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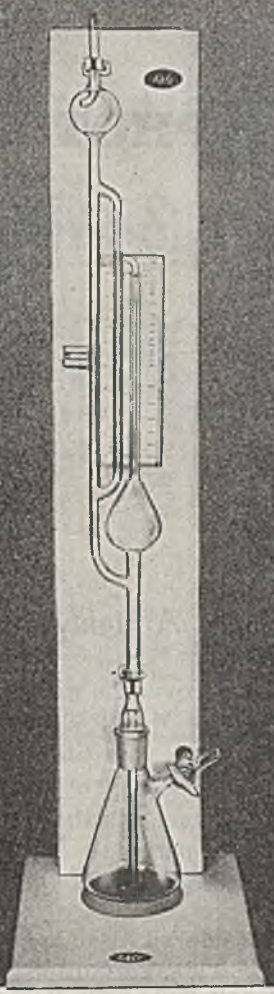

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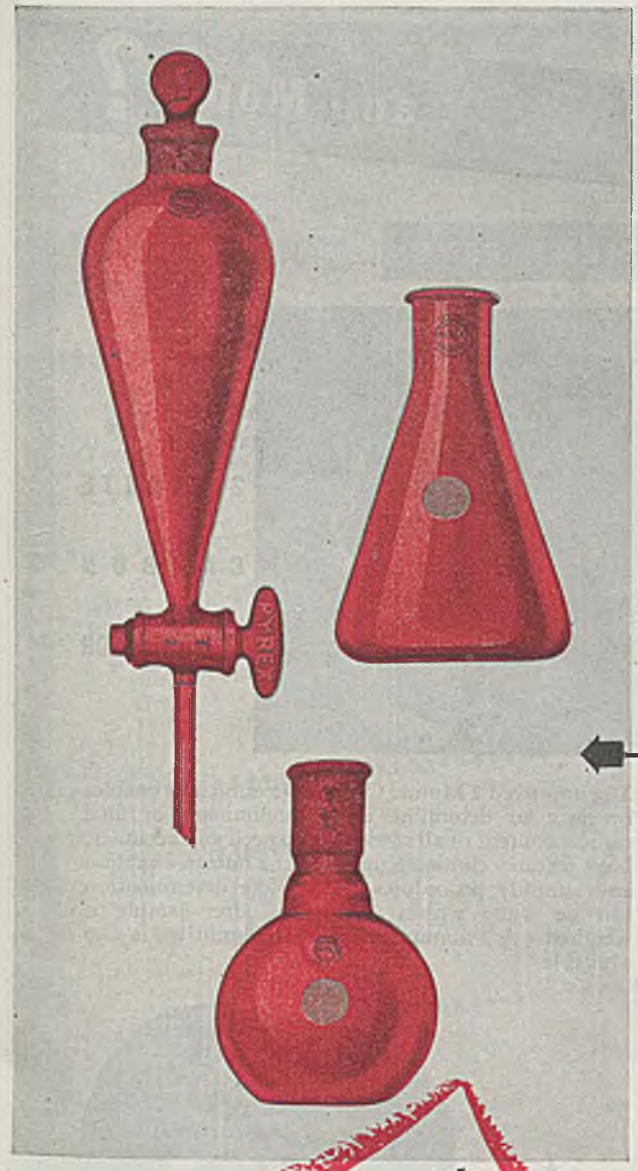

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Style Requirements

In April we discussed editorially the field which the Analytical Edition should endeavor to include in its coverage, emphasizing its desire to print more papers on evaluation of analytical results, statistical treatment of analytical data, principles and theory of analytical chemistry, and college training of personnel. The Analytical Edition is in position to print all such material promptly.

Manuscripts offered for publication differ widely, not only in scientific value but in their presentation. Some are well written, logically developed, concisely written, yet giving all necessary details. When these manuscripts are typed double space, with wide margins to allow room for directions to the printer, and are accompanied by drawings and photographs from which engravings can be made without retouching or relettering, they gladden the editor's heart.

Others, however, fail to meet these specifications, and it is to authors of such papers, as well as to those who have not previously contributed to our pages, that this editorial is directed.

To be of the greatest service the scientific facts must be presented in a clear, logical readable manner. An important point to be kept in mind in writing any article is the type of reader to be reached. Readers of the Analytical Edition are intelligent scientists, who do not need schoolbook directions for laboratory procedure; they do need to be given sufficiently explicit directions so that they can repeat the work reported, if need be.

When a scientist has work to report in a certain journal—the Analytical Edition or any other—he should familiarize himself with that journal and model his report on particularly good papers published there. A little time devoted to thinking the article through before putting pen to paper will pay dividends in producing a logical, concise, and readable presentation that avoids repetition yet gives all the facts. An outline might be set up, for instance, as (1) why the work was done, with a brief reference to previous work, properly documented, (2) a concise account of what was done, with all necessary details as to apparatus, reagents, and procedure followed, and (3) the result of the investigation and conclusions drawn.

A short abstract that can be printed at the beginning of the article is a great help to the busy reader, as it enables him to decide at once whether to read the article in full then and there, to lay it aside for later attention, or to skip it as outside his field of interest.

Too many manuscripts give the impression that the author has dictated a chronological account of what he has done in the laboratory or—worse—has asked his secretary to copy hand-written notes and then has failed to read the manuscript over and check it for accuracy. A good secretary will take care of misspelled words and punctuation, but cannot always interpret carelessly written figures or chemical terms. Footnotes are too often an indication of sloppy thinking. The material there given might better be incorporated in the text or perhaps presented in a separate note.

Literature citations should be carefully checked for accuracy and completeness. Industrial and Engineering Chemistry prints them at the end of each article, arranged alphabetically by authors, and with corresponding numbers inserted in the text at appropriate places. The abbreviations used are given in the "List of Periodicals Abstracted by *Chemical Abstracts*". The spelling and nomenclature approved by the Society's Committee on Nomenclature, Spelling, and Pronunciation are followed. This subject has been recently discussed in the introduction to the 1945 subject index to *Chemical Abstracts*. The Merriam Webster dictionary is followed as the authority for deciding on the spelling of nontechnical words.

Specifications and directions for preparing copy, including illustrations, are given in "Suggestions to Authors", copies of which will be sent on request to any who are interested. A summary is printed on page 340 of this issue.

Having a definite bearing on the question of the preparation of chemical papers is what seems to be a regrettable lack of cooperation between the departments of chemistry and of English in most of our colleges and universities. If the ability to write a clear, concise report is not developed during the college course, it is acquired only with difficulty after graduation. Is not this a subject that should be given greater consideration by our chemical educators?

Improvement of Precision by Repeated Measurements

Application to Analytical Control Methods

JOHN MANDEL[1]
Columbia University, New York, N. Y.

This article critically examines replication of measurements as a means of increasing precision. Formulas are provided for evaluating such improvement of precision in any given case and for computing confidence limits in routine analysis.

IN A previous paper (*2*) it was shown how the estimation of the precision of analytical methods can be accomplished by means of modern statistical theory. This article considers in detail some questions arising in the study of precision.

While the practice of at least duplicating every analysis is well established among analysts, the increase in precision thus brought about has seldom been considered. However, this question appears to be of considerable importance, as can be inferred from the following considerations.

In the practice of routine analysis, it is customary to obtain some indication about the precision of analytical results from the "closeness" of the replicate determinations (generally duplicates) made by the analyst. In some cases the optimistic picture thus obtained is tempered by the knowledge, gained through experience, that this closeness is very often grossly misleading. Moran (*3*) has given some attention to this matter, and has studied it experimentally on the basis of data obtained in his laboratories. Having found that the ratio of the standard deviation of single determinations to that of averages of duplicates was 1.05—appreciably less than the theoretical value $\sqrt{2}$ for the case of true random determinations—Moran (*3*) ascribes this result to the fact that "duplicate analyses made at the same time are not truly random but are rather influenced by slight variations in technique or surrounding conditions".

[1] In a population of variance σ^2, the variance of the mean of a random sample of n is σ^2/n; the corresponding standard deviation is thus $\sigma/\sqrt{n}$. The ratio mentioned above is, therefore, in the case $n = 2$, $\dfrac{\sigma}{\sigma/\sqrt{2}} = \sqrt{2} = 1.41\ldots$

Moran calls this ratio the "ratio of improvement by duplication". It seems preferable to consider the ratio of the variances, the squares of the standard deviations; then the theoretical value for duplication is simply 2; for n-fold replication it will be n. The advantage lies in the intuitiveness of the interpretation. On the other hand, the standard deviations referred to in Moran's "ratio of improvement" are "population parameters"—i.e., fixed though unknown constants characterizing the distribution of errors in the case considered. Now these quantities being unknown, we substitute for them estimates derived from the data available; but such estimates are obviously subject to fluctuations or, in statistical terminology, to "sampling errors". Thus in considering the ratio 1.05 obtained by Moran, it is well to keep in mind that a repetition of the whole experiment might have given a quite different value, perhaps 1.23, which is already much closer to the number 1.41 based on the hypothesis of randomness. For this reason it becomes desirable to infer from the data available not only the estimated ratio, but also an evaluation of the precision with which it is obtained.

This matter, among other things, is discussed in the following pages; formulas are given, based on the theory of statistics, for determining various quantities which are of interest in this connection. The calculations involved in this type of analysis of data, which will be seen to be simple and on an elementary level, are described in Section III and illustrated in Section VI by means of the data given in Moran's paper (*3*). The reader is, however, strongly urged to give careful consideration to Section I, where the chemical situation is described to which the proposed method is applicable. It cannot be expected—as it never can be in the practical application of theoretical considerations—that the conditions of Section I will be exactly fulfilled. It is rather a matter of personal judgment on the part of the chemist to decide whether the conditions in his case correspond sufficiently to the theoretical model described to permit application of the present method. Such subjective judgment can efficiently be supplemented by statistical tests of randomness, of equality of precisions, and the like (cf. *10*). While these tests are valuable in that they offer means of evaluating in an objective and consistent way a variety of important aspects connected with the precision of analytical methods, the author feels that they will in general not be necessary for the application of the method proposed in this paper, provided the analytical procedure in question is well defined in its various steps. This point is brought out more clearly in the following discussion.

I. APPLICABILITY OF THE TEST

Suppose that in order to gain quantitative information about the behavior of an analytical procedure in routine work, the research analyst devises one of the following experiments:

1. A sample, containing ν% of the considered constituent, is stored and analyzed at regular intervals, say every month, for a period of M months, by the same chemist, in N-fold replication each month. The N replicate determinations are to be performed as closely together as they are expected to be made in actual routine work. Thus M series, of N parallel determinations each, are obtained.
2. A similar experiment is performed, involving several analysts, with the understanding, however, that (*a*) every series of N replicates is performed by one analyst only; and (*b*) a random experiment is performed to select the analyst for every series. Thus if 4 analysts are available, 4 slips of paper, one of which is marked, are thoroughly mixed, after which every chemist draws one slip; whoever draws the marked slip performs the series of analyses for this month, regardless of possible repetitions due to chance.
3. The type of experiment is modified, in that M laboratories are selected at random (in so far as this is feasible, considering their suitability for the given type of analysis, etc.). In this case it may be superfluous to space the M series in time: M samples of the same material are sent simultaneously to the various laboratories.
4. Any similar type of experiment can be considered, with the twofold restriction, however, that (*a*) only one analyst makes the N replicates in a given series; and (*b*) the series are more or less random with respect to the factor which is considered to cause marked discrepancies between series, such as laboratory, chemist, weather conditions, etc.

Under these conditions the following assumptions are made

A. To every series, say the ith, corresponds a "population mean" μ_i (which may be interpreted as the mean of all the analyses, in infinite number, which could be performed under the same stability of conditions as prevailed for the N analyses actually performed in this series). As pointed out by Moran, one should not in general expect this μ_i to be equal to ν, even in the case of a very accurate method of analysis, since slight variations in the surrounding conditions will influence similarly all the results of the series.

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B. All series are performed with equal precision. This does not mean, of course, that the estimate of precision (*2*) is to be identical for all M series; it rather implies that no significant difference exists between these estimates. As pointed out previously, this assumption, the validity of which might be questioned by the reader, is believed by the author to hold in general, provided the analytical method under study is described in sufficient detail—i.e., to such an extent that no wide divergence in pH values, temperatures, volumes used, etc., can arise—and provided the analysts are all reasonably competent.

C. Within each series the various results are distributed according to the "normal" ("gaussian") law of errors (cf. any elementary textbook on statistics, e.g., *1*). There can hardly be a question that this is at least approximatively true for most analytical results.

D. The values μ_i themselves are considered as a random sample of a normally distributed population corresponding to the totality of laboratories in a given region, or to the totality of replicate series in a given laboratory, etc., according to the type of experiment as discussed above. The question whether ν is the true mean of all these μ_i is of no concern to us in this connection, since all we are interested in here is the precision of the method.

Let ν' represent the population mean value of all the μ_i and let ν be the "true value" (or the theoretical value in the case of a synthetic sample); if $\nu' = \nu$ the method is accurate; if $\nu' \neq \nu$, $\nu' - \nu$ is the systematic error of the method (cf. *2*).

Here again there may arise serious doubt in the reader's mind concerning the validity of this assumption. However, if one is willing not to generalize beyond reasonable limits the validity of the results obtained by the statistical analysis, there is really no great danger of going far astray by accepting these results. In most cases, common sense will tell where one should draw the limit.

II. PURPOSE OF THE TEST

Our first aim is to estimate what Moran calls the "ratio of improvement" by N-fold replication, or rather its square, which we shall call the "coefficient of improvement by N-fold replication" and denote by the symbol $[CI]_N$. Thus:

$$[CI]_N = \frac{\text{variance of a single determination}}{\text{variance of the average of } N \text{ parallel determinations}}$$

We cannot calculate this quantity, since it contains unknown parameters; therefore we will supplement our sample estimate of it, which we will denote by $Est[CI]_N$, by a lower limit, L_1, and an upper limit, L_2, such that we may have considerable confidence that the interval L_1L_2 includes the true value $[CI]_N$ (*2, 4, 7, 8*).

It is evident that the larger the variability between series, the lower will be $[CI]_N$. As a matter of fact, no amount of replication, however great, within a series, can compensate completely for the loss of precision due to the systematic error that affects the whole series. On the other hand, a single blank will completely eliminate this bias. (Blank is here taken to include the concept of a "control" run on a sample of known composition; the value of the blank is then the difference between the result obtained and the true value.) Therefore it will be of interest to consider two other quantities: (*a*) the coefficient of improvement due to infinite replication (within a series), denoted by $[CI]_\infty$; and (*b*) a coefficient of improvement obtained by running n replicates simultaneously with n' blanks and subtracting the average "blank" from the average "sample". Let us represent the latter coefficient of improvement, which like the others is taken with respect to the precision of a single determination, by the symbol $[CI]_n^{n'}$.

The best choice of n and n', for a given total $n + n'$ of analyses, depends on the ratio of precisions with which the blank and the sample, respectively, are determined: If it appears from the type of analysis that the absolute errors are of the same order of magnitude for sample and blank, the best allocation is $n = n'$. But if there is reason to believe that the absolute errors for the sample are c times as great as those for the blank, the highest precision is obtained by taking $n/n' = c$.

In order to evaluate the practical significance of the calculations described below, it is necessary to distinguish between the task of the research analyst and that of the routine chemist. While for the former any parameter describing the precision of his method will be of value, the latter, on the contrary, will be interested in any new concept only in so far as it may help him in locating in the best possible way the unknown value he is asked to determine by analysis. What contribution our statistical analysis can make to this end is being discussed in Section V. A mathematical justification of the statistical test procedure is given in Section VII.

III. STATISTICAL TEST PROCEDURE

Let there be M series of N replicates each. We can represent them as follows:

1st series: $x_{11}\ x_{12}\ x_{13} \ldots x_{1N}$
2d series: $x_{21}\ x_{22}\ x_{23} \ldots x_{2N}$
. . . . . .
. . . . . .
Mth series: $x_{M1}\ x_{M2}\ x_{M3} \ldots x_{MN}$

In general $x_{i\alpha}$ will denote the αth determination in the ith series.

1. Subtract from every one of these values a same constant, chosen as a round number near a roughly estimated mean of the x-values, and multiply the differences thus obtained by a same factor, say K, chosen in such a way as to make the resulting values small, with at most one decimal place. For example, from 18.22, 18.32, 18.175. 18.26, etc., we obtain first: 0.02, 0.12, −0.025, 0.06, etc., and finally: 2, 12, −2.5, 6, etc. In this case $K = 100$.

Suppose that we thus obtain the values $y_{i\alpha}$, arranged according to the same scheme:

1st series: $y_{11}\ y_{12}\ y_{13} \ldots y_{1N}$
2d series: $y_{21}\ y_{22}\ y_{23} \ldots y_{2N}$
. . . . . .
. . . . . .
Mth series: $y_{M1}\ y_{M2}\ y_{M3} \ldots y_{MN}$

2. Now compute:

a. The sum, T_i, of the elements y in the ith series (row); take the square $(T_i)^2$ of this sum. Do this for all rows (all i) and sum all $(T_i)^2$, thus obtaining: $\sum_{i=1}^{M} (T_i)^2$.

b. The global sum of all y's, denoted by T (obviously $T = \sum_{i=1}^{M} T_i$). Take the square, T^2, of this global sum.

c. The sum of the squares $y_{i\alpha}^2$ of all individual y-values—i.e., $\sum_{i=1}^{M} \sum_{\alpha=1}^{N} y_{i\alpha}^2$.

3. From these three quantities, $\sum_i (T_i)^2$, T^2, and $\sum_i \sum_\alpha y_{i\alpha}^2$, derive the following:

$$A = \left[\sum_i \sum_\alpha y_{i\alpha}^2\right] - \frac{1}{N}\left[\sum_i (T_i)^2\right] \tag{1}$$

$$B = \frac{1}{N}\left[\sum_i (T_i)^2\right] - \frac{1}{MN}\left[T\right]^2 \tag{2}$$

$$D = \frac{A}{B}\frac{M-1}{M} \tag{3}$$

4. By entering, for the proper values of M and N, Tables I and II, two values, F_0 and F_0', are found, respectively. Compute:

$$D_1 = \frac{D}{F_0} \tag{4}$$

$$D_2 = DF_0' \tag{5}$$

Table I. Values of F_0[a]

M \ N	2	3	4	5	6	7	8	9	10
4	28.7	27.5	27.0	26.8	26.7	26.6	26.5	26.5	26.4
5	15.5	14.5	14.2	14.0	13.9	13.8	13.8	13.7	13.7
6	10.7	9.9	9.6	9.5	9.4	9.3	9.3	9.3	9.2
8	6.8	6.3	6.1	6.0	5.9	5.9	5.8	5.8	5.8
10	5.3	4.8	4.6	4.6	4.5	4.5	4.5	4.4	4.4
12	4.4	4.0	3.9	3.8	3.8	3.7	3.7	3.7	3.7
15	3.7	3.3	3.2	3.2	3.1	3.1	3.1	3.1	3.1
20	3.0	2.8	2.7	2.6	2.6	2.6	2.6	2.6	2.6
30	2.4	2.2	2.2	2.1	2.1	2.1	2.1	2.1	2.1

[a] 1% critical point of F for $M(N-1)$ and $M-1$ degrees of freedom.

Table II. Values of F_0'[a]

M \ N	2	3	4	5	6	7	8	9	10
4	16.7	7.6	6.0	5.3	4.9	4.7	4.6	4.5	4.4
5	11.4	6.0	4.9	4.4	4.2	4.0	3.9	3.8	3.8
6	8.8	5.1	4.3	3.9	3.7	3.6	3.5	3.4	3.4
8	6.2	4.0	3.5	3.3	3.1	3.0	3.0	2.9	2.9
10	5.0	3.5	3.1	2.9	2.8	2.7	2.7	2.6	2.6
12	4.2	3.1	2.8	2.6	2.6	2.5	2.5	2.4	2.4
15	3.6	2.7	2.5	2.4	2.3	2.3	2.3	2.2	2.2
20	3.0	2.4	2.2	2.1	2.1	2.0	2.0	2.0	2.0
30	2.4	2.0	2.0	1.9	1.8	1.8	1.8	1.8	1.8

[a] 1% critical point of F for $M-1$ and $M(N-1)$ degrees of freedom.

5. From D, D_1, and D_2 we now obtain:

a.
$$Est\ [CI]_N = 1 + D \tag{6}$$

and the corresponding confidence limits [if $L_2 > N$, take $L_2 = N$ (cf. section IV)]:

$$L_1 = 1 + D_1 \tag{6a}$$

$$L_2 = 1 + D_2 \tag{6b}$$

b.
$$Est\ [CI]_\infty = \frac{(N-1)(1+D)}{N-(1+D)} \tag{7}$$

and the corresponding confidence limits:

$$L_1' = \frac{(N-1)L_1}{N-L_1} \tag{7a}$$

$$L_2' = \frac{(N-1)L_2}{N-L_2} \tag{7b}$$

c. For any n and n' one can also calculate:

$$Est\ [CI]_n^{n'} = \frac{1}{R}\,\frac{N-1}{N}\,\frac{1+D}{D} \tag{8}$$

where

$$R = \frac{1}{n} + \frac{1}{n'c^2} \tag{8c}$$

[c is the ratio of the orders of magnitude of the absolute errors in the sample determination and in the blank. In general, $c = 1$ (cf. Section II)]

and the corresponding confidence limits:

$$L_1'' = \frac{1}{R}\,\frac{N-1}{N}\,\frac{L_2}{L_2-1} \tag{8a}$$

$$L_2'' = \frac{1}{R}\,\frac{N-1}{N}\,\frac{L_1}{L_1-1} \tag{8b}$$

IV. INTERPRETATION FROM THE RESEARCH ANALYST'S POINT OF VIEW

Consider the scheme of analyses of a sample as shown in the previous section, but suppose that the number of rows is considerably extended—i.e., that a very great number of series of N replicates is available. Now suppose that we compare the two following values:

1. The mean value for a given row (N simultaneous determinations), this row being chosen at random.
2. The mean value obtained by choosing N rows at random and selecting at random one value in each of these rows; then taking the average of these N numbers.

Both these means result from N observations; which is the better from the precision point of view? It is easily seen that on account of the bias which may affect all values of a same row, we have a better chance of obtaining a precise mean value by taking the second average than by taking the first. We are therefore justified in expecting, for a same number, N, of determinations, a higher average precision in the second case than in the first; or to obtain the same precision by using, in the second case, a number of determinations N' smaller than N. The coefficient of improvement $[CI]_N$ is precisely this number, N'. Thus from the precision point of view, the mean value of N determinations made in parallel—the usual case in practice—is equivalent to only N' ($\leqslant N$) truly random determinations. As pointed out previously, $[CI]_N$—i.e., N'—is a "population parameter". Formula 6 gives but an estimate of this quantity, subject to sampling fluctuations. Here the confidence limits, L_1 and L_2, will prove of value: the confidence coefficient is 0.98, which means that the probability is 0.98 that the interval L_1L_2 covers the true value $[CI]_N$ (cf. *2*).

It is also obvious that the range of possible values for $[CI]_N$ extends from 1 to N. If, therefore, as may well happen, the interval L_1L_2 extends beyond this range, the outside values must be discarded.

Finally, the value 0.98 given for the confidence coefficient must not be taken in too absolute a sense; indeed, any departure from the ideal conditions as outlined in Section I will affect the value of this probability.

Similar considerations apply to $[CI]_\infty$ and $[CI]_n^{n'}$. Whereas for $[CI]_\infty$ a finite upper confidence limit will in general be obtained (unless $L_2 = N$), the value for $[CI]_n^{n'}$ can, of course, become arbitrarily great for n and n' sufficiently large. The reason for this is apparent: $[CI]_n^{n'}$ is the ratio of the precision (inverse of the variance) of a value corrected for bias to that of a single determination subject to a systematic error; by increasing n and n' the precision of the corrected value necessarily approaches the ideal—i.e., ∞—since the random errors approach 0 and no systematic error vitiates the result. The interest of Formulas 7 and 8 lies in the comparison of their numerical values for small values of n and n' (Section VI).

V. ROUTINE ANALYST'S POINT OF VIEW

Let us suppose that an analytical procedure has been investigated by the method described in this paper. A routine analyst now uses the same analytical procedure for N replicate determinations on an unknown sample, and obtains an average value (arithmetic mean of his N values) which we will denote by Z.

The question confronting him is to determine an interval in which it is reasonable to assume that the true value for his sample lies, allowing for all the imperfections which a study of precision has revealed.

Formulas 9 and 10 give, respectively, the lower limit Z_1 and the upper limit Z_2 for such an interval.

$$Z_1 = Z - \frac{t_{(M)}}{K}\sqrt{\frac{B}{N(M-1)}} \tag{9}$$

$$Z_2 = Z + \frac{t_{(M)}}{K}\sqrt{\frac{B}{N(M-1)}} \qquad (10)$$

where B is given by Formula 2, while K is the factor defined in Section III. For $t_{(M)}$ the first or the second value found in Table III for the proper value of M is taken, according as one wishes a 99% or a 95% confidence coefficient—i.e., according as the probability be 0.99 or 0.95 that the interval Z_1Z_2 covers the true value. The latter statement is true only if the analytical method considered is not affected by a systematic error. If such an error should be present and its value known, this value is subtracted from both Z_1 and Z_2 in order to give a correct interval.

The application of Formulas 9 and 10 requires that the number of parallel determinations made by the routine analyst be precisely the number N of replicates in each of the M series of determinations which were made the basis for the statistical study of the analytical method. If the routine analyst made a number N_0 of replicate determinations ($N_0 \neq N$), he cannot use these formulas; in this case a fair approximation for a 99 or 95% confidence interval may be obtained by applying Formulas 11 and 12, provided that the number $M(N-1)$ be fairly large, say not less than 30. Since this number is entirely the choice of the research analyst, it is reasonable to recommend that in the application of the method proposed in this paper, the experiment be arranged so as to make $M(N-1) \geqslant 30$.

On the other hand it can be seen from Table III that for a value of $M < 5$, even the 95% value of $t_{(M)}$ is fairly large, with the result that the corresponding confidence interval is excessively long. In Table IV are offered a few suggestions for the choice of M and N; evidently a higher precision is obtained by increasing these numbers. More advantage is gained by increasing M than by taking N large.

The formulas referred to above are:

$$Z_1' = Z - \frac{t_{(M)}}{K}\sqrt{V'} \qquad (11)$$

$$Z_2' = Z + \frac{t_{(M)}}{K}\sqrt{V'} \qquad (12)$$

where V' is given by:

$$V' = \frac{B}{N(M-1)}\left\{1 + \frac{D}{N-1}\left(\frac{N}{N_0} - 1\right)\right\} \qquad (13)$$

If, on the other hand, the routine analyst had made n_0 blanks in addition to his N_0 sample determinations, and if Z now represents the difference between average "sample" and average "blank", then Formulas 14 and 15 are correct, even for small values of $M(N-1)$:

$$Z_1'' = Z - \frac{t_{(M_0)}}{K}\sqrt{V''} \qquad (14)$$

$$Z_2'' = Z + \frac{t_{(M_0)}}{K}\sqrt{V''} \qquad (15)$$

where V'' is given by:

$$V'' = \left(\frac{1}{N_0} + \frac{1}{n_0c^2}\right)\frac{A}{M(N-1)} \qquad (16)$$

and

$$M_0 = 1 + M(N-1) \qquad (16a)$$

(If this value of M_0 is not found in Table III, a sufficient approximation will be obtained by interpolation.)

Here again, for a fixed value of $N_0 + n_0$, the smallest value of V'', and consequently the smallest confidence interval and highest precision, are obtained when $N_0 = n_0c$.

Table III. Values of $t_{(M)}$[a]

M	4	5	6	8	10	12	15	20	30	50	>50
$t_{(M)}$ (99%)	5.84	4.60	4.03	3.50	3.25	3.11	2.98	2.86	2.76	2.68	2.58
$t_{(M)}$ (95%)	3.18	2.78	2.57	2.36	2.26	2.20	2.14	2.09	2.04	2.01	1.96

[a] For use with Formulas 14 and 15, replace M in this table by M_0, where M_0 is given by 16a. Interpolate if necessary.

Table IV. Choice of M and N

M	5	6	8	10	12	15	20	30	>30
N	7	6	5	4	4	3	3	2	2

VI. NUMERICAL ILLUSTRATION

In Table VI the simple calculations described in Section III are applied to Moran's data, reproduced in Table V. Not more than 15 to 20 minutes of simple arithmetic, using an ordinary slide rule and perhaps a table of squares, are required. Considering the time usually devoted to the purely experimental work, it is therefore not unreasonable to recommend those few calculations, which lead to a much more objective appreciation of precision than can be gained from mere visual inspection or even by graphical methods.

In order to show this more clearly, the method was also applied to another example found in the recent literature (*9*).

Williams and Haines, in presenting a new determination of sodium in potassium hydroxide, studied the precision of their method under routine conditions by making duplicate deter-

Table V. Precision of Determination of Specific Gravity of CCl_4 under Routine Conditions

Jan.	1.5850	1.5851	July	1.5849	1.5848
Feb.	1.5848	1.5850	Aug.	1.5854	1.5854
March	1.5846	1.5846	Sept.	1.5852	1.5853
April	1.5851	1.5851	Oct.	1.5847	1.5851
May	1.5853	1.5853	Nov.	1.5850	1.5850
June	1.5849	1.5850	Dec.	1.5849	1.5851

Table VI. Estimation of $[CI]_2$, $[CI]_\infty$, and $[CI]_n^a$ for Specific Gravity Determination of CCl_4

Subtracting 1.5850 from all values and taking $K = 10{,}000$

Month	y_{i1}	y_{i2}	T_i	T_i^2	y_{i1}^2	y_{i2}^2	$y_{i1}^2 + y_{i2}^2$
Jan.	0	1	1	1	0	1	1
Feb.	−2	0	−2	4	4	0	4
Mar.	−4	−4	−8	64	16	16	32
Apr.	1	1	2	4	1	1	2
May	3	3	6	36	9	9	18
June	−1	0	−1	1	1	0	1
July	−1	−2	−3	9	1	4	5
Aug.	4	4	8	64	16	16	32
Sept.	2	3	5	25	4	9	13
Oct.	−3	1	−2	4	9	1	10
Nov.	0	0	0	0	0	0	0
Dec.	−1	1	0	0	1	1	2

$T = 6$

$T^2 = 36$ $\quad \sum_i T_i^2 = 212$ $\quad \sum_i \sum_\alpha y_{i\alpha}^2 = 120$

$M = 12$; $N = 2$; hence (cf. Tables I and II): $F_0 = 4.4$ and $F_0' = 4.2$

$A = 120 - (212/2) = 14$ $\quad B = (212/2) - (36/24) = 104.5$

Hence: $D = (14/104.5) \times (11/12) = 0.123$

$D_1 = 0.123/4.4 = 0.03$ $\quad D_2 = (0.123) \times (4.2) = 0.52$

Thus: $Est[CI]_2 = 1 + 0.12 = 1.12$

$L_1 = 1 + 0.03 = 1.03$ $\quad L_2 = 1 + 0.52 = 1.52$

$$Est\,[CI]_\infty = \frac{1 + 0.123}{2 - (1 + 0.123)} = 1.27$$

$L_1' = 1.03/(2 - 1.03) = 1.06$ $\quad L_2' = 1.52/(2 - 1.52) = 3.16$

Assuming $c = 1$, we further obtain:

$$Est\,[CI]_1^1 = \frac{1}{2}\frac{1}{2}\frac{1.123}{0.123} = 2.28$$

$$L_1'' = \frac{1}{2}\frac{1}{2}\frac{1.52}{0.52} = 0.73 \qquad L_2'' = \frac{1}{2}\frac{1}{2}\frac{1.03}{0.03} = 8.6$$

and likewise:

$Est\,[CI]_2^2 = 4.56$ with the confidence limits 1.46 and 17.3.

minations every month over a period of 20 months. The application of the author's formulas to these data (omitting the second of the three determinations made in January, 1942) gives: $M = 20$; $N = 2$; for $K = 1000$, we find $B = 613.25$; $D = 0.786$; $D_1 = 0.26$; $D_2 = 2.32$. Hence: $Est[CI]_2 = 1.79$; $L_1 = 1.26$; $L_2 = 3.32$.

Since of necessity $L_2 \leqslant 2$ (cf. Section IV), we take $L_2 = 2$.

We further find: $Est[CI]_\infty = 8.5$; $L_1' = 1.70$ and $L_2' = \infty$; and—assuming that a blank is made with the same (absolute) precision as a "sample"—$Est[CI]_1' = 0.57$; $L_1'' = 0.50$ and $L_2'' = 1.21$.

A rather striking difference appears when these values are compared with the results of Table VI: there little advantage was gained by infinite replication (a result consistent with the low $[CI]_2$), while only two blanks run in parallel with duplicate determinations resulted in a very definite improvement in precision. (Obviously, infinite replication is a purely theoretical concept. From a practical point of view it is to be considered as the best that can be obtained by running a very great number of replicates under nearly constant conditions.) In the case of the sodium determination, on the contrary, $[CI]_2$ is rather high: replication therefore improves the precision considerably. The running of a blank in the latter case is justified only inasmuch as it improves the accuracy of the method—i.e., inasmuch as it eliminates a constant systematic error inherent in the method—but in contradistinction to the case exemplified in Table VI, a blank made once for all is then perfectly satisfactory.

VII. UNDERLYING THEORY

Referring to the scheme of x-values in Section III, we find for the estimate of the variance σ^2 "within" rows (*4, 5, 6*):

$$\sum_{i=1}^{M}\sum_{\alpha=1}^{N}(x_{i\alpha} - x_i)^2/M(N-1)$$

where x_i is the mean of the ith row. The variability of x_i itself is composed of two parts: the variance σ'^2 of the "true" means of rows and, in addition, the variability resulting from its being a sample mean of N values of variance σ^2—i.e., σ^2/N. Thus the expression $\sum_{i=1}^{M}(x_i - \bar{x})^2/(M-1)$, in which $\bar{x}$ is the grand average of the x-values, is an estimate for $\sigma'^2 + \sigma^2/N$.

From Formulas 1 and 2 it can easily be seen that the two statistics just mentioned are equal, respectively, to $\left(\frac{A}{K^2}\right)\Big/ M(N-1)$ and $\left(\frac{B}{NK^2}\right)\Big/(M-1)$. These two estimates being statistically independent (*2, 4, 8*), the ratio

$$\left[\frac{A/K^2M(N-1)}{\sigma^2}\right]\Big/\left[\frac{B/NK^2(M-1)}{\sigma'^2 + \sigma^2/N}\right]$$

has the F distribution (*2, 4, 5, 6*) with $M(N-1)$ and $(M-1)$ degrees of freedom. [An exact formulation of the theorem involved in this statement will be found in Example 1, on page 115, of (*8*).] Hence:

$$\left(\frac{\sigma'^2}{\sigma^2} + \frac{1}{N}\right)\frac{A}{B}\frac{N(M-1)}{M(N-1)} = F$$

Let $\frac{\sigma'^2}{\sigma^2} = \varphi^2$; using Formula 3 we obtain:

$$F = \left(\varphi^2 + \frac{1}{N}\right) D \frac{N}{N-1} \tag{17}$$

Now, by definition

$$[CI]_N = \frac{\sigma'^2 + \sigma^2}{\sigma'^2 + \sigma^2/N} = \frac{\varphi^2 + 1}{\varphi^2 + \frac{1}{N}} \tag{18}$$

$$[CI]_\infty = \frac{\sigma'^2 + \sigma^2}{\sigma'^2 + \frac{\sigma^2}{\infty}} = \frac{\sigma'^2 + \sigma^2}{\sigma'^2} = 1 + \frac{1}{\varphi^2} \tag{19}$$

$$[CI]_{n'}^{n} = \frac{\sigma'^2 + \sigma^2}{\sigma^2\left[\frac{1}{n} + \frac{1}{n'c^2}\right]} = \frac{1}{\frac{1}{n} + \frac{1}{n'c^2}}(\varphi^2 + 1) = \frac{1}{R}(\varphi^2 + 1) \tag{20}$$

Solving 18 for φ^2, we obtain:

$$\varphi^2 = \frac{1 - [CI]_N/N}{[CI]_N - 1}$$

Hence:

$$\varphi^2 + \frac{1}{N} = \frac{N-1}{N}\frac{1}{[CI]_N - 1} \tag{21}$$

Substitution of 21 in 17 yields:

$$F = \frac{D}{[CI]_N - 1} \tag{22}$$

Now F_0 is the 1% tabular value of the F-statistic (*4, 5, 6*) for $M(N-1)$ and $(M-1)$ degrees of freedom, while F_0' is the 1% tabular value for $(M-1)$ and $M(N-1)$ degrees of freedom. Therefore lower and upper limits for $[CI]_N$, for a 98% confidence coefficient (*2, 4, 8*), are obtained by solving for L_1 and L_2 the equations

$$F_0 = \frac{D}{L_1 - 1} \quad \text{and} \quad \frac{1}{F_0'} = \frac{D}{L_2 - 1}$$

which yield immediately Formulas 6a and 6b, by means of 4 and 5.

A point-estimate (*1, 4, 7, 8*) for $[CI]_N$ is obtained by making $F = 1$ in Formula 22; hence 6.

The groups of Formulas 7 and 8 are similarly obtained by solving, respectively, Equations 19 and 20 for φ^2 and substituting in 17.

The formulas of Section V are immediate applications of "Student's t distribution" (*1, 2, 4, 8*): $t_{(M)}$ is the tabular value for $M-1$ degrees of freedom on either the 1% or the 5% level of significance (*2, 4, 6, 8*). In Formulas 9 and 10 the estimate of the variance is $B/K^2N(M-1)$, corresponding to $\sigma'^2 + \frac{\sigma^2}{N}$.

Formula 13 provides an estimate of the variance $\sigma'^2 + \frac{\sigma^2}{N_0}$, but assumes that σ^2 is exactly equal to $A/K^2M(N-1)$. [It is for this reason that we assumed, in this case, the number of degrees of freedom $M(N-1)$ sufficiently great.] Formula 13 then easily follows from the identity $\sigma'^2 + \frac{\sigma^2}{N_0} = \left(\sigma'^2 + \frac{\sigma^2}{N}\right) + \sigma^2\left(\frac{1}{N_0} - \frac{1}{N}\right)$, remembering that the estimate of $\sigma'^2 + \frac{\sigma^2}{N}$ is $B/K^2N(M-1)$ and introducing 3.

Finally, Formulas 14 and 15 necessitate the variance of $\bar{x}_{N_0} - \bar{b}_{n_0}$ ($\bar{x}_{N_0}$ is the average of N_0 determinations of x, while $\bar{b}_{n_0}$ is the average of n_0 "blanks"), which is $\sigma^2\left(\frac{1}{N_0} + \frac{1}{n_0c^2}\right)$, the estimate of which is V'', as given by 16. Here the number of degrees of freedom is $M(N-1)$; thus $M_0 - 1 = M(N-1)$; hence 16a

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Determining Rates of Deoxidation of Iron Oxide Materials in Reducing Gases

Loss-in-Weight Method

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In determining the rates of deoxidation of iron oxide materials in reducing gases, iron oxide material is heated in an alloy container suspended from a balance, and reducing gas at a controlled rate of flow is passed through the heated material. The loss in weight (oxygen) is recorded at intervals until almost constant weight is obtained. Method is simple, accurate, and rapid.

THE successful use of fluidized solid catalysts for increasing reaction rates in some industrial processes led to the belief that this principle could be used advantageously in the deoxidation of fine iron ores. Fluidization used in this sense means suspension of the finely ground ore in a gas stream of suitable velocity to cause the mixture to behave more or less like a true fluid. The apparatus described in this report was designed and built for studying various factors governing the deoxidation of iron ores fluidized in hydrogen at 575° to 600° C. The results were so satisfactory that the apparatus was modified slightly for use in determining the relative rates of deoxidation of iron oxide materials by direct weighing at any time interval.

LOSS-IN-WEIGHT APPARATUS

The apparatus consists of a reaction tube of Pyrex or heat-resisting alloy 1.7 inches in diameter by 12 inches long, with the lower end closed. Hydrogen is introduced through a heat-resisting alloy tube and distributor extending to the bottom of the reaction tube. A 2-inch layer of −10- +14-mesh mullite grog placed above the metal distributor serves as a heater for the hydrogen and as an additional distributor for the hot hydrogen.

The reaction tube is supported in a stainless steel basket fastened to a disk on the hydrogen inlet tube. An alloy rod serves as a suspension link between the hydrogen inlet tube and a stirrup over one pan of a torsion balance. Unused hydrogen and water vapor from the reaction pass directly into the furnace atmosphere. A plate-type water cooler between the balance and the heater prevents the balance from becoming hot. A schematic drawing of the apparatus is shown in Figure 1 and details are shown in Figure 2.

The maximum temperature at which a Pyrex reaction tube can be used is 700° C.; at higher temperatures it is necessary to use a stainless steel retort. Other parts of the apparatus are unchanged. The rate of deoxidation of iron oxide material not larger than 0.525 inch may be determined at temperatures not exceeding 1000° C. A larger unit, embodying the same principles, has been constructed for determining the rates of deoxidation of larger sizes of ore in water gas.

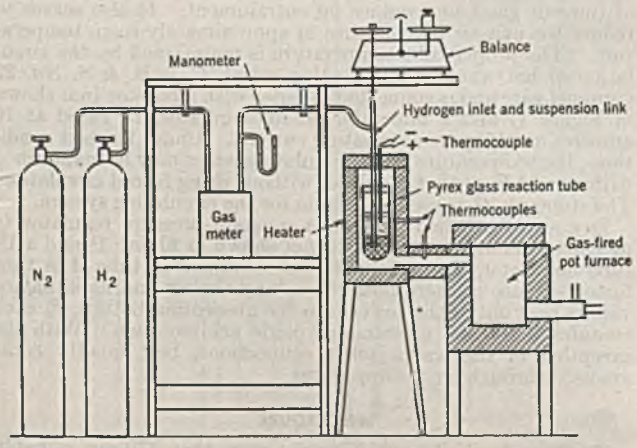

Figure 1. Schematic Drawing of Apparatus for Studying Deoxidation of Iron Ores in Hydrogen

PREPARATION OF SAMPLE FOR DEOXIDATION

Results of numerous tests have shown that the following procedure is suitable for preparing samples of iron ores:

A 100-pound sample of ore is screened on 0.525-inch. The oversize material is crushed to pass 0.525-inch and is added to the original undersize product. The combined −0.525-inch product is wet-screened on 20-mesh; the +20-mesh material is dried at 105° C. and separated into four sizes as follows: −0.525-inch +3-mesh; −3- +6-mesh; −6- +10-mesh; and −10- +20-mesh. A 227-gram (0.5-pound) composite is made of the four sizes and used for the deoxidation test. A duplicate composite is ground to −100-mesh for chemical analysis.

TEST PROCEDURE

The retort assembly containing the oxide sample is suspended from the balance, and the insulating brick top of the heater is replaced. The cover is removed from the pot furnace, and the gas burner is started. When the lining is red-hot the cover is replaced, forcing the hot products of combustion into the heater surrounding the retort. Nitrogen is passed through the retort to facilitate heating the ore charge, to sweep out any volatile components in the ore, and to provide an inert atmosphere. When the desired temperature is attained in the charge and the weight of the charge is constant, the nitrogen flow is stopped and the hydrogen flow is started. A good deoxidation rate is obtained with a hydrogen flow of about 0.25 cubic feet a minute. The loss in weight is recorded at regular intervals, usually every 5 minutes, until constant weight is attained or the deoxidation has proceeded as far as desired. After the gas burner has been shut off, the hydrogen flow is continued until the retort has reached room temperature. Cooling may be expedited by removing the retort from the furnace. When cool, the product is removed from the retort.

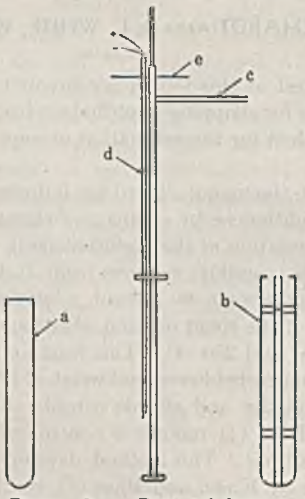

Figure 2. Parts of Loss-in-Weight Apparatus

a. Reaction tube
b. Stainless steel basket
c. Hydrogen inlet and connection to balance
d. Thermocouple
e. Heat deflector

The deoxidation is computed for each period by dividing the weight of oxygen lost during that period by the total available oxygen in the charge. Available oxygen in the charge is computed from the iron and manganese assays of the composite. The total loss of oxygen at constant weight will agree closely with the computed available oxygen if the test has been made properly. The loss in weight of the charge in nitrogen represents the ignition loss at the temperature of the test.

COMPARISON WITH OTHER METHODS

The laboratory method commonly used by the Bureau of Mines at its Minneapolis station for determining the rate of deoxidation

Table I. Log of a Typical Deoxidation Test

Weight of ore	227 grams
Size of ore	−0.525-inch +20-mesh
Temperature of test	900° C.
Rate of hydrogen flow	0.23 cu. ft. per minute
Available oxygen in sample	59.0 grams

Time after Start of H_2	Temperature In heater	Temperature In ore bed	Pressure Drop through Ore Bed	Gross Weight	Deoxidation Cumulative	Hydrogen Utilization
Min.	° *C.*	° *C.*	*Cm. water*	*Grams*	*%*	*%/time interval*
..	25	25	0.0	1,519.3	0.0	0.0
..	890	868	3.3	1,515.4	0.0	0.0
..	916	890	3.3	1,515.4	0.0	0.0
0	916	904	3.4	1,515.4	0.0	0.0
10	912	852	5.8	1,488.0	46.4	63.9
15	912	855	5.6	1,480.0	60.0	37.3
20	920	880	5.5	1,473.0	71.8	32.6
25	920	892	5.4	1,467.2	81.7	27.0
30	920	894	5.3	1,463.2	88.4	18.6
35	920	894	5.3	1,460.3	93.4	13.5
40	916	890	5.2	1,458.6	96.2	7.9
45	912	890	5.1	1,457.2	96.9	2.8
50	914	890	5.1	1,456.5	98.1	2.3
55	916	890	5.1	1,456.3	98.4	0.93
60	918	892	5.1	1,456.2	98.6	0.47
				Over-all H_2 utilization		22.6

of iron oxide materials has been to heat 1 to 20 grams of the oxide in a tube furnace while hydrogen was passed over the heated material. Water formed by the reaction was absorbed and weighed at regular intervals. The apparatus and procedure for determining rates of deoxidation are described in a technical press article (*2*).

After comparing the porosity of a group of iron ores with their respective reducibility, the data (*1*) obtained by the Bureau of Mines indicated that the porosity of an ore could be used as an index of its relative reducibility and of the size to which it should be crushed for the most efficient use in the blast furnace.

The loss-in-weight method has the following advantages:

The larger weight of material used permits more accurate sampling.

The larger weight of oxygen removed minimizes errors in absorbing and weighing small quantities.

Absorption of products of reaction is eliminated.

Errors due to part of the water being condensed in the reaction tube and not absorbed during the period in which it is formed are avoided.

Only one test is necessary to determine the course of deoxidation over its entire range.

Sampling and analyzing the deoxidized products are eliminated.

Rates of deoxidation in hydrogen, in carbon monoxide, or in mixtures of hydrogen and carbon monoxide may be determined.

Utilization of the reducing gas may be determined for each time interval from the beginning to the end of the test and at any degree of deoxidation.

The method is simple, rapid, and accurate.

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Estimation of Naphthalene in Absorbing Oil

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IN CONNECTION with a project at this laboratory involving pilot-plant studies of a process for stripping naphthalene from spent scrubbing oil, a rapid method for the estimation of naphthalene in oil was needed.

The method in general usage in the manufactured gas industry employs volatilization of the naphthalene by means of a stream of hot air, and subsequent precipitation of the naphthalene in a solution of picric acid (*2*). This procedure requires from 15 to 20 hours to perform and is cumbersome to control. Another method requires the distillation of the spent oil, and isolation of the fraction boiling between 195° and 250° C. This fraction is then chilled and the solid naphthalene is filtered and weighed (*3*). This procedure is also rather lengthy and affords considerable opportunity for inaccuracies. Bliss (*4*) reports a control test involving the refractive index of the oil. This method, developed from the work in oil evaluation by Kugel and Bliss (*5*), would have required considerable background study which at the time was not possible. The method of White (*6*), which involves bromination of the oil, solution of the brominated oil in acetone, and subsequent precipitation of naphthalene as the picrate, was not considered satisfactory for the purpose, since it involves considerable manipulation throughout the analysis.

Preliminary attempts were made to effect a separation of naphthalene from the oil by solvent extraction. Tests made by extraction with acetic acid and by shaking the spent oil with saturated picric acid were unsuccessful. It was therefore decided to concentrate on a modification of the volatilization procedure described above, which would effect the separation of naphthalene from a reasonably sized sample of oil in as short a time as possible and with a minimum of analytical manipulation.

REAGENTS

Sodium hydroxide, 0.1 *N*.

Methyl red indicator, 0.1% in ethanol.

Standard Picric Acid. Forty to 50 grams of the analytical reagent are added to approximately 2 liters of distilled water and the mixture is heated to 150° F. The mixture is then cooled to 64° to 66° F. and is filtered. The filtrate is diluted with distilled water until a 100-ml. portion is equivalent to 48 to 50 ml. of 0.1 *N* sodium hydroxide.

Naphthalene-free absorbing oil.

APPARATUS

Figure 1 is a line drawing of the apparatus finally evolved for the test. The apparatus consists essentially of a partly packed glass stripping tower jacketed for circulating hot water, a constant-temperature system for natural circulation of water, a sample container, and a four-bottle absorption train. The sample tube, *A*, may be disconnected from the apparatus and the oil sample weighed directly therein. The stripping tower, *B*, is partly packed with 6-mm. glass beads. The column of packing is 18 cm. high and is supported by projections in the base of the tube. The bulb at the top of *B* is designed to lower the velocity of the exit gases and reduce oil entrainment. It also serves to reduce the exit air temperature to approximately room temperature. The proper tube temperature is maintained by the circulation of hot water. The heating spiral, *C*, is B. & S. No. 22 Chromel wire and is connected in series with a resistor (not shown in Figure 1) and a bimetallic thermoregulator, *D*, rated at 10 amperes at 115 volts alternating current. Under the test conditions, the temperature of the circulating water may be maintained within ±5° F. of that required, without using forced circulation. The stopcock, *G*, is used as a drain for the circulating system.

Dry air is supplied through a constant-pressure regulator (a mercury-column type regulator not shown in Figure 1) and a U-tube flowmeter, *E*, to *A*. The oil contained in tube *A* is thus forced up into the stripping tower, and the air and naphthalene vapors pass out of the tower into the absorption bottles, *F*, each containing 100 ml. of standard picric acid solution. With the exception of the water-jacket connections, best quality corks are used throughout the apparatus.

PROCEDURE

The sample of oil, containing not more than 170 mg. of naphthalene, is weighed directly into the sample tube, and naphtha-

lene-free oil is added, if necessary, to bring the total volume to 10 ± 1 ml. The sample tube is then connected to the base of the stripping tower and the temperature of the circulating water is adjusted to 185° F. Into each of the four absorption bottles are pipetted 100 ml. of standard picric acid solution. The bottles are then connected in series at the outlet of the stripping tower, care being taken to make all connections glass to glass, since naphthalene is appreciably absorbed by rubber tubing. Dry air is then passed through the stripping tower at a rate of 3 cubic feet per hour. At the end of one hour the air rate is increased to 4 cubic feet per hour and is thus maintained for 2 hours. At these rates of air flow, the oil sample is entirely supported in the stripping tower.

The naphthalene is volatilized by the warm air and reacts with the picric acid to form a precipitate of naphthalene picrate, thus reducing the titer of the picric acid. The absorbing solution should be maintained between 65° and 80° F. during the analysis. When the 3-hour stripping period is completed, the contents of the four absorption bottles, including the precipitate, are quantitatively transferred to a 500-ml. volumetric flask and made up to the mark. Only 100 ml. of distilled water are used for washing in order to reduce the solubility of naphthalene picrate to a minimum. It is advisable to use a wide-necked volumetric flask and a powder funnel for the transfer, since the precipitate of naphthalene picrate may be agglomerated because of slight oil carry-over. The contents of the volumetric flask are well mixed and filtered through paper into a clean, dry flask. The first 100 ml. of filtrate are rejected and of the remaining filtrate, 100 ml. are titrated with 0.1 *N* sodium hydroxide using methyl red as indicator. This titer is then corrected to the basis of original undiluted picric acid. The difference in titer of the 400 ml. of picric acid before and after naphthalene absorption is converted to per cent by weight of naphthalene in the original oil sample. This is essentially the method of Sperr and Powell (*1*).

TEST OF ACCURACY

The method was checked using samples of oil containing known amounts of pure naphthalene. The oil used in making up the samples for the tests listed in Table I was Essodiesel 210, marketed by the Standard Oil Company of New Jersey.

The tests listed in Table I are selected from fifty or more tests made during the investigation. As may be expected, the accuracy decreases with decreasing naphthalene concentrations, largely because of the smaller differences in titer at the lower naphthalene concentrations.

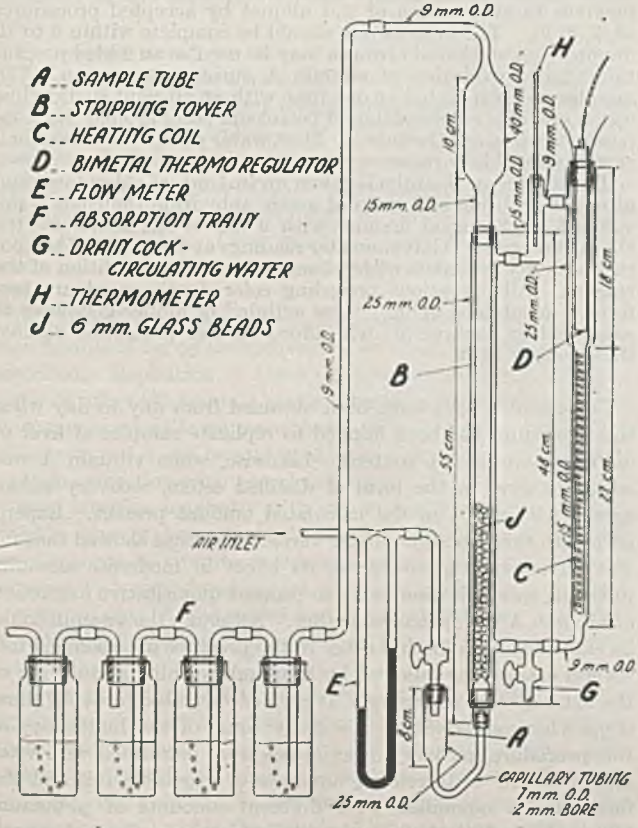

Figure 1. Diagram of Apparatus

Table I. Estimation of Naphthalene

Time of Stripping	Temperature	Total Air	Naphthalene Actual	Naphthalene Test
Hours	*° F.*	*Cu. feet*	*% by weight*	
3.0	185	11	2.00	2.0
3.0	185	11	2.00	2.0
3.0	185	11	2.00	2.0
3.0	185	11	1.48	1.4
3.0	185	11	1.32	1.3
3.0	185	11	1.32	1.2
3.0	188	11	1.01	1.0
2.5	188	9	1.01	1.0
2.5	185	9	1.01	1.1
2.5	185	9	1.01	1.0
2.5	185	9	0.50	0.4
2.5	185	9	0.50	0.4

Several of the tests included in Table I were run in 2.5 hours. When the naphthalene content is on the order of 1% or less, the runs may safely be made in this time. It is not recommended that the time be further reduced, since experience with running times of 2 hours or less has produced low results. However, when analyzing oils containing in excess of 2% naphthalene (when the sample for analysis must be diluted with naphthalene-free oil), it is best to take the full 170 mg. of naphthalene for analysis. This allows a relatively high titer difference and gives greater accuracy.

DISCUSSION

The matter of determining the optimum temperature at which to conduct the stripping procedure was of considerable importance. The amount of naphthalene removed from an oil in a given time varies with the stripping temperature and the volume of air used. Under the test conditions, the air rate is limited by the time of contact required for complete absorption of the naphthalene in the picric acid. It was found that 4 cubic feet per hour was the highest air rate which could be considered. A number of tests were made to determine the proper stripping temperature; temperatures in excess of 200° F. produced erratic results. This was largely due to the type of oil under test. At the higher temperatures a considerable portion of the oil was distilled over with the naphthalene, causing difficulty in carrying through the volumetric procedure. A distillation test of Essodiesel 210 is given in Table II.

Table II. Distillation, A.S.T.M.

	Essodiesel 210 *° F.*
Initial boiling point	419
5% recovered at	...
10%	468
50%	548
90%	640
Maximum temperature	675
Distillation recovery, %	98

Preliminary tests were conducted with other oils used as naphthalene absorbents. These oils distill at temperatures higher than Essodiesel 210 and in a narrower temperature range. Samples containing known amounts of naphthalene were prepared from these oils and check determinations were made on the apparatus. The results were generally satisfactory, although in several instances consistently low values were obtained. This led to the conclusion that, in the case of some oils, a definite percentage of naphthalene is retained in the oil and may not easily be removed by air volatilization. Therefore, when working with oils other than Essodiesel 210, initial runs should be made using samples containing known amounts of naphthalene in order to

determine the extent of recovery possible with air volatilization in the recommended time.

ACKNOWLEDGMENT

The authors wish to thank Ruth Votaw, formerly of this laboratory, for her assistance in the analytical work. They also express appreciation to R. Fram for preparation of the drawing, and to T. L. Robey and J. E. Brewer for helpful suggestions during the course of the investigation. The cooperation of the laboratories of the Consolidated Gas, Electric Light and Power Company of Baltimore, the Eastern Gas and Fuel Associates, the Philadelphia Electric Company, and the Rochester Gas and Electric Corporation in supplying oil samples is gratefully acknowledged.

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Determination of Vitamin A in Liver

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A rapid procedure is described for the colorimetric determination of vitamin A in liver. Vitamin A is quantitatively recovered from saponified 1-gram samples of liver by a single extraction with petroleum ether. Negligible amounts of interfering substances permit direct evaporation of the petroleum ether and treatment of the residue in chloroform with antimony trichloride. Errors due to soap formation, reagent impurities, and light are briefly discussed.

THE vitamin A content of a number of materials can be accurately determined by modified colorimetric methods (*4, 8, 9*) based on the reaction of vitamin A with antimony trichloride in chloroform. Procedures for the extraction of vitamin A and removal of interfering substances previous to color development vary with different materials (*1, 2, 3, 7*) and for the most part require considerable time and careful manipulations. Simplification of these procedures without sacrifice of accuracy in routine testing is desirable, and may be accomplished in the analysis of some materials that are relatively high in vitamin A and low in fat. A simplified procedure similar to that developed for blood (*7*) but with the additional step of saponification (*6*) has been applied to liver after first making a study of the sources of error involved. Among the errors observed are some that have received slight mention in vitamin A methods and others that have been the subject of controversial discussion.

REAGENTS

Potassium hydroxide in aldehyde-free alcohol, 5% solution. The aldehyde-free alcohol is prepared from 95% ethyl alcohol according to the procedure described by Woodman (*11*).

Petroleum ether, boiling point 40° to 60° C. Redistilled Skellysolve F is satisfactory.

Chloroform of reagent grade which meets A.C.S. specifications. If of doubtful quality the chloroform should be specially treated (*8, 9*).

Antimony trichloride reagent, prepared by rapidly weighing about 100 grams of the C.P. salt from a freshly opened bottle and dissolving it in sufficient chloroform to make a 25% solution. The clear solution should be kept in the dark in a glass-stoppered bottle.

Acetic anhydride, C.P.

PROCEDURE

An Evelyn photoelectric colorimeter with a 620-millimicron filter was used in this work. The advantages of this type of instrument have been discussed by Oser and associates (*9, 10*). The instrument was calibrated with a chloroform solution of vitamin A esters (Distillation Products, Inc., Rochester, N. Y.) (*9*). The same vitamin A preparation dissolved in suitable solvents was used to perform recovery experiments. The following procedure was employed for the determination of vitamin A in sheep livers and with slight modification has been used successfully for the determination of both carotene and vitamin A in cattle livers. Greater precision may be expected by introducing in the colorimetric phase of this method the internal standard procedure described by Oser, Melnick, and Pader (*9*).

One gram of liver and 5 ml. of 5% aldehyde-free alcoholic potassium hydroxide are placed in a 50-ml. heavy-walled centrifuge tube having a constricted neck and digested in a water bath at 75° C. until the tissue is completely disintegrated. Ten or more samples can be digested conveniently at one time if the concentric rings of a shallow water bath are replaced with a sheet-lead top having 3.1-cm. (1.25-inch) holes. The saponified sample is cooled, brought to its original volume with alcohol, and diluted with an equal volume of water. This concentration of alcohol and potassium hydroxide in the presence of no more than 50 mg. of original fat permits practically complete extraction of vitamin A (and carotene) by petroleum ether without appreciable change in volume. If large amounts of vitamin A are present, an aliquot of the digest is made to a volume of 10 ml. with 50% alcohol.

The contents of the tube are vigorously shaken for 2 to 3 minutes with 10 ml. of petroleum ether and finally centrifuged. Five milliliters of the upper layer, or smaller amounts for livers high in vitamin A, are transferred to an Evelyn colorimeter tube containing a glass bead and evaporated to dryness at 45° C. with the aid of suction. Carotenoids, when present, are determined previous to evaporation of this aliquot by accepted procedures (*3, 7, 8, 9*). The evaporation should be complete within 5 to 10 minutes. A stream of nitrogen may be used as an added precaution against oxidation of vitamin A during evaporation. Ten samples are evaporated at one time with an efficient suction line which prevents condensation of petroleum ether around the stoppers in the necks of the tubes. Most water pumps have too small capacity for this purpose.

The residue in the tube is taken up in 1 ml. of chloroform and after the addition of 1 drop of acetic anhydride the color is developed in the usual manner with 9 ml. of the antimony trichloride reagent. Galvanometer readings at the point of temporary stability are taken within 5 seconds after the addition of the reagent. All operations preceding color development are performed in subdued light. "Low actinic" or amber glassware as suggested by Embree (*5*) will afford further protection against the effects of light.

Consistent results have been obtained from day to day when this procedure has been applied to replicate samples of liver of unknown vitamin A content. Likewise, when vitamin A was added to liver in the form of distilled esters, recovery values agreed within 5% of the calculated amount present. Experience with this procedure under varied conditions showed that fat was an interfering substance, its effect in moderate amounts following saponification being to prevent quantitative extraction of vitamin A with petroleum ether. Although the vitamin could be extracted with diethyl ether in the presence of saponified fat, several extractions followed by thorough washing and drying of the extract were necessary. It seemed desirable to avoid these steps wherever possible. To study some of the limitations of this procedure, recovery experiments were performed with vitamin A added to increasing amounts of vegetable oils and the fortified oils saponified with different amounts of potassium hydroxide and alcohol previous to extraction. Control experi-

Table I. Reliability of Procedure for Determination of Vitamin A in Lamb Liver

Liver Sample No.	Sample Weight, Grams	Micrograms of Vitamin A per Gram Added	Total	Recovered
4	1.00	0	...	8.55
	1.00	2.43	10.98	10.45
	1.00	5.10	13.65	13.70
	1.00	7.75	16.30	16.20
	1.00	13.45	22.00	21.90
	1.00	18.15	26.70	26.65
8	1.03	0	10.86	...
	1.05	0	11.80	...
	1.12	0	11.57	...
	1.11	0	9.94	...
	1.07	0	11.52	...
	1.07	0	10.97	...
		Av.	11.11 (±0.28)	...
21[a]	1.00	0	1.56	...
28[a]	1.05	0	4.42	...
23[a]	1.09	0	19.00	...

[a] Livers 21, 28, and 23 were from lambs of corresponding numbers that received graded levels of carotene after 6 months on a carotene-free ration. No. 21 received approximately 2 mg. of carotene daily; No. 28 was given twice this amount; No. 23 was given twice this amount but for a longer period and was allowed to graze green grass for a week previous to slaughter.

ments were carried out at the same time without oil. In other experiments vitamin A was added at different steps in the procedure.

RESULTS AND DISCUSSION

In Table I are presented values for the recovery of increasing amounts of vitamin A added in the form of distilled esters to a homogeneous sample of lamb liver. The amounts of vitamin A added to the liver were determined colorimetrically in control experiments by direct treatment of the esters in chloroform with antimony trichloride reagent. The intensity of color produced by the esters was found to be the same as that of their unsaponifiable extract, as previously demonstrated by Oser, Melnick, and Pader (*9*). Good agreement between the observed and calculated values shown for sample 4 in Table I gives evidence of the reliability of the procedure for the estimation of vitamin A in liver.

The values shown for liver 8 in Table I were obtained by analyses of samples taken from different sections of the same liver. These values, by comparison with those of the well-mixed sample 4, are indicative of sampling error attributable to an uneven distribution of vitamin A in different parts of the liver. Where greater accuracy is desired, thorough mixing of random samples with due precautions against loss of moisture during mixing would be advisable. Liver samples 21, 28, and 23 were from lambs that received increasing amounts of carotene in their rations.

In Table II are presented values showing the effect of increasing amounts of fat on the recovery of vitamin A by the procedure described. Repetition of these experiments on other occasions and with other oils and fats has demonstrated that in the presence of soap equivalent to no more than 0.05 gram of oil, over 95% of added vitamin A can be extracted from 50% alcohol solutions by a single extraction with petroleum ether. That larger amounts of soap interfere markedly with extraction of vitamin A by petroleum ether is shown throughout the results in Table II. Material other than vitamin A removed by petroleum ether did not interfere with color development, as shown by the results in series 2 when vitamin A was added to the petroleum ether extract of unsaponifiable material just previous to evaporation and color development.

In the extraction of vitamin A with petroleum ether the best separation of layers occurred when the soap solution contained about 50% alcohol. Varying the percentage of alcohol made separation difficult and failed to improve the recovery of vitamin A. Likewise, variation in the concentration of potassium hydroxide (series 3) and in the volume of soap solution (series 4) previous to extraction failed to improve vitamin A recoveries. When the soap solution was extracted with three 10-ml. portions of petroleum ether (series 5) recoveries were improved and approximately 97% of added vitamin A was recovered from 0.10 gram of oil. However, only 57% of added vitamin A could be recovered from 1.0 gram of oil by the modification. Triple extraction with diethyl ether as recommended in most procedures (series 6) apparently effected good extraction of vitamin A unrelated to the amount of soap present; the recovery values, which ranged from 91.1 to 94%, are probably related to losses of vitamin A that occur during washing and drying the extracts.

PRECAUTIONS

In the progress of this work the significance of various sources of error was investigated. Errors due to relatively large amounts of fat have been discussed in relation to the solvent employed for extraction of unsaponifiable material. This error can be avoided, if the sample is sufficiently high in vitamin A, by taking an aliquot of the saponified sample for extraction with petroleum ether. Certain other errors are mentioned here because, despite indications to the contrary in some recently proposed procedures, they have a marked effect on the accuracy of results.

Aldehydes. Alcohol used in saponification must be free of aldehydes to prevent the formation of yellow resins which dissolve in petroleum ether and chloroform and produce a blue color with antimony trichloride. Galvanometer readings equivalent to 0.5 microgram of vitamin A have been observed in blank determinations carried out with ordinary alcohol. This amount of spurious vitamin A produces errors of from 10 to 50% in some determinations.

Rubber Stoppers. Petroleum ether dissolves from new, and some old, rubber stoppers material which reacts with antimony trichloride to give a blue-green color. The intensity of the color, unlike that of the vitamin A reaction, increases during the first few minutes.

Peroxides. The best grades of diethyl ether frequently contain sufficient peroxides to destroy vitamin A, particularly during

Table II. Effect of Fat and Certain Modifications in Procedure on Recovery of Vitamin A

Series	Modification of Procedure	Cottonseed Oil	Vitamin A Added	Vitamin A Recovered	Recovery
		Gram	*Micrograms per gram*		%
1	None	0.00	4.80	4.81	100.2
		0.01	4.80	4.85	101.0
		0.02	4.80	4.77	99.3
		0.05	4.80	4.53	94.4
		0.10	4.80	4.04	84.2
		0.20	4.80	3.73	77.7
		0.40	4.80	2.70	56.2
		1.00	4.80	1.56	32.5
2[a]	Vitamin A added to unsaponifiable extract	0.20	3.62	3.66	101.1
		0.40	3.62	3.74	103.3
		0.60	3.62	3.70	102.2
		1.00	3.62	3.70	102.2
3[b]	Concentration of KOH varied	0.20	3.60	2.56	71.1
		0.20	3.60	2.49	69.2
		0.20	3.60	2.70	75.0
		0.20	3.60	2.63	73.1
4[c]	Volume of alcohol-soap-water solution increased	0.10	3.51	3.06	87.2
		0.20	3.51	2.63	74.9
		0.40	3.51	2.22	63.2
5[d]	Triple extraction with petroleum ether	0.00	15.70	15.35	97.8
		0.10	15.70	15.20	96.8
		0.40	15.70	13.35	85.0
		1.00	15.70	8.90	56.7
6[e]	Triple extraction with diethyl ether	0.00	15.70	14.30	91.1
		0.10	15.70	14.75	94.0
		0.40	15.70	14.50	92.3
		1.00	15.70	14.75	94.0

[a] Vitamin A was withheld from saponification mixture and added to petroleum ether extract of unsaponifiable material previous to evaporation.
[b] Concentration of potassium hydroxide in alcohol used for saponification of samples in order given was 2.5, 5.0, 10.0, and 20.0%, respectively. Saponified samples were diluted with an equal volume of water previous to extraction.
[c] Volume of saponified fat solution in 50% alcohol was increased to 30 ml. before extraction with petroleum ether.
[d] Saponified fat solution was extracted three times with petroleum ether. Combined extracts were washed and dried and 1/4th aliquot evaporated.
[e] Same as [d] except that diethyl ether was used for extraction.

separatory funnel operations. When an ether solution containing 2.5 micrograms of vitamin A was evaporated in the usual manner in colorimeter tubes in the presence of 0, 2, 4, 6, and 8 ml. of anhydrous ether which gave a positive peroxide test, losses of vitamin A were 0, 6, 18, 27, and 29%, respectively.

LIGHT. Vitamin A solutions are unstable in bright indoor light (*5*). During a 2-hour period, losses of vitamin A were approximately 38% greater when saponified samples were extracted with ether and the extract washed and dried near a north window than when the same operations were performed in subdued light. Losses of vitamin A in ether solutions placed 180 cm. (6 feet) from a north window, in subdued light, and in the dark over a period of 5 hours were 60, 1.2, and 0%, respectively. Losses in petroleum ether solution under similar conditions were 41, 3, and 0%, respectively.

SUMMARY AND CONCLUSIONS

The vitamin A content of liver can be determined by a single extraction of the saponified sample with petroleum ether, followed by treatment of the evaporated extract with antimony trichloride reagent in the usual manner. The procedure eliminates several manipulations and avoids undue exposure of vitamin A solutions to light. Large amounts of soap in alcohol-water mixtures interfere with the extraction of vitamin A by petroleum ether. Approximately 95% of the vitamin can be recovered, however, when the quantity of soap in the alcohol-water phase does not exceed that equivalent to 0.05 gram of fat.

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Determination of Uronic Acids

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A modified method for determining uronic acids has been developed. Theoretical quantities of carbon dioxide were obtained for all uronic acids and their derivatives by heating the sample with 19% hydrochloric acid in an oil bath maintained at 145° C. for 1.5 to 2 hours. The apparatus is simple, compact, easy to construct, and offers a substantial saving of space and time. Analytical results on alginic acid were higher and more reproducible than those obtained by accepted methods.

THE objective of the work reported here was to devise an effective procedure for determining the uronide content of pectic and other polyuronide substances in an appreciably shorter time than that required to carry out this type of analysis by currently recommended modifications (*1–7, 9–15*) of the Lefevre-Tollens (*8*) method. Whereas a minimum time of 4 hours is considered necessary for complete decarboxylation of certain pectic materials with boiling 12.5% hydrochloric acid and even a longer treatment does not suffice for alginic acid, quantitative yields of carbon dioxide were obtained from a variety of pectic substances and alginic acid when they were heated in 19% hydrochloric acid, at a bath temperature of 145° C. for 1.5 and 2 hours, respectively.

APPARATUS AND METHOD

The apparatus used in the investigation is shown in Figure 1. Two assemblies of this type were used simultaneously in the same heating bath.

Air, the carrier gas, passes through an Ascarite or soda-lime column, *A*, which removes traces of carbon dioxide. A mercury valve, *B*, allows gas to pass in one direction through the apparatus. This valve is connected through a side tube, *C*, to a reaction flask, *D*, by means of a rubber connection. *D* is a 100-ml. round-bottomed, long-necked boiling flask, with a 24/40 ground joint attached. The oil bath, *E*, is maintained at 145° C. by means of a thermoregulator and immersion heater.

From the reaction flask the carrier gas passes upward through a 20-cm. reflux condenser, *F*, through a trap, *G*, containing 25 grams of 20-mesh granulated zinc or tin, and finally into the absorption flask, *H*. This 250-ml. Erlenmeyer flask is equipped with a 24/40 ground joint and a side tube attached a little below the ground joint, as shown.

The gas passes upward through an absorption tower, *I*. The lower part of the tower consists of an 18-mm. tube, fitted with a medium fritted Pyrex disk and sealed to the lower end of the inner part of a 24/40 ground joint. The bubbling disk should terminate 1 or 2 mm. above the bottom of the absorption flask when the joint is in place. A bulb of approximately 100-ml. capacity is blown above the ground portion of the joint to serve as a trap to prevent the possible loss of alkali by foaming. The outer portion of a 24/40 ground joint is sealed on above this bulb. The absorption tower, from the bottom of the disk to the top of the ground joint, is approximately 30 cm. in length. The top of the tower is fitted with a hollow, ground stopper, with a short side tube attached. The carrier gas passes from the tower assembly to a soda-lime tower, *J*. A water pump, attached to a capillary-tube regulator, *K*, serves to sweep 1700 to 2000 ml. of carbon dioxide–free air per hour through the apparatus during the heating period.

The sample to be analyzed is placed in the dry reaction flask. (The optimum size of sample used depends upon its uronic acid content—for example, about 250 mg. are sufficient for analyzing pectin.) Thirty milliliters of 19% hydrochloric acid and a small boiling tube are added. The ground joint is lubricated with sirupy phosphoric acid and attached to the reflux condenser and mercury valve. A stream of carbon dioxide–free air is drawn through the reaction flask and reflux condenser to remove traces of carbon dioxide before the absorption tower is attached.

The ground joints of the absorption tower are lubricated with stopcock grease and inserted into the absorption flask, *H*. The

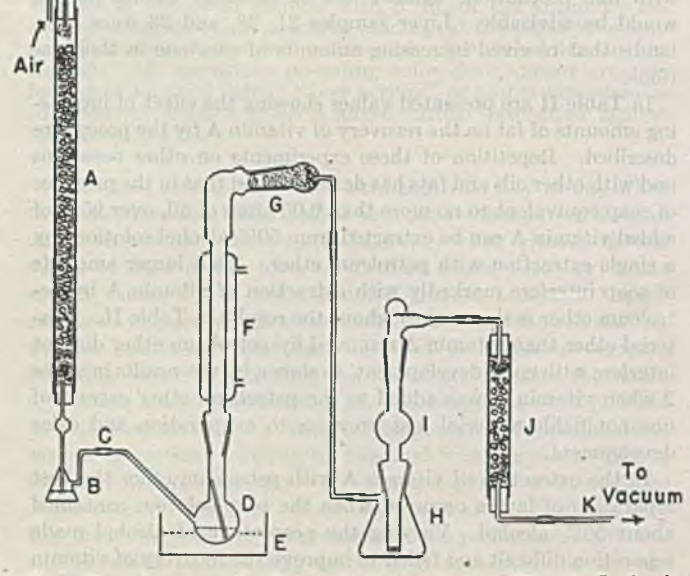

Figure 1. Apparatus for Determination of Carbon Dioxide Evolved from Uronic Acids by Heating with Hydrochloric Acid

flask and tower are swept free of carbon dioxide, and 25 ml. of 0.25 *N* sodium hydroxide and 5 drops of butanol-1 are added to the absorption tower. The side tube of the absorption flask is attached to the zinc trap and the top of the tower to the soda-lime tower, *J*, by means of rubber connections.

The oil bath, previously brought to 145° C., is placed in position, the level of the oil being 1 or 2 mm. below that of the liquid level within the reaction flask. After the initial rapid evolution of gas through the absorption tower has ceased, the capillary tube, *K*, is attached to *J* and to the source of vacuum.

Heating of the oil bath at 145° C. is continued while the apparatus is swept for 1.5 hours. The bath is then removed, the absorption flask and tower are disconnected from the apparatus, and the alkali is washed down from the tower into the absorption flask. Three or four washings are needed to remove the last traces of alkali from the tower. Gentle pressure with carbon dioxide–free air from the top of the tower can be used to hasten the washing.

Ten milliliters of 10% barium chloride dihydrate solution and two drops of phenolphthalein indicator are added to the absorption flask and the excess alkali present is titrated with 0.100 *N* hydrochloric acid. Normal precautions are used to exclude carbon dioxide during the storage, addition, and subsequent titration of the absorption alkali. A control standardization should be run without the sample and used in subsequent calculations of the carbon dioxide evolved from uronic acids.

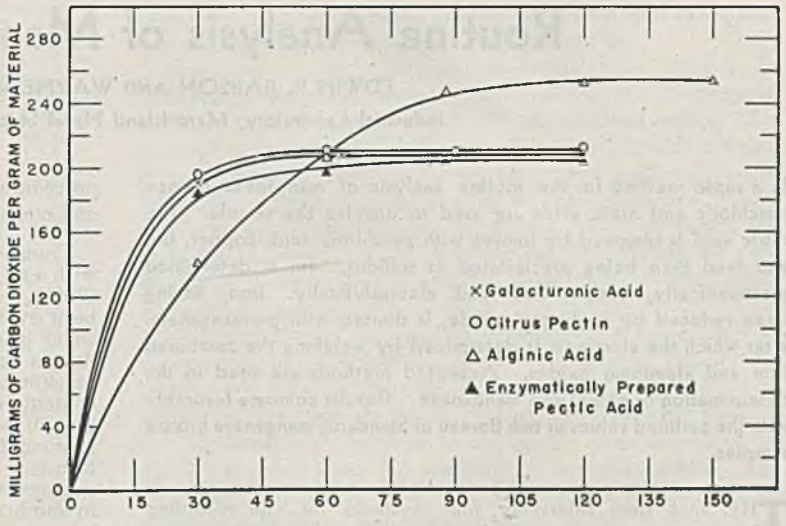

Figure 2. Rate of Evolution of Carbon Dioxide from Citrus Pectin, Galacturonic, Alginic, and Pectic Acids with 19% Hydrochloric Acid in an Oil Bath Maintained at 145° C.

EXPERIMENTAL RESULTS AND DISCUSSION

The results are presented on a moisture- and ash-free basis. Moistures were determined by drying in vacuo for 24 hours at 70° C., and dry samples were heated 3 hours at 600° C. to determine ash. The sample of galacturonic acid monohydrate was dried at 25° C. over phosphorus pentoxide and analyzed without further treatment.

Figure 2 shows the rates of evolution of carbon dioxide from galacturonic acid monohydrate, citrus pectin, alginic acid, and enzymatically prepared pectic acid with 19% hydrochloric acid in an oil bath maintained at 145° C. Pectin and galacturonic acid yielded the calculated quantity of carbon dioxide in one hour. Pectic acids required heating periods of 1.5 hours, while alginic acids required 2 hours for decarboxylation.

Table I shows results obtained with 12.5% hydrochloric acid at an oil bath temperature of 145° C. for 1.5 hours and 12.5% hydrochloric acid at an oil bath temperature of 130° C. for 5 hours, respectively, as compared with the proposed method with 19% hydrochloric acid and an oil bath temperature of 145° C. for 1.5 hours.

Table I. Yields of Carbon Dioxide from 0.250-Gram Samples of Uronic Acids and Their Derivatives

(Heating with 12.5% hydrochloric acid at 130° C. for 5 hours and at 145° C. for 1.5 hours, compared with 19% hydrochloric acid at 145° C. for 1.5 hours)

Sample	Yield of Carbon Dioxide, % 12.5% HCl, 1.5 hours[a]	12.5% HCl, 5 hours	19% HCl	Calculated[b]
Galacturonic acid (monohydrate)	...	20.7 20.7	20.7 20.7	20.75
Citrus pectin (purified)	16.0 16.8	20.7 20.8 21.1	20.8 20.8 20.8	...
Citrus pectin (unpurified)	...	16.5 16.3	16.6 16.7	16.9
Apple pectin	...	21.4 21.2	20.7 20.8	21.2
Pectic acid (enzymatic)	14.5 14.4	20.0 20.0	19.8 19.8	20.0
Alginic acid (purified)	14.2[c] 14.6[c]	21.7 19.2 20.7	23.7[c] 23.8[c] 23.8[c]	24.3
Glucose	...	0.26 0.33	0.22 0.26	0

[a] Heated at bath temperatures of 145° C.
[b] Calculated from equivalent weight corrected for combined ash constituents and methoxyl groups.
[c] Samples heated for 2 hours.

Decarboxylation of citrus pectin (purified), pectic acid, and alginic acid with 12.5% hydrochloric acid at an oil bath temperature of 145° C. for 1.5 hours was approximately 70, 80, and 60%, respectively, complete. Equal quantities of carbon dioxide were obtained from the pectic substances with 12.5% acid for 5 hours and by the proposed method. However, the yields of carbon dioxide from alginic acid were low as compared with those obtained with the proposed method. The data clearly indicate the effectiveness of 19% hydrochloric acid over that of the lesser concentration and a very significant saving of time for uronic acid analyses.

The carbon dioxide evolved from various nonuronide substances such as inulin, gelatin, glucono-δ-lactone, starch, sucrose, and oxalic and mucic acids, when treated either under the reaction conditions herein recommended or those generally used, was similar to that obtained from glucose (Table I). A sample of arabinose yielded 0.7% carbon dioxide when analyzed by the proposed method. These results show that none of these substances would interfere in the analysis of pectic materials when present in moderate amounts as impurities. Quantitative recovery of carbon dioxide was obtained from citrus pectin when 0.020 gram of material was used instead of the recommended 0.250-gram sample.

ACKNOWLEDGMENT

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Routine Analysis of Manganese Bronze

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In a rapid method for the routine analysis of manganese bronze perchloric and nitric acids are used to dissolve the sample. The nitric acid is removed by fuming with perchloric acid, copper, tin, and lead then being precipitated as sulfides. Tin is determined gravimetrically, copper and lead electrolytically. Iron, having been reduced by hydrogen sulfide, is titrated with permanganate, after which the aluminum is determined by weighing the combined iron and aluminum oxides. Accepted methods are used in the determination of nickel and manganese. Results compare favorably with the certified values of two Bureau of Standards manganese bronze samples.

THE fact that relatively few methods for the complete analysis of manganese bronze have appeared in the literature is perhaps evidence that the inherent difficulties have not been satisfactorily overcome. It is known that if a sample of bronze containing considerable iron is decomposed with nitric acid the tin is incompletely precipitated, and contaminated with iron. Tin, if present in the electrolyte, will contaminate both copper and lead deposits if these elements are determined by electrolysis. Unless special precautions are taken the lead peroxide, deposited on the anode during electroplating, is contaminated with manganese dioxide. Also, the separation and determination of aluminum, at best being time-consuming, are complicated by the presence of any tin not previously separated. While accurate methods are available, all are objectionable for one reason or another. The principal objection is the time consumed in avoiding the interferences mentioned. Results of analysis are often needed in 5 or 6 hours, and most methods fail to satisfy this requirement. Some methods involve the use of fluorides to prevent interference from tin in the electroplating of copper and lead (*3*), but for large numbers of samples considerable platinum ware is required in subsequent evaporations. Procedures which require the plating of copper and lead without prior removal of manganese result in high lead values, although in some instances (*3*) the amount of contamination is not excessive for routine work. One of the most acceptable methods for determining aluminum necessitates the use of the mercury cathode (*2*), but is somewhat slow for rapid analysis.

The method described herein avoids these difficulties and is considered novel in some respects. The initial attack is made with perchloric and nitric acids, rendering soluble all constituents with the exception of a small amount of tin. Then, precipitation with hydrogen sulfide in the acid solution separates the copper, lead, and tin from the iron, aluminum, manganese, nickel, and zinc. By this means tin is removed from iron, and lead from manganese. The iron, having been reduced to the ferrous state by hydrogen sulfide, is titrated with permanganate. Later precipitation of mixed iron and aluminum hydroxides with ammonia makes the use of the mercury cathode unnecessary. The accuracy of the method is shown by values obtained with Bureau of Standards samples, and with a secondary standard which had been carefully analyzed by accepted methods (*1*, *4*). Procedures for the determination of manganese and zinc are omitted because present methods (*5*) are satisfactory for the former, and the latter is most accurately obtained by difference.

PROCEDURE

All elements except zinc and manganese are determined from a single weighed sample. After the initial solution and sulfide separation, the precipitate and filtrate are treated simultaneously, in this way decreasing the total time of analysis. The complete procedure for determining tin, copper, lead, iron, aluminum, and nickel is as follows:

Transfer 1.0000 gram of sample to a 400-ml. beaker. Dissolve with 4 ml. of nitric acid (sp. gr. 1.42) and 6 ml. of perchloric acid (70%), cover, and heat until heavy fumes of perchloric acid have been evolved for several minutes. Cool somewhat, wash the cover glass, and dilute to 200 ml. with hot water. Pass hydrogen sulfide into the solution for 20 minutes. Coagulate the precipitated sulfides by adding paper pulp and bringing the contents of the beaker to boiling. Filter through a Whatman No. 30 paper into a 400-ml. beaker, and wash the precipitate thoroughly with hot water, combining the washings and the filtrate. Save both the precipitate and solution.

DETERMINATION OF TIN. Wrap the precipitate and filter in another piece of filter paper, and place in a clean No. 1 porcelain crucible. Ignite completely at 500° C., and, after allowing to cool carefully transfer the ignited material to a 150-ml. beaker. Pour 10 ml. of nitric acid (sp. gr. 1.42) into the crucible and heat on a hot plate for several minutes. Pour the acid into the beaker containing the major portion of the ignited oxides, and wash the crucible with 10 ml. of water, adding the washings to the beaker. Heat until the black copper oxide is dissolved. Dilute to 50 ml. with hot water, add a small amount of paper pulp, and allow to stand just below the boiling point for 30 minutes. Filter on a Whatman No. 42 paper into which has been poured a small amount of paper pulp suspension. Wash the precipitate three times alternately with hot water and dilute nitric acid (1 to 4). Ignite the precipitate in a tared crucible first at 500° C. until the paper is completely charred and finally at 900° C. until fully ignited. Weigh the ignited material as SnO_2, and calculate the percentage of tin.

$$\text{Per cent tin} = \frac{\text{weight of } SnO_2 \times 0.788}{\text{weight of sample}} \times 100$$

DETERMINATION OF COPPER AND LEAD. The filtrate from the tin determination contains the copper and lead. Dilute to 200 ml., and add 2 ml. of dilute sulfuric acid (1 to 1) and a small amount of urea or sulfamic acid. Place on an electroplater and deposit the copper and lead simultaneously. Using the customary gauze cathodes and rotating gauze anodes complete deposition will be obtained in about 60 minutes at 2.5 amperes. Weigh the previously tared electrodes and calculate the percentage of copper and lead. Discard the electrolyte.

$$\text{Per cent copper} = \frac{\text{weight of Cu deposited}}{\text{weight of sample}} \times 100$$

$$\text{Per cent lead} = \frac{\text{weight of } PbO_2 \text{ deposited} \times 0.864}{\text{weight of sample}} \times 100$$

DETERMINATION OF IRON. To the filtrate from the sulfide precipitation add a few glass beads, and boil the solution vigorously for 30 minutes to expel the hydrogen sulfide. Cool the solution in ice water, add 10 ml. of dilute sulfuric acid (1 to 1), and titrate with 0.05 *N* potassium permanganate to a faint pink color that should persist for 10 to 15 seconds. Reserve the solution for the determination of aluminum.

$$\text{Per cent iron} = \frac{\text{ml. of titration} \times 0.05584 \times \text{normality of } KMnO_4}{\text{weight of sample}} \times 100$$

DETERMINATION OF ALUMINUM. To the solution from the iron determination add 10 grams of ammonium chloride and make just alkaline to methyl red with ammonium hydroxide (sp. gr. 0.90). Add a small amount of paper pulp, bring to a boil, add 3 drops more ammonium hydroxide (sp. gr. 0.90), and allow to settle for 5 minutes. Filter the hydroxides on a Whatman No. 31 paper, washing three times with hot ammoniacal ammonium nitrate solution (10% ammonium nitrate made just ammoniacal to methyl red). Remove the beaker containing the filtrate and washings, reserving these for the nickel determination. Dissolve the hydroxides from the paper by pouring hot dilute hydrochloric acid (1 to 1) through the filter into the original beaker. Wash the filter well with cold water and discard. Add 2 grams of ammonium chloride to the solution in the beaker and reprecipitate the iron and aluminum hydroxides as described above. After

Table I. Results of Analysis of NBS Samples

	Sample 62a[a]			Sample 62b[b]		
	Found	Average	Certified	Found	Average	Certified
	%	%	%	%	%	%
Copper	61.47	61.47	61.51	57.36	57.35	57.40
	61.51			57.35		
	61.47			57.31		
	61.43			57.39		
	61.46			57.34		
Tin	0.84	0.84	0.84	1.02	1.01	0.97
	0.84			1.03		
	0.83			0.99		
	0.83			1.00		
	0.84			1.02		
Lead	0.51	0.52	0.50	0.28	0.28	0.27
	0.52			0.29		
	0.52			0.30		
	0.51			0.29		
	0.53			0.26		
Iron	1.02	1.03	1.04	0.80	0.81	0.81
	1.02			0.80		
	1.02			0.82		
	1.03			0.80		
	1.04			0.82		
Aluminum	0.94	0.93	0.92	0.98	1.00	0.97
	0.91			0.97		
	0.93			1.02		
	0.93			0.99		
	0.93			1.02		
Nickel	0.61	0.62	0.61	0.29	0.28	0.27
	0.62			0.27		
	0.61			0.29		
	0.62			0.29		
	0.62			0.28		

[a] Manganese 1.51%. [b] Manganese 1.28%.

filtering and washing the precipitate, place in a tared porcelain crucible, char the paper at 500° C., and ignite at 900° C. Cool the ignited oxides in a desiccator, and weigh the mixture of iron and aluminum oxide (R_2O_3) rapidly. From the weight of the R_2O_3 and the percentage of iron previously determined calculate the per cent aluminum.

$$\text{Per cent aluminum} = \frac{(\text{weight of } R_2O_3 - \text{weight of Fe} \times 1.429)\ 0.529}{\text{weight of sample}} \times 100$$

Determination of Nickel. To the filtrate from the first hydroxide precipitation add 15 ml. of dimethylglyoxime solution (1% alcoholic solution). Warm until the precipitate is well coagulated and filter through a Whatman No. 41 paper, washing eight to ten times with hot water. Wrap the filter in a moistened ashless filter paper and ignite in a tared, covered, porcelain crucible, first at 500° C., and finally at 800° C. Cool in a desiccator and weigh as NiO.

$$\text{Per cent nickel} = \frac{\text{weight of NiO} \times 0.786}{\text{weight of sample}} \times 100$$

EXPERIMENTAL RESULTS

National Bureau of Standards manganese bronze samples 62a and 62b were used to test the accuracy of the method. The results obtained are shown in Table I.

Most samples analyzed in this laboratory have less tin, manganese, lead, and nickel than do the Bureau of Standards samples. A sample of more common alloy, the composition of which had been carefully established by accurate methods (*1*, *4*), was analyzed by the above method. Results are shown in Table II.

DISCUSSION

The properties of perchloric acid make it particularly suitable for this method. Its oxidizing properties when hot and concentrated and the solubility of its salts provide practically complete solution of the sample. Further, the fact that dilute solutions of the acid have no oxidizing power makes it satisfactory for the hydrogen sulfide precipitation and iron titration. It was originally contemplated that the initial attack should be with perchloric acid alone. However, the tin values were found to be consistently low and somewhat erratic. It seemed possible that this might be due to volatilization of the tin during solution of the sample. By using both nitric and perchloric acids, and then evaporating to perchloric acid fumes, this difficulty was overcome. No error attributable to the presence of nitric acid was observed in the subsequent iron titrations.

Aluminum and tin values on sample 62b were found to be consistently high. Spectrographic analysis disclosed the presence of silicon in the ignited tin oxide and in the mixed oxides. This was due to the presence in the sample of 0.047% silicon which is not ordinarily found in manganese bronze analyzed in this laboratory. From a consideration of coprecipitation, hygroscopicity, and solubilities one might not expect to obtain the accuracy shown. However, it is possible that fortuitous compensating errors result in slightly better values than would normally be expected.

With this method one sample of manganese bronze can be analyzed in approximately 5 hours. Six samples require approximately the same time and 12 samples can be completed by one analyst in an 8-hour period. Experience with other methods had indicated that, in order to obtain the same degree of accuracy, about 8 hours are required for a single sample. The equipment required is found in all nonferrous laboratories, no special apparatus or reagents being necessary. All operations are of such simplicity that little is required in the way of special technique and training.

It has been demonstrated that a satisfactory analysis of manganese bronze can be accomplished if tin is separated from iron, if it is not present in the copper-lead electrolyte, and if manganese is separated from lead. The separations described in this method isolate the elements in such a manner that little interference is encountered in each determination. It therefore appears that the method at least partly satisfies the requirements for rapid analysis, and has sufficient accuracy to warrant its use in routine control and inspection work.

Table II. Results of Analysis of Laboratory Standard[a]

	Found	Average	Accepted Value
	%	%	%
Copper	56.03	56.01	56.01
	56.03		
	56.02		
	56.00		
	55.99		
Tin	0.36	0.37	0.37
	0.39		
	0.35		
	0.35		
	0.38		
Lead	0.26	0.24	0.21
	0.25		
	0.24		
	0.26		
	0.23		
Iron	0.82	0.83	0.82
	0.83		
	0.83		
	0.83		
	0.85		
Aluminum	0.64	0.63	0.61
	0.63		
	0.60		
	0.63		
	0.63		

[a] Manganese 0.42%.

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The views expressed in this paper are those of the authors and do not necessarily reflect the opinion of the Navy Department.

Determination of Small Amounts of Aromatic Hydrocarbons in Aqueous Solutions

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A method is described which employs formaldehyde–sulfuric acid reagent for the determination of small amounts (1 to 500 p.p.m.) of aromatic hydrocarbons in aqueous solutions with an accuracy of ±10%. The sensitivity of the test is 0.0001 gram of aromatic hydrocarbon.

THE determination of aromatic hydrocarbons in plant effluents or other aqueous solutions is a problem frequently encountered in laboratories. A reasonably accurate method for such analyses depends upon the development of a brown coloration when concentrated sulfuric acid containing a small amount of formaldehyde is brought in contact with an aromatic hydrocarbon under certain specific conditions.

Sulfuric acid containing formaldehyde has been used for detecting small amounts of benzene in air (*3*, *5*) a brown color being developed when the benzene-containing air is bubbled through the reagent. Toluene and coal-tar naphthas also give the test. Naphthalene is said to interfere by producing a black film. Thiophene and unsaturated hydrocarbons also interfere but can be removed by bubbling the air through sulfuric acid first.

An extension of this method has been reported (*4*, *5*), in which the degree of color development in the reagent is used in connection with a color standard to make a quantitative estimate of the amount of aromatic hydrocarbons present.

In the butanone method for benzene in air (*5*) the benzene is first nitrated to form *m*-dinitrobenzene, which is subsequently estimated colorimetrically with the aid of butanone. This method is both tedious and lengthy.

Another method (*5*) is also based on the nitration of benzene and subsequent separation of the *m*-dinitrobenzene from the nitrating acids by steam distillation. The amount of *m*-dinitrobenzene present is then determined by titration with standard titanous chloride solution. This method is also lengthy.

In the oxidation method for benzene in air (*1*, *2*) the sample is treated with hydrogen peroxide in the presence of ferrous sulfate, and the depth of the brown coloration which develops is taken as a measure of the amount of benzene present. Certain organic materials, particularly the more water-soluble solvents, interfere and must be removed before the test is carried out.

Of all these methods, the one employing the formaldehyde–sulfuric acid reagent seems to offer the best combination of speed, sensitivity, and ease of applicability to aqueous solutions containing aromatic hydrocarbons.

REAGENTS

Formaldehyde–sulfuric acid reagent, prepared by mixing 1.0 ml. of 37% U.S.P. formaldehyde with 100 ml. of C.P. concentrated sulfuric acid.

C.P. carbon tetrachloride (a technical grade may be used if it is washed with sulfuric acid until it remains colorless in contact with the reagent).

METHOD

The formaldehyde–sulfuric acid reagent is used as follows for determining the aromatic hydrocarbon content of aqueous samples: 100 ml. of the aqueous sample to be analyzed are shaken in a separatory funnel with 25 ml. of carbon tetrachloride. After separating, the carbon tetrachloride layer is carefully withdrawn and added to 5 ml. of the formaldehyde–sulfuric acid reagent in a 200-ml. flask. After vigorous shaking for 1 minute, the reactants are allowed to stand for 5 minutes, whereupon a brown discoloration of the acid layer develops.

The depth of this discoloration changes on standing; hence it is important that it be observed at some standard interval of time after shaking. The quantity of aromatic hydrocarbon present is estimated by comparing with a sample prepared at the same time and under exactly similar conditions from an appropriate quantity of an aqueous solution of known concentration of the aromatic hydrocarbon in question. For example, when styrene is being determined it is convenient to have at hand a standard solution of styrene in water. By trial and error the exact amount of the styrene solution of known concentration required to give a perfect color match with the unknown is determined. The styrene concentration in the unknown may then be calculated.

This procedure is actually applied to the determination of the styrene concentration in an aqueous unknown as follows: A standard styrene-in-water solution was made up containing 15 p.p.m. of styrene. Three trials were made in which (*a*) 5.0 ml. of the unknown were compared with 25 ml. of the standard, (*b*) 3.0 ml. of the unknown were compared with 50 ml. of the standard, and finally (*c*) 3.0 ml. of the unknown were compared with 60 ml. of the standard. The last mentioned gave a perfect color match between the unknown and the standard, so the concentration of styrene in the unknown was calculated to be $60/3 \times 15 = 300$ p.p.m.

SCOPE AND APPLICATION

Using the method outlined above, concentrations of aromatic hydrocarbons in water ranging from 1 to 10 p.p.m. may be determined with an accuracy of ±1 p.p.m. However, for higher concentrations it is necessary to dilute the sample with distilled water until it does fall within this range and then multiply the answer by a dilution factor. The inherent error of the method is multiplied by the dilution factor. Thus, for a sample containing 50 p.p.m. of an aromatic hydrocarbon, a fivefold dilution is recommended; and the final answer will be 50 ± 5 p.p.m.

Since the test is capable of detecting 1 p.p.m. of aromatic hydrocarbon in a 100-ml. aqueous sample, the sensitivity is actually 0.0001 gram of aromatic hydrocarbon.

The formaldehyde–sulfuric acid reagent has been applied to aqueous samples containing benzene, toluene, ethylbenzene, and styrene alone or in combination and in concentrations ranging from 1 to 500 p.p.m. It has been found that these aromatic hydrocarbons give different degrees of discoloration of the formaldehyde–sulfuric acid reagent; hence for the most accurate work it is necessary that the identity of the aromatic hydrocarbon be known. This is not necessary if only a rough estimate of concentration is desired.

For routine control work it was found desirable to have available a set of permanent color standards to eliminate the necessity of running a standard along with the unknown. Such a set of color standards was prepared by mixing appropriate quantities of colored inorganic salt solutions to obtain color matches with known samples containing 1, 2, 5, and 10 p.p.m. of aromatic hydrocarbon.

The partition coefficient of aromatic hydrocarbons between water and carbon tetrachloride is such that one extraction with carbon tetrachloride gives a complete removal from the water layer even in those cases where the aromatic content is very high.

Extraction reagents other than carbon tetrachloride may be used to remove the aromatic hydrocarbon from aqueous solution. (Dilution of the reagent prevents its direct application to aqueous solutions.) Diethyl ether results in a much lower sensitivity of the test; this very fact might be of value if the use requirements were such that high aromatic concentrations were frequently encountered.

EFFECT OF FORMALDEHYDE CONCENTRATION IN REAGENT

It has been recommended (*4*, *5*) that the formaldehyde concentration be 5 ml. of 37% formaldehyde per 100 ml. of concentrated sulfuric acid. In order to determine the effect of formaldehyde

concentration, tests were made in which formaldehyde concentrations of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 ml. per 100 ml. of concentrated sulfuric acid were employed on solutions containing 2, 4, and 6 p.p.m. of ethylbenzene. In all cases the low formaldehyde concentrations gave from two to three times the degree of color development obtained with the strong concentrations. Thus, for greatest sensitivity it is recommended that the formaldehyde concentration be kept low. Obviously one formaldehyde concentration must be accepted and maintained as standard to prevent any possible variation from this source.

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Determination of the Solubility of Styrene in Water and of Water in Styrene

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The Karl Fischer reagent and cloud point observations have been applied to the problem of determining the solubility of water in styrene between 6° and 51° C. The formaldehyde–sulfuric acid reagent as well as cloud point observations were utilized for determining the solubility of styrene in water between 7° and 65° C., and data are reported.

DATA on the solubility of styrene in water and of water in styrene and the effect of temperature on these mutual solubilities can be of considerable value when it is necessary to compute organic losses by way of aqueous plant effluent streams. Since no such data are available in the literature, the present investigation was undertaken.

SOLUBILITY OF WATER IN STYRENE

The Karl Fischer reagent (*2*) was applied successfully to the problem of determining water in styrene. The data shown in Table I and Figure 1 are average values of two determinations. The reproducibility of the method is excellent, as the difference between duplicate determinations never exceeded 3% of the average value. Equilibrium conditions were obtained by shaking a large sample of styrene containing a slight excess of water at a given temperature and then allowing it to stand in a water bath at this temperature for 24 hours to ensure complete separation of the two phases before withdrawal of a sample of the styrene phase for titration of the water present with the Karl Fischer reagent. The styrene phase was always clear, and it did not become cloudy even when cooled considerably from a higher temperature at which saturation conditions had existed. Cloud points never occurred when an excess of both phases was present.

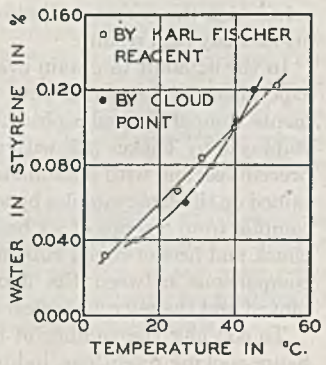

Figure 1. Solubility of Water in Styrene

Several precautions must be observed in applying the Karl Fischer reagent as described above. Samples of styrene stored for several days over anhydrous calcium sulfate showed negligibly small blanks. Since styrene polymerizes readily, fresh samples were used for each determination. The usual polymerization inhibitor, *p,tert*-butylcatechol, interferes even in the small amounts (10 p.p.m.) normally employed. The presence of this inhibitor in dry styrene caused rather high blanks, so styrene samples free of *p,tert*-butylcatechol were used in all the solubility determinations.

Table I. Solubility of Water in Styrene

Temperature ° C.	Solubility of Water in Styrene %
	Karl Fischer Reagent
6	0.032
25	0.066
31	0.084
40	0.101
51	0.123
	Cloud Point
14	0.040
27	0.060
34	0.080
40	0.100
45	0.120

In spite of the fact that during the determination of the solubility of water in styrene by means of the Karl Fischer reagent no cloudiness or visual evidence of phase separation was ever observed (perhaps because of the presence of an excess of both phases), under slightly different conditions it was possible to observe a cloud point and to make use of this phenomenon for solubility determinations. The technique employed was the same as that described below. The data shown in Table I and Figure 1 are averages of two or more determinations. Duplicate determinations gave cloud points agreeing within 1° C., except in the case of 0.120% water in styrene, where the agreement was within about 3° C. Cloud points at 0.040% water were very faint.

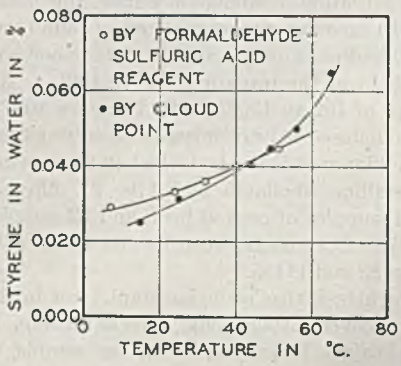

Figure 2. Solubility of Styrene in Water

SOLUBILITY OF STYRENE IN WATER

The solubility of styrene in water was determined by means of the formaldehyde–sulfuric acid reagent (*1*) and cloud point observations. (Table II and Figure 2). The value shown for 7° C. by formaldehyde–sulfuric acid reagent is the mean of three deter-

Table II. Solubility of Styrene in Water

Temperature ° C.	Solubility of Styrene in Water %
	Formaldehyde–Sulfuric Acid Reagent
7	0.029
24	0.033
32	0.036
40	0.040
51	0.045
	Cloud Point
15	0.025
25	0.031
44	0.040
49	0.045
56	0.050
65	0.058

minations. The one shown for 25° C. is the mean of two determinations; all other data are single determinations only.

During the course of the solubility determinations by means of the formaldehyde–sulfuric acid reagent it was observed that the system styrene-in-water exhibited a pronounced and seemingly sharp cloud point whenever the temperature was decreased from a higher value at which equilibrium conditions had existed. Use was made of this phenomenon to obtain further data on the solubility of styrene in water according to the following procedure:

A sample of styrene in water having a known styrene content was prepared and immersed in the water bath. The temperature of the bath was raised slightly above the temperature required for complete solubility of the styrene phase in the water phase and the sample shaken to ensure homogeneity. Then the bath temperature was allowed to decrease slowly and the temperature at which the first cloudiness of the sample occurred was recorded as the cloud point.

The cloud point exhibited by this system appeared to be very sharp, as the samples passed from a state in which they were clear to one in which they were cloudy within 1° C. The cloud point data shown in Table II and Figure 2 are averages of from two to eight separate determinations. Cloud points at 0.025% styrene in water were very faint.

LITERATURE CITED

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Excessively High Riboflavin Retention during Braising of Beef

A Comparison of Methods of Assay

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By comparison of values obtained with several variations in the fluorometric procedure for riboflavin as well as by comparison of fluorometric with microbiological values, no explanation could be found for the excessively high retentions encountered during braising of beef. Critical comparison of several current schemes for calculating cooking retentions in meats disclosed no fallacies in the method employed that could bring about falsely high results. It is suggested that there may be liberation of riboflavin during cooking from a precursor or complex of such a nature that neither the chemical nor the microbiological assays include it as "riboflavin" in the uncooked tissue.

THIS investigation grew out of the results obtained in a series of determinations of retention of riboflavin during the braising of beef roasts. In 19 such experiments on heel-of-round and chuck cuts, Tucker (*25*) had obtained an average total riboflavin retention of 110% with 15 of the retentions above 100%; the latter were in the range of 102 to 129%, with 12 above 105%. Such a trend was too pronounced to be dismissed as resulting from experimental errors in a fluorometric method which in 85 recovery determinations of crystalline riboflavin added to 31 different samples of raw beef, 31 samples of cooked beef, and 23 samples of beef drippings gave 76 recoveries between 90 and 105% with the remaining 9 between 83 and 111%.

It was possible, nevertheless, that some constant error in the method not exposed by recovery experiments, such as incomplete extraction, incomplete enzyme hydrolysis in the raw sample, or inadequate elimination of nonspecific fluorescent material in the cooked muscle or drippings, could account for the trend to excessively high cooking retentions. Furthermore, the method of calculating the cooking retention figures might be responsible for erroneously high results.

In the attempt to obtain evidence on these points two sets of experiments were carried out. In the first, two braising experiments compared results obtained by the fluorometric technique employed by Tucker (*25*) with those from four other fluorometric procedures, and with fluorometric and microbiological results obtained on the same samples by another laboratory. In the second, samples from a series of six braising experiments, including both chuck and heel-of-round cuts, were used to make more extensive comparisons between the fluorometric method originally employed and the microbiological method.

To consider the validity of the scheme of calculation used to figure cooking retentions, indirect evidence is here presented.

The fluorometric method which Tucker had used in her braising studies was a slightly modified procedure of that described for vegetables by Brush, Hinman, and Halliday (*3*), further details of which are reported under "Experimental Methods". It included a digestion of the samples with Clarase; adsorption on Florisil, originally suggested by Ferrebee (*7*) and Conner and Straub (*4*); oxidation of eluates with potassium permanganate as described by these authors; determination of individual eluate blanks made by exposure to light as suggested by Najjar (*15*) and applied to assays of cereals by Wright and Booth (*27*); and determination of "internal" standards as employed by a number of workers when determining fluorescence in digests directly (*8, 13, 16, 19*) or in eluates (*1, 2, 12*).

Since the question of adequate elimination of interfering materials which might either enhance or depress fluorescence readings was to be studied, it was decided to select four methods which combined purification steps such as adsorption and/or per-

manganate oxidation with various procedures for preparing blanks. The methods chosen for comparison were two developed by Andrews *et al.* which, like the original method (Method 1), employed Florisil adsorption but differed either by omission of the oxidation step (Method 2, *2*) or by its application before adsorption (Method 3, *1*), as well as by use of sodium hydrosulfite to prepare blanks; and two which did not include adsorption but varied from each other in the steps for purification of digests and techniques for blanks. These were the procedures of Peterson, Brady, and Shaw (Method 4, *16*) and of Kahler and Davis (*9*) adapted to beef by McLaren, Cover, and Pearson (Method 5, *13*).

In making these comparisons in which the fluorometric techniques per se were under scrutiny, identical digests were used, proceeding separately after enzyme digestion and clearing by centrifugation. Preliminary comparisons had shown no increase in riboflavin values—that is, better extraction—on conducting the acid extraction at 100° instead of 70° C. (used in all earlier work as well as in these experiments), nor had they shown improved extraction or hydrolysis of bound riboflavin when papain and takadiastase instead of Clarase had been used for enzyme digestion. This is in agreement with the results of Peterson *et al.* (*16*) on pork products.

On the other hand preliminary comparison of a 2-hour period (*4*) for Clarase incubation (0.4 gram per 100 ml., pH 4.5, and 48° C.) with "overnight" incubation had left no doubt as to the inadequacy of the 2-hour period, whether resulting from unadsorbability of "combined riboflavin" as convincingly suggested by Rosner, Lerner, and Cannon (*18*), from incomplete extraction, or from a less intensive fluorescence of some bound form. One of the latter two possibilities would seem necessary to explain the same type of result reported on pork by Peterson *et al.* when no adsorption was included. These authors adopted a 24-hour period for Clarase digestion, but in the present study a 20-hour period was chosen as the longest period which was compatible with continuing the assays on the day following the start of the analysis. However, in eight comparisons on older samples, neither 20- nor 24-hour incubation yielded values consistently or significantly higher than the 16- or 18-hour values. In only one such comparison were definitely higher results obtained after the longer periods and they showed no advantage for the raw sample, since the results on the cooked one were increased even more than on the raw. It was tentatively concluded that in the earlier study the high cooking retentions could not have resulted from less complete hydrolysis of the raw than of the cooked muscle and that the 16- to 18-hour incubation periods then used were probably adequate for both.

In this study the case for complete extraction and hydrolysis rests on comparison with the microbiological results submitted by the Wisconsin group in the first experiment and more especially on the more numerous comparisons with microbiological values reported here as the second set of experiments.

EXPERIMENTAL METHODS

Cooking and Preparation of Samples. *First Experiment.* Two raw samples, the paired second rib chuck roasts, were taken from the same animal. The pieces taken for cooking were the contiguous first rib chuck roasts. One, No. 34, was braised without searing and the other, No. 35, was braised after searing. Each was cooked without seasoning until the internal temperature of the roast reached 93° C. By this arrangement it was hoped to determine whether there was any difference in uncooked chucks taken from the right and left side of the carcass as regards the riboflavin value per gram of fat-free dry weight—in earlier experiments on paired roasts one was analyzed raw and one cooked. In addition, evidence was sought as to whether searing might be a factor in bringing about high cooking retention results by fluorometric determinations.

All meat samples were ground twice and mixed with an electric mixer after each grinding to secure homogeneity. Samples of both the raw and cooked meat and of drippings were frozen and sent to the University of Wisconsin for microbiological determination.

Second Experiment. Four boneless chuck and three heel-of-round roasts were used. These had been braised and the raw and cooked parts prepared for sampling by Meyer for an investigation on pantothenic acid and niacin retentions in cooking of beef (*14*).

Calculation for Cooking Retention, Both Experiments. In order to calculate cooking retention it was necessary to determine the fat-free dry residues of all the samples. The fat-free dry weights were used to calculate the total original riboflavin content of the cooked roast in its raw state by the following formula:

$$\text{Total riboflavin before cooking} = \frac{\text{micrograms per gram (raw analysis)}}{\%\text{ fat-free dry weight raw}} \times (\text{cooked roast weight} \times \%\text{ fat-free dry weight cooked plus drippings weight} \times \%\text{ fat-free dry-weight drippings})$$

Cooking retentions were then based on the total riboflavin contents of each of the cooked parts in relation to the above calculated total before cooking.

Fat-Free Dry-Residue Determinations, Both Experiments. These were done in triplicate on raw and cooked meat and on drippings.

Meat. Approximately 10-gram samples weighed by difference from weighing bottles were spread over the walls of Alundum extraction shells and dried to constant weight (within 0.01 gram) in a vacuum oven at 95° C. and less than 100 mm. of mercury pressure for 6 to 12 hours. Then dry samples within the shells were extracted with ether for 40 to 48 hours in Soxhlet extractors.

Drippings. Approximately 10-gram samples of drippings were extracted three times with 10-ml. portions of ethyl ether by thorough shaking in weighed Maizel-Gerson (thiochrome) reaction vessels into which the samples had been weighed. After 5 minutes or more of centrifuging the ether-fat layer could be quantitatively removed with a capillary pipet each time. The water suspensions left were evaporated to dryness by immersion of the vessels in a boiling water bath and finally brought to constant weight in a vacuum oven. Thus the fat-free dry residues were weighed directly.

Preparation of Digests for Fluorometric Riboflavin Determination, Both Experiments. *Meat.* Digests for riboflavin determinations were made by blending 50-gram portions each of raw and of cooked ground meat with 300 ml. of 0.1 *N* sulfuric acid in a Waring Blendor for 3 minutes. Aliquots of these blends equivalent to about 13 grams of meat were weighed into digestion bottles (250-ml. centrifuge bottles) and the volume of acid was brought up to 200 ml. The digests were stirred with electric stirrers for one hour at 70° C., protected from light. They were then cooled and incubated with 0.8 gram of Clarase, making its concentration approximately 0.4% during incubation at pH 4.3 to 4.5 and 45° to 48° C. for at least 20 hours.

Drippings. Digests of the drippings were prepared by first quickly transferring with a wide-ended pipet about 10- or 20-gram aliquots from well-shaken suspensions kept at about 50° C. to weighed 50- or 100-ml. volumetric flasks, respectively, and weighing these after cooling. Thirty or 60 ml. of 0.1 *N* sulfuric acid and sufficient sodium acetate plus Clarase were then added to bring the pH and Clarase concentration to about the same level as used for muscle samples during incubation, with temperature and time the same for all.

Clarification of Digests of Meat and Drippings. Following incubation, 5 ml. of chloroform were added to each bottle or flask to dissolve the fat and take it to the bottom. Then each was brought up to volume (230 to 245 ml. for the meat digests and 50 or 100 ml. for the drippings), shaken thoroughly, and centrifuged to clear. The volume of digest was taken as the capacity of the bottle or flask minus 5 ml. for the chloroform, except that in samples of meat drippings in the second series of experiments, the complete flask contents were transferred to 50-ml. graduated centrifuge tubes and the volumes of the chloroform-fat layers thus determined were used as the corrections on total volumes.

The swelling effect of fat on the chloroform layer would be appreciable only in the case of drippings, and for the dripping samples used in the first series of experiments it was later estimated to be about 3 ml. Since these were made to 100-ml. volumes, riboflavin values of the drippings as determined in this series were probably about 3% too high, resulting in percentage figures for riboflavin retentions in the drippings too high by about 1% and therefore negligible so far as the total picture is concerned. The authors are grateful to E. E. Rice of Swift and Co. for bringing this error to their attention.

Up to this point all operations were carried out in a semidarkened room and the digests were protected from light as

Table I. Riboflavin in Clarase[a]

Sample No.	Method 1, Adsorption, $KMnO_4$ Oxidation, Light Blanks	Method 2, No Oxidation, Adsorption, $Na_2S_2O_4$ Blanks (10–20 Mg.)	Method 3, $KMnO_4$ Oxidation, Adsorption, $Na_2S_2O_4$ Blanks (10–20 Mg.)	Method 4, No Oxidation, No Adsorption, $Na_2S_2O_4$ Blanks (Solution 0.5 Ml.)	Method 5, Filtration at pH 6.8–7.0, No Adsorption, NaOH Blanks
	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
A	2.2	8.5	..	..	..
B[b]	3.2	..	7.1	10.2	..
C	7.0	12.9	9.8	15.4	5.1

[a] Replicate determinations (2 or 3) on samples B and C were carried out in extracts of Clarase of concentrations near 0.013 gram per ml. Determinations on sample A and one additional series on sample C were done on extracts one fourth as concentrated. Average values of replicates are shown.

[b] Two microbiological determinations which were not in good agreement gave values of 3.6 and 4.4 γ per gram on this sample. No microbiological values were obtained on the other two samples.

much as possible. All subsequent operations were done in a dark room except when solutions were in brown bottles.

Fluorometric Methods. *First Experiment.* After centrifuging the digests, aliquots were taken for each method of analysis outlined below:

1. Adsorption, Potassium Permanganate Oxidation, Photolyzed Blanks. Aliquots of 25 ml. were adsorbed on 10- to 11-cm. columns of Florisil (2.0 to 2.3 grams). Elution with pyridine–acetic acid reagent (*4*) was carried to 45 ml. and duplicate 10-ml. aliquots plus 1 ml. of reagent were oxidized with 1 ml. of 4% potassium permanganate followed by 1 ml. of 3% hydrogen peroxide. The preparation of blanks and "internal" standards in separate qliquots as well as the photofluorometry were the same as those described by Brush *et al.* (*3*).

2. No Oxidation, Adsorption, Sodium Hydrosulfite Blanks. The adsorption and elution were the same as in Method 1 but Andrews' (*2*) method for treatment of eluates was followed with a modification of volumes. Three 10-ml. aliquots of the eluate were read, one with 1 ml. of added riboflavin solution for the internal standard, and the other two with 1 ml. each of added reagent. Blanks were obtained by reading each of these immediately after mixing with 10 to 20 mg. of crystalline sodium hydrosulfite.

3. Potassium Permanganate Oxidation, Adsorption, Sodium Hydrosulfite Blanks. The modified method of Andrews (*1*) was followed except that after the prescribed permanganate treatment of 25-ml. aliquots of digests, adsorption and elution were carried out as in Methods 1 and 2. Again 10-ml. instead of 14-ml. aliquots of the eluates were read. After addition of internal standards, volume corrections were adjusted to the volumes employed. Blanks were read without delay as in 2.

4. No Oxidation, No Adsorption, Sodium Hydrosulfite Blanks. The technique of Peterson, Brady, and Shaw (*16*) was followed exactly on 5-ml. aliquots of the beef digests.

5. Removal of Interfering Substances at pH 6.8 to 7.0, No Adsorption, Sodium Hydroxide Blanks. The technique described by McLaren, Cover, and Pearson (*13*) was applied to the Clarase digests of beef but otherwise followed exactly.

Preliminary to analyzing beef by the different methods, a digest of Clarase alone was made, using four times the concentration of Clarase usually employed in the meat digest, and replicate determinations of the Clarase blank values appropriate to each of the five fluorometric methods were made on this.

Second Experiment. The technique for fluorometric assays was the same as Method 1 in the first experiment.

Microbiological Assays. *Second Experiment Only.* The method of Snell and Strong as modified by Strong and Carpenter (*24*) was used, with one slight modification in preparation of neutralized filtrates of the digested samples. Although clear filtrates from the autoclaved digests were obtained at pH 4.5, the aliquots neutralized to pH 6.8 sometimes became slightly cloudy and, in one preliminary experiment, slight drifts were observed in microbiological values which were not eliminated by ether extraction. However, the solutions heated just to the boiling point at pH 6.8, quickly cooled, made to volume, and filtered, were clear again and gave microbiological values without drift. It was not necessary to treat drippings filtrates in this manner.

DISCUSSION OF RESULTS

First Set of Experiments. The results on Clarase alone are given in Table I. Sample C, used in this study, shows the same wide variation obtained by the several techniques in the riboflavin values per gram of Clarase as had been found in previous experiments with other samples of Clarase (A and B, older lots, in use in this laboratory in 1942 and 1943, respectively) using several of these methods. Such results seem to indicate that some of these fluorometric methods are not measuring all the riboflavin or that others are measuring other fluorometric materials in the Clarase. Since such large differences are surprising, Table II is presented to show a typical series of fluorometric readings on sample C, from which the values per gram of Clarase have been calculated. When account is taken of appropriate blank corrections, internal standard readings, and dilution factors, the relation of these readings to final values is obvious.

To make direct comparison of readings in Methods 1, 2, and 3 possible, the readings of Method 1 are also shown calculated to the basis of 11 ml. for final volume. It can then be seen that two thirds of the difference between Methods 1 and 3 results from the different technique in preparing the blanks. Apparently some of the permanganate-stable and light-stable fluorescence of Clarase is not stable to sodium hydrosulfite and is therefore included as "riboflavin" in Method 3. Method 2 includes with the hydrosulfite-stable and with the hydrosulfite-unstable fluorescence some fluorescence which could be destroyed by permanganate, and Method 4 presumably further adds to these some which could be eliminated by adsorption. The readings in Method 4 obviously are not directly comparable to the other three, since not only are the volumes somewhat different but the riboflavin fluorescence is more intense in the buffered water extract than in the pyridine-acetic solution and the instrument was set at a lower sensitivity for these readings.

Further, comparison of "external" standard readings indicates that there is a marked "quenching" effect on the fluorescence of the "internal" (added) riboflavin in all the three methods in which the oxidation step is omitted: Methods 2, 4, and 5.

In the corrected readings for food eluates, however, variations due to Clarase are canceled out by the applica-

Table II. Fluorophotometer Readings on Clarase Sample C[a] by Different Fluorometric Techniques

	Method 1		Method 2	Method 3	Method 4	Method 5
Digest represented in aliquot read, ml.	5.55[b]		5.55[b]	5.55[b]	5	5
Final volumes of aliquots, ml.						
a. Eluate or digest	13		11	11	10	15
b. Eluate or digest + 1 γ standard	13		11	11	10	16
c. Blank	13		11	11	10.5	16.05
Sensitivity setting of instrument, sodium fluorescein standard	70		70	70	55	70
Fluorophotometer readings[c]						
a. Eluate or digest	22.8	26.8[d]	39.1	29.5	66.1	67.0
b. Eluate or digest + 1 γ standard	52.5	61.9	70.0	65.5	98.2	89.4
c. Blank	7.9	9.1	10.5	4.4	34.1	54.2
Calculated						
Reading for riboflavin in eluate or digest ($a - c$)	14.9	17.7	28.6	25.1	31.3[e]	8.8[f]
Reading for 1 γ "internal" standard ($b - a$)	29.7	35.1	30.9	36.0	32.1	26.6[g]
Reading for 1 γ "external" standard under same conditions	30.0	35.4	36.3	36.3	43.9	34.0

[a] Concentration of Clarase in digest, 0.0128 gram per ml.

[b] 10/45 × 25.

[c] Not average but typical; all readings made with optical system suggested by Conner and Straub (*4*); Corning filter 511 only for incident light and No. 351 for fluorescent light.

[d] Calculated to 11-ml. volume for comparison with Methods 2 and 3.

[e] ($a - 1.05\ c$).

[f] ($0.937\ a - c$) for 5 ml. of digest in 16 ml.

[g] ($b - 0.937\ a$) for 1 γ in 16 ml.

Table III. Fluorophotometer Readings on Raw Beef Sample 35[a]

	Method 1	Method 2	Method 3	Method 4	Method 5
Fluorophotometer readings[b]					
a. Eluate or digest	28.7	40.4	37.3 (11 ml.)[b]	51.8	49.9
b. Eluate or digest + 1 γ standard	59.1	75.8	68.5 (12 ml.)	91.0	79.9
c. Blank	5.5	7.7	4.8 (12 ml.)	11.5	22.8
Calculated					
Reading for riboflavin in eluate or digest (*a* − *c*)	23.2	32.7	29.4[c]	39.7[c]	23.9[c]
Reading for 1 γ "internal" standard (*b* − *a*)	30.4	35.4	34.3[d]	39.2	33.2[d]
Reading for 1 γ "external" standard under same conditions	30.5	37.4	36.5 (12 ml.)	43.6	34.4
Correction for Clarase "riboflavin"					
On reading of aliquot of eluate	3.7	...	...	...	...
On calculated γ of "riboflavin" per aliquot of eluate or digest[e]	...	0.23 γ	0.17 γ	0.25 γ	0.083 γ

[a] Concentration of beef in digest, 0.0526 gram per ml.; of Clarase, 0.0032 gram per ml.
[b] Volume of digest represented in aliquot read, final volumes of aliquots, sensitivity setting of instrument, and optical system same as shown in Table II for readings on Clarase except for variation in final volumes in Method 3, indicated above.
[c] For Method 3 (0.917 *a* − *c*), for Method 5 (0.937 *a* − *c*), for Method 4 (*a* − 1.05 *c*).
[d] For Method 3 (*b* − 0.917 *a*), for Method 5 (*b* − 0.937 *a*).
[e] Correction for Clarase not applied directly as reading correction in Methods 2, 4, and 5, because "quenching" effect of 4-fold concentration of Clarase was greater (Table II) than "quenching" in beef digests with 1/4 as much Clarase content. In Method 3 correction also made in γ because of inadvertent variation in final volume in this experiment.

tion of the proper "riboflavin" correction for Clarase in each type of assay. The nonriboflavin fluorescence from the beef itself, at least in eluates, is not so large or so variable by the various techniques. Table III shows a typical set of fluorometric readings on a raw beef sample, No. 35, with appropriate Clarase corrections.

The riboflavin values for beef and drippings did not vary greatly by the different techniques. Table IV includes the average values obtained in this laboratory from four determinations, two on the fresh samples and two after 3 weeks' frozen storage, by each fluorometric method, as well as single microbiological and fluorometric values obtained by the University of Wisconsin laboratory on the frozen samples. [McIntire *et al.* (*12*) have reported the methods of assay in use in that laboratory at about this time. Their fluorometric technique was essentially like Method 3 in these experiments.]

To check for the possible loss of riboflavin and for the adequacy of these methods as a whole, single recovery determinations were included for each sample by each method studied (Table V). Except for Method 1, there was a wider variation in the percentage recovery of riboflavin by these different methods than is desirable, but the lower recoveries were not limited to the methods including adsorption. Good recoveries in analyses of canned vegetables and fruits (*3*) as well as fresh vegetables have also been obtained by this method (*22*, *26*).

From Table IV, it is noted that for both raw and cooked muscle samples, the method including no oxidative step nor adsorption, but using a hydrosulfite blank, Method 4 (Peterson *et al.*), gave definitely higher results than all others. This might indicate that a small amount of nonspecific fluorescent material eliminated by hydrosulfite is included as riboflavin in the analysis of the muscle samples by Method 4, and is eliminated by adsorption in other methods. This seems to be a safe deduction because the standard recovery tests do not indicate an excessive loss of riboflavin itself during adsorption. In Method 5 the use of alkali for a blank determination would seem to account for the difference from Method 4, since no appreciable precipitation was obtained at pH 6.8 to 7.0, and this filtration step thus would hardly seem responsible for removing interfering materials. However, if extra fluorescent materials are so included in Method 4, they seem to be reasonably proportional in cooked and raw samples, since cooking retentions in the roast (Table VI) obtained by using Method 4 are in line with the others.

In this experiment, although there were consistently higher retentions of riboflavin found in both seared muscle and drippings as compared to unseared ones in all fluorometric methods (except in drippings by the Wisconsin fluorometric method), it would be unreasonable to ascribe this trend to extra nonspecific fluorescent substances in the seared, since the microbiological values show a comparable difference in the same direction. Although the two roasts were presumably almost alike in riboflavin content before cooking, judging from the results on the paired raw cuts (Table IV), and although application of the "*t*" test indicated that the difference between the riboflavin retentions of the seared and unseared roasts, though small, is significant, caution must be used in assigning the cause to the searing itself. This seems especially important because in 12 other experiments on quicker methods of cooking beef, such as frying and broiling of steaks, extensive searing was carried out and yet retention of riboflavin exceeded 100% in only 3 instances with only one of these as high as 106% (*25*).

Thus, the comparison of fluorometric techniques exposed no distorted relationships between the assay results on cooked samples, seared or unseared, as compared to raw, and pointed to no solution to the problem of excessively high retentions of riboflavin in cooking.

Second Set of Experiments. The values obtained by both the fluorometric and microbiological assays for the riboflavin contents of four chuck roasts and two heel-of-round roasts are listed in Table VII, including results on raw and cooked muscles and on drippings. Excellent agreement is shown between the values obtained by the two types of assays, although a tendency to slightly higher values by the microbiological method is noticeable, especially in the case of drippings for the chuck roasts. In these, the difference reaches a maximum of 12%, but averages about 9%. The variations between the two assays on all the

Table IV. Comparison of Riboflavin Content in Beef Chuck Analyzed by Different Methods

Sample No.		Fluorometric Methods, Chicago Laboratory					Wisconsin Laboratory	
		Method 1	Method 2	Method 3	Method 4	Method 5	Fluorometric method	Microbiological method
		γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
34	Raw	2.19	2.28	2.27	2.84	2.19	2.15	2.40
	Cooked (unseared)	2.27	2.47	2.30	2.85	2.24	2.40	2.42
							γ/cc.	γ/cc.
	Drippings	2.33	2.65	2.47	2.64	2.40	2.92	2.64
							γ/g.	γ/g.
35	Raw	2.07	2.25	2.17	2.67	2.31	2.45	2.20
	Cooked (seared)	2.38	2.47	2.43	2.95	2.40	2.81	2.55
							γ/cc.	γ/cc.
	Drippings	2.48	3.05	2.57	3.10	2.92	3.08	2.64
	Comparison of values on paired raw samples based on fat-free dry weight							
							γ/g.	γ/g.
34	Raw	10.53	10.96	10.91	13.65	10.53	10.34	11.54
35	Raw	10.40	11.31	10.90	13.42	11.61	12.31	11.06

Table V. Percentage Recovery of Crystalline Riboflavin Added to Beef and Beef Drippings[a]

	Method 1	Method 2	Method 3	Method 4	Method 5
	%	%	%	%	%
Range	88–101	86–121	81–110	79–116	76–126
Average	97	99	94	103	90

[a] Ranges and averages of six determinations, one for each sample of raw and cooked meat and drippings.

Table VI. Retention of Riboflavin during Braising

Sample No.		Fluorometric Methods, Chicago Laboratory					Wisconsin Laboratory	
		Method 1	Method 2	Method 3	Method 4	Method 5	Fluorometric method	Microbiological method
		%	%	%	%	%	%	%
34	Roast (unseared)	71	74	70	69	71	77	69
	Drippings	25	28	26	22	26	33	26
	Total	96	102	96	91	97	109	95
35	Roast (seared)	79	76	77	76	72	79	80
	Drippings	28	31	27	27	29	29	28
	Total	107	107	104	103	101	108	108

chuck meat samples are from 3 to 6%, making an average of about 5% by which the microbiological values exceed the fluorometric, both for raw and cooked samples. In the two heel-of-round roasts all assays show even closer agreement.

As can be readily deduced from these results, the picture of cooking retentions would thus be comparable by the two methods of assay, with any difference being in the direction of higher retentions in the drippings and thus slightly higher total retentions by microbiological assays (Table VIII).

In summary of the total cooking retention picture it can be said that in only one roast out of seven was the retention under 100%, whereas among the other six, ranging from 101 to 122%, four showed total retentions above 105%. These results corroborate earlier findings, that riboflavin retentions above 100% by a significant amount are frequently obtained in beef braising experiments. And since the microbiological assays give similar results, it can be concluded that earlier excessively high cooking retentions obtained in such roasts, with fluorometric analyses, were not due to inadequate extraction or hydrolysis of the raw sample.

SCHEME OF CALCULATION OF COOKING RETENTION

The method of calculating cooking retentions on the basis of total fat-free dry weight of the cooked roast plus drippings assumed no significant change in the weight of the fat-free dry residue during the cooking process. Moreover, the B vitamins were assumed to be present in amounts proportional to the fat-free dry weights in the original raw cuts. These assumptions are probably not strictly valid, but are considered to be less in error for the type of samples used in this study than the supposition that their original total vitamin contents were proportional to their original moist raw weights, implying that the raw cuts of meat contained the same proportion of fat. Moreover, in order to calculate an original raw weight of meat which is cooked while attached to a bone, it must be assumed that the weight of the bone did not change significantly during cooking. The assumption about the homogeneity of the original raw samples obviously would be more valid for finely divided pieces such as boneless stew meat and especially for lean pieces such as liver slices. It is the basis for the method of calculation in which total moist weights before and after cooking are employed and has been used by McIntire *et al.* (*10, 11, 12*) and in most instances by Schweigert *et al.* (*20, 21*) and by Rice and Robinson (*17*). Even though this type of calculation seemed less valid for the beef cuts used in this investigation, it was decided to determine whether its use would greatly change the retention picture. The results so calculated are listed in the last column of Table VIII and although they are lower on the whole, the four highest are still above 105%.

The original method of calculation was also considered as preferable, especially for this type of cooking, to that employed by Cover *et al.* (*5*) in dry roasting of beef rib roasts or that by Rice and Robinson (*17*) in the vitamin retentions during drying of beef. The former used simple ratios of vitamin values per gram of fat-free dry residues after cooking to those before cooking and the latter the same sort of ratio for vitamin concentrations per gram of protein. Such calculations involve the same initial assumptions as those included in the method which was adopted in this laboratory. They differ only in that concentrations of vitamins are dealt with rather than totals. Such a calculation should be entirely valid in methods of processing, like the ones reported in those papers, where no significant loss of fat-free dry residues occurs in the drippings. However, in these braising experiments 7.5 to 10% of the original fat-free solids were found in the drippings and, therefore, the method of "totals" is a more accurate method of calculation.

If the high retentions, in this present study, as obtained by both assay techniques were due to a fallacy in the method of calculation on the total fat-free dry-weight basis, it would seem that other vitamin retentions determined on the same meat and calculated by the same method should tend to be parallel, even though, because of destruction, they run below 100%. On this presumption, pantothenic acid and niacin data by Meyer (*14*) on the same beef cuts were compared and no correlations in per cent retention of the different vitamins on the identical samples were found.

Table VII. Riboflavin Values in Beef Roasts

Sample No.	Fluorometric			Microbiological		
	Raw	Cooked	Drippings	Raw	Cooked	Drippings
	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
Chuck						
10	1.33	1.65	1.69	1.38	1.73	1.82
11	1.52	1.90	2.23	1.60	2.00	2.50
12	1.51	1.52	2.27	1.58	1.59	2.48
13	1.29	1.44	1.88	1.35	1.53	2.06
Av.	1.41	1.63	2.02	1.48	1.71	2.21
Heel-of-round						
1	1.69	1.98	1.75	1.72	2.01	1.77
3	1.58	2.08	1.90	1.57	2.17	2.00
4	..	..	..	1.83	2.30	1.85
Av. of 1 and 3	1.63	2.03	1.82	1.65	2.09	1.89

Moreover, in the frying and broiling experiments by Tucker (*25*), in which excessive retentions were not obtained, the calculations were made by the same scheme and the drippings, though small in volume, were also included.

Therefore, it would seem safe to conclude that the scheme of calculation is not responsible for the excessively high cooking retentions obtained in braising.

POSSIBILITY OF INCREASE IN ASSAYABLE RIBOFLAVIN DURING COOKING

Further support for the idea that the high riboflavin results sometimes obtained in cooking are not due to errors in the analytical procedures can be drawn from a few moderately high results reported but not always commented upon in the literature, most of them by microbiological assays.

Table VIII. Riboflavin Retentions in Braised Beef

Sample No.	Based on Total Fat-Free Dry Weights: Roast, Fluorometric	Roast, Microbiological	Drippings, Fluorometric	Drippings, Microbiological	Total, Fluorometric	Total, Microbiological	Based on Total Moist Weight: Fluorometric	Microbiological
	%	%	%	%	%	%	%	%
Chuck								
10	76	77	34	35	110	112	111	113
11	79	79	35	37	114	116	112	114
12	63	63	33	34	96	97	98	99
13	72	73	29	30	101	103	92	95
Heel-of-round								
1	78	78	26	26	104	104	99	98
3	80	81	39	41	119	122	115	114
4	..	81	..	26	...	107	...	106

McIntire *et al.* (*10*) report retentions of 106 and 105% in one sample each of veal stew and lamb stew and also (*12*) 108 and 109% in one sample each of broiled and braised beef liver including drippings, and Rice and Robinson (*17*) 107% in one sample of pork precooked for dehydration. These retentions were all based on total moist weights. Since, however, those reported by the first group are the exceptions rather than the rule, they could be considered to result from a combination of assay errors. Basing retention calculations on riboflavin values per gram of protein, the latter authors also report 109% for beef precooked for dehydration and 105% in the dehydrated beef, whereas Schweigert *et al.* (*21*) obtain 106 and 108% retentions in 2 out of 4 hams carried through the curing process. Rice and Robinson attribute their high retentions to the formation of stimulating substances (to *L. casei*) during cooking, whereas Schweigert *et al.* conclude that the best method of calculating the per cent of vitamin retention is on the basis of original and final weights of the samples tested because it is the most direct method. One wonders how much this conclusion was influenced by the fact that the alternative method of calculation, based on protein, brought these two riboflavin retentions and one niacin retention above 105%.

In the experiments here reported there was no indication of the presence of stimulatory material in the cooked samples, such as drift in microbiological assays. Standard recovery tests were also run with each microbiological assay and gave uniformly good results—96 to 98%. Moreover, one preliminary comparison of assays done with and without ether extraction of the filtrates from all three types of samples showed no alteration of values for any of them after ether extraction.

A recent report by Feaster *et al.* (*6*) shows that the phenomenon of increased riboflavin as determined by microbiological assay also occurs during storage of processed pork.

High retentions similar to those determined on beef have also been obtained in this laboratory in some cooking of vegetables assayed by the same fluorometric method.

Weinberger (*26*) reported 7 out of 13 determinations of retentions with several household methods of cooking cauliflower which fell between 109 and 129%, and 4 out of 10 on broccoli between 107 and 121%. Smith (*22*) has reported a few such high retentions in institutional boiling of carrots and green beans as well as in small-scale boiling of the latter. Another laboratory has likewise obtained such results on carrots using the microbiological method. Streightoff *et al.* (*23*) report an average riboflavin retention totaling 122% for boiled carrots plus boiling water in ten batches of stored miscellaneous varieties and of 112% in ten batches of fresh Chantenay Coreless carrots, during large quantity preparation. The senior author stated (personal communication), moreover, that the "data on riboflavin were very consistent" which must mean that a large majority of individual cooking experiments gave retentions above 110%, and added that they obtained "much the same sort of result in the case of cabbage".

In the light of all the above investigations it would seem that the probability of the liberation of riboflavin from a precursor or complex in certain types of cooking procedures must be given consideration. Moreover, since chemical and microbiological assays yield similar results, it would be necessary to postulate that the complex from which riboflavin is formed or released during cooking is of such a nature that the vitamin cannot be liberated by digesting or autoclaving under acid conditions as during the preparation of extracts, nor by digesting with Clarase in preparation for chemical assay, and also of such a nature that without release it is unavailable to microorganisms.

Long cooking as compared to short cooking should be further investigated and perhaps the possibility of an enzymic action in the tissue itself during a slow rise in temperature should be considered.

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Correction

In the article entitled "Quantitative Determination of Ergosterol, Cholesterol, and 7-Dehydrocholesterol" by Lamb, Mueller, and Beach [Ind. Eng. Chem., Anal. Ed., 18, 190 (1946)] an error was made in Table IV, where the antimony trichloride value on yeast sample 3 should have been 2.41.

Conductometric Titrations with Organic Reagents

Determination of Copper with Cupferron and Iron with Sulfosalicylic Acid

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The use of certain organic analytical reagents in conductometric titrations has been studied and cupferron found suitable for titrating copper in a sulfuric acid solution. The method determines copper rapidly in brass without removing tin and lead. Sulfosalicylic acid and its ammonium salt were used in the volumetric determination of iron. These compounds illustrate the use of organic reagents in titrations in which the change in conductivity is due to the formation of a soluble metallo-organic complex.

MOST of the analytical methods involving organic reagents are either colorimetric or gravimetric. In this work a study has been made of the use of organic reagents in conductometric titrations. Van Suchtelen and Itano (*21*) in 1914 titrated nitrate with nitron, calcium with oxalic acid, and a number of organic acids with barium acetate. Kolthoff and others (*6–8, 10, 16*) have used the amperometric titration method as a means of utilizing organic reagents in volumetric analysis.

Cupferron (ammonium nitrosophenylhydroxylamine) has been found satisfactory for the conductometric titration of copper. When a standard solution of cupferron is added to an acid solution of copper the very insoluble precipitate of copper-cupferron is formed and ammonium ions replace the copper ions in the solution. The reaction is

$$Cu^{++} + 2C_6H_5N(NO)ONH_4 = Cu[C_6H_5N(NO)O]_2 + 2NH_4^+$$

The release of the fast-moving ammonium ions causes a rapid increase in conductivity before the equivalence point is reached. After the end point, the combination of hydrogen ions with the excess cupferron causes a sharp decrease in conductivity. Typical titration curves are shown in Figure 1. Copper in brass can be titrated with a fair degree of accuracy without the removal of tin, provided the percentage of tin does not exceed 5%. Lead, zinc, nickel, and cobalt do not interfere but iron is precipitated with copper and causes high results.

The solution of cupferron is stable for at least 3 days if kept in a dark bottle. If allowed to stand for longer periods an appreciable quantity of nitrobenzene is formed which can be removed by pouring the solution through a filter paper. Germuth (*4*) has pointed out that the addition of 0.3 to 0.4 gram of *p*-acetophenetide to each liter of cupferron solution aids in its preservation.

APPARATUS

The apparatus consisted of an alternating current Wheatstone bridge arrangement with an electrically driven tuning fork and a Kohlrausch slide-wire. A Knight 7-watt amplifier, modified so that crystal earphones could be used, gives sufficient sensitivity of sound balance. The bridge is connected to the primary of a T-58A37 Thordarson transformer and the secondary of the transformer is connected to the phonograph plug in the amplifier. Apparatus similar to this is used for student conductivity experiments.

A more precise Leeds & Northrup conductivity measuring apparatus built around the Campbell-Shackelton shielded ratio box was used in checking the precision of the method, and a Coleman pH meter was used in following the change in hydrogen-ion concentrations in certain reactions.

The conductivity cell which easily accommodated 200 ml. was large enough so that no correction for dilution was necessary unless more than 10 ml. of titrant was used. Where it was necessary to add more than 10 ml., the corrected conductivity was obtained by multiplying the slide-wire ratio, $\frac{1000-a}{a}$, by the ratio of the final volume of the solution over the original volume.

The calculation of the actual conductance was eliminated by using the slide-wire ratio, $\frac{1000-a}{a}$, as the relative conductivity. Six readings, 3 before and 3 after the end point, were plotted against the number of milliliters of titrant used, and straight lines were drawn through the points before and after the equivalence point. The intersection of these two lines represents the equivalence point on the volume coordinate.

Some increase in precision was attained by calculating the end point according to the method described below.

END POINT BY CALCULATION

The inconvenience of determining the end point graphically can be avoided by a calculation similar to that of Boulad (*3*).

The equation for a straight line, $y = ax + b$, is used with the number of milliliters of titrant represented by x and the corresponding slide-wire ratio, $\frac{1000-a}{a}$, represented by y.

The slope, a, is obtained by picking two sets of readings before the equivalence point, which are far enough from the region of curvature to assure a straight-line relationship. The value of a is then used to calculate the constant, b, for each reading. An aveonge of the b values is used with the value of a to make an equation for the line before the equivalence point. Similar procedure with readings after the end point results in another equation. Simultaneous solution of these two equations for x gives the volume of titrant at the equivalence point.

TITRATION OF COPPER WITH CUPFERRON

SOLUTIONS. Cupferron solutions were prepared by dissolving 30 grams of reagent quality salt in 500 ml. of water. The solution was kept in a dark bottle and its strength was checked against a standard copper sulfate solution about every 3 days.

Standard copper solutions were made by dissolving accurately weighed samples (about 4 grams) of electrolytic copper foil in nitric acid. After the addition of 4 ml. of concentrated sulfuric

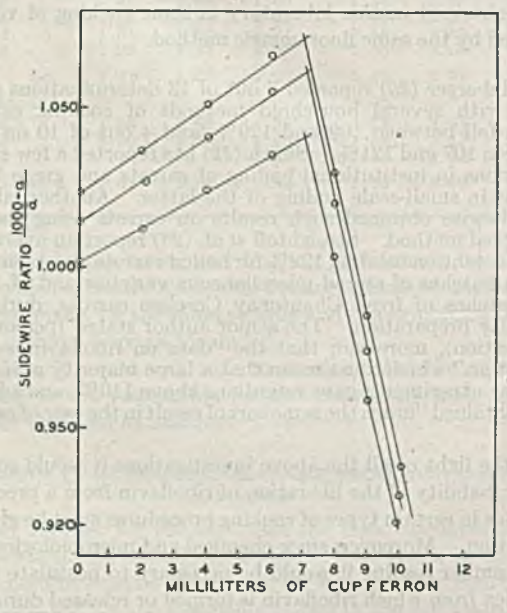

Figure 1. Cupferron *vs.* Bureau of Standards Bronze 52a

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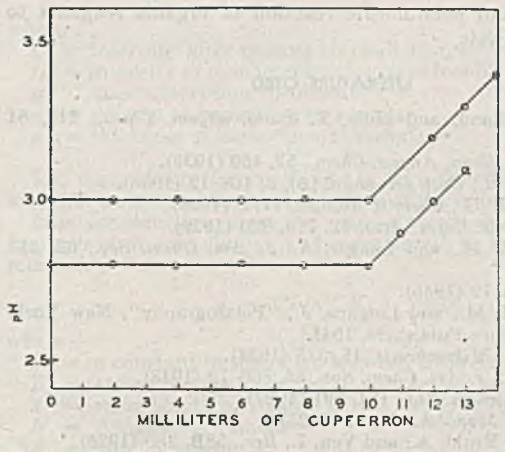

Figure 2. Cupferron *vs.* Bureau of Standards Bronze, Showing pH Change

acid the nitric acid was removed by evaporation to fumes, and the solution was diluted to 1000 ml.

PROCEDURE. Solutions of brass and bronze were prepared by dissolving weighed samples of William S. Murray brass samples and a bronze from the National Bureau of Standards, No. 52a, in nitric acid and proceeding in the same manner as with the standard copper solution. If the tin content of the brass or bronze was below 5%, no constituent or precipitate was removed. The bronze solution of Bureau of Standards 52a, which contains 14% tin, was filtered after the sample had dissolved in nitric acid. Other constituents of the brass or bronze were not removed.

A 50-ml. portion of the standard copper solution or of the brass or bronze was transferred by pipet to the titration cell and sufficient water was added to make approximately 200 ml. The cell was placed in a constant-temperature bath and the conductivity was measured. The standard cupferron solution was added from a microburet in about 2-ml. portions and the conductivity was measured after each addition. As the reaction between cupferron and copper is practically instantaneous, very little time is required for a titration.

Typical titration data, obtained by calculation, are plotted in Figure 1. The pH change at the end point is indicated in Figure 2. The appearance of this curve suggests that accurate pH measurements to three or four significant figures might be used to follow this reaction.

DISCUSSION

The time required for a titration was about 15 minutes and the calculation required about 5 minutes with a calculator, less time than the plotting of curves which would give the same accuracy.

The acid concentration is not a factor in the reaction; it was regulated to the extent that there was enough present to give a good curve after the end point and not so much that the conductivity became too high for measurement. The 4 ml. of concentrated sulfuric acid used in the preparation of the sample, with the subsequent dilutions, made a satisfactory acid concentration.

In accuracy the results show that the largest deviation is 0.8 mg. of copper per 100 mg. of copper taken, and that the average deviation is 0.3 mg. per 100 mg. of copper.

An attempt to analyze a bronze that contained iron, nickel, and antimony gave consistently high results.

The conditions of the analysis allow it to be fitted into the standard gravimetric procedure for brass.

Table I. William S. Murray Samples

No.	Samples Taken	Cu Taken	Cupferron Used	Cu Titer of Cupferron	Cu Found
		Gram	*Ml.*		*Gram*
33	2	0.1450	9.15	0.01583	0.1449
		0.1452	9.39	0.01551	0.1456
35	1	0.1245	8.56	0.01453	0.1243
36	2	0.1415	9.78	0.01453	0.1421
		0.1416	9.82	0.01453	0.1427
Bureau of Standards cast bronze 52a					
	4	0.0883	7.06	0.01258	0.0888
		0.0883	7.02	0.01258	0.0883
		0.0883	7.02	0.01258	0.0883
		0.0883	7.02	0.01258	0.0883
	2	0.0882	7.93	0.01115	0.0884
		0.0882	7.89	0.01115	0.0880

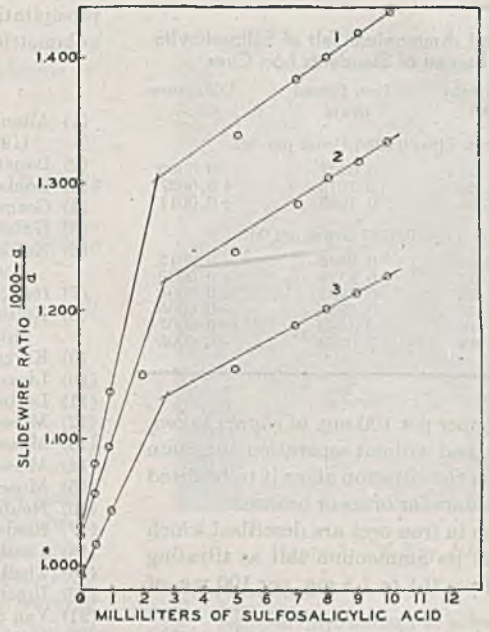

Figure 3. Sulfosalicylic Acid *vs.* Iron and Iron Ore

SULFOSALICYLIC ACID AND ITS AMONIUM SALT AS REAGENTS FOR IRON

Sulfosalicylic acid and its ammonium salt were found to produce usable curves in the titration of ferric iron (Figures 3 and 4). This acid has been used for separating iron from elements such as aluminum, manganese, magnesium, and others (*12–15, 17*) and in colorimetric determination of iron (*1, 2, 5, 9, 11, 18, 19, 20*).

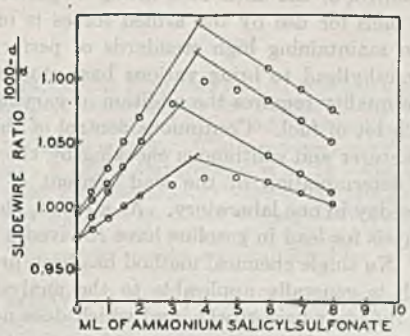

Figure 4. Iron *vs.* Ammonium Salicylsulfonate

Figure 3 shows the results when a 15% solution of sulfosalicylic acid, standardized conductometrically against iron wire, was used in determining iron in iron ore. Figure 4 shows the results when a standardized 10% solution of the ammonium salt of sulfosalicylic acid was used in place of the acid. The standard salt solution was prepared by dissolving the solid acid in ammonium hydroxide until acid to methyl red and titrating it conductometrically against standard iron wire. The calculated results in Table II show the accuracy of the methods. The iron solutions were prepared by the usual methods except that instead of reduction to the ferrous state, the iron was oxidized with either bromine water or Perhydrol to the ferric state.

SUMMARY

A method for the conductometric titration of copper in brass and bronze with cupferron is described, the accuracy of which

Table II. Sulfosalicylic Acid and Ammonium Salt of Sulfosalicylic Acid *vs.* Thorn Smith and Bureau of Standards Iron Ores

Sample	Iron Taken *Gram*	Titrant *Ml.*	Iron Found *Gram*	Difference *Gram*
15% Sulfosalicylic Acid, Iron Titer 0.0380 Gram per Ml.				
T.S. 72	0.0927	2.44	0.0927	0.0000
	0.1048	2.83	0.1075	+0.0027
T.S. 71	0.1072	2.85	0.1083	+0.0011
10% Ammonium Salt, Iron Titer 0.0247 Gram per Ml.				
B.S. 26	0.0813	3.36	0.0828	+0.0015
	0.1126	4.57	0.1128	+0.0002
	0.1126	4.56	0.1127	+0.0001
B.S. 27	0.1356	5.47	0.1351	−0.0005
	0.1356	5.46	0.1349	−0.0007
B.S. 29	0.0905	3.66	0.0902	−0.0003

varies from 0.2 to 0.8 mg. of copper per 100 mg. of copper taken. The method is fast and may be used without separation of certain constituents. The conditions of the titration allow it to be fitted into the usual gravimetric procedure for brass or bronze.

Methods for determining iron in iron ores are described which use both sulfosalicylic acid and its ammonium salt as titrating agents. The accuracy varies from 0.1 to 1.5 mg. per 100 mg. of iron taken.

The conductometric method may be used in applying the precipitation and colorimetric reaction of organic reagents to volumetric analysis.

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Determination of Tetraethyllead in Gasoline by X-Ray Absorption

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RIGID control of the antiknock rating of gasoline and other motor fuels for use by the armed forces is of utmost importance in maintaining high standards of performance. The use of tetraethyllead to bring various base stocks up to a set standard of quality requires the addition of varying amounts of lead to each lot of fuel. Continuous control of this process by the manufacturer and continuous checking by the user may involve the determination of the lead content of hundreds of samples per day in one laboratory. As a result, chemical methods of analysis for lead in gasoline have received a great deal of attention. No single chemical method has been proposed, however, which is generally applicable to the analysis of lead in chemically different base stocks, and which does not require an excessive amount of time for reasonably accurate results (*3*). Aborn and Brown (*1*) employed an x-ray absorption technique for the quantitative analysis of lead in gasoline, but their method was limited in sensitivity by the use of an ionization chamber to detect the radiation.

In the present work a Geiger counter was employed which had a quantum efficiency of roughly 80% for the radiation used. The result is a method for the quantitative determination of lead which is equivalent to the chemical analysis in accuracy, but may be completed by an unskilled worker in 5 minutes.

X-RAY ABSORPTION

Under the conditions necessary for the production of x-rays one observes a spectrum of continuous radiation upon which is superimposed a series of monochromatic characteristic radiations. The continuous spectrum (Figure 1) is composed of all wave lengths down to a certain minimum wave length given by $\lambda_0 = 12395/V$ Ångströms, where V is the peak voltage applied. The intensity of wave lengths longer than this minimum passes

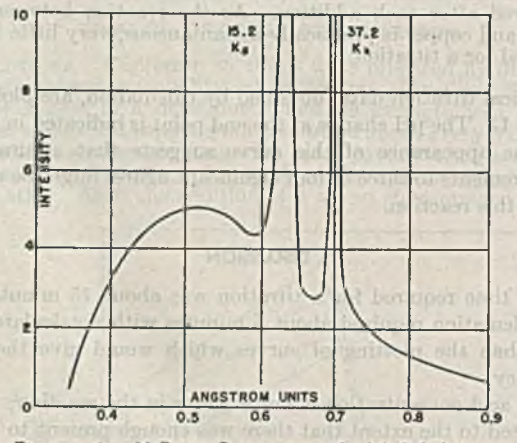

Figure 1. X-Ray Spectrum of Molybdenum Bombarded with 35-Kv. Electrons

through a maximum at about $\lambda = 1.3\ \lambda_0$ and then gradually approaches zero. The intensity of hard radiation in the continuous spectrum increases with atomic number of the target material. The characteristic K series radiations appear only when the peak voltage applied to the tube exceeds the K shell ionization potential of the target atoms. By the proper choice of target, voltage, filters, and possibly a crystal monochromator, almost any range of x-ray wave lengths or monochromatic radiation may be obtained.

When x-rays impinge on matter, the manner in which they are absorbed may be expressed by the relation:

$$I = Ie_0^{-\mu\rho x} \qquad (1)$$

where
I = intensity after passing through sample
I_0 = intensity of monochromatic x-rays incident on sample
μ = mass absorption coefficient
ρ = density of homogeneous sample
x = thickness of homogeneous sample

The mass absorption coefficient, μ, is a function of the wave length of x-rays and the atomic nature of the sample.

It is possible to express the mass absorption coefficient as a function of atomic number and wave length in the approximate relation:

$$\mu = (CZ^4\lambda^3)\ N/A \qquad (2)$$

where
C = a constant in the range between adjacent edges
Z = atomic number
λ = wave length of x-rays
N = Avogadro's number
A = atomic weight

At each critical absorption limit (corresponding to the ionization potential of the atom) the absorption coefficient reaches a maximum value on the short wave-length side of the limit. Where it is desired to identify one component of a mixture by absorption, the optimum contrast will be obtained by working with a wave length slightly shorter than the absorption limit for the particular component being measured. This is not always practical, since it is generally necessary to sacrifice intensity enormously to monochromatize the radiation. Because lead has a high absorption coefficient for practically all x-ray wave lengths compared to any organic material, no attempt was made to utilize an absorption edge in this particular analysis.

According to Equation 2 the mass absorption coefficient increases as the cube of the wave length. In a single-component system, therefore, one employs the longest wave length transmitted by the sample, thus obtaining a maximum change in intensity for a given change in sample thickness. However, leaded gasoline is a two-component system in which the mass absorption coefficients change at different rates as the wave length is varied. As a result an optimum wave length exists above which the advantage of using longer wave lengths disappears because the absorption coefficient for gasoline is increasing more rapidly than that of lead. With an absorption cell length of 15 to 25 cm. the optimum conditions were obtained with the x-ray tube operated at 17 kv.

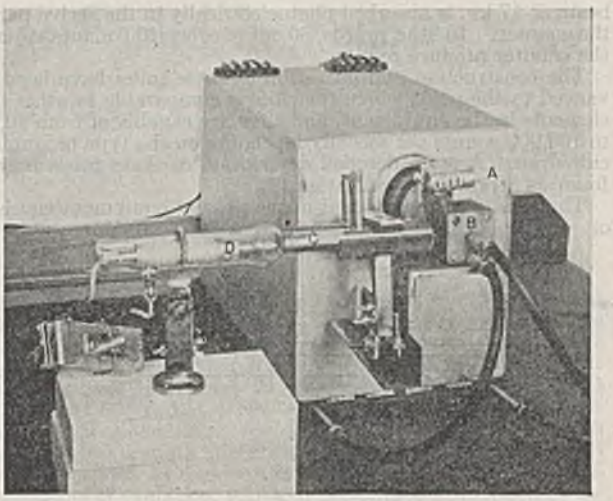

Figure 3. X-Ray Absorption Equipment

A. Power supply
B. X-ray tube
C. Absorption cells mounted on rocker arm for rapid comparison readings
D. Geiger counter

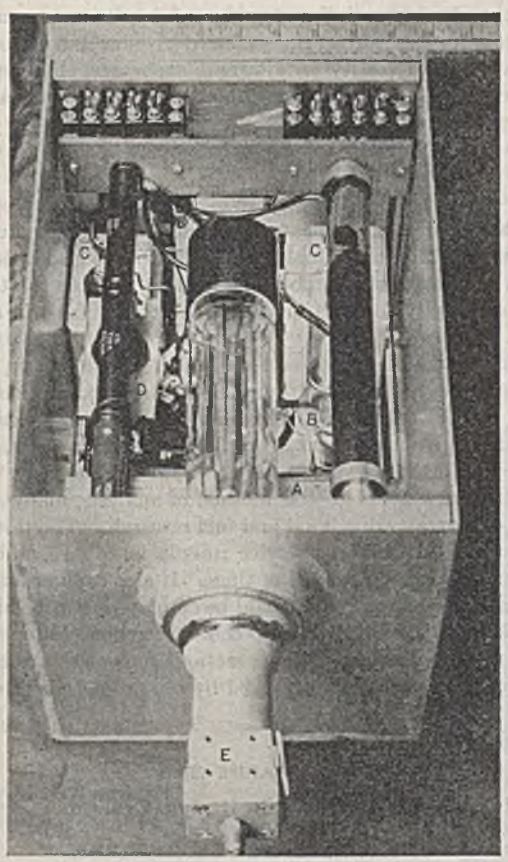

Figure 2. Internal View of X-Ray Power Supply

A. High-voltage transformer
B. 8013A rectifier
C. Capacitor
D. Filament transformer
E. X-ray tube

APPARATUS

The power supply (see Figures 2 and 3) consisted of a 25-kilovolt, 20-milliampere transformer, the output of which was half-wave rectified by an 8013 tube. A 0.05-microfarad condenser reduced the ripple below 10%. The x-ray and rectifier filaments were supplied by a single transformer with two secondaries. A molybdenum target Machlett x-ray tube of the line focus, water-cooled type was used. The entire supply including the x-ray tube was oil-immersed and occupied less than 28.32 liters (1 cubic foot). The power supply was stabilized by a line voltage regulator.

The x-ray supply and tube constitute a shockproof arrangement. The only safety hazard arises from exposure to the x-ray beam. The possibility of such exposure is minimized by ray-proof shielding, and the entire unit may be safely operated in an analytical laboratory.

The radiation detector was a Geiger counter tube developed by this laboratory. The success of the method depends largely on the fact that the Geiger tubes employed in this work were very efficient in the detection of soft x-rays. In its simplest form a Geiger counter consists of a cylindrical cathode and an axial wire anode, enclosed in a glass envelope containing a suitable gas mixture. A potential difference of the order of 1000 volts is applied across the electrodes in the manner illustrated in Figure 4. Under these conditions the passage of an ionizing radiation through the tube triggers a momentary discharge which may be detected as a voltage pulse at the capacitor, C. Following the discharge the counter quickly recovers to its initial condition and is ready to detect the next ionizing event. The high sensitivity of the counter is based upon the fact that a primary ionization of only a single ion pair is sufficient to trigger a momentary discharge of the order of 10,000,000,000 electrons.

In the range of wave lengths employed in these absorption measurements, x-ray quanta are absorbed almost entirely by photoelectric effect. The Geiger counters are constructed so as to permit passage of the radiation down the full 10-cm. length of the counter tube in the gas filling. Under these conditions, if a counter tube is filled with 40 cm. of krypton, 80% of the x-ray

beam at 17 kv. is absorbed photoelectrically in the active part of the counter. In other words, 80 out of every 100 quanta entering the counter produce counts.

The construction and operation of these tubes have been advanced to the point where their life is comparable to other tube elements in the equipment, and they are capable of from 10,000 to 100,000 counts per second, depending on the type of amplifier employed. A more detailed description of these tubes may be found in a previous publication (*2*).

The counting circuits used in the present equipment consisted of an amplifier capable of passing 25,000 random counts per

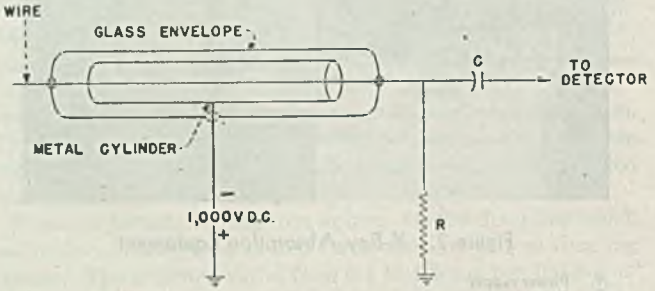

Figure 4. Fundamental Circuit of Geiger Counter

second coupled to a scale of 32, the output of which is registered by a mechanical impulse counter. In order to register 1000 random counts per second without missing more than 4%, the recording system must be able to count at least 25,000 uniform pulses per second. This is true because about 4% of the 1000 random counts arrive at an average rate greater than 25,000 per second. Since all measurements were made at a rate of well under 1000 counts per second, the recorded intensities were very nearly a linear function of the true intensity of radiation reaching the Geiger tube.

Recently the measurement of x-rays by means of a fluorescent screen and a multiplier phototube has been successfully applied in photofluorographic equipment by Morgan (*4*), and to the rapid examination of fuses by Smith (*5*). A comparison test performed at this laboratory showed that at x-ray intensities just sufficient to be observed above the background noise of the photomultiplier tube–fluorescent screen combination, the Geiger counter registered over 1000 counts per second.

EXPERIMENTAL

The unleaded gasoline used was standard aviation-base stock, 62-octane rating, and the fully leaded sample contained 4.6 ml. of tetraethyllead per gallon according to the accompanying chemical analysis. For the purpose of this work it was assumed to be correct and by careful dilution with the unleaded gasoline a series of samples was obtained ranging from 0 to 4.6 ml. per gallon in steps of 10%. The absorption cells were constructed of 15-cm. lengths of brass tubing (inside diameter, 0.5 inch, outside diameter, 0.75 inch) with 0.025-mm. (0.001-inch) aluminum windows.

In the optimum voltage range of 16 to 19 kv., and using a cell length of 15 to 25 cm., a ratio of intensities through leaded and unleaded gasoline of 140 to 1 was obtained. Counting rates of 75 to 1500 per second were registered when the tube current was about 5 milliamperes.

The ethylene bromide present in commercial ethyl fluid accounts for about one fourth of the absorption observed. Since a stoichiometric ratio of lead to bromide (or bromide-chloride mixture) is usually maintained in the ethyl fluid, this need not affect the accuracy of the measurements unless they are made on gasoline stocks whose history suggests that this ratio may have been altered. For special applications it may be desirable to work near an absorption edge of one of the components to minimize such interference. The present work covered only one base stock used with a particular ethyl fluid. Since the x-ray absorption coefficients for most base stocks are very nearly the same and much smaller than that of lead, a single calibration should be applicable to virtually all gasoline stocks using the same type of ethyl fluid.

In obtaining the calibration curve (a straight line), ten alternate 32-second counts were taken for each sample and averaged. Since the values plotted are ratios of counts obtained with clear gasoline to that of leaded gasoline, a total of 640 seconds or 10 minutes was required to locate each point. These results were obtained with an argon-filled counter. A counter filled with 40 cm. of mercury pressure of krypton was 6 times as efficient at 17 kv. and reduced the counting time correspondingly.

PERFORMANCE

By the method of least squares a calibration line was obtained which was more accurate than any one of the points, and by calculating the standard deviation of the points from the line (the square root of the mean of the squares of the delta y deviations from the line), an estimate was obtained of the precision of the determination. It was assumed in these calculations that all points have equal weight in the determination of the calibration line. This treatment is not rigorous, but a more exact treatment is not warranted. The standard deviation was 0.05 ml. of tetraethyllead per gallon of gasoline, or 1%. Similarly a precision of 1% was obtained with samples containing a maximum of 0.46 ml. of tetraethyllead per gallon. This corresponded to the detection of 0.005 ml. of tetraethyllead per gallon, or 0.0002% lead.

The precision attained here in the determination of lead in gasoline is comparable to that obtained by chemical methods. The x-ray absorption method is superior to the chemical method in most respects. It can be carried out in a routine manner by an unskilled operator in a small fraction of the time required for chemical methods. If conditions warrant it, the whole process can be made automatic for a number of simultaneous runs. The simultaneous exposure of the standard gasoline and the unknown will avoid the necessity for alternate exposures, eliminate errors due to fluctuations in x-ray intensity between successive exposures, and materially increase the precision.

OTHER APPLICATIONS

Although all measurements were made on leaded and unleaded gasoline, the method of analysis is applicable to lead in any other media of low atomic number. It will be possible to attain the same sensitivity for lead in any of the oils or fuels in common usage.

The method is not limited to heavy metals, however, because all elements have discontinuities in their absorption curves which may be utilized by careful control of the wave length employed to give maximum absorption for the particular metal desired. Further development of the technique may open up promising new fields for this method of analysis, many of which may be of great value in oil and fuel research. The testing of a good oil or fuel involves service runs in large mechanical units over extremely long periods of time. If a continuous method of analysis for metal particles could be employed, it is possible that "break in" and "break down" could be followed step by step. Wear rates of different metallic sections such as shafts and bearings could be selectively observed by the proper choice of radiation.

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Phenolic Resin Glue Line as Found in Yellow Birch Plywood

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A new technique for the analysis of phenolic resin glue lines is described. The method involves complete removal of the veneers by digestion with chromic acid, the glue being left in an unaltered state suitable for microscopic examination and subsequent stereophotomicrography. The relation of wood structure to the penetration of the adhesive is discussed with suitable illustrations.

PRODUCING stable physical and chemical properties in adhesive which bonds veneers is important at the present time, since it appears both desirable and possible to make strong wood structures possessing both variety in shape and diversity in size.

The fundamental theories of specific and mechanical adhesion were established to a considerable degree by the work of McBain and co-workers (*3*) in England and Browne and Truax (*1*) in the United States during and after the close of World War I. Although these and subsequent investigators have established the importance of specimen analysis strain test methods, and also the usefulness of cross-section analysis, they have left glue line thickness, the relative importance of specific and mechanical adhesion theories, and the variability of strength over limited areas, subjects of lively interest. These problems are discussed in the present paper in the light of work done with a new technique which permits examination of the surface features and structure of the phenolic glue line.

In order to carry out a comprehensive study of a plywood bond, factors such as glue line thickness, moisture content of veneers, gluing pressure, and temperature must be considered.

The glue line has been described as a continuous film of resin between two veneer surfaces and the thickness of this line has been correlated to the bonding pressure. McBain and Lee (*4*) and more recently Maxwell (*5*) claim that the strength increases with decrease of thickness. N. V. Poletika states that the strength varies inversely with the thickness (*6*). Browne and Truax (*1*) disagree with this view and say that the thickness of the film may vary within rather wide limits without affecting the strength of the joint.

The moisture contents of the veneers and of the resin before bonding are critical: when these are high, blistering results, and when low, the resin does not penetrate the superficial openings of the wood. In both cases the resulting adhesion is poor.

The bonding pressure employed depends on the flatness and density of the wood. The degree of polymerization depends on the temperature gradient and bonding time (*7*); hence the control of these conditions is an important factor in plywood manufacturing. The authors have been unable to find any reports relating all these factors with the nature of the resin bond under controlled conditions.

GLUE LINE EVALUATION BY SURFACE ANALYSIS

After careful examination of bleed-through resin removed from plywood by careful dissection it was shown that the resin was a true cast of the wood with which it was in contact. Chemical means were then employed for the removal of the wood. Hot chromic acid was selected as being most satisfactory for this purpose. This reagent has now been employed many times and has proved capable of digesting wood and not digesting phenol-formaldehyde resin within certain time and temperature limits.

The present standard practice is to suspend the test specimen in the hot chromic acid solution (100 grams of chromic acid in 100 cc. of water) with about 2.5 cm. (1 inch) immersed. The remainder of the specimen forms the support and carries the identifying code in notches on the upper edge. Digestion is carried on at 80° to 85° C. for about 20 minutes, which is sufficient to expose completely the glue lines in 3-ply $^1/_{16}$-inch birch veneers. After careful digestion, thorough washing with hot water is necessary to remove the chromic acid and the caramel-like products of oxidation, which, if present, obscure glue line detail. The specimen is then rinsed with acetone and allowed to dry. Though complete removal of this sludge is very difficult in the case of fiber casts, normal washing and drying are rapid and permit immediate examination of the resin.

The gross features of the glue line are examined with a Greenaugh twin objective microscope and areas of particular interest are stereophotomicrographed by a thoroughly tried method (*2*). During these observations and the subsequent photography particular attention is given both to those areas characteristic of a given kind of bonding and to those abnormal ones where failure is likely to occur first.

The real value of the technique depends upon the fact that high-power inspection with the microscope reveals the minute structure of the tracheae and tracheids. This inspection is carried out whenever evidence is required on the extent and thoroughness of the wetting by the unpolymerized resin of the wood.

For this examination the material is mounted in a medium of the same refractive index (normally about 1.66). Köhler illumination with narrow band filters complementary in color to the resin are employed.

MATERIAL EXAMINED

Many types of panels have been examined and their glue lines digested out, but a sufficient number of specimens from closely controlled experiments has not yet been examined to obtain a complete picture of the probable variability of the glue lines. Nevertheless, firm conclusions have been reached, illustrated by examples of glue lines from a set of panels made and tested by the Forest Products Laboratories at Ottawa, under controlled conditions.

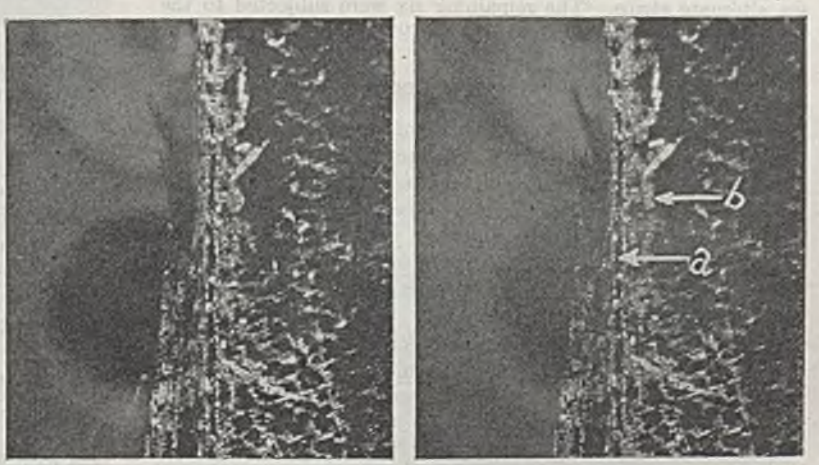

Figure 1. Low-Pressure Panel (× 7.5)

a. Large cavities are clearly visible in glue line. *b*. Very little penetration, as evidenced by hollow resin tendrils

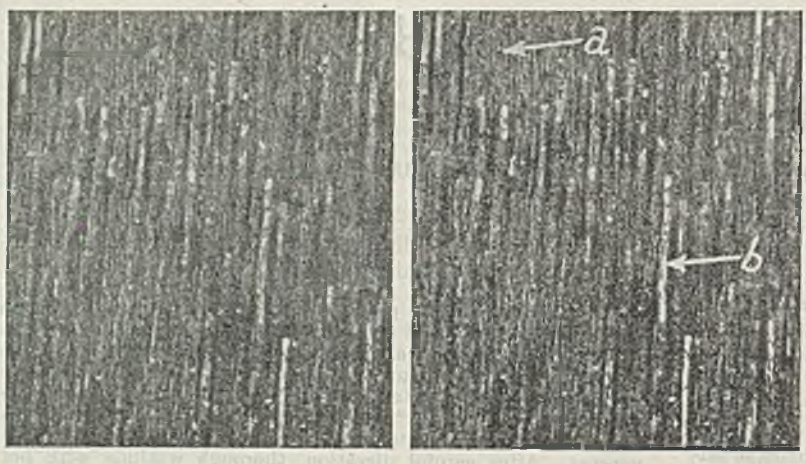

Figure 2 (*Left*). Normal Panel (× 7.5)

Stereophotomicrograph of resin glue line, showing how resin has filled all surface cavities, *a*, of veneer and penetrated into tracheae cells, *b*. Wood has been completely digested away.

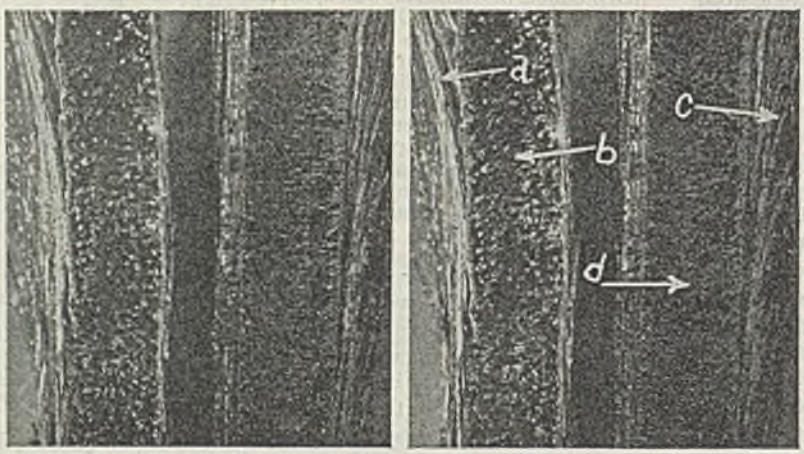

Figure 3. High Moisture Panel (× 7.5)

Stereophotograph showing end view of two specimens of plywood. Left specimen shows extreme penetration into one outside veneer, *a*, and center veneer, *b*. Right specimen shows extreme into one outside veneer, *c*, but almost no penetration into center veneer, *d*. This indicates extreme variation in penetration from point to point, as these two specimens were found within 1 inch of each other.

In all cases $^1/_{16}$-inch birch veneers and phenol-formaldehyde resin were used. The veneers were double-spread and the open assembly time was 1 hour. The veneers were bonded at 285° F. The bonding pressure, bonding time, and moisture content of the veneers were varied.

The panels were cut into 12 strips. Three tension normal to the glue line tests, and two shear tests were carried out on each of six alternate strips. The remaining six were subjected to the digestion treatment and examined microscopically.

LOW-PRESSURE BONDING

Figure 1 is a stereophotomicrograph of a typical edge, indicating the extreme thickness of the glue line and the noticeable lack of solid tracheal penetration present in a low-pressure bonded panel. This panel was made from veneers containing 9% moisture and was bonded under very low pressure for 8 minutes. The average value of the tension tests was 471 pounds per square inch. The shear test results were extremely low. Glue line failure occurred in almost every case.

Examination of the glue line from this panel showed that while wetting had occurred, there were open spaces in the glue line and that the casts formed within the tracheae were hollow tubes rather than solid rods.

OPTIMUM BONDING CONDITIONS

Figure 2 shows the solid character, the typically uneven thickness, and the intimate association of resin and wood which occurs in a good glue line. This illustration was made from a characteristic area of a 9% moisture panel bonded under 200 pounds per square inch for 6 minutes. The average value of the tension tests was 740 and the shear test was 195 pounds per square inch.

The peculiarities shown in Figure 2 were characteristic of not only the whole panel but also many other normal panels. The glue line was nonuniform in thickness, in most areas free from bubbles and composed of solid resin, in other areas discontinuous in nature. The resin had filled the broken surface cavities and passed into the open ends of the tracheae, tracheids, fibers, and medullary rays. The degree of penetration varied from place to place, depending on the orientation of the wood to the glue line and reached a maximum of about 5.0 cm. (Figure 4). Excessive penetration was ordinarily associated with starved areas. In these regions the glue line was very thin, broken, and frequently granular and the tracheae were usually just wetted, leaving thin-walled resin tubes.

HIGH-MOISTURE BONDING

Figure 3 shows a characteristic area in a panel made from veneers containing 14.9% moisture. They were bonded at 200 pounds per square inch for 6 minutes. The average value of the tension tests was 387 and of the shear tests, 132 pounds per square inch.

The very considerable penetration which occurred was characterized by complete wetting. The occurrence of large bubbles in the resin due to blistering resulted in the formation of tubes with thin broken walls. Because of the presence of steam or the ease of penetration under these conditions, the glue line was starved, thin, and discontinuous. When the orientation of the wood permitted, penetration to the surface occurred, and, as a result, produced bleed-through (Figure 4).

DISCUSSION

Many complete casts of the border pits present between tracheae in yellow birch have been found in spite of the fact that the pit diameter was of the order of $^1/_2$ micron (Figure 5). Though botanists believe that pits exist in the fiber wall, these either do not exist or else are too small for the flow of unpolymerized resin. However, many fiber casts have been obtained when the fibers were at the surface of the veneer and cut open during the cutting of the veneer. Wetting of the inner surface of the fiber is not often seen. It would seem that the presence of debris and air within the fiber produces an emulsionlike state which leaves the cast with a bubbly appearance and a rough surface (Figure 6). The surface characteristics of the resin trachea casts were identical with those of sectioned birch. The authors believe this evidence of the intimate contact of the resin and wood firmly sup-

Figure 4 (*Right*). Normal Panel (× 7.5)

Stereophotograph showing extreme penetration into one outside veneer, *a*, and center veneer, *b*. Very little resin has penetrated into outside veneer, *c*. Photograph indicates variation in penetration due to wood structure.

ports the theories of both specific and mechanical adhesion.

On the other hand, failure to confirm the views of McBain and Lee, Maxwell and Poletika, that there is a good negative correlation between the thickness of the glue line and the strength of the bond, appears to be related to the structure of the veneers used in making plywood. The veneer surface, normally variable in character from point to point, is more or less open, more or less superficially fractured, and possesses a chemical character different from metals, rubber, and plastics. These materials are homogeneous in a way in which wood is not, and it is reasonable that the thinness and the strength of the bond should show a good negative correlation.

This technique permits the critical examination of many kinds of phenol-formaldehyde glue lines. Striking variations in the glue line due to wood structure occur within areas smaller than 1 square inch (Figure 3). The stereophotographs reveal that yellow birch is normally wetted and freely penetrated by the unpolymerized resin. Since general wetting is usually observed in both good and bad panels, it is concluded that certain factors rather than the variation in specific adhesion of resin to wood are involved in the variable quality of plywood panels. The relation of these factors to observed types of failure is discussed below, although evidence is limited.

OBSERVED TYPES OF FAILURE

Normal Panel Failure. In panels where the glue line is relatively continuous and moderate penetration of resin into the adjoining vessels has occurred, failure ordinarily takes place within the wood near the glue line. The failure at this point is believed to be related to the fact that the surface structure of the wood in all sectioned material is necessarily damaged during cutting and that in badly cut veneers, the undamaged core may not be very thick. It would appear that a panel bonded under normal conditions cannot attain its maximum strength unless resin penetration has at least reached the undamaged core.

Low Pressure Failure. Examination of panels bonded under low pressure appears to indicate that failure is related to the decrease in contact area between resin and wood and especially to the fact that the wetted wood has probably been weakened during cutting. There is no indication that moderately thick glue lines are not strong. Cracked and brittle resin has not been indicated as a principal cause of failure.

High Moisture Content. Examination of high moisture bonded panels indicates that starvation is the essential cause of failure. The presence of excess moisture results in distribution so extensive that only small amounts of resin are present in the critical zone.

CONCLUSIONS

A new technique for phenolic resin glue line analysis permits the rapid analysis of glue line structure without resorting to the laborious and time-consuming methods of section analysis. It avoids damage to brittle glue line structures which cannot be sectioned even when the best methods of softening are employed. To obtain a good bond the resin must penetrate below the surface, which is usually damaged by the cutting tool.

Observations indicate that adhesion cannot be attributed to

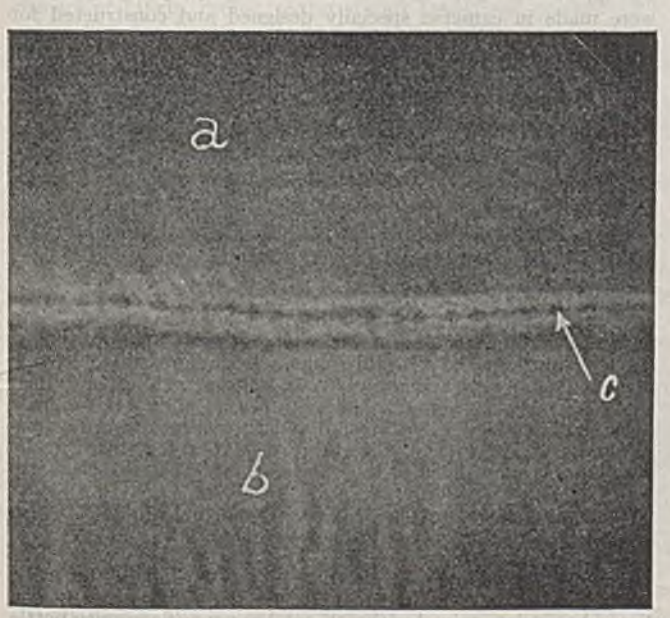

Figure 5. Resin Casts of Pits (× 1060)

Photomicrograph showing resin mold of two tracheae, *a* and *b*, and connecting pits, *c*

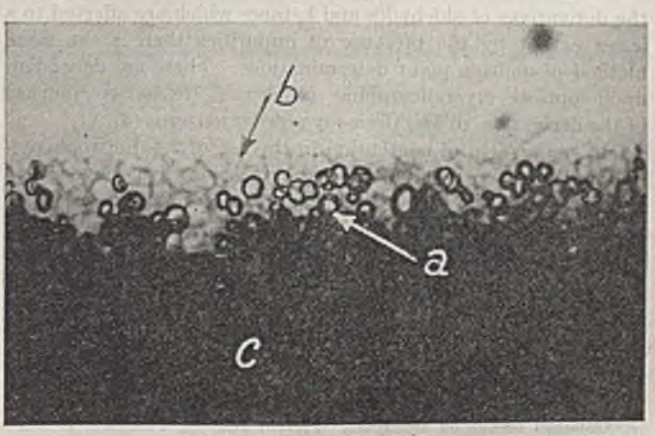

Figure 6. Fiber Casts (× 1240)

Photomicrograph showing granular structure, *a*, and rough surface, *b*, of fiber cast, *c*

either the specific or the mechanical theory but is dependent on the combination of the two.

ACKNOWLEDGMENT

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X-Ray Identification and Crystallography of Aldehydes and Ketones as the 2,4-Dinitrophenylhydrazones

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IN THE past few years a considerable amount of research has been undertaken to work out systematic and practical methods of organic analysis for carbonyl compounds using a solution of 2,4-dinitrophenylhydrazine (*2, 8, 13*).

The reagent is usually prepared by dissolving 2,4-dinitrophenylhydrazine in ethyl alcohol acidified with either hydrochloric or sulfuric acid. The addition of an aldehyde or ketone to such a solution usually results in the rapid formation of crystals of the corresponding 2,4-dinitrophenylhydrazone. If the aldehyde or ketone initially does not contain any great amount of impurities and if the derivative is carefully purified by recrystallization, the aldehyde or ketone undergoing analysis is identified from the color, optical properties, and melting point of the derivative.

When the unknown substance contains a mixture of aldehydes or ketones, the problem of identification becomes difficult since the melting point, which is the most widely used test, is so easily lowered by the presence of impurities. Furthermore, Brandstätter (*4*) has shown recently by thermal analysis the marked tendency of these hydrazones to form solid solutions with one another. Recourse is then usually taken to separation of the aldehyde or ketone by fractional distillation or crystallization before identification with the 2,4-dinitrophenylhydrazine reagent. Such a procedure is time-consuming and any improvement may be considered valuable.

Two instrumental methods have been suggested for identifying the derivatives of aldehydes and ketones which are affected to a lesser extent by the presence of impurities than is the usual method of melting point determinations. These are dependent upon optical crystallographic properties (refractive indices) of the derivative (*6*) and the x-ray powder patterns (*3*).

The major field of contention in the use of 2,4-dinitrophenylhydrazine as a reagent for the identification of aldehydes and ketones lies in the rather large number of modifications which have been reported from time to time. The exact nature of these modifications has caused much speculation. Two modifications of acetaldehyde–2,4-dinitrophenylhydrazone have been clearly shown to exist (*6*) as well as two modifications of derivatives of the furan series (*5*). Still other modifications of these and other compounds seem likely in view of the varied reports of crystalline properties and melting points (*1, 7, 12*).

[1] Obtained results on aldehydes. Present address, Tennessee Eastman Corp., Kingsport, Tenn.

[2] Obtained results on ketones. Present address, Shell Development Co., Emeryville, Calif.

The present x-ray diffraction investigation was undertaken, therefore, with the intention of clarifying uncertainties concerning these derivatives which are involved in this important method of identifying aldehydes and ketones, and of demonstrating the value of x-ray methods in many fields of organic analysis, both qualitative and quantitative, especially in series of related compounds of some complexity. In this paper are presented data on the unit cell dimensions and space groups of a number of aldehyde and ketone 2,4-dinitrophenylhydrazones, several of which are polymorphic; powder pattern data for rapid and certain identification by the organic analyst of aldehydes and ketones, together with techniques of handling micro or semimicro quantities of specimens; and a logical extension to quantitative analysis of pure and mixed aldehydes.

SINGLE CRYSTAL ANALYSES OF UNIT CELLS AND SPACE GROUPS

With single crystals which are ordinarily easily obtained, rotation, oscillation, and Weissenberg patterns have been made, the last type being especially valuable in this study; powder patterns were made in cameras specially designed and constructed for penicillin studies, in which the samples in cellulose acetate capillary tubes are rotated during exposure. Reciprocal lattice projections were made in all cases, but indexing of spots on the equi-inclination Weissenberg patterns was easily possible by inspection of zones. All pertinent data including probable space groups on the derivatives of formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, crotonaldehyde, furfuraldehyde, and dimethyl ketone are assembled in Table I. Typical procedures are illustrated with the case of the formaldehyde compound.

FORMALDEHYDE–2,4-DINITROPHENYLHYDRAZONE PREPARATION. The formaldehyde–2,4-dinitrophenylhydrazone was prepared by adding 40% formaldehyde solution to a hot concentrated solution of 2,4-dinitrophenylhydrazine dissolved in methyl alcohol approximately 2 *N* in hydrogen chloride. When cooled the crystals were filtered and air-dried, then recrystallized once from hot 90% ethyl alcohol and three times from hot isobutanol. The last recrystallization was slow and resulted in the growth of yellow needles and platelets about 2 to 5 mm. in length.

PROPERTIES OF THE CRYSTALS. The formaldehyde–2,4-dinitrophenylhydrazone crystals melted at 166° C. (uncorrected) agreeing with the literature (*6*). The density of the crystals as measured by floating in a mixture of methyl iodide and ethyl bromide was 1.592 ± 0.010 grams per ml., the density of the liquid being determined with a 25-ml. boot specific gravity bottle at 25° C. The needle-shaped crystals are similar to the platelets in being elongated along the *c* axis, the difference being in the

X-ray diffraction data are used to characterize 12 different crystalline aldehyde–2,4-dinitrophenylhydrazones from 6 different aldehydes and 16 ketone derivatives from 15 ketones using Weissenberg, rotation, oscillation, and powder patterns. This series was selected because of its importance in organic analysis of aldehydes and ketones; because there is no previous record of investigation outside of fragmentary optical data; and because it afforded excellent experience in extending the most modern techniques, particularly in distinction between polymorphic forms and in use of micro quantities of specimens. This study has involved the practical reciprocal lattice interpretation of the powder pattern of a triclinic crystal. Polymorphism is very generally observed in the series of hydrazones. Each is uniquely distinguished by its x-ray powder diffraction pattern far better than by color, habit, or melting point. A micromethod of qualitative analysis for pure and mixed carbonyl compounds is described. Aside from the necessary x-ray equipment the apparatus required is readily available. Ordinarily 0.1 mg. is the minimum amount necessary; however, single crystals smaller than 1 microgram may be identified. Tables listing the three most intense powder diffraction lines and the innermost line make possible the identification of 28 hydrazones. This method has the advantage of analyzing mixtures of carbonyl compounds which would be difficult by existing methods. The qualitative micromethod is converted to a quantitative method by accurate measurement of the line densities of known mixtures of the aldehyde derivative with sodium fluoride as an internal standard. Standard intensity ratios are given for a number of the aldehyde derivatives.

growth of the {100} form of the platelets. The angles between the faces parallel to the *c* axis were measured as the angles through which the needle-shaped crystal had to be rotated to bring successive faces into position to reflect a beam of light into a telescope placed on the Weissenberg camera. A cleavage plane making an angle of 52° with the *c* axis in the *ac* plane was observed.

Experimental X-Ray Data. Rotation and Weissenberg patterns for zero, first, and second layer lines were made around the *b* and *c* axes from which b_0 and c_0 unit-cell dimensions are directly measured, while a_0 is calculated from appropriate interferences on the zero layer line pattern from rotation around the *b* and *c* axes. No systematic extinctions are observed for zero, first, or second layer lines.

Since greater precision could be obtained with the powder camera of 7.0-cm. radius, patterns were made both on Agfa nonscreen film and Eastman Type A fine grain film; the latter pattern was then microphotometered with the Leeds & Northrup recording instrument.

The construction of the reciprocal lattice projection of a triclinic unit cell cannot be made with a simple drawing as can be done with orthogonal unit cells. From the projection of the *XY* plane the lines may be indexed as arising from *hk*0 planes; the *XZ* plane projection indexes the lines *h*0*l* and the *YZ* projection indexes the lines as from 0*kl* planes. These reciprocal lattice projections have been constructed from data obtained from the Weissenberg patterns as follows:

Consider first the lines from the planes *hk*0. The interferences from the zero layer line photograph about the *c* axis were tabulated in order of decreasing "d" values. The lines on the powder pattern will occur in this same order and may be rapidly indexed until the planes begin to overlap with the planes having indexes 0*kl*, *h*0*l*, or *hkl*. The intensity measurements serve as a check. The Weissenberg pattern of the zero layer line about the *b* axis was used in a similar manner to construct the *h*0*l* lattice layer. This procedure cannot be followed in indexing the *YZ* plane (0*kl*), since no Weissenberg pattern was obtained with the crystal rotated about the *a* axis. However, it was observed that the 021 spot on the first layer line Weissenberg pattern of the crystal rotated about the *c* axis was very intense. Calculation of the "d" value of this spot on the Weissenberg film leads to a value of 3.08 Å. This calculation was made using the equation

$$\cos 2\theta = \cos \phi \cos \mu$$

where θ is the Bragg angle, ϕ is equal to $(x/r)360/2\pi$ (where x is the measured distance of the spot from the center of the film, and r is the radius of the camera), and $\mu = \tan^{-1} q/r$ (where q is the distance from the zero to the first layer line). Knowing that line 13 on the powder pattern is the 021 line, it is possible to draw the reciprocal lattice projection of the 0*kl* plane.

From the reciprocal lattice projections of the powder pattern together with single crystal data from Laue, rotation, oscillation, and Weissenberg patterns it is possible to describe the unit cell with fair accuracy. We may then say that the unit cell of formaldehyde–2,4-dinitrophenylhydrazone is triclinic:

$a_0 = 10.00 \pm 0.03$ Å. $\quad \alpha = 94°$
$b_0 = 10.41 \pm 0.05$ $\quad \beta = 95°$
$c_0 = 4.23 \pm 0.03$ $\quad \gamma = 87°$

Since there were no systematic extinctions observed, the space group must be P1 or P$\bar{1}$. From the measured density of the crystal, the number of molecules per unit cell is 2.08 according to the formula

$$n = \rho V/1.65 M$$

where ρ is the density, V the volume of the unit cell in cubic Ångströms, and M is the molecular weight. This indicates two molecules per unit cell. The space group P1 has one equivalent position per unit cell, while space group P$\bar{1}$ has two. Since the formaldehyde–2,4-dinitrophenylhydrazone molecule must be asymmetric we may ascribe the probable space group P$\bar{1}$ to this crystal.

POLYMORPHISM OF 2,4-DINITROPHENYLHYDRAZONES

Proceeding in similar fashion 11 other aldehyde and 2 ketone derivatives have been analyzed as summarized in Table I. Only the formaldehyde and crotonaldehyde compounds have only one form; all others are di- or trimorphic. Analyses have indicated that these variations are not the result of impurities. In all cases the original aldehydes and ketones were purified so that any sample had a boiling point range of less than 1°. The powder diffraction spacings which are listed in Tables II and III are highly characteristic and reproducible for each compound. Both

Table I. Crystallographic Data of 2,4-Dinitrophenylhydrazones

Compound	Color	Habit	Solvent	M.P., °C.	System	a_0	b_0	c_0	Axial Angle	Density	n	S.G.
Formaldehyde	Yellow	Tabular (010)	Isobutanol	166	Triclinic	10.00	10.41	4.23	$\alpha = 94°$ $\beta = 95°$ $\gamma = 87°$	1.592	2	P$\bar{1}$
Acetaldehyde I	Yellow	Tabular (001)	*n*-Propanol	166	Tetragonal	7.15	7.15	18.69	.....	1.541	4	$P4_2/n$
Acetaldehyde II	Orange	Acicular[a]	*n*-Propanol	165	Orthorhombic	5.06	10.6	17.3	.....	1.51	4	$P2_12_12$
Propionaldehyde I	Red	Acicular[a]	Xylene	150	Orthorhombic	5.34	11.44	17.35	.....	...	4	$C222_1$
Propionaldehyde II	Orange	Acicular	Ethanol	148	.....	...	...	...	.....	...	..	....
n-Butyraldehyde I	Yellow	Acicular[a]	Methanol	123	Orthorhombic	4.90	17.9	54.0	.....	1.3	..	....
n-Butyraldehyde II	Orange	Acicular[a]	Methanol	122	Orthorhombic	7.0	25.0	25.8	.....	1.3	..	....
n-Butyraldehyde III	Amber	Tabular	Methanol	122	Orthorhombic	7.55	...	25.3	.....	...	..	....
Crotonaldehyde	Red	Acicular[a]	Benzene	190	Orthorhombic	4.63	13.05	18.30	.....	1.43	4	$P2_12_12$
Furfuraldehyde I	Red (deep)	Prisms	Acetone	223	Monoclinic	7.63	13.23	13.07	$\beta = 99°$	...	4	$P2/m$
Furfuraldehyde II	Red (light)	Pyramids	Acetone	218	Monoclinic	?	?	28.3	.....	...	..	....
Furfuraldehyde III	Yellow	Acicular[a]	Ethylene dichloride	199	Monoclinic	3.63	11.7	7.7	$\beta = 95°$	...	1	$P2/m$
Dimethyl ketone I	Yellow	Tabular	Ethanol	126	Triclinic	7.12	8.04	9.91	$\alpha = 66°$ $\beta = 86°$ $\gamma = 102°$	1.42	2	P$\bar{1}$
Dimethyl ketone II	Yellow	Acicular	Ethyl ether	114	Orthorhombic	5.22	11.0	22.6	.....	1.4	4	$P2_12_12$

[a] Elongation parallel to *a* axis.

temperature and solvent are factors in crystallization of a particular type. In two instances two kinds of crystals have appeared together but could be easily separated mechanically. An interesting general property observed is that the acicular crystals were all elongated parallel to the shortest unit cell dimension, and the tabular crystal had planes of flattening which were normal to the longest unit cell dimension.

Several of these modifications have not been reported in the literature as such—namely, acetaldehyde II, propionaldehyde II, butyraldehyde II and III, furfuraldehyde I, and dimethyl ketone II. On the other hand the reported acetaldehyde derivation with stable form from sublimation (*6*) could not be reproduced with as careful repetition of conditions as possible.

QUALITATIVE ANALYSIS WITH MICRO QUANTITIES FROM POWDER DIFFRACTION PATTERNS

Table II, which gives interplanar spacing "d" values and intensities of the three or four most intense lines from the powder x-ray diffraction patterns of the aldehyde–2,4-dinitrophenylhydrazones whose unit cell dimensions and space groups have been determined and listed in Table I, and Table III, of those values for ketone derivatives, provide a system of qualitative analysis for aldehydes and ketones. The amount of 2,4-dinitrophenylhydrazine derivative necessary for an x-ray powder pattern is about 0.1 to 0.5 mg.; however, satisfactory single crystal patterns have been taken of crystals smaller than 10^{-6} cc. (1 microgram). Fortunately, the required degree of purity is not nearly so great as that required for melting point determinations and the impurities themselves may be determined if present in quantities greater than 5%.

Apparatus. The apparatus necessary for preparing the derivative in micro quantities may be easily assembled from glass tubing and commercial semimicro equipment. The essential equipment consists of eye droppers with the ends drawn into short capillary tubes; small centrifuge tubes with constricted end for collecting the precipitate into a compact small space; small centrifuge and x-ray powder cameras.

For the x-ray patterns the powder samples were held in cellulose acetate tubes, prepared in a manner similar to that described by Fricke, Lohrmann, and Schroeder (*9*). For this qualitative analysis of very small amounts, the cellulose acetate tubes prepared on No. 24 Chromel wire have been found more suitable than those on copper wire, since in small sizes Chromel wires are more easily handled than copper wires. After stretching the wire coated with the cellulose acetate, the tubes have an internal diameter of approximately 0.35 mm.

The powder camera was designed for recording diffraction patterns of organic materials. The radius from sample to film is 7.00 ± 0.01 cm. Minimum exposure time is gained by placing the back-defining pinhole of 0.254- or 0.635-mm. (0.010- or 0.025-inch) diameter on the circumference of the camera and a guarded pinhole of 0.635 or 1.02 mm. (0.025 or 0.40 inch) in place 1.25 cm. (0.5 inch) from the sample. With these relatively large front pinholes, exposure times could be shortened without appreciably losing definition through line broadening from angles greater than 20° from the central beam. A conically machined collimating tube holding the camera to the Machlett-type diffraction tube assures positive alignment of the x-ray beam. A small beam trap and central mounting of the specimen rotated or oscillated by a reversing motor permit the registration of spacings of less than 40 Å. and measurement on both sides of the central beam.

Technique of Qualitative Analysis. The sample for such an exposure as that of formaldehyde–2,4-dinitrophenylhydrazone was prepared by adding approximately 1 drop of a 40% formaldehyde solution with an eye dropper to a 0.3 ml. of a methyl alcohol–hydrochloric acid solution of 2,4-dinitrophenylhydrazine in one of the small centrifuge tubes. This solution of 2,4-dinitrophenylhydrazine was prepared by dissolving 1 gram of 2,4-dinitrophenylhydrazine in 25 ml. of methyl alcohol containing approximately 2 equivalents of hydrochloric acid gas per liter of alcohol. Such a solution has been found relatively more stable for high concentrations of 2,4-dinitrophenylhydrazine than ethyl alcohol–sulfuric acid solutions. After allowing the solution of the formaldehyde derivative to stand 0.5 hour the tube was centrifuged, the liquid decanted, and the precipitate washed twice with 95% ethyl alcohol, then centrifuged and decanted again. A bit of filter paper was used to remove most of the wash liquid, then the remainder was removed by attaching the centrifuge tube to a vacuum pump for 15 minutes.

A portion of the dry sample in the constricted end of the centrifuge tube was transferred to a cellulose acetate tube by forcing some of the sample into the open end of the cellulose acetate tube, then tamping the sample solidly into tube with another Chromel wire.

Evaluation of Powder Patterns. Since there is a possibility of contaminating the sample with unchanged 2,4-dinitrophenylhydrazine and its hydrochloride, these powder patterns were taken; also a powder pattern of the hydrochloride after "decomposition" in a vacuum for 2 hours. This decomposition is accompanied by a change in color from yellow to orange red. These together with the derivatives of formaldehyde, acetaldehyde (2 forms), propionaldehyde (2 forms), butyraldehyde (3 forms), crotonaldehyde, and furfuraldehyde (3 forms) are listed in Table II with the three most intense lines in order of their relative intensities in a manner similar to that proposed by Hanawalt, Rinn, and Frevel (*11*). Since the innermost line in all cases is usually the most characteristic, it is also tabulated. Table III similarly lists these data for 16 ketone–2,4-dinitrophenylhydrazones. The use of the tables facilitates the identification of the unknown derivative. Further confirmation of the other weaker lines in the powder pattern of the unknown may be made by reference to the tables of "d" values listed for each standard pattern which in the interest of brevity are not tabulated in this paper.

Table II. "d" Values of Most Intense Lines of 2,4-Dinitrophenylhydrazine and Its Aldehyde Derivatives in Order of Decreasing Intensity

(Relative intensity I/I_0 in parentheses)

Compound	I	II	III	IV[a]
2,4-D.P.H.	3.50 (1.0)	3.15 (0.6)	5.81 (0.6)	8.9 (0.5)
2,4-D.P.H.–HCl	3.18 (1.0)	3.54 (0.8)	2.68 (0.5)	8.0 (0.2)
2,4-D.P.H.–HCl decomposed in vacuum	3.11	3.91	4.51	7.0
Formaldehyde, yellow	3.08 (1.0)	3.62 (0.97)	3.47 (0.88)	10.3 (0.86)
Acetaldehyde I, yellow	3.21 (1.0)	9.35 (0.70)	4.65 (0.37)	9.35 (0.70)
Acetaldehyde II, orange	9.2 (1.0)	3.20 (0.92)	6.8 (0.38)	9.2 (1.0)
Propionaldehyde I, red	9.6 (1.0)	3.24 (0.70)	4.30 (0.39)	9.6 (1.0)
Propionaldehyde II, orange	11.0 (1.0)	3.30 (0.8)	4.75 (0.3)	11.0 (1.0)
n-Butyraldehyde I, yellow	14.0 (1.0)	3.77 (0.32)	3.21 (0.31)	14.0 (1.0)
n-Butyraldehyde II, orange	11.7 (1.0)	3.50 (0.38)	3.48 (0.29)	13.7 (0.16)
n-Butyraldehyde III, amber	12.6 (1.0)	3.28 (0.83)	4.33 (0.26)	12.6 (1.0)
Crotonaldehyde, red	3.22 (1.0)	3.26 (1.0)	10.9 (1.0)	10.9 (1.0)
Furfuraldehyde I, red-black	3.17 (1.0)	3.20 (0.76)	4.20 (0.58)	13.7 (0.4)
Furfuraldehyde II, red	3.24 (1.0)	5.68 (0.3)	5.36 (0.19)	11.7 (0.03)
Furfuraldehyde III, yellow	3.26 (1.0)	6.17 (0.5)	7.7 (0.34)	11.7 (0.29)

[a] Innermost line.

Table III. "d" Values of Most Intense Lines of Ketone–2,4-Dinitrophenylhydrazones in Order of Decreasing Intensity

Compound of Ketone	I	II	III	IV[a]
Dimethyl I, yellow	3.27 (1.0)	5.70 (0.62)	9.30 (0.54)	9.30 (0.54)
Dimethyl II, yellow needles	9.45 (1.0)	11.15 (0.44)	3.03 (0.20)	11.15 (0.54)
Ethyl methyl, orange	12.45 (1.0)	3.25 (0.71)	7.10 (0.54)	12.45 (1.0)
n-Propyl methyl, yellow-orange	3.61 (1.0)	12.50 (0.78)	10.64 (0.66)	12.50 (1.0)
n-Butyl methyl, orange	12.80 (1.0)	7.75 (0.72)	3.47 (0.56)	12.80 (1.0)
n-Amyl methyl, yellow-orange	13.20 (1.0)	3.47 (0.70)	7.48 (0.48)	13.20 (1.0)
Isobutyl methyl, red-orange	13.70 (1.0)	7.60 (0.78)	3.48 (0.42)	13.70 (1.0)
Diethyl, red-orange	12.60 (1.0)	7.10 (0.76)	3.54 (0.37)	12.6 (1.0)
Di-*n*-propyl, yellow	14.9 (1.0)	3.44 (0.56)	7.80 (0.30)	14.9 (1.0)
Diisopropyl, red-orange	13.7 (1.0)	6.90 (0.78)	3.40 (0.62)	13.7 (1.0)
Diisobutyl, orange	11.20 (1.0)	3.72 (0.63)	4.45 (0.42)	11.2 (1.0)
Pinacalone, yellow	14.0 (1.0)	3.16 (0.52)	7.42 (0.50)	14.0 (1.0)
Cyclopentanone, orange	11.1 (1.0)	3.23 (0.58)	5.80 (0.32)	11.1 (1.0)
Benzyl methyl, beet red	3.31 (1.0)	10.5 (0.82)	6.15 (0.78)	10.5 (0.82)
p-Cl-acetophenone, scarlet	3.30 (1.0)	6.10 (0.60)	5.35 (0.42)	12.3 (0.32)
Benzophenone, red-orange	18.2 (1.0)	4.37 (0.80)	8.80 (0.75)	18.2 (1.0)

[a] Innermost line.

Table IV. Quantitative Data on Aldehyde–2,4-Dinitrophenylhydrazones–Sodium Fluoride Patterns

Compound	Weight Ratio, D.P.H. / NaF	Intensities			Intensity Ratios D.P.H.	
		NaF (200)	NaF (220)	D.P.H.	NaF (200)	NaF (220)
Formaldehyde	2.54	0.566	0.273	0.239	0.42	0.87
		0.607	0.295	0.254	0.43	0.86
	1.23	0.950	0.490	0.185	0.195	0.378
		0.968	0.472	0.207	0.214	0.440
	0.915	0.640	0.303	0.090	0.140	0.296
		0.680	0.328	0.100	0.147	0.305
	1.00 (av.)	...	...	...	0.165	0.338
Acetaldehyde I (yellow)	17.70	0.876	0.440	0.549	0.63	1.25
		0.855	0.428	0.506	0.59	1.18
	3.19	0.590	0.281	0.685	1.16	2.44
		0.551	0.274	0.579	1.05	2.11
	2.66	0.992	0.500	0.807	1.81	1.61
		1.11	0.521	0.910	1.82	1.74
	1.00 (av.)	...	...	...	0.345	0.695
Propionaldehyde I (red)	2.33	1.22	0.77	0.72	0.59	0.935
		1.19	0.70	0.72	0.60	1.03
	2.37	1.06	0.65	0.75	0.71	1.15
		1.06	0.67	0.68	0.64	1.01
	1.00 (av.)	...	...	...	0.270	0.438
Butyraldehyde I (yellow)	1.95	0.400	0.239	0.51	1.127	2.13
		0.370	0.203	0.45	1.22	2.21
	1.63	0.845	0.417	0.790	0.935	1.89
		0.727	0.398	0.770	1.06	1.93
	1.00 (av.)	...	...	...	0.597	1.14
Crotonaldehyde	1.90	1.05	0.505	0.458	0.436	0.907
		1.06	0.534	0.447	0.420	0.84
	1.15	1.32	0.715	0.370	0.280	0.519
		1.25	0.630	0.367	0.293	0.580
	1.00 (av.)	...	...	...	0.235	0.465
Furfuraldehyde I (red)	2.08	1.20	0.610	0.540	0.45	0.89
		1.19	0.622	0.470	0.393	0.755
	1.00 (av.)	...	...	...	0.202	0.395
2,4-D.P.H. (uncombined)	1.57	0.885	1.75	0.570	0.325	0.645
		0.970	1.87	0.638	0.340	0.657
	1.60	0.955	2.07	0.635	0.317	0.665
		0.910	1.60	0.560	0.350	0.615
	1.00 (av.)	...	...	...	0.209	0.408

QUANTITATIVE X-RAY ANALYSIS

The quantitative analysis of pure aldehydes by precipitation with 2,4-dinitrophenylhydrazine has been studied carefully by Iddles and Jackson (*12*), who reported yields greater than 95% for simple aliphatic aldehydes. Their method, using standard quantitative procedures, is accurate when the aldehyde may be obtained relatively free of other carbonyl compounds. The use of accurate density measurements of x-ray powder patterns of samples containing some internal standard makes possible quantitative analysis of samples of mixed aldehydes and ketones.

Quantitative analysis by the x-ray powder diffraction method has been reported for the analysis of mineral and metallurgical samples (*10*) using sodium chloride as an internal standard. These articles have dealt rather carefully with the theory of line intensities but lack in the presentation of satisfactory laboratory procedures. These articles report an accuracy of 5% in the determination. This is probably a good estimate for most crystalline compounds, but under favorable circumstances the accuracy may be greater.

The necessary steps for obtaining a good quantitative estimation of a mixture of two known compounds are then:

1. Calibration of the film used.
2. Preparation of a known mixture of the compounds in question with some definite amount of an internal standard such as sodium fluoride. The sample must be prepared in a finely divided state and thoroughly mixed, either by rapid evaporation or fine grinding, using some inert liquid as a dispersing agent.
3. Measurement of the relative line densities of the internal standard lines and the lines of the sample under consideration.
4. Correction of the relative line densities to give line intensities by using the film calibration curve.
5. Calculation of the ratio of line intensities of the internal standard and sample in question for a 1 to 1 mixture.
6. Preparation of another sample containing an unknown amount of the constituent in question with a known percentage of the internal standard.
7. Determination of the relative line densities from the powder pattern.
8. Correction of density to give intensity of the lines.
9. Calculation of the ratio of line intensities of unknown to internal standard. Multiplication of this factor by the ratio determined in step 5 will give the ratio weight of unknown to internal standard. Knowing the amount of internal standard in the sample (step 6) subjected to the x-ray examination, it is possible to calculate the amount of constituent in question present in the original sample.

When a film is used with a linear response to the x-rays, steps 4 and 7 may be omitted.

QUANTITATIVE X-RAY ANALYSIS OF ALDEHYDES. The quantitative analysis of aldehydes was accomplished in this manner. Sodium fluoride was chosen as the internal standard, since it could be obtained in large quantities having a particle size small enough to give smooth sharp diffraction lines as well as produce a relatively simple powder pattern with a minimum of absorption of the x-ray beam.

The samples were prepared by mixing weighed quantities of the aldehyde–2,4-dinitrophenylhydrazone with sodium fluoride. These were ground together carefully in an agate mortar, filled in a 0.35-mm. diameter cellulose acetate tube, and exposed to the x-ray beam in the 7.00-cm. powder camera for 2.5 to 3 hours using Eastman No-Screen film. The motor on the camera was set to rotate through 360° to smooth out any spots on the diffraction lines. The patterns were microphotometered with the Leeds & Northrup recording microphotometer.

Table IV lists the intensity data from the series of aldehyde–2,4-dinitrophenylhydrazone–sodium fluoride patterns. Two different standard lines (200) and (220) are chosen for sodium fluoride and the strongest line, usually corresponding to a "d" spacing near 3.20 Å., for the hydrazones. The two sets of readings for each pattern were obtained from the two sides of the film upon which the powder pattern was registered.

The 1 to 1 ratios of line intensities in the table were calculated by dividing the ratio of intensities of the aldehyde 2,4-dinitrophenylhydrazone to sodium fluoride lines (columns VI and VII) by the weight of the constituents of the sample (column II). These 1 to 1 ratios may now be used to calculate the weight ratio of unknown to sodium fluoride in any given sample, after qualitative identification of the aldehyde.

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Determination of Methyl Chloride in Air

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A rapid and convenient method has been developed for determining low concentrations of methyl chloride in air for safety control purposes. The method consists of passing a measured volume of air through an electric spark between carbon electrodes and absorbing the products of combustion in sodium arsenite solution. The solution is slightly acidified with nitric acid, and silver nitrate is added to develop silver chloride turbidity. The amount of chloride present is determined by comparing the turbidity of the unknown with that of standards similarly prepared. From analyses of synthetic blends in concentration ranges of 50 to 1800 p.p.m. the probable error of a single determination has been calculated to be ±10%.

METHYL CHLORIDE is used in the manufacture of rubber and in other industrial chemical processes, and various amounts inevitably escape at times from the units where it is in use. Because of its toxicity, its presence in the atmosphere in excessive concentrations constitutes a hazard to workers in the area. A method was desired which would determine the amount of methyl chloride in air, so that, if allowable tolerances were exceeded, appropriate precautions might be taken. It was required that the method provide satisfactory accuracy for safety control purposes, be explosionproof for use in areas containing gaseous hydrocarbons, require short elapsed time for a determination, be specific for chlorine compounds, and be portable, so that determinations might be carried out in any designated area of the plant.

Various methods have been described for the determination of low concentrations of methyl chloride and other organic halides in air (*2–4*). Although a high degree of accuracy has been reported for these methods, none seemed to provide the required rapidity, adaptability, or freedom from the interference of such substances as organic sulfur compounds. A method which meets the specifications reasonably well has been developed and is reported herein with experimental results obtained by the method in analyses of known methyl chloride–air blends.

APPARATUS[1]

Diagrams of the reactor tube and of the complete assembly for analysis of methyl chloride are shown in Figures 1 and 2.

The reaction chamber is made of glass, and surgical rubber tubing is used throughout to provide airtight connections. The purpose of the copper collar (*B*, Figure 1) is to prevent loss of air sample by diffusion through the carbon electrodes and to afford electrical contact with the carbon electrodes. The glass plug, *E*, serves to make the apparatus airtight and to aid in spacing the electrode gap. The flame arrester, *G*, which consists of a 2.5-cm. (1-inch) length roll of 100-mesh copper gauze inserted into a glass tube, prevents explosion of hydrocarbon mixtures which might be drawn into chamber *A*, Figure 2, which should be of known volume. A vessel of 250- to 300-ml. capacity should provide a sample large enough to give satisfactory accuracy for most measurements made for safety control purposes; however, for concentrations less than 200 p.p.m., greater accuracy may be provided by use of a larger volume of sample. The chamber is fitted with a three-way stopcock, so that the sample may be drawn into it directly from the area to be tested and later passed through the reactor.

The electrodes are made of 0.25-inch diameter spectrographic carbon rods. A supply of hollow electrodes (*C*, Figure 1) is prepared by the use of 0.125- and 0.063-inch metal drills. The absorber, *D*, is fitted with a fritted-glass disperser to ensure complete scrubbing of the combustion products. The fritted-glass disk should be porous enough so that a pressure of 4 to 6 cm. of mercury is adequate for passage of the sample through the sodium arsenite solution at the desired rate, in order to minimize the possibility of leakage. Experimentation has shown that one absorber is sufficient to ensure complete removal of chlorine and its compounds from the combustion products.

The complete assembly with such accessory parts as Nessler tubes, standard solutions, and buret should weigh less than 40 pounds when built into a compact portable unit. The electrical parts may be installed in this unit in a way which eliminates the explosion hazard of an exposed electric spark. As an alternative method of determining chlorides, a nephelometer may be built into the unit, and undoubtedly will provide greater accuracy of measurement than that provided by visual comparison of the unknown with turbidity standards. Since the apparatus can be used wherever 110-volt alternating current outlet is available, practically any point in the plant is accessible for a determination.

The apparatus must be checked to make sure that all connections are airtight, and any worn rubber connections replaced. Any leakage will, of course, reduce the accuracy of the determination.

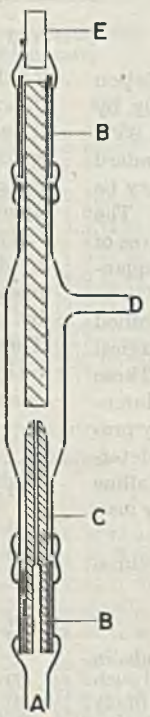

Figure 1. Reaction Chamber

A. Inlet
B. Copper collar
C. Hollow carbon electrode, 1/8 and 1/32 inch diameter
D. Outlet
E. Glass plug

REAGENTS

Sodium arsenite, approximately 0.1 *N*. Dissolve 4 grams of C.P. sodium hydroxide, then 4.9 grams of C.P. arsenic trioxide in 50 ml. of water. Make up to a volume of 1 liter with distilled water.

Standard chloride, 1 ml. containing 0.1 mg. of chloride. Dissolve 1.648 grams of fused C.P. sodium chloride in 1 liter of distilled water. Dilute 100-ml. aliquot of this solution to 1 liter to prepare a solution containing 0.1 mg. of chloride per ml.

Silver nitrate, approximately 1 *N*. Dissolve 17 grams of C.P. silver nitrate in 100 ml. of distilled water.

Nitric acid, concentrated C.P. stock reagent.

Phenolphthalein indicator.

The use of chloride-free reagents throughout is emphasized.

PROCEDURE

The sample to be analyzed is drawn by mercury displacement into chamber *A*. Before closing the stopcock to the outside after drawing in the sample, the stopcock between *A* and *B* is closed, so that the sample in *A* will be at atmospheric pressure.

About 15 ml. of 0.1 *N* sodium arsenite solution are added to absorber *D*. Sodium arsenite is used as the absorption medium, so that any chlorine or oxychlorine compounds that may be formed in the spark will be reduced to chloride.

The spark is started by connecting the primary of transformer *F* to a 110-volt alternating current supply. It is important that the electrodes be properly spaced and in good condition, so that an even spark will be obtained. Electrodes too greatly separated do not spark evenly, while those too close together tend to generate too much heat. This spacing must be determined by trial. Occasionally the electrodes become badly pitted and must be removed and reshaped. The reshaping can easily be done with a pencil sharpener and a knife.

The air sample in *A* is passed through the spark in the reactor and absorber *D* by raising the mercury reservoir, *B*, opening the three-way stopcock to connect *A* and *C*, and opening the stopcock between *A* and *B* enough to permit passage of a 275-ml. sample in 7 to 8 minutes. With experience the operator can learn to adjust the flow quickly to the desired rate of 30 to 40 ml. per minute. When all the air sample has been forced through the reactor, the one-way stopcock below *B* is closed immediately and the electrode spark discontinued. One hundred milliliters of chloride-free air should be used to flush out the reactor after each determination, since there is some retention of reaction prod-

[1] Patent applied for.

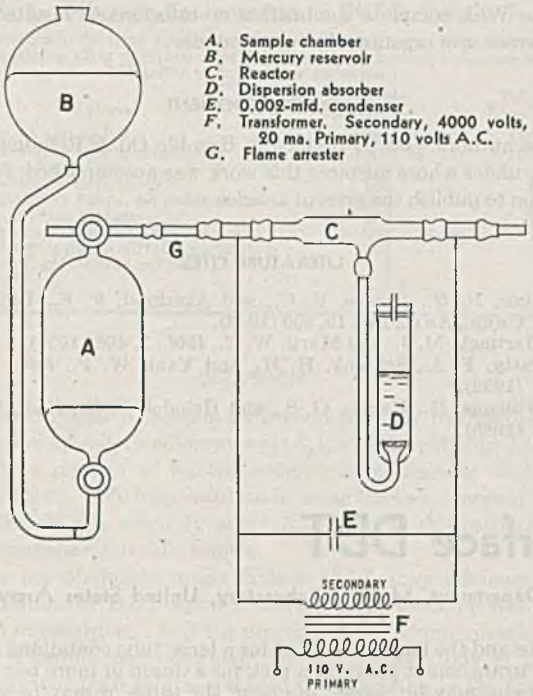

Figure 2. Complete Assembly

ucts in the reactor. A small container of chloride-free air may be used for this purpose. Care must be taken to prevent drawing sodium arsenite solution into the reactor or forcing mercury into it; otherwise the reactor will have to be taken apart and cleaned before it can be used again.

After passage and combustion of the air sample, the sodium arsenite solution from absorber *D* is poured into a 50-ml. beaker and the absorber rinsed with three 5-ml. portions of distilled water which is also added to the beaker. A drop of phenolphthalein indicator is added to the solution, which is then acidified with an excess of 3 drops of concentrated nitric acid. The acidified solution is poured into a 50-ml. Nessler tube, made up to volume with distilled water, and 3 drops of 1 *N* silver nitrate are added. The Nessler tube is stoppered and inverted several times to mix the solution thoroughly. For concentrations of methyl chloride greater than 1000 p.p.m., an aliquot part of the absorber solution should be taken for the chloride determination, so that the turbidity will not be too great to prevent satisfactory comparison with the standards.

Turbidity standards are prepared for comparison with the unknown by adding measured amounts of standard chloride solution to Nessler tubes, each containing 15 ml. of 0.1 *N* sodium arsenite neutralized and acidified with concentrated nitric acid. The standards are made up to volume with distilled water and 3 drops of 1 *N* silver nitrate are added. The amounts of chloride are chosen so that the unknown is matched or closely bracketed by the standard turbidity tubes. The standards are prepared at the same time the unknown sample turbidity tube is being prepared. With practice the operator can learn to complete an analysis in 30 minutes.

CALCULATION

The concentration of methyl chloride in the sample is calculated from the following formula:

$$\text{P.p.m. of methyl chloride} = \frac{N \times 1{,}000{,}000}{13.67 \times V}$$

N is the milliliters of standard chloride equivalent to the test sample in turbidity. *V* is the volume in milliliters of sample tested. The factor is obtained as follows:

The weight of combined chlorine in 1 ml. of methyl chloride at standard pressure and 27° C. is 1.44 mg. Since each milliliter of standard chloride solution contains 0.1 mg. of chloride, 1 ml. of methyl chloride gas is equivalent to 14.4 ml. of standard chloride solution. On multiplying 0.95, a correction factor to compensate for systematic error, by 14.4, the value 13.67 is obtained.

If an aliquot part of the absorber solution has been used to develop the turbidity of the unknown, the appropriate correction is made in calculating the concentration of methyl chloride.

EXPERIMENTAL

To evaluate the present method, synthetic blends for analysis were prepared by introducing methyl chloride, measured at room temperature over mercury in a buret, into a partially evacuated carboy. Analysis by combustion showed the methyl chloride used to be 96% pure; this purity value was taken into account in preparing the blends.

A carboy of 11.5-liter capacity was used to prepare blends containing more than 200 p.p.m. and a carboy of 19-liter capacity for those containing less. After admitting chloride-free air to atmospheric pressure, a 275-ml. sample was withdrawn into chamber *A* for analysis. Two withdrawals of 275 ml. each were made in some cases for samples containing less than 200 p.p.m. of methyl chloride. It was found that the relative error of a determination was less using a 550-ml. sample than using a 275-ml. sample of the same concentration. For safety control purposes the use of larger volumes of sample than 275 ml. will probably be unnecessary.

Distinction between a series of turbidities produced by 0.1-ml. increments of the standard chloride up to 1.5 ml. can be made by direct observation. Distinction between 0.2-ml. increments can be made up to 4.0 ml. of standard chloride, although the distinction is less easily made as the amount of turbidity is increased. These distinctions can best be made by examining the Nessler tubes vertically and against a dark background. As little as 0.005 mg. of chloride can readily be detected when compared with a blank. When a volume of 275 ml. of sample is analyzed, 0.1

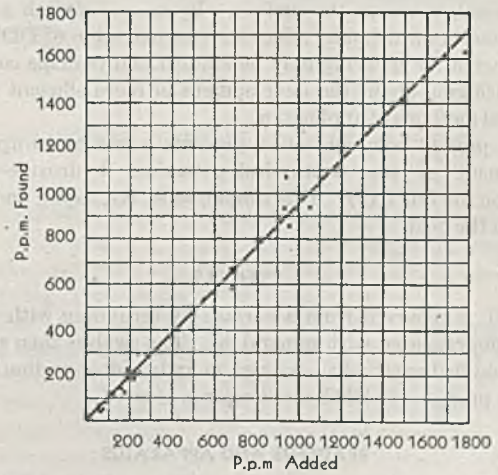

Figure 3. Methyl Chloride Concentration in Air Blends *vs.* Concentration as Found by Analysts

ml. of standard chloride corresponds to a concentration of approximately 26 p.p.m. of methyl chloride. Although measurements of turbidity by direct visual examination may be satisfactory for control purposes, the use of a nephelometer would undoubtedly improve the accuracy of these measurements (*1*).

RESULTS

The results of several determinations made on synthetic blends of high-purity methyl chloride in air containing 50 to 1800 p.p.m. of methyl chloride are shown graphically in Figure 3. Inspection of the data in which "p.p.m. added" values were compared with corresponding "p.p.m. found" values suggested that a systematic error was operative in the method. By application of the method of least squares to the data the slope of the curve of Figure 3 was calculated to be 0.95. This indicates that a detection of 0.95

of the concentration of methyl chloride present in a given sample of air may be expected by the method. Therefore this correction factor is included in the calculation to compensate for the systematic error. It is believed that the systematic error is attributable to the retention in the reactor of combusion products that are not completely removed by rinsing with chloride-free air.

OTHER APPLICATIONS

It is contemplated that the method described in this article will prove useful in the detection and determination of traces of various organic halogen compounds such as refrigerants in air. The method may, with proper modifications, be applied to the determination of traces of methyl chloride in hydrocarbon gases. Although a few determinations of methyl chloride in hydrocarbons containing organic sulfur compounds have been made by this method, no controlled work to evaluate its accuracy has been done. With complete combustion no interference resulted from the presence of organic sulfur compounds.

ACKNOWLEDGMENT

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Field Test for Surface DDT

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A simple field test for surface DDT is presented by which a determination can be completed in 2 to 3 minutes. Conditions which may be encountered in using the test are indicated.

THE application by spray of DDT in solution or in an emulsion, so as to leave a surface residue, presents numerous opportunities for using a fast simple field test to detect the presence of this substance on the surface. By means of such a test the many factors which may affect the concentration of DDT left on a surface after spraying may be studied and perhaps controlled, thus bringing about the development of more efficient and economical methods of application.

The present communication presents a test based upon a development of the xanthydrol–potassium hydroxide–pyridine reaction for DDT (*2*). It is simple, sensitive, rapid, and may be used in the field.

PRINCIPLE

DDT is removed from a surface by scrubbing with a cotton swab impregnated with mineral oil. The swab is then subjected to a modified xanthydrol–potassium hydroxide–pyridine reaction. In the presence of DDT a red color is secured.

REAGENTS AND APPARATUS

Only two reagents are required: a 0.4% solution of xanthydrol in pure anhydrous pyridine, and potassium hydroxide pellets, U.S.P. (either Merck or Baker). Some lots of C.P. pyridine are sufficiently anhydrous for use without purification, but most samples contain water. This may be easily removed by allowing the compound to stand in contact with stick sodium hydroxide for several days and then distilling. The xanthydrol-pyridine solution must be kept in a glass-stoppered bottle, and may be used for several days without deterioration. The hydroxide should be kept in a tightly capped container when not in use, in order that the pellets may absorb as little water as possible.

The apparatus consists of a supply of clean dry 17 × 150 mm. test tubes, a small alcohol lamp, a test tube holder, a 2-ml. pipet, and a number of oil-impregnated swabs. The swabs are prepared by wrapping a bit of cotton tightly around the end of an applicator stick, dipping it in clear liquid petrolatum, and then squeezing out the excess against the side of the container.

The reagents and apparatus may be packed into a suitable box or case for ease in carrying. The set used by the authors consists of a 72-tube wire test-tube rack such as is used for Wassermann tests. Wires were cut out to provide space for the reagent bottles and the lamp, as well as for a large tube containing swabs. This arrangement provides a rack for a dozen or more test tubes. The pipet may be carried in one of the tubes or may be secured to the rear, together with the test-tube holder, by means of clips.

PROCEDURE

An area of about 12 square inches (3 × 4 inches) of the surface supposed to contain DDT is thoroughly scrubbed with the oil-impregnated swab. A test tube is then inserted into the hydroxide bottle, and two pellets are picked up on the lip and allowed to slide to the bottom of the tube. The bottle is immediately recapped to prevent absorption of moisture. Two milliliters of 0.4% xanthydrol in pyridine are then added to the tube by means of the pipet. Using the holder, the tube is kept at a gentle boil over the alcohol lamp by moving it in and out of the flame. When the contents turn green, the swab is inserted, and the tube boiled for a few seconds longer. If DDT is present, the color changes to red. If no DDT is present, the green fades quickly to yellow. The entire procedure takes from 2 to 3 minutes.

EXPERIMENTAL

A large number of different surfaces known not to contain DDT, such as brick, cement, stone, tile, glass, paper, plaster, screen, wood, paint, metal, plastics, canvas, fruit, foliage, grass, etc., were swabbed and tested. No false positives were secured.

DDT in ether solution in concentrations varying by increments of 5 micrograms was measured directly onto a series of swabs. The ether was allowed to evaporate at room temperature and the swabs were subjected to the test. Twenty-five micrograms of DDT were detectable by comparison with a blank, while 75 micrograms gave a very distinct pink color.

DDT in 5% kerosene solution and 5% aqueous triton-xylene emulsion was applied to 3 × 4 inch (12 square inches) surfaces of commonly encountered materials in concentration varying by increments as follows: 1 mg. per square foot from 1 to 10 mg. per square foot, 5 mg. per square foot from 10 to 100 mg. per square foot, 25 mg. per square foot from 100 to 1000 mg. per square foot, 50 mg. per square foot from 1000 to 2000 mg. per square foot. Except in concentrations up to 10 mg. per square foot where 1 to 10 dilutions with kerosene and water were made, the solution or emulsion was applied without dilution. Application was made directly to the surface by means of micropipets, spotting the entire surface so as to obtain a uniform distribution of small drops in the lower concentrations, and a film of liquid in the higher concentrations. This was done to approximate the action of a spray, and at the same time accurately control the amount applied, as accurate control of the amount applied with a spray would have been impossible. After exposure for 48 hours at room conditions, the entire surface was scrubbed with a swab and tested for DDT. A positive test was considered as one which matched the color secured with a swab containing 75 micrograms of DDT. The minimum concentrations at which the test was positive are given in Table I.

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Table I. Minimum Concentration of DDT in Positive Test

(Mg. per square foot, as applied, at which positive tests were secured on various surfaces after application of DDT as a 5% kerosene solution and a 5% triton-xylene-water emulsion.)

Material	Kerosene Solution	Triton Emulsion
Tent canvas, untreated	1500	1500
Celotex building board, unpainted	1000	1000
Rubber matting, ribbed	750	50
Soft pine board, unpainted	100	75
Celotex building board, oil paint surface	250	25
Plaster, oil paint surface	200	25
Soft pine board, oil paint surface	200	15
Plaster, fresh, smooth surface, unpainted	15	25
Sheet metal, galvanized surface	5	5
Glass, plate	3	3

DISCUSSION

The xanthydrol–potassium hydroxide–pyridine reaction is not specific for 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichoroethane but is given by a number of related compounds containing aliphatic halogen (*1*, *2*). With due caution in using the test, however, this factor should not seriously affect its value, as the presence of such compounds is usually known.

There are obviously many factors which may influence the concentration of DDT upon a surface after its application in solution or emulsion. It is the purpose of this communication to present a simple method which may aid in the study of these factors, rather than to give detailed consideration to the factors themselves. Such things as the porosity of the surface to the solvent used, the presence or absence of moisture on the surface or in the material, the size of the droplets deposited by the spray, and even the temperature and relative humidity at the time the spray was applied may be important in determining the amount of DDT left on a surface. For this reason a statistical evaluation of the application of the test is impossible at the present time, and the data are presented only to convey an idea as to what may be expected in using the method. On the same basis, it is impossible to control all the conditions of experimental work, so that the figures are more a representation of order or magnitude than exact data. One rather definite conclusion can be drawn from the experiments, however. If conditions are such that more than 0.075 mg. of DDT can be picked up on the swab by scrubbing the surface, a positive test will be secured.

The experimental data presented deal with the surfaces of building materials. These materials were used because of their availability and because they suited the conditions of the experiment. Application of the test to fruit, grass, vegetation, and foliage leads the authors to believe that the conditions encountered in testing building materials apply to these surfaces also. In general, fruit and green foliage, being relatively nonporous, will retain a high concentration of DDT on the surface, while dry vegetation, absorbing more of the solution or emulsion, will have less of a surface residue.

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Colorimetric Determination of Fatty Acids and Esters

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A rapid colorimetric method for determination of fatty acids and esters is based on the formation of hydroxamic acid from fatty esters by the use of hydroxylamine hydrochloride in alkaline media. On the addition of an acidified solution of alcoholic ferric perchlorate, a stable red colored complex of ferric hydroxamate is formed, proportionate in intensity to the esters present. Fatty acids are first quantitatively methylated in an anhydrous ether solution with diazomethane or thionyl chloride. The method has been applied to the quantitative determination of oil on tin plate The results are in good agreement with a gravimetric determination.

EXISTING methods for the estimation of fatty acids and their esters are time-consuming and tedious and often are inaccurate when interfering substances are present. This investigation was undertaken to develop a rapid and accurate method for the determination of small amounts of palm oil, cottonseed oil, dibutyl sebacate, and lanolin applied to tin plate and other sheet metal surfaces for rust prevention, lubrication, or other purposes in subsequent manufacturing operations.

EQUIPMENT USED

Coleman Model 11 spectrophotometer and Cenco-Sheard-Sanford filter photelometer.

EXPERIMENTAL

The spot tests for carboxylic acids and esters are the basis for the proposed method (*1*). When an ester is warmed in an alkaline media with hydroxylamine, hydroxamic acid is formed. Feigl (*1*) reports the following reaction:

$$R.CO.OR + NH_2OH = R.CO(NHOH) + ROH$$

Ferric iron forms a bright red or a lavender complex with hydroxamic acids in acid media according to the reaction (*1*):

$$R.CO(NHOH) + Fe^{+++} = R{-}C\begin{matrix}\diagup \\ \diagdown\end{matrix}\begin{matrix}N{-}H{-}O \\ O\ldots Fe/3\end{matrix}$$

The red colored complex is readily soluble in aqueous ethanol, isopropanol, or methanol, being least stable in the isopropanol. The stability decreases with increased water content, about 5% of water being optimum for the three alcohols.

The ferric hydroxamate complex gains about 0.1% transmittancy per minute for the first 20 minutes but becomes rather stable beyond this, the rate being less than 0.05% per minute. A rise in temperature produces a gain of 0.2% transmittancy per degree at room temperature. The addition of a small amount of sodium carbonate increases the color stability beyond the figures shown above.

Hydrochloric acid and ferric chloride are used in a spot test by Feigl (*1*) to form ferric hydroxamate. The complex follows Beer's law when perchloric acid and ferric perchlorate are substituted for hydrochloric acid and ferric chloride.

Fatty acids do not form hydroxamic acid directly but are first quantitatively methylated in an anhydrous ether solution according to one of the following procedures and the esters determined in the usual way.

With thionyl chloride:

$$R.CO.OH + SOCl_2 = R.COCl + SO_2 + HCl$$

$$R.CO.Cl + CH_3OH = R.CO.OCH_3 + HCl$$

Or with diazomethane:

$$R.CO.OH + CH_2N_2 = R.CO.OCH_3 + N_2$$

Table I. Determination of Oil

Sample No.	Grams of Oil per Base Box	
	Gravimetric	Colorimetric
1	0.67	0.67
2	0.21	0.21
3	0.14	0.15
4	0.18	0.20
5	0.12	0.13
6	2.34	2.30
7	1.67	1.70
8	1.77	1.78
9	2.63	2.50
10	2.10	2.10

SOLUTIONS REQUIRED

Hydroxylamine hydrochloride 2.5% in 95.0% ethanol, made fresh daily.

Sodium hydroxide, 2.5% in 95.0% ethanol, made fresh daily. Saturated with sodium carbonate.

Solution A. Dissolve 0.4 gram of iron or equivalent amount of iron as ferric chloride in 5 ml. of concentrated hydrochloric acid. Add 5 ml. of 70% perchloric acid, evaporate almost to dryness, and dilute to 100 ml. with water. Pipet 10 ml. of this solution to a 1-liter volumetric flask, add 5 ml. of 70% perchloric acid, and make up to mark with 95% ethanol. Prepare weekly.

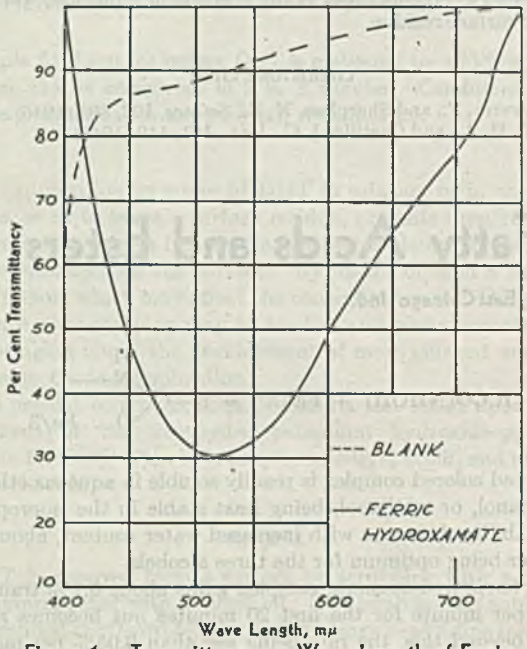

Figure 1. Transmittancy *vs.* Wave Length of Ferric Hydroxamate in 95% Ethanol

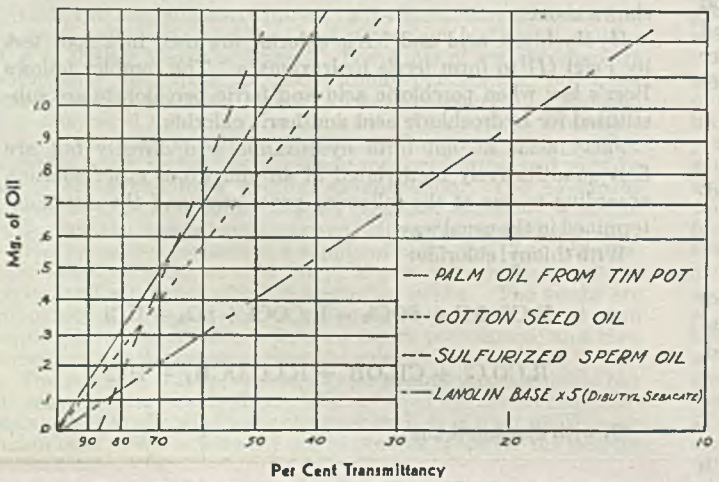

Figure 2. Concentration *vs.* Transmittancy for Five Oils

Solvent for fatty acids and esters, absolute ethyl ether or isopropyl ether free from acids or esters.

Thionyl chloride or a 5% ethereal solution of diazomethane.

Absolute methanol.

METHOD

For Esters. Obtain an absolute ethereal solution in a 250-ml. wide-mouthed Erlenmeyer flask containing from 0.05 to 1 mg. of the esters. Add from an accurate transfer pipet 0.3 ml. of 2.5% sodium hydroxide in ethanol and 0.3 ml. of 2.5% hydroxylamine hydrochloride in ethanol. Evaporate to dryness on a water bath at 60° to 70° C. and heat just 5 seconds longer. Add 10 ml. of Solution A, and let stand a few minutes until all salts have dissolved. Prepare a blank in a similar manner. Adjust the temperature to 27° C. and compare transmittancy in a 1.0- to 1.5-cm. cell at 520 mμ with blank. Obtain the amount of esters from a previously prepared ester *vs.* transmittancy curve.

For Fatty Acids. Obtain an ethereal solution containing 0.05 to 1 mg. of fatty acids in 10 ml. of ether, add about 3 ml. of thionyl chloride, and evaporate almost to dryness. Add a few drops of absolute methanol and heat briefly. Evaporate the excess reagents by blowing a gentle current of air into the warm beaker. Add about 20 ml. of anhydrous ether and complete the determination as for esters.

DISCUSSION

Comparison of wave length *vs.* transmittancy against a blank was made on a Coleman Model 11 spectrophotometer having a 35-mμ band. Figure 1 also shows the curve for ferric perchlorate blank. The measurements were made in a 1.5-cm. tube using 10 ml. of solution. Maximum absorption is at 520 mμ.

In adapting the method to Cenco-Sheard-Sanford photelometer, a 515-mμ filter was used with a 1-cm. cell. Sample sizes ranged from 0.05 to 1 mg. of cottonseed oil.

Because presence of water or alcohol in the ether solution interferes with the color development, anhydrous ether solutions should be used. Technical isopropyl ether was found to contain esters, but was readily purified by saponification with glycerol–potassium hydroxide solution followed by simple distillation.

Esterification of fatty acids may be carried out by adding an excess of diazomethane in ether and boiling off the excess. Diazomethane was prepared in sufficiently good purity by the method of Staudinger and Kupfer (*2*). Isopropanol was used instead of absolute ethanol. Since esterification with thionyl chloride was satisfactory, this method was adapted because of its economy despite the superior method of esterification with diazomethane.

The method may be applied to lubricating oils containing fatty esters without preliminary separation. Sulfurized sperm oil was determined in a heavy mineral oil by employing a suitable blank.

Preliminary work has been done on the determination of hydroxyl groups, the esterification being carried out by the use of acetyl chloride in ether solution. After the ether is evaporated, the excess acetyl chloride is destroyed with a few drops of water. After drying, the esters are determined in the usual way.

No attempt has been made to apply the method to the more volatile esters such as ethyl or butyl acetate but indications are that such determinations may be made by the use of isopropyl ether and a suitable reflux condenser.

RESULTS

The method has been applied in the determination of cottonseed oil on electrolytic tin plate and palm oil on hot-dip tin plate. Lanolin-base oil on black plate has also been determined. Comparative results between gravimetric and colorimetric methods are shown in Table I.

In the gravimetric method, 3 strips 10 cm. wide and 50 cm. long (4 inches wide and 20 inches long, 240 square inches of plate) were formed in a coil and extracted with carbon tetrachloride in a 1-liter Soxhlet extractor for 30

minutes. The extract was filtered and the oil was weighed in a tared platinum dish after evaporating off the solvent in a steam bath. For the colorimetric method the oil was extracted from the plate by agitating 9 disks of 26 sq. cm. (4 square inches) each in a 250-ml. beaker with three 15-ml. portions of ether at room temperature. The reagents were added directly to the ether extract. The whole determination was carried out in less than 10 minutes.

Figure 2 shows the transmittancy curves for cottonseed oil, palm oil from tin pots, dibutyl sebacate, sulfurized sperm oil, and a lanolin-base oil used as a rust preventive.

It is apparent that transmittancy values may be plotted in terms of saponification number, acid value, ester value, or acetyl value.

Larger samples may be used if the corresponding quantities of reagents are used and dilutions made to a greater volume. Thus, a 10-mg. sample of cottonseed oil was determined on the same curve as the 0- to 1-mg. curve by using 1 ml. each of saturated hydroxylamine hydrochloride solution and saturated sodium hydroxide solution and subsequently diluting to 100 ml. with Solution A.

Accuracy of determinations are within ±0.01 mg. of cottonseed oil.

LITERATURE CITED

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(2) Staudinger and Kupfer, *Ber.*, 45, 505 (1912).

Determination of Nitrogen, Phosphorus, Potassium, Calcium, and Magnesium in Plant Tissue

Semimicro Wet-Digestion Method for Large Numbers of Samples

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A procedure is described for the determination of nitrogen, phosphorus, potassium, calcium, and magnesium in a single acid-digested sample of plant material. Plant tissue is digested with a salicylic acid–sulfuric acid solution. Nitrogen is determined by nesslerization of the ammonia. Phosphorus is determined by adaptation of the Fiske and Subbarrow (3) method. Potassium is determined by the cobaltinitrite method. The separation of manganese, iron, aluminum, and phosphorus for the determination of calcium and magnesium is made by the method of Peech (7). Calcium is determined by the oxalate method and titrated. Magnesium is precipitated as ammonium phosphate and the phosphate determined as indicated above for phosphorus. An evaluation is made of the analytical errors involved in the determination of the various elements indicated.

INVESTIGATIONS conducted by the Soils Division of the Guayule Research Project on the interrelationships of soil moisture, mineral nutrition, and other factors which affect the growth and rubber formation of guayule have necessitated an extensive study of the nutrient status of guayule plants. Despite the fact that it would be desirable to know the content of all the mineral elements that affect plant growth, it has been necessary to limit the analytical program to the determination of the more common elements—nitrogen, phosphorus, potassium, calcium, and magnesium. No satisfactory method for the determination of all these elements in one sample was found in the literature.

Pepkowitz and Shive (*8*) describe an excellent method for a rapid wet-digestion microdetermination of Kjeldahl nitrogen. They suggest that other elements can be determined in the digest, but give no reference to the elements considered or to the methods which would be suitable for their determination. Parks *et al.* (*6*) describe a method for the microdetermination of 12 elements on one plant sample, but nitrogen is not included. Lindner (*5*) has recently described a method for the determination of nitrogen, phosphorus, potassium, calcium, and magnesium on the same sample of plant tissue, following a digestion in an Erlenmeyer flask with sulfuric acid and hydrogen peroxide. He suggests that care must be taken in the addition of the hydrogen peroxide to prevent the oxidation of ammonia to nitrate.

The authors have found that this is difficult for routine workers to accomplish. The C.P. hydrogen peroxide which is commercially obtainable has the additional disadvantage of containing such large amounts of nitrogen and phosphorus as to necessitate the subtraction of large blanks, or the purification of the hydrogen peroxide. To the authors the use of the sulfuric acid–hydrogen peroxide digestion was not so satisfactory for rapid quantitative work by routine analysis as the method described below.

Numerous methods have been described for the individual determinations of nitrogen, phosphorus, potassium, calcium, and magnesium. Several of the methods for individual determinations have been adapted to a system such that these five elements can be determined in one digest by a rapid and inexpensive procedure. This paper presents a description of this procedure, together with data which make possible the evaluation of the analytical errors involved.

REAGENTS

All reagents are of reagent grade unless otherwise indicated.

DIGESTION. Concentrated sulfuric acid, nitrogen-free.

Ranker's solution, 32 grams of salicylic acid per liter of concentrated sulfuric acid.

Selenium oxychloride, 2.4 grams per liter of concentrated sulfuric acid.

Sodium thiosulfate monohydrate, 50 grams per 100 ml. of distilled water.

Perchloric acid, 10%, diluted from 70% with distilled water.

DETERMINATION OF NITROGEN. Sodium hydroxide, 1 *N*. Gum ghatti, 1%.

Nessler's Reagent. Dissolve 150 grams of potassium hydroxide in 200 ml. of water. Dissolve 50 grams of mercuric chloride in 500 ml. of water. Dissolve 61.75 grams of potassium iodide in 200 ml. of water. Pour the mercuric chloride solution cautiously into the potassium iodide solution until a precipitate begins to form, then add mercuric chloride solution dropwise until a permanent bright red color is produced. Dissolve 0.75 gram of potassium iodide in the red solution, stir well, and transfer to a 1-liter flask. Add the potassium hydroxide solution, shake well, cool, and make to volume. Transfer to a brown bottle and keep in cool, dark place. Centrifuge before using.

DETERMINATION OF PHOSPHORUS. Ammonium molybdate, 2.5% in 5 *N* sulfuric acid.

1,2,4-Aminonaphtholsulfonic Acid. Add 0.125 gram of 1,2,4-aminonaphtholsulfonic acid to 49 ml. of filtered 15% sodium bisulfite, then add 1.25 ml. of 20% sodium sulfite. Mix well.

DETERMINATION OF POTASSIUM. Ethyl alcohol, 95 and 70% by volume.

Sodium Cobaltinitrite. Mix together 46.2 grams of sodium

cobaltinitrite, 18.9 grams of sodium acetate, 120 ml. of distilled water, and 18 ml. of glacial acetic acid. Prepare 48 hours before using. Keep stoppered and in a cold, dark place. Before using, centrifuge to remove any precipitate.

Ceric sulfate, approximately 0.02 *N* in 1 *N* sulfuric acid. Standardize against ferrous ammonium sulfate or sodium oxalate.

Ferrous ammonium sulfate, approximately 0.02 *N* in 0.3 *N* sulfuric acid. Standardize against ceric sulfate.

Indicator, *o*-phenanthroline ferrous complex, 0.025 *M*. Dissolve 0.173 gram of ferrous sulfate and 0.125 gram of *o*-phenanthroline in 25 ml. of water.

Separation of Manganese, Iron, Aluminum, and Phosphorus. Ammonium chloride, 25%; ammonium hydroxide, 0.6 *N*; bromine water, saturated.

Ferric chloride, 1 mg. of iron per ml. Dissolve 1.22 grams of ferric chloride hexahydrate in 250 ml. of water containing 1 ml. of concentrated hydrochloric acid.

Sodium acetate, 10%.

Sodium hydroxide, 0.1 *N*.

Methyl red indicator, 0.02%.

Determination of Calcium. Ammonium acetate, 60%.

Ammonium oxalate, saturated solution.

Ammonium hydroxide, 1%, saturated with calcium oxalate. Centrifuge or filter before using.

Ceric sulfate, ferrous ammonium sulfate and *o*-phenanthroline ferrous complex indicator, as described above under Determination of Potassium.

Sulfuric acid, 1 to 1.

Determination of Magnesium. Ammonium chloride, 25%.

Ammonium dihydrogen phosphate, $NH_4H_2PO_4$, 5%.

Wash Solution. Mix 20 ml. of ammonium hydroxide, 80 ml. of water, 100 ml. of ethyl alcohol, and 100 ml. of ether.

Ammonium molybdate and 1,2,4-aminonaphtholsulfonic acid, as described under Determination of Phosphorus.

2 *N* sulfuric acid.

ANALYTICAL PROCEDURES

Digestion. The digestion of plant material is made by an adaptation of the rapid wet-digestion micro-Kjeldahl method for nitrogen determination described by Pepkowitz and Shive (*8*).

Solution A. Weigh into a 100-ml. Pyrex volumetric flask a suitable sample of the material to be analyzed—for example, 200 mg. of dry guayule tissue. Add 3 ml. of the salicylic acid–sulfuric acid solution, mix it with the tissue, and allow to stand in the cold for about 30 minutes. Add 4 drops of the sodium thiosulfate and 5 ml. of the selenium oxychloride solution. Mix thoroughly. Heat gradually to boiling on a hot plate under a hood and digest until clear. Cool the flasks and wash the necks down with a small stream of water. Cool. If the solution is not colorless, add 2 drops of 10% perchloric acid and shake immediately. Heat again, at low temperature first, and digest until colorless, but do not boil. Cool and make to volume.

Solution B. Shake Solution A thoroughly to resuspend all precipitated material. (Calcium is likely to be precipitated as the sulfate.) Immediately withdraw a 25-ml. aliquot and transfer to a 50-ml. beaker. Evaporate to dryness on a hot plate, and heat in a muffle furnace at a low red heat, to drive off ammonia salts and excess sulfuric acid. Cool and take up with 2 drops of concentrated hydrochloric acid and 10 ml. of water. Transfer quantitatively to a 25-ml. volumetric flask and make to volume.

Determination of Nitrogen. Nitrogen is determined by the nesslerization of ammonia according to the procedure described by Fraps and Sterges (*4*).

After the aliquot for Solution B has been removed and the suspended material in Solution A has settled, quantitatively transfer 2 ml. Solution A to a 100-ml. volumetric flask. Add 50 to 60 ml. of water, then 2 ml. of 1 *N* sodium hydroxide, and shake thoroughly. Add 2 ml. of 1% gum ghatti and shake again. Add 2 ml. of Nessler's reagent, shake thoroughly, and make to volume. Using a 420-mu filter, compare in a colorimeter with a series of standard ammonium hydroxide solutions treated similarly.

Determination of Phosphorus. Phosphorus is determined by an adaptation of the method of Parks, Hood, Hurwitz, and Ellis (*6*) and Fiske and Subbarrow (*3*).

After the aliquot for Solution B has been removed and Solution A has settled until clear, transfer 5 ml. of Solution A to a 50-ml. volumetric flask. Add 5 ml. of ammonium molybdate solution and shake. Add 2 ml. of 1,2,4-aminonaphtholsulfonic acid solution, make to volume, and shake well. Prepare a set of standards simultaneously, since the slope of the standard curve is not constant (*6*). Allow the color to develop 30 minutes. Read in a photoelectric colorimeter, using a filter of 660-mu wave length for color comparison.

Determination of Potassium. Potassium is determined by a modification of the method of Brown, Robinson, and Browning (*2*).

To 1.5 ml. of 95% ethyl alcohol in a 15-ml. centrifuge tube add a 5-ml. aliquot of Solution B and mix throughly. Place the tube in a water bath at 20° C. and allow to come to equilibrium. Add slowly, with continuous shaking, 2 ml. of sodium cobaltinitrite solution, and allow to stand for exactly 1 hour at a temperature of 20° ± 1° C. Centrifuge for 10 minutes at 2000 r.p.m., decant the supernatant liquid, invert the tube at an angle of about 45°, and let drain for several minutes. Wash the precipitate with 5 ml. of 70% alcohol, centrifuge for 5 minutes, and drain as before. Dry the precipitate at 80° C. until all the alcohol is removed. Add 5 ml. of 0.02 *N* ceric sulfate (if more than 0.5 mg. of potassium is present, more than 5 ml. of ceric sulfate is required) and 1 ml. of 1 to 1 sulfuric acid. Heat in a water bath at 90° to 95° C. until all the precipitate is oxidized, cool, and titrate the excess ceric sulfate with 0.02 *N* ferrous ammonium sulfate, using 1 drop of the *o*-phenanthroline ferrous complex indicator. The end point is very sharp, the color of the solution changing from pale blue to red.

A set of standard potassium solutions, containing approximately the same amount of potassium as is present in the aliquots of Solution B, should be carried through the procedure at the same time. Assuming complete recovery of potassium in the standard solution, calculate a factor for the equation:

$$\text{Mg. of potassium} = \text{ml. of } Ce(SO_4)_2 \text{ used in oxidation of precipitate} \times \text{normality of } Ce(SO_4)_2 \times \text{factor}$$

Calculate the potassium content of the digested plant material (Solution B), using the calculated factor in the same equation.

Separation of Manganese, Iron, Aluminum, and Phosphorus. Manganese, iron, aluminum, and phosphorus are removed according to the method of Peech (*7*).

Solution C. Transfer a 5-ml. aliquot of Solution B to a 15-ml. centrifuge tube, and add 0.2 ml. of ferric chloride solution, 3 ml. of water, and 2 ml. of 10% sodium acetate solution. Mix, add 1 ml. of 0.1 *N* sodium hydroxide, and mix again. Place in a water bath at 95° C., add 1 ml. of bromine water, and maintain this temperature for at least an hour. Add 2 ml. of 25% ammonium chloride solution and digest about 15 minutes longer. Add 1 drop of methyl red and if the color of the indicator persists, indicating complete expulsion of bromine, remove the tube from the water bath, cool, add 0.6 *N* ammonium hydroxide from a buret until the color of the solution changes from slightly red to deep yellow, and then add 2 drops in excess. Make to volume of 13 ml., add 5 drops of water in excess to allow for evaporation, mix the contents with a stirring rod, and digest in a water bath at 80° C. for 5 minutes to flocculate the precipitate. While hot, centrifuge for 10 minutes.

Determination of Calcium. Calcium is determined by a modification of the method of Peech (*7*).

Solution D. Without disturbing the precipitated manganese, iron, aluminum, and phosphate, transfer 10 ml. of Solution C to a 15-ml. centrifuge tube, and add 1 ml. of 60% ammonium acetate. Place the tube in a water bath at 70° C. Mix the contents by spinning the tube, add 2 ml. of saturated ammonium oxalate solution, mix thoroughly, and digest for 30 minutes. Cool, make to volume of 13 ml., and centrifuge for 10 minutes at 2000 r.p.m. Decant the supernatant liquid into a dry test tube and save for the determination of magnesium.

Allow the centrifuge tube, inverted at about a 45° angle, to drain for several minutes. Wipe the lip of the tube with filter paper. Wash the precipitate with 5 ml. of a 1% ammonium hydroxide solution saturated with calcium oxalate and centrifuge again. Decant and discard the supernatant liquid. Allow the tube to drain again for several minutes. Add 3 to 5 ml. of 0.02 *N* ceric sulfate (depending upon the amount of calcium present) and 1 ml. of 1 to 1 sulfuric acid. Mix, heat to 70° C. in a water bath, and titrate with 0.02 *N* ferrous ammonium sulfate, using 1 drop of *o*-phenanthroline ferrous complex indicator.

Determination of Magnesium. Magnesium is determined by an adaptation of the method of Reitemeier (*9*).

Transfer 5 ml. of Solution D to a 15-ml. centrifuge tube. Add 1 ml. of 25% ammonium chloride, 1 ml. of 5% ammonium dihydrogen phosphate, and 1 drop of phenolphthalein. Mix, heat to 90° C. in a water bath, and add concentrated ammonium hydroxide until pink. Cool, add 2 ml. of concentrated ammonium hydroxide, and stir with a glass rod. Withdraw the rod and rinse it with a very small stream of water. Stopper the tube and let it stand overnight (preferably in a cool place). Centrifuge, decant, invert the tube at about a 45° angle, and let drain several minutes. Wash the precipitate and sides of the tube with 5 ml. of the ammonia-alcohol-ether wash solution. Decant and drain.

Table I. Determination of Nitrogen, Phosphorus, Potassium, Calcium, and Magnesium on Standard Guayule Plant Tissue[a] with Added Increments of These Elements

Sample	Nitrogen				Phosphorus				Potassium				Calcium					Magnesium			
													Found								
	Added	Source	Total present	Found	Recovery	Added	Total present	Found	Recovery	Added	Total present	Found	Recovery	A	B	C	Mean	Recovery	Added	Total present	Found	Recovery
	Mg.		*Mg.*	*Mg.*	%	*Mg.*	*Mg.*	*Mg.*	%	*Mg.*	*Mg.*	*Mg.*	%	*Mg.*	*Mg.*	*Mg.*	*Mg.*	%	*Mg.*	*Mg.*	*Mg.*	%
Plant tissue	0	....	6.50	...	...	0	..	0.69	...	0	...	8.00	...	5.22	5.14	5.43	5.26	...	0	..	1.11	...
	0	....	6.35	...	...	0	..	0.69	...	0	...	8.34	...	5.39	5.47	5.34	5.40	...	0	..	1.15	...
	0	....	6.40	...	...	0	..	0.69	...	0	...	8.12	...	5.39	5.43	5.30	5.37	...	0	..	1.25	...
	..	....	...	...	...	...	..	..	...	...	...	...	...	5.34	5.43	5.59	5.45	...	0	..	1.25	...
	..	....	...	...	...	...	..	..	...	0	...	...	...	5.47	5.63	5.39	5.50	...	0	..	1.28	...
	..	....	...	...	...	...	..	..	...	0	...	...	...	5.14	5.55	5.34	5.34	...	..	..	..	...
Av.	0	....	6.42	...	100	0	..	0.69	100	0	...	8.15	100	5.33	5.44	5.40	5.39	100	0	..	1.21	100
Plant tissue	3.00	KNO_3	9.40	9.90	105	1.01	1.70	1.68	99	2.80	10.95	11.14	102	2.00	7.39	7.30	7.75	7.51	7.52	102	1.00	2.21	2.40	109
	3.00	KNO_3	9.40	9.80	104	1.01	1.70	1.69	99	2.80	10.95	11.12	102	2.00	7.39	7.84	7.59	7.34	7.42	100	1.00	2.21	2.27	103
	3.00	KNO_3	9.40	10.10	107	1.01	1.70	1.67	98	2.80	10.95	11.08	101	2.00	7.39	7.22	7.34	7.34	7.30	99	1.00	2.21	2.26	103
	..	....	...	...	...	...	..	..	...	...	...	...	...	...	...	...	...	...	...	...	1.00	2.21	2.37	107
Av.	3.00	KNO_3	9.40	9.90	105	1.01	1.70	1.68	99	2.80	10.95	11.11	101	2.00	7.39	7.29	7.56	7.40	7.41	100	1.00	2.21	2.31	105
Plant tissue	6.00	KNO_3	12.42	12.70	102	0.68	1.37	1.35	98	8.30	16.45	16.30	99	4.00	9.39	9.10	9.18	9.38	9.22	98	3.00	4.21	4.33	103
	6.00	KNO_3	12.42	13.00	105	0.68	1.37	1.35	98	8.30	16.45	16.40	100	4.00	9.39	9.10	9.38	9.38	9.29	99	3.00	4.21	4.20	100
	6.00	KNO_3	12.42	12.60	101	0.68	1.37	1.37	100	8.30	16.45	16.25	99	4.00	9.39	9.18	9.02	9.06	9.09	97	3.00	4.21	4.17	99
	..	....	...	...	...	...	..	..	...	...	...	...	...	...	...	...	...	...	...	...	3.00	4.21	4.53	108
	..	....	...	...	...	...	..	..	...	...	...	...	...	...	...	...	...	...	...	...	3.00	4.21	4.40	105
	..	....	...	...	...	...	..	..	...	...	...	...	...	...	...	...	...	...	...	...	3.00	4.21	4.33	103
Av.	6.00	KNO_3	12.42	12.80	103	0.68	1.37	1.36	99	8.30	16.45	16.32	99	4.00	9.39	9.13	9.19	9.27	9.20	98	3.00	4.21	4.33	103
Plant tissue	2.50	NH_4Cl	8.92	8.80	98	0.34	1.03	1.02	99	16.70	24.85	24.60	99	6.40	11.79	11.51	11.51	11.71	11.58	98	..	..	..	..
	2.50	NH_4Cl	8.92	8.65	97	0.34	1.03	1.03	100	16.70	24.85	24.00	97	6.40	11.79	11.14	11.63	11.73	11.50	98	..	..	..	..
	2.50	NH_4Cl	8.92	8.80	98	0.34	1.03	1.02	99	16.70	24.85	22.80	92	6.40	11.79	11.42	11.26	10.89	11.19	95	..	..	..	..
Av.	2.50	NH_4Cl	8.92	8.72	98	0.34	1.03	1.02	99	16.70	24.85	23.80	96	6.40	11.79	11.36	11.47	11.44	11.42	97	..	..	..	..
Plant tissue	5.00	NH_4Cl	11.42	10.50	92	0.17	0.86	0.84	98	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	5.00	NH_4Cl	11.42	11.75	103	0.17	0.86	0.85	99	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	5.00	NH_4Cl	11.42	11.60	102	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Av.	5.00	NH_4Cl	11.42	11.28	99	0.17	0.86	0.84	98	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

[a] 200 mg. of plant tissue used in each determination.

Repeat this washing and draining once. Dissolve the precipitate with 5 ml. of 2 *N* sulfuric acid, transfer to a 25-ml. volumetric flask, and develop the phosphate color as directed above under Determination of Phosphorus. Prepare a photometric calibration curve by simultaneously carrying a series of standard magnesium sulfate solutions through the same operations each time a group of samples is analyzed. From this curve calculate the amount of magnesium present in the unknowns.

RESULTS AND DISCUSSION

The length of time required for the digestion of any given sample of plant material will depend upon the temperature at which the digestion takes place. If kept boiling vigorously, the digestion will usually be completed within 1 to 2 hours. Pepkowitz and Shive (*8*) reported that 10 to 25 minutes were required for the complete digestion of samples of 10 to 15 mg. of plant material. The time required for complete digestion of 200-mg. samples of guayule tissue for a routine analysis in this laboratory is usually 3 to 6 hours. If boiling temperatures are not maintained, 8 to 16 hours may be required. The sulfuric acid digestion can be continued until the digest is colorless, thus eliminating the use of perchloric acid. This usually requires 3 to 6 hours longer and is not a recommended practice. As is pointed out by Pepkowitz and Shive, the digest should not be boiled after the perchloric acid has been added. Boiling at this stage may result in a loss of nitrogen through the oxidation of ammonia to nitrate.

At first thought it may appear that a method which requires 3 to 6 hours' digestion is not a rapid method for the analysis of plant tissue. Little attention is required during the digestion process, however, and in the analysis of large numbers of samples the time required for digestion is of little consequence if the work is properly planned. Only one weighing and digestion is required for the determination of the five elements most commonly determined. (In this laboratory rapid weighing of samples is facilitated by the use of a Roller-Smith microtorsion balance of 0- to 1500-mg. capacity.) The analyst can proceed with determinations on the digested material while a second set of samples is digesting. The gas hot plate used in this laboratory is 45 × 90 cm. (18 × 36 inches) in dimension and will accommodate 105 100-ml. volumetric flasks. Chemical determinations on the digests that can be made with a hot plate of this size will more than keep two good routine analysts busy.

In this laboratory a digest and reagent blank, and duplicates of a standard guayule sample, are carried through the entire analytical procedure with each lot of samples. The digestion and reagent blank allow for correction for impurities in the reagents, which may vary with changes in sources. The standard guayule sample which is analyzed with each run serves as a check on accuracy. For this purpose it is preferred to a synthetic standard.

It is necessary to shake Solution A thoroughly before taking the 25-ml. aliquot for the determination of potassium, calcium, and magnesium. It is equally essential that that aliquot be taken before any other aliquots are removed from Solution A, unless the solution be thoroughly shaken before such aliquots are taken. Because of the low solubility product of calcium sulfate, and the high concentration of sulfuric acid in the digest, calcium is likely to be present as precipitated calcium sulfate. If this precipitate is not uniformly suspended throughout the solution by shaking, the aliquot for potassium, calcium, and magnesium will not be representative. Likewise, if the aliquot for nitrogen or phosphorus be taken from the flask without thoroughly mixing the

contents there will be a concentration of calcium sulfate in the remaining solution; and any subsequent sample, if then representative of what remains in the flask, will contain a greater concentration of calcium than the original digest. For these reasons it is advisable to take the aliquot for potassium, calcium, and magnesium first and then allow the suspended material in Solution A to settle before the aliquots for nitrogen and phosphorus are taken.

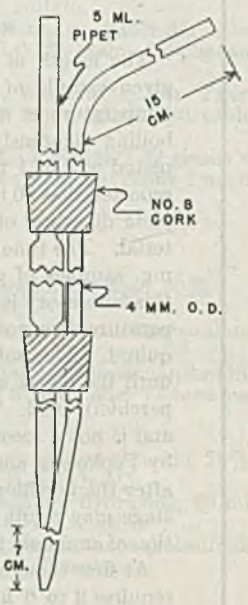

Figure 1. Pipet Assembly for Washing Compacted Precipitates in Centrifuge Tubes

The reliability of the procedures used is indicated by the data of Table I. Varying amounts of nitrogen, phosphorus, potassium, calcium, and magnesium were added to standard guayule tissue, before digestion, and the recovery of each was determined. The data given are typical of several sets of determinations.

The recovery of nitrogen added to guayule tissue varied from 92 to 107%. These were extreme values, however, and in most cases the recovery was between 97 and 103%.

The recovery of added increments of phosphorus was very satisfactory. In the described procedure silica is not removed. If silicates are present to any considerable extent, it may be necessary to transfer an aliquot of Solution A to a platinum dish and treat it with hydrofluoric acid to remove the silica before proceeding with the determination of phosphorus.

The recovery of potassium was very good except where large amounts were added. In nearly all cases a recovery of 97 to 103% was obtained. The determination of potassium by the cobaltinitrite method must take place under standardized conditions. It is necessary that the precipitation be carried out at nearly constant temperature, preferably 20° ± 1° C. The potassium content of the aliquot for precipitation should be between 0.2 and 1.0 mg. During centrifugation the precipitate compacts tightly in the bottom of the centrifuge tube. It is rather difficult to wash such a compacted precipitate well. The authors have found that a pipet assembly constructed as shown in Figure 1 aids considerably in the washing of precipitates compacted in the bottom of conical centrifuge tubes.

The pipet is filled with the wash solution and the small glass tube is placed in contact with the compacted precipitate. As the wash liquid drains from the pipet, air is blown through the glass tube, thus thoroughly mixing the precipitate with the wash solution. Before all the wash solution has drained from the pipet the glass tube is raised clear of the liquid in the centrifuge tube. The remaining liquid draining from the pipet then washes the small tube free of any precipitate or solution that may have adhered to it. The side of the centrifuge tube may also be washed by focusing the wash liquid around the top inside edge. This type of pipet assembly is used with all precipitates being washed by centrifugation.

The composition of the precipitate obtained in the volumetric determination of potassium by the sodium cobaltinitrite method varies, depending upon the conditions of precipitation, particularly temperature, concentration of alcohol, and concentration of sodium (*10*). Brown, Robinson, and Browning (*2*) used an empirical factor of 6.52 in the calculation of the potassium in the precipitate. In the authors' studies seldom did calculations made with the use of this factor indicate 100% recovery of the potassium present in standard solutions of potassium nitrate. In this laboratory the procedure was adopted of carrying standard potassium solutions through the precipitation and titration procedure simultaneously with each set of unknowns. Assuming 100% recovery of potassium from the potassium standards, an empirical factor was calculated for the conditions under which the precipitation was carried out. This factor was then used in the calculation of the potassium content of the unknowns. Although it has been demonstrated (*2*) that calcium, magnesium, iron, sulfate, chloride, phosphate, and certain other ions do not interfere in the volumetric determination of potassium with cobaltinitrite, it is advisable to have the standard solution contain these ions in about the same ratios as they are present in the plant material.

In Table I are presented the data obtained in the analysis of guayule with added increments of calcium as calcium chloride. Determinations were made on three aliquots from each of six digestions of guayule with no added calcium, and from each of three or more digestions with increments of calcium. It is evident that the variation between aliquots from the same digestion is of about the same magnitude as that between digestions, and that this variation is not great. For the 18 determinations made on guayule tissue alone the coefficient of variation is 2.5%.

The maximum variation in the percentage recovery of the various elements added to the standard guayule tissue occurred in the determination of magnesium. It is thought that most of the error involved is in the actual determination of magnesium and is not in any way due to the digestion procedure. The authors have not found a completely satisfactory method for the colorimetric determination of small amounts of magnesium.

The accuracy of the determination of phosphorus and potassium by the digestion procedure described in this paper was checked by the ashing methods of the A.O.A.C. (*1*). Five samples were analyzed in duplicate by each method (Table II). The phosphorus data obtained by the two methods are in good agreement, the maximum variation amounting to about 6%. The potassium data agree satisfactorily, but are somewhat more variable than those for phosphorus, the maximum difference being about 8%.

Table II. Comparison of Ashing Method of A.O.A.C. and Digestion Method for Determination of Phosphorus and Potassium in Guayule Tissue

Sample No.		Per Cent P, Dry Basis Ash (A.O.A.C.)	Per Cent P, Dry Basis Digestion	Per Cent K, Dry Basis Ash (A.O.A.C.)	Per Cent K, Dry Basis Digestion
1		0.21	0.21	6.7	7.0
		0.22	0.22	6.7	6.9
	Av.	0.22	0.22	6.7	7.0
2		0.28	0.29	7.0	7.1
		0.30	0.30	7.3	7.3
	Av.	0.29	0.30	7.2	7.2
3		0.27	0.27	7.0	6.5
		0.26	0.25	6.8	6.2
	Av.	0.27	0.26	6.9	6.4
4		0.20	0.21	7.1	6.6
		0.20	0.22	7.1	7.0
	Av.	0.20	0.22	7.1	6.8
5		0.19	0.20	5.8	6.4
		0.20	0.21	6.3	6.4
	Av.	0.20	0.21	6.1	6.4

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Use of an Ion-Exchange Resin in Determination of Traces of Copper

With Special Reference to Powdered and Fluid Milk

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A simple and rapid determination of copper in fluid milk, powdered milk, and ice cream mix is described. Ignition or acid digestion of the sample is eliminated, the copper being made available by reducing the pH with perchloric acid. Copper is then concentrated on an ion exchanger in the hydrogen cycle, stripped from the ion exchanger with dilute hydrochloric acid, and determined polarographically using an additive standard technique.

THE chief difficulty encountered in determining the copper content of milk is the extremely small concentration of the metal present. Raw milk has been reported from several sources to contain from 0.1 to 0.5 p.p.m.; the Quartermaster Corps limits the copper content of premium grade powdered whole milk to 1.5 p.p.m. and nonfat milk solids to 2.0 p.p.m. The usual copper content of premium grade powdered whole milk is now found to be less than 1.0 p.p.m.

After exhaustive investigation of applicable methods, a spectrophotometric method employing dithizone (*2*) has been selected as the official army specification method of analysis, as being the most sensitive and specific. However, the specification method lacks precision. Three laboratories determined copper on six samples of powdered whole milk ranging from 0.6 to 20.0 p.p.m. Each laboratory made ten determinations on each sample and the coefficient of variation was determined for each sample for each laboratory. Figure 1 demonstrates that the coefficient of variation values becomes excessively large at very low concentrations, particularly at the critical range below 2.0 p.p.m. Thus is demonstrated the necessity of making replicate determinations on all borderline samples. Seven points were not plotted. Three represented data obtained from less than ten determinations each, and four represented high coefficient of variation values on 0.5 and 1.0 p.p.m. samples that were above 22 and off the scale. However, all seven points not plotted appeared to fit the curves well.

By and large, the limiting factor in the determination of the copper content in dairy products or, for that matter, in food products in general is the size of the sample that can be adequately handled. In practically all methods of analyses it is necessary to destroy the organic matter as the primary step. Whether the destruction is accomplished by acid digestion or ignition, the procedure when applied to low copper content foods of high protein and fat content becomes both laborious and time-consuming. A further handicap is that only a relatively small sample is practical, and the resulting concentration of copper in the prepared sample is also exceedingly small. The ideal procedure would be one in which preliminary ashing is eliminated and a sufficiently large sample can be taken to ensure an adequately measurable concentration of copper.

Recently it has been shown (*1*) that copper proteins lose their copper in acid solution below pH 3.0. Both cupric and cuprous copper are lost, the latter being oxidized in the acid aqueous solution (*6*). It has also been demonstrated that copper in powdered whole milk is concentrated in the milk protein (*10*). Presumably a similar condition exists in raw milk. It is therefore sufficient to transfer the copper in milk to the electrovalent form of cupric ions by reducing the pH to less than 3.0. This accomplishes a secondary advantage, in that under optimum conditions the protein is precipitated and fat will be carried into the curd. Thus by simple filtration it is possible to make the copper available and to eliminate interfering protein and fat. The filtrate so obtained, when corrected for added acid, solids and fat loss, contains copper in the same concentration as the original solution whether it was raw milk or reconstituted powdered milk. Synthetic ion-exchange resins offer a satisfactory method of concentrating the copper from the milk filtrate with the minimum effect of residual organic constituents remaining in the milk filtrate.

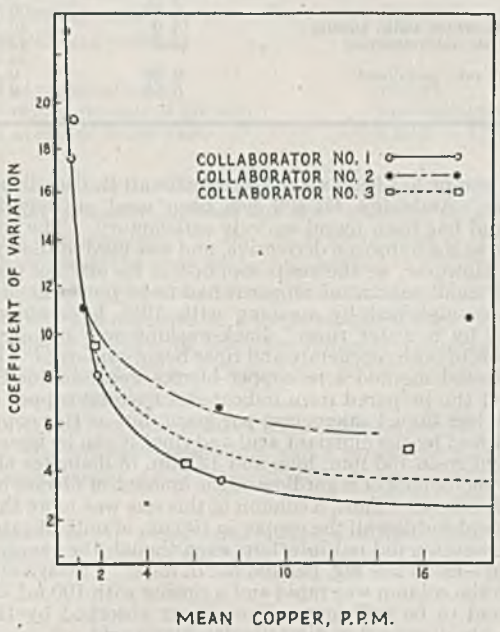

Figure 1. Reproducibility of Data Obtained by Bendix-Grabenstetter Procedure, Employing 5-Gram Samples

While there has been a considerable amount of work published on the general behavior of ion-exchange resins on cations in solution (*4, 8*), very little has been published on concentrations for analytical procedures. Zeo-Carb has been used (*3*), but it was not considered satisfactory for trace work because it cannot be purified sufficiently. In so far as the authors are aware there have been no publications describing the use of ion-exchange resins as a means of concentrating metals in microgram concentrations. The general equation applicable to the separation of cations from anions by any ion exchanger in the hydrogen cycle (*3*) may be represented as:

$$2H^+ A^- + Cu^{++} + SO_4^{--} \rightleftharpoons Cu^{++} A^-_2 + 2H^+ + SO_4^{--}$$

EXPERIMENTAL

The usual protein precipitants such as metaphosphoric, trichloroacetic, and tungstic acids were unsatisfactory, owing to

the difficulty involved in removing traces of copper from these reagents. Glacial acetic acid, although a satisfactory precipitant, could not be used because of buffering capacity of the system. An unreasonably large amount was required to reduce the pH substantially below pH 3.5. An ideal precipitant was found in perchloric acid (7); an almost negligible amount was required to reduce the pH to 1.0 with a resulting precipitate that was easily filtered. The data presented in Table I were obtained from samples by varying the contact time of the perchloric acid with the milk from 30 minutes to 16 hours and demonstrate the rapidity with which the copper is removed from protein complexes at low pH. Complete liberation is obtained in 30 minutes or less.

Table I. Spectrophotometric Data Obtained by Acid Digestion of Samples and Prepared Filtrates

Sample	Copper Content of 5-Gram Sample	Copper content of 75-Ml. Prepared Filtrate
	P.p.m.	*P.p.m.*
Raw fluid milk	0.15	0.10
	0.15	0.15
Powdered whole milk	0.85	0.70
	0.80	0.80
	0.95	0.85
	0.95	0.90
	0.60	0.70
Powdered whole milk, special	15.0	15.5
Duplicate determination	15.2	15.0
	...	15.2
Ice cream mix, powdered	0.20	0.35
	0.65	0.45

No attempt has been made to compare all the possible ion exchangers. Amberlite IR-100 has been used entirely for this study and has been found entirely satisfactory. The resin was supplied as the hydrogen derivative, and was used in the hydrogen cycle. However, as the resin supplied in its original form contained a small amount of copper it had to be purified. This was easily accomplished by washing with 10% hydrochloric acid followed by a water rinse. Back-washing was not necessary, down flow of both regenerate and rinse being employed. In using the proposed method zero copper blanks were obtained. Dry-ashing of the prepared resin indicated a residual copper content of much less than 1 microgram per gram, but as this copper was not removed by the elutriant acid and rinse it can be ignored. A column of resin 150 mm. long and 12 mm. in diameter absorbed up to 1 mg. of copper regardless of the amount of filtrate in which it was dissolved. Thus, a column of this size was more than adequate for absorbing all the copper in 100 ml. of milk filtrate. Calcium absorption did not interfere, even though the concentration was high—up to 250 mg. per 100 ml. of milk. Passage of filtrate through the column was rapid and a rinsing with 100 ml. of water was found to be sufficient. The copper absorbed by the resin was readily dissolved in dilute hydrochloric acid.

Polarographic analysis of the elutriant was employed as offering the most rapid and simple method. This does not preclude spectrophotometric methods of analyses, however.

METHOD OF APPLICATION

Apparatus. Suitable polarograph with cells of approximately 10-ml. capacity.

Pyrex Ware. All Pyrex ware should be thoroughly cleaned by washing with concentrated nitric acid, and rinsed thoroughly with tap water, with distilled water, and finally several times with redistilled water. Columns can be prepared from Pyrex condenser tubes, chromatographic separation tubes, filter tubes, etc. It is essential only that a column should be approximately 150 mm. × 10 mm. in diameter. The lower end of the column should be plugged loosely with just sufficient glass wool to retain the resin. The elutriant should be withdrawn through a small length of amber gum rubber tubing equipped with a screw clamp to shut off and to adjust flow of elutriant.

Reagents. Amberlite IR-100, analytical grade, low in copper, obtained from Resinous Products and Chemical Company, Philadelphia, Pa.

Redistilled Water. Distilled water redistilled from Pyrex.

Perchloric Acid, 72%, double vacuum-distilled, obtained from G. Frederick Smith Chemical Company, Columbus, Ohio.

Filter Paper. Wash with dilute hydrochloric acid and rinse with redistilled water to remove any traces of copper.

Cresol Purple Indicator. Dissolve 0.1 gram of cresol purple in ethyl alcohol.

Ammonia, Dilute. Dilute 10 ml. of concentrated ammonium hydroxide with 100 ml. of redistilled water.

Hydrochloric Acid, 6%. Dilute 1 volume of concentrated hydrochloric acid with 5 volumes of redistilled water.

Electrolyte. 3 *N* ammonia and ammonium chloride with gelatin as a maxima suppressor. Prepare as follows:

NH_4OH (28%)	200 ml.
NH_4Cl	40 grams
Gelatin	10 ml. of a 1% solution

Make up to 1000 ml. with redistilled water.

Standard Copper Solutions. Stock Solution. Dissolve 1.0000 gram of electrolytic sheet copper in 20 ml. of concentrated nitric acid and make up to 1000 ml. with redistilled water. One milligram of this solution is equivalent to 1 mg. of copper.

Working Standard. Dilute a volume of the stock solution with redistilled water so that 1 ml. of the working standard is equivalent to 20 micrograms of copper. Prepare a fresh working standard daily.

Wash the untreated Amberlite IR-100 in a 1000-ml. Erlenmeyer flask with a fairly strong stream of tap water. Allow flask to overflow, adjusting the water pressure so that any undue amount of fine material will be carried off with the overflow. Transfer the washed resin to a suitable column of such capacity that a considerable quantity of resin may be freed of copper at one time. The column must be filled with water prior to the addition of the resin. Fill column so that it is about three-quarters full of resin. Drain water in the column so that only a few millimeters height remains above the resin bed.

Slowly pass hot 10% hydrochloric acid through the resin, allowing the fluid level almost to reach the surface of the resin before adding the next portion. Continue the acid wash until 75 ml. of the effluent acid wash show a negative copper content when tested with a copper-specific reagent such as carbamate or dithizone. Wash free of hydrochloric acid with redistilled water as indicated by a negative chloride test with silver nitrate.

Store the prepared resin under redistilled water.

A suitable column is easily prepared if it is first filled with redistilled water and the resin added. Never allow the resin to become dry. In all operations make certain that the fluid level never passes below the upper level of the resin. Properly cleansed resin will give zero copper contents when washed with a water–perchloric acid solution at pH 5.0.

Sample Preparation. Whole Milk. Mix well to ensure uniform distribution of fat.

Powdered Milk and Ice Cream Mix. Reconstitute 25 grams in redistilled water and make up to 250-ml. volume with redistilled water. Reconstitution is best accomplished by adding the milk slowly to water with constant agitation.

Procedure. Transfer the prepared sample to a 400-ml. beaker, add 4.00 ml. of double vacuum-distilled 72% perchloric acid, and mix thoroughly by stirring. Allow to stand at least 0.5 hour. Stir thoroughly but gently and filter. Allow the precipitated curd to drain; the curd should retain no more than 25% of the filtrate. If the filter paper is washed free of copper just prior to use, discard the first few milliliters of filtrate which will be diluted with water from the paper. Transfer 100 ml. of filtrate to a 150- to 250-ml. beaker, and add 3 drops of cresol purple indicator. Add dilute ammonia reagent slowly with thorough mixing until the addition of one drop yields a change from yellow to gray or gray-purple (approximately pH 5.0). At this point there will be formed a slight but permanent precipitate, which may be utilized as a further indication of the end point.

Pass the neutralized filtrate through a prepared ion-exchanger column, adjusting the flow so that the dropping rate is 2 to 3 drops per second. Never allow the ion exchanger to become dry. Always stop flow so that a small amount of fluid remains above the surface. Rinse residual filtrate from the beaker into the column with redistilled water and wash the column with about 200 ml. of redistilled water. Stop flow on final wash, so that about 5 mm. of water remain above the resin.

Wash the absorbed copper from the column with a total volume of 40 ml. of 6% hydrochloric acid, using four approximately equal portions. Never allow fluid level to pass below top level of resin bed. On the final wash allow the column to drain as completely as possible. Evaporate the combined washings to dryness on a water bath and cool. Add 5 ml. of the electrolyte, stir, and allow to stand 10 minutes. Prepare two polarographic cells. To cell *A* add 1 ml. of redistilled water and 2 ml. of the prepared sample. To cell *B* add 1 ml. of the working copper standard (1 ml. = 20 micrograms of copper) and 2 ml. of the prepared sample. Eliminate oxygen by bubbling hydrogen or nitrogen

through the cells for 5 minutes and record polarograms. Run a reagent blank daily on redistilled water and perchloric acid and correct for any copper found in the reagents. Run the blank in the same manner as the sample.

CALCULATION. Let a represent the step height due to the concentration of copper in cell A (unknown + water) and b the step height due to the concentration copper in cell B (unknown + standard). The difference $(b - a)$ is then due to the added standard which represents a concentration of 1/2 of 20 or 6.67 micrograms of copper per ml. Then $\left(\frac{a}{b-a}\right) \times \left(\frac{20}{3}\right)$ is the concentration of the copper in the unknown in micrograms per ml. $\left[\left(\frac{a}{b-a}\right) \times \frac{20}{3} \times \frac{3}{2} \times 5\right]$ or $\left[\left(\frac{a}{b-a}\right) \times 50\right]$ represents the total micrograms copper in the aliquot of milk filtrate taken for analysis. (It is assumed, of course, that the analyst will determine a and b at the same sensitivity.)

Determine the micrograms of copper in the milk filtrate as directed above. Deduct for a reagent blank and report copper on a p.p.m. basis of the original sample.

Example: Make up 25 grams powdered milk to 250 ml. and take 100 ml. of filtrate for analysis; 100 ml. of filtrate would then represent 10 grams of sample. If step heights a and b are found to be 55 and 75, respectively, the total micrograms of copper in the 100 ml. of filtrate would be $\left[\left(\frac{55}{75-55}\right) \times 50\right]$ or 137.57 micrograms of Cu.

If the reagent blank was found to contain 5 micrograms of copper, the copper expressed as p.p.m. would be $\left(\frac{137.57-5.0}{10}\right)$ or 13.3 p.p.m. (micrograms per gram).

Express copper in powdered products as p.p.m. (mg. per kg.) and in fluid milk as p.p.m. (mg. per liter).

Table II. Precision of Proposed Method

(Single sample of high copper content powdered whole milk)

Aliquot of Prepared Filtrate	Added Copper	Sensitivity	Step Height	Total Copper Found in Aliquot	Copper Originally Present in Filtrate
Ml.	γ		1/10 *in.*	γ	γ/*ml.*
100	0.0	1/5	73	114.50	1.14
50	40.0	1/5	62	97.25	1.14
100	0.0	1/10	35	112.00	1.12
50	40.0	1/10	30	96.00	1.12

Recovery of copper from aqueous perchloric acid solution, pH 5.0

Copper passed through column	80.6 γ
Copper recovered from column	79.2 γ
Copper passed through column	10.0 γ
Copper recovered from column	9.9 γ

DISCUSSION

When the procedure was first tried, an external standard was employed, but the amount of copper recovered was erratic. This condition was corrected by using an internal standard or "standard addition" technique as described by Hohn (*5*). The failure of the external standard method was traced to the fact that small but varying quantities of precipitated casein redissolved at the very low pH employed. On neutralization of the filtrate to pH 5.0 the casein would again be precipitated, remain in the resin column, and again be redissolved by the elutriant acid. In addition, sugars and salts of the milk filtrate are carried through the resin. The varying quantities of these materials markedly influenced the diffusion current. However, with the method of standard addition the diffusion current was found to be strictly a linear function of the copper concentration. Although two polarograms must be recorded for each analysis, it is no serious time-consuming burden on the analyst—for example, with the instrument used in this work (Heyrovský micropolarograph, Model XIX) the two polarograms can be completed in 6 minutes.

The gross volume change between prepared sample and deproteinized and defatted milk filtrate due to change in total solids and fat elimination has a negligible effect on the concentration of trace components. Furthermore, the addition of perchloric acid compensates largely for this loss. This is well evidenced in Table I, which shows comparative data between spectrophotometric determinations of copper in fluid milk, powdered whole milk, and ice cream mix and their respective filtrates prepared in accordance with the proposed method. In all cases where spectrophotometric data are presented the mono-color dithizone procedure of Bendix and Grabenstetter (*2*) was employed. Seventy-five milliliters of filtrate were employed for all determinations made on filtrates. The aliquots were concentrated and digested with sulfuric and nitric acids. Five-gram samples of the powdered milk samples were prepared for analysis by acid digestion with sulfuric, nitric, and perchloric acids as described by Thompson (*9*) for preparation of samples for iron.

The necessity of buffering the filtrate is eliminated, as the pH need not be too critically controlled. Excellent copper recoveries may be obtained from filtrates ranging from pH 3.5 to 6.5. The neutral point of the ammonium hydroxide–perchloric acid system is pH 5.0, and the indicator titration described will with reasonable care yield a filtrate well within the pH limits. During the passage of neutralized filtrates (pH 3.5 to 6.5) through the resin, the pH will not vary appreciably from 1. Water washing of a prepared resin column will yield an effluent of pH 5 to 6. Thus, if the following conditions are adhered to, excellent copper recoveries will be obtained:

pH of prepared resin	5 to 6
pH during passage of filtrate	Approximately 1
pH at end of water wash	5 to 6

When the pH of the filtrate is greater than 7, the residual acid of the resin is rapidly depleted and the "copper-grabbing" properties are also depleted. If the pH is lower than 1, the copper will pass through the resin without absorbing.

Precision of the proposed method is demonstrated in Table II, which shows data obtained on a single sample of high copper content powdered whole milk, but using different volumes of filtrate and different sensitivities. Copper was added prior to the addition of the perchloric acid.

Table III shows comparative data obtained by the proposed method, the dithizone procedure, and a procedure in which the sample was first dry-ashed (ignited) and determined polarographically.

If polarographic equipment is not available, spectrophotometric methods may be employed. A suggested procedure is as follows:

Recover the elutriant acid and washings from the resin in a 300-ml. Kjeldahl flask. Concentrate by boiling. Cool and add 4 ml. of concentrated sulfuric acid and few milliliters of redistilled nitric acid. Complete destruction of organic matter in the usual

Table III. Comparison of Methods for Determining Copper

Sample	Spectrophotometric Dithizone Procedure, Sample Wet-Ashed	Polarographic Procedure, Sample Dry-Ashed	Proposed Method
	P.p.m.	*P.p.m.*	*P.p.m.*
Raw fluid milk	0.15	.....	0.15
	0.15	.....	0.20
Powdered whole milk[a]	.....	11.3	11.7
	.....	11.4	11.7
	.....	10.8	10.7
Duplicate determination	.....	.....	10.8
Powdered whole milk[a]	15.2	.....	15.0
Duplicate determination	15.1	.....	15.5
	15.0	.....	15.2
Powdered whole milk	0.80	.....	0.80
	0.60	.....	0.70
Ice cream mix	0.65	.....	0.45
	0.20	.....	0.35
Nonfat milk solids	.....	1.1	1.1
	.....	1.2	1.2

[a] Special high copper content samples.

manner, make the prepared solution up to 50 ml., and determine the copper in accordance with the method of Bendix and Grabenstetter (*2*).

In this manner a concentration of four times that possible by direct digestion of the sample is accomplished. Phosphates will have been eliminated as anions in passing through the resin the first time.

It is anticipated that the proposed method, or modifications thereof, will have wide application to the determination of traces of copper in a number of food products, particularly those foods which are in liquid form or can be readily dissolved or blended to a liquid form. In the latter case correction for volume changes will undoubtedly be of significance.

Investigations are now being undertaken in application of the concentration techniques described for the determination of traces of metals other than copper.

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Measuring the Distribution of Particle Size in Dispersed Systems

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An existing method for particle size measurement has been simplified. An apparatus and a technique are described which bring the investigation of particle size distribution and related research within the scope of laboratories having limited facilities. A consistent technique is suggested for testing all dispersions in general and concentrated emulsions in particular.

MOST of the methods now in use for particle size determination involve apparatus too complex and specialized to be feasible for the occasional needs of many investigators. A simplified procedure, useful for every system from concentrated emulsions to dilute suspensions and requiring only apparatus which can be assembled in any laboratory, would be advantageous.

Significant data on globule size distribution in emulsions having a high percentage of internal phase are difficult to obtain, since most methods used for particle size measurements make use of the laws of settling which do not apply under such conditions. Stoke's friction law, for instance, fails when the emulsifying agent itself constitutes a continuous phase within the emulsion, or when the external phase ceases to represent a homogeneous mass compared to the mean distance between internal phase globules.

A constant velocity of settling is assumed in most laws governing the fall of particles in a fluid medium. This condition can be obtained only in dilute systems. Addition of excess external phase until a dilution is reached, where it is known that the laws of settling do apply, may be the most straightforward way of handling concentrated emulsions.

The testing of all dispersed systems under the same conditions tends to increase the significance of the results. This is particularly evident when systems having nearly the same range of particle size but different concentrations are considered, for when these are diluted to a standard concentration for measurement, the probability of any particle suffering interference equivalent to a definite lengthening of its period of settling is approximately the same for each system.

In view of these considerations, the author has improved an existing micromanometric method (*2*, *3*, *5*, *6*, *9*) by simplifying calculations, minimizing work, and introducing a more consistent technique which extends the usefulness of the method.

THEORETICAL

Let

θ = angle inclined part of capillary manometer makes with horizontal
M = hypothetical membrane at juncture of manometer with sedimentation tube
A = cross-sectional area of sedimentation tube of uniform cross section
H = vertical distance from mid-point of M to surface in sedimentation tube
s = mean cross-sectional area of capillary manometer
d_i = density of internal phase of dispersed system
d_x = density of external phase (dispersion medium)
y = distance meniscus retreats in manometer during time t
w = grams of internal phase settling or rising past M during time t

A dispersed system is put into the large tube after the manometer has been filled with the dispersion medium (by filling the whole system with the dispersion medium, closing the stopcock, and pouring out the contents of the sedimentation tube). As particles of the internal phase move past the mid-point of M, the density of the system above this point decreases and the pressure which its weight exerts upon M diminishes, causing a receding of the meniscus in the manometer. As w grams of the dispersed phase settle or rise past M, a volume of dispersion medium weighing $w\frac{d_x}{d_i}$ grams is displaced upwards or downwards.

[1] Present address, 1530 Park Ave., Richmond, Va.

The accompanying change in pressure, Δp_1, on the sedimentation tube side of M is given by

$$\Delta p_1 = -\frac{\left| w - w\frac{d_x}{d_i} \right|}{A} \quad (1)$$

To compensate this change, the meniscus falls in the manometer a distance y, producing a change in pressure, Δp_2, on the manometer side of M.

$$\Delta p_2 = -d_x X y \sin\theta \quad (2)$$

However, the fall in level in the manometer introduces into the main tube a quantity of dispersion medium. This amount of liquid added to the quantity in the tube increases the pressure on that side of M by Δp_3 which is given by

$$\Delta p_3 = \frac{sXyXd_x}{A} \quad (3)$$

Equating the pressures on both sides of M—i.e., letting $\Delta p_1 + \Delta p_3 = \Delta p_2$ and simplifying:

$$w = y\frac{d_x d_i}{|d_i - d_x|}(A\sin\theta + s) \quad (4)$$

Inspection of Equation 4 shows that, in order to measure the distribution of particle size in emulsions where $(|d_i - d_x|)$ is small, the term $(A \sin\theta + s)$ should have a value of about 0.05. Thus, for a 5% benzene in water emulsion the meniscus would retreat over 40 cm. in the manometer during a run on a 50-ml. sample. When testing dispersions of most solids, $(A \sin\theta + s)$ should approach 0.1 for an apparatus of the dimensions discussed below. Since y can be measured to the nearest 0.1 mm. with simple equipment, a precision of 1% is obtained on all but 2.5% or less of the dispersed phase.

The capillary should have an inside diameter of about 0.75 mm. with 8-mm. outside diameter for rigidity. The manometer gage should be about 50 cm. long. Sin θ should lie between 0.01 and 0.05. The diameter of the main tube should not exceed 16 mm. and it should be long enough to contain 50 ml. below and above M. It is not wise to use a capillary with much smaller bore than that mentioned, for capillarity and lack of uniformity over any useful length would introduce errors. The influences of temperature variations, inertia, evaporation, and contamination of the walls of the manometer contribute to make 1% the limiting accuracy for this method. However, excepting carefully controlled conditions, the errors which arise in most applications of the equations governing fluid friction do not permit greater accuracy.

If we let P denote the percentage of the total mass of internal phase settling or rising past M during time t, while W represents the total mass of internal phase in the original system

$$P = \frac{w}{W} \times 100 \quad (5)$$

If Y represents the total recession of the meniscus in the manometer during a run, it follows from Equation 4 that

$$P = \frac{y}{Y} \times 100 \quad (6)$$

When a run is made, the position of the meniscus as read on a scale strapped under the manometer must be recorded at measured time intervals. Also, H must be known to the nearest millimeter. Equation 6 is used to compute values of P corresponding to observed values of y.

The actual amount of material represented by any value of P is a heterogeneous system composed of (*a*) particles constituting size groups the whole of which have passed M during the associated time elapsed, and (*b*) smaller particles belonging to all the other size groups present in the polydispersed system. The rate of sedimentation (up or down) of all these classes together is dP/dt. Thus, $t \times dP/dt$ gives the percentage of dispersed phase in these latter size ranges. Therefore, if S denotes the percentage of internal phase in size ranges which have, in their entirety, passed M during time t

$$P = S + (t \times dP/dt) \quad (7)$$

If P is plotted against t, the ordinate intercept on the P axis of any tangent to the resulting curve will be a value of S (*1*, *4*, *7*, *8*). Thus, the difference between the ordinate intercepts of any two tangents to this P,t curve gives the percentage, ΔS, of the total dispersed phase corresponding to the size range $r \ldots r_n$ calculated from Stoke's law for the time interval between the abscissas at these points of tangency. The distribution of particle size can now be tabulated for all the material in the dispersed phase.

The following form of Stoke's law should be used in this calculation:

$$r^2 = \frac{9 \times \eta \times H}{2 \times D \times g \times t}$$

where

r = radius of particles moving with constant velocity
η = viscosity of dispersion medium, poise
D = absolute density of internal phase, $|d_i - d_x|$
g = acceleration of gravity
t = time of sedimentation, seconds

In the author's experience, most emulsions having a percentage of internal phase not greater than the close-packing ratio for spheres of uniform size (74.048%) do not break upon dilution with excess of their pure external phase. In true emulsions the protecting films of emulsifying agent (no matter whether they are composed of solid particles, polar molecules, electrically charged ions, etc.) surrounding a globule of internal phase can exist unchanged completely apart from any other such films on any other globule. The same characteristics of the protecting layer which prevent globules from coalescing when they are almost in contact with one another will function equally well when the same globules collide in a diluted emulsion.

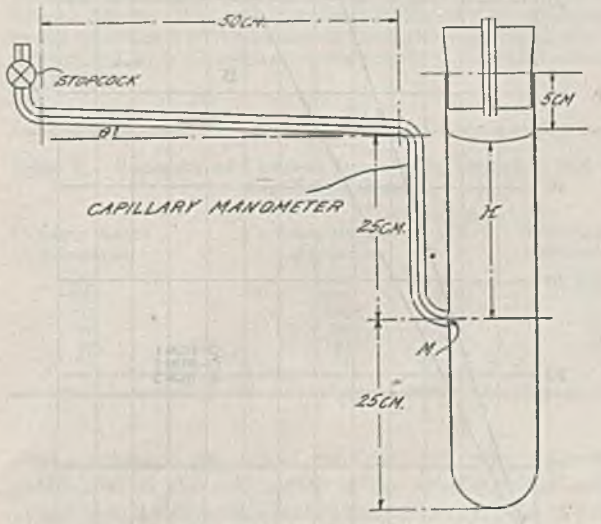

Figure 1. Sedimentation Apparatus

Emulsions which break upon dilution are generally those having more than 74.048% of internal phase, and usually, these are partially pseudo-emulsions—that is, they contain internal phase globules which are not covered by emulsifying agent but are pocketed mechanically among protected globules. Upon dilution these unprotected globules coalesce to form a layer of free internal phase which destroys the whole emulsion. When the close-packing ratio is exceeded, the films of emulsifying agent are in continuous contact over large areas of their surfaces and often become actually a separate continuous phase. In such systems, dilution destroys the continuity of the network of emulsifying agent and such emulsions often break spontaneously.

However, for most emulsions having concentrations of internal phase between 20 and 74%, dilution may be the only practical means by which they can be reduced to a form where the dis-

tribution of particle size can be measured. Furthermore, the cases other than the exceptions discussed above give little evidence that dilution will influence the size of dispersed particles.

EXPERIMENTAL

The dimensions of the apparatus have been discussed and are repeated in Figure 1. The capillary is selected for its uniformity by measuring the length of a piece of clean mercury at various portions of the length of the tube to be used as the manometer. Available in most laboratories is a room or bath where a fixed temperature can be maintained to within ±0.5° C., which is sufficient even when liquids having high coefficients of expansion are used in this apparatus. No calibration of the apparatus is necessary.

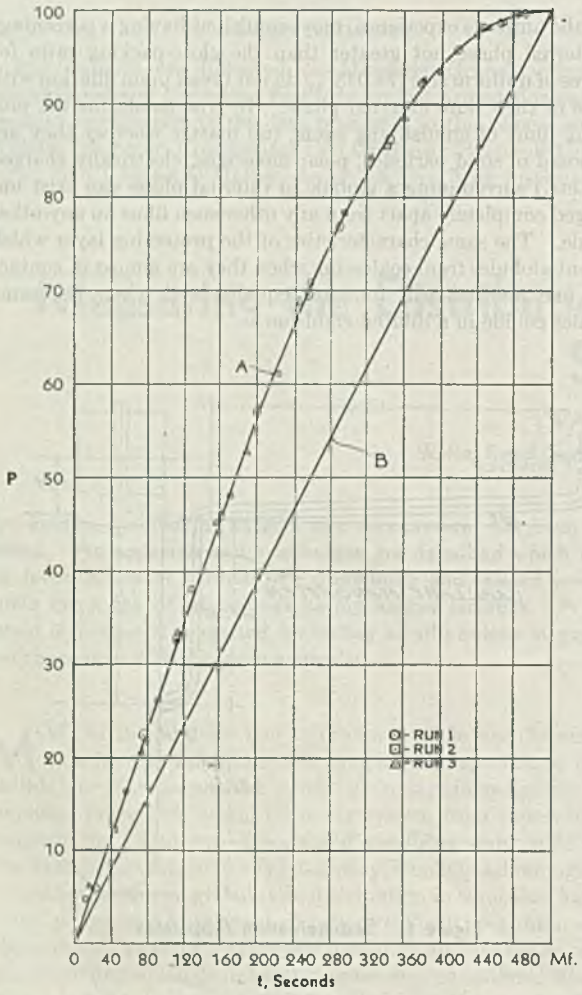

Figure 2

If any difficulty arises in obtaining the desired sin θ, a trial run can be made, during which the positions of the clamps are adjusted to bring the manometer as close to the horizontal as necessary to obtain the desired accuracy. With the manometer full of dispersion medium and the main tube empty, a run is begun by pouring a dispersion into the main tube to a predetermined mark where the meniscus in the manometer is supported near the beginning of the manometer scale close to the stopcock. The timer is started immediately and, simultaneously, the stopcock is opened. A few readings must be taken at close intervals, so that the position of the meniscus at zero time can be accurately obtained by a short extrapolation. The rate of recession of the dispersion medium in the manometer due to evaporation via the stopcock and in the main tube should be determined for each liquid used. Volatile liquids will give a recession of only a fraction of a millimeter per hour. Correction can be made for this on the finest dispersions. Normally, readings should be stopped when the rate of recession approaches this value.

Table I. Particle Size Distribution

$r \times 10^4$ cm.	ΔS (%)
> 10.1	2.9
10.1 – 8.97	5.4
8.97 – 8.24	5.6
8.24 – 7.74	7.6
7.74 – 7.51	5.4
7.51 – 7.16	4.3
7.16 – 6.74	12.4
6.74 – 6.42	16.5
6.42 – 6.14	14.7
< 6.14	25.2

Curve *A* in Figure 2 shows the mean P,t curve for three runs on identical suspensions of 1.80% Fe_3O_4 in 1,4-dioxane (diethylene dioxide). The density and viscosity at 25° of the *p*-dioxane were 1.03 grams per ml. and 1.20 cp., respectively. The ordinate deviations of the points from the curve are 1% or less in all but the initial values. Results which are reproducible to within 1% for such coarse systems show that higher precision is possible when dispersions having longer settling times are tested. Curve *B*, included for comparison, represents a monodispersed system composed of only the smallest particles in *A*. Table I gives the weight percentages of dispersed phase corresponding to various size ranges.

This method is suggested for measurements upon emulsions of all concentrations at various ages to determine the effects of aging upon the distribution of globule size. Samples of creams of such emulsions may be diluted with dispersion medium to a concentration, for example, of approximately 5% internal phase as the standard form to be tested. While some doubt may exist as to whether or not such dilution affects the distribution of globule size, there is no doubt that, for a series of similar emulsions all tested under approximately the same conditions, significant results for comparison may be obtained. Systems of mixed pigments where photographic or colorimetric methods give dubious results are readily tested with this apparatus.

ACKNOWLEDGMENT

The author wishes to express his gratitude to M. W. Green and the officers of the American Pharmaceutical Association, who generously extended the facilities of their laboratories for this work.

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Collective Index to Analytical Edition

Type of the Collective Index to the ANALYTICAL EDITION has been held since the second printing of the book several months ago, but the present need for type metal is such that it cannot be held much longer. Any who want to secure copies are advised to send orders now to the AMERICAN CHEMICAL SOCIETY, 1155 Sixteenth St., N. W., Washington 6, D. C.

NOTES ON ANALYTICAL PROCEDURES

High-Speed Filter Aid for Chromatographic Analysis

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SINCE a specially prepared heat-treated siliceous earth (Hyflo Super-Cel) sold by Johns-Manville was first recommended by Strain (*5*) to preserve the advantages inherent in the use of finely divided adsorbents in chromatographic analysis and to increase the rate of flow of liquid through the adsorbent column, it has found wide application for these purposes. It may be used with various adsorbents in addition to that originally recommended (*4*).

At the time Hyflo Super-Cel was first recommended by Strain, this product allowed the highest rate of flow of liquids through a column of any of the filter aids commercially available. Several years ago, however, several filter aids became available which allow higher rates of flow. Nevertheless, Hyflo Super-Cel continues to be the filter aid in general use for chromatographic analysis, as shown by recent articles (*1*, *2*, *3*, *7*, *8*). Celite No. 545 allows the highest rate of flow of any filter aids presently available. It is white in color and therefore suitable for use as a filter aid and diluent in chromatographic analysis. Table I shows the rates of flow resulting from using Celite No. 545 in place of Hyflo Super-Cel in adsorption columns containing some typical adsorbents mixed with filter aids.

The magnesia used in the tests was Micron Brand No. 2641, made by Westvaco Chlorine Products Co., Newark, Calif., which is widely used for chromatographic analysis. The materials were weighed in the proportions listed and thoroughly mixed by passing through a kitchen-type flour sifter three or four times. In all cases the adsorption columns were 3.3 cm. in diameter. The adsorption columns were filled with adsorbent mixture to the height listed in Table I, and fitted to modified side-arm separatory funnels. A battery of six modified side-arm separatory funnels was connected to a water pump capable of developing a maximum vacuum of 2 cm. of mercury, and the solvents were drawn through the columns under the full force of the pump. The solvents were run concurrently through comparable adsorbent columns using Hyflo Super-Cel and Celite No. 545. All determinations were in triplicate, and the averages of three determinations are shown in Table I.

The data in Table I show that substituting Celite No. 545 for Hyflo Super-Cel is a diluent or filter aid in chromatographic adsorption analysis will result in greatly increased flow rates through the adsorbent columns. The saving in time can be appreciable when large numbers of chromatographic analyses are made during a day, or when large volumes of solvent must be passed through an adsorption column.

Table I. Rates of Flow of Solvents through Adsorbent Columns

Adsorbent	Solvent	Column Height	Time for Solvent to Pass through Column: First drop	Time for Solvent to Pass through Column: 100 ml. after first drop
		Cm.	*Min.*	*Min.*
1 part Super-Cel to 1 part MgO	Petroleum ether	10	3.5	9.5
1 part Celite 545 to 1 part MgO	Petroleum ether	10	2.5	6
3 parts Super-Cel to 1 part MgO	Petroleum ether	15	4.25	6.25
3 parts Celite 545 to 1 part MgO	Petroleum ether	15	1.25	2.25
1 part Super-Cel to 1 part fuller's earth	Water	7.5	4.5	29
1 part Celite 545 to 1 part fuller's earth	Water	7.5	2.25	12

These heat-treated siliceous earths will adsorb chlorophylls and carotenols (*6*), but Celite No. 545 is less strongly adsorbent than Hyflo Super-Cel for these pigments, as shown by the greater width of pigment bands on the Celite No. 545 columns when equal amounts of pigments are adsorbed on the two siliceous earths. Some lots of Hyflo Super-Cel slightly adsorb carotenes, but not sufficiently to prevent petroleum ether from readily washing the carotenes through any column of the sizes normally used. Adsorption of carotene by Celite No. 545 has not been observed.

To test the loss of carotenes upon passage of a solution of carotenes through the two siliceous earths, aliquots of a solution of carotenes in low-boiling petroleum ether (boiling range 30° to 60° C.) were run through adsorption columns of Hyflo Super-Cel and Celite No. 545. The columns were 3.3 cm. in diameter and were filled to a height of 25 cm. with the siliceous earths. The aliquots were diluted to 75 to 100 ml. with petroleum ether, the solutions were poured on to the dry columns which were under suction, the carotenes were washed through the columns with fresh petroleum ether, and the solutions were made up to a volume of 200 ml. The total carotenes in the original solutions and in the solutions which had passed through the columns were then determined in a photoelectric colorimeter. Results are shown in Table II.

Table II. Recovery of Carotene from Hyflo Super-Cel and Celite No. 545 Columns

Carotene Added	Carotene Recovered: Celite No. 545	Carotene Recovered: Hyflo Super-Cel
Micrograms	*Micrograms*	*Micrograms*
402	407	402
207	207	201
102	100	100
89	89	83
39	39	37
14	14	14

The minimum amount of carotene that could be definitely determined in the colorimeter at the concentrations involved avried from 2 micrograms for the most dilute solution to 5 micrograms for the most concentrated solution. Within these limits, no significant loss of carotenes could be detected when the carotene solutions were passed through the Celite No. 545 columns. A small but significant loss of carotenes occurred in some instances with the Hyflo Super-Cel columns.

Thompson *et al.* (*7*) report that a contaminant absorbing light in the region of 310 to 370 millimicrons dissolved from a mixture of calcium hydroxide and Hyflo Super-Cel with petroleum ether and benzene mixtures. It has been observed in this laboratory that passing methanol or water through a column of Hyflo Super-Cel or Celite No. 545 will wash out a yellow pigment. Less of this pigment has been obtained from Celite No. 545 than from equal volumes of Hyflo Super-Cel in the samples tested, but sufficient data are not available to determine if this is a general relationship. This pigment will cause a marked light absorption on a photoelectric colorimeter using a Corning No. 554 (Signal Blue) glass color filter as in the colorimetric determination of riboflavin. This pigment will not normally interfere with the determination of riboflavin if riboflavin is first adsorbed on a column of mixed

Table III. Recovery of Riboflavin from Hyflo Super-Cel and Celite No. 545 Columns

Riboflavin Added	Riboflavin Recovered	
	Hyflo Super-Cel	Celite No. 545
Micrograms/ml.	*Micrograms/ml.*	*Micrograms/ml.*
2.8	2.5	2.9
5.5	5.3	5.4
9.8	9.3	9.6
15.0	14.7	14.8
19.8	19.8	19.6

adsorbent and siliceous earth and then eluted, as the pigment is normally discarded with the first washings from the adsorption column. Celite No. 545 is, however, preferable to Hyflo Super-Cel in the preparation of adsorbent columns for chromatographic analysis. Although riboflavin does not form adsorption bands on columns of these siliceous earths, a small amount of riboflavin appears to be lost upon passage through these materials. This amount is generally not significant for Celite No. 545, but may be significant for Hyflo Super-Cel.

To determine the loss of riboflavin in Hyflo Super-Cel and Celite No. 545, columns 3.3 cm. in diameter were filled to a height of 12 cm. with these materials. A solution was made of synthetic U.S.P. riboflavin in water containing 5 ml. of glacial acetic acid to the liter. Aliquots were taken of this solution for determination of the riboflavin content in a photoelectric colorimeter. Similar aliquots were poured on the dry columns, the solutions drawn through the columns under suction, and the columns washed free of pigment with water containing acetic acid. The riboflavin solutions were diluted to a volume of 100 ml., and the riboflavin content was determined in the photoelectric colorimeter. The results were corrected for the interfering pigment which equaled 1.40 micrograms per ml. of riboflavin for Hyflo Super-Cel and 0.54 microgram per ml. of riboflavin for Celite No. 545. These results are shown in Table III.

The concentration of riboflavin that could be definitely determined varied from 0.1 microgram per ml. for the most dilute solution to 0.3 microgram per ml. for the most concentrated solution tested. The loss of riboflavin in the Celite No. 545 column is, therefore, barely significant. An appreciable loss of riboflavin was experienced upon passage of the solutions through the Hyflo Super-Cel columns.

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Determination of Hydrogen in Highly Fluorinated Carbon Compounds

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A PROCEDURE for analyzing compounds of the type C_nF_m for carbon and fluorine has been previously described (*2*). A method is described in this paper for the determination of hydrogen in similar highly fluorinated carbon compounds. The method consists of decomposing the sample with magnesium in an evacuated quartz tube at about 700° C., passing the residual hydrogen over hot copper oxide, and absorbing the water formed in a weighed phosphorus pentoxide tube.

Mention has been made (*1*) of an experiment in which the hydrogen content of fluoroform was determined by repeatedly passing the sample over heated sodium metal until it was completely decomposed and then measuring the volume of the residual hydrogen. Attempts were made in this work to use sodium or potassium in the decomposition, but the results were erratic and consistently low. While the temperature in the hottest portion of the tube was sufficiently high to decompose sodium or potassium hydrides, it is believed that combination of the metal and hydrogen took place in cooler portions of the tube. When magnesium was used no difficulties of this nature were encountered.

The apparatus, Figure 1, consists essentially of alternate sample bulbs, *C* and *D*, a quartz tube, *G*, in which the nickel boat containing the magnesium is placed, a copper oxide container, *J*, the absorption tube, *I*, which contains phosphorus pentoxide, and an automatic gas pump, *H* (*3*) to recycle unreacted hydrogen and sample. *G* is heated by a two-piece resistance furnace which can be removed without disconnecting the tube. *J* is wound with a Nichrome coil which serves as a heating element.

Experimental Procedure. The sample, in the case of substances boiling below about 50° C., was weighed by introduction into the previously evacuated and weighed bulb, *D*. For higher boiling substances the sample was weighed directly into a small glass cup which was then placed in tube *C;* it was necessary to freeze and degas these samples before analysis. The samples were of such size that between 30 and 40 mg. of water would be formed.

The nickel boat, filled with approximately 1 gram of magnesium turnings, is introduced into the quartz tube and the system sealed and evacuated. The furnace, previously heated to 650° to 700° C., is lowered and clamped about the quartz tube. The copper oxide is heated to about 300° C. Evacuation is continued until no further gas is given off by the magnesium. The sample is then admitted to the magnesium and the circulating

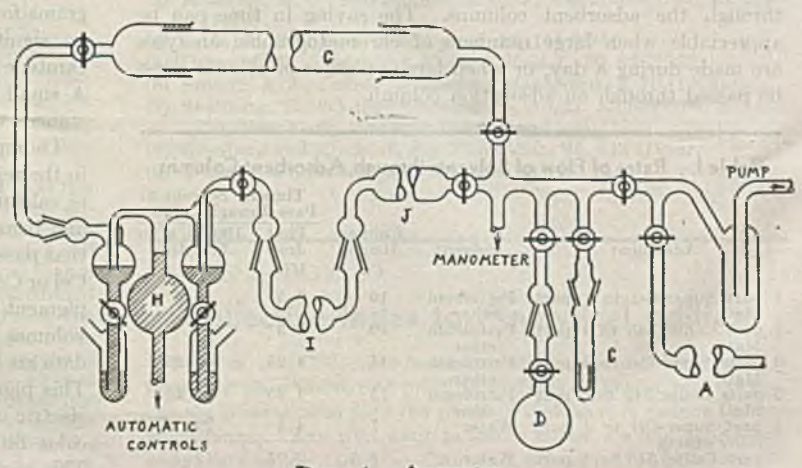

Figure 1. Apparatus

pump started. Pumping is continued until the pressure falls to approximately 3 mm., the lower operating limit of the pump. By withdrawal of the mercury from the valves, the residual gas trapped in the pump is released to the combustion system.

When the pressure has fallen to essentially zero, dry air is admitted through tube *A* which contains phosphorus pentoxide. The absorber tube, *I*, is then removed, protecting ground-glass caps are placed over the ends, and the tube is reweighed. The amount of hydrogen present in the original sample can then be readily calculated.

The procedure was checked by analysis of methyl fluoride and tank hydrogen. Calculated for CH_3F: H, 8.89. Found: H, 8.51. Calculated for H_2: 0.357 gram of H_2O. Found: 0.0345, 0.0336 gram of H_2O.

Three of the compounds analyzed are presented as examples.

Compound	Hydrogen Calculated	Found
C_2F_7H	0.59	0.55
$C_2F_6H_2$	1.32	1.29
C_2F_6HBr	0.43	0.40

This work was done under the sponsorship of Minnesota Mining and Manufacturing Company.

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Electronic Make and Break for Relay Operation

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IN THE operation of a constant-temperature air bath where mechanical circulation was not permissible and convection provided the only stirring, the problem of supplying heat in small increments to prevent overregulation of the bath temperature was met. If the heat source remained "on" continuously, enough heat to raise the bath some 2° or 3° had been supplied by the time the thermoregulator responded to the temperature increase. By the make and break arrangement heat was supplied for 3 to 4 seconds only of each 30 seconds, to allow dissipation by convection, and regulation to ±0.2° was obtained.

The first device employed to effect this was a clock with a sweep second hand brushing over a metallic strip, so that an electrical contact was maintained for 3 to 4 seconds in each 30 seconds. A very reliable apparatus is described by Booth and Martin (*1*). It was thought that these mechanical devices might be simplified by the use of an adaptation of the relaxation oscillator. However, the necessity for the auxiliary power supply equipment was undesirable and a modification of the Serfass regulator (*3*), employing one electron tube and a few resistors and condensers, was chosen as the simplest solution. The principle of a pickup and dropout action has been described by Henney (*2*).

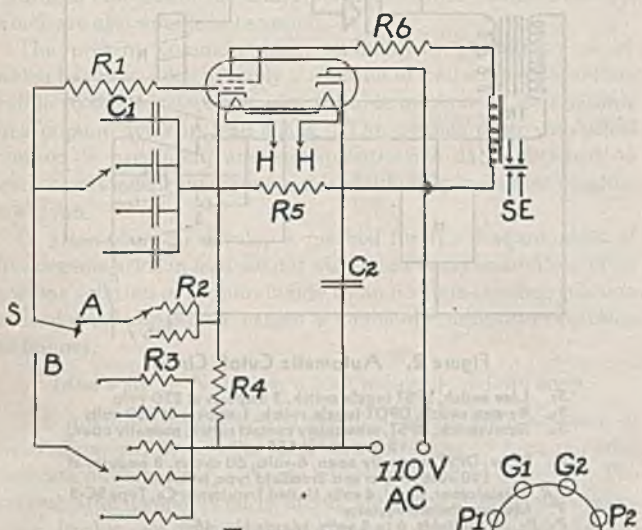

Figure 1

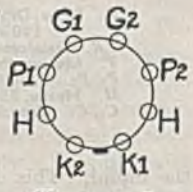

Figure 2

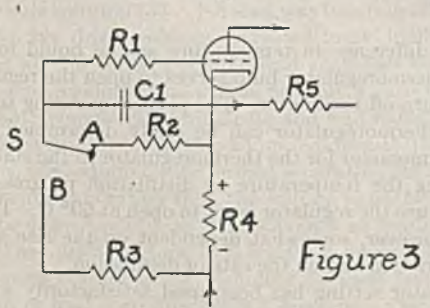

Figure 3

Resistor and Condensers

R_1. 1 meg. 0.5 watt
R_3. 1 meg. 0.5 watt
20 meg. 0.5 watt
R_4. 1 meg. 0.5 watt
2 meg. 0.5 watt
5 meg. 0.5 watt
10 meg. 0.5 watt
20 meg. 0.5 watt
R_5. 1000 ohms 5 watts
R_6. 15,000 ohms 2 watts
R_7. 10,000 ohms 2 watts
C_1. 0.1 mfd. 250 volts
0.25 mfd. 250 volts
1.0 mfd. 250 volts
4 mfd. 250 volts
These are paper condensers.
C_2. 8-mfd. 250-volt dry electrolytic condenser

The basic circuit is that of Serfass, modified by a condenser to introduce time delay into the rise and fall of the grid voltage, with a mechanical device for opening and closing the grid circuit. This latter was accomplished by using a double-pole double-throw relay switch, one pole of which acted as a make and break in the grid circuit, *S*, and the other pole as an off-on switch for outside auxiliary apparatus, *E*. Switch *S*, appearing in the grid circuit of Figures 1 and 3, and switch *E* of Figure 1, are the two halves of the double-pole double-throw relay contacts. Current in the relay coil activates switches *E* and *S* simultaneously.

Figure 3 is a simplified version of the grid circuit. Electrons flow up through R_4 to the cathode of the tube. Voltage is maintained across resistance R_4 by the by-pass resistor, R_5. If section *S* of the double-pole double-throw switch is in position *B* electrons will flow through R_3 to charge condenser C_1. Initially *S* is in position *A*, and the grid is, in effect, short-circuited to cathode by R_1 and R_2. Current flows in the tube and causes the relay to close, switching *S* from *A* to *B* positions. Electrons flow through R_3, charging C_1 so that the grid side becomes negative. As C_1 charges the plate current of the tube decreases, and when it has reached a value too weak to hold the relay closed, *S* is switched from *B* to *A*. The time delay before the relay opens is controlled by the capacity of C_1 and the value of resistor R_3.

C_1 can now discharge through R_2 and does so at a rate controlled by the condenser capacity and the resistance of R_2. As C_1 discharges, the grid potential approaches that of the cathode and the plate current rises. At a value of plate current sufficient to close the relay, *S* is switched from *A* to *B* again and the cycle repeats. The time of charge and discharge is regulated by condenser C_1. The ratio of off to on periods can be varied by changing the ratio of the values of R_3 and R_2.

When R_2 and R_3 are equal the relay will be open and closed roughly for equal periods of time. If R_3 is greater than R_2 the time for charge of C_1 will be greater than for discharge and the relay contacts will be closed for a longer period than they are open. If the power fails the contacts open. When R_2 is greater

than R_3 the relay contacts are open for a longer time than they are closed. In order to vary the off-on ratio, a number of resistors from 1 to 20 megohms on a multiple contact switch were substituted for R_3. In order that the period of time for which the contacts were closed might be either greater or less than the time period they were open, a single-pole double-throw switch was used for R_2 with two resistors of 1 and 20 megohms. Changing from one resistor to the other in R_2 inverts the open-closed time ratio.

The complete circuit is shown in Figure 1, and the tube base (117L7/M7 GT) is shown in Figure 2. The relay is a double-pole double-throw Guardian (Guardian Series 5, D.C. coil 7.5 ma., 800 ohms). A 10,000-ohm resistor, R_4, was put in the plate circuit to reduce the plate current to fit the relay. This resistor should be adjusted to the relay coil used. The tension of the relay spring had to be increased to get reliable operation on the pullout action. The pullout action may manifest a certain vibration or chattering due to the half-wave rectification of the vacuum tube, but this has not affected the operation of the device. The rapidity of the cycles is governed only by the response of the relay.

This apparatus has been in operation for some months and has functioned quite satisfactorily. The device may also be used for operation of an enclosed magnetic stirrer where the cycle or rate of stirring might be varied over wide limits, or any apparatus where electrical impulses are required periodically.

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Automatic Cutoff for Electrically Heated Water Still

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AN ELECTRICALLY heated glass water still equipped with the constant-level device described by Holmes (*1*) has been installed in this laboratory to furnish redistilled water for microchemical and other analytical work. The still is entirely automatic and requires no attention except for periodic removal of sediment in the still pot and refilling of the water reservoir. Since it is frequently desirable to allow the still to run without attention, a means has been devised for automatically shutting off the heater when the reservoir is empty, to prevent breakage of the distillation flask. This device is simply constructed and positive in action.

A ⅌ 14/20 outer joint was sealed onto the standpipe about 60 mm. above the constriction (7 mm. in inside diameter) as shown in Figure 1. A mercury thermoregulator was sealed through a ⅌ 14/20 inner joint, so that its bulb extended as nearly as practical across the standpipe. The thermoregulator was made from 6-mm. capillary tubing having a bore of approximately 0.5 mm. An expansion chamber equivalent to at least 25° C. must be left at the top of the regulator.

Equally good results were obtained with an 11-mm. glass side tube on the standpipe in place of the ⅌ 14/20 outer joint, and with a regulator, also without ⅌ joint, held in place by a short sleeve of rubber tubing. Small thermoregulators, both fixed- and variable-setting, are commercially available in the right-angle style.

The circuit (Figure 2) consists essentially of a two-pole normally open relay, R; one pole and the holding coil are in series with the mercury thermoregulator, T, in the secondary circuit of transformer TR, and the other pole is in series with the line switch, S_1. S_2 is used to by-pass the thermoregulator until the water in the standpipe is warm enough to close the thermoregulator contacts and also to operate the pilot lights, P_1 and P_2, which indicate whether or not the heater, H, is under the control of the thermoregulator. S_3, a momentary contact switch, serves as a reset for the relay; one pole completes the 115-volt circuit and the other completes the secondary circuit. Capacitors C_1 and C_2 are placed across the mercury thermoregulator and the relay contacts to reduce sparking when contact is made or broken.

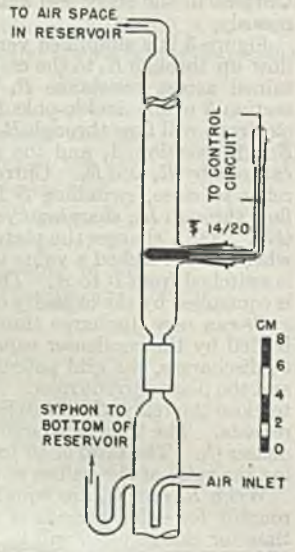

Figure 1. Standpipe and Mercury Thermoregulator

The air above the liquid in the standpipe is much cooler than the liquid. This difference in temperature as the liquid level drops below the thermoregulator bulb serves to open the regulator contacts and turn off the heater. The proper operating temperature for the thermoregulator can be easily determined by substituting a thermometer for the thermoregulator in the standpipe and observing the temperature as distillation progresses. In the unit now in use the regulator is set to open at 50° C. This temperature is, however, somewhat dependent on the size and shape of the constriction and on the rate of distillation.

The same regulator setting has been used satisfactorily with reservoirs having capacities of 1, 10, and 20 liters. The bottom of the reservoir should not be lower than the constriction in the standpipe. The reservoir is then completely empty when the heater is turned off.

To operate the still, line switch S_1 is closed, by-pass switch S_2 is thrown to position 1, and reset switch S_3 is closed momentarily. The heater is now on and pilot light P_1 burns to serve as a warn-

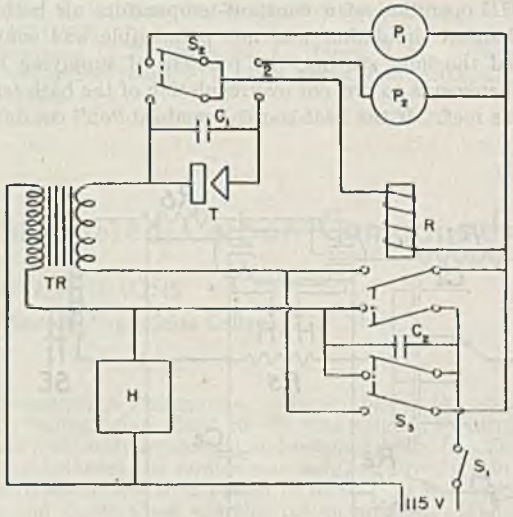

Figure 2. Automatic Cutoff Circuit

S_1. Line switch, SPST toggle switch, 3 amperes at 250 volts
S_2. By-pass switch, DPDT toggle switch, 1 ampere at 250 volts
S_3. Reset switch, DPST, momentary contact switch, normally open, 12 amperes at 125 volts
R. Relay, DPST, normally open, 6-volts, 60 cycles, 8 amperes at 110 volts, Potter and Brumfield type MRA-4
TR. Transformer, signal, 4 volts, United Transformer Co. Type SC-3
T. Mercury thermoregulator
P_1, P_2. Pilot lights, 6 to 8 volts, Mazda No. 46
H. Heater, 550 watts, Precision Scientific Co. Type H
C_1, C_2. Capacitors, 0.1-mfd., Cornell-Dubilier Type DT-4P1

ing that the heater is not under the control of the regulator. After the water has warmed sufficiently to close the regulator contacts (20 to 30 minutes with the unit now in use), the by-pass switch is thrown to position 2 and S_3 is closed momentarily. Pilot light P_2 now burns to show that the heater is controlled by the regulator. The temperature drop due to the change from liquid to gas phase at the thermoregulator bulb as the reservoir empties causes the regulator contacts to open. This break in the secondary circuit immediately opens the relay and shuts off the heater and primary coil of the transformer; thus, continued arcing at the regulator points and relay chatter, which are common when alternating current is used with mercury regulators, are eliminated. It is not possible to turn the heater on again until the relay is reset, either manually or electrically.

The control system can be somewhat simplified as follows:

The primary coil of the transformer can be wired directly to the line switch, S_1, instead of to the relay as shown in Figure 2. (The heater must, of course, remain wired to the relay as shown in Figure 2.) The primary coil of the transformer is then energized whenever S_1 is closed. S_3 can then be replaced by a doorbell-type push button in parallel with the secondary pole of the relay. Signal transformers are designed for continuous duty and it does no harm if the primary of the transformer remains energized after the heater is turned off.

S_2 can be a shorting switch ("make before break") instead of one of the common toggle type. Such a switch would eliminate the necessity for resetting the relay after changing the position of S_2.

The signal lights can be eliminated. The thermoregulator is then connected directly to the coil of the relay and a single-pole single-throw toggle switch is placed in parallel with the thermoregulator. The switch is "on" during the preliminary warm-up and "off" after contact is made at the thermoregulator.

The entire cost of materials listed under Figure 2, exclusive of the thermoregulator and the heater, is about $9. These makes, types, and capacities were used because they were immediately available. Other materials of similar characteristics could have been used.

This device should find use in other applications where change in level of a liquid results in lowered temperature.

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A Reaction of Lead Soaps with Sodium Iodide

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PREVIOUS investigators (*1*, *4–7*) have shown that reactions of the double-decomposition type can take place in insulating solvents such as dry benzene between heavy metal soaps and dry hydrogen chloride or metallic chlorides, such as stannic chloride, which are also soluble in benzene.

The present communication summarizes preliminary work which has been done to apply a reaction of lead soap with sodium iodide to the development of a suitable method for determining free organic acids in lead soaps. The essence of an analytical method is presented, and semiquantitative data, obtained on lead soaps made from organic acids of differing molecular weights, are given.

In attempting to develop a method for the determination of free organic acids in lead soap it was found that the addition of an acetone solution of sodium iodide to an acetone-benzene solution of lead soap apparently causes a double-decomposition reaction as follows:

$$\text{Lead soap} + \text{NaI} \longrightarrow \text{a lead iodide} + \text{sodium soap}$$

The mixture turns a bright greenish yellow upon addition of the sodium iodide solution and flocs of soapy-looking material separate out when the solvents are evaporated to dryness. The residue after evaporation is bright yellow, and gives an opalescent, frothy, soapy extract with water, which is alkaline to phenolphthalein and gives a curdy precipitate with barium chloride solution (*3*). No lead was found in the benzene extracts from five dried residues extracted individually. This indicates that all the benzene-soluble lead soap had been converted to a benzene-insoluble lead iodide, possibly a basic iodide.

Both commercial lead oleate and lead naphthenates have been treated in solution with sodium iodide with the same general results, the sodium oleate formed separating out more quickly upon solvent evaporation than the sodium naphthenate—i.e., seeming to be more insoluble in these solvents, as would be expected.

Warm 95% acetone (5% water) is an excellent solvent for extracting organic acids, including oxidized fatty acids, from lead soap or lead soap lubricants. However, a small amount of lead soap is dissolved concurrently, which invalidates the usual titration for free organic acids of the combined acetone extracts, because the heavy metal soap is titrated as free acidity (*2*).

Some work has been done in using the foregoing lead soap-sodium iodide reaction to transform such dissolved lead soap in combined acetone extracts to sodium soap and a lead iodide. By extracting the dried residue left after evaporation of the acetone with hot benzene it is possible to isolate the free organic acids (together with a small amount of sodium soap), leaving behind the lead iodide, excess sodium iodide, and most of the sodium soap. The extracted material taken up in alcohol can then be titrated for free organic acids in the usual manner, as the sodium soap present does not interfere.

Semiquantitative results for free acids on lead naphthenates made from naphthenic acids of different molecular weights, using the above technique, indicate a much higher free acid content for the soap made from high molecular weight acids, the following comparative figures being obtained:

Lead Soap	% Organically Combined Pb	% Free Fatty Acids as Oleic
From 176 saponification value acids (318 calculated molecular weight)	18.5	10
From 282 saponification value acids (199 calculated molecular weight)	41.3	3

The greater reactivity of the lower molecular weight acids with lead may account for the lower free acidity in this soap.

Indications have also been obtained that sodium iodide in acetone solution does not react with lead oxide (which may be present in lead soaps) to give free caustic alkali, which would, of course, invalidate the determination of free acidity. However, this reaction does occur when lead oxide is heated with aqueous sodium iodide solution.

It is proposed for the future to ascertain by analysis whether the lead iodide compound formed is a basic salt or the normal diiodide, and thus gain some idea of the structure of the dissolved lead soap. It is also proposed to complete work on the analysis of lead soaps containing weighed additions of free organic acids, using the technique described above, in order to define the accuracy and reproducibility of this method as a means of determining free organic acids in lead soaps.

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New Standard for Use in Ultimate Analysis of Organic Compounds Especially Suited for Microprocedures

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FOR the past three years *S*-benzylthiuronium chloride (benzyl-isothiourea hydrochloride, listed by Eastman Kodak Company in Catalog 34 of Organic Chemicals) has been used in this laboratory as a standard material in the ultimate analysis of organic compounds. It more nearly fulfills the criteria established by Hillebrand (*2*) for judging the fitness of material used as a standard to check on analytical procedures than most of the standards in common use.

Since *S*-benzylthiuronium chloride contains the five elements most commonly encountered in organic analysis—namely, carbon, hydrogen, nitrogen, chlorine, and sulfur—and since it also contains a benzene ring, a thio ether linkage, amine and imine groups, and an ionizable chlorine atom, it is suitable for checking the methods of analysis of any or all of the five elements in a wide variety of compounds. Its use as a standard for carbon and hydrogen not only serves to prove that combustion is complete but also shows the effectiveness of the removal of the acid-forming gases of the three elements most likely to interfere with this determination.

The synthesis and recrystallization of the pure compound (*1*) require no unusual apparatus or techniques and its purity is readily established by confirming analyses for any two or more of the five elements. After drying at 110° C., no precautions are required for its storage, since under ordinary conditions it is stable and nonhygroscopic.

Table I. Analysis of *S*-Benzylthiuronium Chloride

Element	Methods	Percentage Composition Found (Range)	Theory
Carbon	Dry combustion	47.22–47.55	47.40
Hydrogen	Dry combustion	5.36– 5.53	5.47
Sulfur	Catalytic combustion and peroxide bomb	15.71–15.89	15.82
Chlorine	Catalytic combustion	17.41–17.59	17.49
Nitrogen	Dumas and Kjeldahl	13.68–13.88	13.82

The standard has been analyzed repeatedly for the different elements by the following procedures: dry combustion carbon and hydrogen, catalytic combustion and peroxide bomb sulfur, catalytic combustion chlorine, and Dumas and Kjeldahl nitrogen. Representative analytical data obtained on the compound are shown in Table I.

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PRESENTED at the Spring Meeting of the Philadelphia Section, AMERICAN CHEMICAL SOCIETY, June 13, 1945.

Determination of Sulfur in Sulfur Compounds with Benzidine Hydrochloride

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IN ORDER to determine total sulfur by the benzidine method in waters containing bisulfites, sulfites, hyposulfites, and thiosulfates, it was necessary to oxidize these compounds to simple sulfates previous to adding the benzidine hydrochloride reagent.

Oxidizing agents such as alkaline potassium permanganate (*15*), the Parr oxygen bomb (*1*, *19*), or some of the strong oxidizing methods developed for blood, plasma, or urine (*2*, *7*, *10*, *14*, *21*) were found too lengthy and cumbersome to be practical for determinations of water samples on a large scale, especially under field conditions.

Since the publication of the benzidine method (*12*) and its subsequent modification (*6*, *9*, *17*), many variations of the method have appeared, both colorimetric (*4*, *8*, *20*, *22*) and titrimetric (*3*, *5*, *11*, *13*, *16*, *18*). In the present application to total sulfur determination, recently published data for quantitative precipitation of benzidine sulfate have been incorporated. The volumes, concentrations, and time allowed for precipitation have been found necessary under the conditions of this method.

THE METHOD. Oxidation of the sulfur compounds with 30% hydrogen peroxide (Merck's Superoxol) was found most satisfactory, with no detectable interference with the benzidine method.

Into an evaporating dish containing a sample of water carrying between 3 and 20 mg. of sulfur, put 2 drops of 1% alcoholic phenolphthalein, add dilute hydrochloric acid if alkaline or sodium hydroxide if acid until the turning point of the indicator, then make the sample definitely alkaline with sodium hydroxide. Evaporate to about 5 ml., add 30% hydrogen peroxide, a drop at a time, and allow to simmer. After a few minutes, repeat addition of the peroxide until the solution becomes colorless or foaming ceases. Throughout this period maintain the volume of approximately 5 ml. by addition of distilled water.

Add 50 ml. of distilled water, heat the sample to 50° to 60° C., and add 10 ml. of benzidine reagent (11.2 grams of benzidine hydrochloride reagent grade, Eastman, and 11.6 ml. of concentrated hydrochloric acid, made up to 500 ml.). After a few minutes add 5 ml. of 99.5% acetone and let stand until cooled to room temperature. Filter through sulfate-free filter paper. Wash the evaporating dish twice with 5 ml. of 50% acetone and pour over the precipitate. After complete drainage, further wash the precipitate twice with 3 ml. of 99.5% acetone and let drain but do not allow to dry. Remove filter paper and wash the precipitate from the filter paper into the evaporating dish in which the precipitation took place. Heat to boiling and add 3 drops of 1% alcoholic phenolphthalein. With a microburet, titrate while hot with 0.01 *N* sodium hydroxide until near the end point, then boil again, and continue titration to first permanent pink color. Determine a blank on the reagents used.

DISCUSSION

Recoveries of known quantities of sulfur in various forms by this method are compared with unoxidized recoveries in Table I. The accuracy has been found to be ±0.2 mg. if the amount of sulfur present in the sample is between 3 and 20 mg.

Compounds such as *p*-aminodimethylaniline sulfate, potassium aluminum sulfate, and hydrazine sulfate have been tried with similar success. Mixtures of organic and inorganic sulfur compounds as found in paper-mill wastes lend themselves well to this method, providing all the material in suspension is first filtered out.

The sulfur content of pyrite waters from coal-mining operations can be determined as simple sulfate, with the reservation that iron in excess of 2 p.p.m. be removed from solution by precipitation. The iron, if not removed, oxidizes from ferrous to ferric and results in a fine dark precipitate, the presence of which alters the sulfate values.

ACKNOWLEDGMENT

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Table I. Determination of Sulfur in Solutions Containing Known Amounts of Sulfur Compounds

Compound	Recovery before Oxidation with Superoxol			Recovery after Oxidation with Superoxol		
	Determined	Theory	Difference	Determined	Theory	Difference
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Na_2SO_3	2.62	10.16	−7.54	9.96	10.16	−0.20
$NaHSO_3$	0.96	9.12	−8.16	9.24	9.12	+0.12
$Na_2S_2O_3.5H_2O$	0.21	10.32	−10.11	10.15	10.32	−0.17
$Na_2S_2O_4.2H_2O$	1.47	12.17	−10.70	12.29	12.17	+0.12
$Na_2S_2O_5$	10.01	10.76	−0.75	10.57	10.76	−0.19

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Separation of 2,4-Dinitrophenylhydrazones by Chromatographic Adsorption

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IN DETERMINING the structure of a number of unsaturated compounds by ozonization, considerable difficulty has been experienced in separating small amounts of low molecular weight carbonyl compounds. The most satisfactory method of isolation is through the 2,4-dinitrophenylhydrazones, as these are readily formed and are insoluble in water, but in many cases separation of the derivatives by fractional crystallization is unsatisfactory.

Separations of a number of 2,4-dinitrophenylhydrazones by chromatographic adsorption have been reported by Strain (*4*), by Lucas, Prater and Morris (*2*), and by Buchman, Schlatter, and Reims (*1*), but only a few of the aliphatic derivatives have been investigated. In the present work, the adsorption of 2,4-dinitrophenylhydrazine and the 2,4-dinitrophenylhydrazones of acetaldehyde, propionaldehyde, acetone, and methyl ethyl ketone was studied on columns of silicic acid–Super Cel.

Procedure. The dinitrophenylhydrazones (*3*) were adsorbed from solutions in 1 to 2 benzene-ligroin (60° to 90°). The adsorbent was Merck's silicic acid (reagent grade) mixed with Super Cel in a 2 to 1 ratio by weight to increase the rate of flow of solvents through the column. The most satisfactory solvent for development of the chromatograms was ligroin (60° to 90°) containing 4% ether by volume. Mixtures of benzene and ligroin gave more diffuse bands. After the column was extruded and sectioned, the derivatives were eluted with ether and crystallized from ethanol.

In a typical experiment a mixture of about 5 mg. each of acetone and methyl ethyl ketone 2,4-dinitrophenylhydrazones was chromatographed on a 3.4 × 17 cm. column. After development the column consisted of the following bands: 4 cm. colorless, 4 cm. yellow, 2.5 cm. colorless, and 3.5 cm. yellow. The upper zone yielded the acetone derivative, m.p. 122–124.5° C. The lower band gave the methyl ethyl ketone derivative, m.p. 115° C.

The 2,4-dinitrophenylhydrazones were found to be adsorbed in the order: acetaldehyde, acetone, propionaldehyde, and methyl ethyl ketone. Each of these derivatives could be separated from mixtures with the others and 2,4-dinitrophenylhydrazine (strongly adsorbed) except for the hydrazone of acetone, which could not be separated from that of propionaldehyde.

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Arrangement for Multiple pH Determinations

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IN AN attempt to minimize the work required in making several hundred pH determinations a day, an arrangement has been worked out which facilitates this routine task to a marked degree.

The table in the assembly (Figure 1) is of desk height, in order to permit the operator to be seated while making the pH determinations. Figure 2 shows the construction and dimensions (in inches) of the table. For easier reading, the pH meter is set on a shelf at an angle of 15° to the table top.

The electrodes used were the No. 270 calomel electrode and No. 290 glass electrode as supplied with a Beckman Laboratory Model G pH meter. The leads on both the glass and calomel electrodes have been extended, so that they can be used at a distance from the meter case. The lead to the glass electrode must be shielded and the shield grounded to the case of the meter.

The design of the electrode cup is shown in Figure 3. The purpose of the paraffin around the electrodes is to fill up the space between the electrodes and the cup wall, in order that less solution (only 2 ml.) will be required for the measurement and its subsequent removal facilitated. The hot paraffin is put in place with an eye dropper, the first arm being allowed to cool before the other is filled.

Figure 4 shows the details of the electrode cup assembly. Depressing the lever at the right causes the sample in the cup to be drawn by vacuum into the waste flask situated on the shelf at the back of the table (Figure 1). When the left lever is depressed, a jet of distilled water flows into the electrode cup. The washings are removed by depressing the right lever again. The copper pan is provided with a drain to permit easy washing and should be grounded electrically. When not in use the electrode cup is filled with water and covered with a small glass cap.

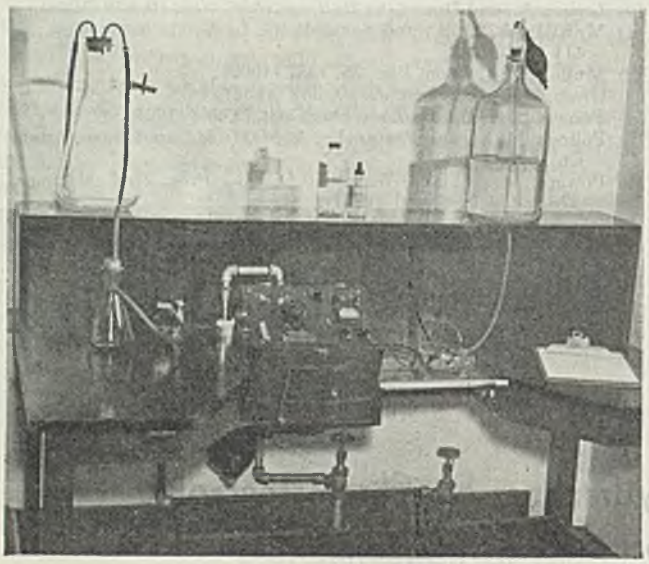

Figure 1. Assembly for pH Determinations

An indication of the accuracy of the equipment described is presented in Table I. Glycine–hydrochloric acid and boric acid–potassium chloride–sodium hydroxide buffer solutions were prepared (*1*). The pH of each of these solutions was first determined by the conventional procedure and then by use of the equipment described. These latter determinations were made in the order shown and as rapidly as possible. The number of rinses between determinations is indicated in the table.

Before each series of determinations the pH meter was standardized against 0.05 *M* potassium acid phthalate buffer solution (pH 4.00) and a standard buffer of pH 7.00 obtained from the National Technical Laboratories.

It is apparent from the values shown that the results obtained can be as accurate as the reading of the meter will allow. With but a single distilled water rinse between samples of widely differing pH, the largest difference noted was 0.05 pH, which is acceptable for most work.

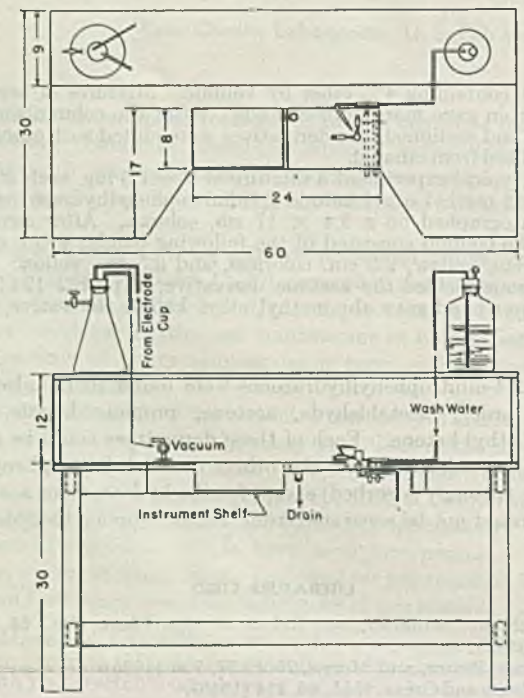

Figure 2. Table

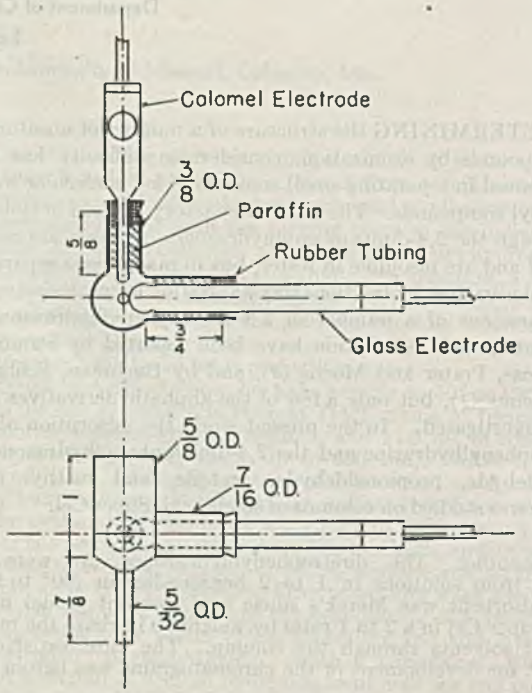

Figure 3. Electrode Cup Assembly

Dimensions in Inches

Table I. pH at 25° C.

Buffer Solution No.	Given	Determined by Conventional Method	Determined by Present Method: One water rinse before each determination	Determined by Present Method: Three water rinses and three rinses with sample before each determination
1	10.0	9.97	9.90	9.95
2	3.99	3.97	3.98	3.95
3	8.8	8.77	8.74	8.75
4	1.93	1.97	1.92	1.90
5	7.8	7.75	7.72	7.73

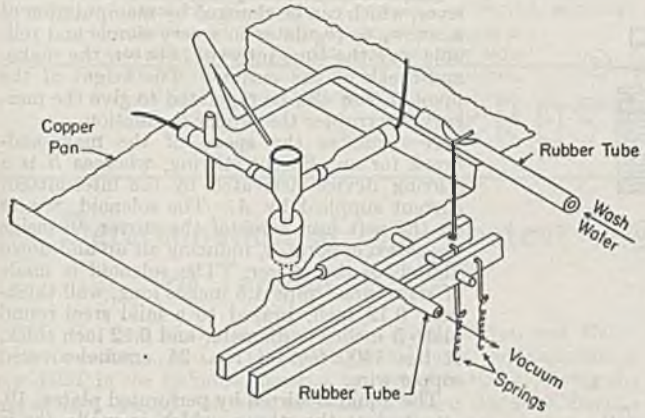

Figure 4. Electrode Cup Assembly

The electrodes were mounted in a horizontal position below the top of the electrode cup in order to obtain a clear working space above the electrode cup assembly. It was realized that contamination of the liquid junction solution might result with the calomel electrode mounted in this position. In order to establish accuracy of the instrument after use for 6 months, the system was checked against five buffer solutions prepared in accordance with the recommendations of the Bureau of Standards. Three of the solutions were prepared from chemicals supplied for this purpose by the Bureau of Standards. The other two solutions were prepared from A.C.S. reagent grade chemicals without further purification. The system was calibrated against buffer solution A and the results obtained on measurement of the pH of the other solutions are shown in Table II.

These data indicate that there has been little deterioration of the electrodes in the period of time they have been used. It is recommended that regular attention be given the saturated potassium chloride liquid junction solution in order to avoid errors due to dilution or contamination. To test the system for linearity, it may be calibrated against buffer solutions B and C, as recommended by Manov (*2*). The use of buffers of pH above 7.00 demands that precautions be taken to prevent absorption of atmospheric carbon dioxide. For normal use the system should be calibrated against a single buffer solution having a pH approximately that of the sample to be examined. Solution A or B will serve well for most purposes.

Table II. pH Measurements

Buffer Solution	Description of Solution	pH at 25° C. Given	pH at 25° C. Determined	Error
A	0.025 *M* potassium dihydrogen phosphate, disodium hydrogen phosphate, B.S. No. 186	6.86	...	...
B	0.05 *M* potassium acid phthalate, B.S. No. 84b	4.00	4.03	+0.03
C	0.01 *M* borax, B.S. No. 187	9.18	9.17	−0.01
D	0.05 *M* potassium acid phthalate, A.C.S. reagent grade	4.00	4.02	+0.02
E	0.01 *M* borax, A.C.S. reagent grade	9.18	9.18	0.00

The use of this equipment permits an unskilled operator to make from 100 to 150 pH determinations per hour. The electrode cup and electrodes may be rinsed well between determinations, and there is little danger of breaking them.

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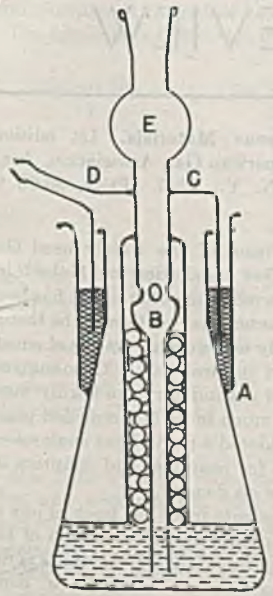
(1) Gortner, "Outlines of Biochemistry", 2nd ed., pp. 122–3, New York, John Wiley & Sons, 1938.
(2) Manov, *J. Assoc. Official Agr. Chem.*, 28, 597–9 (1945).

Modified Gas-Absorption Apparatus

ARCHIE N. BOLSTAD AND RALPH E. DUNBAR, North Dakota Agricultural College, Fargo, N. Dak.



RECENT modifications and improvements have been added to the gas-absorption apparatus recently described (*1*). Some difficulty has been encountered in adjusting the level of the absorption column to the changing level of the liquid. If the connection at the opening of the flask is sufficiently tight to prevent leakage of gas, it is almost impossible to make the necessary adjustments in level. This difficulty has been largely eliminated by adding the familiar liquid seal to the apparatus.

A small Pyrex beaker, with bottom removed, is sealed to the upper portion of a wide-necked Erlenmeyer flask as shown at *A*. This operation is not so difficult as it might appear, for the bottom of a small beaker can be readily removed by heating and blowing. The original pourout provides a convenient means for removing any sealing liquid used, provided the Erlenmeyer flask is first closed with a solid cork or stopper. The absorption portion of the apparatus, *B*, is identical with that previously described (*1*). A smaller beaker, *C*, is attached to the upper portion of the absorption tube, in an inverted position, as shown. A small inlet tube, *D*, is provided for introduction of the gas being employed. Mercury or any other heavy inert liquid may be employed for the seal. Obviously the liquid used must not react chemically with the gas employed. If a bulb, *E*, is constructed above the absorption tube as shown, it will largely eliminate the danger of overflow due to bumping or refluxing, and will also care for some increase in volume of the liquid.

With this modified gas-absorption apparatus the necessary adjustments can be made by raising or lowering the Erlenmeyer flask only. This is a decided advantage, since no other portion of the entire train of equipment is disturbed. The size of the equipment can be varied and proportioned to meet various needs.

LITERATURE CITED

(1) Bolstad, Luther, and Dunbar, R. E., IND. ENG. CHEM., ANAL. ED., 15, 498 (1943).

Electromagnetic Stirring Device

WILLIAM N. McINTOSH
Department of Physics, Howard University,
Washington, D. C.

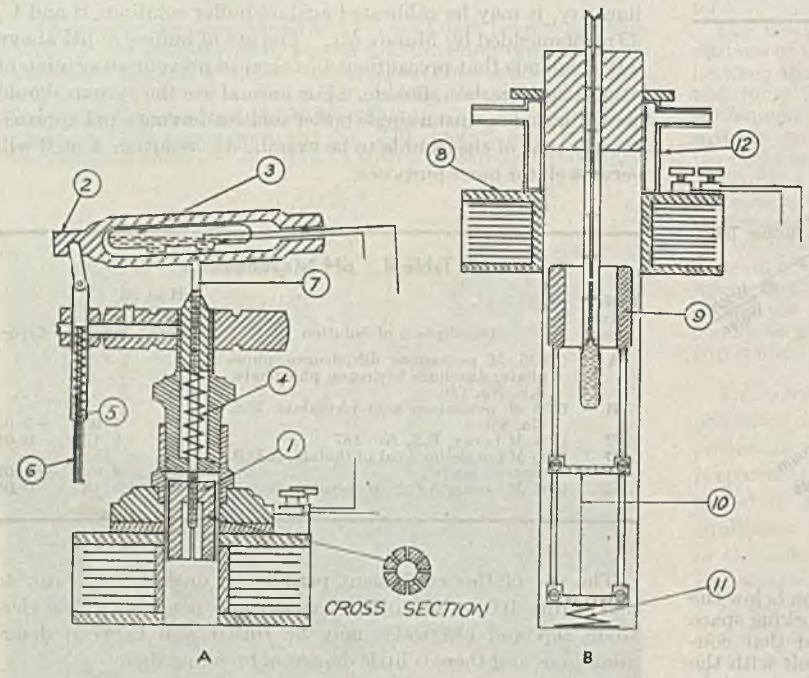


Figure 1. Diagram of Stirring Device

THE application of an electromagnetic stirring device is especially advantageous for agitating a liquid under pressure or in a vacuum.

Several articles describing such devices have been published most recently (*1–4*). The electromagnetic stirrer devised by the writer embodies simplicity of construction, reliability of operation, safety in use because of sealed contacts, and low operating temperature and cost. It can be used on 110-volt direct or alternating current, requiring 0.4 ampere and a 75-ohm line resistance on direct current and 0.7 ampere on alternating current with no line resistance.

The stirrer consists of two parts, *A* and *B*. The base of *A* is a solenoid directly connected to the 110-volt alternating current line or through the 75-ohm resistance to the 110-volt direct current line. The solenoid is made of a brass spool with 2333.4 feet of No. 24 enamel-covered copper wire (0.022-inch diameter) which operates a plunger made of solid soft iron (height 1.25 inches, diameter $^{15}/_{16}$ inch).

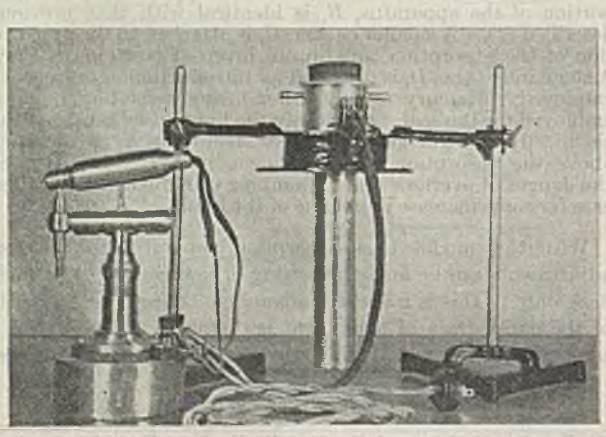


Figure 2. Stirrer

When the plunger, 1, is pulled down by the solenoid it lowers a lever, 2, consisting of a brass tube in which an evacuated mercury interrupter, 3 (available at laboratory supply houses as made by General Electric, Minneapolis-Honeywell Regulator Co., and American Instrument Co., Washington, D. C.), is situated, which breaks the current when the lever reaches its lowest point, thus allowing the lever and plunger to be pushed up by a coil spring, 4.

The tension of coil spring 5 at the end of the lever, which can be changed by manipulation of a screw, 6, regulates in a very simple and reliable way the time interval between the make-and-break of the current. The height of the pivot, 7, can also be regulated to give the mercury interrupter the proper inclination.

A regulates the speed of the make-and-break for an efficient stirring, whereas *B* is a stirring device activated by the intermittent current supplied by *A*. The solenoid, 8, acts on the soft iron cores of the stirrer, 9, inside the closed apparatus, inducing an up and down motion of the stirrer. The solenoid is made of short brass pipe 1.5 inches long, wall thickness 0.12 inch, brazed to a mild steel round plate 5 inches in diameter and 0.12 inch thick. It has 1400 feet of No. 24 enamel-covered copper wire.

The liquid is stirred by perforated plates, 10, attached to the stirrer, which is made (as is the whole apparatus) from nonmagnetic metal (copper or silver). This method of stirring is more efficient than rotating motion which has a tendency to move the entire liquid.

A spiral spring, 11, situated at the bottom of the apparatus intermittently touches the lower perforated plates, enhancing the regularity of the stirring.

A jacket, 12, through which liquids are circulated provides temperatures required by various experiments.

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(1) Lamb, A., and Haring, M. M., *J. Chem. Education*, 17, 577 (1940).
(2) Rowley, H. H., and Anderson, R. B., IND. ENG. CHEM., ANAL. ED., 11, 397 (1939).
(3) Simons, J. H., *Ibid.*, 10, 638 (1938).
(4) Simons, J. H., and Powell, M. G., *J. Am. Chem. Soc.*, 67, 75 (1945).

BOOK REVIEW

Gas Analysis and Testing of Gaseous Materials. 1st edition *V. J. Altieri.* xi + 567 pages. American Gas Association, Inc., 420 Lexington Ave., New York, N. Y., 1945. Price, $5.00 to members; $7.50 to nonmembers.

The book has evolved from a revision of the widely used Gas Chemists Handbook of the American Gas Association and is the third of a series of volumes into which the former one-volume text has been expanded. It covers in a thorough manner that portion of the theory and practice of gas analysis customarily encountered by the chemists of gas companies (manufactured and natural gas). Gas analyses of types not often involved in the fuel gas industry are hardly more than mentioned. The book contains much in its 500 crowded pages that is related to but not usually considered a part of gas analysis—a concise outline of the kinetic theory, for example, and chapters on the measurement and flow of gases and on density.

The dominant impression the reader gets from the book is one of admiration for the thoroughness and rigidly logical operation of the author's mind and for his vast capacity for work. Everything is defined, classified, described, and pigeonholed with exactness, com-

pleteness, and finality. No chance is left for misunderstanding or error, and nothing essential to the discussions has been left out, no matter how obvious or well known it may seem to the reader. For example, a full page, containing both dimensional and differential equations, is devoted to "the concept of pressure". It is extremely doubtful that the discussion will be understood by any one who does not already have an entirely adequate concept of pressure. For this reason the book is tedious reading.

In his preface, the author shows that he set himself the task of writing a book that would meet the needs of every type of person concerned with gas analysis in the fuel-gas industries—to make "instantly available...detailed but concise directions, thoroughly indexed, for laboratory and plant technicians; theory, mathematical concepts, and literature references for chemists, physicists, and engineers; and matters of general interest for executives and persons who usually do not want highly specialized technical information". Perhaps the author's way is the only one in which this ambitious and very difficult objective can be attained.

Gas analysts are usually individualists, who tend to employ only apparatus and procedures to which they are accustomed and rarely publish data regarding the relative merits of competing analytical processes. The lack of such data, which is the principal defect of this very valuable text, is consequently not entirely the author's fault; but in the reviewer's opinion it should have been possible to give more information of the sort than has been included. The omission of some of the less useful equipment and procedures and the curtailment of some of the more generalized discussions would not be regretted.

E. R. Weaver

CORRESPONDENCE

Determination of *p,p'* Content of Technical DDT

Sir: In view of the publication of Cristol, Hayes, and Haller [Ind. Eng. Chem., Anal. Ed., 17, 470 (1945)] on the determination of *p,p'*-DDT in the technical material by crystallization involving the use of 75% ethanol, we wish to place on record the work done in England in the first half of 1943.

During our investigations on a suitable method for the manufacture of DDT, we succeeded in preparing material which could be readily isolated as a fine colorless powder, but which had a melting point range of 76° to 89° C., and although this was satisfactory for commercial purposes it was very desirable to work out a method which would give us the *p,p'* content, and therefore a measure of its purity. This was accomplished by a direct crystallization from 99.5% alcohol, under a set of standard conditions.

The method was communicated to the Ministry of Supply on Feb. 2, 1944, and in due course was incorporated in the British specification for technical DDT. Subsequently, work carried out by the Chief Chemical Inspectorate, Ministry of Supply, led to the use of 99.5% alcohol saturated with pure *p,p'*-DDT and also to an increase of the volume of alcohol employed, but fundamentally the method was unaltered.

Ten grams of the sample were treated with 30 ml. of 99.5% ethanol in a 100-ml. conical flask and brought to the boil to effect solution without loss of solvent. The flask was then set aside and cooled for 2 hours at room temperature (20° C.). The material was collected on a weighed No. 3 sintered-glass crucible, washed twice with two 5-ml. portions of 75% ethanol, and dried to constant weight at 80° C.

The following results were obtained:

Sample No.	*p,p'* Content, %	Melting Point, ° C.[a]
1	69.5	105–7
	69.5	105–7
	70.0	105–7
2	77.1	107–9
	77.8	107–9
	74.8	106–8
3	69.5	105–7
	69.9	105–7

[a] We had previously found that pure DDT had a melting point of 108.5–109.5° C. (corrected). Zeidler, *Ber.*, 7, 1191 (1874), recorded melting point as 103–105° C.

Pharmaceutical Laboratory
Geigy Co., Ltd.
Trafford Park
Manchester 17, England

I. E. Balaban
R. D. Calvert

Sir: The work described by us was conducted in the latter half of 1944 and makes use of 75% ethanol saturated with *p,p'*-DDT as recrystallization solvent. This gave more consistent results, in our hands, than that using stronger ethanol. We are happy, however, at this time to acknowledge the prior use by Balaban and Calvert of a recrystallization technique for the determination of the *p,p'* isomer content of technical DDT, earlier publication of which was barred by security restrictions.

Bureau of Entomology and
Plant Quarantine
U. S. Department of Agriculture
Beltsville, Md.

Stanley J. Cristol
Robert A. Hayes
H. L. Haller

Determination of Ethylene

Sir: We have tested the method of Francis and Lukasiewicz [Ind. Eng. Chem., Anal. Ed., 17, 703 (1945)] for the determination of ethylene and, while we found that the reagent they described absorbs olefins completely and rapidly, we also found that the reagent reacts at an appreciable rate with carbon monoxide to form carbon dioxide.

Samples of pure carbon monoxide and a mixture of 50% carbon monoxide and 50% nitrogen were tested with the mercuric sulfate solution in a pipet packed with vertical tubes, leaving the sample in the pipet 20 seconds between passes. After 20 passes had been made into the solution the sample was passed into 50% potassium hydroxide solution. The results of these tests are given here:

Passes	Pure CO *Ml.*	Pure CO *Ml.*	Mixture (50% N_2) *Ml.*
0	98.8	98.4	97.6
5	98.8	98.4	97.6
10	98.7	98.3	97.6
15	98.6	98.1	97.5
20	98.3	97.9	97.4
Passed into 50% KOH	93.1	92.3	95.4

The action of this reagent on carbon monoxide, forming carbon dioxide, may lead to erroneous results when an alkaline absorbent is used after the mercuric sulfate (such as alkaline pyrogallol solution for determining the oxygen content of a sample) or when the residual gas after absorption in mercuric sulfate solution is used for the determination of paraffin content by measurement of the carbon dioxide produced by combustion analysis. Errors of this type can, of course, be avoided by passing the gas into caustic solution after it is passed into the mercuric sulfate reagent and correcting any subsequent carbon monoxide determination by the amount of carbon dioxide removed.

Shell Development Co.,
Emeryville, Calif.

F. R. Brooks
P. Benjamin
V. Zahn

Sir: We appreciate the tests made by Brooks, Benjamin, and Zahn of our reagent for determining ethylene, indicating a possible error in its use, and also a means for avoiding the error. We believe their note will add to the value of the method.

Socony-Vacuum Laboratories,
Paulsboro, N. J.

A. W. Francis
S. J. Lukasiewicz

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Examples. (1) Comings, E. W., and Egley, R. S., IND. ENG. CHEM., 32, 714–18 (1940).
(2) Li, K. C., and Wang, C. Y., "Tungsten", A.C.S. Monograph 94, p. 75, New York, Reinhold Publishing Corp., 1943.
(3) Smallwood, H. M. (to U. S. Rubber Co.), British Patent 533,669 (Feb. 19, 1941); Canadian Patent 403,992 (April 7, 1942).

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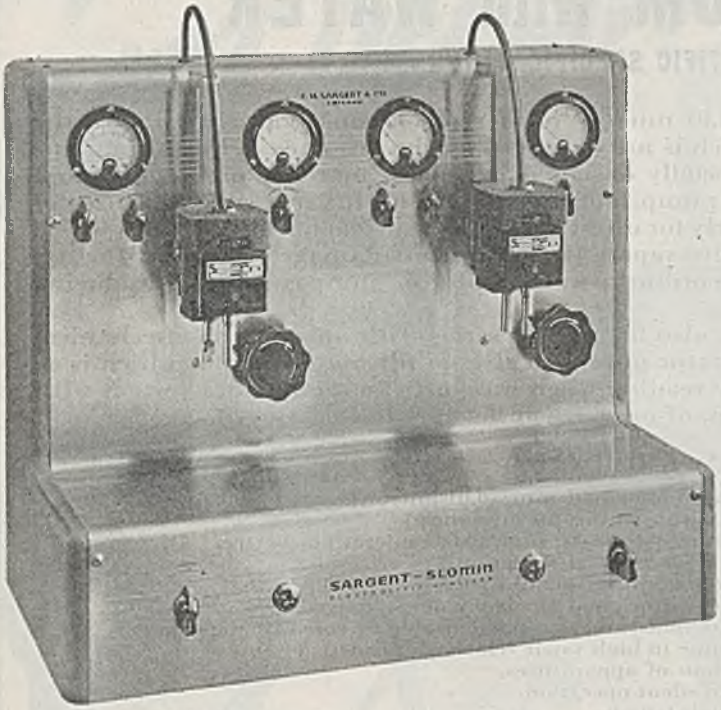

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Current Developments in INSTRUMENTATION IN ANALYSIS

Discussed by Ralph H. Müller

We came away from the recent national meeting of the American Chemical Society with exultation and high hopes for the future of analytical chemistry. Not only the aims, but the positive accomplishments of some segments of industry are breathtaking. Superlatives are rarely in order in scientific circles, but they may be forced upon one by circumstances. With no difficulty at all we found evidence of the extensive use of optics, nuclear physics, electronics, and sundry phases of engineering in the solution of analytical problems. That which impressed us most was the enthusiasm and optimism about the revival of analysis as one of the most important contemporary subjects.

The Academic Situation

Our own misgivings about the academic situation were quite uniformly confirmed. We came to the conclusion that the analytical needs of modern science and industry are veering tangentially from present academic training at approximately the fourth power of the elapsed time.

Instruction in instrumental methods is increasingly popular in many institutions, but of necessity it means different things in different places. In one department it means spectroscopy, in another electronics, and in a third polarography. He who would master the contemporary techniques would find himself at Minnesota with Kolthoff or at Harvard with Lingane, then at Ann Arbor with Willard and at Princeton with Furman. He would seek Mellon at Purdue and Brode at Ohio State. These rounds would be supplemented by extended sessions in large industrial laboratories with the Ashleys, Barneses, Clarkes, Halletts, and those other exponents of modern techniques, whose talents are less hampered by budgetary considerations. The dusty wanderer would emerge cum laude, cum itineris as a modern analyst. Truly, industry has unwittingly posed a perplexing problem in establishing complex techniques, the complete training for which becomes increasingly difficult.

Role of Automatic Analysis

These difficulties are compounded by another situation in industry. We find numerous examples of distinct "bottlenecks" in analytical or control laboratories which have arisen from changes in manufacturing practice. The use of automatic controls and regulators has speeded up production in many cases to such an extent that the ordinary facilities for analysis or inspection are no longer adequate. It is true that some improvement in quality of the product has resulted from the better control maintained during manufacture, but if the product must meet rigorous specifications or if certification is required by law, an analysis must still be performed. In such cases, automatic analysis becomes mandatory, because the cost of conventional methods might well exceed the savings effected by the improved process.. A further advantage ultimately arises in this step because the "autoanalyzer" may just as well control the process itself. If the characteristics of the ultimate product are used to control the process, we may expect to get better results than those afforded by the initial regulation of the reaction variables. This will be recognized as an extreme case of the closed-loop servomechanism, and its success does require that the product can be tested at high speed. Otherwise, the corrective measures would be applied to the process with excessive delay and a condition of oscillation or hunting would be set up.

There is no scarcity of automatic methods for the continuous indication of a single constituent, or for the empirical analysis of binary or even ternary mixtures. The techniques which have been used include thermal conductance, the heat of catalytic conversion, electrolytic conductance, absorption in the visible, ultraviolet, and infrared, and on rare occasions, acoustical methods. Most of these involve standard physical or physical-chemical techniques which are re-engineered to suit plant conditions.

We are thinking, more particularly, about an analysis for half a dozen or more impurities which would involve sampling, weighing, solution, precipitation, and subsequent estimation. Each final step would, of necessity, involve objective measurements which could be recorded. The choice of method and procedure would be a problem for the analyst; the rest of the problem would best be delegated to a mechanical engineer and electronics expert. They would not be frightened by the assignment because a machine filling these requirements would hardly be as complicated as one designed to knit fashioned hosiery or to make cigarets.

It is not certain that the "autoanalyzer" would necessarily duplicate the conventional operations of the analyst. It is conceivable that, in certain cases, the simultaneous determination of several properties of the system would yield data, which after treatment by suitable computers would furnish an unmistakable criterion of acceptance.

In all this, we may seem insensitive or oblivious to the practice of random sampling and the treatment of the resulting data by statistical methods. We also recognize the great value of these methods in the critical examination of a given procedure for the proper allocation of error sources. We do object to their use in procedures which have not been improved or replaced by better methods. As an analyst, we shall continue to rebel against the "Gallup Poll", at least until the full resources of high-speed automatic inspection have been exhausted.

One might gain the impression that these problems concern the factory and production line exclusively, but, to a limited degree, the same trend is to be found in the research laboratory. Much data gathering is required and under a great variety of conditions. It is not too surprising then, to find research directors in progressive laboratories insisting upon the automatic recording of data and their assimilation as well, whenever possible. A policy of this kind is definitely autocatalytic because it often permits the staff to undertake investigations which would be prohibitive in manpower requirements.

These are still data-gathering times, but the process is no longer regarded as either interesting or exalted. There are too many exciting things to be done with the information.

Typical Automatic Analytical Operation

It might be in order to see what would be involved in converting a typical analytical operation to its automatic analog. The primary considerations are speed, adequate precision, and an equivalent substitute for operator judgment. It is easy to set

Instrumentation

up a semiautomatic "gadget" to demonstrate a principle, but to replace a skilled operator is another matter, and may be expected to involve considerable complexity and expense.

Let us assume that a large number of titrations is to be performed entirely automatically with the speed and precision equal to that of a skilled operator. Assume also that the control of the process shall be photoelectric. The conventional buret is replaced by a motor-driven syringe. The motor drive will be provided with two forward speeds and a fast reverse for reloading. The bulk of the reagent will be added at high speed and of course we shall overshoot the end point, but by borrowing a trick from differential titration procedure we may keep a portion of the solution trapped under a bell. The preliminary end point stops the high-speed delivery and simultaneously raises the bell. A brief time delay permits the trapped solution to mix with the main portion and the titration now proceeds to completion at the slow delivery rate. The color change at each end point, preliminary and final, is detected photoelectrically with a pulse amplifier. This responds only to changes in light transmission and not to the particular level of illumination which prevails.

The motor-driven syringe has now advanced, in one fast and one slow stage, to a point indicative of the titration value. This motion has rotated a dial, or print wheel, through a magnetic clutch. The signal resulting from the second or final end point illuminates the dial so that it may be read; or, better, prints the answer along with a consecutive serial number, time of day, or other pertinent designation. The clutch which drives the dial or print wheel is of the variable-ratio type. It is therefore readily adjustable for a new batch of stock reagent or change in the titration factor.

The next cycle of operations restores the entire system to the stand-by condition ready for the next sample. (Without these provisions we merely have a "gadget" of no practical use.) The dial or print wheel now returns by declutching to its zero point. The solution is vented to waste and the reaction vessel is spray-rinsed for a stated interval. Greater speed and economy in this operation may be obtained by a simple conductance test of entrant and effluent rinse water. A single ratio bridge with electronic control supervises this operation. During these operations the syringe is being reloaded, if necessary, through a by-pass valve from reagent storage. The photoelectric controls are inactive during these operations. The titration vessel is now reloaded automatically to the correct level with the appropriate stock solution (acid, buffer, or indicator) and is then ready for the injection of a new sample. If a process line is being sampled, this can be accomplished by a precise cam-driven syringe.

The correct sequence of operations is best achieved electronically and this includes the necessary time delays, interlocks, etc. Alternative methods would employ stepping relays or cam-driven selectors. Hydraulic or pneumatic controls are also satisfactory.

One might well ask, "Will this machine occupy half the space of the laboratory?" From what we have seen in the packaging of airborne equipment, our guess would be that the essential controls (excluding reagent storage, etc.) would fit in a shoe box. A practical installation would require duplicate units, operating in sequence or in tandem. Some simplification would then result from the sharing of individual components, power supplies, reagent tanks, and motor drives.

We hope some one will undertake the description and classification of the work which has been done in the field of automatic analysis. The nearest approach to this treatment, as far as we know, is "Der Chemie-Ingenieur" by Eucken and Jacob, published in 1935. We believe that analysts will have to give increasing attention to the problems presented by modern production methods, even while they are concerned with the more fundamental questions of the newer research techniques. The engineer and instrument man are always available to put their ideas in practical form, but the analyst must provide the essential information. He still retains the burden of assimilating dozens of new techniques and must continue to worry about the new generation of analysts and the means for their training.

The latter continues to be our principal problem.

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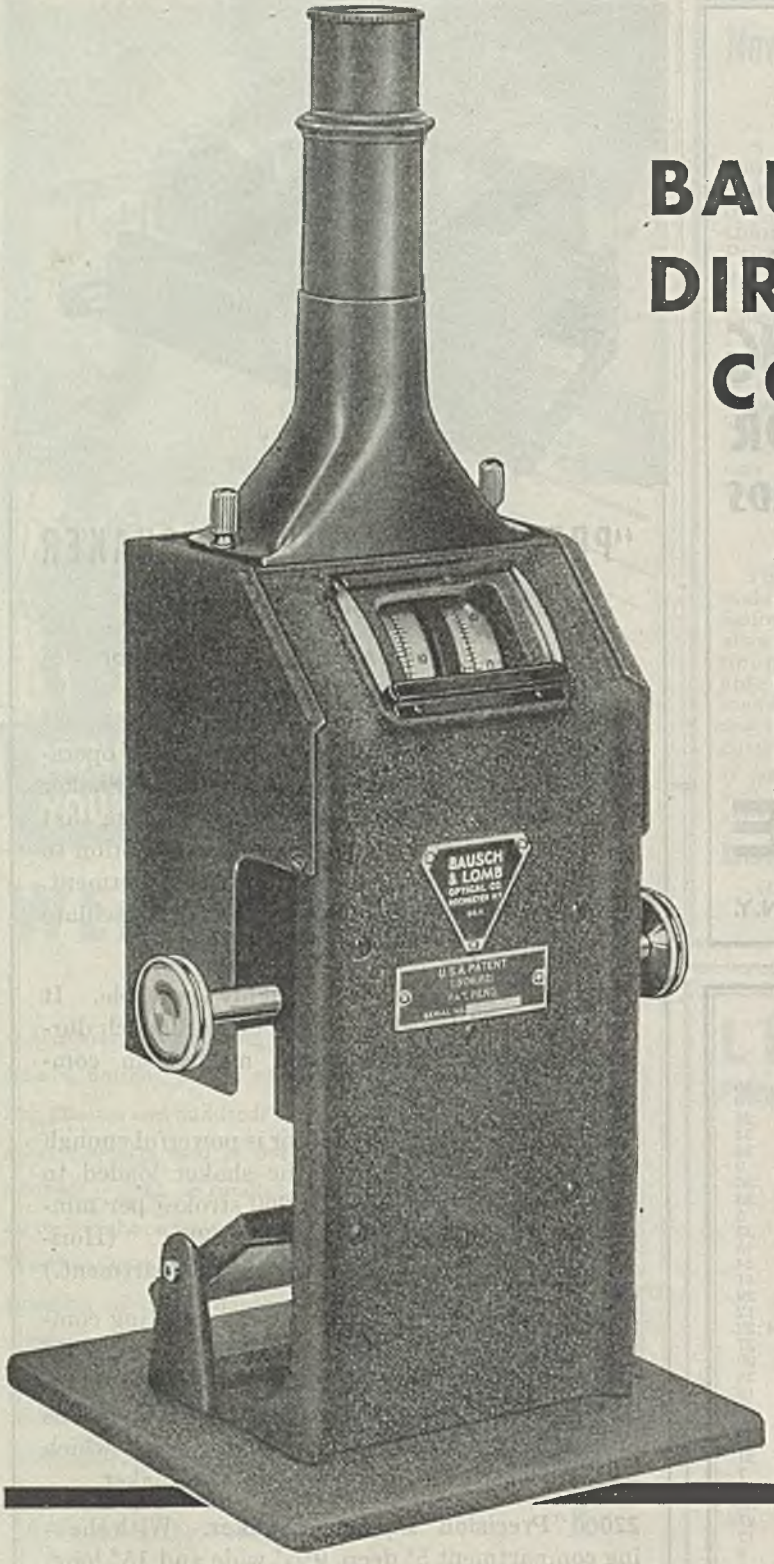

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