussed by E. Creuz and C. R. Schwob, respectively, both of the Carnegie Institute of Technology. F. Marshall of Westinghouse discussed the protection of personnel in this work.

The installation of instruments at Oak Ridge was perhaps the greatest of its kind in history, the best criterion of which is the statement that it contained eleven miles of control panels.

Exhibits

Among the many items which justified the slogan "Instrumentation for Tomorrow" was the transient recorder shown by the Brush Development Co. of Cleveland. This instrument is designed to record and represent graphically varied transient phenomena of 0.2 second or less. As such, it meets an urgent need for something intermediate between slow mechanical recorders and the high speed cathode ray oscillograph. The device has been described in detail [*Proc. Inst. Radio Engrs.*, **33**, 753 (1945)]. It will record phenomena having a frequency range of 0.02 to 1000 cycles per second. The transient is recorded on a loop of magnetic tape and played back synchronously every 0.1 second on an oscilloscope screen. Thus, a steady image of the transient is obtained. A high signal-to-noise ratio (40 decibels) is obtained by the use of a 10-kilocycle carrier, which is frequency-modulated by the phenomenon under observation. The recording can be obliterated by simply pressing a button, which clears the tape. This has advantages over the use of persistent screen oscillographs, which are often used for transients, and will do some jobs not easily solved by the slow decay screens.

An imposing group of new devices has been developed by the Victoreen Instrument Co. of Cleveland. In addition to an extensive line of instruments for nuclear research including Geiger-Müller counters, scaling circuits, and radiation-protection devices, the company has developed a series of miniature vacuum tubes with unusual characteristics, which promise to revolutionize many electronic techniques. Of special interest is the VX-41 electrometer tube. For many applications such as pH, photoelectric photometry, and any phenomena in which e.m.f.'s from high impedance sources are to be measured, there has been a constant demand for suitable electrometer tubes. This tube is 1.125 inches long and 0.366 inch in diameter. Its filament voltage is 1.25, filament current 10 ma., plate resistance 100,000 ohms, mutual conductance 10 microhms. These and following characteristics refer to an accelerator grid connection. The current to this grid under normal operating conditions is less than 10^{-14} ampere and the input resistance is greater than 10^{16} ohms.

Detailed characteristics and operating parameters are defined in a pamphlet by this company. Another tract on electrometer circuits and a careful study on stabilization of tubes having oxide coated cathodes are also worthy of careful study by anyone interested in electrometer tube circuits.

Other tubes in this series include the VX-32 low-mu triode, the VX-21 high voltage diode, and the VX-10 vacuum switch. An appropriate series of Hi-meg resistors has been developed for use with these tubes, the characteristics of which are also unusual. The type 249 resistor is stable and accurate over a wide range of climatic and atmospheric conditions. Some pertinent characteristic values are: maximum resistance 10^8 megohms, minimum resistance 1 megohm, temperature coefficient (neg.) 0.18% per °C.; voltage coefficient (neg.) 0.026% per volt, maximum operating temperature 50° C. These resistors are noninductive, vacuum-sealed in glass, and are treated to withstand 100% humidity.

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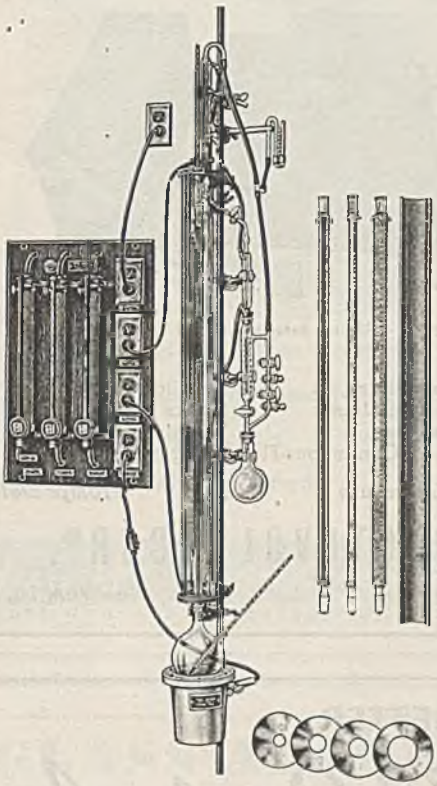

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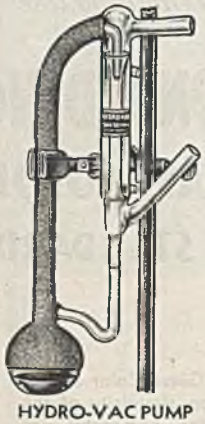

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