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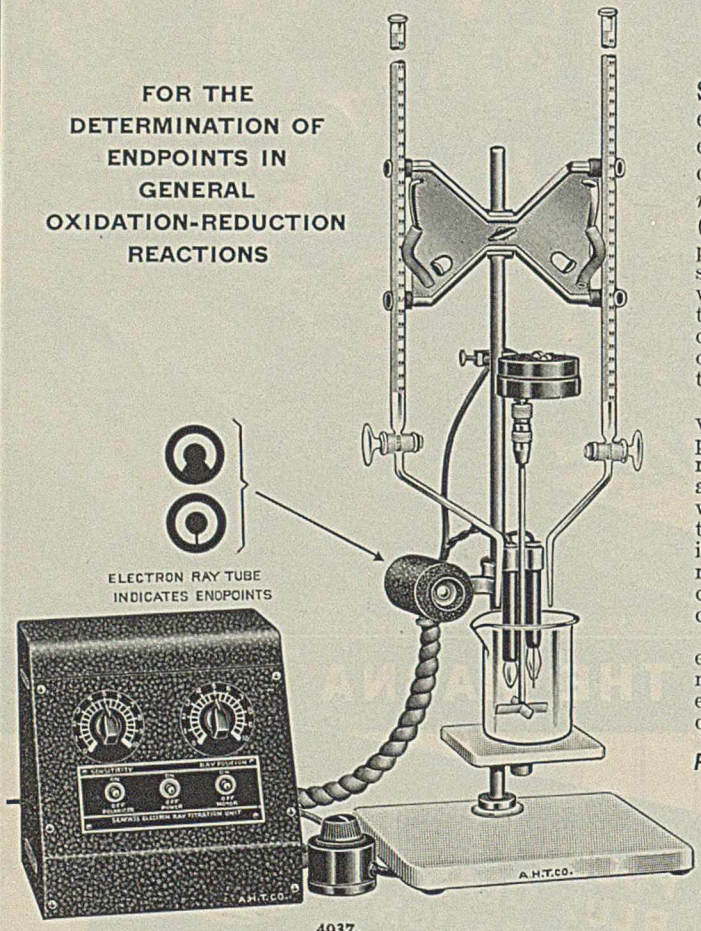

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Factors to Be Considered in Quantitative Polarography

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IN A RECENT monograph (*3*) the field of voltammetry (polarography) and of amperometric titrations was comprehensively reviewed. Since the literature was covered in this monograph until the end of 1940, the author has not endeavored to give a condensed review of the entire subject here, but proposes to concentrate on those factors which have to be considered in quantitative (and sometimes qualitative) polarography. Some of these factors are often overlooked in the literature. This may not be of any consequence in a particular study, but neglect of these factors may bring polarography into discredit as a general analytical method. Some of the factors have been discussed at length by Kolthoff and Lingane (*3*), and these will only be summarized in the present paper, which will concentrate on those factors that are of primary importance when dealing with mixtures of electroreducible substances.

Factors of Primary Importance

1. The diffusion current measured is an apparent diffusion current, i_{d_a}. In order to find the true diffusion current, i_d, which is proportional to the concentration, C, the residual current of the medium, i_r, at the potential where the apparent diffusion current is measured must be subtracted from i_{d_a}:

$$i_d = i_{d_a} - i_r = kC \qquad (1)$$

The residual current in a medium free of oxygen and other reducible substances should be zero at a potential of −0.55 volt (against S. C. E.). It increases with increasing negative potential, but should never be larger than a few tenths of a microampere. When the concentration of the electroreduced substance is greater than about 0.001 N, i_d becomes very large with respect to i_r, and the latter can be neglected from a practical viewpoint. However, the correction becomes increasingly more important the smaller the concentration of the reducible substance and the more negative its reduction potential. The most exact way of finding the residual current is by determination of the current-voltage curve of the supporting electrolyte in the absence of the reducible substance(s). The residual current can be found with a fair degree of approximation by extrapolating the part of the current voltage curve before the wave starts to the potential at which the apparent diffusion current is read.

2. Quite generally, oxygen should be removed from the solution before electrolysis is started. This can be done by passing nitrogen or hydrogen through the solution in the cell for 10 to 20 minutes. In neutral or alkaline medium oxygen is completely removed by the addition of some solid sodium sulfite. Oxygen yields two waves. The first, corresponding to a reduction to hydrogen peroxide, is fairly steep and has a half-wave potential of about +0.15 volt *vs.* the S. C. E. The second wave, corresponding to a reduction of peroxide to hydroxyl ions, is very flat, and extends over a potential range between −0.6 and −1.2 volt, the half-wave potential being −0.94 volt. Since a solution which is saturated with air is about 0.001 N in oxygen, it is evident that oxygen waves will interfere with the determination of the diffusion current of another reducible substance. This interference becomes very pronounced at low concentrations of the substance being determined.

3. The factors which determine the diffusion current are given by the fundamental Ilkovic (*1*) equation:

$$i_d = 605nD^{1/2}Cm^{2/3}t^{1/6} \qquad (2)$$

in which i_d is the diffusion current in microamperes, n is the number of faradays of electricity required per molar unit of the electrode reaction, D is the diffusion coefficient of the reducible (or oxidizable) substance in cm.^{2}/sec.$^{-1}$, C is its concentration in millimoles per liter, m is the weight of mercury in milligrams flowing out of the capillary per second, and t is the drop time in seconds. Experimentally, the Ilkovic expression has been found to hold when the drop time is greater than 3 seconds.

4. In order to get reproducible results with the same capillary and the same solution the pressure on the dropping mercury should be kept constant. From the Ilkovic equation and Poiseuille expression it follows that

$$i_d = k'\sqrt{h}$$

where h is the height of the mercury column above the tip of the dropping electrode.

5. With all other factors constant Equation 1 follows from Equation 2. From a practical viewpoint it is of importance to know how the value of k in Equation 1 is affected by a change of the concentration of the indifferent electrolyte in the supporting medium. The relative change of k is equal to the relative change of the square root of the diffusion coefficient of the reducible (or oxidizable) substance. It is the author's experience that, in general, the concentration of the indifferent electrolyte can be varied between about 0.02 and 0.5 M without affecting the value of k, provided that the indifferent electrolyte does not form a complex with the reducible substance or react with it in some other chemical way. When an aquo

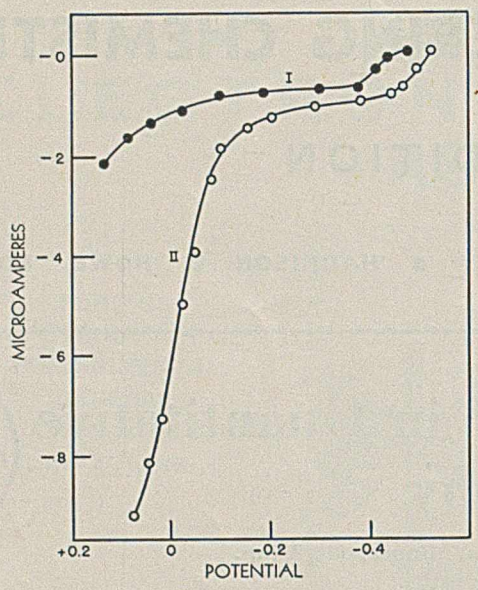

FIGURE 1. CURRENT-VOLTAGE CURVES OF CYSTEINE IN A BUFFER SOLUTION WITH A pH OF 6.0

I. 2.5×10^{-4} M cysteine (true diffusion current)
II. 2.0×10^{-3} M cysteine (false diffusion current)

TABLE I. VALUE OF $k = i_d/c$

(Obtained by E. F. Orlemann in electrolysis of lead nitrate in potassium nitrate solutions)

Concentration of $Pb(NO_3)_2$ M	Value of k In 0.1 N KNO_3	In 1 N KNO_3	In 0.1 N KCl
1×10^{-3}	7.02	7.00	7.10
5×10^{-3}	7.32	7.02	7.42
1×10^{-2}	7.60	7.06	..

ion is transformed into a complex ion, the diffusion coefficient (and therefore k) is affected. Metal ion complexes with tartrate or nitrate, for example, have a smaller D than the aquo ions. On the other hand, some ammino ions have a larger D than the corresponding aquo ions.

6. The value of k (D) is considerably affected by a change of the solvent. In water-alcohol mixtures k is much smaller than in water.

7. The diffusion currents of most substances increase by 1.3 to 2.0 per cent per degree increase in temperature, owing chiefly to the increase in the diffusion coefficient with increasing temperature.

8. In order to find the true diffusion current the migration current has to be eliminated. This is done by making the concentration of the indifferent electrolyte about 50 times greater than that of the reduced ion. If the migration current is not eliminated, there is no longer proportionality between i_d and the concentration; in other words, Expression 1 no longer holds. The complete elimination of the migration current may be easily overlooked when the concentration of the reduced ion becomes relatively large. As a demonstration of the effect, some results obtained in the electrolysis of lead nitrate in solutions of potassium nitrate or chloride are given.

If Equation 1 is valid, the value of k in a particular supporting electrolyte should be constant and independent of the lead concentration. From Table I it is seen that the value of k obtained with a 0.001 M solution of lead in 0.1 N potassium nitrate is the same as in 1 N nitrate (see 5 above). Even in the 0.1 N nitrate solution the migration current was eliminated when the lead concentration was 0.001 M. However, the value of k was found to increase markedly in the 0.1 N nitrate solution when the lead concentration was increased. This is due to an incomplete elimination of the migration current. On the other hand, the value of k was found to be independent of the lead concentration in the 1 N nitrate solution, since the concentration of the indifferent salt was large enough to eliminate the migration current even in the 0.01 M lead solution.

9. When the electrolysis product forms an insoluble film at the surface of the mercury drop, the film may have a large resistance and interfere badly with further electrolysis. In such instances a false diffusion current may be obtained which is no longer proportional to the concentration, and may even become independent of the concentration. A false diffusion current was found in the determination of anodic waves of cysteine (*2*). This substance can be determined polarographically by measuring the anodic diffusion current in 0.1 N perchloric acid. When the pH becomes greater than about 2, a film of mercurous cysteinate is formed at the electrode, which interferes with the further electrolysis. This is demonstrated by the current voltage curves in Figure 1, which were obtained in a buffer solution with a pH of 6.0. At cysteine concentrations smaller than about 3×10^{-4} M a true diffusion current is found. At higher concentrations a false diffusion current is found which is independent of the cysteine concentration. It was not possible to eliminate the film by the addition, for example, of a little gelatin.

In Figure 2 are shown anodic current voltage curves obtained with solutions of 0.002 N bromide in 0.1 N potassium nitrate (*6*); curve I refers to the solution in the absence of gelatin, curve II to the same in the presence of 0.01 per cent of gelatin. The anodic wave starts out in a normal way. However, when the current has become equal to about 4 microamperes a film of mercurous bromide interferes with the further electrolysis. The current increases only slightly as the potential is made more positive, until it becomes equal to the diffusion current at a potential of about +0.3 volt.

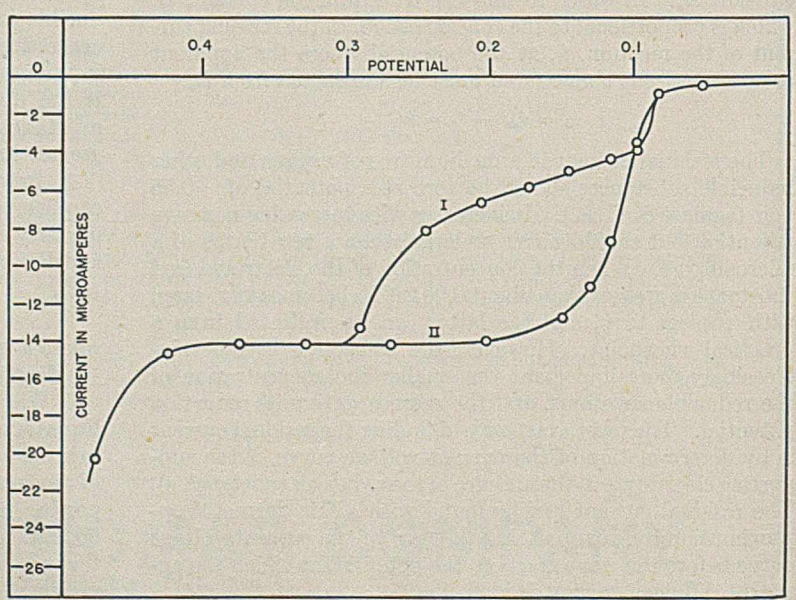

FIGURE 2. CURRENT-VOLTAGE CURVES OF 2×10^{-3} N BROMIDE IN 0.1 N POTASSIUM NITRATE

I. No gelatin added
II. In presence of 0.01 per cent gelatin

The addition of a trace of gelatin eliminates the interference by the film, as is evident from curve II.

10. The importance of a proper reference electrode is not generally appreciated. Heyrovský recommended the use of a pool of pure mercury in the cell as an internal reference electrode. In general, we (*3*) prefer an outside standard half-cell, ordinarily the saturated calomel electrode. Connection with the electrolysis cell is made by means of an agar salt bridge. This involves an extra resistance which has to be considered when the waves are analyzed for the relation between current and potential. Aside from this slight disadvantage, the use of an outside reference electrode is preferable to the internal pool of mercury for the following reasons:

The potential of the pool of mercury is often poorly defined. In the presence of depolarizers, like halides, and soluble hydroxides, the mercury adopts a potential close to that of the half-cell saturated with the particular mercurous halide or mercuric hydroxide. Relatively small amounts of oxygen in the solution are sufficient to oxidize enough mercury to yield the insoluble compound. In the presence of depolarizers which form soluble complexes or slightly dissociated compounds with mercury like cyanide and sulfide, the potential of the mercury will vary, dependent upon the amount of interaction between mercury and oxygen. When the indifferent electrolyte has no depolarizing effect the potential of the mercury varies and is of the order of +0.4 volt (*vs.* S. C. E.). The potential of the mercury referred to the S. C. E. has, roughly, the following values in different solutions: 0.1 *N* Cl^-, +0.09; 1 *N* Cl^-, +0.04; 0.1 *N* NaOH, −0.08; 1 *N* NaOH, −0.11; 0.1 *N* I^-, −0.3; 0.1 *N* CN^- (in NaOH), −0.6; 0.1 *N* sulfide, −0.85 volt. When half-wave potentials are measured it is necessary to determine the potential of the mercury pool separately. This is not necessary when a depolarized external half-cell is used.

When solution contains constituents which oxidize mercury—e. g., ferric chloride or vanadic acid—the internal mercury pool cannot be used. The diffusion current of these oxidizing agents can be found at the dropping electrode if the drops are collected in a suitable container (*1*) and prevented from interacting with the oxidant in the solution. When the solution contains constituents which form soluble stable complexes or slightly dissociated compounds with mercury—e. g., cyanide and iodide—the speed of interaction between mercury and oxygen is greatly enhanced. Thus, when the pool of mercury is introduced into the cell containing the solution to be electrolyzed, appreciable amounts of mercury (and usually of hydrogen peroxide) may be introduced into the solution when the mercury is left in contact with the solution before the oxygen is removed. This is well demonstrated (*9*) in Figure 3. The rate of interaction between mercury and oxygen can be studied conveniently by the polarographic method and in the case of interaction between mercury, oxygen, and cyanide, equivalent amounts of mercuric cyanide and hydrogen peroxide are formed:

$$Hg + O_2 + 2CN^- + 2H_2O \longrightarrow Hg(CN)_2 + H_2O_2 + 2OH^-$$

The first wave of the current voltage curve shows the reduction of mercury in the cyanide solution and the second flat wave that of hydrogen peroxide. The peroxide wave is very prominent in Curve VI of Figure 3. After the indicated times of contact, the oxygen was removed from the solution and then the current-voltage curves were determined.

In the interaction between mercury, oxygen, and iodide, only a mercury wave was found, since the peroxide reacts with the iodide to form iodine which in turn oxidizes mercury.

When a mercury pool is used as an anode in solutions containing cyanide, it is commendable to remove the oxygen from the solution before the mercury is added to the cell.

In connection with the above discussion a statement may be made regarding the addition of a "pilot" ion in cases where a mercury pool electrode is used. The pilot ion should be such that its half-wave potential is unaffected by the medium. Thallous thallium satisfies this requirement, its half-wave potential (−0.46 volt *vs.* S. C. E.) being the same in solutions containing hydroxide, tartrate, etc., as in solutions of ordinary indifferent electrolytes. Thus, upon addition of a small (but unknown) amount of thallous salt, a corresponding thallium wave is found and its known half-wave potential serves as a standard value on the potential axis. However, this method does not work, for example, when the solution contains cyanide. The anodic cyanide (0.1 *N*) wave starts at a potential of −0.58 volt and no cathodic wave can appear at potentials more positive than −0.58 volt. For example (*4*), in the electrolysis of a mixture which was 0.001 *N* in thallium, 0.1 *N* in sodium hydroxide, and 0.1 *N* in cyanide, an "apparent" thallium wave was obtained with a half-wave potential of −0.58 volt.

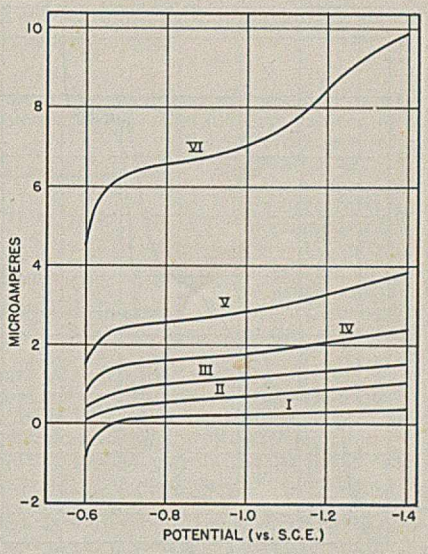

FIGURE 3. INTERACTION BETWEEN MERCURY AND AN AIR-SATURATED SOLUTION

Solution 0.1 *N* in potassium cyanide and 0.1 *N* in sodium hydroxide
I. Immediately air free
II. 10 minutes' contact
III. 30 minutes' contact
IV. 90 minutes' contact
V. 5 minutes' contact with stirring
VI. 30 minutes' contact with stirring

Mixtures of Reducible Substances

The following factors are of special importance in dealing with mixtures of reducible substances:

11. In the first place we briefly consider maxima on current-voltage curves. When, after the maximum is reached, the potential is made more negative the current decreases more or less rapidly, finally becoming equal to the diffusion current. When dealing with one reducible substance or with two reducible substances whose waves are widely separated it is not necessary to eliminate the maximum, as a potential range is easily found at which the diffusion current can be measured. However, when the waves of the two reducible substances are fairly close together it is necessary to eliminate the maximum of the first wave. If the maximum is not eliminated the diffusion current of the constituent which is reduced first may not be attained before the wave of the second constituent starts. In a mixture—e. g., of 0.001 *M* lead and cadmium ions in 0.1 *N* potassium chloride—a "false" diffusion current of lead is measured. This current is much greater than the true diffusion current which is found when the maximum is eliminated (*3*). Maxima are generally eliminated by the addition of small amounts of capillary-active substances, such as dyes, camphor, naphthalene, thymol, and especially gelatin and tylose.

12. When dealing with mixtures, the reaction product formed in the electrode reaction of the most easily reduced constituent may react with the second constituent; also, the reaction product of the second constituent may react chemically with the more easily reduced constituent. In the former case the diffusion current of the second constituent will be reduced by an amount which corresponds to the concentration of the second constituent which has been removed at the electrode. In the latter case the apparent diffusion current of the

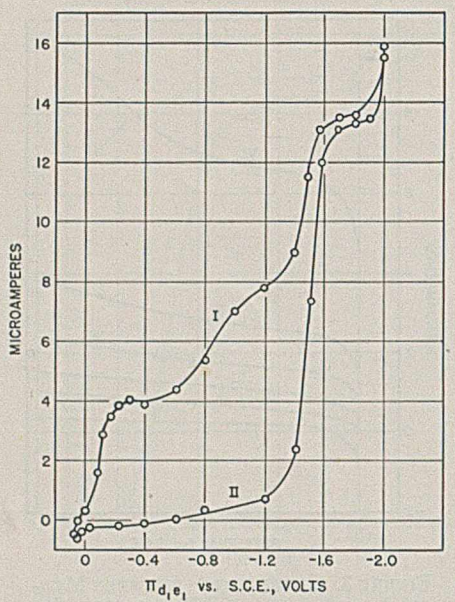

FIGURE 4. DECREASE OF DIFFUSION CURRENT OF HYDROGEN IONS BY HYDROXYL IONS PRODUCED IN REDUCTION OF OXYGEN

I. After saturating solution with air
II. Hydrogen wave from air-free 0.001 *N* HCl containing 0.1 *N* KCl and a trace of methyl red

second constituent also will be decreased, because chemical interaction does not take place until the second constituent is being reduced. The total diffusion current measured after reduction of the second constituent is equal to the sum of the first and the second, and from the polarogram it is impossible to decide whether the first or second diffusion current has been reduced.

An instructive example of interaction is found with a mixture of oxygen and very dilute hydrochloric acid in an excess of potassium chloride (*5*). The current-voltage curves reproduced in Figure 4 were obtained in the presence of a trace of methyl red to suppress oxygen maxima. Curve 2 is the current-voltage curve of an air-free, 0.001 *N* hydrochloric acid solution in 0.1 *N* potassium chloride. Curve 1 was obtained after saturating the solution with air. The two oxygen waves correspond to the reductions:

$$O_2 + 2H_2O + 2e \longrightarrow H_2O_2 + 2OH^-$$
$$O_2 + 2H_2O + 4e \longrightarrow 4OH^-$$

It is seen that in the presence of oxygen the diffusion current of the hydrogen discharge is greatly decreased and becomes equal to its original value minus the total diffusion current of oxygen. If the original diffusion current of the hydrogen ions had been smaller than the total one of oxygen, no hydrogen wave would have been found in the air-saturated solution. We make use of this decrease of the diffusion current of hydrogen ions to prove the formation of hydroxyl ions in some other electrode reactions.

The formation of hydroxyl ions in some electrode reduction is of importance when the solution contains metal ions which form slightly soluble hydroxides. Great interference by oxygen is encountered, for example, in the analysis of neutral air-saturated solutions containing copper, lead, cadmium, zinc, etc. The interference is eliminated when the electrolysis is carried out in weakly acid medium (say a suitable buffer) or in the presence of a complex former (ammonia, or tartrate) which dissolves the hydroxide formed. However, the author prefers to remove oxygen from the solution before the electrolysis is started (see 2 above).

An interesting example of interference by the formation of hydroxyl ions at the electrode is met with in the polarographic analysis of neutral mixtures of iodate or bromate and heavy metals.

The reduction of iodate or bromate occurs according to the net reactions:

$$IO_3^- + 6H^+ + 6e \longrightarrow I^- + 3H_2O$$
$$BrO_3^- + 6H^+ + 6e \longrightarrow Br^- + 3H_2O$$

Lingane (*8*) found that the diffusion current of iodate was apparently decreased, and under certain conditions almost eliminated by the preceding discharge of cadmium. In reality, however, it is the cadmium diffusion current which is decreased through the formation of cadmium hydroxide during the reduction of iodate. The cadmium is reduced at a more positive potential than the iodate. Hence the cadmium wave is unaffected by the iodate, but the diffusion current of the cadmium is decreased during the discharge of iodate. That the "apparent" decrease of the iodate current in the presence of cadmium is actually due to the formation of cadmium hydroxide at the surface of the mercury drop was proved by Orlemann (*10*). By means of a special microscopic arrangement he was able to observe a film (of cadmium hydroxide) on the surface of the mercury drop when a mixture of 0.001 *M* cadmium sulfate and 0.001 *M* potassium iodate was electrolyzed in a neutral unbuffered 0.1 *M* potassium chloride solution. The film did not appear until the iodate was also reduced.

To study the hydroxyl-ion effect from a quantitative viewpoint Orlemann (*10*) electrolyzed mixtures of cupric copper and iodate in neutral and ammoniacal solutions and in acid buffers. The results obtained are presented in Table II. All currents have been corrected for the residual current. The diffusion current of copper ($i_{d_{Cu}}$) was measured at a potential of −0.8 volt, the total diffusion current—(i. e., the sum of the diffusion currents of copper and iodate)—at a potential of −1.5 volts. In order to find the apparent diffusion current of iodate, the diffusion current of copper has to be subtracted from the total diffusion current. However, the diffusion current of copper is smaller at a potential of −1.5 volts than at −0.8 volt where it had been measured. This change is mainly due to a decrease of the drop time, as explained in section 13. The value of the diffusion current of copper at a potential of −1.5 volts has been calculated from the value measured at −0.8 volt in the way described in section 13. From Table II it is seen that the correct value of the diffusion current of iodate in the presence of copper is found in ammoniacal solution or in the buffer with a pH of 5. However, in neutral medium the apparent decrease of the iodate wave is approximately equal to the diffusion current of copper. Actually, the iodate reduction takes place to the same extent as in the absence of copper, but the hydroxyl ions produced in the reduction of iodate precipitate the cupric copper and prevent it from being reduced. If the iodate concentration in the mixture had been such that its diffusion current, when present alone, would have been less than that of the copper, no (apparent) iodate wave would be found at all. Actually, at potentials where iodate alone gives a wave a reduction of iodate occurs in the mixture, but the corresponding current would be equal to the decrease of the copper current.

The results indicate clearly that current-voltage curves obtained in mixtures of iodate or bromate or other substances, which upon reduction yield hydroxyl ions, and metals forming insoluble hydroxides, yield unambiguous results only when a medium is used in which the metal hydroxides are soluble.

13. The factors which determine a diffusion current are given by the Ilkovic expression (Equation 2, section 3).

TABLE II. DIFFUSION CURRENTS

[Obtained in mixtures of copper and iodate in various media at 25.0°. Concentration of iodate 5×10^{-4} *M* ($=3 \times 10^{-3}$ *N*)].

Medium[a]	Concentration Cu × 10^3 *M*	$i_{d_{Cu}}$ at −0.8 Volt	i_{total} at −1.5 Volts	$i_{d_{IO_3}}$ Apparent at −1.5 Volts	Error $i_{d_{IO_3}}$ %
Neutral	0	0	11.2	11.2	0
Neutral	1.00	6.63	11.2	4.85	−57
0.5 *M* NH_3	1.00	7.80	18.7	11.2	0
Buffer, pH = 5.0	0.90	6.02	17.0	11.3	+1

[a] All solutions were 0.2 *M* in potassium nitrate.

TABLE III. EFFECT OF POTENTIAL ON i_d. I_d AND $i_d/m^{2/3}t^{1/6}$ IN ELECTROLYSIS OF CUPRIC COPPER

Potential (*vs.* S. C. E.)	−0.3	−0.5	−1.00	−1.30	−1.50	−1.60	−1.70
i_d, microamperes	6.12	6.14	6.10	5.98	5.88	5.84	5.70
$i_d/m^{2/3}t^{1/6}$	6.13	6.14	6.16	6.16	6.15	6.18	6.13

TABLE IV. RELATIVE VALUES OF $m^{2/3}t^{1/6}$ AT DIFFERENT POTENTIALS

(Value at −0.5 volt is taken equal to 1)

π	0	−0.2	−0.5	−1.0	−1.1	−1.3	−1.5	−1.6	−1.7	−1.8	−1.9
Relative value (*r. v.*)	0.985	0.99	1.00	0.985	0.98	0.97	0.95	0.94	0.93	0.92	0.91

For our present purpose it is of interest to consider the change of the diffusion current of a given solution obtained with a capillary of known characteristics (m and t in Equation 2) with the applied potential.

The mass of mercury, m, flowing out per second is hardly affected by the applied potential. On the other hand, the drop time, t, which is roughly proportional to the surface tension of the mercury, is greatly dependent upon the potential. It has a maximum value at a potential of −0.55 volt (*vs.* S. C. E.), the so-called electrocapillary zero point. Its value decreases when the potential is made more positive or negative. For example, when the potential is zero, one may find a drop time—e. g., of 2.75 seconds—at −0.5 volt 3.10, at −1 volt 2.7, at −1.5 volts 2.2, and at −1.9 volts 1.6. Fortunately, the diffusion current is not proportional to t but to $t^{1/6}$. The result is that between potentials of zero and 1 volt, i_d changes only very slightly with the potential. However, the effect becomes more marked when the potential is made increasingly more negative than 1 volt. According to the Ilkovic equation i_d should change with the potential, but $i_d/m^{2/3}t^{1/6}$ should remain constant. This was verified again recently by Kolthoff and Orlemann (*7*), who measured the diffusion current of cupric copper at different potentials. From the known values of m and t of the capillary used they calculated the ratio $i_d/m^{2/3}t^{1/6}$. Table III shows that this ratio is constant within the experimental error.

Let us now consider a mixture of two reducible substances. In the proper supporting electrolyte a current-voltage curve is obtained as shown diagrammatically in Figure 5. The diffusion current, i_{d_1}, of substance 1 is measured at a potential π_1, while the total current i_t is measured at potential π_2 (all corrected for the residual current). The diffusion current, i_{d_2}, of substance 2 then is equal to the total current minus the diffusion current which substance 1 would have at a potential π_2. From the above, it is evident that the diffusion current of substance 1 at a potential π_2 [denoted as $(i_{d1})_{\pi 2}$] is:

$$(i_{d1})_{\pi_2} = (i_{d1})_{\pi_1} \frac{(m^{2/3}t^{1/6})_{\pi_2}}{(m^{2/3}t^{1/6})_{\pi_1}} \qquad (3)$$

and

$$i_{d_2} = i_t - (i_{d1})_{\pi_1} \frac{(m^{2/3}t^{1/6})_{\pi_2}}{(m^{2/3}t^{1/6})_{\pi_1}} \qquad (4)$$

Kolthoff and Orlemann (*7*) compared the relative value of $m^{2/3}t^{1/6}$ at different potentials for four different capillaries which had quite different characteristics. They found that the relative value was virtually independent of the characteristics of the capillary. The data given in Table IV allow the calculation of the change of i_d with the potential without determining the characteristics of the capillary. From Table IV it is seen that if the diffusion current of a substance measured at a potential of −0.5 volt is 10 microamperes it is equal to 9.4 at a potential of −1.6 volts and 9.1 at a potential of −1.9 volts.

Denoting the relative value at a certain potential π_1 by $(r.v.)_{\pi_1}$ Equation 4 can be rewritten as follows:

$$i_{d_2} = i_t - (i_{d1})_{\pi_1} \frac{(r.v.)_{\pi_2}}{(r.v.)_{\pi_1}} \qquad (5)$$

To illustrate the significance of Equation 5 some figures obtained in the polarographic analysis of a mixture which was 0.001 M in copper, 0.001 M in manganese, and 0.1 N in potassium chloride are given.

The diffusion current of copper measured at π = −0.5 volt was 6.19 microamperes. The diffusion current of manganese in the absence of copper at a potential of −1.7 volts was 6.00 microamperes. In the mixture a total current of 11.85 microamperes was measured at π = −1.7 volts. If we did not correct for the $m^{2/3}t^{1/6}$ effect we would find a value for the diffusion current of manganese in the mixture of 11.85 − 6.19 = 5.66, corresponding to an error of −5.6 per cent. However, if the value of i_d of copper at π = −1.7 volts is calculated with the aid of Equation 5 and Table IV we find for i_d of manganese: 11.85 − 5.75 = 6.10, corresponding to an error of +1.7 per cent. The larger the ratio of copper to manganese, the greater becomes the error if the $m^{2/3}t^{1/6}$ effect is not considered. For example, in a mixture which was about 0.002 M in copper and 0.001 M in manganese the following figures were found: i_d of copper at π of −0.5 volt = 12.76 microamperes; i_t at π of −1.7 volts = 18.00. Uncorrected for the $m^{2/3}t^{1/6}$ effect this would yield i_d of manganese = 18.00 − 12.76 = 5.24 microamperes, or an error of −12 per cent. However, if corrected for the effect we find i_d of manganese = 6.10 or an error of +1.7 per cent. It is not possible to eliminate the discussed effect by any of the empirical procedures which have been proposed in the literature for finding diffusion currents or the relation between i_d and the concentration.

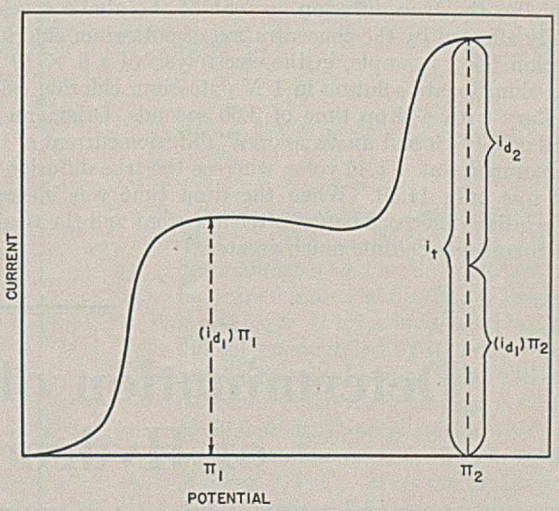

FIGURE 5. SCHEMATIC PICTURE OF CURRENT-VOLTAGE CURVE OF A MIXTURE OF TWO REDUCIBLE SUBSTANCES

14. Recently (*11*) the discovery has been made that an anomalous "wave" occurs in solutions with a relatively high concentration of indifferent electrolyte, approximately 0.5 M or larger, when a current due to the reduction of some constituent passes through the solution. For example, when a thallium solution is electrolyzed in 1 to 4 N potassium chloride we first obtain the diffusion current of thallium. At a potential of about −0.9 volt a new wave starts which is flat and reaches a maximum at −1.35 volts. When the potential is made more negative the decrease in current is much greater than corresponds to the decrease of $m^{2/3}t^{1/6}$. However, before it becomes equal to the diffusion current of thallium, the discharge of potassium sets in. The residual current in 1 to 4 N solutions of potassium chloride or other indifferent electrolytes does not show any irregularities, and is of the same order of magnitude as it is in 0.1 N solutions. The

anomalous wave is due to a discharge of water under the specified conditions:

$$H_2O + e \longrightarrow H + OH^-$$

The water current at a potential of −1.35 to −1.4 volts is proportional to the total current flowing. Suppose, for example, that under certain conditions the thallium wave is equal to 5 microamperes and the water wave to 4. When the thallium concentration is doubled, the water wave becomes equal to 8.

In addition to being dependent upon the total current, the concentration and kind of indifferent electrolyte present, the water wave depends greatly upon the characteristics of the capillary. The water current decreases markedly with increasing drop time.

From the analytical viewpoint the "water wave" can be the cause of serious errors in the interpretation of current-voltage curves, especially in the determination of constituents, the waves of which yield diffusion currents at potentials more negative than about 1 volt. Under such conditions only one wave is found, since the water wave overlaps with the wave of the reduced constituent. Consequently, the diffusion current measured is not equal to that of the reduced constituent, but is equal to the sum of the latter and the water wave. For example, in the electroreduction of zinc, iodate, or bromate a marked increase in the "apparent" diffusion current is found with increasing concentration of potassium chloride, if the concentration of the chloride is made greater than 0.5 *N*. Actually, the true diffusion current of the above species is hardly affected by the concentration of potassium chloride in solution. For example, in the electrolysis of a 5×10^{-4} *M* potassium iodate solution in 1 *N* potassium chloride using a capillary with a drop time of 3.60 seconds, Orlemann and Kolthoff (*11*) found an "apparent" diffusion current of 14.20 microamperes at −1.45 volts, whereas the true diffusion current was only 11.30. When the drop time was increased, the relative difference between the apparent and the true diffusion currents became much greater.

In quantitative polarographic work solutions which contain a high concentration (1 to 2 *N*) of indifferent electrolyte—(e. g., in steel analysis)—are often electrolyzed. When only one reducible constituent is present it is not essential to eliminate the water wave because a calibration line with known concentrations of the constituent can be obtained in the particular medium. However, when the solution contains a mixture of reducible substances, it is not easy to eliminate the effect of the water current upon the results by a set of calibrations, because the water current is proportional to the total current. In the polarographic analysis of a mixture in a medium of high salt concentration it becomes essential to eliminate the water wave entirely. This can be done by the addition of 0.01 per cent of gelatin to the solution. Other capillary-active substances, which are often used as maximum suppressors, also affect the water wave; they do not eliminate it completely as gelatin does, but shift the wave to more negative potentials. Next to gelatin the most effective of the capillary active substances investigated is tylose, which shifts the wave to a potential of −1.6 volts.

In order to avoid any complications by the water wave in work with the dropping electrode, the author recommends that 0.01 per cent of gelatin be added to solutions, the salt concentration of which is larger than 0.5 *N*.

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Determination of Mixed Aniline Points of Hydrocarbon Solvents

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DURING the past few years, considerable attention has been given to methods of determining the solvency characteristics of paint, varnish, and lacquer thinners. While the viscosity method represents an ultimate means of determining the relative value of various thinners, a more simple test, such as aniline point, probably will continue to be used for controlling the uniformity of a given thinner.

With the development of petroleum naphthas of substantial aromatic content, it has been necessary to use the mixed aniline point test. The mixed aniline point has been defined as the minimum miscibility temperature in degrees centigrade of a mixture of 10 cc. of anhydrous aniline, 5 cc. of the thinner under test and 5 cc. "of any naphtha whose aniline point is 60° C." (*3*). McArdle (*1*, *4*) states that the nonaromatic diluent shall be "a mineral spirit (regardless of boiling point)" having a 60° C. aniline point. Experiments described herein indicate the necessity for defining the diluent more closely.

The mixed aniline points, as defined above, have been determined on a number of experimental high-solvency naphthas of varying aromatic content. Two sets of tests were made, one using A. S. T. M. precipitation naphtha as the diluent and the other using mineral spirits. The inspection tests of the two diluent naphthas are shown in Table I.

Figure 1 shows the relationship obtained between the two sets of tests after classifying the high-solvency naphthas under

Table I. Inspection Tests

	A. S. T. M. Precipitation Naphtha	Mineral Spirits
Gravity, °A. P. I.	71.9	48.9
A. S. T. M. distillation D-86		
Initial b. p., ° F.	140	305
10% recovery, ° F.	150	323
50% recovery, ° F.	164	345
90% recovery, ° F.	195	388
End point, ° F.	234	423
Aniline point, ° C.	59.6	59.7

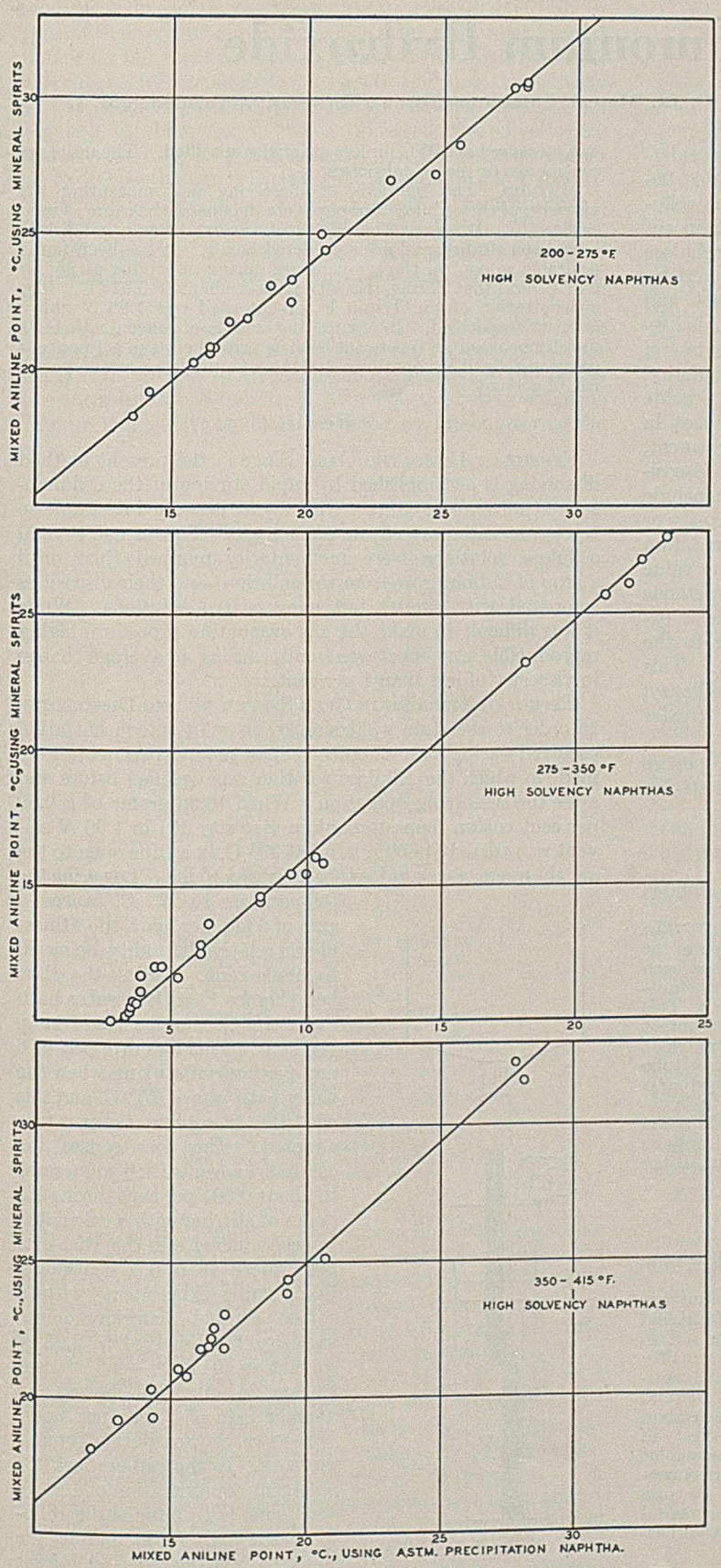

FIGURE 1. EFFECT OF DILUENT ON MIXED ANILINE POINTS OF HIGH-SOLVENCY NAPHTHAS

consideration into three boiling point ranges—200° to 275°, 275° to 350°, and 350° to 415° F., respectively. It will be noted that the mixed aniline points determined with A. S. T. M. precipitation naphtha are appreciably lower than when the mineral spirits are employed. These differences are not constant, but vary with the concentration of aromatics and with the boiling range of the solvent naphthas as shown by the following data taken from the curves:

Mixed aniline point with mineral spirits	10° C.	15° C.	20° C.
Mixed aniline point (° C.) with precip. naph.			
For 200–275° F. naphthas	..	9.5	15.5
For 275–350° F. naphthas	3	8.7	14.5
For 350–415° F. naphthas	..	9	14.4

Mixed aniline point with mineral spirits	25° C.	30° C.
Mixed aniline point (° C.) with precip. naph.		
For 200–275° F. naphthas	21.4	27.3
For 275–350° F. naphthas	20.3	
For 350–415° F. naphthas	20	25.7

This same effect of the variation of the paraffinic diluent on the mixed aniline point is observed with C. P. toluene. Baker's C. P. toluene, having an n_D^{20} of 1.4951 shows the following mixed aniline points:

	° C.
Using A. S. T. M. precipitation naphtha	−0.9
Using mineral spirits	+7.6

From the above discussion it is evident that the paraffinic diluent used for determining mixed aniline points should be defined more rigidly than it has been in the past. It is recommended that this diluent be identified by its aniline point and by its mixed aniline point with C. P. toluene. Some users employ a diluent equivalent to mineral spirits which has a mixed aniline point with C. P. toluene of about 7.5° C. McArdle (*2*) proposes that this diluent have an aniline point of 60° C. and a mixed aniline point of 10 ± 0.5° C. with C. P. toluene. It is suggested by the authors that the manufacturers and consumers of high-solvency naphthas cooperate to define the specifications for the diluent used to determine mixed aniline points.

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PRESENTED before the Division of Petroleum Chemistry at the 102nd Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Viscosity Determination of Cotton in Dimethyl Dibenzyl Ammonium Hydroxide

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IN AN EARLIER study (*6*) it was found that over a considerable viscosity range a nearly linear relation existed between the viscosities of 0.5 per cent solutions of cotton celluloses in dimethyl dibenzyl ammonium hydroxide and in cuprammonium hydroxide, respectively. The cotton cellulose samples in various states of degradation were produced by oxidation in alkaline medium. Recently Brownsett and Clibbens (*3*) have investigated cotton celluloses degraded by a number of different oxidizing and hydrolyzing agents and find for the group no unique relationship between dimethyl dibenzyl ammonium hydroxide and cuprammonium hydroxide viscosity. However, these authors find in most instances, in comparing celluloses degraded by a given chemical treatment, that substantially linear relationships exist between viscosities in the two solvents. More important still, they conclude that the dimethyl dibenzyl ammonium hydroxide viscosity or fluidity measures the total (apparent and latent) degradation which a cellulose has suffered while the cuprammonium value gives only the apparent degradation. The two methods yield comparable information if the cellulose sample is extracted with dilute sodium hydroxide solution prior to the cuprammonium hydroxide viscosity determination. This extraction is unnecessary in the case of the dimethyl dibenzyl ammonium hydroxide, presumably because of the greater basicity of the latter.

In order to secure more information about the properties of celluloses dissolved in the newer solvent the earlier studies (*6*) have been continued and are reported in this paper.

Apparatus

VISCOMETER. Cannon and Fenske viscometers were calibrated and used as in the earlier work (*6*).

APPARATUS FOR DISSOLVING THE CELLULOSE SAMPLE. The same principle has again been used to secure rapid solution of the sample but, as is apparent from Figure 1, a glass bell has been added whose lower end is sealed by the liquid of a constant-temperature bath in which the dissolving tube is immersed. This simple addition makes it possible to dissolve the sample in contact with any desired gas. The bell is secured to the stirrer shaft by a No. 5 rubber stopper through which a small-bore glass tube carrying a stopcock is passed. Through the latter the system is flushed and the desired gas atmosphere introduced. If it is desired to exclude water vapor from the water bath an additional cup for a mercury seal, shown in dotted lines in Figure 1, may be added. The glass bell and water seal have been used in all the present work unless otherwise noted.

Materials and Method

GASES. Cylinder oxygen and nitrogen of high quality were used without further purification.

SOLVENT. The source of dimethyl dibenzyl ammonium hydroxide was the commercial product Triton F, furnished through the courtesy of D. H. Powers of Röhm & Haas. Two different lots were used. As received, the first lot had a normality of 1.96, and at 25° C. a density of 1.072 and viscosity of 8.92 centipoises. When concentrated to 2.12 *N* the density rose to 1.078 and the viscosity to 11.24 centipoises. The second lot as received had a normality of 2.08, and at 25° a density of 1.081 and a viscosity of 13.90 centipoises. Comparable data for dilution of the second lot to 1.96 *N* are density 1.078 and 10.79 centipoises, and for concentration to 2.25 *N*, density 1.088 and 20.14 centipoises. Because of these small differences in Triton F specifications experimental data are compared only for the same lot of solvent unless otherwise noted.

COTTON SAMPLES. Samples of cotton of known cuprammonium fluidities were obtained from several different sources. The cuprammonium viscosity or fluidity values given in this paper always refer to a 0.50 per cent solution of cellulose whose viscosity was measured at 20° C. unless otherwise specified. The units are centipoises or reciprocal poises.

METHOD. The methods of dissolving and measuring the viscosity of the cellulose samples were in general the same as used earlier (*6*). However, the effects of varying certain conditions have been studied and are considered below. It has been found desirable to control the temperature during dissolving at 25° ± 0.5° C. and that during viscosity measurement to 0.02° C. The concentration of the Triton F solvent used was 1.96 *N* unless otherwise specified. In computing cellulose concentrations, a moisture content of 6 per cent has been assumed, and a 1 per cent solution defined as containing 100 mg. of anhydrous cotton per 10 ml. of solvent.

Results

EFFECT OF DISSOLVED GAS. Since in the present method dissolving is accomplished by rapid stirring of the cellulose-solvent mixture in contact with a gas, it seemed desirable to try to ascertain any effects of such gas. To this end several cellulose solutions were momentarily pumped (just until a crop of bubbles rose from the solution) and their viscosities compared with similar but unevacuated solutions. While it was difficult to make the gas evacuation procedure highly reproducible, any effect was small, causing an average change in viscosity of less than 1 per cent.

EFFECT OF STIRRING IN OPEN SYSTEM DURING DISSOLVING. In order to ascertain what changes in solution concentration occurred during dissolving in an open tube, experiments were made in which the cellulose solution was weighed before and after the dissolving operation. When 10.66 grams of a 0.50 per cent cotton (cuprammonium viscosity 19) in 1.96 *N* solvent was stirred at 650 r. p. m. at 30° C. in a tube open to the air, the average weight loss per hour was 15 mg. Lowering the temperature to 22° C. caused a gain of 6 mg. per hour, the atmospheric relative humidity being 80 to 90 per cent. Adding the glass bell (Figure 1) with a water bath seal at 21° C. caused a loss of 8 mg. per hour. This loss dropped to 6 mg. per hour of stirring when the water bath was at 25° C. and this was the dissolving temperature adopted. This loss causes the cellulose concentration to increase to about 0.501 per cent during 3.5 hours of stirring with a calculated viscosity increase in this case of a little more than 0.5 centipoise. Comparable experiments with a cotton sample (cuprammonium viscosity 22) dissolved in 2.25 *N* solvent to 0.45 per cent concentration in nitrogen showed an average gain of 5 mg. per hour.

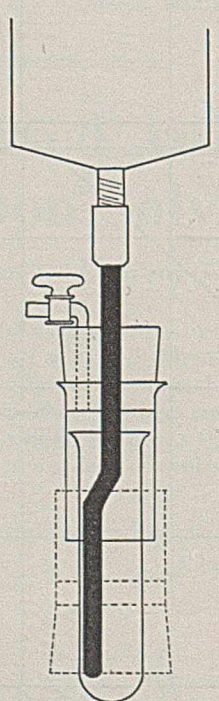

FIGURE 1. APPARATUS FOR DISSOLVING COTTON SAMPLE

EFFECT OF SOLVENT CONCENTRATION. In the earlier work (*6*) a solvent concentration of 1.96 *N* was found adequate for the celluloses studied. In extending the work to less degraded celluloses produced by various means, a somewhat larger concentration between 2.1 and 2.25 *N* has been

found to possess greater dissolving capacity for very slightly modified cellulose—e. g., cuprammonium viscosity 30 to 50. In spite of their greater solvent capacity these more concentrated liquids do not swell cellulose as quickly as the 1.96 *N* solvent. The effect of increasing solvent concentration upon the viscosity of the cellulose solution produced is shown graphically in Figure 2, in which viscosity in centipoises is plotted vertically and solvent normality horizontally. In the viscosity region up to 500 centipoises it is clear that the viscosity of the cellulose solutions rises with increasing solvent concentration at a rate which is greater the more viscous the solution. The rising solvent viscosity, however, causes the specific viscosities of the cellulose solutions to fall with increasing solvent normality.

Effect of Air and Oxygen during Dissolving. The ease with which cellulose is oxidized in the presence of alkalies made it of interest to ascertain the oxygen sensitivity of the present solutions of cellulose. Although the bell attachment (Figure 1) made it possible to vary the gas present during the

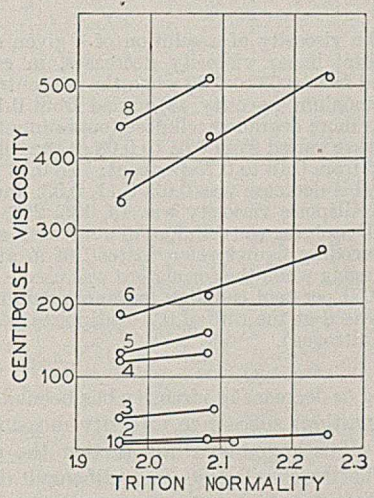

Figure 2. Effect of Solvent Concentration upon Viscosity

1. Triton F solvent, first lot
2. Triton F solvent, second lot
3. Sample (cuprammonium viscosity 19) at 0.25% concentration
4. Sample (cuprammonium viscosity 5.4) at 0.75% concentration
5. Sample (cuprammonium viscosity 11) at 0.50% concentration
6. Sample (cuprammonium viscosity 19) at 0.50% concentration
7. Sample (cuprammonium viscosity 22) at 0.45% concentration
8. Sample (cuprammonium viscosity 31) at 0.45% concentration

dissolving of the cellulose, the viscosity measurements were always made in contact with air. Experiments with a cotton yarn sample (cuprammonium viscosity 19) at 0.50 per cent concentration in 1.96 *N* solvent showed, when nitrogen was present during the dissolving period of 3.5 hours, a viscosity of 189.0 centipoises. With air present the value was 182.0, while oxygen still gave a viscosity of 181.8. For the same sample at 0.25 per cent concentration the figures were 45.4 (air) and 47.7 (nitrogen). This behavior is in marked contrast to cellulose dissolved in cuprammonium solution which deteriorates rapidly upon exposure to air or oxygen (*2*). In view of the above and other similar evidences of some atmospheric attack upon the cellulose during dissolving, nitrogen has been used in all the work reported here, unless otherwise noted. Because the nitrogen used was not specially purified, and some contamination from foreign gases dissolved in the water of the seal may have occurred, and furthermore exposure to air took place during viscosity measurements, it is

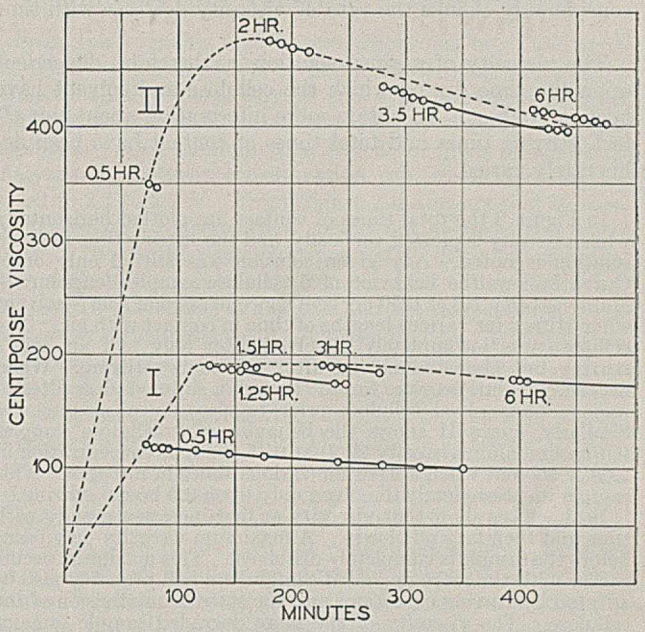

Figure 3. Effect of Time upon Viscosity

Duration of stirring given in hours

I. Sample (cuprammonium viscosity 19) at 0.50% concentration in 1.96 *N* Triton F

II. Sample (cuprammonium viscosity 22) at 0.45% concentration in 2.08 *N* Triton F

possible that if oxygen is rigidly excluded in all operations evidence of greater oxygen sensitivity will be found.

Effect of Temperature. In certain experiments the sample was dissolved to 0.25 per cent concentration in contact with air at different water bath temperatures. Thus a sample (cuprammonium viscosity 19) gave a centipoise viscosity of 50.6 when the dissolving temperature was 20° C., and 45.4 at 25° C. A less degraded sample showed a somewhat greater viscosity loss between 25° and 30° C. In other experiments the viscosity of a cellulose solution was measured at both 20° and 25° C. in order to determine the temperature dependence. In Table I are given data for four cellulose samples whose cuprammonium viscosities range from 5.3 to 31. The temperature coefficient increases with viscosity, varying from 1.23 with no cellulose present in the 2.08 *N* solvent to 1.30 for the solution of greatest viscosity. The average centipoise change per degree in viscosity between 20° and 25° C. ranges from 2.42 to 28.74 for the samples and concentrations studied, thus indicating a marked temperature sensitivity for the more viscous solutions.

Effect of Time. As was pointed out earlier (*6*) the viscosity of a solution of cellulose in dimethyl dibenzyl ammonium hydroxide decreases with time. This factor becomes the more noticeable the greater the viscosity of the solution examined and/or the smaller the viscometer constant. Carrying out the dissolving and the viscometer measurements in an atmosphere of not specially purified nitrogen did not

Table I. Effect of Temperature upon Viscosity of Triton F Solvent and Solutions of Cellulose

Sample	Cuprammonium Viscosity	Triton Normality *N*	Cellulose Concentration	Triton Solution Viscosity 25° C.	Triton Solution Viscosity 20° C.	Ratio $\frac{\eta\ 20°}{\eta\ 25°}$
	Centipoises		%	*Centipoises*		
11	31	2.08	0.45	485.2	628.9	1.30
13	19	2.08	0.50	210.5	270.6	1.29
15	5.4	2.08	0.75	133.2	171.0	1.28
16	5.3	1.96	0.50	51.35	63.45	1.24
		2.08	0	13.90	17.14	1.23
		1.96	0	10.79	13.20	1.22

considerably change the rate of viscosity decrease with time (*1*).

The viscosity of a given solution is, therefore, dependent upon the time during which the cellulose and solvent have been in contact. To obtain more information about this effect, stirring times and total times of contact have been deliberately varied.

In Figure 3 the total times of contact are plotted horizontally while viscosity is plotted vertically, and the stirring time for each solution is noted. Any given solution was stirred only once. Curve I shows the behavior of 5 cellulose samples (cuprammonium viscosity 19) at 0.50 per cent concentration in 1.96 *N* solvent when stirred for various lengths of time in contact with air. The cellulose was incompletely dissolved after only half an hour's stirring but almost dissolved after 1.5 hours' stirring. When necessary, solutions were forced through a sintered-glass filter to remove undissolved cellulose prior to viscosity measurements. Similarly, curve II shows the behavior of 4 cellulose samples (cuprammonium viscosity 22.2) at 0.45 per cent concentration in 2.08 *N* solvent when stirred for various times in nitrogen. This sample was completely dissolved only upon 3.5 hours' stirring.

Both curves show that viscosity at first increases rapidly with time and then falls off slowly. A maximum viscosity may occur before the sample is completely dissolved. This maximum occurs earlier with the more degraded sample and will probably also be affected by stirring conditions and the state of subdivision of the cellulose. The viscosity of the more degraded sample remains substantially constant for stirring times from 1.25 to 3 hours. This is probably explained by more cellulose dissolving to offset the aging effect. The sharper maximum in curve II is probably due to the slower dissolving and more rapid aging of the less degraded cellulose sample. The experimental points on curves I and II show the aging effects with the individual solutions as given by successive viscometer measurements. In general the viscosity decreases at a nearly linear rate for an hour or so, but on long standing the rate of aging decreases. This is observed in following the aging of the various solutions shown in curves I and II. The slope of curve I as it passes beyond the last point was determined by measuring the viscosity of the last solution after 12 hours when its viscosity had dropped to 140 centipoises.

The aging rate of a cellulose solution was found to be greater the greater the concentration of a given cellulose in solution, and the greater the viscosity of a solution of a given concentration. Thus the initial linear viscosity decreases, in centipoises per minute, varied from 0.03 to 0.90 when the concentration of cellulose (cuprammonium viscosity 44) varied from 0.10 to 0.45 per cent. With a more degraded cellulose (cuprammonium viscosity 5.4) this decrease varied from 0.01 to 0.08 as the cellulose concentration varied from 0.15 to 0.75 per cent. In 0.50 per cent cellulose solution this decrease was 0.02, 0.11, 0.33, and 3.1 for solutions whose centipoise viscosity was 43, 135, 269, and 1540, respectively. Dissolving the cellulose in a nitrogen atmosphere, to which the preceding figures refer, instead of in air, produced a lower rate of aging when the former gas was used. Thus the viscosity of a 0.50 per cent cellulose (cuprammonium viscosity 19) solution decreased at the rate of 0.2 centipoises per minute (air) against 0.12 (nitrogen).

Table II. Dependence of Viscosity upon Cellulose Concentration and Quality

[Viscosity in centipoises (η). Cuprammonium viscosity (η_{Cu}) for 0.50 per cent cotton at 20° C. Triton F (1.96 *N*) viscosity (η_T) at 25° C.]

Sample	10	11	12	13	14	15
(η_{Cu})	(43.3[a])	(31)	(25)	(19[a])	(11)	(5.4)
Cotton concentration	η_T	η_T	η_T	η_T	η_T	η_T
%						
0.05	17.3	...	...	...	...	...
0.076	23.9	...	...	...	...	...
0.10	31.8	30.1[b]	27.4[b]	...	20.1[b]	...
0.15	...	...	...	30.7[b]	...	19.6[b]
0.20	88.5	66.3[b]	53.6[b]	...	35.8[b]	...
0.25	...	...	...	47.7	...	...
0.30	246	...	...	...	...	...
0.35	397	189	136	88.1	61.7	30.6
0.40	598	...	...	...	...	...
0.45	861[b]	454[b]	...	...	...	...
0.50	1520	...	267	189	135[b]	54.2
0.75	...	...	...	...	...	123[b]

[a] Cuprammonium viscosity determined at 25° instead of 20° C.
[b] Second lot of Triton F.

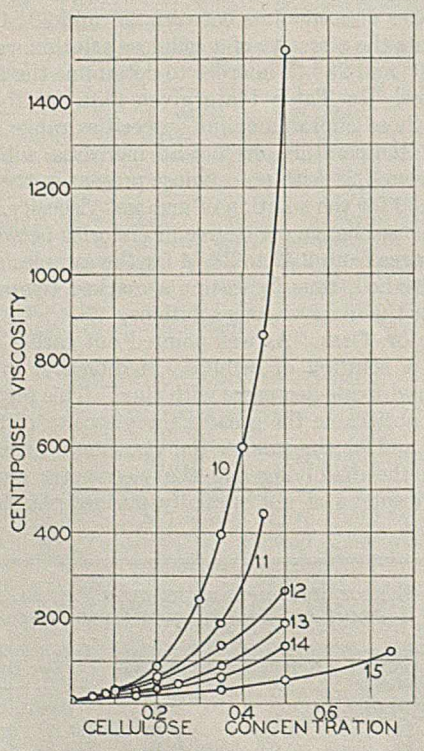

Figure 4. Relation between Viscosity and Cellulose Concentration
1.96 *N* Triton F
Concentration of cellulose in per cent

Some aging or decrease in viscosity has been observed under whatever conditions successive viscosity measurements have been made. It is hoped to study further the nature of the interaction between cellulose and dibenzyl dimethyl ammonium hydroxide which appears responsible for the aging. Because of this interaction it is necessary to specify the age of a solution in considering its viscosity. In this paper all data have been interpolated (or sometimes extrapolated) to the same "age" before being compared.

Variation of Viscosity with Cellulose Concentration. Since the manner in which viscosity changes with cellulose concentration affords an insight into the nature of cellulose solutions, several cellulose samples of very different viscosities and prepared by different methods have been studied in 1.96 *N* solvent. All samples were dissolved by stirring in contact with nitrogen for 3.5 hours and the viscosity has been interpolated (occasionally extrapolated) to an age of 275 minutes. The data are recorded in Table II and shown graphically in Figure 4, in which cellulose concentration is plotted horizontally and viscosity in centipoises vertically. It is clear that the viscosity increases exponentially with solute concentration. A similar relation has been found for cuprammonium solutions of cellulose (*5*) and nitrocelluloses in organic solvent (*4*).

In Figure 5 the dotted curves indicate how the specific viscosity of these samples changes with cellulose concentration. It appears that a linear relation between specific viscosity and solute concentration will occur only below a cellulose concentration of 0.05 per cent for the least degraded sample (10) although an essentially linear relation already occurs below 0.30 per cent for the most degraded sample (15). This low concentration region is of interest because of its possible theoretical significance (*7*). In Figure 5 the solid curves show fluidities of these samples in reciprocal poises plotted

vertically and solute concentrations horizontally. This plot emphasizes the nearly linear character of the fluidity variation for sample 15 and the increase of curvature with decreasing cellulose degradation. Also it is clear from this plot that the fluidity range of the celluloses studied varies widely with solute concentration, and passes through a maximum near 0.25 per cent.

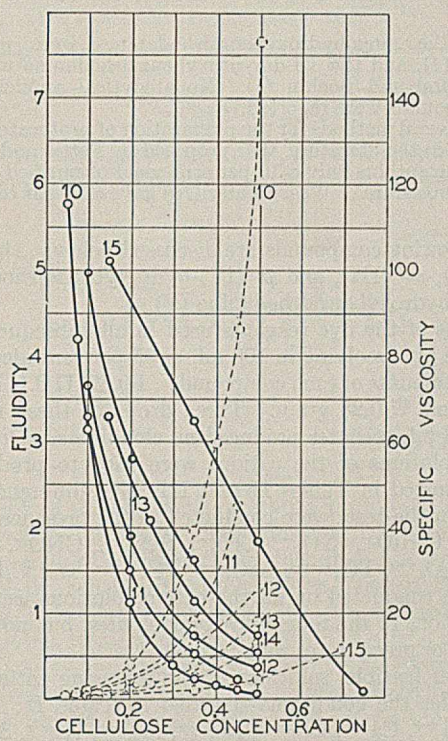

FIGURE 5. RELATION OF FLUIDITY AND SPECIFIC VISCOSITY TO CELLULOSE CONCENTRATION IN 1.96 *N* TRITON F

Solid curves, fluidity
Dotted curves, specific viscosity

In view of the recent work of Brownsett and Clibbens (*3*), no unique relation between cuprammonium viscosity and Triton F viscosity is to be expected except in the cases of celluloses modified by the same or equivalent chemical attack. In the present work samples 10 and 13 were chemically unmodified celluloses, sample 12 was bleached, while samples 11, 14, and 15 were bleached with sodium chlorite–sodium hypochlorite, peroxide, and sodium hypochlorite, respectively, so that no unique relation would necessarily be expected. The simple "ten" relation found earlier (*6*) involved a series of celluloses similarly degraded—namely, by a succession of commercial laundry treatments—to cuprammonium fluidities between 4 and 25. However, present samples 12 to 15, inclusive, fit into the former series. Brownsett and Clibbens (*3*) find for hydrocellulose, and oxycelluloses prepared with dichromate–oxalic acid, periodic acid, and alkaline bromate, respectively, that for 0.5 per cent solutions the cuprammonium fluidity is approximately 6.5 times the Triton F fluidity. This figure is 5.8 and 4.6 for oxycelluloses prepared by dichromate–sulfuric acid and neutral hypochlorite, respectively. After extraction with dilute sodium hydroxide, however, all the celluloses give the 6.5 ratio. The discrepancy between this lower value and value of 10 found in the earlier work (*6*) is probably explained for the most part by the considerable aging which occurred during the long dissolving time of 18 hours used by Brownsett and Clibbens.

Summary and Analytical Considerations

From the foregoing certain conclusions can be drawn as to conditions which should be favorable to viscosity analyses for cellulose quality when carried out with dimethyl dibenzyl ammonium hydroxide as solvent. When slightly degraded celluloses (cuprammonium viscosity 25 or greater) are to be analyzed the solvent concentration can advantageously be raised to 2.1 to 2.2 *N*. From Figure 2 it appears that so raising the solvent concentration should not decrease the sensitivity of the method even for moderately degraded samples. It is desirable to control the temperature of the dissolving sample to a few tenths of a degree and the temperature of the viscometer measurements to a few hundredths. This becomes the more important the more viscous the solution.

To reduce changes in solution weight (concentration) to a few milligrams per hour during dissolving it is an easy matter to employ a glass bell (Figure 1) to ensure an atmosphere of fixed water vapor pressure. Using a mercury or oil seal eliminates any water vapor. If a nitrogen atmosphere is maintained in the bell the viscosity values are some 4 to 5 per cent higher and the solution ages somewhat more slowly. Because the viscosity of cellulose in dimethyl dibenzyl ammonium hydroxide solution changes with time it is necessary to know how long the cellulose and solvent have been in contact and it is desirable to employ known stirring times. Since after dissolving this aging occurs at an approximately linear rate, for an hour or so, extrapolations are easy.

In general, unless the cellulose has a cuprammonium viscosity in excess of 20 and/or the cellulose concentration is greater than 0.5 per cent the solution ages no faster initially than about 0.2 centipoise per minute. Also under these conditions a considerable variation in dissolving time appears permissible in view of the very flat maximum in curve I of Figure 3. While a cellulose concentration of 0.5 per cent is satisfactory for samples with a cuprammonium viscosity of 25 or less, for less degraded celluloses a smaller concentration is desirable to avoid excessively viscous solutions. From the fluidity curves in Figure 5 it appears that a cellulose concentration of 0.25 per cent should give the analysis maximum sensitivity for celluloses with cuprammonium viscosities falling between 5 and 43 centipoises. In technical analysis a cellulose concentration of 0.35 per cent may prove most generally useful.

If it is desired to compute cuprammonium viscosities (or fluidities) from measurements made upon celluloses dissolved in dimethyl dibenzyl ammonium hydroxide, it becomes necessary to have conversion data. A certain amount of such data (*3*, *6*) is now available. Thus far conversion relations have proved simple over considerable ranges of cellulose degradation, making possible the use of conversion factors. However, the present method of itself appears to give different information about cellulose quality than the cuprammonium method, and according to Brownsett and Clibbens (*3*) the dimethyl dibenzyl ammonium hydroxide method measures total cellulose (apparent and latent) degradation while the former method measures only apparent degradation. The present method, therefore, appears to merit consideration upon additional grounds.

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Analytical Possibilities of Some Hydroxybenzalrhodanines

o-, *m*-, and *p*-Hydroxybenzalrhodanines and 3,4-Dihydroxybenzalrhodanine

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SINCE the use of an organic compound for inorganic analysis by Griess (*5*) in 1879 and especially since the excellent results obtained with dimethylglyoxime (*9*) in 1905, the use of organic reagents in inorganic analysis has increased steadily.

Rhodanine was first prepared by Nencki (*7*) in 1877 and the analytical applications of a number of its derivatives were recently investigated by Feigl (*2*), who found that rhodanine itself gave yellow precipitates with silver, mercury, gold, palladium, and platinum, and that *p*-dimethylaminobenzalrhodanine was the most sensitive reagent yet discovered for the detection of silver (*3*). Because of its aqueous insolubility, alcohol or acetone solutions of the reagent are used. Rhodanine itself is precipitated from such solutions when added to appreciable quantities of water, which restricts its wider use as an analytical reagent.

Table I. Physical Constants

	Color	Solubility[a] Water	Solubility[a] 95% Alcohol	Melting Points Cited	Melting Points Found
		G./100 cc.		° *C.*	° *C.*
Rhodanine	Yellow	0.4032	3.1444	269.70	267.69
o-Hydroxybenzalrhodanine	Yellow	0.0136	2.1932	200 and 218–219	213–14
m-Hydroxybenzalrhodanine	Greenish yellow	0.0292	1.2436	244–45	245–46
p-Hydroxybenzalrhodanine	Orange	0.0216	2.0620	260	270–75
3,4-Dihydroxybenzalrhodanine	Yellow	0.0115	0.5280	270–80	265 decomposed

[a] Grams per 100 cc. of solvent at 25° C. Values are averages of three parallel determinations, made by making a saturated solution at 60° C., allowing to cool to 25° C. in presence of undissolved solid, pipetting off 25-ml. portions, evaporating solvent, drying residue at 110° C., weighing, and recalculating on basis of 100 ml. of solvent.

Since the introduction of a hydroxyl group into benzaldehyde increases the water-solubility from 4- to 8-fold and the introduction of a second hydroxyl makes the product even more water-soluble; and since a number of aromatic hydroxy compounds themselves possess analytical possibilities, the present investigation was undertaken to find whether or not both of these properties might be carried over into the condensation products.

A wide variety of methods for the condensation of rhodanine and aldehydes is given in the literature. Concentrated sulfuric acid (*11*), acetic acid (*6*), and an alkaline medium (*11*) seemed to be most generally employed. All three methods were used by the present authors in the preparation of their reagents and in every instance the alkaline medium gave better yields and a purer initial product. Continued agitation throughout the period of condensation was found to be very advantageous.

o-Hydroxybenzalrhodanine was made by Zipser (*11*). All three of the monohydroxybenzalrhodanines were made by Borgellini (*1*), and the 3,4-dihydroxybenzalrhodanine was made by Rosemand and Boehm (*8*). No analytical applications of these compounds were reported.

Of the several methods for the preparation of protocatechualdehyde listed in the literature, that proposed by Fettig and Remsen (*4*) gave the authors only a 10 per cent yield of purified product, while the procedure of Wegscheider (*10*) gave a 75 per cent yield.

The following compounds are discussed below: rhodanine (I), *o*- (II), *m*- (III), and *p*- (IV)-hydroxybenzalrhodanines, and 3,4-dihydroxybenzalrhodanine (V).

Solutions of the five reagents used in all subsequent tests were made by dissolving in 100 ml. of 95 per cent alcohol the following amounts of each compound: I 0.82, II 0.1, III 0.23, IV 0.05, and V 0.28 gram. Three drops of these reagents added to 3 ml. of water produced no cloudiness. One molar aqueous solutions of the cations were used to produce the results reported in Tables II and III. No differences were noted when the tests were conducted in the presence of Cl^-, Br^-, I^-, $C_2H_3O^-$, NO_3^-, ClO_3^-, SO_4^{--}, CNO^-, CNS^-, $S_2O_3^{--}$, $C_2O_4^{--}$, PO_4^{---}, AsO_4^{---}, CO_3^{--}, and $C_4H_4O_6^{--}$. Tests were conducted on all the cation solutions using alcoholic solutions of the four hydroxyaldehydes, but no distinctive colors or precipitates were formed.

Wherever feasible, solutions of the following cations were tested under the conditions specified in Table II: Al^{+++}, As^{+++}, Ba^{++}, Ca^{++}, Cr^{+++}, Mg^{++}, Mn^{++}, Ni^{++}, Sn^{++++}, and Zn^{++}. No precipitates were formed. Very little difference was obtained when dilute hydrochloric or acetic acid solutions of the cations were used.

The only reagent producing a distinctive deep red color in alkaline solution was 3,4-dihydroxybenzalrhodanine (Table III). One drop of the alcoholic reagent solution was added to 3 cc. of the aqueous cation solution and the mixture made basic with an excess of sodium hydroxide, ammonium hydroxide, or pyridine. In some cases an excess of ammonium salts interfered with the test.

3,4-Dihydroxybenzalrhodanine was by far the most sensitive of the reagents tested but it was also the least water-soluble. The alkaline nickel test was definite and characteristic.

Table II. Color and Sensitivity[a] of Precipitates from Neutral[b] Solutions of Cations

Cation	Rhodanine Color	Rhodanine Sensitivity	*o*-Hydroxybenzalrhodanine Color	*o*-Hydroxybenzalrhodanine Sensitivity	*m*-Hydroxybenzalrhodanine Color	*m*-Hydroxybenzalrhodanine Sensitivity	*p*-Hydroxybenzalrhodanine Color	*p*-Hydroxybenzalrhodanine Sensitivity	3,4-Dihydroxybenzalrhodanine Color	3,4-Dihydroxybenzalrhodanine Sensitivity
Cd^{++}	...	...	...	...	...	...	...	...	Red	9,000
Ag^+	Dark green	10,000	Red brown	10,000	Yellow	10,000	Brown	10,000	Greenish black	100,000
Co^{++}	...	...	...	...	...	...	...	...	Orange	17,000
Cu^{++}	Green	16,000	Red brown	160,000	Red brown	160,000	...	...	Brown	160,000
Au^{+++}	Cream	5,000	Red brown	5,000	Red brown	50,000	Red brown	5,000	Red brown	50,000
Fe^{+++}	...	...	...	...	Yellow	18,000	Yellow	18,000	Brown	180,000
Pb^{++}	...	...	...	...	Yellow	50,000	...	...	Red brown	50,000
Hg^+	Cream	5,000	Yellow	5,000	Yellow	5,000	...	...	Brown	5,000

[a] Limiting sensitivities were calculated from data obtained by adding known volumes of distilled water to a definite amount of molar solution of cations until a dilution was reached at which test was still distinctive but beyond which it became questionable. Figures express number of grams of solution in which 1 gram of cation was detectable.

[b] Salt of cation dissolved in distilled water.

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TABLE III. LAKES[a] AND COLORS OF 3,4-DIHYDROXYBENZALRHODANINE IN ALKALINE SOLUTIONS OF CATIONS

Cation	Excess NaOH	Excess NH_4OH	Excess Pyridine
Al^{+++}	.....	Red-brown lake	Brown lake
Co^{++}	Purple-red lake	.....	.....
Mg^{++}	Purple-red lake	Red lake	.....
Mn^{++}	Purple-red lake	Pink ppt.	.....
Ni^{++}	Lavender lake	Lavender lake	Deep blue soln.
Ag^{+}	.....	Violet ppt.	Violet ppt.
		Purple soln.	Blue soln.

[a] Lake formation was taken to be affixing of characteristic color to precipitate, so that a clear solution resulted upon filtering.

Summary

The analytical possibilities of *o*-, *m*-, and *p*-hydroxybenzalrhodanines and 3,4-dihydroxybenzalrhodanine were studied. More metals were precipitated by the hydroxybenzalrhodanines than by rhodanine itself. The very slight water-solubility of the compounds materially restricts their use as analytical reagents.

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Electrolytic Determination of Iron

A Modified Moore Method

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MANY of the volumetric methods for iron are highly satisfactory for routine determinations. However, they all require standard solutions, and, when iron is determined only occasionally, considerable time is often spent in standardizing the required solution. The electrolytic method presented here requires stock solutions which can be stored indefinitely, and should be particularly convenient in laboratories where electroanalysis of other metals is routine. Both accuracy and precision of 1 to 2 parts per thousand are readily attained in the presence of the usual substances in iron ores. Only the customary silica separation, followed by decomposition with hydrofluoric and sulfuric acids to recover the iron combined as silicate, is necessary; chloride does not interfere.

The method is based on the deposition of metallic iron from the complex formed by the addition of excess ammonium carbonate solution to an acid solution containing ferric iron and orthophosphate ions. The hot solution is electrolyzed at a high current density without stirring.

Moore (*3*) suggested the use of a ferric phosphate–ammonium carbonate solution for the electroanalysis of iron. Brand (*2*) used pyrophosphate instead of phosphate. He stated that the last traces of iron were difficult to deposit and that the solution dissolved the metal when current was not flowing.

Avery and Dales (*1*) criticized Moore's method. They found that 5 hours were required to deposit 200 mg. of iron. Their deposits were high in weight, although deposition was incomplete. They found a slight amount of carbonaceous material and some phosphorus in their deposits.

Experimental

SELECTION OF AMMONIUM CARBONATE STOCK SOLUTION. The addition of excess ammonium carbonate solution to an acidic ferric phosphate solution forms a soluble complex, which is much more soluble in cold than in hot, and in concentrated than in dilute ammonium carbonate. Since electrolysis of the cold solution is unsatisfactory, it is necessary to prepare a solution sufficiently concentrated to hold moderate amounts of the ferric complex in solution at 70° C. (just below the ebullition temperature). When it precipitates before or during electrolysis, many hours are usually required for resolution and complete deposition of the iron. This precipitation is possibly the cause of the criticism of Moore's method (*1*).

When the concentration of ammonium carbonate in the electrolyte is at least 3.5 *M*, the solubility of the complex is such that no precipitation of iron occurs at 70° C. from a solution containing as much as 200 mg. of iron in a volume of 200 ml. In order to prepare conveniently an electrolyte of such concentration, a 5.0 *M* stock solution is necessary.

PREPARATION OF STOCK SOLUTIONS. The 5.0 *M* ammonium carbonate stock solution, $NH_3/CO_2 = 2.05$–2.1, was prepared by dissolving 1570 grams of Eimer & Amend reagent quality lump ammonium carbonate, assay 31.8 to 32.5 per cent ammonia, in 700 ml. of clear reagent quality concentrated ammonium hydroxide, specific gravity 0.90, and sufficient water to make 4 liters. A higher concentration of free ammonium hydroxide is undesirable. With only occasional shaking, several days were required for complete solution. Heating to 65° C. and/or stirring facilitate solution but must be employed with suitable precautions because of the volatile nature of ammonium carbonate.

The 2.0 *M* ammonium dihydrogen phosphate stock solution was prepared by dissolving 230 grams of the low-arsenic salt in water and diluting to 1 liter. Other sufficiently pure alkali phosphate salts or phosphoric acid also were found to be satisfactory.

PREPARATION AND STANDARDIZATION FERRIC AMMONIUM SULFATE TEST SOLUTION. In 2 liters of water containing 278 ml. of concentrated sulfuric acid 890 grams of ferric alum were dissolved. After filtering, the solution was diluted to 10 liters and used in all the analyses reported; it was 1.0 *N* with sulfuric acid. It was standardized by the stannous chloride–potassium dichromate procedure of Willard and Furman (*4*). The reduction and titration were carried out at 25° C. The averaged result of four determinations was 0.1033 gram of iron in 10 ml.

APPARATUS. The cathode was a 10-gram cylindrical platinum wire gauze, surface area 80 sq. cm. The anode was a 1.25-mm. diameter platinum wire loop, surface area 4 sq. cm. The electrolytic stand was arranged so that the electrodes could be removed without interruption of the current. Current was taken from a lead storage bank capable of supplying 6 amperes at 10 volts. Larger electrodes and a higher current are desirable.

A 400-ml. Pyrex brand tall-form beaker was used as electrolyte container. A 1.5 × 15 cm. indented upright glass column (similar to that of the familiar Claisen distillation flask) sealed just below the rim served as a spray trap, while evolved ammonia was drawn off by gentle suction through a side tube near the top. This device was not necessary, but prevented escape of ammonia into the laboratory during electrolysis, since it was not convenient to use a hood. The beaker was covered with a suitably notched split watch glass.

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TABLE I. EXAMINATION OF ANODIC DEPOLARIZERS

Depolarizer Added	Time of Electrolysis	$(NH_4)_2S$ Test	Weight of Fe Deposit	Error in Fe
Grams	*Min.*		*Gram*	*Mg.*
5 Welch's Na_2SO_3	35	Negative	0.2104	+3.8
5 E. & A. c. p. Na_2SO_3	30	Green	0.2094	+2.8
5 ml. 30% SO_2 NH_4HSO_3	35	Negative	0.2098	+3.2
5 Merck's $Na_2SO_3.7H_2O$	35	Green	0.2073	+0.7
5 E. & A. reagent $NaHSO_3$	35	Negative	0.2115	+4.9
7 Baker's $K_2SO_3.2H_2O$	45	Pale green	0.2078	+1.2
5 Hydrazine sulfate	80	Negative	0.2074	+0.8
5 Ammonium oxalate[a]	75	Pale green	0.2063	−0.3
5 Urea + 1 gram KI	105	Negative	0.2069	+0.3
5 Sodium formate	60	Pale green	0.2066	0.0
5 Sodium hypophosphite	40	Negative	0.2123	+5.7
5 Ammonium phosphite	75	Pale green	0.2071	+0.5

[a] Did not completely dissolve during electrolysis.

General Procedure

A sample containing 100 to 200 mg. of iron was weighed and dissolved in the clean electrolytic beaker. All iron was oxidized to the ferric condition with persulfate or hydrogen peroxide, the excess being destroyed by boiling. Nitrate must be absent. The solution was cooled, and 15 ml. of stock phosphate solution were added to the cold weakly acidic solution and mixed thoroughly; the volume should now be 50 ml. or less. There must be 3 to 5 moles of phosphate for every gram-atom of metal other than the alkalies.

Then 150 ml. of stock ammonium carbonate solution were added, the first part cautiously from a buret to avoid spattering. Not more than 10 ml. should be required for neutralization. The clear tan solution was covered with the split watch glass and heated to 67–70° C. (not higher). Both electrodes were placed against the bottom of the beaker. If this is not done, the slowness of upward diffusion of metal ions into the effective electric field will greatly prolong the time required for complete deposition. The solution was electrolyzed for 90 minutes with a current of 6 or more amperes ($N.\ D._{100}$ = 5 to 10) or until deposition was complete. Incomplete deposition is indicated by a green or black color formed upon addition of clear ammonium sulfide to a small portion of the electrolyte. Electrolysis was continued 15 minutes after the first negative test, the deposit being kept covered during electrolysis by use of wash water on the cover glass and sides of the beaker.

The current was reduced to 1 ampere and the electrodes were removed without interruption of the current while being continuously washed with a heavy stream of water. The cathode was washed thoroughly with water, then with 95 per cent ethyl alcohol, dried 30 to 35 cm. above a spread burner flame, and weighed.

Results of Analyses

In all the analyses described below, unless otherwise noted, the same conditions were employed—namely, 20 ml. of standard ferric alum solution (0.2066 gram of iron), 15 ml. of stock phosphate solution, 150 ml. of stock carbonate solution, a 200-ml. volume of electrolyte, and 90 minutes of electrolysis at 67–70° C. with a current of 6.0 amperes at 5.2 volts. The temperature, current, and voltage remained nearly constant during electrolysis. The ammonium sulfide test was made on the entire electrolyte after removal of the electrodes. Under these conditions, it is sensitive to 0.1 mg. of iron.

The iron deposits obtained, unless otherwise stated, were always bright, dense, adherent, highly pure, and readily washed and dried without rusting. Hydrogen sulfide must be absent from the laboratory atmosphere to avoid contamination of the deposit with sulfur. When present, arsenic partly codeposits with iron.

EXAMINATION OF ANODIC DEPOLARIZERS. Since nitrate formed by anodic oxidation of ammonia depolarizes the cathode and retards iron deposition, various reducing agents were examined in an attempt to eliminate this difficulty. The results are shown in Table I.

Of all the depolarizers examined, only sulfite and hypophosphite were effective in reducing the time required for complete deposition, but neither was satisfactory because impure iron deposits were obtained, sulfide being present in the sulfite and arsenic in the hypophosphite. The deposits from the sulfite solutions were dark, contained sulfur, and were slightly rusty. Obviously, the depolarizers were added after the ammonium carbonate, since many of them would reduce ferric ion in acidic solution.

SEPARATION OF IRON FROM OTHER MATERIALS BY ELECTROLYSIS. Table II shows that the method is satisfactory in the presence of large amounts of ammonium chloride and ammonium sulfate. The upper limits for noninterference of aluminum, chromium, and titanium are 50 mg. of aluminum trioxide, 40 mg. of chromium, and 20 mg. of titanium dioxide.

In Table III it is shown that moderate amounts of manganese interfere. Crystals of manganese ammonium phosphate, formed during electrolysis, in part adhere to the cathode, causing high results. The time when these crystals were first detected is shown. Ten minutes elapsed between the addition of carbonate and start of electrolysis. It is obvious that it is necessary to deposit the iron completely and stop electrolysis before this crystallization starts in order to obtain accurate results for iron. The presence of other metals, such as aluminum, chromium, and titanium, apparently helps in holding manganese in solution. This effect was not studied quantitatively. An iron ore containing up to about 1 per cent of manganese may be analyzed without interference.

TABLE II. SEPARATION OF IRON FROM OTHER MATERIALS BY ELECTROLYSIS

Material Added		$(NH_4)_2S$ Test	Weight of Fe Deposit	Error in Fe
Mg.			*Gram*	*Mg.*
....	[a]	Green	0.2062[b]	−0.4
....	[a]	Negative	0.2066[c]	0.0
10,000 NH_4Cl	[a]	Negative	0.2066[c]	0.0
10,000 NH_4Cl	[a]	Negative	0.2065	−0.1
10,000 $(NH_4)_2SO_4$	[a]	Green	0.2060[c]	−0.6
10,000 $(NH_4)_2SO_4$	[a]	Negative	0.2065	−0.1
70 Al_2O_3	[d]	Negative	0.2059	−0.7
50 Al_2O_3	[a]	Negative	0.2066	0.0
50 Al_2O_3		Negative	0.2065	−0.1
50 Cr	[d]	....	0.2058	−0.8
40 Cr		....	0.2066	0.0
40 Cr		....	0.2067	+0.1
20 TiO_2	[e]	Negative	0.2069	+0.3
10 TiO_2		Negative	0.2066	0.0
10 TiO_2		Negative	0.2068	+0.2

[a] 10 ml. of stock phosphate used.
[b] Electrolyzed 60 minutes.
[c] Electrolyzed 75 minutes.
[d] Added as potassium alum solution.
[e] Added as 5% sulfuric acid solution.

TABLE III. SEPARATION OF IRON FROM MANGANESE BY ELECTROLYSIS

Mn Added (as $MnSO_4$)	Fe Added	$MnNH_4PO_4$, Appeared after:	Time of Electrolysis	$(NH_4)_2S$ Test	Weight of Fe Deposit	Error in Fe
Mg.	*Gram*	*Min.*	*Min.*		*Gram*	*Mg.*
10	0.2066	50	90	Neg.	0.2104	+3.8
5	0.2066	75	90	Neg.	0.2078	+1.2
5	0.1033	60	60	Neg.	0.1034	+0.1
5	0.1033	60	60	Neg.	0.1033	0.0
4	0.2066	75	90	Neg.	0.2069	+0.3
4	0.2066	75	75	Neg.	0.2065	−0.1

It is probable that magnesium would not interfere to the same extent as manganese, since magnesium ammonium phosphate is appreciably soluble in hot ammoniacal solution.

If it is desired to determine other metals remaining in the electrolyte after separation of the iron, the ammonium carbonate may be boiled off and the precipitated metallic phosphates dissolved by cautious addition of mineral acid. Any silica is removed by filtration before proceeding.

Codeposition of Iron and Other Metals

In Table IV data are given for the codeposition of iron with nickel, cobalt, molybdenum, and tungsten. With nickel or cobalt, the iron alloy deposited more rapidly than nickel or

TABLE IV. CODEPOSITION OF IRON AND OTHER METALS

Weight of Second Metal Added[a]	Weight of Fe Added	Dimethylglyoxime Test	$(NH_4)_2S$ Test	Weight of Deposit	Total Error
Gram	*Gram*			*Gram*	*Mg.*
0.0126 Ni	0.1033	Positive	Negative	0.1153	− 0.6
0.0084 Ni	0.1033	Negative	Negative	0.1116	− 0.1
0.0084 Ni	0.1033	Negative	Negative	0.1114	− 0.3
0.0042 Ni	0.1033	Negative	Negative	0.1077	+ 0.2
0.1091 Co	..	..	Negative	0.1037	− 5.4
0.1091 Co	0.1033	..	Negative	0.2114	− 1.0
0.1091 Co	0.2066	..	Negative	0.3154	− 0.3
0.1091 Co	0.2066	..	Negative	0.3159	+ 0.2
0.0218 Mo	0.1033	[b,c]	Black	0.0950	−30.1
0.0109 Mo	0.2066	[c]	Negative	0.2181	+ 0.6
0.0109 Mo	0.1033	[c,d]	Negative	0.1144	+ 0.2
0.0075 W	0.1033	[c,e]	Negative	0.1072	− 3.6
0.0075 W	0.1033	[c,e]	Negative	0.1068	− 4.0

[a] Second metals added as $NiSO_4$, $CoSO_4$, Na_2MoO_4, and Na_2WO_4 standard solutions to ferric alum before addition of phosphate.
Electrolyzed:
[b] 140 [d] 75, and [e] 60 min., respectively.
[c] Used 10 ml. of stock phosphate.

cobalt alone. Ammonium sulfide is still only a test for iron; it does not precipitate small amounts of the other metals. The quantitatively codeposited alloys may be dissolved in acid and one metal determined by another procedure; the second may then be calculated by difference.

Up to 10 mg. of molybdenum are quantitatively codeposited with 100 mg. or more of iron. Amounts of molybdenum above about 20 mg. depolarize the cathode and prevent complete deposition of iron. The range between 10 and 20 mg. of molybdenum in 200 ml. of electrolyte was not examined. In this range, the transition from the platable to the depolarizing concentration occurs. It might be profitable to examine the possibility of plating molybdenum alone from a suitable electrolyte containing a very low concentration of molybdate.

Summary

Iron can be quantitatively determined as metal by electrodeposition from a hot complex ferric phosphate–ammonium carbonate solution at a high current density without stirring. The method is accurate and reasonably fast. The highly sensitive ammonium sulfide test is available to prove completeness of deposition. The method may be applied to low manganese iron ores without removal of hydrochloric acid.

Small amounts of nickel or molybdenum are quantitatively codeposited with large amounts of iron, and cobalt with at least twice as much iron. Tungsten must be absent.

Large amounts of chloride, sulfate, and phosphate do not interfere. Nitrate and all strong oxidizing agents must be absent.

Acknowledgment

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FROM a thesis presented by William H. Armistead, Jr., to the Graduate School of Vanderbilt University in partial fulfillment of the requirements for the degree of doctor of philosophy.

Determination of Ash in Coals Unusually High in Calcite and Pyrite

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IN DETERMINING ash in coals containing unusually large amounts of calcite and pyrite, difficulty in obtaining satisfactory results by the standard A. S. T. M. procedure (*3*) may be experienced, because of the varying amounts of sulfur that are retained as calcium sulfate in the ash. Variations in the heating procedure used in the ash determination influence the amount of sulfur retained. Lower ash and lower sulfur in the ash are obtained by slow rates of heating.

This paper is a report of cooperative work done by the Analytical Laboratory of the Illinois State Geological Survey and the Pittsburgh Laboratory of the United States Bureau of Mines for the purpose of studying modified procedures for the determination of ash in such troublesome coals.

Experimental Tests

For this work five sizes of coal prepared by screening from a large sample of 1¼ inch × 0 screenings from the No. 2 bed, Woodford County, Ill., were used. The larger sizes were crushed to pass a No. 4 sieve and after mixing and riffling all sizes, two 1-quart samples of each were cut out. One set of samples was sent to the U. S. Bureau of Mines Laboratory and one set was retained in the Illinois State Geological Survey Laboratory.

The samples are designated as follows:

Sample	Size	Sample	Size
1	1¼ × ¾ inch	4	10 × 48-mesh
2	¾ × ⅜ inch	5	−48-mesh
3	⅜ inch × 10-mesh		

Each laboratory prepared its own samples for analysis by the usual A. S. T. M. procedure (*1*). Analyses in the two laboratories indicated that the two samples of each size were satisfactory duplicates, with the exception of sample 3. Because the two 1-quart portions differed in ash content by too large an amount, the ash values reported by the Geological Survey laboratory were obtained on the 60-mesh coal prepared in the Pittsburgh laboratory.

The coals were analyzed for total sulfur (Eschka method), and for forms of sulfur and carbon dioxide by methods of the U. S. Bureau of Mines (*7*). Moisture at 105° C. was determined by each laboratory on the samples according to the A. S. T. M. standard method (*2*).

Bureau of Mines Determinations

METHOD A. The dried samples were heated on the hearth of a hot muffle furnace for 8 minutes to drive off volatile matter, then heated at 725° C. to constant weight (within 0.001 gram).

METHOD B. The dried samples were placed in a cold furnace and heated to 725° C. in 1.5 hours. The temperature was kept at 725° C. to constant weight.

METHOD C (Parr's sulfated ash method, *5*, *6*). The dried samples were burned by Method A and after cooling were moistened with a few drops of 1 to 1 sulfuric acid and dried on an air bath until the fumes were largely driven off. The samples were then heated at 725° C. to constant weight. This treatment produces a sulfated ash in which it is assumed that all calcium is present as the sulfate. To correct to a calcium oxide basis the percentage of sulfur trioxide, coal basis, determined in the sul-

fated ash is subtracted from the sulfated ash or 1.82 times the mineral carbon dioxide, coal basis, is subtracted from the sulfated ash. In the latter method of correction it is assumed that the mineral carbon dioxide is a measure of the calcium carbonate present in the original mineral matter of the coal.

TABLE I. SULFUR FORMS AND CARBON DIOXIDE

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
	%	%	%	%	%
Sulfate sulfur					
U. S. B. M.	0.06	0.08	0.07	0.11	0.12
I. G. S.	0.01	0.02		0.04	0.07
Pyritic sulfur					
U. S. B. M.	0.67	1.03	1.30	2.28	3.14
I. G. S.	1.02	1.10		2.56	3.50
Organic sulfur					
U. S. B. M.	0.72	0.69	0.58	0.55	0.52
I. G. S.	0.53	0.49		0.40	0.19
Total sulfur					
U. S. B. M.	1.45	1.80	1.95	2.94	3.78
I. G. S.	1.56	1.61		3.00	3.76
Carbon dioxide					
U. S. B. M.	0.29	0.53	1.59	4.24	3.63
I. G. S.	0.47	0.64		4.10	3.64

TABLE II. DETERMINATION OF ASH

(Per cent of dry coal)

Sample	Ash	SO_3 in Ash	Sulfide in Ash	SO_3- and S-Free Ash	Ash	SO_3 in Ash	Sulfide in Ash	SO_3- and S-Free Ash
	Data by Method A				Data by Method E			
1	9.28	0.46	..	8.82	9.40	0.29	0.04	9.09
	9.27	0.40	0.00	8.87	9.50	0.30	0.02	9.19
Av.	9.28			8.85	9.45			9.14
2	14.37	0.62	..	13.72	13.80	0.22	0.02	13.57
	14.20	0.57	0.03	13.60	13.66	0.31	0.03	13.34
Av.	14.29			13.66	13.73			13.46
3	18.59	1.52	..	17.06	18.06	..	..	..
	18.70	1.56	0.01	17.13	17.87	..	..	..
Av.	18.65			17.10	17.97			
4	31.27	3.64	..	27.61	29.26	1.73	0.02	27.52
	31.15	3.49	0.02	27.64	28.92	1.28	0.02	27.63
Av.	31.21			27.63	29.09			27.58
5	34.93	3.14	..	31.73	34.83	1.86	0.32	32.81
	35.12	3.03	0.06	32.03	34.65	1.59	0.27	32.92
Av.	35.03			31.88	34.74			32.87
	Data by Method B				Data by Method F			
				SO_3-Free Ash				SO_3-Free Ash
1	9.13	0.29		8.84	9.32	0.23		9.09
	9.09	0.26		8.83	9.33	0.24		9.09
Av.	9.11			8.84	9.33			9.09
2	14.09	0.40		13.69	13.77	0.23		13.54
	14.06	0.34		13.72	..	..		..
Av.	14.08			13.71	13.77			13.54
3	17.87	0.85		17.02	17.53	..		..
	17.80	0.68		17.12	17.85	..		..
Av.	17.84			17.07	17.69			
4	29.04	1.39		27.65	28.81	0.70		28.11
	28.91	1.07		27.84	28.14	0.68		27.46
Av.	28.98			27.75	28.48			27.79
5	33.19	1.01		32.18	34.73	1.45		33.28
	33.01	0.83		32.18	34.03	1.24		32.79
Av.	33.10			32.18	34.38			33.04
	Data by Method D							
1	9.12	0.29		8.83				
	9.10	0.24		8.86				
Av.	9.11			8.85				
2	14.03	0.38		13.65				
	14.05	0.34		13.71				
Av.	14.04			13.68				
3	17.58	0.53		17.05				
	17.71	0.61		17.10				
Av.	17.65			17.08				
4	28.53	0.86		27.67				
	28.49	0.76		27.73				
Av.	28.51			27.70				
5	32.95	1.02		31.93				
	33.16	0.95		32.21				
Av.	33.06			32.07				

METHOD D. The dried samples were placed in a cold furnace and heated to 400° C. in 0.5 hour. They were held at this temperature for a further 0.5 hour, then transferred to another furnace at 725° C. and heated to constant weight.

One ash sample from each test was analyzed for total sulfur by the sodium carbonate fusion method (*7*). The duplicate ash was tested for sulfide sulfur by the evolution method used for coke (*8*). Sulfate sulfur was determined in the hydrochloric acid solution from the sulfide tests. Ammonium hydroxide was added in slight excess and the precipitated iron together with any insoluble ash was removed by filtration. The filtrate was made slightly acid and the sulfur precipitated in the regular manner.

Geological Survey Determinations

METHOD E. The dried samples were heated on the hearth of a hot muffle furnace for 10 minutes to drive off volatile matter, moved just inside the furnace for 5 minutes, and then moved back to the hot portion of the furnace where they were heated to constant weight at 750° C.

METHOD F. The dried samples were placed in a cold muffle furnace and heated to 750° C. in 1.75 hours. They were heated to constant weight at this temperature.

METHOD G (Parr's sulfated ash method). The dried samples were burned by Method F and after cooling were treated with 1 to 1 sulfuric acid, the excess acid was fumed off on a hot plate, and the samples were heated to constant weight at 750° C.

The ashes obtained by all three methods were analyzed for sulfur trioxide by extraction with dilute hydrochloric acid, precipitation and removal of R_2O_3 with ammonium hydroxide, with subsequent precipitation of sulfate with barium chloride in acid solution. Sulfide sulfur determinations were made on the ashes obtained by Methods E and F by the procedure mentioned above (*8*).

The effect of using furnaces of different sizes, in which the rate of change of atmosphere varied, for duplicate determinations was studied in the Geological Survey laboratory. One duplicate was ashed in the larger Hoskins F. D. 204 furnace whose heating chamber is $7^1/_2$ inches wide, $5^1/_4$ inches high, and 14 inches long while the other was ashed in the smaller Hoskins F. D. 202 furnace whose heating chamber is $4^1/_4$ inches wide, 3 inches high, and 10 inches long. Both furnaces were equipped with thermocouples placed loosely through the back, so that air flow through the furnace would take place.

All tests in both laboratories were made in electrically heated muffle furnaces.

Results of Tests

Results of analyses of the coals for forms of sulfur and mineral carbon dioxide are given in Table I. Ash values as determined by the various methods, together with amounts of sulfur as sulfur trioxide retained in the ashes, are listed in Tables II and III. Sulfide sulfur was found only in ashes obtained by Methods A and E. Table IV presents additional data showing the effect of a slower rate of heating and ashing in furnaces of different sizes in which air circulation was different. Table V presents a comparison of average results obtained by different methods.

Discussion

The tolerances for permissible differences between ash determinations in the same laboratory and between different laboratories by the A. S. T. M. standard method (*4*) are ±0.3

TABLE III. DETERMINATION OF ASH

(Per cent of dry coal)

Sample	Sulfated Ash	SO_3 in Sulfated Ash	SO_3-Free Ash	Sulfated Ash Less 1.82 × Mineral CO_2	Sulfated Ash	SO_3 in Sulfated Ash	SO_3-Free Ash	Sulfated Ash Less 1.82 × Mineral CO_2
	Results by Method C				Results by Method G			
1	9.81	1.05	8.76	9.28	10.15	0.76	9.39	9.29
	9.82	1.00	8.82	9.29	10.23	0.84	9.39	9.37
Av.	9.82		8.79	9.29	10.19		9.39	9.33
2	15.15	1.54	13.61	14.22	14.86	1.21	13.65	13.70
	15.18	1.50	13.68	14.25	14.99	1.25	13.74	13.83
Av.	15.17		13.65	14.24	14.93		13.70	13.77
3	20.43	3.43	17.00	17.56	...	...	...	...
	20.49	3.49	17.00	17.62	...	...	...	...
Av.	20.46		17.00	17.59	...	...	...	...
4	35.62	8.09	27.53	27.88	35.95	7.85	28.10	28.49
	35.68	8.15	27.53	27.94	35.71	7.77	27.94	28.25
Av.	35.65		27.53	27.91	35.83		28.02	28.37
5	39.38	7.42	31.96	32.78	40.68	7.16	33.52	34.06
	39.59	7.50	32.09	32.99	40.67	7.21	33.46	34.05
Av.	39.49		32.03	32.89	40.68		33.49	34.06

TABLE IV. COMPARISON OF ASH VALUES

Obtained by varying time necessary for furnace to reach 750° C., by ashing in different sized furnaces, and by applying the modified Parr method where first burning off took place in different sized furnaces. Data by Illinois Geological Survey.

Sample	Per Cent of Ash Starting with Cold Furnace, Heating to 750° C. 1.5 hours	2.25 hours	Per Cent Ash, Starting with Cold Furnace, Heating to 750° C. in 1.75 Hours Large furnace, Hoskins F. D. 204	Small furnace, Hoskins F. D. 202	Per Cent Ash, Sulfated Modified Parr Method Large furnace, Hoskins F. D. 204	Small furnace, Hoskins F. D. 202	Per Cent Ash, Modified Parr, Sulfated Ash Less 1.82 × Mineral CO_2
1	9.14	9.08	9.00	9.24	9.59	9.53	9.03
2	14.08	14.05	14.02	...	14.76	14.89	13.87
3	17.89	17.60	17.46	17.73	20.08	20.15	17.23
4	29.07	28.52	28.66	29.91	35.46	35.70	27.87
5	33.28	32.98	32.76	33.16	39.16	39.39	32.68

TABLE V. COMPARISON OF AVERAGE ASH VALUES OBTAINED BY METHODS A, B, D, E, AND F WITH METHODS C AND G

(Per cent of dry coal)

Sample	Data by U. S. Bureau of Mines: Method A, hot muffle	Method B, cold muffle	Method D, two muffles	Method C, Parr's method[a]	Difference from Method C: Method A	Method B	Method D
1	9.28	9.11	9.11	9.29	−0.01	−0.18	−0.18
2	14.29	14.08	14.04	14.24	+0.05	−0.16	−0.20
3	18.65	17.84	17.65	17.59	+1.06	+0.25	+0.06
4	31.21	28.98	28.51	27.91	+3.30	+1.07	+0.60
5	35.03	33.10	33.06	32.89	+2.14	+0.21	+0.17

Sample	Data by Illinois Geological Survey: Method E, hot muffle	Method F, cold muffle	Method G, Parr's method[a]	Difference from Method G: Method E	Method F
1	9.45	9.33	9.33	+0.12	0.00
2	13.73	13.77	13.77	−0.04	0.00
3	17.97	17.69	17.23	+0.74	+0.46
4	29.09	28.48	28.37	+0.72	+0.11
5	34.74	34.38	34.06	+0.68	+0.32

[a] Parr's sulfated ash less 1.82 × mineral CO_2.

and 0.5 per cent, respectively, on coals containing carbonates. For coals with more than 12 per cent ash containing carbonate and pyrite these are 0.5 and 1.0 per cent, respectively.

For the most part results obtained by the various methods checked within these tolerances, both in the same laboratory and between the two laboratories (Tables II and III), particularly the results corrected for retained sulfur. In a few cases the results did not check within these tolerances but, with one exception, these results were for the last two samples (samples 4 and 5) which are high in mineral matter containing large amounts of mineral carbon dioxide and pyritic sulfur. The ash of these samples is considerably higher than would normally be encountered in commercial samples.

The ash values obtained by Methods A and E, uncorrected for retained sulfur, are higher than those obtained by other methods with the exception of the Parr sulfated ashes. Methods A and E correspond to a more rapid rate of heating and the ashes contain considerably greater amounts of retained sulfur than those obtained by Methods B, F, and D. Results obtained by Method D probably show the best agreement between duplicate determinations, with comparatively lower amounts of sulfur being retained in the ashes. Results obtained by the Parr sulfated ash method check reasonably well for the most part, but this procedure is rather long and requires mineral carbon dioxide values for use in correcting the sulfated ash obtained.

In Table IV are presented further data on the effect of different rates of heating, not corrected for sulfur retained in the ash. Lower results are obtained with the slower rate of heating. In addition, information is presented on the use of different sized furnaces for ashing. The higher results obtained in the smaller F. D. 202 furnace indicate that sulfur trioxide as formed was not removed as rapidly as in the larger furnace and was therefore fixed in the ash to a larger extent. The adequate removal of sulfur trioxide therefore becomes important. Use of the Parr sulfated ash method appears to smooth out these differences to some extent.

Table V presents a comparison of average ash values obtained by the procedures tried. For those samples containing larger amounts of ash and mineral carbon dioxide the slower rates of heating give definitely better results when compared to those obtained by the Parr sulfated ash method. However, differences appear which are outside A. S. T. M. tolerances and in such cases the ash values should be corrected for retained sulfur or determined by the modified Parr method. It is not likely that such samples would be encountered in commercial samples but they might be encountered in certain special studies.

Summary

The preliminary hearth heating method (A and E) gave results within the A. S. T. M. tolerances for all duplicates obtained in the same laboratory. Checks between different laboratories within A. S. T. M. tolerances were obtained for coals containing up to about 3.6 per cent mineral carbon dioxide, but these ashes contained larger amounts of retained sulfur.

The determination of ash by the cold furnace method (B and F) gave duplicate results within A. S. T. M. tolerances for all samples in the U. S. Bureau of Mines laboratory and for samples up to about 1.6 per cent mineral carbon dioxide content in the Illinois Geological Survey laboratory. Checks between average values from the two laboratories were within A. S. T. M. tolerances for all samples.

Results obtained by the modified Parr method were all well within A. S. T. M. tolerances for duplicates in the same laboratory. With the exception of results on one sample (5), the checks between the two laboratories were within A. S. T. M. tolerances.

The determination of ash by Method D gives duplicates checking within the 0.3 per cent tolerance for all samples.

The adequate removal of sulfur trioxide from the furnace during ashing is important.

As compared to results obtained by the modified Parr procedure, Methods A and E gave results within the A. S. T. M. 0.3 per cent tolerance for samples containing up to 0.6 per cent of carbon dioxide; Method B for coals up to 3.6 per cent of carbon dioxide; Method F for coals up to 4.2 per cent of carbon dioxide (with one exception); and Method D for coals containing up to 3.6 per cent of carbon dioxide.

Sulfur trioxide–free ash values were similar for all methods.

The slow heating method starting with a cold furnace appears to be satisfactory for determining ash in commercial samples of coal containing unusually large amounts of calcite and pyrite.

Conclusions

Methods A and E are not recommended for determining ash in coals high in calcite and pyrite, because too much sulfur is retained in the ashes.

Methods B, D, and F give most consistent results, with Method D apparently giving the best results of the three.

Methods C and G appear to give good results, especially for coals high in calcite and pyrite where other procedures studied are not so satisfactory. This procedure requires more work than other procedures.

Adequate removal of sulfur trioxide from the furnace in which ashing takes place is necessary.

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Measurement of Color and Turbidity in Solutions of White Granulated Sugars

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A practical method for the measurement of color and turbidity in solutions of granulated sugars is presented, a modification of the method of Keane and Brice. The two assumptions on which their method is based are shown to be not entirely justified. Color measurements with the new method are free from the influence of turbidity, and vice versa.

A STRONG need for an adequate method for the measurement of color and turbidity in white granulated sugar solutions has been felt for some time. The best practical method has been that of Keane and Brice (*2*), which as presented, however, has suffered from certain errors arising from the basic assumptions made.

Mathematical Relationships

The transmittancy of a turbidity-free sugar solution will be represented by T_c and that of a colorless sugar solution by T_t. For a solution containing both color and turbidity, the transmittancy, T, is given by the fundamental expression

$$T = T_c \times T_t \tag{1}$$

If the transmittancy measurements are made with light passed by different filters, say a blue-green and a red filter, then

$$T_g = T_{gc} \times T_{gt} \tag{2}$$

and

$$T_r = T_{rc} \times T_{rt} \tag{3}$$

The notation is obvious.

Keane and Brice assume that T_{rc} is constant and equal to 1.00 for solutions of white sugars, stating that there is virtually no light absorption in the red part of the spectrum by the small amount of coloring matter present. From this assumption and Equation 3 they obtain

$$T_r = T_{rt} \tag{4}$$

and set the turbidity index equal to the per cent absorbency of red light:

$$I_t = 100(1 - T_{rt}) = 100(1 - T_r) \tag{5}$$

They also set the ratio T_{gt}/T_{rt} equal to 1.00, although they state that it is only an approximation. Then from Equations 2 and 3, they obtain

$$T_g/T_r = T_{gc}/T_{rc} \times T_{gt}/T_{rt} = T_{gc}/1 \times 1 = T_{gc} \tag{6}$$

The color index is thus taken as the per cent absorbency of the blue-green light in a turbidity-free solution and by the assumptions above is expressed as

$$I_c = 100(1 - T_{gc}) = 100(1 - T_g/T_r) \tag{7}$$

Nees (*3*) was not able to substantiate the assumption of Keane and Brice that both T_{rc} and T_{gt}/T_{rt} equal 1.00. Nees suggested that experimentally determined factors be applied to correct the difficulty and proposed expressing color and turbidity in terms of percentage absorption of blue light. Nees' method is not satisfactory, in that the use of an additive

TABLE I. TRANSMITTANCIES OF FILTERED SOLUTIONS

Sugar Sample	Run	T_{rc}	T_{gc}
1	1	0.954	0.786
	2	0.949	0.786
	3	0.950	0.783
2	1	0.890	0.769
	2	0.888	0.764
3	1	0.875	0.669
	2	0.875	0.676
4	1	0.852	0.625
	2	0.855	0.637
5	1	0.884	0.731
	2	0.877	0.723
	3	0.886	0.723

relationship of the absorbencies due to color and turbidity cannot be employed if a long cell is used. If A is the absorbency, from Equation 1

$$1 - A = (1 - A_c)(1 - A_t) \tag{8}$$

whence

$$A = A_c + A_t - A_cA_t \tag{9}$$

If the absorbencies are low—as was the case with the short cell used by Nees—the term A_cA_t can be neglected. However, if a long cell is used and the absorbencies vary from roughly 0.15 to 0.50, the term A_cA_t cannot be neglected. The absorbencies obtained with a cell of the length used by Nees are not large enough to permit adequate photometric accuracy to be obtained (*5*).

It would seem that the best solution to the problem is to determine experimentally the relationships between T_{rc} and T_{gc} and between T_{rt} and T_{gt}. By means of these relationships and Equations 2 and 3, the color and turbidity indices can be calculated and expressed as

$$I_c = 100(1 - T_{gc}) \tag{10}$$

$$I_t = 100(1 - T_{rt}) \tag{11}$$

Thus the indices are expressed by equations of the same form as employed by Keane and Brice.

Description of Apparatus

A Lumetron photoelectric colorimeter, Model 402E, manufactured by the Photovolt Corporation, New York, N. Y., was used for the transmittancy measurements. The instrument was altered to take a 25-cm. cell and the light source was replaced by a 6- to 8-volt, single-filament automobile headlamp which was lighted by storage batteries.

The two filters used were the same as employed by Keane and Brice (Corning light shade blue-green, No. 428, 3.4 mm. thick, and Corning traffic red, No. 245, 3.05 mm. thick. The No. 428 filter used in this work was from melt 194). Data supplied by the manufacturer indicate that the transmission curves for the No. 428 filter are practically identical for different melts of glass. The No. 245 filter covers a narrower spectral range and should be readily reproducible. If necessary, slight adjustments in filter thickness can be made to correct for any difference in the melts.

The color temperature of the light source was arbitrarily set at 2485° Kelvin. It was measured with an Eastman color temperature meter. For convenience in routine work, the lamp temperature adjustment is made by noting the galvanometer deflection with the No. 428 filter in position. This was checked from time to time against the Eastman meter. Experiments showed that the measured transmittancies were not dependent to any marked extent on the color temperature of the lamp. A change of 160° in the temperature did not change the measured transmittancy with the red filter a measurable amount and changed the blue filter reading 1.9 per cent. The color temperature can probably be adjusted to within 15°, which is equivalent to a transmittancy variation of 0.2 per cent.

Experimental Procedure

The primary transmission standard used in this work was a colorless, turbidity-free 50 refractometer dry substance (R. D. S.) sugar solution. It was prepared from confectioners' sanding sugar by adding Darco decolorizing carbon to the hot solution, allowing it to cool, filtering on a Büchner funnel through No. 40 Whatman paper, and filtering through asbestos according to the recommendations of Peters and Phelps (*4*). In this work "turbidity-free" is applied to any solution which was filtered through the specially prepared asbestos. There has been much discussion in the literature regarding color adsorption of asbestos and other filtering media (*1*, *4*, *6*). However, experiments made by the authors indicate that any adsorption of coloring matter from solutions of granulated sugar by asbestos is slight and can be neglected without introducing serious error.

For convenience, a secondary transmission standard was prepared and standardized against the primary standard. Two 5-cm. squares of thin glass from photographic plates were bound together with the inner surfaces separated by a border mask of thin cardboard. The transmittancies of this absorber for red light and blue-green light were determined, taking the transmittancy of the standard sugar solution in the 25-cm. absorption cell as 1.000 in each case. The secondary standard was checked in this manner several times during the course of the work. In routine runs, the circuit of the Lumetron was readily balanced by using the secondary standard without the necessity of having standard sugar solutions always on hand.

Solutions of granulated sugar (50 R. D. S.) were prepared by mixing equal weights of sugar and boiling double-distilled water. After cooling to room temperature, the transmittancies of the solutions were measured in the 25-cm. cell using first the blue-green and then the red filter. The solutions were next filtered through asbestos and the transmittancies again determined.

Duplicate and triplicate runs made with several sugar samples show that the transmittancies of the asbestos-filtered solutions are reproducible (Table I).

A considerable number of colorless but turbid 50 R. D. S. sugar solutions were also run in the colorimeter. The turbidizing agent was prepared by adding a little fuller's earth to a colorless sugar solution, stirring well, and allowing to settle overnight. The supernatant liquid was decanted and used to turbidize other colorless sugar solutions. A few measurements were carried out on solutions made turbid with finely divided amorphous sulfur.

Transmittancy Measurements

Table II contains the results of the transmittancy measurements made before and after filtration. A large number of

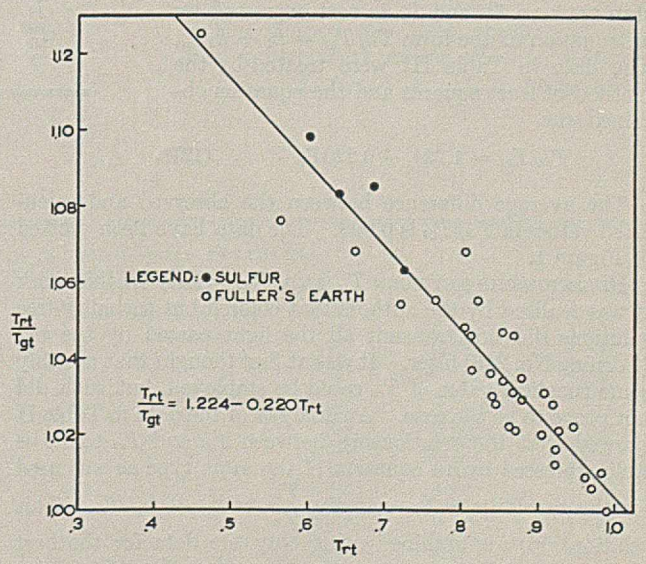

FIGURE 1. TRANSMITTANCY RELATIONSHIP FOR TURBID BUT COLORLESS SUGAR SOLUTIONS

samples of beet sugar from three beet factories and a lesser number of cane samples from a cane sugar refinery were used in the work. All the beet sugar samples were of white granulated sugar. The cane sugar samples were of varieties designated in Table II. In a number of cases transmittancy measurements were not made before filtration of the solutions through asbestos.

The ratio T_{rt}/T_{gt} was calculated from the relationship

$$T_{rt}/T_{gt} = \frac{T_r}{T_g} \times \frac{T_{gc}}{T_{rc}}$$

The results of the measurements on the turbid but colorless sugar solutions are given in Table III.

RELATIONSHIPS BETWEEN T_{rt} AND T_{gt}. The data in Tables II and III show plainly that T_{rt}/T_{gt} cannot be set equal to 1.00 if accuracy is desired. In cases of high turbidity the ratio departs markedly from 1.00, as is shown with samples 18, 20, and 32 from Factory 1, where the ratio is about 1.2. It is therefore necessary to establish from the experimental data the relationship between T_{rt} and T_{gt}. This relationship can be obtained from the data in Table II, but it is more advantageous to use the data in Table III which were obtained directly with artificial turbidity in colorless sugar solutions. In the first place, the use of artificial turbidity allows one to cover a greater range of turbidity and with greater uniformity. Secondly, it permits the use of a more nearly true turbidity rather than having to depend upon chance in using natural sugar turbidity. Often the low transmittancies observed with sugar solutions are due to fibers, large dust particles, and the like, especially if the samples have been stored in cloth bags. Even what might be called true turbidity is not constant in nature or particle size, but depends on its source and other factors.

It was found empirically that the relationship between T_{rt} and T_{gt} is best expressed by an equation of the form $T_{rt}/T_{gt} = k_1 + k_2 T_{rt}$. The data in Table III were treated by the method of least squares and the equation obtained was

$$T_{rt}/T_{gt} = 1.224 - 0.220 T_{rt} \qquad (12)$$

The average difference between the observed and calculated values of T_{rt}/T_{gt} is 0.008. The data have been plotted in Figure 1.

RELATIONSHIP BETWEEN T_{rc} AND T_{gc}. Early in this work it was realized by the authors that colored but turbidity-free solutions did not transmit all the light passed by the red (Corning No. 245) filter. It was at first thought that possibly some constant value of T_{rc} could be employed, but such did not prove to be the case. An analysis of the data in Table II showed that the relationship between T_{gc} and T_{rc} could be well expressed by an equation of the same type as was used for the turbidity ratio—namely, $\frac{T_{gc}}{T_{rc}} = k_3 + k_4 T_{gc}$. This relationship was obtained using only the data for the beet sugar samples, inasmuch as it was found that no significant difference was obtained between the equations for the individual beet plants, but that they did differ considerably from the equation obtained for the cane sugar samples. Thus it seems advisable to use one set of coefficients for beet sugar and one set for cane sugar. Most of the samples investigated in this work were beet sugar samples (107 beet, 16 cane) and the equation presented applies to beet sugar. Many more cane samples should be run to obtain a truly representative equation for cane sugar. The final equation obtained for beet sugar was

$$T_{gc}/T_{rc} = 0.310 + 0.673 T_{gc} \qquad (13)$$

and the average difference between the observed and calculated values of the ratio is 0.007.

TABLE II. TRANSMITTANCIES

Sugar Sample	Transmittancies Before Filtration T_r	Transmittancies Before Filtration T_g	Transmittancies After Filtration T_{rc}	Transmittancies After Filtration T_{gc}	T_g/T_r	T_{gc}/T_{rc}	$\frac{T_{gc}}{T_{rc}} - \frac{T_g}{T_r}$	T_{rt}/T_{gt}
Beet Sugar Factory A								
1	....	....	0.888	0.688	....	0.775	....	...
2	....	....	0.889	0.703	....	0.791	....	...
3	....	....	0.893	0.682	....	0.764	....	...
4	....	....	0.916	0.720	....	0.786	....	...
5	....	....	0.924	0.775	....	0.839	....	...
6	....	....	0.937	0.775	....	0.827	....	...
7	....	....	0.906	0.704	....	0.777	....	...
8	....	....	0.880	0.661	....	0.751	....	...
9	....	....	0.926	0.771	....	0.833	....	...
10	0.807	0.632	0.925	0.759	0.783	0.821	0.038	1.05
11	0.680	0.465	0.901	0.670	0.684	0.744	0.060	1.09
12	0.807	0.637	0.931	0.752	0.789	0.808	0.019	1.02
13	0.672	0.486	0.911	0.730	0.723	0.801	0.078	1.11
14	0.722	0.527	0.897	0.693	0.730	0.773	0.043	1.06
15	0.630	0.437	0.874	0.669	0.694	0.765	0.071	1.10
16	0.726	0.561	0.912	0.735	0.773	0.806	0.033	1.04
17	0.811	0.600	0.909	0.697	0.740	0.767	0.027	1.04
18	0.601	0.383	0.876	0.678	0.637	0.774	0.137	1.22
19	0.687	0.517	0.854	0.612	0.753	0.717	−0.036	0.95
20	0.461	0.284	0.852	0.625	0.616	0.734	0.118	1.19
21	0.803	0.603	0.896	0.711	0.751	0.794	0.043	1.06
22	0.776	0.599	0.909	0.719	0.772	0.791	0.019	1.03
23	0.618	0.427	0.876	0.684	0.691	0.781	0.090	1.13
24	0.742	0.552	0.897	0.719	0.744	0.802	0.058	1.08
25	0.766	0.572	0.917	0.721	0.747	0.786	0.039	1.05
26	0.821	0.654	0.909	0.733	0.797	0.806	0.009	1.01
27	0.680	0.482	0.888	0.689	0.709	0.776	0.067	1.09
28	0.671	0.466	0.855	0.649	0.694	0.759	0.065	1.09
29	0.857	0.669	0.928	0.748	0.781	0.806	0.025	1.03
30	0.724	0.532	0.897	0.698	0.735	0.778	0.043	1.06
31	0.482	0.292	0.852	0.651	0.606	0.764	0.158	1.26
32	0.803	0.602	0.911	0.718	0.750	0.788	0.038	1.05
33	0.798	0.605	0.912	0.730	0.758	0.800	0.042	1.06
34	0.563	0.419	0.900	0.701	0.744	0.779	0.035	1.05
35	0.541	0.406	0.889	0.702	0.750	0.790	0.040	1.05
Beet Sugar Factory B								
1	0.411	0.285	0.942	0.753	0.693	0.799	0.106	1.15
2	....	....	0.940	0.756	....	0.804	....	...
3	....	....	0.934	0.759	....	0.813	....	...
4	....	....	0.910	0.765	....	0.841	....	...
5	....	....	0.893	0.749	....	0.839	....	...
6	....	....	0.975	0.868	....	0.890	....	...
7	....	....	0.943	0.847	....	0.898	....	...
8	....	....	0.921	0.813	....	0.883	....	...
9	....	....	0.888	0.771	....	0.864	....	...
10	....	....	0.890	0.769	....	0.864	....	...
11	....	....	0.920	0.755	....	0.821	....	...
12	....	....	0.951	0.837	....	0.880	....	...
13	....	....	0.904	0.742	....	0.821	....	...
14	....	....	0.914	0.758	....	0.829	....	...
15	....	....	0.945	0.809	....	0.856	....	...
16	....	....	0.919	0.741	....	0.806	....	...
17	....	....	0.938	0.808	....	0.861	....	...
18	....	....	0.936	0.795	....	0.849	....	...
19	....	....	0.940	0.782	....	0.832	....	...
20	....	....	0.947	0.785	....	0.829	....	...
21	....	....	0.952	0.816	....	0.857	....	...
22	....	....	0.953	0.817	....	0.857	....	...
23	....	....	0.943	0.805	....	0.854	....	...
24	....	....	0.889	0.673	....	0.757	....	...
25	....	....	0.918	0.759	....	0.827	....	...
26	....	....	0.908	0.732	....	0.806	....	...
27	....	....	0.921	0.776	....	0.843	....	...
28	0.900	0.750	0.963	0.828	0.833	0.860	0.027	1.03
29	....	....	0.948	0.793	....	0.836	....	...
30	....	....	0.952	0.808	....	0.849	....	...
31	....	....	0.945	0.760	....	0.804	....	...

Derivation of Equations for Color and Turbidity Indices

If the color of a sugar solution is to be expressed as the per cent absorbency of blue-green light by the turbidity-free

OF VARIOUS SUGAR SOLUTIONS

Sugar Sample	Transmittancies: Before Filtration T_r	Before Filtration T_g	After Filtration T_{rc}	After Filtration T_{gc}	T_g/T_r	T_{gc}/T_{rc}	$\frac{T_{gc}}{T_{rc}} - \frac{T_g}{T_r}$	T_{rt}/T_{gt}
Beet Sugar Factory B (*Cont'd*)								
32	....	....	0.948	0.837	....	0.883	....	...
33	....	....	0.940	0.822	....	0.874	....	...
Beet Sugar Factory C								
1	0.849	0.688	0.931	0.764	0.810	0.821	0.011	1.01
2	0.862	0.702	0.933	0.772	0.814	0.827	0.013	1.02
3	0.846	0.682	0.932	0.763	0.806	0.819	0.013	1.02
4	0.871	0.711	0.944	0.767	0.816	0.813	−0.003	1.00
5	0.833	0.661	0.948	0.792	0.794	0.835	0.041	1.05
6	0.853	0.708	0.939	0.785	0.830	0.836	0.006	1.01
7	0.863	0.705	0.950	0.794	0.817	0.836	0.019	1.02
8	0.885	0.733	0.954	0.786	0.828	0.824	−0.004	1.00
9	0.876	0.707	0.942	0.773	0.807	0.821	0.014	1.02
10	0.842	0.672	0.930	0.762	0.798	0.819	0.021	1.03
11	0.846	0.695	0.923	0.757	0.822	0.820	−0.002	1.00
12	0.882	0.723	0.930	0.770	0.820	0.828	0.008	1.01
13	0.863	0.684	0.917	0.734	0.793	0.800	0.007	1.01
14	0.852	0.662	0.918	0.732	0.777	0.797	0.020	1.03
15	0.863	0.671	0.926	0.728	0.778	0.786	0.008	1.01
16	0.876	0.693	0.922	0.743	0.791	0.806	0.016	1.02
17	0.842	0.643	0.915	0.706	0.764	0.772	0.008	1.01
18	0.656	0.464	0.892	0.693	0.707	0.777	0.070	1.10
19	0.810	0.617	0.900	0.695	0.762	0.772	0.010	1.01
20	0.860	0.678	0.916	0.737	0.788	0.805	0.017	1.02
21	0.862	0.700	0.933	0.763	0.812	0.818	0.006	1.01
22	0.859	0.695	0.929	0.753	0.809	0.811	0.002	1.00
23	0.870	0.722	0.942	0.795	0.830	0.844	0.014	1.02
24	0.881	0.725	0.936	0.787	0.823	0.841	0.018	1.02
25	0.904	0.750	0.948	0.798	0.830	0.842	0.012	1.01
26	0.872	0.702	0.932	0.763	0.805	0.819	0.014	1.02
27	0.889	0.725	0.934	0.765	0.816	0.819	0.003	1.00
28	0.873	0.686	0.923	0.760	0.786	0.823	0.037	1.05
29	0.902	0.719	0.933	0.759	0.797	0.815	0.017	1.02
30	0.859	0.688	0.920	0.739	0.801	0.803	0.002	1.00
31	0.877	0.717	0.918	0.756	0.818	0.824	0.006	1.01
32	0.894	0.731	0.942	0.792	0.818	0.841	0.023	1.03
33	0.850	0.697	0.930	0.784	0.820	0.843	0.023	1.03
34	0.880	0.702	0.922	0.754	0.798	0.818	0.020	1.03
35	0.857	0.685	0.941	0.765	0.799	0.813	0.014	1.02
36	0.842	0.673	0.923	0.747	0.799	0.809	0.010	1.01
37	0.648	0.480	0.897	0.696	0.741	0.776	0.035	1.05
38	0.371	0.257	0.878	0.686	0.693	0.781	0.088	1.13
39	0.828	0.632	0.902	0.703	0.763	0.779	0.016	1.02
Cane Sugar Factory								
Cane granulated	....	....	0.884	0.731	....	0.827	....	...
Cane granulated	....	....	0.877	0.723	....	0.824	....	...
Cane granulated	....	....	0.886	0.723	....	0.816	....	...
Cane cubes	....	....	0.974	0.946	....	0.971	....	...
Extra hard cubes	....	....	0.981	0.945	....	0.963	....	...
Cocktail cubes	....	....	0.975	0.945	....	0.969	....	...
Tablets	....	....	0.985	0.962	....	0.977	....	...
No. 1. wet	0.885	0.773	0.962	0.870	0.873	0.904	0.031	1.04
No. 3 wet	0.849	0.703	0.935	0.797	0.828	0.852	0.024	1.03
No. 4 wet	0.822	0.602	0.916	0.717	0.732	0.783	0.051	1.07
Wet cube	0.948	0.928	0.983	0.968	0.979	0.985	0.006	1.01
Confectioners' A	0.886	0.827	0.967	0.933	0.933	0.965	0.032	1.03
Confectioners' AA	0.920	0.870	0.983	0.938	0.946	0.954	0.008	1.01
Sanding	0.907	0.868	0.985	0.953	0.957	0.968	0.011	1.01
Bar	0.768	0.624	0.895	0.747	0.813	0.835	0.022	1.03
Baker's	0.772	0.595	0.873	0.718	0.771	0.822	0.051	1.07

solution, it is necessary to calculate T_{gc} from the measured values of T_g and T_r, employing Equations 2, 3, 12, and 13. Similarly, the turbidity index can be calculated by finding T_{rt}.

After performing the algebra the following equations for T_{gc} and for T_{rt} are obtained:

$$T_{gc}^2 + T_{gc}[0.461 + T_g(0.220 - 1.819/T_r)] + 0.101T_g = 0 \quad (14)$$

$$T_{rt}^2 + T_{rt}[-5.564 - T_r(0.673 - 1.409/T_g)] + 3.744T_r = 0 \quad (15)$$

Only one root in each equation is significant. T_{gc} must be greater than or equal to T_g, but less than 1.00. Also T_{rt} must be greater than or equal to T_r, but less than 1.00.

A table was prepared by substituting various values of T_g and T_r in Equations 14 and 15 to obtain the transmittancies and then these were converted readily to per cent absorbencies, the units of the color and turbidity indices.

Comparison of Observed and Calculated Color and Turbidity Indices

A practical test of the method can be made by comparing the observed and calculated color and turbidity indices. From the data in Table II the color index is given as $100(1-T_{gc})$ and the turbidity index is $100(1 - T_r/T_{rc})$. In Table IV these indices are compared with the indices calculated from Equations 14 and 15, using the observed values of T_g and T_r.

The average difference between the calculated and observed color indices is 1.9 units; for the turbidity indices, it is 1.4 units. The agreement is especially gratifying in the cases of high turbidity.

Practical Application

The method has been applied for practical control work at the Woodland factory, and has functioned in a very satis-

TABLE III. TRANSMITTANCIES OF TURBID SUGAR SOLUTIONS

Type of Turbidity	T_{rt}	T_{gt}	T_{rt}/T_{gt}
Sulfur	0.726	0.682	1.064
	0.687	0.633	1.085
	0.641	0.592	1.083
	0.604	0.550	1.098
Fuller's earth	0.867	0.841	1.031
	0.841	0.817	1.030
	0.814	0.785	1.037
	0.804	0.767	1.048
	0.767	0.727	1.055
	0.983	0.973	1.010
	0.962	0.953	1.009
	0.947	0.927	1.022
	0.918	0.893	1.028
	0.854	0.826	1.034
	0.822	0.779	1.055
	0.742	0.704	1.054
	0.662	0.620	1.068
	0.564	0.524	1.076
	0.460	0.409	1.125
Fuller's earth (*Cont'd*)	0.990	0.990	1.000
	0.970	0.964	1.006
	0.908	0.881	1.031
	0.853	0.815	1.047
	0.805	0.754	1.068
	0.878	0.853	1.029
	0.868	0.830	1.046
	0.923	0.912	1.012
	0.927	0.908	1.021
	0.871	0.853	1.021
	0.863	0.844	1.022
	0.845	0.822	1.028
	0.813	0.777	1.046
	0.879	0.849	1.035
	0.924	0.913	1.012
	0.923	0.900	1.026
	0.838	0.809	1.036
	0.905	0.887	1.020

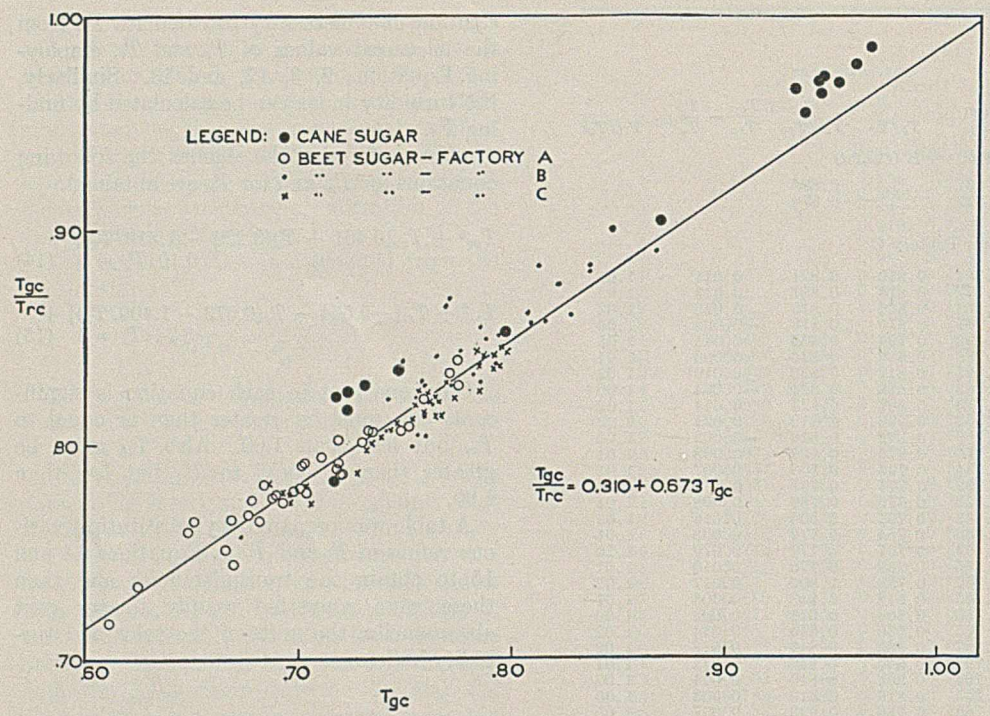

FIGURE 2. TRANSMITTANCY RELATIONSHIPS FOR SUGAR SOLUTIONS CONTAINING COLOR BUT NOT TURBIDITY

factory manner. About 7 minutes are required for each complete analysis.

One hundred and fifty grams of sugar are dissolved in an equal weight of hot distilled water, and stirred into solution. The hot solution is poured through a heat exchanger, from which it emerges at approximately room temperature. The cooled solution is poured into the absorption cell.

The color temperature of the light source is checked. The blue-green filter and secondary standard are inserted and the Lumetron is balanced to the required value. The secondary standard is removed, the absorption cell inserted, and a reading made of the transmission.

The same procedure is carried out with the red filter. From the two readings, the color and turbidity indices are obtained from tables. (Complete directions and copies of the tables will be furnished at cost to anyone interested.)

Discussion

The color and turbidity indices in this method are given as per cent absorbencies due to color or turbidity for an absorption cell 25 cm. in length. Naturally the values will differ for another cell length. It has been found, however, that a cell of this length is necessary for optimum absorption (*5*).

With control of the color temperature of the illuminant, and some care in duplication of light filters, there should be little difficulty in obtaining substantially the same results with different instruments.

In order to save time, the sugar samples are dissolved in

TABLE IV. COMPARISON OF OBSERVED AND CALCULATED COLOR AND TURBIDITY INDICES

Sugar Sample, Factory A	Color Index Observed	Color Index Calculated	Turbidity Index Observed	Turbidity Index Calculated
10	24	27	13	12
11	33	38	25	19
12	25	25	13	12
13	27	31	26	24
14	31	32	20	19
15	33	36	28	27
16	26	27	20	20
17	30	34	11	8
18	32	47[a]	31	25
19	39	28[a]	20	24
20	37	47[a]	46	42
21	29	32	10	10
22	28	28	15	14
23	32	36	30	28
24	28	31	17	17
25	28	33	17	14
26	27	25	10	11
27	31	36	23	21
28	35	36	22	22
29	25	29	7.7	5.4
30	30	32	19	19
31	35	48[a]	43	39
32	28	32	12	10
33	27	32	13	11
34	30	24[a]	37	38
35	30	22[a]	39	41
Factory C				
1	24	23	8.8	8.7
2	23	23	7.6	7.6
3	24	25	9.2	9.0
4	23	23	7.7	6.6
5	21	25	12	10
6	21	19	9.2	9.9
7	21	21	9.2	8.1
8	21	22	7.2	5.7

Sugar Sample, Factory C	Color Index Observed	Color Index Calculated	Turbidity Index Observed	Turbidity Index Calculated
9	23	24	7.0	5.4
10	24	25	9.5	8.7
11	24	21	8.3	10
12	23	22	5.2	5.5
13	27	27	5.9	6.2
14	27	29	7.2	5.8
15	27	29	6.8	4.7
16	26	28	5.0	4.0
17	29	31	8.0	5.8
18	31	37	27	25
19	30	29	10	9.7
20	26	27	6.1	5.5
21	24	23	7.6	7.6
22	25	23	7.5	7.2
23	20	21	7.6	7.4
24	21	21	5.9	6.0
25	20	21	4.6	4.3
26	24	25	6.4	5.7
27	23	22	4.8	4.0
28	24	27	5.4	4.2
29	24	26	3.3	1.6
30	26	25	6.6	6.7
31	24	22	4.5	5.5
32	21	22	5.1	3.6
33	22	21	8.6	9.3
34	25	26	4.6	3.7
35	23	25	8.9	6.9
36	25	25	8.8	9.0
37	30	29	28	28
38	31	29	58	54
39	30	31	8.2	6.7

[a] Solutions of these samples contained a variety of foreign matter, such as fibers and large and fine particles. Probably this is the reason for the large differences between calculated and observed values. Equations 14 and 15 cannot be expected to hold under such circumstances.

hot water. Tests have shown that this causes a slight increase in color, but as it is roughly constant, it is ignored.

There is no definite assurance that the exact relationships given in this paper will hold for coloring matter and turbidity present in beet sugars from other districts. If they do not, the correct relationships may readily be established by the methods described.

The problem is one of unusual complexity, and unfortunately no simple solution seems possible, barring the perfection of a method for rapid optical filtration of sugar solutions.

Acknowledgment

The advice of E. M. Hartmann is gratefully acknowledged.

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Determination of K_2O in Commercial Fertilizers

Using 95 and 80 Per Cent Alcohol and Acid-Alcohol

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FOR several years many fertilizer chemists have believed that potash determinations made by the official method have given low results due to the use of 80 per cent alcohol and acid-alcohol in the determinations. The method to date has not been changed to correct this, even though the general referee on fertilizer of the A. O. A. C. has often recommended the investigation of the solubility of potassium chloroplatinate in acid-alcohol and alcohol.

Pierrat (*5*) gave the solubility of potassium chloroplatinate in various concentrations of alcohol at 14° C. but made no reference to the solubility at higher temperatures or under the conditions of the determination of the potash in fertilizers by the official method. Allen (*1*) reported a greater solubility of potassium chloroplatinate in 80 per cent than in 95 per cent alcohol but made no direct reference to the temperature, nor were the conditions identical with those involved in the determination of potash in fertilizers by the official method. Hughes and Ford (*4*) reported the solubilities of potassium chloroplatinate in 83 per cent alcohol and acid-alcohol at two different temperature levels, 18° and 38° C., but this work was done on potassium chloroplatinate precipitated from pure potassium chloride and was therefore not carried out under conditions exactly similar to those used in the determination of potash in fertilizers by the official method. Archibald, Wilcox, and Buckley (*2*) gave the solubilities of potassium chloroplatinate in alcohol-water mixtures at 20° C. but made no reference to acid-alcohol. Thus their results are not directly comparable to those obtained by the official method for potash in fertilizers. The above references, while important in themselves, are not general enough to include all the conditions to be met in the determination of potash in fertilizers by the official method.

The work reported in this study is a comparison of the potash contents of commercial fertilizers using 80 and 95 per cent alcohol. Since Hughes and Ford (*4*) reported increased solubility with rise of temperature, all work herein reported was done at 18° C. for both 80 and 95 per cent acid-alcohol and alcohol. Determinations were made on fertilizers of various analyses. A higher potash content was generally obtained when 95 per cent rather than 80 per cent acid-alcohol and alcohol were used.

Procedure

The study is divided into three steps:

1. Comparison of the K_2O values in mixed fertilizers by the official method (*3*, section 41, a), using aliquots of the same solution for both 80 and 95 per cent acid-alcohol and alcohol.
2. Comparison of the K_2O values in potash salts by two procedures.

Determination of the K_2O in potash salts by the method for potash salts (*3* section 41, b) using both 80 and 95 per cent acid-alcohol and alcohol.

Determination of the K_2O in potash salts, adding to the aliquot prepared for determination 1 gram of sodium chloride, by the method for potash salts (*3*, section 41, b) using both 80 and 95 per cent acid-alcohol and alcohol.

3. Comparison of the K_2O values in a high-analysis complete fertilizer using a collaborative check sample (*3*, section 41, a) with 80 and 95 per cent acid-alcohol and alcohol.

The same sintered-glass crucible (either Jena BG 3 or Pyrex M) was used in the filtration of the two aliquots of the same sample, using the concentrations of alcohol mentioned above. It was believed that this laboratory technique would avoid variations in filtration conditions that might occur had a padded Gooch crucible been used. All samples were washed with approximately the same amount of alcohol (125 ml.). All K_2O

Table I. Comparison of Averages of Potash Determinations of Mixed Fertilizers Using 80 and 95 Per Cent Alcohol

	Low Complete Fertilizers, 12–18 Units[a]		Medium Complete Fertilizers, 19–24 Units[b]		High Complete Fertilizers 25–40 Units[c]		Medium Phosphate and Potash Fertilizers, 19–24 Units[d]		High Phosphate and Potash Fertilizers, 25–40 Units[e]		Potash Salts 50 Units[f]	
	95% alcohol	80% alcohol	95% alcohol	80% alcohol	95% alcohol	80% alcohol	95% alcohol	80% alcohol	95% alcohol	80% alcohol	95% alcohol	80% alcohol
No. of analyses averaged	10	10	51	51	63	63	37	37	44	44	16	16
Average result, % K_2O	3.80	3.67	6.52	6.40	13.91	13.73	11.40	11.21	20.94	20.78	50.96	50.73
Average difference between alcohols, %	0.13	....	0.12	....	0.18	...	0.19	...	0.16	...	0.23	...
Indivdual sample difference between alcohols, %	0.02	....	0.00	....	0.02	...	0.03	...	0.02	...	0.17	...
	−0.33	....	−0.32	....	−0.31	...	−0.31	...	−0.34	...	−0.31	...
Percentage K_2O increase	3.54	....	1.88	....	1.36	...	1.70	...	0.77	...	0.45	...

Units distributed among the following analyses: [a] 3-8-6, 2-10-4, 2-12-2, 1-8-3, 2-8-6, 1-12-2, 1-14-2. [b] 2-12-6, 4-10-6, 4-12-4, 5-10-5, 6-8-6, 4-8-8, 4-16-4. [c] 3-12-12, 17-8-8, 2-12-12, 2-8-16, 3-6-18, 3-8-16, 3-9-18, 4-24-12, 3-18-9, 2-16-8. [d] 0-14-6, 0-12-12, 0-10-10, 0-8-16. [e] 0-21-9, 0-8-24, 0-20-20, 0-10-20, 0-16-24, 0-10-30. [f] 0-0-50.

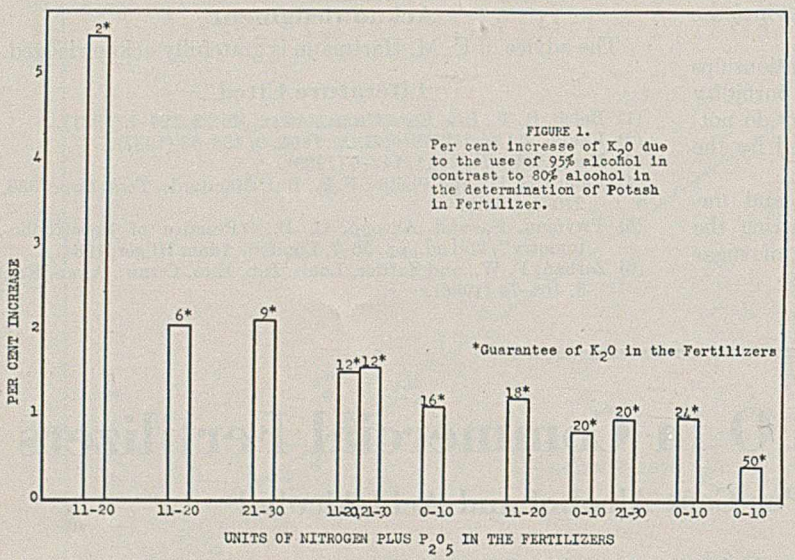

FIGURE 1.
Per cent increase of K_2O due to the use of 95% alcohol in contrast to 80% alcohol in the determination of Potash in Fertilizer.

TABLE II. COMPARISON OF AVERAGES OF POTASH IN POTASH SALT DETERMINATIONS

(With and without the addition of sodium chloride using 80 and 95 per cent alcohol)

	Potash Salts, 50 Units		Potash Salts, 50 Units, NaCl Added	
	95% alcohol	80% alcohol	95% alcohol	80% alcohol
No. of analyses averaged	16	16	16	16
Average result, % K_2O	50.96	50.73	52.03	51.19
Difference between alcohols, %	0.23	...	0.84	...
Individual sample difference between alcohols, %	0.17– 0.31	...	0.21– 1.05	...
Percentage K_2O increase	0.45	...	1.64	...

TABLE III. COMPARISON OF POTASH VALUES ON A COLLABORATIVE CHECK SAMPLE

	Collaborative Fertilizer, Check Sample, 42 Units	
	95% alcohol	80% alcohol
No. of analyses averaged	12	12
Average result, % K_2O	17.52	17.36
Difference between alcohols, %	0.16	...
Individual sample difference between alcohols, %	0.04– 0.28	...
Percentage K_2O increase	0.92	...

values were determined by dissolving the potassium chloroplatinate in hot water and reweighing the crucible.

Discussion of Results

Table I shows the comparison of the averages of determinations of potash, using 80 and 95 per cent acid-alcohol and alcohol. These results were obtained on low-, medium-, and high-grade complete fertilizers which contained 12 to 40 units of plant food; medium- and high-grade phosphate-potash mixtures which contained 20 to 40 units of plant food; and potash salts containing about 50 units of water-soluble potash.

The differences between results reported using 80 per cent as compared to those using 95 per cent alcohol range from 0.13 per cent in the case of low-grade complete analyses to 0.18 per cent for the high-grade complete analyses (Table I). A gain of 0.19 per cent was observed in the case of medium phosphate and potash fertilizers and of 0.16 per cent in the case of high phosphate and potash fertilizers due to the use of 95 per cent acid-alcohol and alcohol. An average difference of 0.23 per cent in favor of the use of 95 per cent alcohol was noted in the potash salt determinations.

Figure 1 shows the percentage increase of K_2O due to the use of 95 per cent acid-alcohol and alcohol in contrast to 80 per cent in the determination of K_2O in the fertilizers. Data reported in this graph show that there is a greater K_2O recovery with 95 per cent alcohol in the case of fertilizers having a low potash content than in the case of those having a high potash content. At the same time there is some indication that where the salt content is high in a potash determination the use of 95 per cent alcohol may give too high potash values, owing to the insolubility of the salts in the 95 per cent alcohol. This explanation is supported by the data in Table II, which gives the results obtained when sodium chloride is added.

Table II reports the comparison of the averages of determinations of potash in potash salts and on aliquots of the same salt to which has been added an equal amount of sodium chloride. Whereas a difference of 0.23 per cent greater was found when 95 per cent alcohol was used in place of 80 per cent, with the addition of the sodium chloride the difference increases to 0.84 per cent. This difference will usually be encountered unless the sample is washed with a larger volume of alcohol and it logically follows that it would require a larger volume of 95 per cent than of 80 per cent alcohol to wash out the excess sodium salt if the difference can be entirely attributed to some sodium salt.

Table III reports the averages of 12 potash determinations on a collaborative check sample representing a high-analysis complete fertilizer containing more than 40 units of plant food. In this case 0.16 per cent more potash is obtained with 95 per cent alcohol than with 80 per cent alcohol, in agreement with results obtained on fertilizers of similar analyses reported in Table I.

A study of the individual sample differences between alcohols of the three tables indicates that in some cases 95 per cent alcohol gave little if any increase in potash content when compared to 80 per cent alcohol, but in most cases a significant increase was evident.

Summary

There is definitely a greater potash value obtained with 95 per cent in place of 80 per cent alcohol in the determination of potash in fertilizers by the official method. This difference for fertilizers of 12 to 40 units of plant food ranges from 0.13 to 0.19 per cent K_2O. For muriates of potash there is an average difference of 0.23 per cent in favor of the use of 95 per cent alcohol.

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Derivatives of Amytal, Pentobarbital, and Dial

An Optical Crystallographic Study

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IN A PREVIOUS study the optical properties of certain derivatives of barbital and luminal were presented (*3*). Since chemical tests are not entirely satisfactory for the identification of some of the newer derivatives of barbituric acid, the optical crystallographic study has been extended to include three additional barbiturates: amytal (5-ethyl-5-isoamyl barbituric acid), pentobarbital (5-ethyl-5-alpha-methyl butyl barbituric acid), and dial (5,5-diallyl barbituric acid). Benzyl and phenacyl derivatives were prepared from each of the compounds under investigation. None of the derivatives presented in this paper, however, has been reported in the literature with the exception of *p*-nitrobenzyl compounds. These derivatives for amytal and dial have been prepared by Lyons and Dox (*5*), Hargreaves and Nixon (*2*), and Jesperson and Larsen (*4*), and for pentobarbital by Hargreaves and Nixon.

Each new compound was analyzed for halogens or nitrogen. The melting points were run by both the tube method and the "Bloc Maquenne" method. A determination for water of crystallization was made on each of the compounds prepared.

The *p*-nitrobenzyl, *p*-bromobenzyl, and *p*-chlorobenzyl derivatives were prepared with each of the medicinal substances. The analytical results are presented in Table I.

Optical Crystallographic Data

The optical properties of each compound listed in Table I were determined by methods used in the previous study for compounds of barbital and luminal (*3*), and are presented in Table II. The optical properties of the derivatives of the three substances differ sufficiently from each other to allow the use of the optical data in the identification of the original barbituric acid derivative. No previous optical crystallographic data have been reported for the derivatives of amytal, pentobarbital, or dial. Incomplete data on dial itself have been listed by Haas (*1*).

TABLE I. ANALYTICAL DATA

Compound	Formula	Crystal Habit	Color	Nitrogen Calcd. %	Nitrogen Found %	Halogen Calcd. %	Halogen Found %	Melting Points Uncorrected Tube	Melting Points Uncorrected Block
Amytal	$C_{11}H_{18}N_2O_3$	Rods	White	...	... ...	...	... ...	156.5	157
p-Nitrobenzyl derivative	$C_{25}H_{28}N_4O_7$	Plates	Pale yellow	11.29	11.18, 11.25	...	... ...	169	172
p-Bromobenzyl derivative	$C_{25}H_{28}N_2O_3Br_2$	Rods and plates	White	...	... ...	28.33	28.47, 28.29	133	134
p-Chlorobenzyl derivative	$C_{25}H_{28}N_2O_3Cl_2$	Rods and plates	White	...	... ...	14.92	14.87, 15.03	102–105	102
Pentobarbital	$C_{11}H_{18}N_2O_3$	Needles	White	...	... ...	...	... ...	130	130
p-Nitrobenzyl derivative	$C_{25}H_{28}N_4O_7$	Rods and plates	Pale yellow	11.29	11.28, 11.23	...	... ...	153	151
p-Bromobenzyl derivative	$C_{25}H_{28}N_2O_3Br_2$	Thick plates	White	...	... ...	28.33	28.28, 28.40	114	114
p-Chlorobenzyl derivative	$C_{25}H_{28}N_2O_3Cl_2$	Thick plates	White	...	... ...	14.96	15.02, 15.20	111	111
Dial	$C_{10}H_{14}N_2O_3$	Plates	White	...	... ...	...	... ...	171.5	173
p-Nitrobenzyl derivative	$C_{24}H_{24}N_4O_7$	Plates	Pale yellow	11.67	11.50, 11.64	...	... ...	191.5	192
p-Bromobenzyl derivative	$C_{24}H_{24}N_2O_3Br_2$	Rods and plates	White	...	... ...	29.16	28.95, 29.11	132.5	133
p-Chlorobenzyl derivative	$C_{24}H_{24}N_2O_3Cl_2$	Flat rods	White	...	... ...	15.45	15.60, 15.46	125–134	125–134

TABLE II. OPTICAL CRYSTALLOGRAPHIC DATA

Compound	Optical Sign	Elongation	Refractive Indices at 25° Alpha	Beta	Gamma	Rhombic Dispersion	Crystal System
Amytal	−	+	1.467	1.533	1.560	None	Monoclinic
p-Nitrobenzyl derivative	−	±	1.510	1.640	1.656	V > P	Monoclinic
p-Bromobenzyl derivative	+	±	1.559	1.573	1.662	V > P	Monoclinic
p-Chlorobenzyl derivative	+	±	1.539	1.574	1.694	None	Monoclinic
Pentobarbital	−	−	1.469	1.528	1.569	None	Monoclinic
p-Nitrobenzyl derivative	?[a]	+	1.548	?[a]	1.696	?[a]	Monoclinic
p-Bromobenzyl derivative	−	±	1.527	1.638	1.702	None	Monoclinic
p-Chlorobenzyl derivative	−	±	1.523	1.631	1.680	None	Monoclinic
Dial	−	−	1.518	1.567	1.610	None	Monoclinic
p-Nitrobenzyl derivative	−	+	1.511	1.658	1.699	V > P	Monoclinic
p-Bromobenzyl derivative	+	−	1.569	1.589	> 1.700	None	Monoclinic
p-Chlorobenzyl derivative	+	±	1.560	1.592	> 1.700	None	Monoclinic

[a] Since no optic axis interference figures could be seen, these values could not be determined.

Preparation and Analysis

The benzyl and phenacyl derivatives were prepared in the following manner:

Equivalent amounts of the barbituric acid derivative and potassium or sodium carbonate were dissolved in a little more than enough boiling water to make a saturated solution. This solution was added to a solution of the benzyl or phenacyl halide (two molecular quantities) dissolved in an amount of alcohol twice as large as the amount of water used to dissolve the barbituric acid salt. The resulting mixture was refluxed until the reaction was completed. Crystals of the derivative usually separated out of the solution during the heating. After being cooled, the derivatives were separated by filtration and purified by recrystallization from a mixture of chloroform and alcohol until there was no change in the melting point of the substance. The crystals were dried in air, and none of the compounds contained water of crystallization.

Acknowledgment

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Type Analysis of Hydrocarbon Oils

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ORDINARILY, a sample of petroleum oil above the gasoline range contains so many individual hydrocarbons that their separate identification amounts to a lengthy program of research. However, such detailed knowledge is not necessary, since the usefulness of the oil is usually governed by the relative distribution of types—i. e., by average constitution. In industrial operations such as cracking or acid treatment the aim is the transformation of such types rather than of individual compounds. In the control of such operations, use of an accurate type analysis offers distinct advantages.

While there are in use a number of methods for analyzing gasolines and other light fractions in terms of paraffins, naphthenes, olefins, and aromatics, the only method which is generally applied to heavy oils—i. e., through the lubricant range—is that of Vlugter, Waterman, and van Westen (*15*). However, the relation of aromaticity to aniline point, whose assumed constancy is one of the bases of the original method, has been recently found to be variable (*16*). Moreover, the method is not intended for oils whose unsaturation is olefinic rather than aromatic, and hence is not applicable to many cracked oils.

The purpose of the present work was to attempt to provide a more accurate and widely applicable system of analysis by strictly defining the terms expressing composition and developing new correlations through which values of them may be derived from physical tests. The general procedure of analysis, which the method developed here shares with that of Waterman and his colleagues, is:

1. Evaluation of aromaticity or unsaturation of the sample.
2. Determination of the structure of the sample when completely saturated. This combined with 1 permits a segregation of the carbon atoms as aromatic, naphthenic, and paraffinic.

The more critical step is the evaluation of aromaticity or other unsaturation. If this is not correct, the predicted structure of the saturates, which depends on it, cannot be correct. It is shown below that unsaturation (whether olefinic or aromatic) may be calculated from physical measurements by the use of two empirically established relations. The analysis may then be completed by purely mathematical processes.

Unsaturation or Aromaticity

The Lorenz-Lorentz molecular refraction is defined as

$$Mr = M \times \frac{n^2 - 1}{n^2 + 2} \times \frac{1}{\mathrm{d}}$$

in which M is molecular weight, d is specific gravity (here at 20° C. referred to water at 4° C.), and n is refractive index (here at 20° C., for sodium D line).

A recent brief discussion of this function was given by Brode (*6*). Brühl (*3*), Eisenlohr (*4*), and others established from much evidence on pure compounds that the molecular refraction for any individual saturated hydrocarbon may be obtained by additively combining constant increments for the carbon and hydrogen atoms present, providing there are no strains from ring structures other than 5- or 6-membered. (These are believed to be the predominant ring structures in petroleum, *2*, *14*.) Further, there is a certain theoretical basis for this additivity, as discussed by Hückel (*7*), Nernst (*13*), etc. A mathematical statement of the relationship for a hydrocarbon C_nH_{2n+x} is

$$Mr = An + Bx \qquad (1)$$

in which A and B are constants.

The usefulness of this relationship for our purposes depends on its validity when applied to actual oils—that is, multicomponent mixtures of hydrocarbons. It has therefore been tested and its constants established in such an application. For this to be done it was convenient to transform Equation 1 to

$$r = a + by \qquad (2)$$

in which a and b are new constants and y is weight per cent of hydrogen, defined by

$$y = \frac{1.008\ (2n + x)}{M} \times 100$$

If a plot of r against y for a number of saturated oils yields points falling closely about a straight line then Equation 2, and hence also 1, are indicated to be generally valid.

Figure 1 shows such a plot; the data used were compiled from the work of a number of investigators, and represent a thorough search of the chemical literature. Sources are listed in the caption with the reasons, in brief, for accepting each group as fully saturated. As may be seen, the points fall within a narrow zone. It appeared justifiable, from the evidence presented in this figure, to select a line down the center of the zone of scatter as the practical locus of all saturates. The equation of this line (shown on the figure as a full line) is

$$r = 0.2084 + 0.008421y \qquad (3)$$

The line derived from the slightly different molecular refraction constants used by Waterman is also indicated.

It was then necessary to extend the expression for specific refraction to double-bond unsaturation, and thus make it generally applicable to all hydrocarbons which occur in appreciable amounts in petroleum. It has been recognized that an additive increment of molecular refraction can be assigned to each double bond, though the value of the term varies with the kind of double bond. Denoting the variable increment by k, Equation 1 may be amplified by writing

$$Mr = An + Bx + kz$$

in which z is the number of double bonds per molecule. Then on dehydrogenating any saturated oil whose specific refraction is originally expressed, to a very close approximation, by Equation 3, the specific refraction changes to

$$r = 0.2084 + 0.008421y + \frac{kz}{M}$$

Or, expressing by h the unsaturation in grams of hydrogen per 100 grams of sample, so that

$$h = \frac{201.6z}{M} \qquad (4)$$

then

$$r = 0.2084 + 0.008421y + \frac{hk}{201.6} \qquad (5)$$

Because the quantity k has been left variable, with no restrictions imposed on it, Equation 5 is fully as accurate as 3 and is a defining equation for k.

As mentioned above, the first step in a type analysis is the determination of unsaturation or aromaticity. (Olefins are treated as partial aromatics for the present.) Equation 5 provides an expression for h in terms of r, y, and k. Of these three quantities the first two may be measured by familiar

TABLE I. VALUES OF k FOR REPRESENTATIVE HYDROCARBONS

Hydrocarbon	k at 20° C.
Cyclopentene	1.46
Alkyl cyclohexenes	1.40–1.85
Aliphatic monoolefins	1.60–1.90
Alkyl benzenes	1.60–1.90
Indene	1.89
Biphenyl	1.91
Alkyl naphthalenes	2.10–2.25
Alkyl anthracenes	2.60–2.90

methods. Only an evaluation of k is needed, then, to complete the determination.

Study of a large body of data from the literature on individual hydrocarbons shows each series to yield a characteristic range of values of k (as calculated from Equation 5). To each series a mean may be assigned which remains substantially constant with increasing M, except for generally low values for the first member or two members of a homologous series. Some of these values are given in Table I.

In the special case in which the class of unsaturates present in a sample is known with certainty, an appropriate value of k can be assigned from these values. However, in general, not enough is known about the composition of a sample to permit this assignment to be made. A means of making a separate measurement of k for each particular sample is needed.

In this connection, possible uses of the refractive dispersion were considered. A convenient expression of this property is that of Gladstone and Dale

$$S = \frac{n_F - n_C}{d} \times 10^4$$

Here n_F and n_C are refractive indices for the F and C hydrogen lines, and d is the specific gravity referred to water at 4° C. The temperatures of measurement should be the same, though the absolute value is not especially important.

Trends in various dispersion functions have long been recognized; Auwers (*1*) has exhaustively studied the dispersions of various hydrocarbon types. Some relation between dispersion and constitution has been indicated, but no satisfactory formulation of this relation has hitherto been made. In the course of the present work, it was found impossible to assign additive increments of molecular dispersion analogous to the additive increments of molecular refraction and the effort was

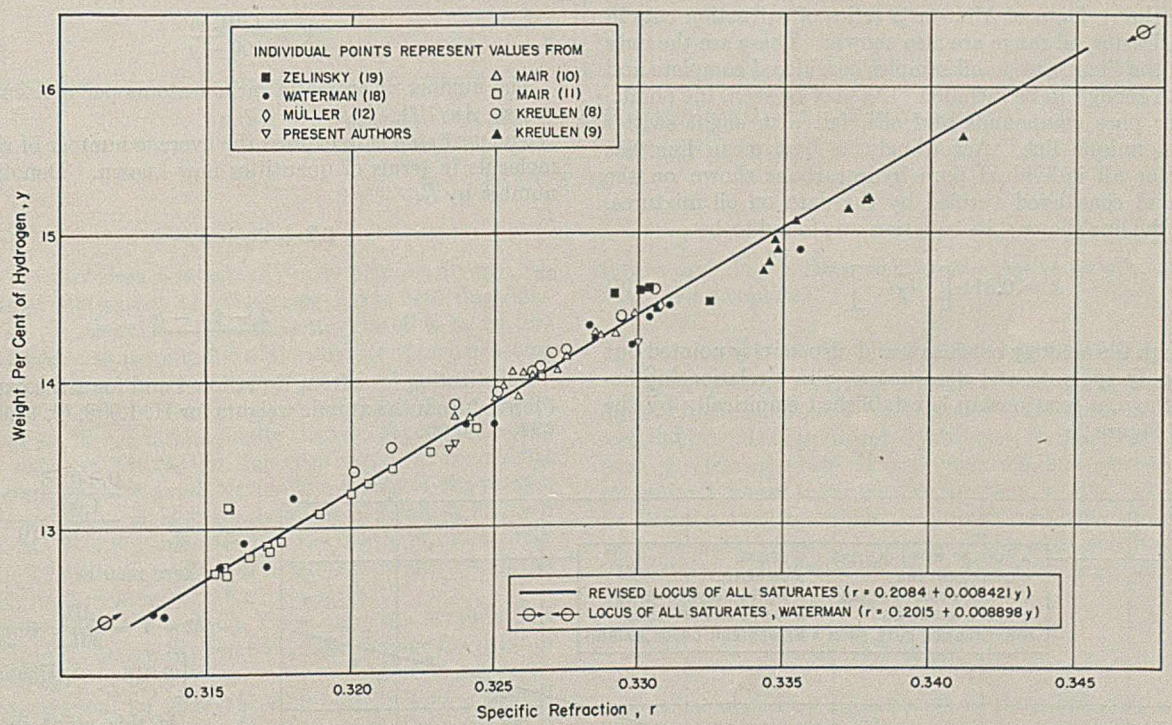

FIGURE 1. SPECIFIC REFRACTION–COMPOSITION RELATIONSHIP FOR SATURATED OILS

Zelinsky and Kazansky (*19*). Baku petroleum fractions were freed from aromatics by acid treatment, dehydrogenated, and acid-treated once more until, assertedly, only paraffins and 5-membered saturated rings remained. All fractions boiled within the range 160° to 200° C.

Waterman and Leendertse (*18*). Data on a large variety of hydrocarbon mixtures are summarized; all have been reported previously. Polymerization products from such varied hydrocarbons as isobutylene, tetralin, and pinene, as well as a number of natural oil fractions, were "completely hydrogenated". Complete saturation is stated to be proved by specific dispersions and, in some cases, by aniline points. The authors' rough conversions of the dispersions (stated for all but two samples), in yielding values of S consistently under 100, seem to confirm this. Molecular weights range from 238 to 645.

Müller and Neyman-Pilat (*12*). A Pennsylvania oil was repeatedly extracted with nitrobenzene and the raffinate was hydrogenated seven times, yielding a product of 479 molecular weight.

These laboratories (unpublished). Several oil fractions were exhaustively extracted, yielding raffinates with values of S under 100. Molecular weights range from 270 to 380. Source materials were Poso, Calif., and Rodessa, Texas, crudes.

Mair and Schicktanz (*10*). The water-white oil obtained from silica-gel absorption of a mid-continent crude fraction was subjected to fractional distillation and the resulting fractions were intensively extracted with acetone. All extraction cuts represented in Figure 1 are among those judged by the authors, on the basis of S values (100 or less), to be saturated. Molecular weights range from 385 to 537.

Mair, Willingham, and Streiff (*11*). Narrow cuts from the methyl cyanide extraction of an extract portion of the same crude were repeatedly hydrogenated to values of S of approximately 100. The molecular weights of the hydrogenated fraction range from 309 to 455.

Kreulen (*8*). A number of gas oils, of undesignated origins, were given "complete" hydrogenations. Authors' corrections of the dispersion data yield values of S varying between 96 and 100. Molecular weights range from 184 to 261.

Kreulen (*9*). Borneo paraffin was cracked ("care was taken that no cyclization occurred") in the presence of hydrogen, and the residue was separated into fractions by distillation. The value of S for each fraction (by authors' conversion) is 100 or 101. Aniline point-molecular weight data seem to confirm the fact that the samples are wholly paraffinic (they are asserted to be normal-paraffin mixtures). No data for y were obtained, but the evidence is considered good enough to justify calculating this quantity from molecular weights, on the assumption of the formula C_nH_{2n+2}. Molecular weights range from 138 to 498.

TABLE II. SPECIFIC DISPERSIONS OF REPRESENTATIVE HYDROCARBONS

Compound	S
1-Hexene	121
Benzene	189
Toluene	185
Indene	224
Biphenyl	269
Naphthalene	303
1-Methylanthracene	521

abandoned. Table II, giving values for certain unsaturated hydrocarbons, illustrates this difficulty.

Accordingly, another approach to the use of dispersion was tested. It is generally accepted that the Gladstone-Dale specific dispersion of all saturates is approximately 98 (*5*, *17*). For any unsaturate, then, the increase of molecular dispersion is $M(S - 98)$; the increment per double bond is $\frac{M(S-98)}{z}$. From Equation 4, this is a constant multiple of $\frac{(S-98)}{h}$, which we have designated the dispersivity. The increment of molecular refraction per double bond is, of course, k.

In Figure 2 the dispersivity is plotted against k for the large body of hydrocarbons for which values of k were tabulated earlier. Data of Mair on a series of extraction cuts in the lubricating oil range are also shown. These are the only data in the literature on oil samples considered complete and accurate enough to be included. As may be seen, the points, both for pure compounds and oils, fall with slight scatter about a unique line. Accordingly, a best mean line was drawn for all individual pure hydrocarbons shown on the figure and considered verified by the data on oil mixtures. This is the line shown; its equation was found to be

$$k = 0.811\left[\frac{S-98}{h}\right]^{0.292} \qquad (6)$$

Though the analogy between k and dispersivity pointed out above only suggests and does not require a relationship between them, a relationship is established empirically by the data of Figure 2.

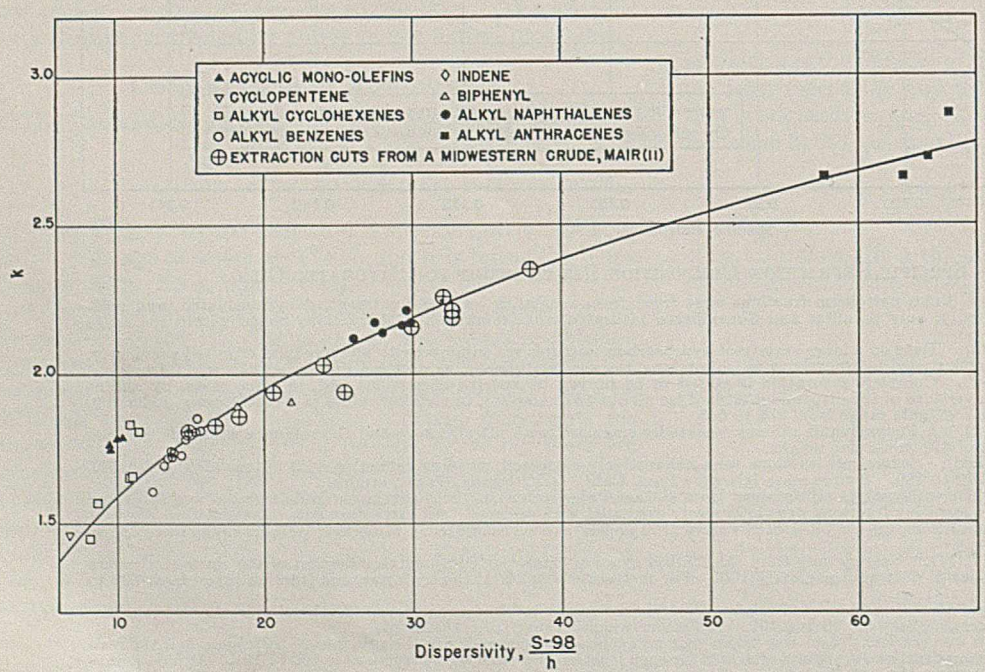

FIGURE 2. RELATIONSHIP BETWEEN k AND DISPERSIVITY FOR UNSATURATED HYDROCARBONS

For a complete expression of h in terms of measurable quantities r, y, and S, it is thus necessary only to eliminate k between Equations 6 and 5. Algebraic treatment is lengthy; a pair of nomographs by which the equations may be solved is provided in Figure 3. The analysis is hereafter designated the nomographic analysis.

Completion of Analysis

The preceding section developed a method of deriving the unsaturation, h, in terms of the measurable quantities, r, y, and S. To complete the plan of analysis, expressions of the percentages of aromatic, naphthenic, and paraffinic carbon atoms per molecule are required. For these a further measurement, that of molecular weight, is needed. Assuming for the moment that the unsaturates are wholly aromatic, the percentage of aromatic carbon atoms per molecule, for a hydrocarbon mixture of average formula C_nH_{2n+x}, is defined by $A = 100\left[\frac{2z}{n}\right]$. Then from the further simple constitutional relations (*4*) and

$$n = \frac{M(100 - y)}{1201.0}$$

there results

$$A = \frac{1191.5h}{100 - y} \qquad (7)$$

The number of aromatic carbon atoms per molecule is, of course, $An/100$.

The next step is to express the average number of rings per molecule in terms of quantities now known. Denoting this number by R,

$$2R + 2z + x = 2$$

or

$$R = \frac{2 - 2z - x}{2}$$

Substituting for z from Equation 4 and for x the expression (derived from the atomic weights for H, 1.008, C, 12.010, and CH_2, 14.026)

$$x = \frac{\frac{0.14026}{1.008}y - 2}{12.010}M$$

there results

$$R = 1 - \frac{Mh}{201.6} + \frac{M}{24.212} \times \left[2.016 - 0.14026y\right] \qquad (8)$$

At this point in the development an inevitable ambiguity arises. We can determine the number of rings per molecule but we cannot say how many carbon atoms there are per ring. If, for example, the molecule contains the naphthalene or decalin group there are ten carbon atoms in two rings or five per ring, while in benzene, diphenyl, or cyclohexane there are six per ring. While there is an accumulation of circumstantial evidence that petroleum contains no substantial

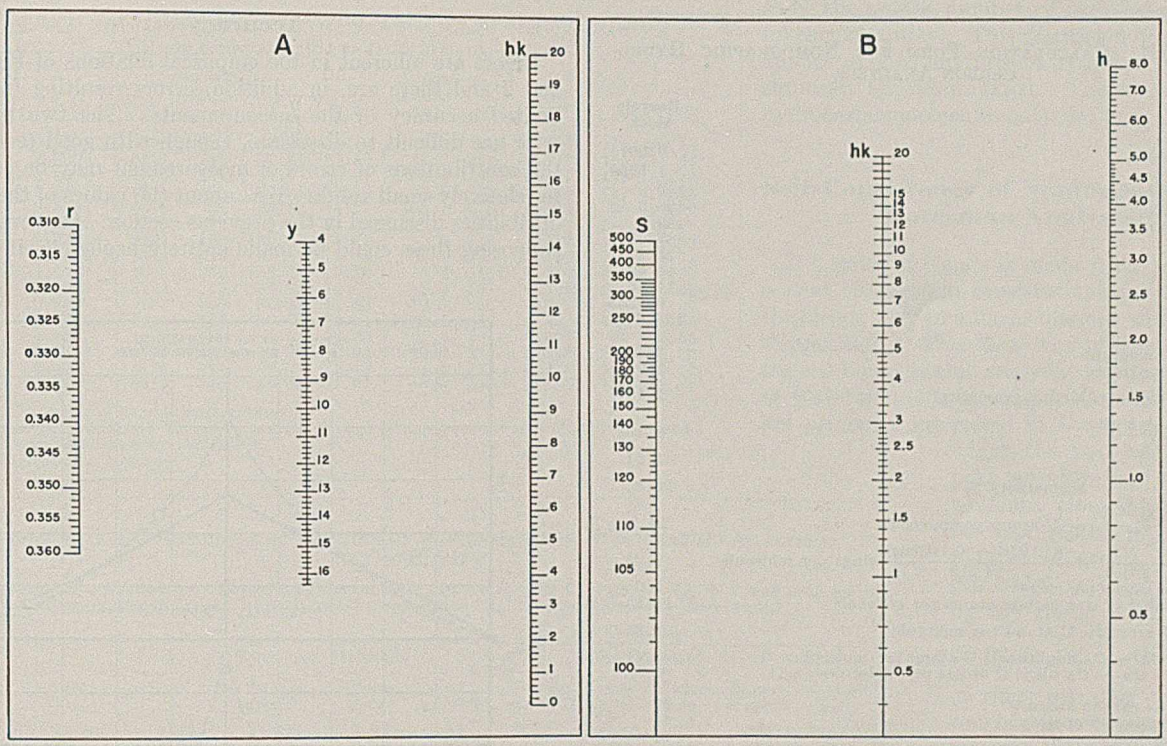

FIGURE 3. NOMOGRAPHIC CHARTS FOR SOLUTION OF GENERAL EQUATIONS

$$A,\ hk = 201.6r - 1.6978y - 42.02$$

$$B,\ h = \left[\frac{hk}{0.811\ (S - 98)^{0.292}}\right]^{1.412}$$

amount of other than 5-membered or 6-membered rings, we do not know the extent to which ring fusion and ring linkage occur. If, therefore, the analysis of the oil is to be formally completed an assumption of the number of carbon atoms per ring must be made at this point.

The number will be variable, obviously, depending on the ring types present. It was finally decided that it is best to make a separate assumption for each sample. In making this assumption, any available foreknowledge of the sample should be considered; likewise the value of k, which Figure 2 shows to be distinctly influenced by the degree of fusion, may be used—for example, values of about 2.5 denote a high degree of fusion, values below 2.0 a low degree.

In the method of Waterman the assumption is made that all the naphthenes present are 6-membered rings fused up to the limit imposed by the molecular weight of the sample. (It is assumed that no carbon atom is buried—that is, shared among more than two rings. In other words, the progression cyclohexane, decalin, perhydroanthracene . . . is assumed.) In effect this is an assumption of nearly the minimum possible number of carbon atoms per ring (if all are 6-membered rings), which is on the whole unlikely. It has the further disadvantage that it assumes that the number of carbon atoms per ring declines continuously in all oils as the molecular weight increases.

Having so ascertained the number of ring carbon atoms, the number of naphthenic carbon atoms can be calculated as the difference between this and the number of aromatic carbon atoms previously derived. This assumes, as noted previously, that all unsaturation is aromatic. Then, only the number of alkyl carbon atoms is needed further. This is, obviously,

$$n - \text{naphthenic C atoms} - \text{aromatic C atoms}$$

With these expressions of the numbers of aromatic, naphthenic, and alkyl carbon atoms converted to percentages, the analysis is complete.

Expression of Olefin Content

The presence of olefins in a sample is shown by a bromine number determination. The various techniques in use are well known. It is to be noted, however, that all methods become far less accurate as molecular weight increases. Expressed in terms of the total unsaturation, h, that portion due to olefins alone, h_0, equals the bromine number divided by 79.3, the ratio of atomic weights of bromine and hydrogen. Then the remaining portion, $h_A = h - h_0$, is due to aromatics alone. The true aromaticity is then

$$A = \frac{1191.5h_A}{100 - y}$$

R is calculated by Equation 8, as described above. It is important to note that this expression is independent of the distribution of unsaturation between h_0 and h_A. Then, including the aliphatic olefins with the saturated aliphatic part and the cyclic olefins with the naphthenes, we may write as before

$$\text{naphthenic C atoms} = \text{total cyclic C atoms} - \frac{nA}{100}$$

$$\text{alkyl C atoms} = n - \text{total cyclic C atoms}$$

The change from numbers to percentages of aromatic, naphthenic, and alkyl carbon atoms is readily made, and these quantities, together with h_0, comprise the solution of the nomographic analysis for the general case in which unsaturation is partly aromatic, partly olefinic. The olefins must be included with the naphthenes and paraffins, instead of being expressed separately, because it is impossible to distinguish cyclic from acyclic olefins. Thus, the percentage of naph-

TABLE III. CALCULATION FORM FOR NOMOGRAPHIC HYDROCARBON ANALYSIS

Sample	Recycle stock
d_4^{20} (vacuum)	0.9014
n_D^{20}	1.5009
$(nF - nC) \times 10^4$	140.2
Bromine No.	36.0
M (extrap.)	189.
C, wt. %	87.93
H, wt. %	11.47
C + H, wt. %	99.40
y	11.55
r	0.3268
S	155.5
hk from nomograph	4.22
h from nomograph	1.96
$k = \frac{hk}{h}$ (indicates kind of aromatics)	2.15
$h_0 = \frac{\text{bromine no.}}{79.3}$	0.45
$h_A = h - h_0$	1.51
$A = \frac{1191.5h_A}{(100 - y)}$ = aromaticity %	20.4
$n = \frac{M(100 - y)}{1201}$ = C atoms per molecule	13.9
$R = 1 - \frac{Mh}{201.6} + \frac{M(2.016 - 0.14026y)}{24.212}$ rings per molecule	2.25
C_R carbon atoms per ring[a]	5.0
$R.C_R$ number of ring carbon atoms per molecule	11.25
$C_A = \frac{nA}{100}$ aromatic C atoms per molecule	2.84
$C_N = R.C_R - C_A$ naphthenic C atoms per molecule	8.41
$C_P = n - C_A - C_N$ alkyl C atoms per molecule	2.65
% aromatic carbon atoms	20
% naphthenic carbon atoms	61
% alkyl carbon atoms	19

[a] Describing assumption used.

thenic carbon atoms, in the sense of including all cyclic olefins, is explicit.

AN EXAMPLE. Use of the method is illustrated in the analysis of a recycle stock from a refinery cracking furnace given in Table III, which shows the calculations in their proper sequence on a form convenient for routine use. In a sample contaminated slightly by nonhydrocarbon impurities, y is taken as one hundred times the ratio of hydrogen to hydrogen plus carbon, rather than the absolute weight per cent of hydrogen from combustion analysis. Other measurements are not altered to take account of the impurity. It is thus implied that the contaminants have average properties identical with those of the hydrocarbon mixture. This is certainly not exactly the case, in general, but no better assumption is apparent.

Reproducibility

When a Pulfrich refractometer is used for the optical measurements and the best combustion analysis technique is observed, determinations of the properties required by this analytical method are usually reproducible within the limits 0.0001 in r, 0.04 in y, and 2 in S. Assuming that the maximum values listed above re-enforce each other, the resulting maximum deviations in h, for values of k covering the usual range of variation for oil samples, are those shown in Table IV.

Since no method of obtaining bromine numbers is specified, it is not possible to discuss the reproducibility of h_0.

The quantity R depends on h and one additional measurement, M. From tests of routine methods used in these laboratories, M is usually reproducible within 4 per cent for a given sample. Then, using the extreme deviations of h noted above, the largest possible deviations in R, for some typical values of k, R, and M, are as shown in Table V. These figures represent the extremely unlikely case of simultaneous maximum deviations in all measurements, all re-enforcing.

Accuracy

Errors are inherent in the empirical relations of Figures 1 and 2 and there are, in addition, errors resulting from the limited accuracy of the measurements. The two types of error are difficult to dissociate, though with good techniques the contributions of errors of measurement may be confined to relatively small values—i. e., about the values of the reproducibilities discussed in the previous section. However, even supposing these could be made entirely negligible, it is very

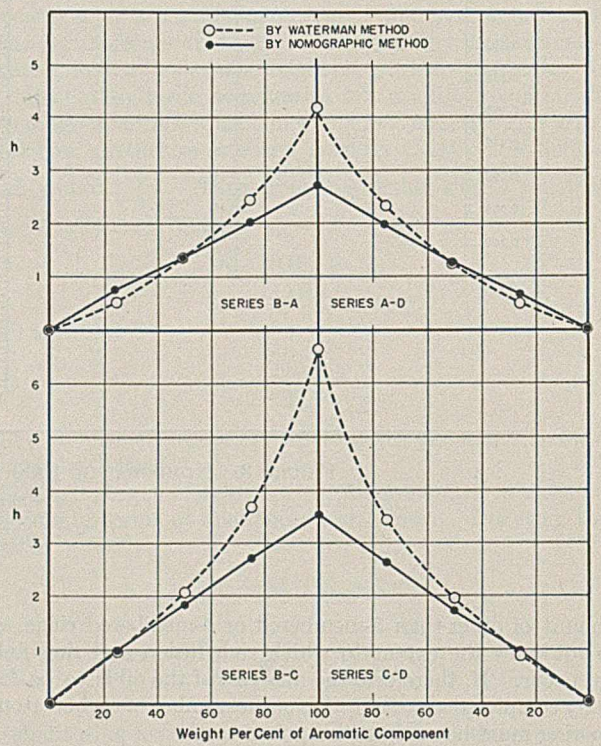

FIGURE 4. DEVIATIONS FROM LINEAR BLENDING OF h
Calculated by Waterman and nomographic methods

TABLE IV. MAXIMUM DEVIATIONS IN h DUE TO EXPERIMENTAL ERRORS

k	δh
1.7	0.14
2.0	0.10
2.5	0.07

TABLE V. MAXIMUM DEVIATIONS IN R DUE TO EXPERIMENTAL ERRORS

k	R	M	δR
1.7	2	200	0.22
2.0	4	500	0.48
2.5	4	500	0.40

TABLE VI. COMPARISON OF VALUES OF h OBTAINED FROM NOMOGRAPH AND BY HYDROGENATION

Oil	From Nomograph	By Hydrogenation	Difference
B-12	3.95	4.02	−0.07
B-16	3.35	3.28	+0.07
B-19	3.06	3.01	+0.05
C-13	3.46	3.43	+0.03
C-20	2.86	2.72	+0.14
C-30	1.71	1.70	+0.01
C-33	1.60	1.58	+0.02
C-35	1.54	1.50	+0.04
C-37	1.53	1.34	+0.19
C res.	0.03[a]	0.04	−0.01

[a] k = 2.0 assumed.

TABLE VII. MEASUREMENTS ON OIL BLENDS, USED FOR COMPARING NOMOGRAPHIC AND WATERMAN ANALYSES

Sample	d_4^{20}	n_D^{20}	$(n_F - n_C) \times 10^4$ (Abbé)	% C	% H	Aniline Point[a], °C.	M[b]	r_D^{20}	S	y
AAAA	0.9679	1.5451	166	87.60	10.54	8.0	263	0.3267	171	10.74
AAAB	0.9309	1.5214	140	87.27	11.50	41.6	264	0.3273	150	11.64
AABB	0.8969	1.4995	121	86.76	12.37	64.6	269	0.3277	135	12.48
ABBB	0.8651	1.4794	101	86.24	13.29	81.8	271	0.3280	117	13.35
BBBB	0.8356	1.4606	82	85.88	14.21	95.7	275	0.3281	98	14.20
BBBC	0.8801	1.4919	121	86.39	12.97	81.1	304	0.3296	137	13.05
BBCC	0.9304	1.5271	162	86.87	11.72	62.0	316	0.3305	174	11.89
BCCC	0.9842	1.5650	210	87.51	10.53	35.1	333	0.3309	213	10.74
CCCC	1.0460	1.6088	266	88.03	9.23	−15.4	346[c]	0.3309	254	9.49
CCCD	1.0012	1.5735	213	87.63	10.40	42.2	361[c]	0.3293	213	10.61
CCDD	0.9590	1.5407	168	87.27	11.53	70.0	380	0.3275	175	11.67
CDDD	0.9233	1.5129	128	86.86	12.51	88.9	386	0.3255	139	12.59
DDDD	0.8857	1.4835	88	86.19	13.63	105.5	392	0.3227	99	13.65
DDDA	0.9048	1.4976	106	86.86	12.93	90.4	356	0.3238	117	12.96
DDAA	0.9247	1.5126	124	87.08	12.12	71.4	317	0.3248	134	12.22
DAAA	0.9452	1.5279	144	86.98	11.31	48.0	295	0.3258	152	11.51

[a] Equal volumes of sample and aniline.
[b] Cryoscopic in benzene, except as noted. Trend of apparent M with changing concentration extrapolated to 0 concentration.
[c] Ebullioscopic in benzene.

TABLE VIII. COMPARISON OF RESULTS OF WATERMAN AND NOMOGRAPHIC ANALYSES OF OIL BLENDS

For uniformity, the Waterman analysis assumption that number of ring C atoms = $4R + 2$ was used for all nomographic analyses, although better assumptions might conceivably have been made.)

Blend	Nomographic h	Nomographic k	Rings per Molecule, R: Nomographic	Rings per Molecule, R: Waterman	Per Cent Carbon: Aromatic Rings, Nomographic	Aromatic Rings, Waterman	Naphthenic Rings, Nomographic	Naphthenic Rings, Waterman	Paraffinic Chains, Nomographic	Paraffinic Chains, Waterman
AAAA	2.70	2.12	3.02	3.25	36	55	36	23	28	22
AAAB	2.01	2.09	2.54	2.45	27	33	36	28	37	39
AABB	1.34	2.12	2.17	1.85	18	18	36	30	46	52
ABBB	0.73	2.00	1.63	1.47	10	7	33	34	57	59
BBBB	0.00	..	1.27	1.20	0	0	36	35	64	65
BBBC	0.97	2.37	1.87	1.47	13	13	30	23	57	64
BBCC	1.84	2.40	2.66	1.95	25	28	29	15	46	57
BCCC	2.70	2.40	3.55	2.87	36	49	29	6	35	45
CCCC	3.51	2.45	4.75	4.45	46	85	35	−7	19	22
CCCD	2.61	2.44	4.19	3.15	35	45	35	10	30	45
CCDD	1.70	2.46	3.75	2.75	23	26	38	20	39	54
CDDD	0.93	2.44	3.20	2.55	13	12	40	31	47	57
DDDD	0.00	..	2.63	2.70	0	0	44	44	56	56
DDDA	0.62	2.08	2.81	2.65	8	6	43	43	49	51
DDAA	1.25	2.16	2.99	2.65	17	16	43	37	40	47
DAAA	1.95	2.14	3.04	2.90	26	31	39	32	35	37

difficult to check the relations directly with actual oil samples. The direct measurement of h by hydrogenation is laborious in the extreme, and if synthetic mixtures are used for a test, there is always the objection that they are not typical of naturally occurring oils. Recourse must be had to indirect methods for an estimation of the accuracy of the relationships.

The unsaturated extraction cuts of Mair and associates represented in Figure 2 were carefully hydrogenated by those authors, and values of y, M, etc., were measured (*11*) on the saturates obtained (represented in Figure 1); these measurements enable the authors to calculate values of h. In Table VI these calculated values are compared with values from the nomograph. Since these oils were considered in the construction of the mean line of Figure 1 (though not of the line of Figure 2), they do not afford a wholly independent test of error, nor can errors of measurement be separated from those inherent in the relations. Nevertheless, the comparison does suggest magnitudes likely to be encountered.

Summarizing the results of such indirect evidence so far available, it is concluded that for substantially uncontaminated hydrocarbon mixtures the absolute error in h, the unsaturation in grams of hydrogen per 100 grams of oil, is not likely to exceed 0.2 (2.5 per cent in aromaticity), whatever the value of h, and the absolute error in R, the number of rings per average molecule, is not likely to exceed 10 per cent. In control testing of related samples, differences smaller than these magnitudes may be significant, because of the systematic nature of some of the contributing errors. In the example of Table III, the authors have used what they consider to be the proper number of significant figures for each quantity. This discussion of accuracy applies only to mixtures of hydrocarbons, substantially uncontaminated. The effects of nonhydrocarbon molecules have not been studied.

Self-Consistency of Nomographic and Waterman Analyses

If a series of blends is made from two component oils, certain constitutional properties of the blends vary in a linear manner with weight composition. This fact was utilized in testing the nomographic analysis, and the analysis of Waterman (*15*) in comparison with it. We are primarily interested in the accuracy with which the two analyses report unsaturation and ring content. Unsaturation may be represented by h, the number of grams of hydrogen which may be taken up by 100 grams of oil, and ring structure by $\frac{R}{M}$, the number of rings per unit molecular weight; in a two-component mixture these quantities are by definition linear functions of weight composition. The latter may readily be known without error; hence, plots of h and $\frac{R}{M}$ as obtained from the two analyses, against weight composition, afford a check on the self-consistency of each analysis. Percentages of aromatic, naphthenic, and alkyl carbon atoms are not linear functions of weight composition, and hence cannot be used for an accurate check.

Four series of two-component blends were prepared, utilizing two highly aromatic (A and C) and two completely saturated (B and D) petroleum oils as components. All measurements necessary to conduct both analyses on each blend were made. A complete record of these measurements is presented in Table VII. In Table VIII are recorded the results of the two analyses based on these measurements. Throughout each series of blends the value of k obtained in the nomographic analysis remains substantially constant, as it should if k is a function of the kind of aromatics present only, and independent of the quantity.

In Figure 4 values of h are plotted against weight composition for all series. Since h is not stated directly in the Waterman analysis, it was calculated from

$$h = \frac{201.6}{M}\left[\frac{M - 2.016 + 2.016R}{\frac{2805}{A} - 2.016}\right]$$

The quantities M, R, and A are explicitly stated in the analysis. As may be seen in considering the plots of h against composition, the deviations from linearity of values from the nomographic analysis are extremely small. For each series of blends, a straight line can be drawn through the origin so as to agree with all points to less than 0.1 in h (corre-

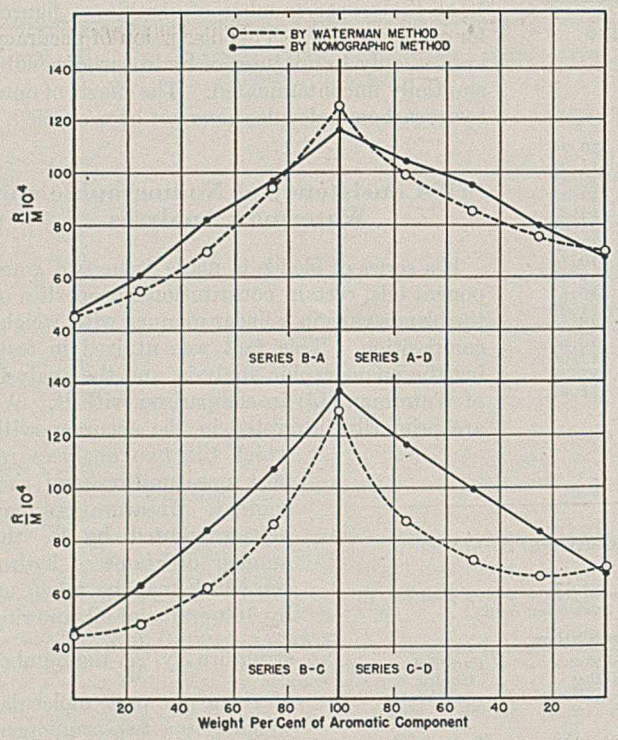

FIGURE 5. DEVIATIONS FROM LINEAR BLENDING OF $\frac{R}{M}$
Calculated by Waterman and nomographic methods

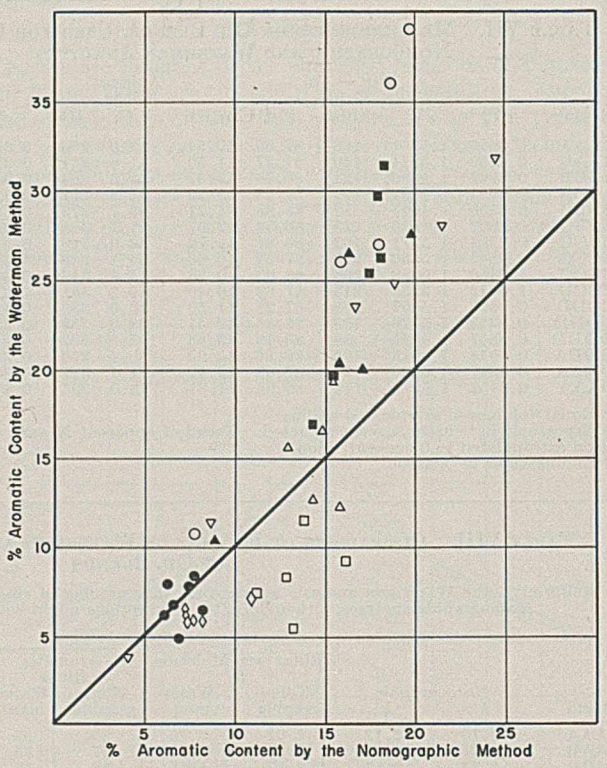

FIGURE 6. COMPARISON OF WATERMAN AND NOMOGRAPHIC AROMATICITIES

sponding to about 1 per cent aromatics). The deviations from linearity of values from the Waterman analysis are considerably larger. It is realized that the latter analysis is not intended for highly aromatic samples, and the agreement of the two methods for low aromaticities—i. e., $h < 1.5$—is fairly good. As aromaticity increases, however, the deviation becomes marked. In the extreme case, that of the pure component C, the Waterman analysis finds so high an aromatic content that a negative content of naphthenes (Table VIII) results. Considering next the plots of $\frac{R}{M}$ against composition, Figure 5, we see that, except for one series, there are only small consistent deviations from linearity in values calculated from the nomographic analysis, while all the values from the Waterman analysis yield lines with pronounced curvatures.

That the relatively higher aromaticities of the Waterman analysis are not due to peculiarities of the oils selected for blending, but to differences inherent in the two methods, is shown by Figure 6. This figure compares the results of analysis, by the two procedures, of distillation cuts from several petroleum crudes of widely different origin and character. The range of molecular weights represented in the various cuts is approximately 190 to 670. For every oil for which the nomographic analysis yields an aromaticity of 17 per cent or more, the Waterman analysis yields a higher aromaticity.

Acknowledgment

The authors wish to express their gratitude to Harry Sutherland for assistance in the preparation of the manuscript, and to D. C. Crowell for his painstaking work in the preparation and measurement of physical properties of samples.

Summary

A new type analysis of petroleum oils has been developed. In its development this method is consistent with the known data on both pure hydrocarbons and hydrocarbon oils, and appears to be equally applicable to straight-run and cracked products and to synthetic oils over all the range in which the requisite measurements are possible. An absolute check could not be made, but estimates indicated the errors to be relatively small and random. The analysis yielded self-consistent results, while that of Waterman, the only other generally used for the same purpose, did not.

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Evaluating Starches for Textile Purposes

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ALTHOUGH the starching of textile fabrics has long been a major operation in the textile and laundry industries, it is still a moot question as to how the efficiency of this process may be judged. Uniform methods for evaluating starched fabrics and starch mixtures have not been generally adopted. This has undoubtedly contributed to the tendency of the processors, when buying starching materials, to stress price considerations rather than quality of product. The result seems to be that the manufacturer of a new kind of starch finds himself in an unfavorable trade position. The way has not been cleared for him to prove with any degree of certainty and dispatch the superiority of his product over another for any particular use. Without doubt, the initial step to overcome this handicap would be to make available technical test methods by which it will be possible to measure certain of the desirable properties imparted to fabrics by the starching operation, and to measure certain properties of starch mixtures which affect the starching operation.

It is the purpose of this paper to describe test methods for appraising the properties of starched fabrics and starch mixtures, and to give data obtained by application of these methods.

Stickiness of Starched Fabrics during Drying

During either the ironing or drying over "dry cans" of starched fabrics, it is essential that these fabrics do not stick excessively to the heating surfaces. If sticking occurs, there is a tendency for the quality and the uniformity of the fabric to suffer—i. e., the number of surface imperfections may be increased and the fabric construction distorted. Then, too, the wear life of the fabric may be adversely affected by the pulling action required to remove the fabric from the heated surface. Lastly, excessive sticking may make it necessary to clean the drying surfaces so frequently that both the plant operating efficiency and capacity will be reduced.

To determine the relative stickiness which may be expected from a starch mixture, the apparatus developed in this laboratory and described in this paper is suggested. The method, in brief, consists in pressing a heated metallic surface against a starched sample prepared for ironing and subsequently determining the pull necessary to free the sample from that surface.

Method for Measuring Stickiness

The apparatus used consisted of a rotary electric ironer and a Jolly balance with a pulley setup so arranged that a horizontally exerted pull may be measured (Figure 1).

Fabric samples 20 × 7.5 cm. (8 by 3 inches) were starched according to a definite procedure. It may be preferable in some instances to simulate the particular practice of a given plant. In the work of this laboratory, a starch mixture was cooked with live steam, at a fixed steam valve setting, for exactly 30 minutes. The mixture was made up to volume with boiling water and thoroughly mixed.

The fabric samples were first immersed in water (50° C.) for 5 minutes, passed through a wringer having a given pressure setting, and then immersed for 3 minutes in the cooked starch mixture (90° C.). Each sample was separately "whizzed" in a small basket centrifuge for a given length of time—e. g., 10 seconds for samples which had been treated with thin-boiling starches and 30 seconds for those starched with thick-boiling starches. After centrifuging, the samples were placed in a small stoppered bottle to keep the moisture content fixed; they were then ready to be tested for stickiness.

It is suggested that the samples be starched and tested in sets of 10 and that after each set has been tested, but not until then, the heated shoe surface be thoroughly cleaned.

The sample to be tested was placed in a fixed position on or over the roll of the ironer, so that when the shoe was lowered it covered the center portion of the sample, leaving uncovered equal lengths on both sides of the shoe surface. When the shoe had been heated so that the thermometer, T (Figure 1), registered 150° C., the shoe was automatically lowered and allowed to rest under its own weight on the sample for 10 seconds, when the shoe carrying the sample with it was raised from the roll. During the drying interval, a hook was attached to the free end of the sample nearest to the balance; the hook in turn was attached to a cord, K, which had been brought under pulley A over movable pulley B under pulley C and fastened to reel E.

As soon as the shoe was raised, the reel was slowly turned, thus winding or shortening the length of the cord and, hence, exerting a horizontal pull on the sample. This pull was simultaneously transmitted to the Jolly balance and could be accurately determined from the extension of the balance spring. The reel was turned until the fabric sample had been pulled free from the shoe. At the moment of separation the elongation of the spring was read and considered as the final reading. The difference between the zero reading of the spring and the final reading,

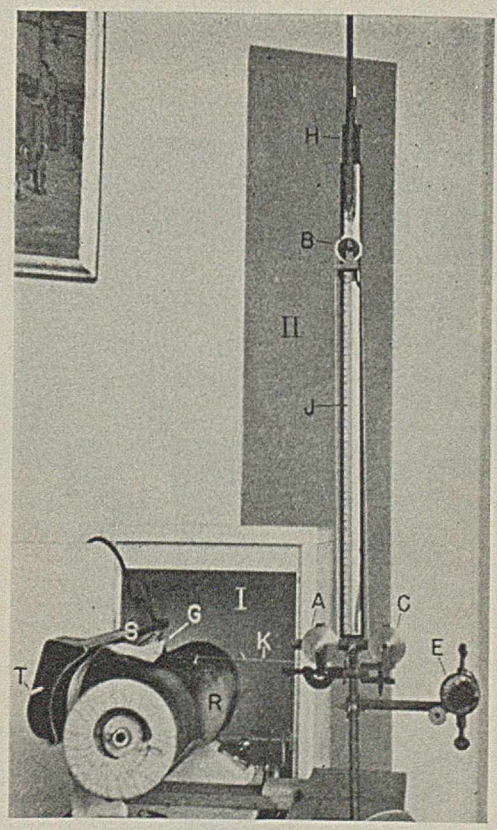

FIGURE 1. ROTARY ELECTRIC IRONER (I) AND JOLLY BALANCE SETUP (II)

A, C. Fixed-position low-fraction pulleys
B. Movable pulley
E. Reel
H. Spring
J. Jolly balance scale
K. Cord
R. Ironing roll
S. Heating shoe
T. Thermometer

TABLE I. STICKINESS DATA

Starch[a]	Stormer Viscosity	C. P. R. No.	Force Required to Release Sample[b]	Moisture Analysis[c]	Starched Fabrics Starch[d]
			Grams	%	%
A	75	2	598	205	8.4
B	170	1	940	231	8.7
C	38	45	231	156	5.2

[a] Starch concentration, 40 grams per liter of water.
[b] Mean deviation 10 sets of 10 determinations, 29 grams.
[c] Dried at 105° C. to constant weight.
[d] Desized with desizing agent, heated with water under reflux 1 hour, washed with hot water, and dried at 105° C.

after conversion into grams' pull from the calibration curve of the spring, was considered to be the stickiness factor for the starch mixture used or the force required to release the sample.

Table I gives data on stickiness.

STORMER VISCOSITY METHOD (used by the Laurel starch plant). Three grams of starch (dry basis) were wetted with 10 ml. of water in a 200-ml. Erlenmeyer flask, 100 ml. of boiling water were added with mixing, and the flask was fitted to an air condenser and immersed in a boiling water bath for 1 hour. Measurement was made at 90° C. in a Stormer viscometer. Values were expressed as the number of $^1/_5$ seconds required for spindle to make 100 revolutions when actuated by a 70-gram weight.

CORN PRODUCTS REFINING COMPANY FLUIDITY DETERMINATION. Starch (4.5 grams dry weight) was wetted with 10 ml. of water (23.89° C., 75° F.), stirred for 3 minutes after the addition of 90 ml. of 1 per cent sodium hydroxide (75° F.), allowed to stand 27 minutes (75° F.), and transferred to a funnel having a standardized orifice, and the milliliters which passed through the orifice in 70 seconds were determined. This volume was considered to be the C. P. R. number. The orifice opening had been made so that 100 ml. water passed through that opening in 70 seconds.

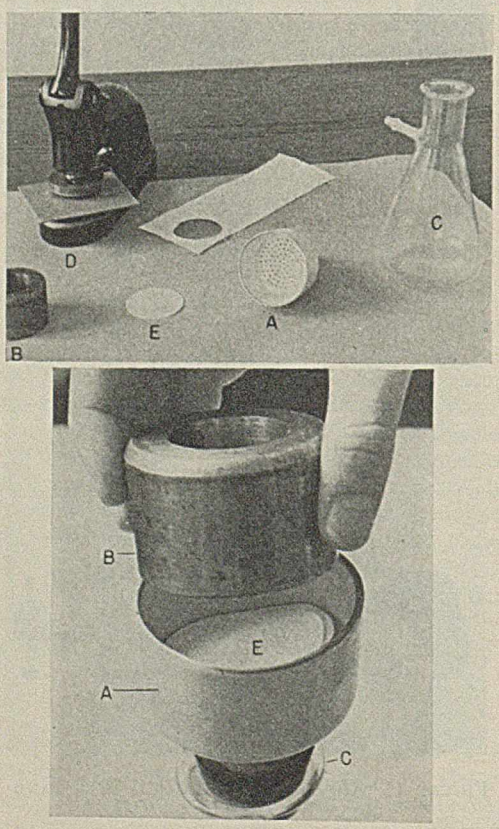

FIGURE 2. APPARATUS FOR DISK PENETRATION METHOD

A. Büchner funnel
B. Weight
C. Flask
D. Die to cut samples
E. Sample

Penetration of Starch Mixtures

The speed with which a starch mixture will penetrate a fabric is of considerable importance to the starching operator.

Analysis of cloth used (desized with desizing agent)
Warp, 62 ends per inch; count, 20.4
Filling, 48 pick per inch; count, 19
Selvage, 80 ends
Ends in body, 4530
Body of cloth reeded, 2 per cent
No. of ends per beam, 2305
Width of each beam 36.75 inches
Total possible number of ends, 4646
Width of reed 73.25 inches (30 dents per inch)

Plant production capacity is more or less dependent upon how quickly a starch mixture will be taken up by and pass through a fabric. As an aid in evaluating this factor the two methods described below are suggested. The first or disk method is a modification of the procedure described by the Laundry Owners' National Association (*2*).

DISK METHOD (for measuring penetration through interstices of fabric and by capillary flow). This method consists in determining under fixed conditions the number of wet fabric disks through which a given volume of starch will penetrate in a specified time (Table II).

Apparatus (Figure 2). *A*, Büchner funnel, 66 mm. in outside diameter, 56 mm. in inside diameter. *B*, weight, 510 grams. Outside diameter 56 mm., diameter of center opening 38 mm.

Procedure. Twelve desized fabric disks, *E*, 5 cm. (2 inches) in diameter, were placed in funnel *A*, and weight *B* was placed over the disks. Fifty milliliters of boiling water were poured through the opening in the weight onto the disks. After 3 minutes were allowed for the water to drain, 10 drops of the starch mixture (82.22° C., 180° F.) were added through the opening. At the end of another 3 minutes, the weight was removed, the disks were separated, and each was tested for starch with an iodine solution. The number of disks which gave a test for starch was considered to be the penetration number for that particular starch.

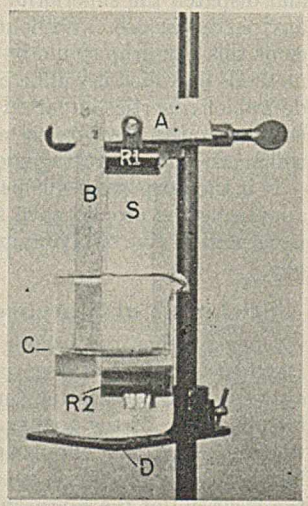

FIGURE 3. CAPILLARY PENETRATION APPARATUS

A. Rack to hold sample and ruler
B. Measuring ruler
C. Starch mixture level
D. Adjustable platform
S. Fabric sample
R_1, R_2. Clamps

METHOD FOR DETERMINING PENETRATION OF STARCH MIXTURES CAUSED BY CAPILLARY PULL. *Apparatus* (Figure 3). *A*, rack to hold sample, *B*, measuring scale, and *D*, adjustable platform.

Procedure. A strip of fabric 2.5 × 11.25 cm. (1 by 4.5 inches), which had been previously desized and then humidified at 65 per cent relative humidity and 21.1° C. (70° F.), was used as the test sample. Two small paper clamps were fastened to the strip, one at each end, and the sample was hung vertically from rack *A* parallel to ruler *B*.

A beaker containing a hot starch mixture (88.22° C., 180° F.) was raised under the free-hanging strip until the starch mixture reached a definite level—i. e., until the lower end of the strip had become immersed to about 1.25 cm. (0.5 inch) above the lower clamp. The platform, *D*, on which the beaker rested was made fast in this position and an initial reading of the liquid level on the fabric was taken immediately. At the end of 3 minutes the platform was lowered and several drops of an iodine solution were applied to the test sample. The iodine solution made possible an accurate reading of the vertical creep or rise of the starch mixture during a 3-minute period. The difference between the initial and the final reading was considered to be a measure of the penetration.

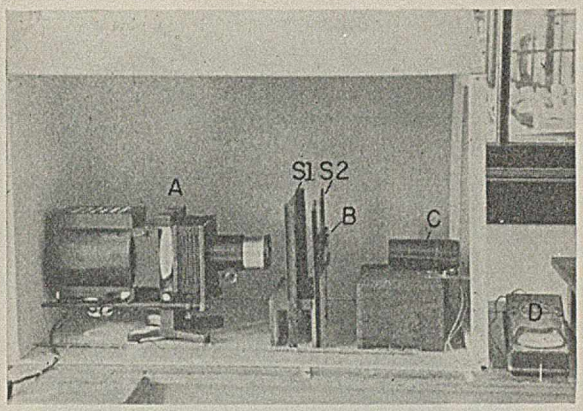

FIGURE 4. TRANSPARENCY DEVICE
A. Projector
B. Rack for holding sample slide
C. Photoelectric cell
D. Galvanometer
S_1, S_2. Cardboard shelf with slide

Transparency of Starch Films

A more or less continuous starch film is acquired on the surface of a fabric during the starching or finishing operation. In some types of fabrics it is almost imperative that this film be clear and transparent. A nontransparent film covering on a dyed fabric may change the tone of a previously matched shade, may detract from the brightness of a shade, or may partially hide the color of a delicate shade.

METHOD FOR DETERMINING TRANSPARENCY OF STARCH FILMS. The method suggested for measuring this property is based on a determination of the loss in light intensity caused by placing a starch film between a light of constant and known intensity, and a light intensity–measuring device. For convenience the starch film may be mounted on a microscope slide glass. The method makes use of a photoelectric cell, a galvanometer, and a 40-watt light bulb enclosed in a projector (Figure 4). The entire setup, with the exception of the galvanometer, is enclosed in a light-tight box. A thickness gage, calibrated in units of 0.0001 inch, is used to measure the thickness of the starch film.

Procedure. The thickness of a blank slide glass, at its center, was measured.

A galvanometer reading was taken when the blank slide had been placed in the rack between the light source and the photoelectric cell and considered to be the initial reading. [All but a 1.25-cm. (0.5-inch) portion at the center of the slide was screened from the light by the rack.]

The slide glass was coated by dipping in a hot starch mixture and the films on the slide were dried overnight at 65 per cent relative humidity and 21.1° C. (70° F.). (It may be advantageous to use a drying temperature corresponding to that used in the particular plant in which one may be interested.)

The coated slide was returned to the rack between the light and the cell and a final galvanometer reading was taken. [It is suggested that all determinations be made at fixed conditions of humidity and temperature, preferably at 65 per cent relative humidity and 21.1° C. (70° F.).]

The combined thickness of the two starch films at the center of the slide was determined by measuring total thickness and subtracting that of the glass.

TABLE II. PENETRATION OF STARCH MIXTURES THROUGH FABRIC INTERSTICES AND BY CAPILLARY FLOW

Starch[a]	Stormer Viscosity	C. P. R. No.	No. of Disks[b]
A	75	2	5
B	170	1	4
C	38	45	8

[a] 40 grams of starch per liter of water.
[b] Mean deviation was less than 0.3 disk.

TABLE III. PENETRATION CAUSED BY CAPILLARY PULL

Starch[a]	Stormer Viscosity	C. P. R. No.	Capillary Rise in 3 Minutes	Mean Deviation
			Mm.	*Mm.*
A	75	2	16.3	1.3
B	178	1	14.0	1.3
C	38	45	22.5	1.8

[a] 40 grams of starch per liter of water.

TABLE IV. TRANSPARENCY OF STARCH FILMS

Starch	Stormer Viscosity	C. P. R. No.	Light Intensity Loss	Film Thickness
			%	*Inch*
A	75	2	10.4	0.0040
B	170	1	26.3	0.0040
C	38	45	11.0	0.0040

Effect of Crushing Starched Fabrics

Of several fabrics, the one which is affected least by a given crushing or crumpling action will probably remain "fresh" for the longest time when worn in the form of a garment. In short, the method suggested consists in crushing a folded sample and then measuring the effect of that crushing action by determining the increase in the angle of bend from the normal caused thereby.

EXPERIMENTAL METHOD FOR MEASURING EFFECT OF CRUSHING. The apparatus was made up of a crushing device and a means for measuring the angle with the horizontal made by a freely hanging portion of a sample. More specifically, the former consisted of a modified dissecting microscope chassis, having as a fixed stage a piece of pressed board and as the adjustable stage a metal plate. A mirror graduated in millimeter squares was mounted directly in back of the device. By the use of this mirror it was possible accurately to adjust the height of the movable stage. The means for measuring the angle consisted of a smooth metal plate under the sample, a glass plate partially to cover the sample, and a mirror mounted vertically which was calibrated in degrees from 0° to 90° C.

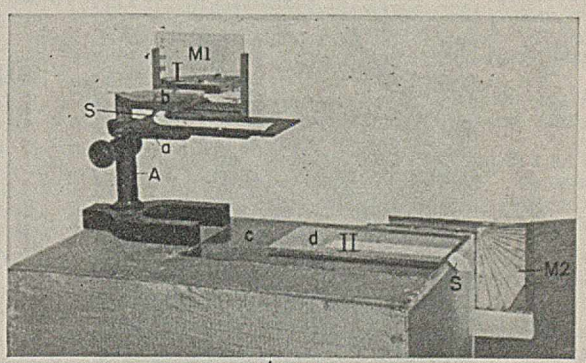

FIGURE 5. CRUSHING DEVICE (I) AND ANGLE-MEASURING DEVICE (II)
A. Modified dissecting microscope
a. Fixed stage
b. Adjustable stage
c. Metal plate under sample
d. Glass plate, partially covers sample
M1. Mirror
M2. Mirror calibrated in degrees
S. Sample

The test sample consisted of a regular piece of cloth cut 2.5 × 15 cm. (1 by 6 inches), one end of which had been trimmed to a point by cutting back from the center of the end to points 2.5 cm. (1 inch) along the sides of the strip. It was placed flat on the stage (*a*, Figure 5) with the marked side up and the pointed end towards *A*. The movable plate, *b*, was brought down to a position 1 mm. distant from *a*. The sample was allowed to remain in this position for 1 minute, after which the plate was lifted. The sample strip was moved to the metal plate, *c*, with the marked side down and the pointed end toward the end of the box having the extending mirror. The edge of the square glass plate, *d*, was placed along the 2.5-cm. (1-inch) line on the sample and thus all but the 2.5-cm. (1-inch) length, including the pointed end, was covered.

TABLE V. EFFECT OF CRUSHING STARCHED FABRICS

Starch[a]	Stormer Viscosity	C. P. R. No.	Angle of Bend	Mean Deviation
A	75	2	29.1	1.3
B	170	1	29.4	1.3
C	38	45	24.7	2.0

[a] 40 grams of starch per liter of water.

TABLE VI. STIFFNESS OF STARCHED FABRICS[a]

Starch[b]	Stormer Viscosity	C. P. R. No.	Stiffness Factor	Mean Deviation
A	75	2	33.11	2.4
B	170	1	31.39	1.7
C	38	45	30.10	1.3

[a] Fabrics were conditioned and tested at 65 per cent relative humidity and 70° C.
[b] 40 grams of starch per liter of water.

The sample and the glass plate were slid along the metal plate until the edge of the glass and, hence, the 2.5-cm. (1-inch) line coincided with the edge of the metal plate. In this position 2.5 cm. (1 inch) of the sample extended freely beyond the end of the box. After 10 seconds the angle of bend which the extending fabric made with horizontal was read directly from the mirror, M_2, mounted in back of the box. This angle was considered to be the zero or normal reading of the strip.

Again the sample was placed on stage *a*. The pointed end was lightly folded up and over, forming a U-shaped bend along the line 2.5 cm. (1 inch) from the tip. Plate *b* was then lowered to a position 1 mm. from *a*, creasing the sample along the 2.5-cm. (1-inch) line, and held in this position for one minute. The sample was then laid flat and pressed by lowering *b* to within 1 mm. of *a* and holding it there for one minute. Again the angle of bend made by the sample with the horizontal was determined as described above. The difference between the two readings, before and after the sample had been exposed to the crushing action, was considered to be the angle of bend caused by that action.

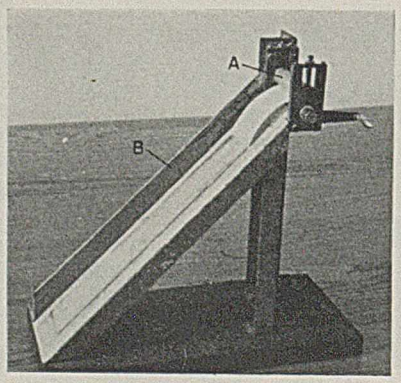

FIGURE 6. STIFFNESS TESTER
A. Set of rubber rolls
B. Shelf, set at 45° angle with vertical

The angle of bend here given represents the angle of deformation from the normal caused by a given crushing action on the fabric—i. e., when the adjustable stage was lowered to point 1 mm. from the fixed stage. It follows that the smaller this angle the less it has been affected by this action.

Stiffness of Fabrics

One of the factors which, to a large extent, determines the so-called "body" and "handle" of a fabric is stiffness. This property may be judged by the results obtained by the use of the method of Peterson and Dantzig (*1*, *4*).

EXPERIMENTAL METHOD FOR MEASURING STIFFNESS. This method (*4*) depends upon the deformation of a supported strip bent under its own weight. The apparatus consisted of a set of rubber-covered rolls through which samples passed in a horizontal plane and a shelf fastened at a 45-degree angle with the vertical at the zero reading of the scale (Figure 6).

A fabric sample 5 × 10 cm. (2 by 4 inches) was inserted lengthwise between the rollers and slowly fed through those rollers in the direction of the scale until the end of the sample barely touched the metal shelf. The distance in millimeters from the nip of the rolls to the point on the shelf where the strip made contact multiplied by 0.43 was considered to be the stiffness of the fabric tested. It follows that the larger the reading the greater will be the stiffness.

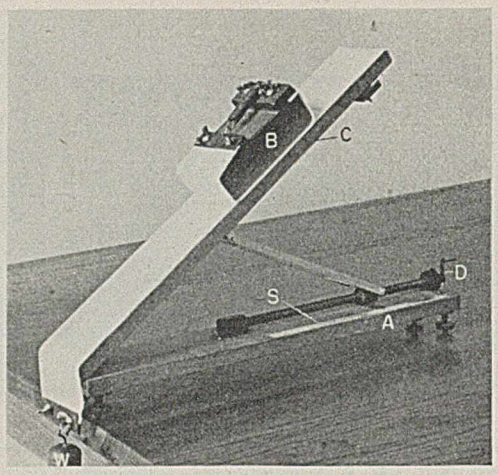

FIGURE 7. SMOOTHNESS TESTER
A. Base board
B. Sliding block, covered with sample
C. Adjustable board, covered with sample
D. Crank to adjust angle between *A* and *C*
S. Scale measuring between *A* and *C*
W. Weight to hold sample under tension

TABLE VII. SMOOTHNESS OF STARCHED FABRICS

Starch[a]	Stormer Viscosity	C. P. R. No.	Smoothness Factor	Mean Deviation
A	75	2	0.6395	0.0209
B	170	1	0.5459	0.0152
C	38	45	1.0724	0.0262

[a] 40 grams of starch per liter of water.

Smoothness of Fabrics

The surface characteristics of a fabric are undoubtedly made up of a number of contributing properties, which, in turn, have a bearing upon the "feel", appearance, and, in some instances, the utility of the fabric. One of these properties is smoothness. The method of Mercier (*3*) for measuring the coefficient of friction of a fabric may be used to evaluate the smoothness of a fabric.

METHOD FOR MEASURING SMOOTHNESS OF STARCHED FABRICS (*3*). Two boards, each 55 cm. (22 inches) long and 15 cm. (6 inches) wide, are hinged together at one end. One of the boards rests on three small supports which can be adjusted to bring the board to a horizontal position. The angle between the boards can be changed by a screw arrangement, and this angle or its tangent read on a scale on the upper surface of the horizontal board. The block of wood 20 cm. (8 inches) long and 15 cm. (6 inches) wide weighs about 560 grams (1.25 pounds). This block of wood and the inclined plane are covered with the fabric to be tested. Clamps for holding the fabric on the block and on the inclined plane are shown in Figure 7. A spring keeps the fabric under tension on the block and a small weight clamped to the lower end of the fabric keeps the fabric taut on the inclined plane during the test.

The wooden block, covered with sample of fabric to be tested, was placed on the inclined plane which had also been covered with another piece of the same fabric. The angle between the inclined plane and the horizontal was then increased until, with the assistance of light tapping on the inclined board, the block began to slide. A reading of the tangent of the angle thus made by the inclined plane was considered to be the smoothness factor of the fabric tested. The smaller the angle, and, hence, the smaller the tangent, the greater will be the smoothness.

Summary

Methods are described for evaluating starches for use on fabrics; these include methods for judging the starch mixtures from a processing or plant operating viewpoint and methods for evaluating the quality of starched fabrics. The former may be used for measuring the stickiness of starch mixtures during ironing, and the penetration of such mixtures; the latter may be used to measure the transparency of starch films, and to determine the smoothness, stiffness, and resistance to crushing of starched fabrics.

These methods may also be used to advantage in evaluating other sizing or finishing agents.

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Use of Enzymes in the Refractometric Method for Egg Solids

A Production Control Method

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IN ORDER to prepare eggs of definite solids content, the frozen egg industry requires a rapid method of determining egg solids. Ordinary drying methods, such as the official A. O. A. C. vacuum method (*3*), require too long a time to serve as production control methods. Among the shorter procedures developed, the refractometric method (*2*, *4*) has become important. In applying this method, the refractive index of the egg is determined and, from suitable charts previously prepared on eggs of known solids content, the refractive index is converted into per cent solids. Egg white solids can be determined by this method, but when whole eggs or yolks are examined, the line of demarcation between the light and dark fields of the refractometer is indistinct, and accurate determinations cannot be made (*4*).

Recently, Cahn and Epstein (*6*) reported that the addition of an electrolyte renders the egg optically homogeneous, with the result that sharp contrast between the light and dark fields is obtained, permitting accurate determinations. The use of an electrolyte for this analytical purpose is covered by a patent to Cahn and Epstein (*6*).

The difficulty of determining solids of whole eggs and yolks with a refractometer is occasioned by the turbidity of these products. This turbidity causes a reduction in the amount of light passing through the sample and, in addition, causes scattering of the light into the dark portion of the refractometer field, thereby reducing the contrast between the two portions of the field and rendering the line of demarcation indistinct. Any suitable agent which will remove this turbidity should make it possible to obtain accurate readings.

The turbidity of eggs resides chiefly in the yolk. To some extent it is due to an inhomogeneity resulting from the presence of globules of yellow and white yolk which are probably associated with the laminar structure of the yolk (*7*). In addition to this, the chief protein of the yolk lecithovitellin, is probably not completely in solution in the yolk, since it is not a readily soluble protein (*5*). It is well known, however, that lecithovitellin is soluble in the presence of electrolytes (*1*, *5*), and this probably accounts for the clarifying action of electrolytes in the method of Cahn and Epstein (*6*). Since solution of protein material is involved, it seems obvious that a proteolytic enzyme would be capable of accomplishing the desired clarification by a process of enzymatic digestion.

The choice of a particular enzyme for this purpose is governed by availability, ranges of pH and temperature in which it exerts its action, and speed of reaction. The purpose of this paper is to present refractometric methods of determining solids of whole eggs and yolks in which essential optical homogeneity of sample is obtained by digestive action of the enzymes trypsin and papain.

Materials and Apparatus

The trypsin was a dry preparation of the Difco Laboratories, Detroit, Mich., designated as Trypsin 1:250. The papain was a dry preparation obtained from Parke, Davis and Company, Detroit, and was the dried fruit juice of the papaw tree (*carica papaya* Lin).

Eggs were obtained in the local markets as shell eggs and broken out as needed. Varying solids contents were obtained by changing the ratio of yolks to white.

A Zeiss sugar refractometer having the range 1.33 to 1.54 was employed. A constant-temperature water bath, operating either at 30° or 50° C. ± 0.1°, supplied water to the instrument for uniform temperature. A 25-watt incandescent lamp provided the illumination for the refractometer.

Trypsin Methods

WHOLE EGGS. The dry enzyme was dissolved in water containing sodium hydroxide according to the following formula:

Trypsin Solution A

Trypsin	500 grams
Water	770 cc.
0.25 *N* NaOH	800 cc.

This solution had a refractive index of about 1.377 at 30° C., approximately that for whole eggs. It was found that 1.8 cc. of this solution, added to 10 grams of whole eggs, gave a sharp line on the refractometer field. A reasonable quantity of solution in excess of this does not affect the refractive index reading; less than this quantity will yield a distinct field with time, but since in practice the minimum possible time is desirable, an amount was chosen (1.8 cc. per 10 grams of egg) which would yield a clear field in about 15 seconds. The incorporation of sodium hydroxide in trypsin solution A rendered the mixture of enzyme and egg more alkaline—(i. e., brought it closer to the range of optimum activity for trypsin)—and in this way materially accelerated the digestion. The amount of sodium hydroxide used in this way was not sufficient by itself to render the egg optically homogeneous.

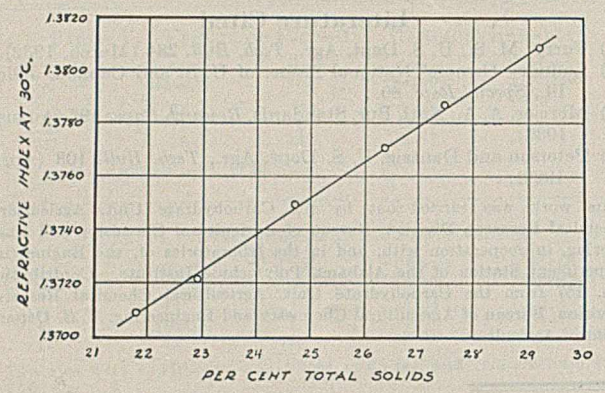

FIGURE 1. CHANGE OF REFRACTIVE INDEX WITH TOTAL SOLIDS FOR WHOLE EGGS WITH TRYPSIN SOLUTION A

That a true enzymatic digestion occurred in as short a period as one minute was demonstrated as follows:

It was assumed that enzymatic digestion of the egg would increase the water-soluble nitrogen. To 25 grams of whole egg 4.5 cc. of trypsin solution A were added. The mixture was stirred for one minute at 27° C. and then diluted with 100 cc. of water. To this were added 100 cc. of a 5 per cent solution of trichloroacetic acid. After one-half hour, the precipitated proteins were filtered off and a Kjeldahl nitrogen determination was made on the filtrate. The same procedure was repeated with heat-inactivated trypsin solution, with water in place of the trypsin solution, and with sodium chloride instead of the trypsin solution. In addition, in place of the egg, 25 grams of water were used with 4.5 cc. of active trypsin solution A and with 4.5 cc. of heat-activated trypsin solution A.

TABLE I. WATER-SOLUBLE NITROGEN DATA ON WHOLE EGG MIXTURES

Mixture	Soluble N in Filtrate	
	%	%
1. 4.5 cc. of trypsin solution A, 25 grams of egg	8.24	7.98
2. 4.5 cc. of trypsin solution A, 25 grams of water	5.81	5.68
3. 4.5 cc. of water, 25 grams of egg	1.52	1.44
4. 4.5 cc. of water, 0.6 gram of NaCl, 25 grams of egg	1.52	1.46
5. 4.5 cc. of heat-inactivated trypsin solution A, 25 grams of egg	8.14	8.40
6. 4.5 cc. of heat-inactivated trypsin solution A, 25 grams of water	6.58	7.00
Increase in soluble N due to active trypsin (mixture 1 minus sum of 2 and 3)	0.91	0.86
Increase in soluble N due to inactive trypsin (mixture 5 minus sum of 3 and 6)	+0.04	−0.04

The data of Table I show that a true increase in water-soluble nitrogen occurs only when active trypsin is used. In arriving at the amount of the increase, it is necessary to deduct the contributions of soluble nitrogen made by the enzyme and by the egg, which are not a result of enzymatic digestion of the egg protein. It is noteworthy that sodium chloride, which may be taken as a typical electrolyte, does not increase the water-soluble nitrogen of the egg protein under the conditions of this test. While the electrolytes may cause solution of proteins, such as lecithovitellin, this action does not alter the proteins and, upon dilution, they are precipitated intact. The action of enzymes, however, is to dissolve the proteins by hydrolyzing them to smaller units, which are soluble.

Heat-inactivated trypsin solution A does not act to yield a distinct field in the refractometer.

The curve shown in Figure 1 gives the variation of the refractive index at 30° C. with thatof the total solids for whole eggs treated with 1.8 cc. of trypsin solution A per 10 grams of egg. Oven moisture solids were obtained by vacuum-drying the samples for 2 to 3 hours at 100° C. (Approximately two dozen eggs were used in obtaining the curve shown in Figure 1.)

The data of Table II show the temperature variation of the refractive index of a sample of whole eggs. From these data, a temperature coefficient of 0.00016 unit per degree centigrade is computed. To obtain an accuracy of 0.15 per cent solids, the temperature of the refractometer must, therefore, be kept within 1.3° of 30° C. (0.00021 refractive index unit equivalent to 0.15 per cent whole egg solids).

TABLE II. CHANGE OF REFRACTIVE INDEX OF A WHOLE EGG SAMPLE WITH TEMPERATURE

Temperature, °C.	Refractive Index	Temperature, °C.	Refractive Index
26.2	1.3784	31.0	1.3775
27.0	1.3782	33.0	1.3772
29.0	1.3778	35.0	1.3770

The procedure in making a solids determination on whole eggs is as follows:

Trypsin solution A (1.8 cc.) is added to 10 cc. (roughly equivalent to 10 grams) of whole egg, the two are intimately mixed, and several drops of the mixture are placed on the prisms of the refractometer. The prisms are immediately closed and after 30 seconds (to allow for temperature adjustment), the refractive index is read to the nearest 0.0001 unit. The solids figure is obtained by reference to Figure 1. In practice the refractometric determination yields results agreeing with vacuum-oven method within 0.15 per cent solids.

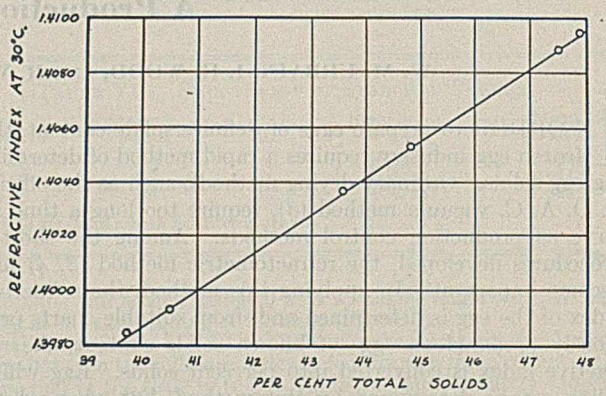

FIGURE 2. CHANGE OF REFRACTIVE INDEX WITH TOTAL SOLIDS FOR YOLKS TREATED WITH TRYPSIN SOLUTION B

YOLKS. The reagent for yolks differs from the whole egg reagent chiefly in its refractive index which has been adjusted to a higher value in order to be approximately the same as that of the yolks. The following approximate formula has been devised:

Trypsin Solution B			
Trypsin	500 grams	0.25 *N* NaOH	800 cc.
Water	700 cc.	Dextrose	1100 grams

The reagent should have a refractive index of about 1.403 at 30° C. It may be necessary to vary the amount of dextrose to obtain this value. Treatment of 10 grams of egg with 1.5 cc. of this solution yields a refractometer field with a sharp line of demarcation in about 15 seconds.

Table III lists data for yolks corresponding to those of Table I for whole eggs, demonstrating that true enzymatic digestion occurs with the active enzyme.

TABLE III. WATER-SOLUBLE NITROGEN DATA ON YOLK MIXTURES

Mixture	Soluble N in Filtrate	
	%	%
1. 3.6 cc. of trypsin solution B, 25 grams of egg	6.72	6.08
2. 3.6 cc. of trypsin solution B, 25 grams of water	4.14	4.00
3. 3.6 cc. water, 25 grams of egg	1.40	1.30
4. 3.6 cc. of water, 0.6 gram of NaCl, 25 grams of egg	1.40	1.26
5. 3.6 cc. of heat-inactivated trypsin solution B, 25 grams of egg	6.24	6.08
6. 3.6 cc. of heat-inactivated trypsin solution B, 25 grams of water	4.80	4.84
Increase in soluble N due to active trypsin (mixture 1 minus sum of 2 and 3)	1.18	0.78
Increase in soluble N due to inactive trypsin (mixture 5 minus sum of 3 and 6)	+0.04	−0.06

The curve shown in Figure 2 gives the variation of the refractive index at 30° C. with that of the total solids for yolks treated with 1.5 cc. of trypsin solution B per 10 grams of egg. Each experimental point represents the determination upon approximately 120 grams of mixed yolks.

The temperature variation of the refractive index of yolks is shown in Table IV. From these data, a temperature coefficient of 0.00019 unit per degree can be computed. To obtain an accuracy of 0.15 per cent solids, the temperature of the refractometer must, therefore, be kept within 1.1° of 30° C. (0.00021 refractive index unit equivalent to 0.15 per cent yolk solids).

The procedure of making a solids determination on a yolk sample is identical with that given for whole eggs, except that 1.5 cc. of trypsin solution B per 10 cc. of yolk are required.

Papain Methods

Papain differs from trypsin in several respects. The commercially available papain is not so active as trypsin 1:250; its optimum activity lies in a more acid range; and the temperature of optimum activity is considerably higher. These facts alter the procedure in using papain.

Whole Eggs. A suitable reagent for whole egg determinations may be prepared according to the following formula:

Papain Solution A		Papain Solution A	
Papain	40 grams	2 *N* H_2SO_4	23.4 cc.
Water	100 cc.	Dextrose	15.6 grams

Acid is added to this reagent to lower the pH of the reagent-egg mixture more nearly to approximate the pH range for optimum activity of papain. Commercial papain contains appreciable insoluble material which it is advantageous to remove by centrifuging before using this reagent.

Table IV. Change of Refractive Index of Yolks with Temperature

Temperature, ° C.	Refractive Index	Temperature, ° C.	Refractive Index
27.0	1.4072	33.0	1.4061
29.0	1.4070	35.0	1.4057
31.0	1.4065		

In order to obtain reasonably rapid digestion with papain it is necessary to raise the temperature to about 50° C. A simple way to accomplish this is to raise the temperature of the refractometer to 50° C. and permit the digestion of the sample to occur on the prisms. Treatment of 10 grams of whole egg with 1 cc. of papain solution A will yield a distinct refractometer field in 3 to 4 minutes at 50° C. When the temperature of the refractometer is as high as 50° C., extreme care must be exercised to avoid loss of moisture from the sample on the prisms.

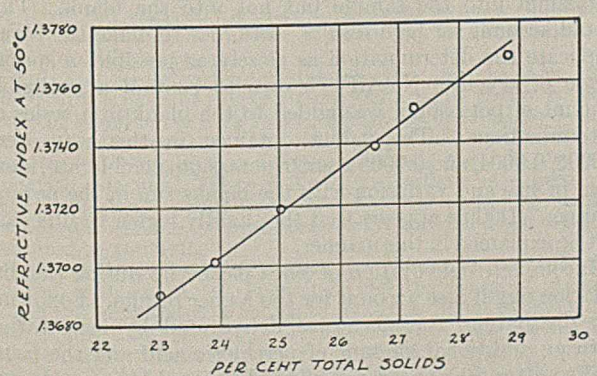

Figure 3. Change of Refractive Index with Total Solids for Whole Eggs Treated with Papain Solution A

Digestion data, similar to those given for trypsin, have demonstrated that the removal of the turbidity of whole eggs involves a true enzymatic digestion.

The curve shown in Figure 3 gives the variation of the refractive index at 50° C. with total solids for whole eggs treated with 1 cc. of papain solution A per 10 grams of whole egg. By controlling the temperature of the refractometer within the limits given for whole eggs under trypsin methods, solids determinations can be made with an accuracy of 0.15 per cent solids by using papain solution A and the curve of Figure 3.

Yolks. A suitable reagent for yolks may be prepared according to the following formula:

Papain Solution B	
Papain	40 grams
Water	100 cc.
Dextrose	Sufficient to yield a refractive index for the solution at 50° C. of 1.403

Treatment of 10 grams of yolk with 1 cc. of this reagent will yield a distinct refractometer field in 3 or 4 minutes at 50° C. Because of the more acidic nature of the yolks, no added acid is required. Digestion data here, too, support the belief that the process is dependent upon enzymatic digestion.

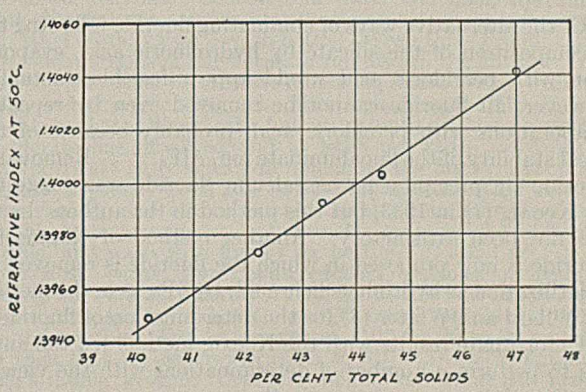

Figure 4. Change of Refractive Index with Total Solids for Yolks Treated with Papain Solution B

Figure 4 shows the solids–refractive index curve for yolks at 50° C. using this reagent. With proper temperature control, an accuracy of 0.15 per cent solids can be obtained.

Summary

A refractometric method of determining solids of whole eggs and yolks is described in which these products are rendered essentially optically homogeneous by enzymatic digestion, so that their refractive indices can be determined with ease and accuracy.

Specific procedures are given employing either trypsin or papain.

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Direct Determination of Potassium in Silicate Rock

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THE advent of the triple acetate methods for the determination of sodium has made possible the direct determination of sodium in silicate materials. The silicate may be decomposed by treatment with hydrofluoric acid, the fluoride removed by evaporation with sulfuric acid, and the sodium precipitated immediately as magnesium or zinc sodium uranyl acetate, in which form it is weighed. Unfortunately, sulfate cannot be present in the determination of potassium when it is weighed as perchlorate. The Berzelius method, in which the sulfate is removed by precipitation as barium sulfate and the excess barium and other heavy metals are removed by precipitation with ammonia and ammonium carbonate, is long, and the coprecipitation of potassium with the barium sulfate makes the results somewhat uncertain. The J. Lawrence Smith method, in which the decomposition is accomplished by a fusion with ammonium chloride and calcium carbonate, is extremely time-consuming but yields results beyond reproach.

Of the alternative ways of eliminating fluoride following the decomposition of the silicate by hydrofluoric acid, evaporation with perchloric acid would appear feasible; actually, however, all fluoride cannot be removed even by repeated evaporations with perchloric acid, probably because of the great stability of the fluoaluminate ion, AlF_6^{---}. Removal of fluoride by precipitation as calcium fluoride was suggested by Koenig (*1*) in 1933, but this method in the authors' hands has not been satisfactory. Another method of eliminating fluoride is now proposed in which the fluoride is removed by volatilization as hydrofluosilicic acid, H_2SiF_6, as in the method of Willard and Winter (*5*) for the determination of fluoride.

Unfortunate results with the Koenig method led the authors to perform a number of determinations with the view of locating the sources of error in the procedure.

In a series of determinations of the sodium plus potassium in Bureau of Standards Sample No. 70, a high-potassium feldspar, the results obtained were highly erratic and invariably low, sometimes by as much as 50 mg. in the 0.2440 gram of sodium plus potassium chlorides which should have been derived from a 1-gram sample. The mixed chlorides obtained were also found to contain appreciable quantities of fluoride as measured by the bleaching effect on pertitanic acid. Treatment with calcium hydroxide does not remove fluoride completely in the analysis of a silicate, again probably because of the stability of the fluoaluminate ion. The calcium oxide used was prepared by ignition of the special grade of calcium carbonate usually used for J. Lawrence Smith fusions. In some experiments the calcium oxide was slaked slowly by treatment with steam prior to use, with the object of improving the physical character of the calcium hydroxide. This had no beneficial effects on the results, nor did more extensive washing during the filtrations. Finally, weighed portions of pure potassium chloride and mixtures of pure potassium chloride and pure sodium chloride were treated with hydrofluoric acid, the solutions evaporated to dryness, dissolved, added to the calcium oxide, and carried through the procedure exactly as in the silicate analysis. The results were erratic and low by as much as 30 mg., samples of about 1 gram having been taken. No fluoride was found in the chlorides obtained. It thus appears that the alkalies are retained somewhere in the process, undoubtedly with the calcium hydroxide. In view of these results the authors considered the problem as still lacking an adequate solution.

The separation of fluoride by distillation as hydrofluosilicic acid has received considerable study since it was originally proposed and the conditions and completeness of the separation have been confirmed. As the distillation may be effected with either sulfuric or perchloric acid to elevate the temperature of distillation, the obvious choice is perchloric acid, which yields directly the solution of perchlorates needed for the determination of potassium by the perchlorate method and also furnishes a method of dehydrating any residual silica prior to the determination of the potassium. As no ammonium salts are introduced during the analysis their removal is obviated.

Since in this case it is immaterial whether some perchloric acid is distilled with the hydrofluoric acid, the distillation was carried out at temperatures of 140–150° C.—that is, somewhat above the 135° recommended by Willard and Winter—and the time required for the distillation was thus shortened. The distillation is best effected by injecting steam into the distillation flask and regulating the flame below the flask to give the desired temperature.

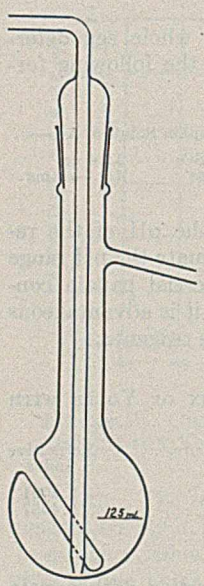

FIGURE 1

In final form the all-Pyrex distillation vessel shown in Figure 1 was employed. If the bulb were attached to the stem by a ground joint, the removal of the contents would be somewhat facilitated. A volume of distillate of 400 to 500 ml. is sufficient to remove all fluoride. As is customary in the analysis of the alkali metals, blanks must be run concurrently. Since the apparatus used is platinum and Pyrex and the time required for the determination is not great, the blank is very small and consistent. Twenty blank determinations run during the course of the work gave an average value of 0.0005 gram of potassium perchlorate, the minimum and maximum values being 0.0003 and 0.0009 gram.

Table I indicates that the method tends to give slightly high results. This was thought to be due possibly to the fact that fluoride was taken into the distilling flask in the samples but not in the blank, in which no aluminum was present; this fluoride might then attack the distilling flask, introducing potassium into the sample but not into the blank. This would account for high results. In order to make the blank duplicate the determination as closely as possible, a special grade of cryolite, Na_3AlF_6, known to contain a negligible amount of potassium, was added to the blank in a series of determinations. The values obtained in this way were 0.0005, 0.0004, and 0.0008 gram of potassium perchlorate, identical in size and variation with the blanks run in the normal manner. It thus appears that the slightly higher results cannot be explained in this manner.

Incomplete dehydration of silica picked up during the distillation might also account for the higher results. Following the distillation, the silica was dehydrated by evaporation with an additional amount of perchloric acid and the usual boiling after the appearance of perchloric acid fumes. It was improbable that any silica contaminated the potassium salt, but as a check the potassium perchlorate finally obtained and

TABLE I. DETERMINATION OF POTASSIUM IN BUREAU OF STANDARDS SAMPLES

Analysis No.	Weight of Sample	Weight of $KClO_4$	K_2O Present Found	K_2O Present B. of S.
	Grams	*Gram*	%	%
	B. of S. No. 70 Feldspar			
1	1.0000	0.3707	12.59	12.58
2	1.0004	0.3708	12.59	
3	1.0000	0.3730	12.66	
4	1.0011	0.3741	12.70	
5	1.0000	0.3676	12.48	
6	1.0000	0.3680	12.49	
7	1.0000	0.3680	12.49	
8	1.0010	0.3676	12.48	
	B. of S. No. 99 Soda Feldspar			
1	3.000	0.0396	0.44	0.41
2	3.000	0.0412	0.46	
3	3.000	0.0400	0.45	
4	3.000	0.0336	0.38	
	B. of S. No. 91 Opal Glass			
1	2.000	0.1959	3.32	3.25
2	2.000	0.1936	3.28	
3	2.000	0.1952	3.31	
4	1.0028	0.0988	3.34	
5	1.0006	0.0955	3.23	
6	1.0010	0.0948	3.21	
	B. of S. No. 89 Lead-Barium Glass			
1	2.000	0.4908	8.33	8.40
2	2.000	0.4965	8.43	
3	2.000	0.4915	8.34	
	B. of S. No. 97 Flint Clay			
1	1.0015	0.0176	0.58	0.54
2	1.0008	0.0174	0.57	
3	1.0016	0.0178	0.59	
	B. of S. No. 98 Plastic Clay			
1	2.0000	0.1896	3.22	3.17
2	2.0010	0.1891	3.21	
3	1.5000	0.1411	3.19	
4	1.5015	0.1439	3.25	

weighed was dissolved in water and the crucible dried and weighed. In a few cases in earlier work some silica was found but none if the dehydration was properly performed.

Results by students on the silicate samples used as unknowns at the University of Michigan and at Iowa State College have also shown that the method tends to give slightly higher values for potassium than the J. Lawrence Smith fusion followed by the perchloric acid method. The method is more rapid than the Smith method and requires less applied time. Sulfates must, of course, be absent.

Procedure

Weigh 1 to 2 grams of the sample, depending on the potassium content, into a 20-ml. platinum crucible, moisten with water, and add 3 ml. of 70 per cent perchloric acid and 10 ml. of hydrofluoric acid. Stir well with a platinum wire or the rubber end of a policeman, taking care not to let the acid touch the glass; rinse, remove the stirring rod, place in a Hillebrand evaporator in a good hood, and allow to evaporate to dryness. Moisten with water, add 2 to 5 ml. more of hydrofluoric acid, depending on the size of the sample taken, and 3 ml. of perchloric acid, stir well, and again evaporate to dryness. Transfer the salts to a 125-ml. Pyrex distilling flask, using a long-stemmed funnel and making sure that all insoluble material is transferred to the flask. Blanks should be started with the samples and carried through the entire procedure.

The use of a two-hole rubber stopper carrying the thermometer and steam inlet tube is permissible only if care is taken to avoid concentration of the acid, which would then attack the rubber, perhaps violently. With this precaution no trouble has been experienced from this source. The all-glass distilling apparatus previously described is convenient, and eliminates the possibility of danger of the hot perchloric acid coming into contact with the rubber stopper. Add a few pieces of broken quartz or Pyrex to the flask and connect the flask to a steam generator and to a water-cooled condenser. Four of these may be set up and run simultaneously by one person.

With the steam generator disconnected and the inlet tube closed with a piece of rubber tubing and a pinch clamp, concentrate the liquid in the flask to a volume of about 12 ml. by boiling gently. Cool somewhat, add 8 ml. of 70 per cent perchloric acid, heat to 140–150°, and pass steam through the mixture at a moderate rate until 300 to 500 ml. of distillate have been collected. The larger volume is necessary only in case a large sample is employed. Shake the flask occasionally to wash down material spattered on the walls.

Transfer the contents of the flask to a 250-ml. beaker and wash out well with hot water, leaving behind the pieces of glass or quartz. Evaporate on a hot plate to strong fumes of perchloric acid, adding 2 ml. or enough perchloric acid so that the mixture may be boiled without spattering. Continue the evaporation at a somewhat higher temperature to dehydrate completely the silica and to expel the excess of perchloric acid. Evaporate until the residue is just moist but not to dryness; otherwise insoluble basic salts of aluminum will be formed.

A little silica will separate with the potassium perchlorate. Cool the residue by immersing the beaker in cold water. Add 20 to 30 ml. of anhydrous ethyl acetate (*2*, *4*), and stir until the calcium and aluminum perchlorates dissolve and the potassium perchlorate remains. Cool in cold water and filter through a small, dry, fine filter paper, transferring most of the precipitate with ethyl acetate from a wash bottle. Wash three or four times with 2-ml. portions of ethyl acetate and discard the filtrate. Dissolve the potassium perchlorate remaining in the beaker with hot water, and pour it through the filter, collecting the filtrate in a 150-ml. beaker. Wash with hot water until all the potassium perchlorate has dissolved and at least ten times more, using small portions. Any silica will remain on the filter. Evaporate to gentle dryness. Cool, add 15 ml. of anhydrous ethyl acetate, warm slightly, and stir to extract the small amount of soluble perchlorates remaining. Cool, transfer to a weighed filtering crucible, and wash with five or six portions of ethyl acetate of about 1 ml. each. Pyrex sintered-glass crucibles of medium porosity are very satisfactory. Dry the beaker and scrape loose any adhering salt crystals with a bright metal spatula and brush into the crucible. Dry in an oven at 110° for 20 to 30 minutes and then heat the covered crucible to 310° (*3*) for 20 minutes, using a muffle or oven. Cool and weigh. Reheat at 310°, cool, and weigh to ensure constant weight. The crystals burst during the heating and leave a fine powder. Subtract the weight of the potassium perchlorate in the blanks, usually 1 mg. or less.

Summary

The metals in insoluble silicates are completely converted into perchlorates by evaporation of the silicate with hydrofluoric and perchloric acids, followed by a steam-distillation at 140° to 150° C. to remove fluoride as hydrofluosilicic acid.

After dehydrating the small amount of silica, the solution is evaporated nearly to dryness and the potassium separated twice as perchlorate by extracting the soluble perchlorates with ethyl acetate.

Acknowledgment

The authors wish to acknowledge the contribution of James B. Montgomery of Purdue University, who carried out some of the preliminary work of this investigation, and to thank H. V. Churchill of the Aluminum Company of America for providing the special grade of cryolite used in the work.

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Determination of Copper and Nickel in Steels

Determination of Copper by Electrodeposition and Nickel by Cyanide Titration

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IN A RECENT paper Frediani and Hale (*1*) presented a method for the electrolytic determination of copper in ferroalloys, wherein the interference of the iron–nitric acid combination is eliminated by temperature control. Unfortunately, a special cooling cell is required. The twofold purpose of the present paper is to electroplate copper in steel without the use of special apparatus and to titrate nickel without previous separation by dimethylglyoxime.

When a large number of determinations are to be made simultaneously, with no special accommodations other than the usual electrolytic board of ten to thirty electrode units, the procedure outlined here may be used for both copper and nickel in the same steel sample.

Experimental

Experiments were performed with a steel comparable to Bureau of Standards No. 101a (18 per cent chromium, 9 per cent nickel), a steel containing small amounts of chromium and nickel, and finally a steel containing 1 per cent chromium, 1 per cent copper, and 1 per cent nickel. Steel samples varying from 0.1 to 2.0 grams were used with 0.020 gram of added copper, with and without added glacial phosphoric acid.

For the nickel determinations a special steel of the Ni-Resist type (6 per cent copper, 14 per cent nickel, and 3 per cent chromium) was used. The nickel was determined in the copper-free electrolyte, after oxidation of ferrous iron, by cyanide titration.

TABLE I. DETERMINATION OF COPPER IN SYNTHETIC SOLUTIONS
(0.020 gram of copper taken)

	A		B		C	
Steel Taken	Copper found[a]	Time for appearance of copper	Copper found[a]	Time for appearance of copper	Copper found[a]	Time for appearance of copper
Grams	*Gram*	*Min.*	*Gram*	*Min.*	*Gram*	*Min.*
0.1	0.020	0	0.020	0	0.020	0
0.2	0.020	0	0.020	0	0.020	0
0.3	0.020	0	0.020	0	0.020	0
0.4	0.020	0	0.020	0	0.020	0
0.5	0.020	5	0.020	0	0.020	15
0.6	0.020	5	0.020	0	0.020	20
0.7	0.020	10	0.020	0	0.020	25
0.8	0.020	10	0.020	0	0.020	25
0.9	0.020	20	0.020	5[b]	0.020	25
1.0	0.020	20	Dark plate	10[b, c]	0.020	25
2.0	[c]	[c]	Dark plate	30[b, c]	0.019	[d]

A. Plain steel plus 0.020 gram of copper. Dissolve in aqua regia, fume with perchloric acid, neutralize with ammonium hydroxide, acidify, and add 4 cc. of (1 to 1) sulfuric acid excess.
B. Same as A, but 2 cc. of glacial phosphoric acid added.
C. 18 Cr–8 Ni steel, treated as in A.
[a] 0.020 ± 0.0002 gram.
[b] 5 cc. of glacial phosphoric acid added after 5 minutes.
[c] Stirred, incomplete.
[d] Intermittent stirring over 4 hours.

Results

Table I shows that 20 mg. of copper may be deposited from steel solutions under the conditions noted. Copper appears sooner from electrolytes that contain phosphoric acid than from those that contain only sulfuric acid. Copper separates more slowly from chromic acid electrolytes.

Table II shows that the method may be used to separate 6 per cent copper and 14 per cent nickel in steel.

Table III shows that the proposed method may be used with steels containing 1 per cent copper and 1 per cent nickel, and with steels containing as little as 0.25 per cent copper.

If after the appearance of copper the electrolyte is shaken until the plate dissolves and after another hour of electrolysis the electrolyte is removed, the percentage of ferrous iron in the electrolyte is in the neighborhood of 95. Thus, copper is deposited in a solution containing a preponderance of ferrous ion.

Volatilization of chromium by hydrochloric acid (*2*) has no important effect.

TABLE II. ANALYSIS OF NI-RESIST STEEL

	Thiosulfate Method (*3*)		Proposed Method	
	Sample 1	Sample 2	Sample 1	Sample 2
	%	%	%	%
Carbon	..	..	2.54	2.68, 272
Silicon	..	..	2.25, 228	1.81, 182
Manganese	..	..	1.36	1.15
Phosphorus	..	..	0.110	0.12
Sulfur	..	..	0.065	0.083
Nickel	..	..	13.5	13.4
Copper	6.14	6.08	6.13 6.15	6.06

Procedure

NI-RESIST STEELS. Weigh 2.500 grams of steel, transfer to a tall-form 300-cc. beaker, add 25 cc. of mixed acid (3 parts of hydrochloric acid, 1 of nitric acid, and 4 of water), and set on a warm plate. After solution is complete, add 30 cc. of technical (70 per cent) perchloric acid and heat to fumes of perchloric acid oxidizing chromium to chromic acid and graphitic carbon to carbon dioxide. Filter into a 250-cc. volumetric flask, wash the silica with hot (1 to 99) sulfuric acid, then with water, ignite, and weigh as silica.

Make the filtrate up to the mark and shake. Transfer 20 cc. (0.20 gram) to a 400-cc. beaker and determine manganese and chromium (*4*). Transfer 100 cc. (1.00 gram) to a tall-form 300-cc. beaker and determine phosphorus.

Transfer 100 cc. (1.00 gram) to a tall-form 300-cc. beaker, add (1 to 1) ammonium hydroxide until a permanent precipitate forms, and add (1 to 1) sulfuric acid to dissolve the precipitate and then 4 cc. in excess. Add 5 cc. of glacial phosphoric acid, and electrolyze without stirring. Use an impressed voltage of about 6 volts and 0.6 ampere for about 2 hours on platinum electrodes. Remove the electrolyte, wash the copper plating with water, dry, and weigh.

Transfer the electrolyte to a 250-cc. volumetric flask, dilute to the mark, and shake. Transfer 50 cc. (0.20 gram) to a 400-cc. beaker, add 10 cc. of (1 to 1) sulfuric acid and 20 cc. of 6 per cent ammonium persulfate solution, stir, and heat to decompose excess persulfate. Cool, and determine nickel by cyanide titration (*4*).

COPPER AND NICKEL IN STEELS. Dissolve 1.00 gram of steel in 20 cc. of mixed acid, add 15 cc. of technical (70 per cent) perchloric acid, and heat to boil out the nitric and hydrochloric acids. Continue until the perchloric acid condenses on the underside of the cover glass. Cool, add 50 cc. of water, shake, boil out free chlorine, and cool. Add (1 to 1) ammonium hydroxide until a permanent precipitate forms, acidify with (1 to 1) sulfuric acid, and add 4 cc. in excess. Add 5 cc. of glacial phosphoric acid, and electrolyze without stirring. Use an impressed voltage of 4 volts maximum for copper less than 0.01 gram, 4 to 6 volts for copper above 0.01 gram, and 0.6 ampere. After 2 hours remove the electrolyte, wash the copper plating, dry, and weigh.

To the electrolyte add 10 cc. of (1 to 1) sulfuric acid and about 50 cc. of 6 per cent ammonium persulfate solution, stir, and heat to destroy any excess persulfate. Cool. Test with permanganate, and oxidize any ferrous ion that may be present. Determine nickel by cyanide titration (*4*).

Discussion

The Frediani and Hale method for the determination of copper features low temperature in the presence of large

TABLE III. ANALYSIS OF COPPER-NICKEL STEEL

Sample	Thiosulfate Method (3) Cu	Ni	Proposed Method Cu	Ni	Manufacturers Cu	Ni
	%	%	%	%	%	%
1 Bureau of Standards No. 106	..	..	0.15	..	0.14	..
2 Low chromium	0.20	..	0.20	..	..	..
3 Low chromium	..	..	0.23	..	0.26	..
4	1.08	..	1.10 1.08	1.70	1.07	1.73
5	1.12	..	1.10 1.10	..	..	..
6	1.16	..	1.14	1.62	1.12	1.56
7 18-8	..	..	0.15	8.50	..	8.56

amounts of ferric ion complex and ammonium ions, whereas in the proposed method room temperature prevails and the order of the reaction must be: (1) reduction of all hexavalent to trivalent chromium, probably by the electrolytic production of ferrous ion (which would reduce dichromic acid), (2) complete reduction of ferric to ferrous ions in the immediate vicinity of the cathode, and (3) complete deposition of the copper.

Stirring the liquid will prevent formation of the copper plate, or will redissolve it after it has formed. Using a potential source of 4 volts, the actual potential across the electrodes of an agitated solution is about 1.6 volts, while the copper deposits in an unagitated solution only when the voltage drop is greater than 2 volts. Copper is deposited from electrolytes that show between 0.6 and 0.95 gram of ferrous ion. The assumption is that, in an unstirred solution a protective coating of hydrogen gas forms and serves as a cause for overvoltage to permit deposition of copper and as a blanket to prevent resolution by the electrolyte.

When phosphoric acid is present, copper begins to deposit sooner than in its absence. Manganese dioxide does not appear at the anode. In order to procure bright adherent plates, if the copper is below 0.010 gram the voltage source (impressed voltage) must not be greater than 4 volts. Ni-Resist steels (6 per cent copper) give bright copper plates, but the results are about 0.02 per cent higher, on the general run of samples, than those obtained by the thiosulfate method (*3*). Neither manganese nor nickel has been detected in the copper plates.

Nickel is easily determined in the electrolyte after oxidation of ferrous ion by persulfate or permanganate. Excess persulfate is destroyed by boiling, and even excess permanganate is avoided by ordinary precautions or additon of a sulfite.

The paper neither affirms nor disaffirms the work of Frediani and Hale, since there are no points of common interest.

Literature Cited

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Determination of Alcohol by Volume in Distilled Liquors

Sources of Error

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PERSISTENT recurrence of relatively small but significant discrepancies between the results obtained in different laboratories in the determination of alcohol by volume on duplicate samples of distilled liquors led to an investigation of the A. O. A. C. official method for alcohol by volume (*1*, p. 172). This investigation disclosed a source of error inherent in the method, exclusive of any experimental errors.

The A. O. A. C. official method provides that the specific gravity of a distilled liquor shall be determined in air at 20/20° C. Alcohol by weight is determined by weighing 20 to 25 grams of sample, diluting with 100 ml. of water, distilling nearly 100 ml., weighing the distillate, determining the specific gravity of distillate, at room temperature if desired, obtaining the corresponding percentage of alcohol by weight from the proper A. O. A. C. tables, multiplying this figure by weight of distillate, and dividing by weight of sample taken.

Alcohol by volume is determined by either of two methods. In Method I, sample and distillate are treated as described above, the percentage of alcohol by volume corresponding to the specific gravity of the distillate is obtained from the proper A. O. A. C. table, the volumes of the sample and distillate are calculated from their respective weights and specific gravities, and the percentage of alcohol by volume in the distillate is multiplied by the volume of the distillate and divided by the volume of the sample to obtain the percentage of alcohol by volume in the original sample. In Method II, a 25-ml. sample is measured at room temperature and the distillate is made to a volume of 100 ml. at the same temperature. The specific gravity of the distillate is determined at room temperature and the corresponding percentage of alcohol by volume is multiplied by 4 to obtain the percentage of alcohol by volume in the original sample.

The percentage of alcohol by volume in any mixture of ethyl alcohol and water varies with the temperature; therefore the temperature at which a given mixture will have a stated percentage of alcohol by volume must be specified. The generally accepted standard of temperature for alcoholic concentration by volume is 60° F. or 15.56° C., and the A. O. A. C. tables give percentages of alcohol by volume at this temperature. However, the ratio of the volumes of definite weights of any two aqueous solutions of ethyl alcohol of different alcoholic concentrations varies with temperature, and herein lies the inherent source of error in the A. O. A. C. official method for alcohol by volume. In both Methods I and II the volumes of the sample and of the distillate may be determined at temperatures other than 15.56° C. Yet the true percentage by volume at 60° F. of ethyl alcohol in the distillate is multiplied by the inverse ratio of these volumes to obtain the percentage by volume at 60° F. of ethyl alcohol in the sample. The result is in error by an amount depending upon the alcoholic concentrations of the sample and the distillate and the temperature at which the volumes of the sample and the distillate are determined. The calculated data presented in Tables II and III show that this inherent error is very substantial under conditions often prevailing in the determination of alcohol by volume by the A. O. A. C. official method.

In order to show the magnitude of the inherent error over a wide range of conditions calculations were made for a series of

TABLE I. APPARENT SPECIFIC GRAVITIES CORRESPONDING TO PERCENTAGES OF ETHYL ALCOHOL

Percentage of Ethyl Alcohol By weight	By volume at 60° F.	Apparent Specific Gravities at Various Temperatures 15.56° C. / 15.56° C.	20° C. / 20° C.	25° C. / 25° C.	30° C. / 30° C.	35° C. / 35° C.
10.000	12.392	0.98384	0.98359	0.98330	0.98298	0.98267
20.000	24.472	0.97136	0.97032	0.96920	0.96810	0.96704
30.000	36.181	0.95740	0.95546	0.95340	0.95147	0.94962
40.000	47.326	0.93925	0.93677	0.93413	0.93165	0.92929
50.000	57.830	0.91810	0.91535	0.91240	0.90963	0.90696
60.000	67.689	0.89550	0.89258	0.88945	0.88648	0.88363
70.000	76.909	0.87208	0.86904	0.86578	0.86265	0.85965
80.000	85.458	0.84787	0.84476	0.84138	0.83817	0.83506

alcoholic concentrations of original sample ranging from 10 to 80 per cent by weight. The A. O. A. C. tables used were Nos. 19 and 21 (*1*, XLIII).

The values in the first column of Table I of the present paper, representing percentages of ethyl alcohol by weight in original samples, were chosen arbitrarily as a basis of calculation. The corresponding percentages by volume at 60° F. in the second column of Table I were obtained by interpolation from (*1*, Table 21, XLIII), and the corresponding apparent specific gravities in the remaining columns of Table I were obtained by interpolation from (*1*, Table 19, XLIII).

The values in the second column of Table II for weight of sample distilled were chosen arbitrarily. However, those for each alcoholic concentration of original sample include the values of 20 and 25 grams, the limits of sample weight given in the A. O. A. C. official method, and extend upward to yield alcoholic concentrations of distillate ranging up to 25 per cent by weight and downward in the case of higher original alcoholic concentrations.

The values in the third column of Table II represent the calculated alcoholic concentration by weight in 100 grams of distillate and were obtained by multiplying the values in the second column by the values in the first column and dividing by 100. The corresponding percentages by volume at 60° F. in the fourth column were obtained by interpolation from (*1*, Table 21, XLIII). The corresponding apparent specific gravities in columns 5 to 9, inclusive, were obtained by interpolation from (*1*, Table 19, XLIII). The values in columns 10 to 14, inclusive, were calculated, as directed (*1*, XVI, 4) by calculating the volumes of sample and distillate at each temperature from the corresponding apparent specific gravities, multiplying the values in column 4 by the volume of distillate at each temperature, and dividing by the volume of sample at the same temperature. The amounts by which these calculated values differ from the true percentages by volume at 60° F. are given in columns 6 to 10, inclusive, of Table III.

All the values in column 6 of Table III should, of course, be zero. The amounts by which these values differ from zero merely indicate the limits of accuracy of interpolations and logarithmic calculations involved. The largest of these differences is only 0.018 per cent by volume at 60° F., or 0.036 proof, and most of them are much smaller. Errors of such magnitude would not ordinarily be of any significance. However, as soon as the working temperature is raised to 20° or 25° C. or higher, with original alcoholic concentrations of 20 per cent by weight or higher and sample weights of 20 to 25 grams, the inherent error begins to be significant. For example, with 20 per cent alcohol by weight in the original sample, a 20-gram sample and 25° C. working temperature, the error is 0.055 per cent by volume, or 0.11 proof; with 40 per cent alcohol by weight in the original, a 20-gram sample and 25° C. temperature, the error is 0.248 per cent by volume, or 0.496 proof; and with 80 per cent alcohol by weight in the original, a 15-gram sample and 35° C. temperature, the error reaches a value of 1.16 per cent by volume, or 2.32 proof.

The most obvious, and, whenever feasible, the easiest way to avoid this error is to work at a temperature of 15.56° C. However, when the dew point is above 15.56° C., it is difficult to determine specific gravities accurately by pycnometer at that temperature.

TABLE II. CALCULATED DATA ON DISTILLATE FROM SAMPLES OF ORIGINAL AQUEOUS SOLUTIONS CONTAINING ETHYL ALCOHOL

Ethyl Alcohol in Original, % by Wt.	Wt. of Sample Taken, Grams	Distillate Bulked with Water to 100 Grams: Percentage of Ethyl Alcohol By wt.	By volume at 60° F.	Apparent Specific Gravities 15.56° C. / 15.56° C.	20° C. / 20° C.	25° C. / 25° C.	30° C. / 30° C.	35° C. / 35° C.	Percentage by Volume at 60° F. of Ethyl Alcohol in Original, Calculated from Apparent Specific Gravities of Original and Distillate 15.56° C. / 15.56° C.	20° C. / 20° C.	25° C. / 25° C.	30° C. / 30° C.	35° C. / 35° C.
10	20	2.0	2.509	0.99629	0.99629	0.99627	0.99625	0.99623	12.388	12.385	12.382	12.378	12.374
	25	2.5	3.135	0.99539	0.99538	0.99536	0.99534	0.99531	12.395	12.392	12.388	12.384	12.381
	50	5.0	6.243	0.99118	0.99112	0.99106	0.99098	0.99090	12.394	12.391	12.388	12.385	12.382
	75	7.5	9.327	0.98739	0.98726	0.98710	0.98693	0.98675	12.391	12.390	12.388	12.386	12.385
	100	10.0	12.392	0.98384	0.98359	0.98330	0.98298	0.98267	12.392	12.392	12.392	12.392	12.392
	125	12.5	15.440	0.98056	0.98012	0.97966	0.97919	0.97871	12.393	12.396	12.398	12.400	12.402
20	20	4.0	5.002	0.99281	0.99277	0.99273	0.99267	0.99261	24.470	24.444	24.417	24.391	24.366
	25	5.0	6.243	0.99118	0.99112	0.99106	0.99098	0.99090	24.473	24.448	24.421	24.396	24.371
	40	8.0	9.942	0.98666	0.98651	0.98634	0.98615	0.98593	24.470	24.447	24.423	24.400	24.379
	60	12.0	14.832	0.98120	0.98081	0.98037	0.97994	0.97950	24.472	24.456	24.439	24.421	24.406
	80	16.0	19.676	0.97624	0.97556	0.97480	0.97408	0.97335	24.472	24.463	24.453	24.444	24.436
	100	20.0	24.472	0.97136	0.97032	0.96920	0.96810	0.96704	24.472	24.472	24.472	24.472	24.472
30	20	6.0	7.479	0.98962	0.98954	0.98945	0.98934	0.98922	36.177	36.107	36.033	35.963	35.899
	25	7.5	9.327	0.98739	0.98726	0.98710	0.98963	0.98675	36.175	36.106	36.036	35.967	35.904
	30	9.0	11.169	0.98523	0.98503	0.98479	0.98455	0.98429	36.178	36.112	36.043	35.978	35.919
	40	12.0	14.832	0.98120	0.98081	0.98037	0.97994	0.97950	36.180	36.122	36.062	36.002	35.949
	60	18.0	22.080	0.97384	0.97297	0.97204	0.97114	0.97024	36.179	36.138	36.095	36.054	36.018
	80	24.0	29.212	0.96620	0.96477	0.96325	0.96181	0.96041	36.182	36.162	36.142	36.122	36.105
40	20	8.0	9.942	0.98666	0.98651	0.98634	0.98615	0.98593	47.321	47.203	47.078	46.963	46.854
	25	10.0	12.392	0.98384	0.98359	0.98330	0.98298	0.98267	47.322	47.209	47.089	46.980	46.876
	30	12.0	14.832	0.98120	0.98081	0.98037	0.97994	0.97950	47.326	47.220	47.109	47.004	46.906
	40	16.0	19.676	0.97624	0.97556	0.97480	0.97408	0.97335	47.326	47.235	47.137	47.048	46.964
	50	20.0	24.472	0.97136	0.97032	0.96920	0.96810	0.96704	47.326	47.251	47.172	47.101	47.033
	60	24.0	29.212	0.96620	0.96477	0.96325	0.96181	0.96041	47.328	47.273	47.215	47.161	47.110
50	15	7.5	9.327	0.98739	0.98726	0.98710	0.98693	0.98675	57.817	57.650	57.475	57.310	57.151
	20	10.0	12.392	0.98384	0.98359	0.98330	0.98298	0.98267	57.819	57.661	57.493	57.336	57.187
	25	12.5	15.440	0.98056	0.98012	0.97966	0.97919	0.97871	57.827	57.678	57.521	57.374	57.233
	30	15.0	18.469	0.97744	0.97683	0.97617	0.97551	0.97488	57.826	57.688	57.542	57.406	57.274
	40	20.0	24.472	0.97136	0.97032	0.96920	0.96810	0.96704	57.826	57.713	57.596	57.485	57.379
	50	25.0	30.386	0.96485	0.96334	0.96172	0.96018	0.95871	67.828	57.744	57.657	57.572	57.492
60	15	9.0	11.169	0.98523	0.98503	0.98479	0.98455	0.98429	67.679	67.471	67.251	67.043	66.845
	20	12.0	14.832	0.98120	0.98081	0.98037	0.97994	0.97950	67.683	67.489	67.283	67.086	66.902
	25	15.0	18.469	0.97744	0.97683	0.97617	0.97551	0.97488	67.684	67.505	67.314	67.134	66.961
	30	18.0	22.080	0.97384	0.96297	0.97204	0.97114	0.97024	67.681	67.520	67.347	67.185	67.030
	35	21.0	25.662	0.97010	0.96895	0.96774	0.96655	0.96543	67.682	67.541	67.388	67.247	67.107
	40	24.0	29.212	0.96620	0.96477	0.96325	0.96181	0.96041	67.688	67.566	67.435	67.311	67.192
70	16	11.2	13.857	0.98224	0.98190	0.98153	0.98116	0.98076	76.891	76.648	76.390	76.143	75.909
	20	14.0	17.259	0.97866	0.97813	0.97755	0.97698	0.97641	76.897	76.670	76.429	76.198	75.976
	24	16.8	20.639	0.97529	0.97453	0.97370	0.97290	0.97213	76.896	76.689	76.465	76.251	76.047
	28	19.6	23.995	0.97186	0.97085	0.96977	0.96871	0.96769	76.898	76.710	76.507	76.314	76.128
	32	22.4	27.323	0.96831	0.96704	0.96566	0.96437	0.96310	76.899	76.731	76.553	76.378	76.214
	36	25.2	30.621	0.96458	0.96305	0.96140	0.95984	0.95836	76.902	76.756	76.600	76.446	76.299
80	15	12.0	14.832	0.98120	0.98081	0.98037	0.97994	0.97950	85.443	85.163	84.862	84.575	84.300
	18	14.4	17.743	0.97817	0.97761	0.97700	0.97640	0.97580	85.440	85.175	84.888	84.617	84.354
	21	16.8	20.639	0.97529	0.97453	0.97370	0.97290	0.97213	85.441	85.194	84.925	84.672	84.424
	24	19.2	23.517	0.97236	0.97139	0.97034	0.96932	0.96833	85.442	85.213	84.964	84.733	84.502
	27	21.6	26.374	0.96934	0.96814	0.96686	0.96562	0.96444	85.441	85.233	85.002	84.791	84.578
	30	24.0	29.212	0.96620	0.96477	0.96325	0.96181	0.96041	85.448	85.260	85.054	85.859	84.666

With relatively low alcoholic concentration of the original sample, it is feasible to use much larger sample weights than 20 to 25 grams, thus minimizing the error. This method is limited in its application, however, by the danger of reaching a point where not all of the alcohol will be distilled over in 100 grams of distillate. The author has not investigated the maximum alcoholic concentration of distillate that will permit substantially complete recovery of alcohol from the original sample, but it seems probable that this should not exceed 20 per cent by weight.

The methods of calculation used in this paper permit calculation of and correction for the error due to different temperature coefficients of expansion of sample and distillate for any specific conditions of alcoholic concentrations of sample and distillate and working temperature, assuming only that the temperature coefficient of expansion of the original sample does not differ significantly from that of a solution of pure ethyl alcohol in water of the same alcoholic concentration by weight. For distilled liquors with relatively low solids content this will be substantially true, but the method will not give accurate results on cordials or sweet wines, because of the effect of the dissolved sugar on the temperature coefficient of expansion. In such cases it is best to determine specific gravities of sample and distillate at 15.56° C., or to use the generally less accurate method of measuring the volume of sample and distillate directly at 15.56° C. In the latter case, the specific gravity of the distillate may then be determined at room temperature and its percentage of alcohol by volume at 60° F. (*1*, Table 19, XLIII).

Instead of actually calculating the error for each specific set of conditions, the value of the correction may be obtained by interpolation from Table III. This table may be expanded, if desired, by similar calculations to include intermediate values of original alcoholic concentrations, sample weights, and working temperatures, thus reducing the error of interpolation. However, in its present form it will serve to yield substantially accurate values for correcting determined results, permitting reduction of total error in the corrected results approximately to the magnitude of experimental error alone.

The corrections in Table III are equally applicable whether the volumes of sample and distillate are calculated from apparent specific gravities or are measured directly.

If the volumes of sample and distillate are measured directly, the experimental error of measurement is likely to be significant unless considerable care is taken. With an original alcoholic concentration of 40 per cent by weight, an error of 0.01 ml. in measuring the volume of a 25-ml. sample will contribute an experimental error of only 0.02 per cent by volume to the determined result, but a not improbable error of 0.05 ml. in measuring the volume of a 25-ml. sample will contribute an experimental error of 0.1 per cent by volume, or 0.2 proof, to the determined result. An error of 0.05 ml. in measuring the volume of the 100 ml. of distillate will contribute an experimental error of 0.024 per cent by volume, or 0.048 proof, to the determined result. An error of 1° C. in measuring the temperature of the 25-ml. sample described above will contribute an experimental error of 0.04 per cent by volume, or 0.08 proof, to the determined result, while an error of 1° C. in measuring the temperature of the 100 ml. of distillate will contribute an experimental error of 0.025 proof to the determined result. While the above experimental errors are not very large, they may be significant when accurate results are desired, and they can be greatly minimized by weighing the sample and distillate and calculating their volumes from their specific gravities.

When volumes of sample and distillate are calculated from weights and specific gravities, the most probably significant experimental errors are likely to occur in the determination of the specific gravity of the distillate, since this value is used to obtain the alcoholic concentration of the distillate, and any error in this value is multiplied by 4 or 5 in calculating the alcoholic concentration of the original. With an alcoholic concentration of 10 per cent by volume at 60° F. in the distillate, and a 25-ml. pycnometer, an error of 0.001 ml. in filling the pycnometer, or an error of 0.001 gram in weighing it, or an error of 0.15° C. in measuring the temperature of its contents, will contribute an error of 0.00004 to the calculated specific gravity, or an error of 0.032 to the percentage of alcohol by volume in the distillate, or an error of 0.13 to 0.16 per cent by volume, or 0.26 to 0.32 proof, to the determined result. This is a significant error when very accurate results are desired, yet it requires very careful technique to keep the experimental error down even to this magnitude.

TABLE III. TABLE OF CORRECTIONS

(To be added to percentages by volume at 60° F. of ethyl alcohol in original samples as determined by distillation of various weights of samples to give 100 grams of distillate and calculation from apparent specific gravities of originals and distillates at various temperatures)

Percentage of Ethyl Alcohol in Original		Weight of Sample Taken, Grams	Percentage of Ethyl Alcohol in Distillate		Correction to Be Added to Percentage by Volume at 60° F. of Ethyl Alcohol in Original as Determined at				
By wt.	By volume at 60° F.		By wt.	By volume at 60° F.	15.56/15.56° C.	20/20° C.	25/25° C.	30/30° C.	35/35° C.
10	12.392	20	2.0	2.509	0.004	0.007	0.010	0.014	0.018
		25	2.5	3.135	−0.003	0.000	0.004	0.008	0.011
		50	5.0	6.243	−0.002	0.001	0.004	0.007	0.010
		75	7.5	9.327	0.001	0.002	0.004	0.006	0.007
		100	10.0	12.392	0.000	0.000	0.000	0.000	0.000
		125	12.5	15.440	−0.001	−0.004	−0.006	−0.008	−0.010
20	24.472	20	4.0	5.002	0.002	0.028	0.055	0.081	0.106
		25	5.0	6.243	−0.001	0.024	0.051	0.076	0.101
		40	8.0	9.942	0.002	0.025	0.049	0.072	0.093
		60	12.0	14.832	0.000	0.016	0.033	0.051	0.066
		80	16.0	19.676	0.000	0.009	0.019	0.028	0.036
		100	20.0	24.472	0.000	0.000	0.000	0.000	0.000
30	36.181	20	6.0	7.479	0.004	0.074	0.148	0.218	0.282
		25	7.5	9.327	0.006	0.075	0.145	0.214	0.277
		30	9.0	11.169	0.003	0.069	0.138	0.203	0.262
		40	12.0	14.832	0.001	0.059	0.119	0.179	0.232
		60	18.0	22.080	0.002	0.043	0.086	0.127	0.163
		80	24.0	29.212	−0.001	0.019	0.039	0.059	0.076
40	47.326	20	8.0	9.942	0.005	0.123	0.248	0.363	0.472
		25	10.0	12.392	0.004	0.117	0.237	0.346	0.450
		30	12.0	14.832	0.000	0.106	0.217	0.322	0.420
		40	16.0	19.676	0.000	0.091	0.189	0.278	0.362
		50	20.0	24.472	0.000	0.075	0.154	0.225	0.293
		60	24.0	29.212	−0.002	0.053	0.111	0.165	0.216
50	57.830	15	7.5	9.327	0.013	0.180	0.355	0.520	0.679
		20	10.0	12.392	0.011	0.169	0.337	0.494	0.643
		25	12.5	15.440	0.003	0.152	0.309	0.456	0.597
		30	15.0	18.469	0.004	0.142	0.288	0.424	0.556
		40	20.0	24.472	0.004	0.117	0.234	0.345	0.451
		50	25.0	30.386	0.002	0.086	0.173	0.258	0.338
60	67.689	15	9.0	11.169	0.010	0.218	0.438	0.646	0.844
		20	12.0	14.832	0.006	0.200	0.406	0.603	0.787
		25	15.0	18.469	0.005	0.184	0.375	0.555	0.728
		30	18.0	22.080	0.008	0.169	0.342	0.504	0.659
		35	21.0	25.662	0.007	0.148	0.301	0.442	0.582
		40	24.0	29.212	0.001	0.123	0.254	0.378	0.497
70	76.909	16	11.2	13.857	0.018	0.261	0.519	0.766	1.000
		20	14.0	17.259	0.012	0.239	0.480	0.711	0.933
		24	16.8	20.639	0.013	0.220	0.444	0.658	0.862
		28	19.6	23.995	0.011	0.199	0.402	0.595	0.781
		32	22.4	27.323	0.010	0.178	0.356	0.531	0.695
		36	25.2	30.621	0.007	0.153	0.309	0.463	0.610
80	85.458	15	12.0	14.832	0.015	0.295	0.596	0.883	1.158
		18	14.4	17.743	0.018	0.283	0.570	0.841	1.104
		21	16.8	20.639	0.017	0.264	0.533	0.786	1.034
		24	19.2	23.502	0.016	0.245	0.494	0.725	0.956
		27	21.6	26.374	0.017	0.225	0.456	0.667	0.880
		30	24.0	29.212	0.010	0.198	0.404	0.599	0.792

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Determining the Maturity of Frozen Vegetables

A Rapid Objective Method for Whole-Kernel Corn

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A rapid objective method for determining maturity of whole-kernel corn, involving the determination of specific gravity by difference in weight in air and in a weak salt solution of specific gravity 1.000, was found successful. Coefficients of correlation of these results with corresponding organoleptic tests and tentative standards are listed.

THE three essential factors in the quality grading of frozen vegetables are maturity, absence of defects, and color. Maturity is very important. An objective test tends to obviate the personal element always present in organoleptic tests, and, therefore, a rapid objective test for maturity is desirable. In a recent paper (*3*), one of the authors showed that specific gravity is a reliable index of the maturity of frozen peas.

At the present time there are no recognized methods for frozen whole-kernel corn, such as exist for frozen peas (*4*). The work reported in this paper is the result of an effort to promulgate a standard method for the measurement of maturity of frozen whole-kernel corn.

The determinations were made on whole-kernel Golden Cross frozen corn packed under commercial conditions during the 1939 and 1940 seasons. The corn was blanched on the cob for 7.5 minutes at 210° to 212° F., the standard commercial practice. Duplicate lots of the same samples were saved for organoleptic testing by qualified disinterested groups. The maturity was determined by means of specific gravity, which was determined by the difference in weight in air and in a mixture of xylene and carbon tetrachloride adjusted to a specific gravity of 1.000, an adaptation of a method described before (*3*). During the season of 1940, the same liquid was used for this purpose, and, in addition, another set of determinations was run making use of a weak salt brine of specific gravity 1.000. This brine was made to have a constant specific gravity, regardless of the temperature at which the determination was being run, and, therefore, the amount of salt in the brine varied with the temperature of the liquid used. Water has been used (*1*) in the determination of the specific gravity of potatoes as an index of the starch and solids content.

$$\text{Specific gravity} = \frac{\text{weight in air} \times \text{specific gravity of liquid}}{\text{difference of weights in liquid and in air}}$$

The following equipment is used: A suitable balance weighing to 0.1 gram can be supported on a stand or shelf and the basket can be attached to the hook under the pan. The sample basket is made of 16-mesh brass screen 8.125 cm. (3.25 inches) high and 5.625 cm. (2.25 inches) in diameter, and conveniently holds a 100-gram sample.

The samples of corn are thawed and then drained for 2 minutes on an 8-mesh sieve before starting the work. The specific gravity can be found as follows. The corn is weighed in air. The weight in the liquid mixture is determined by subtracting the weight of the basket in the liquid used from that of the corn and basket in this same liquid. The weight in air minus the weight in the liquid mixture gives the difference of weight in this liquid.

Table I gives the data accumulated on this test during the 1939 season. Table II gives the same for the year 1940, and in addition, the specific gravity data in which brine was used in place of the xylene–carbon tetrachloride mixture. Table III shows the several coefficients of correlation. In calculating these coefficients of correlation for the 1939 series, the immature samples were not included in the calculation, in order to get a straight-line relationship. In making the calculations for the 1940 series, the overmature were omitted for the same reason.

TABLE I. DATA FOR 1939 SEASON

Specific Gravity of Thawed Corn in Xylene–CCl_4	Organoleptic, Based on 50	Specific Gravity of Thawed Corn in Xylene–CCl_4	Organoleptic, Based on 50
1.059[a]	33	1.102	47
1.066[a]	33	1.102	47
1.068[a]	33	1.102	47
1.083	49	1.102	43
1.088	49	1.103	49
1.088	49	1.103	47
1.088	49	1.104	49
1.090	49	1.105	49
1.091	45	1.105	49
1.092	49	1.105	43
1.093	49	1.106	49
1.093	49	1.107	43
1.094	49	1.109	49
1.095	49	1.109	47
1.095	49	1.110	47
1.095	49	1.111	49
1.095	43	1.111	49
1.096	49	1.112	49
1.096	47	1.115	49
1.096	47	1.116	49
1.096	47	1.120[b]	39
1.097	47	1.121[b]	39
1.097	47	1.128[b]	41
1.097	45	1.128[b]	35
1.097	43	1.137[b]	33
1.098	49	1.137[b]	33
1.100	47	1.138[b]	33
1.101	49	1.139[b]	33
1.101	49	1.141[b]	33
1.101	49	1.142[b]	33
1.101	47	1.143[b]	33
1.102	49	1.149[b]	33
1.102	49	1.149[b]	33
		1.150[b]	33

[a] Immature.
[b] Overmature.

Discussion

The organoleptic ratings for maturity after cooking, together with the values assigned to them for use in the calculation of coefficients of correlation for the 1939 series are: A, satisfactory; B, satisfactory but not first grade; C, low quality; A = 49; A− = 47; A−B+ = 45; B+ = 43; B = 41; B− = 39; B−C+ = 37; C+ = 35; C = 33. Samples were added to one-half cup of boiling water and removed 2 minutes after the second boil.

The organoleptic ratings after cooking (*2*) for the 1940 series are based upon a numerical basis of 100 points. Scores of 0 to 40 are designated as C grade or unfit for food, 40 to 70 as B grade or good food but not the highest quality, and 70 to 100 as A grade or high quality. This difference in scoring is accounted for by the fact that two different, disinterested groups judged the samples.

The point will always arise in the determination of maturity of whether the seasonal variation affects the quality of the product. This work on corn was done in two successive seasons of opposite climatic conditions. In 1939 the season was hot and dry, while in 1940 it was cool and wet. Little variation, if any, was found in the indexes of quality for the two seasons. Although the length of ripening periods may vary from season to season, the specific gravity determinations

[1] Died October 9, 1940.

show no variation for the corn harvested in prime condition. It is evident, therefore, that restandardization is not necessary from year to year. Check tests indicate that the figures given here are applicable to all similar sweet corn varieties.

Table II indicates that the specific gravities as determined by means of the xylene–carbon tetrachloride mixture are somewhat higher than those determined by means of the water containing enough sodium chloride to give a specific gravity of 1.000. This is possibly caused by the fact that water adhering to the vegetable is weighed when it is immersed in the xylene–carbon tetrachloride mixture because of its failure to dissolve in this mixture. When the vegetable is weighed in the aqueous mixture, only the vegetable itself is weighed. The final result would be a slight error in both methods, and a different set of standards would have to be prepared for each method.

The brine method is somewhat the better of the two because of its low cost, because it is easier to prepare, and because it is less likely to change in use, although it should be tested with the hydrometer from time to time to be sure the specific gravity is 1.000.

TABLE II. 1940 SERIES

Specific Gravity of Thawed Corn In brine	In xylene–CCl_4	Organoleptic, Based on 100
1.042[a]	1.063	58
1.048[a]	1.053	45
1.050[a]	1.051	46
1.053[a]	1.062	55
1.058[a]	1.065	57
1.059[a]	1.065	48
1.062[a]	1.059	53
1.066[a]	1.074	56
1.066[a]	1.081	57
1.070[a]	1.076	66
1.071[a]	1.078	55
1.073[a]	1.058	69
1.074[a]	1.084	59
1.076[a]	1.082	60
1.080	1.089	73
1.084	1.098	69
1.085	1.096	62
1.086	1.103	80
1.087	1.087	74
1.088	1.094	77
1.089	1.093	85
1.091	1.098	77
1.092	1.101	71
1.096	1.101	70
1.096	1.102	75
1.097	1.101	77
1.097	1.102	77
1.097	1.111	77
1.098	1.101	77
1.098	1.101	68
1.098	1.106	80
1.098	1.106	80
1.098	1.109	74
1.098	1.111	91
1.099	1.103	70
1.099	1.105	68
1.099	1.106	82
1.100	1.097	79
1.100	1.102	75
1.101	1.105	76
1.101	1.110	86
1.102	1.100	89
1.102	1.105	72
1.102	1.110	71
1.102	1.111	74
1.104	1.103	70
1.106	1.112	87
1.106	1.114	82
1.107	1.110	78
1.107	1.114	71
1.108	1.110	88
1.108	1.116	79
1.109	1.111	82
1.109	1.112	92
1.109	1.116	84
1.110	1.120	89
1.112	1.116	89
1.113	1.110	80
1.113	1.115	80
1.117	1.113	80
1.118	1.117	77
1.121[b]	1.128	64
1.122[b]	1.122	64
1.130[b]	1.134	63
1.149[b]	1.147	34
1.150[b]	1.154	39
1.151[b]	1.155	35

[a] Immature.
[b] Overmature.

TABLE III. COEFFICIENTS OF CORRELATION FOR SPECIFIC GRAVITY OF THAWED CORN

Correlation between:	Coefficient
Xylene–carbon tetrachloride method and organoleptic tests (1939)	-0.8592 ± 0.0221
Brine method and organoleptic tests (1940)	$+0.8456 \pm 0.0246$
Xylene–carbon tetrachloride method and organoleptic tests (1940)	$+0.8408 \pm 0.0253$

The following tentative standards are suggested, based upon the results using the brine solution for the determination of the specific gravity:

Fancy	1.080 to 1.118
Reject, immature	1.079 and lower
Reject, overmature	1.119 and higher

These standards can be revised if and when other grades are generally packed.

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Determination of the Maturity of Frozen Peas

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A REVISION of the method for determining the maturity of frozen peas (*1*) has been worked out, in which the mixture of xylene and carbon tetrachloride is replaced by water containing enough sodium chloride to give a liquid of specific gravity 1.000. This liquid is considerably less expensive than the former, is easier to prepare, and has the added advantage of undergoing less change in specific gravity in use.

The following set of standards is suggested to replace those published before:

Fancy	1.072 and lower
Standard	1.073 to 1.084
Substandard	1.085 and higher

These standards can be revised if and when an extra standard grade is generally packed.

Literature Cited

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Separation of Lithium from Potassium and Sodium

By Treatment of the Chlorides with Higher Aliphatic Alcohols

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OF THE various methods for the quantitative separation of lithium from potassium and sodium by extraction of the mixed chlorides with organic solvents, the isoamyl alcohol method of Gooch (*2*) appears to have had the widest use. Because the boiling point of isoamyl alcohol is considerably above that of water, the aqueous solution containing the alkali chlorides may be evaporated in the presence of this solvent; the water is not only evaporated away without much loss of solvent but the salts are completely dehydrated in the process. Moreover, the lithium chloride gradually dissolves in the isoamyl alcohol while the other alkali chlorides are gradually precipitated out. Hence, for quantitative separation by this general method such a solvent is better than one, such as dioxane, whose boiling point is only slightly above that of water, and much better than one, such as acetone, whose boiling point is below that of water. The only other solvent of relatively high boiling point that has been advocated for this separation is pyridine (*3*), but its disagreeable odor and the difficulty of obtaining it readily in a highly pure state have apparently restricted its use.

The method of Gooch has the disadvantage that neither potassium chloride nor sodium chloride is quantitatively insoluble in isoamyl alcohol, so that relatively large corrections must be applied for the amounts of these salts that dissolve along with the lithium chloride. Furthermore, these corrections differ in accordance with the particular combination of salts present. Though the corrections may be applied with considerable confidence, there is, nevertheless, some uncertainty involved, especially in regard to the amounts of salts dissolved in the washings, and for this no corrections are attempted. Furthermore, the measurement of the volume of solvent present after extraction in order to apply these corrections is somewhat inconvenient in practice. A solvent that required no such corrections and yet had the high boiling point and other desirable characteristics of isoamyl alcohol would manifestly be better from the standpoint of both accuracy and convenience.

Since the solubilities of lithium, potassium, and sodium chloride in the aliphatic monohydric alcohols are known to decrease progressively in ascending the series from methyl to amyl alcohol, it seemed apparent that the solubilities of potassium chloride and sodium chloride in similar alcohols of more than five carbon atoms might become very small, whereas the solubility of lithium chloride might still remain large enough for analytical separation. Experiment showed this to be true. The higher alcohols chiefly considered were *n*-hexanol and 2-ethylhexanol, since these are now readily available in a technical grade at low cost. For the purpose of this separation no advantage would probably result from the use of a grade of these alcohols entirely free from isomers. By reason of these considerations, the experiments here described were all made with the so-called practical or technical grades of these two alcohols.

Solubility Experiments

The solubilities of lithium, sodium, and potassium chloride in the two alcohols at 25° C. are shown in Table I. These results were obtained by conventional procedures.

Before being used for these determinations the solvents were boiled to ensure their freedom from water, and the salts were carefully dehydrated. With one exception each result shown is the result of several concordant determinations. The result for the solubility of potassium chloride in 2-ethylhexanol is merely an estimate based upon the probable limit of measurement of the analytical method used for the determination, since the solubility of the salt in this solvent was so slight that the attempted measurements gave the same results as blank determinations. For convenient comparison the solubilities of the three salts in isoamyl alcohol are also given, calculated from data published by Turner and Bissett (*4*).

With the increase in number of carbon atoms the solubility of sodium chloride in these three alcohols decreases more than the solubility of lithium chloride, and the solubility of potassium chloride decreases much more. From the standpoint of probable sharpness of separation, the *n*-hexanol is obviously better than isoamyl alcohol, and the 2-ethylhexanol obviously much better. However, since the solubility of lithium chloride in octyl alcohol is considerably less than the solubility of this salt in either amyl or hexyl alcohol, it seemed possible that in actual practice hexyl alcohol might be a more satisfactory solvent. Therefore, analytical experiments were made with both solvents.

ANALYTICAL EXPERIMENTS. The general procedure recommended by Gooch was followed except for certain minor variations. The potassium and sodium were weighed out in the form of their chlorides, and the lithium in the form of carbonate, the lithium carbonate in the weighed test mixture then being converted to chloride by the addition of slightly more than the calculated amount of hydrochloric acid before evaporating the aqueous solutions of the salts with the alcohol. After evaporation and dehydration, the residual potassium chloride, sodium chloride, or both, was collected on a weighed sintered-glass crucible, and washed thoroughly with successive small portions of the cold alcohol. No attempt was made to restrict the total volume of alcohol used in washing, as is the usual practice in using amyl alcohol for this separation. After washing, the crucible and salts were dried in an air oven at a temperature sufficient to volatilize rapidly the residual solvent. In separations made with *n*-hexanol a temperature of 180° C. was used, and in those with 2-ethylhexanol, a temperature of 210° C.

The accuracy of separation was judged from the agreement or discrepancy between the weights of the residual salts and their original weight. The lithium chloride that dissolved in the alcohol was not converted to sulfate and weighed, as is the usual procedure in actual analysis, since this seemed unnecessary for measuring the accuracy of separation. However, in order to make sure that the observed results were not due to a compensation of errors, the weighed residual salts were all tested for lithium with a spectroscope. Except when the lithium was present in unusually large amounts, these experimental separations were made by

TABLE I. SOLUBILITIES OF CHLORIDES OF LITHIUM, SODIUM, AND POTASSIUM

(In certain amyl, hexyl, and octyl alcohols at 25° C., expressed in grams dissolved by 100 ml. of solvent)

Salt	Isoamyl Alcohol	*n*-Hexanol	2-Ethylhexanol
LiCl	7.3	5.8	3.0
NaCl	0.0016	0.0008	0.0001
KCl	0.0006	0.00004	<0.00001

TABLE II. SEPARATIONS OF SIMILAR AMOUNTS OF LITHIUM AS CHLORIDE BY *n*-HEXANOL AND 2-ETHYLHEXANOL

Solvent	NaCl Taken	KCl Taken	LiCl Present	Weight of Extraction Residue	Error of Separation in Terms of: NaCl	Na	KCl	K	LiCl	Li
	Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
n-Hexanol	0.2507	None	0.0608	0.2500	−0.0007	−0.0003	.....	.....	+0.0007	+0.0001
	0.2475	None	0.0629	0.2470	−0.0005	−0.0002	.....	.....	+0.0005	+0.0001
	None	0.2213	0.0592	0.2208	.....	.....	−0.0005	−0.0003	+0.0005	+0.0001
	None	0.2641	0.0663	0.2637	.....	.....	−0.0004	−0.0002	+0.0004	+0.0001
	0.1613	0.1524	0.0743	0.3122	.....	.....	.....	.....	+0.0015	+0.0002
	0.1702	0.1754	0.0755	0.3447	.....	.....	.....	.....	+0.0009	+0.0001
2-Ethylhexanol	0.2072	None	0.0640	0.2069	−0.0003	−0.0001	.....	.....	+0.0003	±0.0000
	0.2378	None	0.0636	0.2375	−0.0003	−0.0001	.....	.....	+0.0003	±0.0000
	None	0.3077	0.0548	0.3073	.....	.....	−0.0004	−0.0002	+0.0004	+0.0001
	None	0.2134	0.0679	0.2132	.....	.....	−0.0002	−0.0001	+0.0002	±0.0000
	0.1583	0.1049	0.0570	0.2628	.....	.....	.....	.....	+0.0004	+0.0001
	0.1147	0.1056	0.0435	0.2198	.....	.....	.....	.....	+0.0005	+0.0001
	0.1099	0.0791	0.0587	0.1884	.....	.....	.....	.....	+0.0006	+0.0001
	0.0714	0.1202	0.0621	0.1918	.....	.....	.....	.....	−0.0002	±0.0000

TABLE III. SEPARATIONS BY 2-ETHYLHEXANOL WITH LITHIUM PRESENT IN UNFAVORABLE RATIO OR AMOUNT

Analysis No.	No. of Extractions	NaCl Taken	KCl Taken	LiCl Present	Wt. of Extraction Residue	Error of Separation in Terms of: LiCl	Li
		Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
1	1	0.0493	0.0561	0.1584	0.1210	−0.0156	−0.0026
2	1	0.0547	0.0524	0.1366	0.1270	−0.0199	−0.0033
3	1	0.0605	0.0744	0.1010	0.1372	−0.0023	−0.0004
4	2	0.0568	0.0556	0.1278	0.1118	+0.0006	+0.0001
5	2	0.0757	0.0612	0.1240	0.1364	+0.0005	+0.0001
6	2	0.0699	0.0569	0.1113	0.1261	+0.0007	+0.0001
7	1	0.2296	0.2187	0.0546	0.4475	+0.0008	+0.0001
8	1	0.1673	0.2449	0.0541	0.4117	+0.0005	+0.0001
9	1	0.2269	0.2413	0.0510	0.4677	+0.0005	+0.0001
10	1	0.2831	0.1224	0.0502	0.4058	−0.0003	±0.0000
11	1	0.0829	0.1117	0.0122	0.1943	+0.0003	±0.0000
12	1	0.0968	0.0807	0.0118	0.1769	+0.0006	+0.0001
13	1	0.0886	0.0812	0.0118	0.1705	−0.0007	−0.0001
14	1	0.0812	0.0951	0.0117	0.1766	−0.0003	±0.0000

means of a single extraction only, and no solubility corrections were applied to any of the results obtained.

In Table II are shown typical results obtained in separations with *n*-hexanol and with 2-ethylhexanol under similar conditions. The results of the separations with *n*-hexanol are fairly satisfactory, though as might be expected from the solubility data, the results for sodium are slightly low, and those for potassium slightly less so. With both sodium and potassium present, the slightly high results for lithium reflect the combined noticeable solubility of the sodium and potassium chlorides. However, these uncorrected results are obviously much better than could be obtained by the use of isoamyl alcohol without application of solubility corrections, and not very much worse than those ordinarily obtained by the isoamyl alcohol method when such corrections are applied. The results obtained with 2-ethylhexanol are obviously better than those obtained with *n*-hexanol, and for an analytical method of this type every one of these results is very satisfactory. In accordance with the solubility data, 2-ethylhexanol is therefore a better solvent than *n*-hexanol for the separation of lithium chloride from admixtures with potassium chloride and sodium chloride, and good results are obtained in spite of the low actual solubility of lithium chloride in this octyl alcohol.

In order to test further the value of 2-ethylhexanol as a solvent in this separation, a number of analyses were made of mixtures in which lithium was present either in relatively large amount or in small proportion. Typical results are shown in Table III.

As might be expected from the known inaccuracy of the isoamyl alcohol method when an attempt is made to separate 100 mg. or more of lithium chloride by a single extraction, poor results are also obtained with 2-ethylhexanol when an attempt is made to separate such quantities by a single extraction. This is shown by the first three results in Table III. However, the results with 2-ethylhexanol, especially analyses 1 and 2, are much poorer than analogous results obtained by Gooch (*2*) with isoamyl alcohol. This difference is apparently not due to the difference in the solubility of lithium chloride in the two solvents, for in the experiments there was always more 2-ethylhexanol than was needed to dissolve the lithium chloride present. It is probably due to difference in temperature of final dehydration when this dehydration is performed at the boiling points of the two solvents, a greater proportion of insoluble lithium hydroxide being formed by hydrolysis at the high boiling point of 2-ethylhexanol.

The remedy, of course, is to avoid dehydrating the salts with 2-ethylhexanol at too high a temperature. Dehydration at a temperature 50° C. below its boiling point will remove the water as well as dehydration with boiling isoamyl alcohol. That better results are obtained when the temperature of dehydration is not too high is shown by analysis 3 of Table III. However, by a double extraction, which is the usual procedure, satisfactory results are obtained when 2-ethylhexanol is used to separate these larger amounts of lithium, even when not much attention is given to the temperature used for final dehydration. This is shown by analyses 4, 5, and 6. That satisfactory results are obtained by even a single extraction when lithium is present in small amount and proportion is shown by the other analyses of Table III.

All the salt residues from which the lithium chloride had been extracted with 2-ethylhexanol were tested for the presence of lithium by means of a hand spectroscope and a special light filter. With the exception of those from analyses 1, 2, and 3 of Table III, no lithium was detected in these residues. From this evidence of complete separation and from the solubility data it seems likely that the so-called errors of separation found by the test analyses are in large part only errors of manipulation.

Applicability of Ammonium Stearate Reaction

In a previous paper by the senior author (*1*) it was shown that minute amounts of lithium present in an isoamyl alcohol extract of mixed alkali chlorides could be detected or estimated by means of a reagent consisting of solution of ammonium stearate in this solvent. The same reaction may be applied in 2-ethylhexanol solution, even though ammonium stearate is considerably less soluble in 2-ethylhexanol than in isoamyl alcohol. Only about 0.55 gram of ammonium stearate dissolves in 100 ml. of 2-ethylhexanol at room temperature. In spite of this low solubility a sufficient concentration of salt is present in the saturated reagent to give precipitates or turbidities with very dilute solutions of lithium chloride in 2-ethylhexanol. Lithium stearate is apparently somewhat less soluble in 2-ethylhexanol than is isoamyl alcohol, since

the sensitivity of the reaction appears, in spite of the more dilute reagent, to be slightly greater in the octyl alcohol solution. From a series of experiments it was found that when 1 or 2 ml. of a saturated solution of ammonium stearate in 2-ethylhexanol was added to like volumes of a solution of lithium chloride in this same solvent, a slight but definite reaction was still obtained when the amount of lithium present was as low as 0.01 to 0.02 mg.

Summary

These experiments show that 2-ethylhexanol yields results equal to or better than those obtained by the use of isoamyl alcohol with the advantage that no solubility corrections are necessary and therefore no attention need be paid to the exact volume of solvent used in the extraction or for washing. Another advantage of 2-ethylhexanol over isoamyl alcohol for this separation is that the former need not be boiled in order to dehydrate the salts properly. As a consequence the danger of loss from bumping is less, and quantities of disagreeable fumes are not evolved, which some chemists regard as a serious objection to the isoamyl alcohol method of Gooch. On the whole, 2-ethylhexanol appears to be the best solvent yet proposed for the separation of lithium from potassium and sodium by extraction of the mixed alkali chlorides. The ammonium stearate reaction for the detection or estimation of lithium may be applied with at least equal success in 2-ethylhexanol solution.

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PRESENTED before the Division of Analytical and Micro Chemistry at the 102nd Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J. Constructed from a senior thesis submitted by Herbert D. Axilrod to the Department of Chemistry, Princeton University, 1941.

Improved Manometer Design

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IN CHEMICAL and metallurgical plants there are numerous applications for vertical manometers, to indicate pressure, vacuum, liquid level, or fluid flow rate. The diagram illustrates a manometer that can be made in any plant having ordinary machine shop and welding facilities. It is inexpensive, can be made in any length desired, is easily maintained, and will not lose any appreciable amount of manometer liquid even when pressures greatly in excess of the maximum scale reading are accidentally applied.

The body of the manometer consists of two pieces of standard $1^1/_2$-inch wrought iron pipe separated by a 1.56-cm. (0.625-inch) mild steel partition to which they are welded. A length of standard $^1/_4$-inch wrought iron pipe is inserted through a central hole in the partition plate and welded to it. This tube extends 6.25 cm. (2.5 inches) above the plate and down to a point 1.25 cm. (0.5 inch) above the bottom outlet hole to the gage glass. The top outlet hole to the gage glass is drilled so that the bottom of this hole is flush with the top of the partition plate. Bosses are welded to the sides of the manometer body and are drilled and tapped for the high- and low-pressure connections and the filling plug. The high- and low-pressure connections can be spaced for convenient installation of an equalizing valve. The open ends of the meter body are closed by welding on end plates, which are drilled and tapped for $^1/_4$-inch pipe plugs for ease of cleaning and draining. A 0.16-cm. (0.0625-inch) thick mild steel baffle plate is welded to the top end plate before it is put in place.

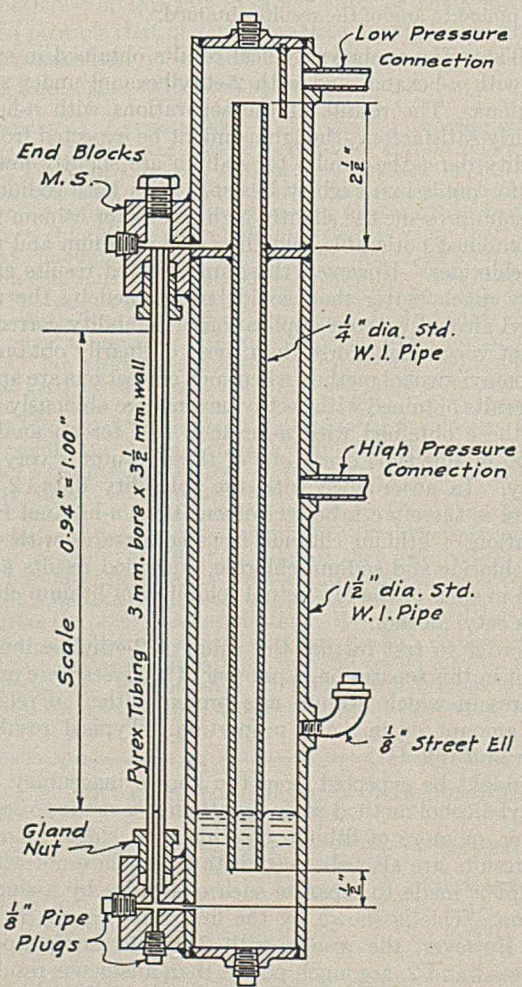
The end blocks for the gage glass are drilled and tapped as shown for the gland nuts and clean-out plugs, and welded to the manometer body. Care must be taken to hold these blocks rigidly in correct alignment where they are being welded in place, as gage glasses are easily broken by tightening up incorrectly aligned gland nuts.

A satisfactory gage glass is Pyrex tubing of 3-mm. bore and 3.5-mm. wall.

An aluminum or plastic scale with zero adjustment is mounted behind the gage glass. In this type of manometer a correction must be made on the scale for the depression of the liquid level in the high-pressure chamber. In the manometer described above the correction factor is 0.94.

OPERATION. When a pressure in excess of the maximum scale reading is applied, the liquid seal in the lower chamber is broken at the bottom of the central tube. The manometer liquid in this tube is blown into the upper chamber, where it collects in the annular space surrounding the tube. The baffle plate prevents this liquid from being blown out of the manometer. The central tube is then free of liquid, and permits continued passage of gas with no further disturbance of the liquid. When the excess pressure is released, the liquid in the upper chamber drains back to the lower chamber through the gage glass and restores the seal.

This type of manometer is particularly suitable for use in chemical plants on materials with high crystallizing temperatures, as the body of the instrument can readily be maintained at a temperature above the point of crystallization by means of a steam jacket or electric heater.



Chromatographic Adsorption Analysis

HAROLD H. STRAIN, Carnegie Institution of Washington, Stanford University, Calif.

The separation of mixtures by passage of their solutions through towers or columns of powdered adsorbents (Tswett's chromatographic adsorption analysis) is applicable to the detection, preparation, and estimation of both inorganic and organic substances. This method, in macro and micro modifications, is useful for concentration of solutes from dilute solution, for comparison of substances suspected of being identical, and for estimation of the structure of organic molecules. The new technique is beginning to find application in industrial operations.

IN 1906 at the then Russian city of Warsaw, a botanist named Tswett invented a unique adsorption method of chemical analysis that was destined to find wide application in many branches of the natural sciences (*52*). Results obtained through improvement and application of the method have broken the hardpan in barren fields and have made virgin regions tillable by the chemist.

Separation of Colored Compounds

In the course of his experiments on the pigments of green leaves, Tswett performed a simple experiment that has formed the basis for chromatographic adsorption technique.

In the narrow end of a constricted glass tube he placed a wad of cotton, and above this he tamped successive portions of finely powdered, adsorptive material such as precipitated chalk. This packed tube or adsorption column (Figure 1) was attached to a suction flask, and then a green, petroleum ether extract of dried leaves was passed through it. Under these conditions, some of the pigments, particularly the yellow carotene hydrocarbons, were weakly adsorbed and passed rapidly through the column. Other more strongly adsorbed pigments, the green chlorophylls and yellow xanthophylls, were held near the top of the adsorbent. In this way, the migrating pigments gradually separated from one another, forming a series of colored bands or zones, called a "chromatogram". The bands always occurred in the same sequence, analogous to the colors in the spectrum.

Tswett observed that the separation of the several bands was increased if only a small quantity of the solution was passed into the column and if this was then followed with fresh solvent. The completed separation of the leaf pigments is shown by Figure 2.

Utilization of fresh solvent to complete the separation of the bands is now recognized as an essential step in the resolution of mixtures. It is known as the "development" of the chromatogram. A diagrammatic representation of the development of a chromatogram is provided by Figure 3.

In order to recover the leaf pigments separated on the adsorption column, the development with fresh solvent was continued until the pigments in each band were carried successively into the percolate. This provided solutions of the pure pigments. Another procedure was to push the moist adsorbent from the tube. The bands in the cylinder of cohesive adsorbent were then separated from one another with a knife. Each colored band or zone of adsorbent was agitated with a little alcohol or other polar solvent (now called the "eluant"). This liberated the adsorbed pigment. Solutions of the eluted pigment were separated from the adsorbent by filtration. Still another procedure was to dig out the bands separately from the column with a spatula and to elute the pigments from the respective portions with a polar solvent.

As indicated by Figure 2, Tswett separated one yellow carotene, three or four yellow xanthophylls, and two green chlorophylls from leaf extracts. However, only minute quantities of these pigments were obtained. Isolation of crystalline leaf xanthophyll at this time by use of other methods provided a single pigment and gave credence to the assumption that Tswett's adsorption method had produced alteration of a single leaf xanthophyll (*54*, *55*). As a consequence, the adsorption method fell into disrepute, and not until 1931 was its great usefulness rediscovered.

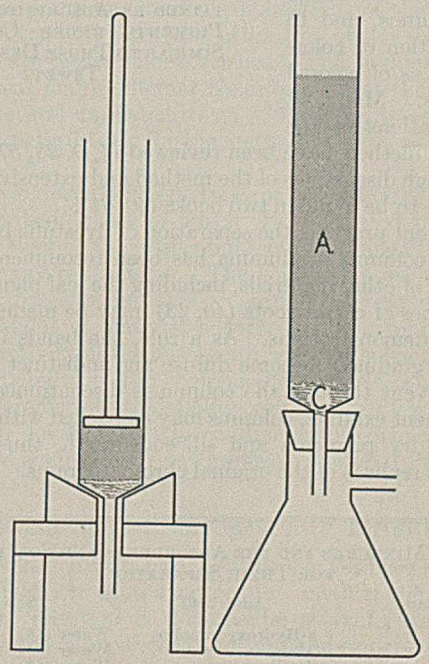


FIGURE 1. (*Left*) CORK RING SUPPORT AND METAL PLUNGER USED FOR PACKING ADSORPTION COLUMN. (*Right*) COMMONEST ARRANGEMENT FOR CHROMATOGRAPHIC ADSORPTION ANALYSIS (*40*)

A, adsorbent; *C*, cotton

Separation of crystalline, presumably homogeneous, carrot root carotene into several isomeric hydrocarbons by adsorption on columns of specially prepared adsorbents marked the beginning of a new era in chromatographic investigations (*19*, *22*, *26*, *43*). It demonstrated the great selectivity of the method because the carotenes were found to differ only in the position of one double bond in molecules containing 40 atoms of carbon. It proved that sufficient quantities of material for subsequent chemical analysis could be prepared quickly and in good yield. It indicated that the method of preparation and activation of the adsorbent exerted a profound effect upon the applicability of the method.

Because of the importance of the carotenes as provitamins A (*14*), their resolution by adsorption attracted the attention of workers in many different fields. One result was the substantiation of Tswett's observations pertaining to the resolution of the leaf pigments. With the most selective adsorb-

ents, about a dozen xanthophylls were finally separated from the leaf extracts (*45*). These advances stimulated a great deal of work on the preparation of various adsorbents for the separation of mixtures of many different types of compounds ranging from the elements themselves to the most complex materials found in organic nature. This in turn led to many new applications of the method, to the perfection of macro- and microcolumns, to improvement in the design of the columns, and to the separation of colorless as well as of colored compounds. Many of the applications of the adsorption method have been reviewed (*4, 9, 28, 57, 62, 63*), and thorough discussions of the method and extensive bibliographies are to be found in two books (*40, 61*).

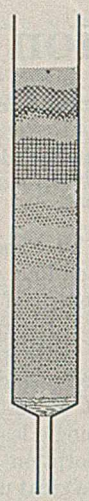


FIGURE 2. CHROMATOGRAM OBTAINED BY ADSORPTION OF LEAF PIGMENTS UNDER CONDITIONS SIMILAR TO THOSE DESCRIBED BY TSWETT

For student practice, the separation of dyestuffs by adsorption upon columns of alumina has been recommended (*36*). Separation of other materials, including the leaf pigments and the carotenes of carrot roots (*40, 43*) may be made the basis of lecture demonstrations. As a rule, the bands in a chromatogram gradually become diffuse and indistinct when the flow of solvent through the column is discontinued; hence, for permanent exhibits, columns may be packed with mixtures of stable, dry pigments and siliceous earth, thus yielding permanent replicas of the original chromatograms.

TABLE I. MIXTURES AND THE ADSORBENTS AND SOLVENTS USED FOR THEIR SEPARATION

Mixture	Adsorbent	Solvent
Cations	8-Hydroxyquinoline	Water (*13*)
	Alumina	Water (*38*)
Lithium isotopes	Zeolites	Water (*47*)
Halogens	Magnesium silicate	Carbon tetrachloride (*40*)
Carotenes	Magnesia	Petroleum ether (*43*)
	Lime	Petroleum ether (*22*)
	Alumina	Petroleum ether (*26*)
Vitamins A_1 and A_2	Lime	Petroleum ether (*19*)
Chlorophylls	Sucrose	Petroleum ether (*59*)
	Inulin	Petroleum ether (*30*)
	Magnesium citrate	Ether and petroleum ether (*30*)
Xanthophylls	Magnesia	1,2-Dichloroethane (*45*)
	Calcium carbonate	Carbon disulfide (*60*)
Fatty acids	Charcoal	Petroleum ether (*8*)
Fats	Alumina	Petroleum ether (*51*)
Polycyclic hydrocarbons	Alumina	Petroleum ether (*58*)
Thiamin and riboflavin	Decalso and Supersorb	Water (*11*)

Separation of Colorless Substances

Colorless compounds are separable on the adsorption columns, but special methods must be employed to locate these invisible materials. According to one procedure, successive portions of the percolate are collected and analyzed by chemical, optical, or biological methods. In order to use the Toepler *Schlieren* optical method for determination of colorless substances in the percolate, the solution may be passed upward through the adsorbent (*49*). By another procedure, the column containing the colorless compounds is sectioned empirically and the compounds eluted from the respective sections are examined as just described. Colorless, fluorescent compounds may be observed on the adsorption columns in ultraviolet light (*20, 58*).

Previous to adsorption some colorless compounds may be converted into colored derivatives that can then be separated on the columns (*27, 41*). Other colorless compounds may be located on the columns by means of reagents with which they produce color changes. These reagents may be passed through the column (*38*) or they may be applied to the cylinder of moist adsorbent after it has been pressed from the tube (*63*). A clever innovation is the adsorption of cations on columns of 8-hydroxyquinoline, with which they form colored compounds and thus become visible (*13*). Adsorption of a colorless compound with a colored indicator serves to locate the former with respect to the latter (*5, 6*). The closer together the two are adsorbed, the more precisely one can locate the colorless compound.

TABLE II. SUBSTANCES PREPARED BY ADSORPTION OF EXTRACTS OF PLANT AND ANIMAL PRODUCTS

Substance	Adsorbent	Solvent	Source
Vitamin A	Lime	Petroleum ether	Fish liver oil (*18*)
Vitamin B_1	Decalso	Water	Rice hulls (*10*)
Vitamin B_2 (acetylated)	Alumina	Ethyl acetate	Alfalfa (*25*)
Vitamin D	Alumina	Petroleum ether	Fish liver oil (*6*)
Vitamin E	Alumina	Petroleum ether	Wheat germ oil (*15, 50*)
Vitamin K_1	Decalso	Petroleum ether	Fish meal (*3*)
Vitamin K_2	Decalso	Petroleum ether	Putrified fish meal (*29*)
Sex hormones	Alumina	Carbon tetrachloride	Human urine (*7*)
Glucosidase and chitinase	Bauxite	Water	Snails (*64*)
Anthocyanins	Alumina	Water (*21*)	.....
Pterins	Frankonit	Water	Insects (*2*)
Carotenes	Magnesia	Petroleum ether	Leaves (*30, 44*)

Applications of the Method

From the analyst's point of view it is important to know what can be accomplished by use of the adsorption method. To this end it has seemed desirable to classify the various procedures and to illustrate them with selected experiments.

One of the principal uses of adsorption columns is the resolution of mixtures into their constituents. Here all the components are recovered after qualitative or quantitative separation of the mixture. Some examples are summarized in Table I.

Very often it is necessary to use the adsorption method for the preparation of only one or two substances from complex mixtures. Many examples of this application of the method are to be found among the procedures utilized for preparation of specific substances from extracts of biological materials. Minute quantities of enzymes, hormones, vitamins, pigments, and similar materials have been prepared from extracts containing large quantities of other materials. Unwanted materials in these extracts frequently complicate the adsorption procedure, necessitating the use of relatively large quantities of adsorbent. Examples of important biological products prepared or investigated by adsorption methods are reported in Table II.

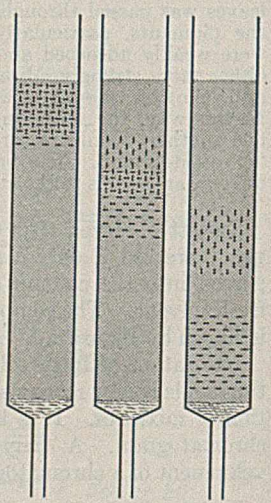


FIGURE 3. SUCCESSIVE STAGES IN DEVELOPMENT OF A CHROMATOGRAM

A solution of substances + and − is passed into the column. When the column is washed with fresh solvent, + and − move through the tube at different rates, finally separating completely from each other (*40*).

TABLE III. ADSORBENTS AND SOLVENTS FOR SEPARATION OF CAROTENES FROM OTHER LEAF PIGMENTS

Adsorbent	Solvent
Inulin	Petroleum ether (*35, 53*)
Sucrose	Petroleum ether (*53, 59*)
Calcium carbonate	Petroleum ether (*53*)
Starch	Petroleum ether (*46*)
Calcium phosphate	Petroleum ether (*33*)
Soda ash	Petroleum ether (*23*)
Magnesia (partly activated)	Petroleum ether (*17*)
Magnesia (activated)	1,2-Dichloroethane (*45*)

If quantitative separations of one or two components from complex mixtures are to be made, the adsorption method is often modified so that the materials to be estimated pass through the column without being adsorbed while the contaminants remain on the column. This reduces losses that often result from incomplete elution of adsorbed compounds. One of the best examples of this application of the adsorption method is the separation of the carotenes from other pigments in leaf extracts. A number of adsorbents and solvents have been utilized for this purpose, as is illustrated in Table III. The method is not restricted to use in this way, because it has been shown that riboflavin and thiamin (vitamins B_2 and B_1) in food products can be adsorbed and eluted quantitatively (*11*).

Some materials containing traces of impurities may be purified by passage of the solutions through adsorption columns that retain the impurities. This application of the method is similar to the well-known decolorization of solutions by percolation through towers of charcoal or fuller's earth, as in the industrial clarification of sugar solutions and lubrication oils.

Chromatographic adsorption is often used to determine the purity of chemical compounds. Substances are said to be chromatographically homogeneous if they cannot be purified further with adsorption columns.

Still another use of the adsorption technique is the concentration of solutes from dilute solution, thus eliminating or supplementing the tedious and sometimes destructive process of evaporation. Materials adsorbed by passage of the dilute solution through the column are recovered in greatly concentrated form upon elution from the adsorbent. This procedure has been utilized for the concentration of certain alkaloids and urinary pigments (*16, 24*).

Two substances suspected of being identical may be compared quickly by use of the chromatographic method. A solution of each compound is adsorbed on separate columns to make certain that the materials yield only one band and are therefore homogeneous. Solutions of the two compounds are then mixed and adsorbed on a fresh column of adsorbent. Formation of a single band indicates that the two substances are very similar or identical. Formation of two bands proves that the compounds are different (*28, 37, 40, 61*).

The great selectivity of the chromatographic adsorption method makes it extremely useful for the detection of adulterants in technical products. It has been used to prove the presence of artificial coloring in wine and in butter (*32, 48*).

Determination of the molecular structure of certain types of organic molecules has been facilitated by the use of adsorption methods. Experience has shown that there is a relation between the adsorbability of organic molecules and the architecture of their molecules. The more polar the molecule, the greater the number of polar groups, and the greater the number of double bonds the more strongly is the compound adsorbed. Chemical reactions that alter the molecule also change its relative position upon the adsorption column. The greatest use of these relationships has been made in studies of the carotenoid pigments (*40*).

Chromatographic adsorption has found some use for the separation of hydrocarbons and sterols from their addition compounds with trinitrobenzene or picric acid. The nitro compounds are held by the adsorbent and the liberated materials pass into the percolate (*34, 35*).

For the separation of water-soluble, ionized, or charged compounds the adsorption method may be combined with the electrophoretic method. When electrical potential is applied to the ends of a column upon which ionized substances have been adsorbed, the materials migrate over the surface of the adsorbent although no liquid is permitted to flow through the tube. Thus far, the method has been applied only to the separation of a few dyes (*42*).

Recent developments indicate that the chromatographic adsorption method may find considerable use in industrial processes (*1*). Alumina has been utilized for the dehydration of organic solvents (*12*), and magnesia has been recommended for continuous purification of dry cleaning solvents (*39*).

Apparatus and Procedure

In spite of the hundreds of applications of the adsorption method in the past ten years, utilization of this technique remains an art rather than a science. This is due to the fact that a great many different conditions affect the procedure, often in several unrelated ways. Moreover, there is no adequate theoretical basis for the method to serve as a guide for its use (*8, 56*).

Some two dozen different types of adsorption columns have been described, a few of which are illustrated in Figure 4. The diameters of the adsorption columns range from 1 or 2 mm. to many centimeters. The lengths are usually 6 to 12 times the diameter, but this proportion is by no means fixed.

The amount of material adsorbed on the columns varies with the activity of the adsorbent, with the solvent, and with the adsorbability of the compound itself. It ranges from a few micrograms with the smallest columns to a few tenths of a gram with columns several centimeters in diameter. With still larger columns, several grams of material may be prepared.

Adsorbents, Solvents, and Eluants

No universal adsorbent or solvent has yet been discovered. Each application of the adsorption method depends upon the careful selection of adsorbents, solvents, and eluants, upon the basis of experience and trial and error. Adsorbents and solvents useful for adsorption of a given substance are

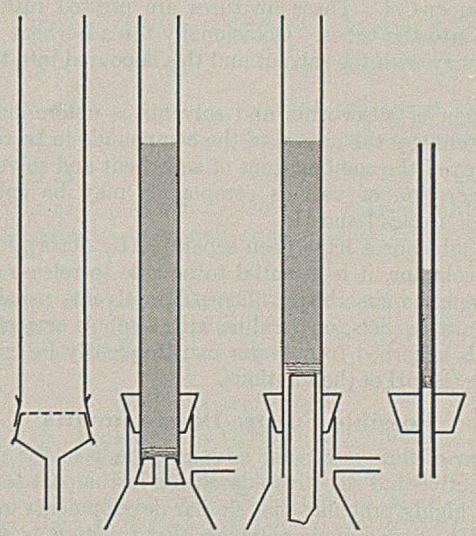


FIGURE 4. VARIOUS TYPES OF ADSORPTION TUBES (*40, 61*)

usually suitable for adsorption of other materials of the same class.

Particular attention must be paid to the properties of the adsorbent. The adsorptive capacity of most solids varies enormously with the method of preparation and with the presence of impurities and polar solvents such as water. To a considerable extent it is characteristic of the solid itself, as is indicated by the following list of adsorbents arranged in approximate order of their adsorption capacity, the most active recorded first (*40*):

Activated alumina, charcoal, and magnesia (Micron brand)	Calcium carbonate
Lime	Talc
Magnesia (Merck)	Magnesium citrate
Magnesium carbonate	Inulin
Calcium phosphate	Sucrose, starch

The adsorption capacity of all solids varies with the solvents that are employed. Adsorption is strongest from aliphatic hydrocarbons such as petroleum ether, and is progressively weaker from more and more polar solvents, as illustrated by the following list of solvents arranged in approximate order of their effect on adsorption (adsorption is greatest from those listed first):

Petroleum ether, b. p. 30–50°	1,2-Dichloroethane
Petroleum ether, b. p. 70–100°	Alcohols
Carbon tetrachloride	Water
Cyclohexane	Pyridine
Benzene	Acids

Substances adsorbed from a nonpolar solvent may be caused to move through the column at a faster rate by the addition of a more polar liquid to the solvent. This accelerates the development of the chromatogram (*51*).

For each application of the adsorption technique, the adsorbent must be of just the right activity. If it binds the adsorbed compounds too firmly, a chromatogram cannot be developed. If it is too weakly adsorptive, sufficient quantities of material will not be adsorbed.

The particle size of the adsorbent is of importance because this determines the rate of percolation of the solvent through the column, the definition and evenness of the bands, and the method to be used in packing the adsorption column. Granular adsorbents (about 200-mesh) can be poured into the glass tube and tamped gently. More finely divided materials such as precipitated chalk must be tamped firmly into the column in small portions. Very fine powders should be mixed with a filter aid such as the heat-treated siliceous earth known as Hyflo Super-Cel. These mixtures are pressed rather than tamped into the tubes. Occasionally the adsorbent is made into a slurry with the solvent and this is poured into the glass tube.

Selection of adsorbents and solvents is determined, to a large extent, by the nature of the compounds to be resolved. Some idea of the combinations of adsorbent and solvent used for preparation of various compounds may be gained by perusal of Tables I and II.

Once substances have been separated by adsorption upon Tswett columns, it is essential to be able to release or elute them from the adsorbent. The polar solvents usually used for this are alcohols, or pyridine with alcohols or acetic acid. Materials adsorbed from water can frequently be eluted by changing the pH of the solutions.

Possible Future Developments

In view of the spectacular advances made in the preparation of active and selective adsorbents in the past ten years, it seems highly probable that similar developments may continue. The new organic polymers that function as exchange adsorbents have yet to be tested in adsorption columns. Future investigations may be expected to reveal methods whereby adsorbed compounds can be eluted in good yield, thus extending the usefulness of the chromatographic adsorption technique in quantitative analysis. Much remains to be done in order to perfect the methods for location of colorless compounds on the adsorption columns. With the clarification of concepts regarding the process of adsorption itself, further useful applications of the chromatographic method will undoubtedly be made.

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Sodium Salt of Quinizarin-6-Sulfonic Acid as an Acid-Base Indicator

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THE red sodium salts of quinizarin sulfonic acid have been used as dye intermediates by the I. G. Farbenindustrie (*3*), and for the formation of metallic lakes by Bayer & Co. (*1*). The author observed that when the sodium salt of quinizarin-6-sulfonic acid was treated with acid, the yellow quinizarin-6-sulfonic acid was formed, while treatment with alkali produced a blue-violet coloration. As these reactions indicated that the sodium salt of quinizarin-6-sulfonic acid might be used as an indicator in acidimetry-alkalimetry, this investigation was undertaken.

Preparation of Reagents

The quinizarin is prepared according to the method of Bigelow and Reynolds (*2*). To make the sodium salt of quinizarin-6-sulfonic acid (*4*) quinizarin, boric acid, and 20 per cent fuming sulfuric acid are heated to 90–100° C. for 1.5 hours, after which 60 per cent fuming sulfuric acid containing mercuric sulfate is added; the temperature is raised to 170–180° C., and kept constant until the melt becomes soluble in water. The mass is then poured into water, and the quinizarin-6-sulfonic acid is neutralized with sodium carbonate and salted out with sodium chloride. Red crystals are obtained, which are purified by recrystallization from alcohol. The sodium salt of quinizarin-6-sulfonic acid is soluble in water and concentrated sulfuric acid.

Approximately 0.1 *N* solutions of hydrochloric acid, sulfuric acid, nitric acid, acetic acid, oxalic acid, sodium hydroxide, and sodium carbonate, and a 0.2 *N* solution of sodium hydroxide were prepared, as was the universal buffer mixture of Prideaux and Ward (*5*), which gives pH values between 2 and 12. A 0.1 per cent aqueous solution of the sodium salt of quinizarin-6-sulfonic acid is used.

Color Ranges and Constants of Indicators

This indicator changes from a yellow color under distinctly acid conditions to pink and finally to blue-violet as alkali is added. The change from yellow to pink is sharp, and that from pink to blue-violet is gradual. The color ranges were obtained through the use of a series of buffer solutions, made by titrating the Prideaux and Ward universal buffer solution with 0.2 *N* sodium hydroxide. All buffer solutions employed in this investigation were checked by means of a Beckman pH meter. The pH range for the change from yellow to pink was found to be 7.1 to 9.1, and from pink to blue-violet 9.1 to 11.5 at 20° C.

The ionization constants were determined by using a Bausch & Lomb hydrogen-ion colorimeter. The pKa for the first hydrogen was found to be 8.2 and for the second hydrogen 10.7 at 20° C.

Titration

Acid and base titrations were made by using the appropriate indicator in each case. These were compared with similar titrations made by using a 0.1 per cent aqueous solution of the sodium salt of quinizarin-6-sulfonic acid as the indicator, the end point being the color change from yellow to pink.

Discussion

The preparation of the indicator required no special apparatus. The cost of preparation of the compound was less than that of phenolphthalein. The author found that in titrating acids with bases, the color change was sharper when using the sodium salt of quinizarin-6-sulfonic acid than when using phenolphthalein. In titrating bases with acids, the sodium salt of quinizarin-6-sulfonic acid gave a sharp color change, whereas phenolphthalein cannot be used so efficiently. This indicator can be used for colorimetric determinations of pH over the range from 7 to 11.5.

TABLE I. TITRATIONS OF ACIDS WITH BASES[a]

Acid	Base, NaOH	Acid-Base Ratio	Mean Deviation	Indicator
Ml.	*Ml.*			
HCl				
29.00	27.81	0.960	0.000	Na salt of quinizarin-6-sulfonic acid
28.66	27.47	0.959	0.000	Phenolphthalein
29.40	27.91	0.951	−0.001	Methyl orange
H_2SO_4				
27.32	30.86	0.896	−0.001	Na salt of quinizarin-6-sulfonic acid
27.98	31.19	0.898	−0.001	Phenolphthalein
27.98	31.01	0.902	0.000	Methyl orange
HNO_3				
28.32	27.03	1.047	+0.001	Na salt of quinizarin-6-sulfonic acid
28.99	27.61	1.050	+0.001	Phenolphthalein
29.00	27.47	1.055	0.000	Methyl orange
$HC_2H_3O_2$				
28.66	29.77	0.962	+0.001	Na salt of quinizarin-6-sulfonic acid
28.99	30.24	0.959	−0.001	Phenolphthalein
$H_2C_2O_4$				
28.66	32.43	0.884	−0.001	Na salt of quinizarin-6-sulfonic acid
28.32	32.02	0.883	0.000	Phenolphthalein

[a] Values are means of several titrations, using volumes between 20 and 40 ml.

The sodium salt of quinizarin-6-sulfonic acid can be used as an indicator in the titration of strong acids with strong bases, and weak acids with strong bases.

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The High-Vacuum Still in Medicine

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THE place of the high-vacuum still in medical research is largely a matter of definition. Various organic substances of high molecular weight used in the preparation of drugs have been purified in the high-vacuum still and most of the materials which are produced commercially by molecular distillation are applied in popular medicine—the vitamins (*23*), for instance. When, however, we examine the acceptance which the latest kind of molecular distillation apparatus and method has found in laboratories devoted to medical research we find that the technique has not yet come into widespread use, which is a pity because the methods would seem to offer much to the biochemist and pharmacologist.

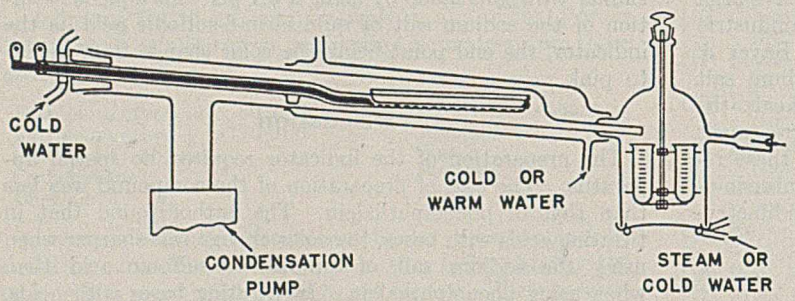

FIGURE 1. BURCH TRAY STILL

Molecular distillation is a term applied as loosely as its real meaning is restricted. In the narrow sense it refers to distillation between a parallel evaporator and condenser, the gap between the two being held under high vacuum. In the wider terminology of the literature almost any kind of a distillation device, whether pot or tube or a falling-film apparatus, is referred to as a molecular still if the operating pressure is believed to be less than 0.001 mm. Operating pressures are generally quoted, not from measurements made during and at the site of distillation but from the theoretical pressure which the pump attached to the apparatus could produce under ideal conditions when the apparatus contained nothing to be distilled. This state of affairs must be the subject of a separate paper, since it is in urgent need of airing. For the purpose of this review we may cover the distillations which have been done in pot stills, tubular stills, falling-film stills, and revolving-plate stills.

Figure 1 shows the first tray still of Burch (*4*) and Figures 2 and 3 show various pot stills after Waterman (*25*), Washburn (*24*), Hickman (*19*), Nelson and Haller (*21*), Carothers (*5*), and others. The material is placed in the pot, which may have a detachable lid; vacuum is applied and the material is warmed while the lid is cooled or frozen. The distillate is removed at intervals by dismantling the still or allowing drippings from the lid to pass out through a side arm into a receiver. This kind of still has been in operation since about 1929 in laboratories throughout the world.

The tubular stills, shown in Figure 4, are of two general varieties, the simpler kind having a single stationary source of heat, the others having a graduated or moving evaporative zone. The material is placed at the far closed end of the tube which is maintained nearly horizontal. In the simple type, heat is applied gently below the material and later the sublimate is scraped off the walls near the mouth after dismantling. In the compound type (*2, 10*) the material is volatilized at the far end and the vapor allowed to condense fractionally by controlling the heat throughout the length of the tube. The proper description for this kind of apparatus would seem to be a high-vacuum diffusion still, since it is not truly molecular no matter how low the pressure of operation.

In the falling-film molecular still, Figure 5, the substance, generally dissolved in oil, is allowed to fall in a thin film over a heated surface which is maintained parallel to a cooled condenser. The revolving plate still, Figure 6, employs centrifugal force to whirl the liquid in a thin layer across the surface of a heated plate held in front of a cooled condenser. The thermal exposure in a falling-film still is perhaps a hundred times less than in a pot still. In the centrifugal still the exposure may be a thousand or ten thousand times less and the distillation method is extended in corresponding degree to the separation of unstable substances.

Reviewing the substances of interest in medicine, calciferol (*3*), or vitamin D_2, was first distilled free from ergosterol in a tubular diffusion still. Vitamin K was separated by Almquist (*2, 10*) in a compound tube still and by Dam (*6*) in a pot still. Progesterone (*1*) was separated from an extract of pigs' ovaries in a tiny pot still in the early 1930's. Farmer and van den Heuvel (*13*) investigated the marine fatty acids in glass pot stills. Vitamins A (*20*), D (*18*), and E (*17*) have been produced industrially during the last few years in relatively large quantities in the falling-film stills. A valuable but by no means complete bibliography of high-vacuum distillation has been compiled by Detwiler and Markley (*7, 8*); and a revised compilation is reported to be near completion.

At the present time the molecular still can serve medicine in three capacities: as a manufacturing process for vitamins A and E and sterols; as an occasional adjunct to organic research; and as a quantitative tool in the central focus of medicinal research. The usefulness as an adjunct to organic research has existed for about twelve years, and as a manufacturing process for the last three or four years, but this application is expanding rapidly. During the year 1941 more than five tons of vitamin A in the form of ester concentrate (largely palmitate) will have been distilled from over 100,000 gallons of crude fish liver oils. About half a ton of mixed tocopherols will have been distilled from vegetable sources in the same period. However, the importance of molecular distillation is not its past accomplishments, which are insignificant compared with those

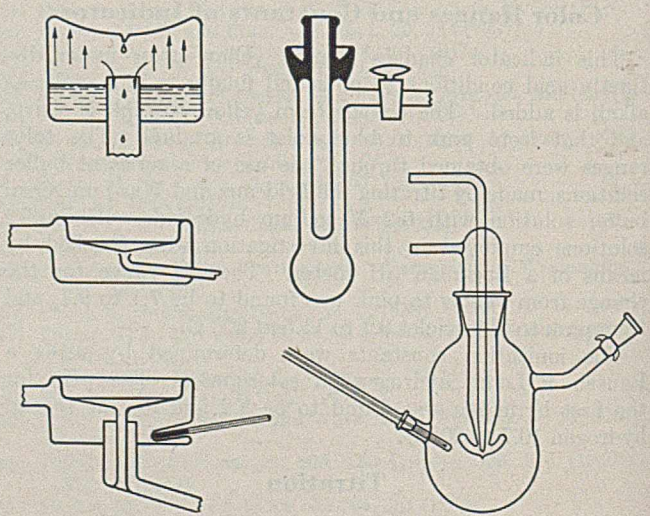

FIGURE 2. POT STILLS

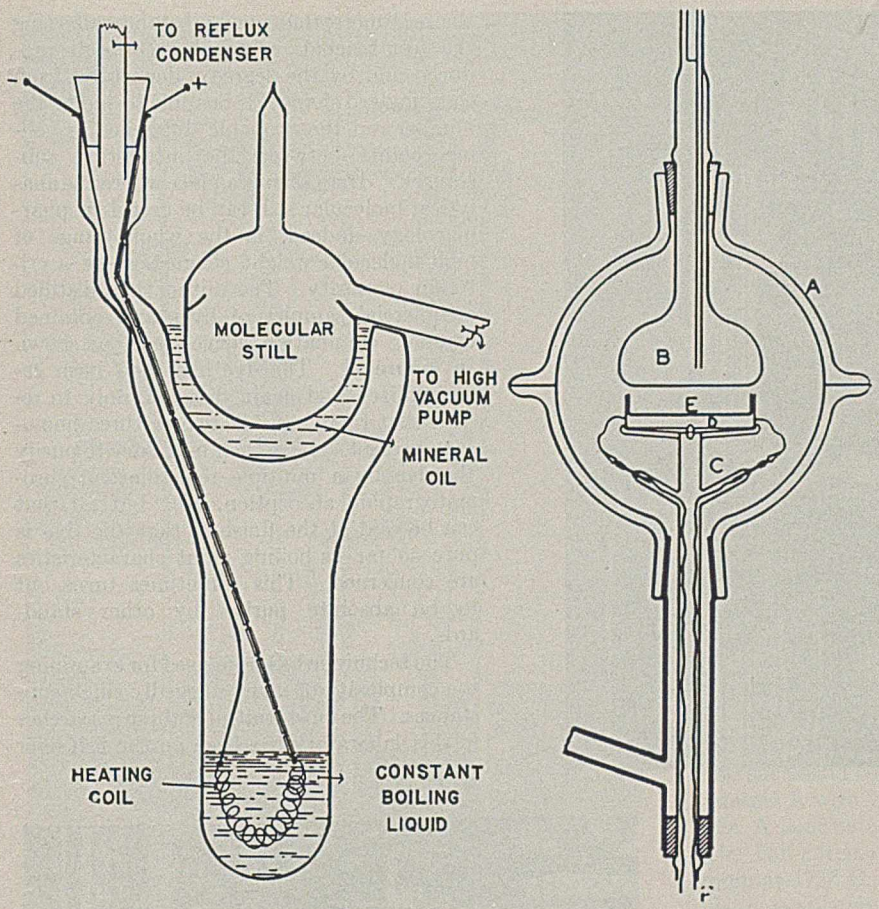

FIGURE 3. POT STILLS

of the Twsett column or the polarograph, but its future promise. How can it be useful in the reader's laboratory? The brightest prospects are as a quantitative tool in medicinal research.

In the distillation of one or more compounds from a mixture the first requirement is a sharp and complete degree of separation and the second is the power to identify the fractions and follow the course of separation. Ordinary distillation methods employ fractionating columns for the first and boiling point measurements for the second. In the molecular still there is no sharp separation and, ordinarily speaking, there is no boiling point. Our first concern, therefore, is to find an analog of boiling point. As a rough generalization we may equate separating power, S, and identifying power, T, with utility, U, as follows: $S \times T = U$. Without going into the reasons why the separation is poor and why there is no boiling point we may say that the conventionalized distillation described below grants us so much power under T that we are able to accept severe compromises with S for separation.

The conventionalized method uses a cyclic molecular still (*16*). That this apparatus has reached some finality of design is evidenced by the fact that Figure 7 is a photograph of apparatus built five years ago and in the meantime few modifications in the essential layout have been required. The cyclic still of the near future will have a centrifugal plate instead of a falling-film column and may operate automatically; otherwise major changes are not foreseen. The material under investigation is for convenience dissolved in a carrier fluid. This at present limits the method to working with oil-soluble substances, but fortunately these comprise more than half the field of interest in current biochemical research. The oily solution is given a series of distillations at succeeding temperatures, the process being known as stepwise distillation. The time of passage through the still is controlled accurately and the temperatures are raised by known and constant amounts, generally 5° or 10° C. over a range of 80° to 260° C., though these limits are purely arbitrary. From 15 to 30 fractions result and each fraction is weighed and analyzed for the wanted constituent. The product of weight and concentration is plotted graphically against temperature and the line connecting the points forms an elimination curve (*11*). This elimination curve is merely a grand name for a special kind of distillation curve and distillation curves, of course, are as commonplace and old as manipulative chemistry itself. The elimination curve, however, because of its specialized mode of production tells us very much more than a common distillation curve. From it we may learn some or all of the following: (1) the exact fractionating power of the apparatus; (2) the efficiency with which the apparatus is being employed; (3) the purity of the substance under investigation; (4) the nature of the substance, whether polar or nonpolar, whether stable or labile, high or low latent heat, etc.

Embree (*11*) of this laboratory has worked out from theoretical considerations the expected shape of the elimination curve for a substance of average latent heat of evaporation. The curve looks like Figure 8. From the falling-film still have been obtained the curves for various volatile dyes (Figure 9), the concentration of which can be measured in the fraction with the minimum of labor. The curves are similar to the

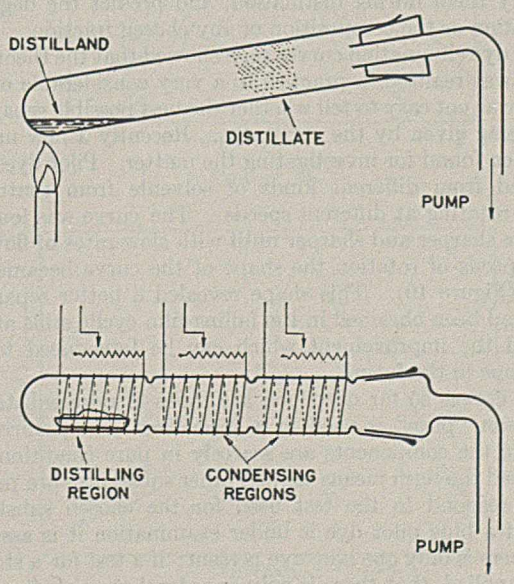

FIGURE 4. TUBULAR STILLS

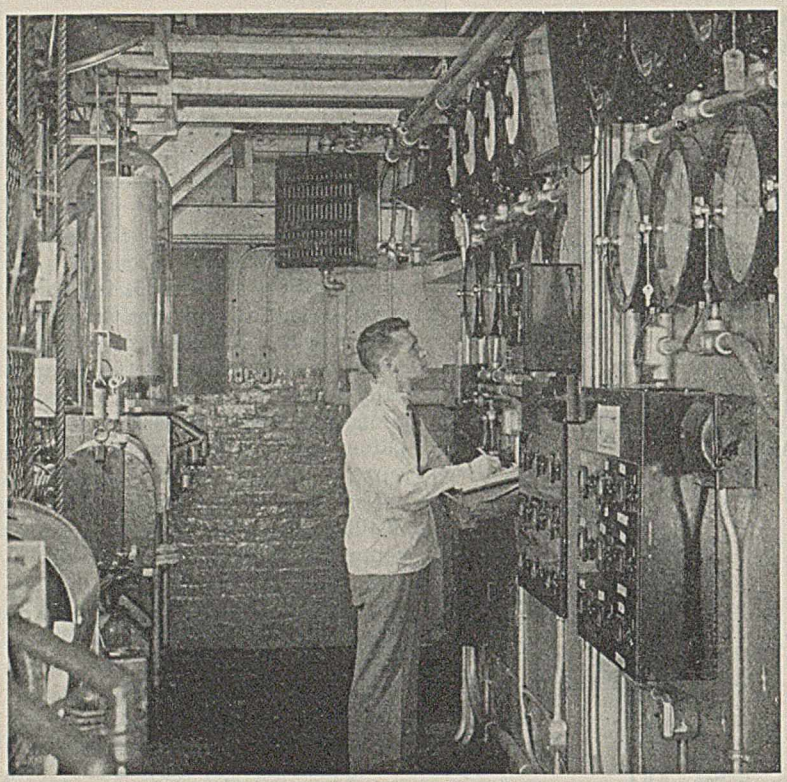

FIGURE 5. FALLING-FILM MOLECULAR STILL

well-known probability distribution curve and possess a sharply defined maximum situated along the distillation temperature axis. This maximum would seem to be a good substitute for boiling point. Unfortunately the position of the maximum depends on the kind and details of the apparatus, the rate at which distillation is done, and the relative affinity of the test substance and the solvent oil. It is, therefore, not a measure of absolute boiling point. However, comparative scales have been constructed using colored indicators as arbitrary standards and in this way it is possible to determine with some exactness the relative boiling points of substances, identify them during distillation, and predict the degree of separation or the composition of any chosen fraction.

The dye elimination curves soon showed that the theoretical shape was realized in practice to a very considerable extent; but it was not easy to tell whether the best possible separation was being given by the apparatus. Recently a new method has been found for investigating the matter. Pilot dyes were distilled from different kinds of solvents from centrifugal plates rotating at different speeds. The curve was found to become sharper and sharper until with slow rates of flow and high speeds of rotation the shape of the curve became constant (Figure 10). This shape revealed a better separation than had been obtained in the falling-film cyclic stills and indicated the improvement which can be introduced to this technique in the future.

The curves so far described are those of pure substances. The term "pure" is used in a specialized sense, since in a mixture the components are scarcely in pure condition. As here used the term means that no other substances are present which respond to the test used for the chosen substance. Thus if a blue pilot dye is under examination it is assumed that there is only one blue dye present; if a test for a sterol is being applied, that there is only one sterol present, the other substances in solution not affecting the sterol test. When there is more than one substance affecting the test reagent, a complex curve is produced and by the degree of departures from the standard shape it is possible to judge the number and the probable difference in boiling points between the interfering substances. Here then is a place where the analytical molecular still can be useful in pharmacology—indeed, in the whole range of high molecular weight chemistry—as a criterion of purity. The author has distilled commercial samples of dyes and obtained complex elimination curves such as shown in Figure 11. The dye has then been recrystallized and again distilled, only to reveal that there are still two or three homologs present. It is then necessary to purify the dye by a multiple redistillation, chromatographic absorption, etc., but all that can be said at the finish is that the dye is pure so far as boiling point characteristics are concerned. This sometimes turns out to be absolute purity by other standards.

The technique has been used for examining the complexity of many allegedly single substances. The most fruitful of these researches in this laboratory has been on the fish liver

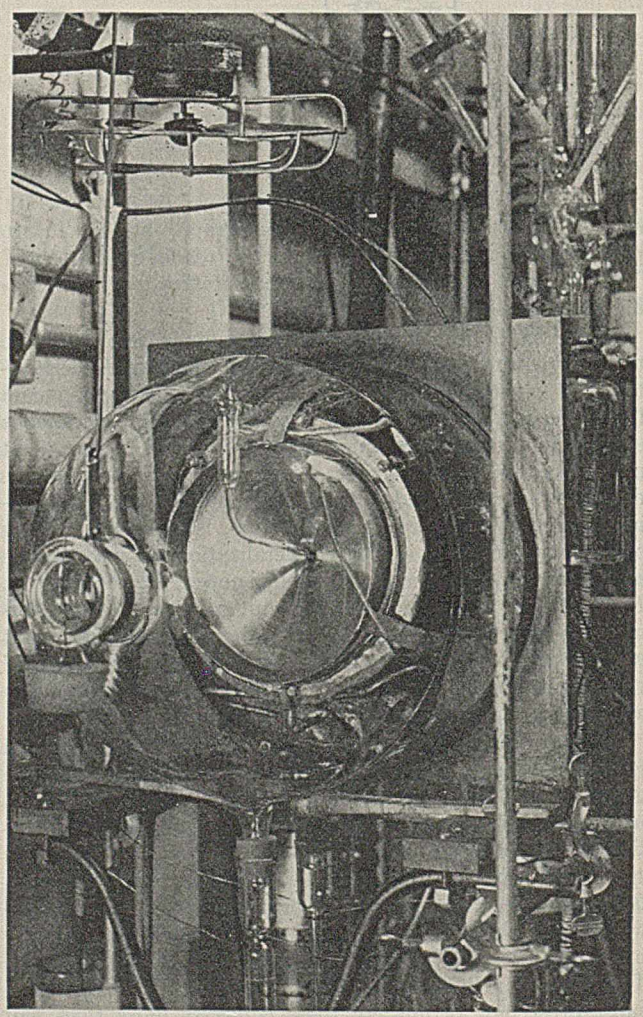

FIGURE 6. REVOLVING-PLATE STILL WITH CONDENSER REMOVED

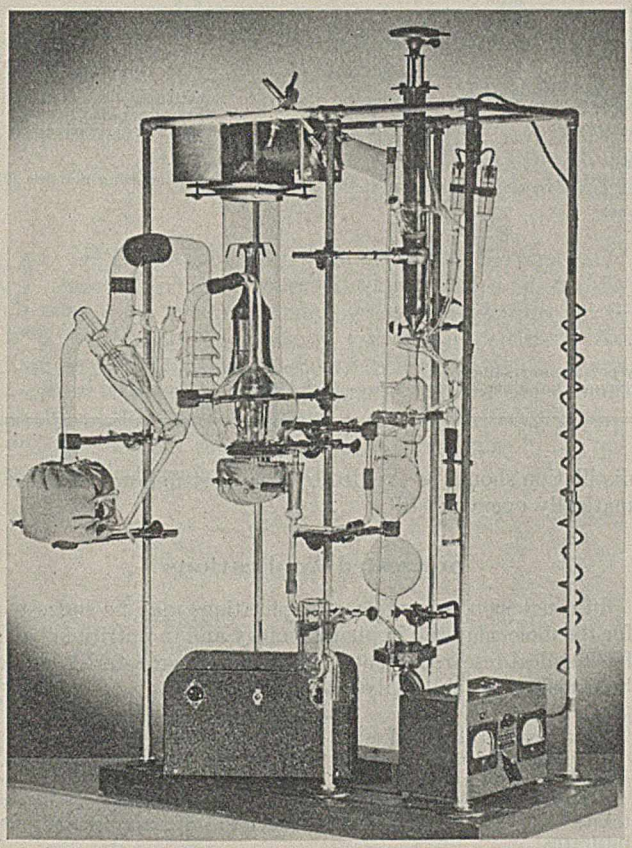

FIGURE 7. CYCLIC MOLECULAR STILL

oils to determine the complexity of vitamins A and D. Vitamin A is found to have a double elimination curve (*11*), from which it has been deduced that the vitamin is present in the free form, maximum 123° C., and as a mixture of esters, maximum 190° to 230° C. Natural vitamin D has been shown to be a mixture of at least four, probably six, antirachitic substances existing partly in free and partly in esterified condition (*18*). Table I lists various substances for which the elimination curves have been traced and for many of which the technique of molecular distillation has been largely responsible for their separation and the knowledge which we have about them today. (For more exact data concerning

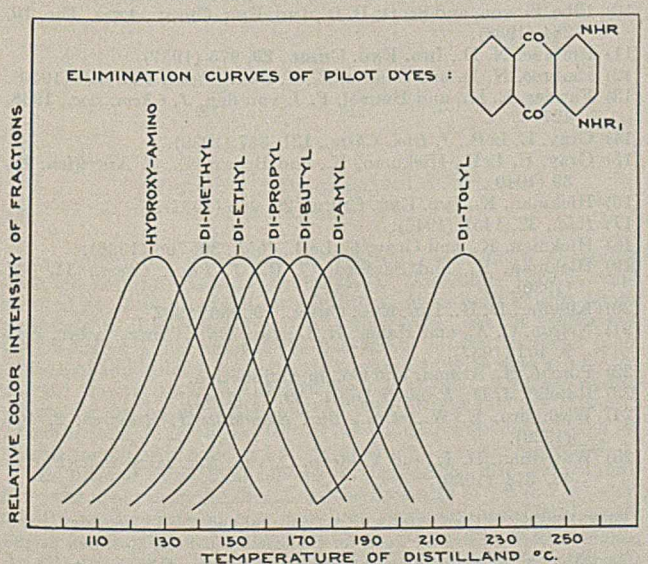

FIGURE 9

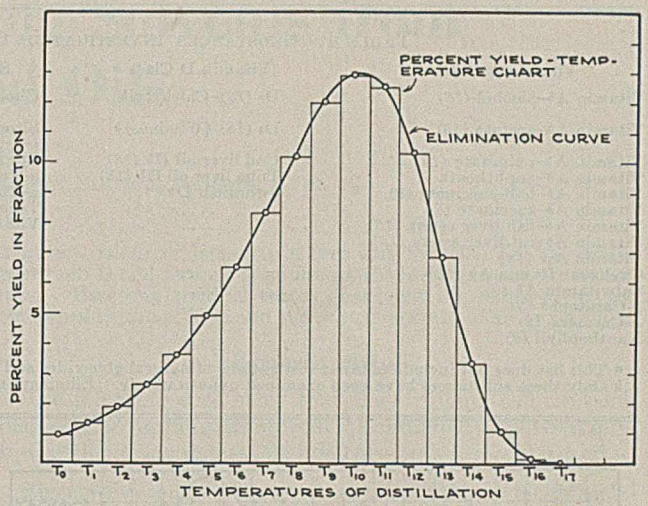

FIGURE 8

these and other substances reference must be made to a paper to appear in *Chemical Reviews*.) Through the aid of the molecular still it has been possible to determine the constitution of vitamin A compounds metabolized in the animal body under normal circumstances or after administration of large doses of the vitamin. The still has been used to trace the progress and successful completion or otherwise of the esterification of vitamin A with various substances. The preparation of vitamin A succinate and half-succinate is a case in point (*9*). The half-succinate gave an elimination curve with a maximum at 173° C., the double succinate of vitamin A failed to distill completely at 250° C., and the submaximum at 120° C. showed that not all of the reaction mixture had become esteri-

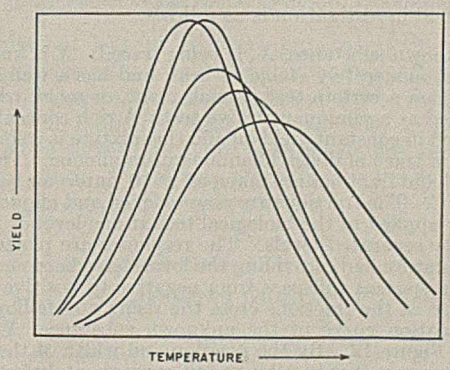

FIGURE 10

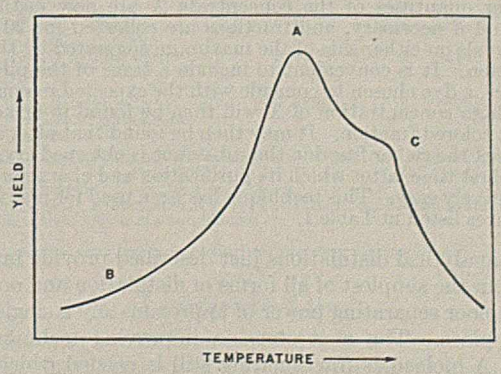

FIGURE 11

TABLE I. SUBSTANCES INVESTIGATED[a] QUANTITATIVELY[b] BY THE MOLECULAR STILL

Vitamin A Class	Vitamin D Class	Sterol Class	Hormone Class	Dye Class
Vitamin A[b]–alcohol (*16*)	D_2 (*18*)–Calciferol[b]	Cholesterol (*17*)	Progesterone (*1*)	Substituted diamino anthraquinones[b] (*16*)
Vitamin A[b]–acetate (*15*)	D_3 (*18*) (Windaus)[b]	Sitosterols[b] (*17*)	Bone marrow anemia factor (*22*)	Indigo and derivatives[b] (*16*)
Vitamin A[b]–palmitate (*15*)	Cod liver oil *D*[b] (*18*)	Stigmasterol[b] (*17*)	....	....
Vitamin A[b]–naphthoate	Tuna liver oil D[b] (*18*)	α-Tocopherol[b] (*17*)	....	Butter coloring matters (*9*)
Vitamin A[b]–half-succinate (*9*)	Swordfish D[b] (*18*)	γ-Tocopherol	....	....
Vitamin A[b]–succinate (*9*)	....	Tocoquinones	....	....
Vitamin A[b]–fish liver esters (*16*)	....	Vitamin K	....	....
Vitamin A[b]–rat liver esters (*15*)	....	....	....	....
Vitamin A_2[b] (*14*)	....	....	....	....
Cyclized vitamin A[b] (*12*)	....	....	....	....
Subvitamin A[b] (*9*)	....	....	....	....
β-Carotene[b] (*9*)	....	....	....	....
α-Carotene (*9*)	....	....	....	....
Xanthophyll (*9*)	....	....	....	....

[a] This list does not include many investigations of natural glycerides and heavy petroleum residues.
[b] Only these substances have been examined quantitatively. Elimination maxima are not given because figures do not yet give a concordant series.

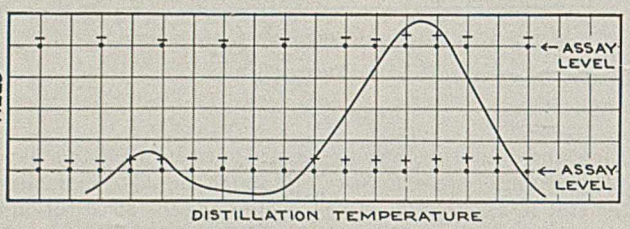

FIGURE 12. METHOD OF SETTING ASSAY LEVELS

fied. When both esters were hydrolyzed and redistilled a maximum at 120° C. only was obtained, showing that vitamin A had been liberated from what must have been a true A-ester.

The elimination technique used with the tocopherols has been applied to the separation of the tocopherols, their quinones, and various other oxidized forms, but with no great success.

A useful method of applying the molecular still in medical or biological investigation is as follows.

An unknown substance, *X*, is being traced. *X* is known to be oil-soluble, moderately stable to heat, and has a well-characterized effect on a certain test animal, plant, or mold, which effect can be used as a semiquantitative test. A rich concentrate of *X* is dispersed in constant yield oil and the mixture is colored faintly blue with a trace of diethyldiaminoanthraquinone. The mixture is distilled and fractions are taken off at 10° intervals between 80° and 260° C. The fractions are now weighed and aliquot portions are fed or applied to the biological test at one level or preferably two widely separated levels. The responses are plotted against the temperature and, providing the levels have been chosen properly, the responses will pass from negative to positive and back to negative as the fractions cross the rising and falling limits of the elimination curve of the unknown substance, *X*. This is shown in Figure 12. By the position and width of the region of positive response it is possible to tell approximately how much of *X* is present and indicate its boiling point relative to the pilot dye.

Larger quantities of the concentrate *X* are now distilled, in carrier oil if necessary, and fractions are collected for 20° C. in 5° intervals on either side of the maximum suggested by the pilot distillation. It is convenient to include a trace of the pilot dye, or better, a dye chosen to coincide with the expected maximum of *X*. A high concentration of *X* will then be found in or near the deepest colored fraction. It may then be found that after saponification of the richer fraction the substance is obtained crystalline for the first time, after which its purification and characterization are relatively easy. This technique has been used for many of the substances listed in Table I.

The analytical distillations just described provide information from the simplest of all forms of distillation and one having the poor separating power of approximately a single theoretical plate. The next steps in improving technique are plain. A molecular fractionating still is needed which gives better separations and the long and tiresome task of cyclic distillation should be avoided by the construction of an automatically operated still.

Suggested Applications

In conclusion, a number of applications may be suggested for the molecular still in pharmacology and in nutritional and biochemical research. Those marked with an asterisk would be benefited by the analytical technique.

Investigating essential fatty acids
Investigating fats, alcohols, sterols, and vitamins recovered after feeding to animals
* Testing for homogeneity or purity of new drugs, plant extracts, dyes, etc., with special reference to sulfonamides and barbiturates
* Separation and characterization of steroids and other lipids from bone marrow, pituitary, suprarenal, and other glands
* Extending the chemistry of the carotenoids
Analysis of fecal, visceral, and tissue extracts, etc.

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PRESENTED before the Division of Agricultural and Food Chemistry, Symposium on New Analytical Tools for Biological and Food Research at the 102nd Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J. Communication No. 28 from the laboratories of Distillation Products, Inc.

Column for Stripping Solvents from Extracted Oils

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OLCOTT (*1*) has recently pointed out that the removal of traces of solvent from laboratory-extracted oils is difficult even when vacuum is employed at temperatures up to 50° C. He found that traces of solvent still persisted. No adequate device to facilitate the removal of the small amounts of solvent is generally available; hence, the stripping column described below meets a definite need. It has been successfully used without causing any detectable change in the unsaturated oils given in Table I.

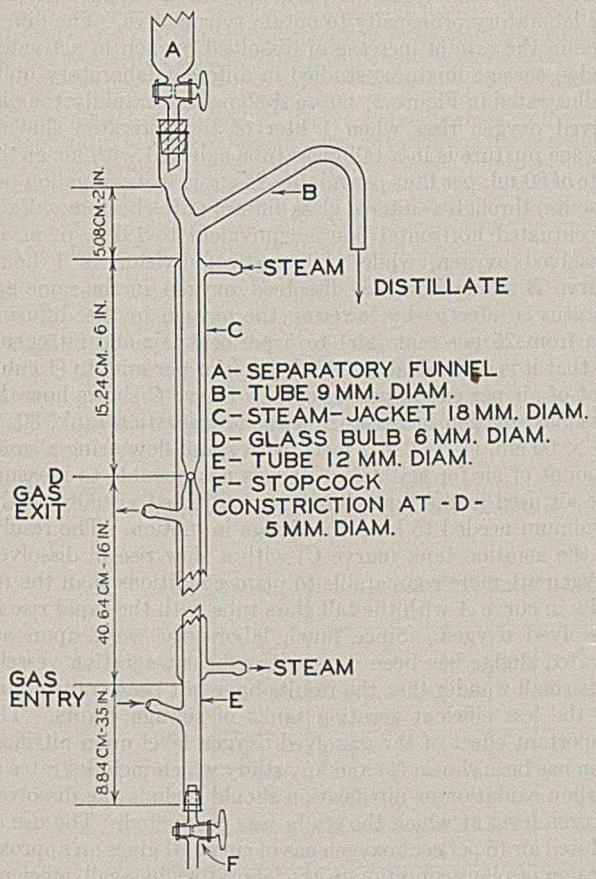

FIGURE 1. DIAGRAM OF COLUMN

The apparatus illustrated in Figure 1 consists of a steam-jacketed column made of Pyrex glass. The inner tube is constricted at *D* and a small loosely fitting glass bulb at this point permits the oil to pass through at a slow rate. At the same time the oil forms a seal against the passage of solvent vapors. The section of the inner tube below *D* is provided with side arms. A current of air is drawn in through the lower arm, or nitrogen under pressure may be admitted, at the rate of 800 ml. per minute. The stopcock, *F*, at the bottom of the column is opened enough to permit the oil as it collects here to drain into a receiving vessel.

The filtered extract oil-solvent mixture is fed into the column from the separatory funnel, *A*, at the flow rate of 1.6 to 2 ml. per minute for solvents such as ethyl or petroleum ether, and more slowly for the higher boiling solvents such as ethylene dichloride. As the oil-solvent mixture enters the area surrounded by the steam jacket, the solvent volatilizes and passes out at *B* through a water-cooled condenser to a receiving vessel. The oil drains past bulb *D* and forms a thin film on the lower portion of the column. Here any residual traces of solvent are removed by the combined action of heat and the countercurrent of air or nitrogen.

Experimental

The following data show that the stripping column completely removes the solvent from the extracted oil-solvent mixture without any detectable influence on the recovered oil.

Seventy-five milliliters (69 grams) of refined corn oil (Mazola) were mixed with 400 ml. of petroleum ether, Skellysolve F, boiling range 30° to 60° C. This mixture was passed through the stripping column with a recovery of 68 grams of oil and 340 ml. of solvent.

To confirm the completeness of separation of solvent from the stripped corn oil, charges of about 21 grams of the original and of the stripped oil were subjected to drying in a vacuum oven 6 hours at 30° C. at about 1-mm. pressure, followed by 4 hours' drying at 50° C. at 20-mm. pressure. The changes in weight on the charges of each oil were 0.0115 and 0.0122 gram, respectively. These losses are insignificant and indicate that the solvent had been completely removed by the stripping column. Further, the organoleptic tests of odor and taste for the presence of solvent in the recovered oil were negative, and the values of the fat constants of specific gravity, refractive index, and iodine number of the corn oil both before and after treatment remained the same and showed no change in unsaturation (Table I).

A sample of crude soybean oil, No. 2537, was extracted from Tokyo soybeans with petroleum ether and stripped free of solvent by passing through this apparatus. Sample 2537a was extracted from the same lot of soybeans with ethylene dichloride. The fat constant gave no indication that the unsaturation of these oils had been changed. The odor and taste of the stripped oils gave no detectable evidence of the presence of any remaining solvent.

While efficient solvent recovery in laboratory stripping operations is generally considered unimportant, the amounts of solvent recovered were recorded because the column might be adapted to industrial usage by suitable modification. The amount of solvent recovery from 5 samples varied from 85 to 93 per cent. A cold trap placed in the gas exit line below *D* did not recover any more solvent.

TABLE I. DATA ON EXTRACTED OILS STRIPPED OF SOLVENTS

Sample No.	Oil	Solvent Used	Solvent Recovery	Specific Gravity, 25°/25° C.	Refractive Index, $n_D^{20°}$	Iodine No. (Hanus)	Extract Mixture Flow Rate
			%				*Ml./Min.*
2707	Refined corn oil[a]	....	...	0.9190	1.4743	123.6	...
2707a	Refined corn oil[b]	Petroleum ether	85.0	0.9188	1.4741	123.5	1.6
2537	Crude Tokyo soybean oil	Petroleum ether	93.0	0.9203	1.4751	131.5	1.6
2537a	Crude Tokyo soybean oil	Ethylene dichloride	88.0	0.9229	1.4753	130.8	0.7

[a] Mazola; 21.1804 grams of refined corn oil lost 0.0115 gram when dried in a vacuum oven 6 hours at 30° C. at 1-mm. pressure, followed by 4 hours' further drying at 50° C. at 20-mm. pressure.

[b] A charge of 21.2922 grams of stripped corn oil lost 0.0122 gram in weight when dried under the same conditions.

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Dissolved Oxygen Recordings with the Dropping Mercury Electrode

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THE importance of dissolved oxygen in several phases of water and sewage chemistry has long been recognized. The satisfactory use of the dropping mercury electrode for determining dissolved oxygen in sewage and activated sludge–sewage mixtures has been demonstrated by the author (*2*). Petering and Daniels (*5*) have used the dropping mercury electrode for the determination of dissolved oxygen in studies upon photosynthesis, while Manning (*4*) has adapted this method to the study of the dissolved oxygen cycle in lake water throughout a 24-hour period. Manning was the first to demonstrate that dissolved oxygen can be determined continuously in water by using a constant voltage and noting the shift in current flow with a sensitive recording galvanometer. This paper will demonstrate the use of the constant voltage method to obtain continuous dissolved oxygen values in laboratory experiments and during the regular operation of the aeration tank of activated sludge–sewage plants.

Instrument

The instrument supplied by the Cambridge Instrument Company, New York, N. Y., for this study uses a simple potentiometer circuit with a sensitive, suspension, mirror galvanometer to measure the current flow in the dropping mercury electrode circuit. A light beam impinging upon the mirror is reflected to a photoelectric cell. The current from the photoelectric cell is amplified and then recorded at minute intervals with a recording thread galvanometer. The electrodes consist of a calomel half-cell as the inert reference electrode and the dropping mercury electrode consisting of a separatory funnel with a long stem and a short length of capillary tubing of 0.04-mm. bore to obtain a drop rate of approximately 1 drop per second. For outside work both were encased in a steel shell to protect the electrical connections and prevent contamination of either reservoir. A cup was placed 5 cm. (2 inches) below the capillary to catch the used mercury. A long, heavy, high-conductance cable was used to connect the electrodes to the instrument for outdoor work. The unit is illustrated in Figure 1.

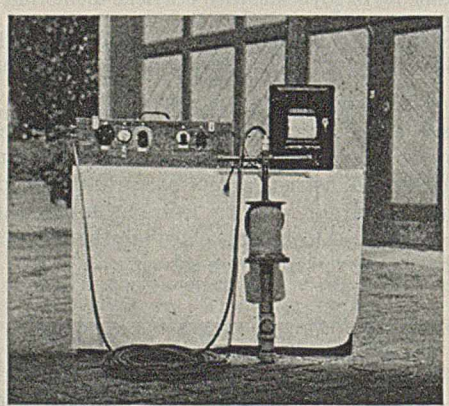

FIGURE 1. DISSOLVED OXYGEN RECORDING INSTRUMENT

Results

The dissolved oxygen recording apparatus has been used in the laboratory principally to obtain type curves. The difference in the rate of increase of dissolved oxygen in activated sludge–sewage mixtures studied in different laboratory units is illustrated in Figure 2; curve *A* shows how rapidly the dissolved oxygen rises when 1 liter of the activated sludge–sewage mixture is in a tall glass tube agitated with air at the rate of 80 ml. per liter per minute (4 cubic feet per gallon per 6 hours) through a sintered glass diffuser at the bottom. Each accentuated horizontal line is equivalent to 1.0 p. p. m. of dissolved oxygen, while each vertical division is 1 hour. Curve *B* shows how the dissolved oxygen in the same apparatus is affected by lowering the oxygen in the diffusing gas from 25 per cent (air) to 5 per cent (air and nitrogen), so that it is equivalent to 20 ml. per liter per minute (1 cubic foot of air per gallon per 6 hours). Curve *C* shows how the dissolved oxygen increases in a shallow aeration tank, 30 × 30 × 60 cm. (1 × 1 × 2 feet), with spiral flow using a small amount of air for agitation. It was not possible to measure the air used in the tank, but it was adjusted visually to the minimum needed to keep the sludge in motion. The results in the aeration tank (curve *C*) with a slow rise in dissolved oxygen are more comparable to plant conditions than the results in curve *A* with the tall glass tube with the rapid rise in dissolved oxygen. Since much laboratory work upon activated sludge has been done with efficient aeration vessels, it is small wonder that the results have not been transferable to the less efficient aeration tanks of sewage plants. The important effect of the dissolved oxygen level upon nitrification has been shown (*2*) and any study which includes rates of carbon oxidation or nitrification should include the dissolved oxygen level at which the study was conducted. The use of diluted air (5 per cent oxygen gas of curve *B*) gives an approximation of plant condition in the laboratory in small, efficient, more readily controlled apparatus which does not use too much sample or space.

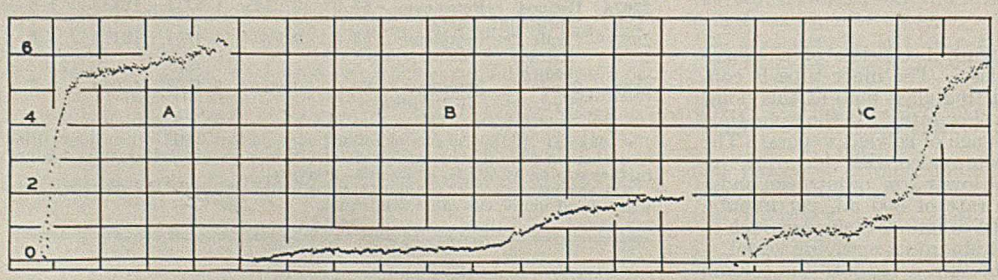

FIGURE 2. DISSOLVED OXYGEN CURVES OBTAINED IN LABORATORY UNITS WITH ACTIVATED SLUDGE PLUS SEWAGE

A. 1 liter of sewage-sludge mixture in cylinder agitated with air
B. In same cylinder agitated with 5 per cent O_2 gas
C. In tank 1 × 1 × 2 feet, agitated with minimum amount of air

The glass tubes and 5 per cent oxygen gas as shown in curve *B* were used to study the effect upon the dissolved oxygen of the addition of different quantities of food to activated sludge. Return activated sludge was brought into the laboratory and diluted with balanced mineral dilution water, described previously (*3*), to 1500 p. p. m. of suspended solids; 100 p. p. m. of sodium propionate were added to the sludge mixture.

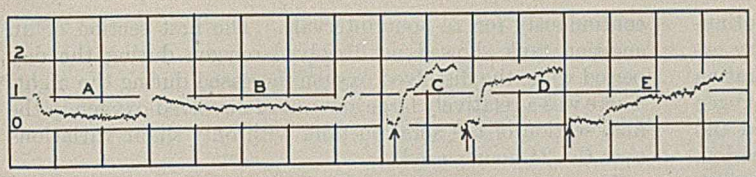

FIGURE 3. DISSOLVED OXYGEN CURVES

Different amounts of food fed to activated sludge.
A. 100 p. p. m. sodium propionate on 1st dose
B. 200 p. p. m. sodium propionate for 2nd dose
C. 10 p. p. m. sodium propionate after 3 doses
D. 30 p. p. m. sodium propionate
E. 100 p. p. m. sodium propionate

The solution was agitated with nitrogen till the instrument showed no further drop, indicating a zero dissolved oxygen and the 5 per cent oxygen gas was then used, producing curve *A* (Figure 3). Between curves *A* and *B*, 200 p. p. m. more of sodium propionate were added to the activated sludge, but the dissolved oxygen levels in *A* and *B* indicate that the food in sodium propionate is not sufficiently available to the sludge organisms to use all the available oxygen at first and that increasing the quantity of food does not increase the quantity of oxygen required. The nitrogen necessary for the utilization of sodium propionate was supplied by the addition of urea to yield a carbon-nitrogen ratio of 1 to 5. The sludge was aerated overnight with more sodium propionate and then settled. The supernatant was withdrawn and more mineral dilution water added. Curve *E* was obtained by the addition of 100 p. p. m. of sodium propionate to the conditioned sludge and indicates by the absence of dissolved oxygen how much higher the oxygen demand is over a shorter period of time. Curves *C* and *D* were obtained with 10 and 30 p. p. m. of sodium propionate additions, respectively, and indicate that increasing the amount of food increases the period of high oxygen demand.

Studies of the effect of feeding different amounts of food to activated sludge with methods to indicate either oxygen consumption or carbon dioxide production produce curves the reverse of those shown. The effects of different types of laboratory equipment upon dissolved oxygen level are best studied with the dropping mercury electrode which measures the dissolved oxygen directly.

An opportunity presented itself at an activated sludge plant where the air supply was temporarily impeded, resulting in deterioration of sludge and a turbid effluent, to test changes in the liquor under these conditions. The electrodes were placed in the aeration tank within 90 cm. (3 feet) of the outfall weir and several curves were obtained, of which the lowest curve in Figure 4 is typical. There was no dissolved oxygen present in the liquor during the 24-hour period. Spot tests in other parts of the aeration tank indicated that dissolved oxygen was absent.

In order to show positive variations in the dissolved oxygen of aeration tanks, tests were made at the activated sludge plant at Bernardsville, N. J. (*1*). The electrodes were placed in the first of eight compartments of the aeration tank and the center curve of Figure 4 was obtained. The onset of the rise in dissolved oxygen agrees with the shift from day to night flow. The sudden drop and subsequent rapid rise in the dissolved oxygen at 8:30 A.M. are due to the use of one-third of the air supply to pump sludge from the primary settling tank to the digester for a 15-minute period. The curve indicates that operation might be altered somewhat, without greatly increasing the cost, by increasing the flow of air during the day and decreasing it at night, if the operator has a positive indication of the actual conditions in the aeration tanks and could follow immediately and continuously the effect of any changes in operation. During the dissolved oxygen rise in the curve, a wider band of dots is shown, indicating that the liquid is more variable. When the electrodes are placed nearer the diffusers, the variability in the current flow increases, owing to the surging of some better or poorer aerated liquid past the electrodes.

When the electrodes are placed in the final compartment of the aeration tank, the variations in dissolved oxygen shown in the top curve of Figure 4 are obtained. The curve shows approximately 2 parts per million dissolved oxygen throughout the day, and a rise in the dissolved oxygen with the lower sewage flow during the night. During the strong day flow, there is a rise of 2 parts per million in the dissolved oxygen through the aeration tank as purification progresses and oxygen demand decreases. Because there is dissolved oxygen present during the day in the final compartment of the aeration tank, there is less variation in the dissolved oxygen between the day and night flow than between the day and night flow in the first compartment.

Summary

The importance of maintaining dissolved oxygen in the activated sludge process of sewage treatment is well known.

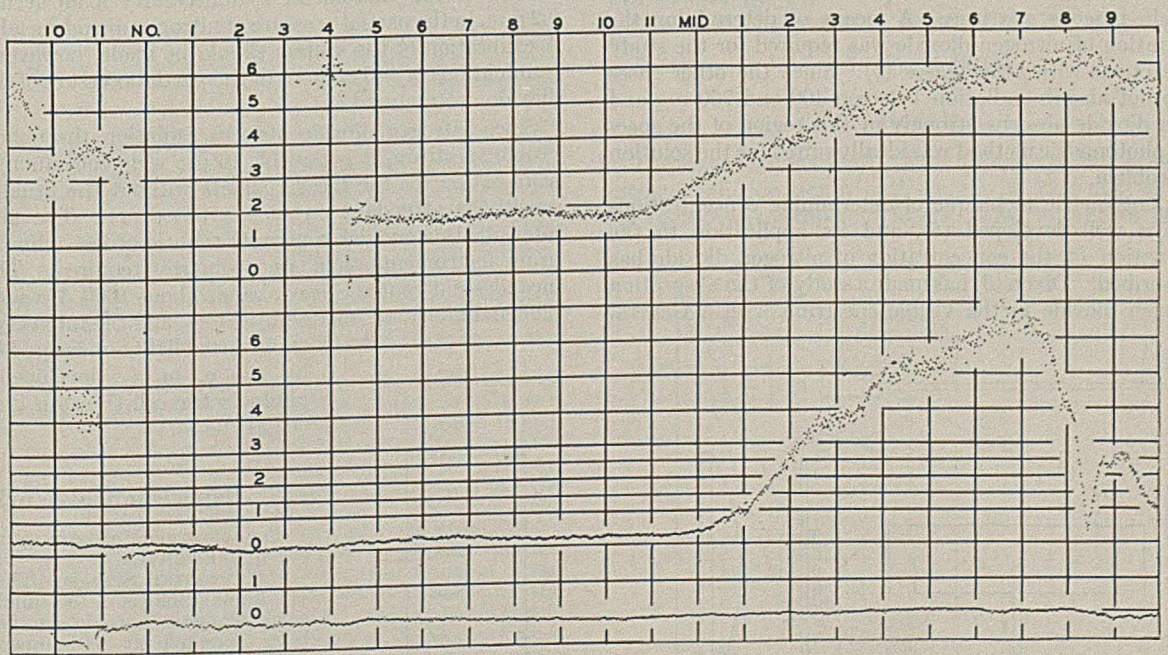

FIGURE 4. DISSOLVED OXYGEN CURVES OBTAINED IN AERATION TANK

Top. In final compartment of aeration tank at Bernardsville
Middle. In first compartment of tank at Bernardsville
Bottom. In aeration tank at plant with temporary inadequate blower capacity

An instrument to record the dissolved oxygen level continuously is briefly described and some results of laboratory experiments and activated sludge–sewage plant operation studies are given. The variations in the dissolved oxygen produce changes in the current flow which are amplified and recorded upon a thread galvanometer for a 24-hour period. The curves showing the dissolved oxygen in activated sludge–sewage mixtures in the laboratory under different conditions demonstrate that the shape of the vessel can largely determine the efficiency of the gas used for aeration. The effect upon the dissolved oxygen of the addition of different quantities of the same food to activated sludge indicates that when more food is added there is no effect upon the dissolved oxygen unless or until the enzyme capacity is large enough to handle the increased load. The electrodes were placed in the aeration tank of an activated sludge–sewage treatment plant, and the dissolved oxygen levels at several points in the tank recorded continuously for 24-hour intervals. The first section of an aeration tank showed no dissolved oxygen during the day period while the dissolved oxygen increased during the night. There was a relatively large amount of dissolved oxygen in the final section of the aeration tank with only slight variations over the 24-hour period.

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A Simplified Photometer for Determining Nitrogen Dioxide Concentrations

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Massachusetts Institute of Technology, Cambridge, Mass.

The concentration of nitrogen dioxide in gaseous mixtures is conveniently determined with a simple photometer. The installation and calibration of the system are described here. The photometric system permits measurement of the partial pressures of nitrogen dioxide to within 0.05 mm.

THE authors have developed a simple photometric method for the determination of the partial pressure of nitrogen dioxide in gaseous mixtures. A means of determining the concentration of nitrogen dioxide was required for the study of its reactions with other gases (*4*). Since the other gases used do not absorb radiation between 400 and 700 mμ and nitrogen dioxide absorbs strongly in this region of the spectrum, a photometric method was ideally suited for the solution of the problem.

The photometric technique of determining concentrations of gases is well developed (*5*), and its application to the determination of the concentration of nitrogen dioxide has been described. Dixon (*3*) has made a study of the absorption of nitrogen dioxide in the visible spectrum with a General Electric recording spectrophotometer. Narrow spectral regions (15 and 40 Å. wide) were isolated with the "built-in" monochromator. Willey and Foord (*6*) used a filtered incandescent source and measured the intensity of light transmitted with a potassium photocell and an amplifying system.

The system described here has the advantage of being more easily adaptable to problems involving the determination of nitrogen dioxide concentrations than those heretofore described. The necessary parts are readily available, inexpensive, and easy to install. The reproducibility of the measurements exceeds that previously reported and recalibration of the photometer is unnecessary if an accuracy of 0.2 mm. in the partial pressure of nitrogen dioxide is adequate. A calibration of the system should be made, however, if determinations of ±0.05 mm. in the partial pressure of nitrogen dioxide are required.

Since nitrogen dioxide absorbs radiation throughout the visible spectrum, it is possible to use wide continuous bands of radiation for the measurements without the usual loss in sensitivity attendant on such a procedure. The increased intensity thus available permits the use of less refined measuring instruments than are ordinarily required. Thus, an incandescent source is used, bands about 1000 Å. wide in the blue and green are isolated by filters, and the intensity of the transmitted light is measured directly by the e. m. f. developed by a photoelectric cell (Weston Photronic cell, Type 1).

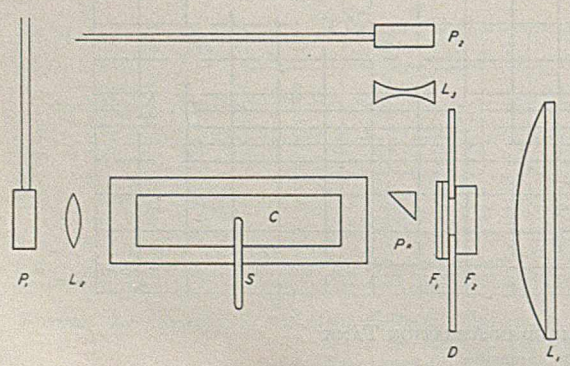
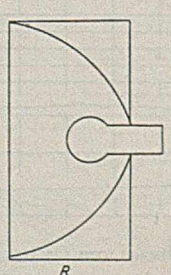


FIGURE 1. EXPERIMENTAL ARRANGEMENT



Experimental Work

The experimental arrangement is shown in Figure 1.

ILLUMINATING SYSTEM. The source of illumination is a 50-candlepower single-filament automobile headlight bulb operated at 6.0 amperes (5.6 volts) with a steady current supplied by storage batteries of large capacity. The optical system consists of a parabolic, chromium-plated reflector, *R* (diameter 11 cm.), housing the

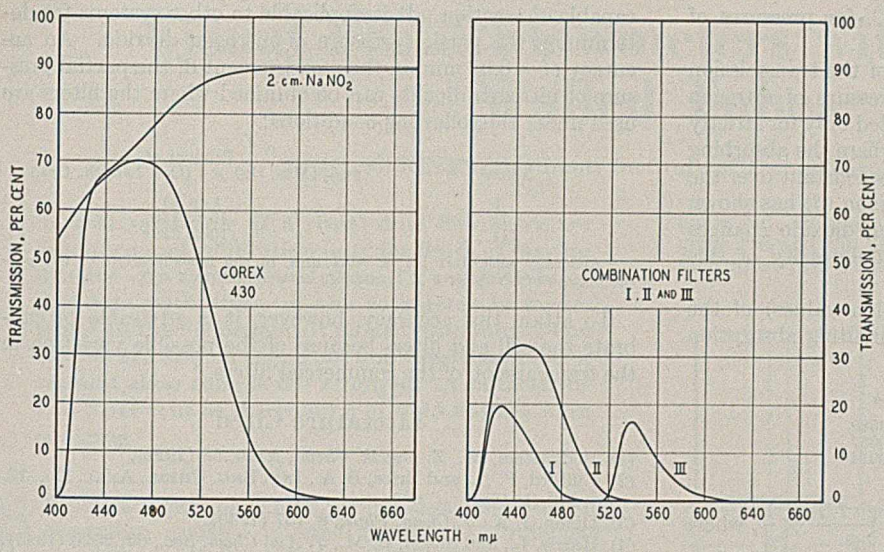


FIGURE 2. TRANSMISSION OF FILTERS

lamp, an inexpensive plano-convex lens, L_1 (focal length 25 cm., diameter 11 cm.), placed 1.4 meters in front of the reflector, and a diaphragm with a 2-cm. aperture, D, placed 3 cm. in front of the lens. This optical arrangement produces a beam which does not diverge more than 3 cm. for a distance of 40 cm. beyond the diaphragm.

FILTERS. There are three filters, each composed of three components, all held at F_1 and F_2 by the diaphragm which has been designed to hold the filters and make their interchange simple. Two components are common to all three filters: a cell 2-cm. long containing a saturated aqueous solution of sodium nitrite, and a Corning Corex filter No. 430.

The three compound filters used are:

I. Corex filters, Nos. 585 and 430, and 2 cm. of $NaNO_2$ (saturated solution)
II. Corex filters, Nos. 554 and 430, and 2 cm. of $NaNO_2$ (saturated solution)
III. Corex filters, Nos. 351 and 430, and 2 cm. of $NaNO_2$ (saturated solution)

A set of filters transmitting sharp bands covering the visible spectrum has been listed by Clifford and Brice (*2*). A set of filters could be chosen from this list which would give transmission bands similar and sharper, but which have a smaller per cent transmission and narrower bands than the ones used by the authors. These factors would reduce the response, and since the calibrations had been completed on the above set of filters before the publication of the set by Clifford and Brice, no measurements have been made with their filters.

It is only necessary to interchange the single filters Nos. 585, 554, and 351 to obtain any one of the three compound filters. The transmission of the filters is shown in Figure 2. Since the incandescent source gives much more radiation in the green than in the violet, bright brass screens are used with filters II and III to produce approximately equal e. m. f. for each of the three filter combinations.

MEASUREMENT OF LIGHT INTENSITY. The beam, having passed through the diaphragm aperture and filters, is split by a right-angle prism, P_R, into two beams which are adjusted to give approximately the same e. m. f. (1.5 millivolts) from the photronic cells, P_1 and P_2, when the absorption cell, C, is empty. The e. m. f. response of the photronic cells is measured on a Type K Leeds & Northrup potentiometer to the nearest microvolt. The absorption cell has parallel windows 22.3 cm. apart.

Certain precautions are necessary when photronic cells are used for quantitative measurements. Here the response of the cells was calibrated with screens and found to be linear for the low intensity of radiation used (about 0.1 to 0.2 lumen). It was found advisable to illuminate the cells for 5 to 10 minutes before recording the e. m. f. to allow for the large decay in the response of the cells. In addition, the beam of light was diffused over the whole face of the photronic cells by lenses L_1 and L_2, but the more sensitive outer edges of the cells, at which the copper contacts are exposed, were covered with opaque rings. The photronic cells were housed in aluminum boxes to prevent any sudden temperature changes.

The photronic cell, P_2, is used as a check both of any variation in the intensity of the incident beam and the decay and fluctuation of response, for the decays in the two cells are similar. Measurements of the ratio $\frac{\text{e. m. f. } P_1}{\text{e. m. f. } P_2}$ with the absorption cell empty are constant within 1 per cent.

CALIBRATION OF PHOTOMETER. Nitrogen dioxide which had been prepared by the decomposition of lead nitrate and stored with an excess of oxygen was dried by passing it slowly over phosphorus pentoxide. The residual oxygen was pumped off the nitrogen dioxide, maintained at −78° C. The nitrogen dioxide was charged into the quartz absorption cell, C, through an all-Pyrex system with glass valves; so that at no time did the nitrogen dioxide come in contact with any stopcock grease or mercury. The cell itself was surrounded by a lagged copper sheath.

An excess of nitrogen dioxide was sublimed into the cell (a small amount of liquid remained in the side arm at room temperature), and the cell was sealed off just above the side arm of the cell. The total pressure (of the nitrogen dioxide plus nitrogen tetroxide) in the cell was varied by controlling the temperature of the liquid or solid phase in the side arm. The pressures were measured with a mercury manometer and Société Génevoise cathetometer, pressure balance being indicated by a quartz spiral between the absorption cell and the mercury manometer. The partial pressure of the nitrogen dioxide was calculated from the measurements of total pressure and from the equation of Bodenstein (*1*) for the equilibrium $N_2O_4 \rightleftharpoons 2NO_2$. The cell temperature was recorded to 0.1° C.

The transmission of light at each nitrogen dioxide pressure was calculated from four measurements:

E_{1f}, e. m. f. of photocell P_1 when the absorption cell contains nitrogen dioxide.

E_{1e}, e. m. f. of photocell P_1 when the absorption cell contains no nitrogen dioxide, all the nitrogen dioxide having been frozen in the side arm to −195° C.

E_{2f}, e. m. f. of photocell P_2, measured just before and just after E_{1f} (and averaged).

E_{2e}, e. m. f. of photocell P_2, measured just before and just after E_{1e}.

Transmission $I/I_0 = E_{1f}/E_{1e} \times E_{2e}/E_{2f}$

Results

The transmissions at 23° C. for different partial pressures of nitrogen dioxide, for a path length of 22.3 cm., are given for each of the compound filters. The calibration is not af-

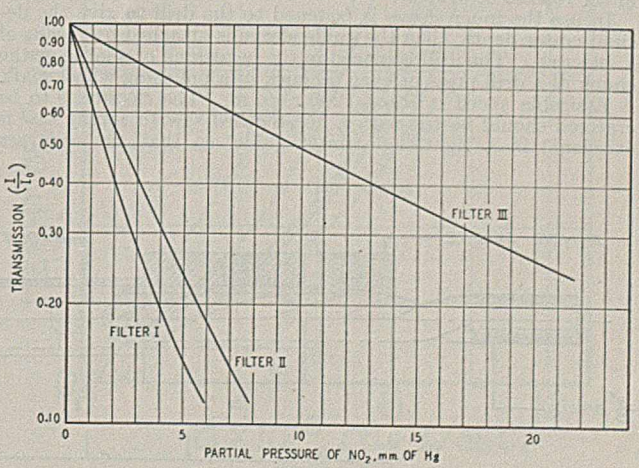


FIGURE 3. TRANSMISSION OF NITROGEN DIOXIDE

fected by temperature changes of ±3° C. for pressures of nitrogen dioxide below 10 mm.

It will be observed that the logarithm of the transmission is not a linear function of the partial pressure of nitrogen dioxide. The linear relation is to be expected only for strictly monochromatic radiation or for the case where the absorbing species has an absorption coefficient that is constant over the whole spectral band used for analysis. Dixon (*3*) has shown that the absorption coefficient of nitrogen dioxide changes rapidly with wave length, so that the nonlinearity of this function is to be anticipated.

The limiting slopes (at high transmission values) of the curves of Figure 3 lead to the following limiting absorption coefficients, k:

Filter	k
I	0.0089
II	0.0057
III	0.0014

k is calculated from the relation $k = -\frac{\log_{10} I/I_0}{p} \times \frac{1}{l}$, where p is the partial pressure of nitrogen dioxide (reduced to 0° C.) in millimeters of mercury, and l = 22.3 cm., the cell length.

The authors have used the procedure described to follow the nitrogen dioxide partial pressure in various gas mixtures capable of reaction. It is applicable to other systems for determining the partial pressure of nitrogen dioxide. An accuracy of ±0.05 mm. in the measurement of the partial pressure of nitrogen dioxide can be obtained where the filters are used under the following conditions:

Filter	Product of p_{NO_2} (Mm. Hg) × l (Cell Length, Cm.)
I	1 to 90
II	45 to 130
III	110 to 220[a]

[a] Accuracy with filter III is ±0.1 mm. in the measurement of the partial pressure of NO_2 for $p \times l$ products between 220 and 450.

To attain this accuracy, however, it is advisable to calibrate the cell and filters because of the possible variation in the transmission of the commercial filters.

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A Cutter for Spectroscopic Electrodes

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MANY workers using carbon electrodes for spectrographic analysis employ a small crater to hold either a dry solid sample or a small liquid volume to be evaporated. Two devices for cutting such craters, described previously (*1*, *2*), require either a lathe of special design or a drill press capable of turning the tool and holding the carbon in line in a chuck. The tool described here is simpler than either of these, both in manufacture and in use. Its use requires neither a lathe nor a drill press with a stationary chuck whose axes must be kept aligned.

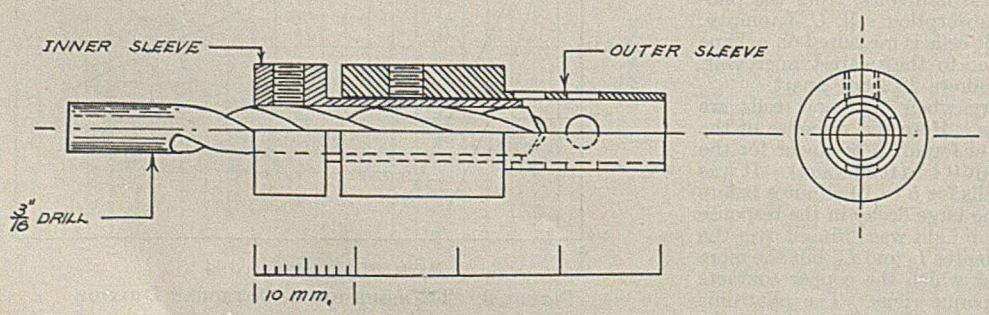
The accompanying figure shows an inner sleeve which is fastened by a setscrew to an ordinary twist drill of the size of the crater to be made. The depth of the crater is regulated by the position of the sleeve along the drill. An outer sleeve for guiding and centering the electrode is fastened to the inner one. The inside diameter of this outside sleeve is the same as the diameter of the rods to be drilled.

In use the inner sleeve is fastened to the drill to give the desired crater depth, then the guide sleeve is attached by means of a setscrew. The drill is placed in the headstock of a lathe or the chuck of a drill press or even a chuck of a polishing wheel shaft. A desirable speed is about 1200 r. p. m. The electrodes to be cratered should be cut with a fine-toothed saw (a coping saw is excellent) in a right-angle miter guide, so that the ends are smooth and perpendicular to the axis of the rod. Any roughness should be taken off with emery paper. The uniformity of depth in the crater will be determined by the flatness and the right-angle surface of the electrode. A carbon rod is drilled by slowly pushing it into the rotating tool until the maximum depth is cut.

In preparing craters on the spectroscopic carbons and special graphite electrodes a sharp drill holds its edge very well. However, because of the hardness of the special carbon spectroscopic electrodes the drills become dull and it is desirable to have several well-sharpened drills of the desired size at hand. Drills should be replaced after 20 to 30 cuttings, or when the crater walls begin to break. It is important to use a sharp drill and not to attempt to turn down the crater wall.

This device has been found especially satisfactory since it does not require that two axes be kept aligned. By using drills only for this purpose a regular shop drill press can be used without fear of contamination of the electrodes, even those of the highest purity. While similar devices are known to have been used for some time by Cholak, Mankovich, and others, this is presented as a very satisfactory means of cutting the harder special spectroscopic carbon electrodes.

The writer wishes to thank Fred Mangelson of the College of Engineering, University of Kentucky, for technical assistance.



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THE investigation reported here is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

Visual Fluid Flowmeters with Straight-Walled Tubes

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Several variations of a visual fluid flowmeter are described and calibration data for fluids of varying density and viscosity are given. A comparison of the data with those of the rotameter is made, based on the Schoenborn-Colburn equations. The meters are easy to construct and inexpensive, as a tapered glass tube is not required. Accuracies of 1 to 3 per cent of the volume of fluid flowing were observed.

SEVERAL papers have been published on the visual fluid flowmeter, usually referred to as the "rotameter". Stout and Rowe (*3*) described a meter which operated on similar principles and which involved a straight-walled glass tube and a central cone in its construction. This paper describes several variations of a meter of the visual flow type which has been developed in this laboratory. Calibration data for fluids of varying viscosity and density are included and a comparison with the rotameter, based on the equations of Schoenborn and Colburn (*2*), is given. The meters are easy to construct and inexpensive, as a tapered tube is not required.

Apparatus

TYPE P. In constructing the meter, shown diagrammatically in Figure 1, two parallel-sided slots were cut along the 180° axes of a piece of standard 0.75-inch brass pipe, after which the pipe was reamed to a uniform internal diameter. Above, but connected with the slots, a number of large holes were cut through the pipe walls to allow the fluid to return to the pipe after passing around a float. It was found that unless the area of these holes was made many times the entire area of the slots, the upper readings would be useless. A rubber stopper was fitted snugly around the pipe above these holes and a second stopper placed below the bottom of the slots. The two stoppers held a glass tube 2 inches in internal diameter in place to confine the flow, through which the position of the float could be noted with reference to a scale inscribed on the slotted brass pipe. Four tie rods, not shown on the diagram, were used to hold the stoppers securely in place. Two types of floats were used: A simple sphere of glass or molybdenum of slightly smaller diameter than the interior of the slotted pipe, and an aluminum cylinder modified as shown in Figure 2, held in place by a central guide rod.

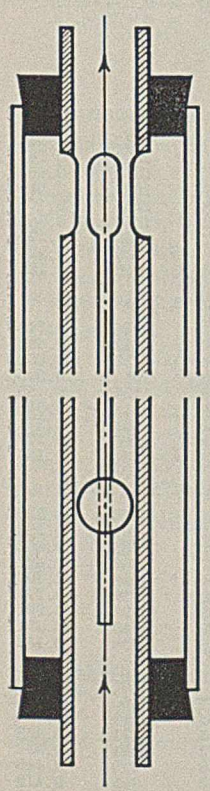

FIGURE 1. DIAGRAM OF TYPE P METER

The fluid rises in this instrument in the metal pipe until it meets the float, passes out through the slots into the annular space between the pipe and the glass jacket, and reenters the metal pipe above the float, both through the slots and through the large holes provided for the purpose. The whole may be said to act like a variable-sized orifice as set forth by Schoenborn and Colburn (*2*), the available orifice being the annular ring between the float and the interior surface of the metal pipe together with the area of the slots below the float. In the case of floats using a central guide rod, an additional small annular ring exists between the guide rod and the float. Since the kinetic energy of the flowing fluid must just balance the potential energy of the float, there must be an equilibrium position of the float for each rate of flow because of the increasing area provided by the slots at higher levels.

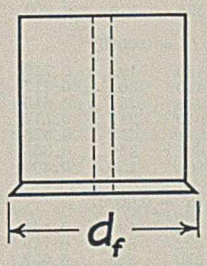

FIGURE 2. TYPE OF CYLINDRICAL FLOAT

TYPE V meters were made similar in all respects to the Type P meters, except that the slots in the metal tube were V-shaped, varying in width from zero at the bottom to 0.4375 inch at a height of 9.375 inches. This was done in order to provide a smaller available area for flow per unit of height at the lower readings and a relatively larger area per unit of height at the higher readings.

Experimental

Water was allowed to flow from a constant-head tank through the instrument to a weighing tank. A number of runs were made and the results averaged for each position of the float. For the runs on liquids other than water, the fluid was pumped at constant rate from a broad shallow tank through the instrument to the weighing tank.

The results of these tests are presented in tabular form in Tables I to IV. A typical calibration curve of rate of flow for each meter reading is shown in Figure 3.

Correlation of Data

Let us consider the parallel-slot meter designated as Type P. We might think of the slot as a weir, the height of the float being a measure of the head of fluid above the bottom of the weir. The construction of the meter violates all the re-

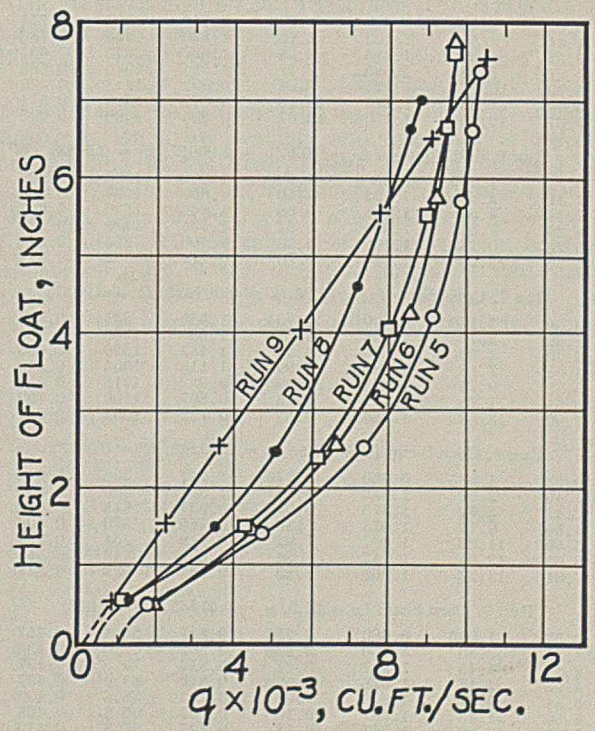

FIGURE 3. CALIBRATION CURVES FOR METER P-2

Table I. Calibration Data

Meter P-1, Spherical Float
d_t = 0.8075 inch d_f = 0.7615 inch
w = 0.063 inch ρ_f = 151.7

x (Inches)	$\frac{S_0}{1000}$	$\frac{D}{100}$	$\frac{q}{1000}$	V	Re	C
Run 1, Water (ρ = 62.36, μ = 0.00074, H = 0.0604)						
0.67	0.65	0.1284	1.010	1.554	168	0.788
3.07	2.75	0.211	3.745	1.36	242	0.690
5.67	5.02	0.231	6.02	1.199	234	0.608
7.72	6.88	0.239	6.74	0.988	199	0.501
9.53	8.40	0.2425	7.52	0.896	183	0.454
12.05	10.60	0.247	7.54	0.711	148	0.360
15.28	13.43	0.250	8.03	0.598	126	0.303
Run 2, Water (ρ = 62.26, μ = 0.000614, H = 0.0606)						
0.71	0.683	0.1317	1.013	1.480	198	0.750
3.11	2.78	0.211	3.94	1.416	303	0.717
5.47	4.85	0.231	5.91	1.218	284	0.616
7.79	6.97	0.239	6.95	1.010	245	0.512
10.31	9.09	0.244	7.64	0.841	208	0.426
13.70	12.05	0.248	7.97	0.661	166	0.335
15.58	13.70	0.250	8.26	0.604	153	0.306
Run 3, Water (ρ = 62.10, μ = 0.000506, H = 0.0609)						
0.315	0.398	0.0967	0.664	1.668	198	0.843
2.60	2.34	0.203	3.33	1.422	355	0.719
5.32	4.72	0.230	5.91	1.250	353	0.632
8.27	7.30	0.240	7.17	0.983	290	0.497
10.82	9.54	0.246	7.60	0.797	240	0.403
11.92	10.50	0.247	7.76	0.738	223	0.373
15.23	13.40	0.250	8.11	0.605	186	0.306
Run 4 Water (ρ = 61.86, μ = 0.000414, H = 0.0613)						
0.827	0.788	0.141	1.176	1.492	314	0.753
2.875	2.58	0.208	3.58	1.390	431	0.701
5.67	5.02	0.231	6.12	1.218	420	0.614
8.35	7.37	0.240	7.46	1.012	363	0.510
9.92	8.75	0.246	7.49	0.856	312	0.432
12.68	11.16	0.248	7.84	0.703	260	0.354
15.40	13.54	0.250	8.20	0.606	226	0.305

Table II. Calibration Data

Meter P-2, Cylindrical Float
d_t = 0.8297 inch d_f = 0.8140 inch
w = 0.125 inch ρ_f = 167.9
d_h = 0.073 inch v_f = 0.3068 cu. in.
d_r = 0.0682 inch S_f = 0.516 sq. in.

x (Inches)	$\frac{S_0}{1000}$	$\frac{D}{100}$	$\frac{q}{1000}$	V	Re	C
Run 5, Water (ρ = 62.23, μ = 0.000631, H = 0.0843)						
0.50	1.011	0.638	1.770	1.750	1105	0.751
1.41	2.58	1.102	4.72	1.830	1990	0.785
2.53	4.52	1.385	7.35	1.627	2225	0.695
4.21	7.28	1.585	9.125	1.254	1970	0.538
5.70	10.05	1.697	9.84	0.979	1643	0.420
6.60	11.62	1.742	10.13	0.877	1500	0.374
7.37	12.96	1.770	10.37	0.800	1400	0.343
Run 6, Salt Solution (ρ = 70.6, μ = 0.00087, H = 0.0683)						
0.51	1.000	0.65	1.900	1.900	1003	0.905
1.47	2.68	1.122	4.50	1.680	1530	0.800
2.54	4.55	1.288	6.66	1.464	1530	0.697
4.24	7.50	1.595	8.51	1.135	1470	0.540
5.76	10.15	1.700	9.20	0.906	1250	0.431
7.76	13.65	1.785	9.70	0.711	1032	0.338
Run 7, Corn Sirup (ρ = 72.25, μ = 0.000925, H = 0.0657)						
0.56	1.100	0.690	1.704	1.550	835	0.753
1.50	2.75	1.135	4.275	1.555	1380	0.755
2.40	4.30	1.360	6.16	1.433	1520	0.696
4.05	7.18	1.580	7.99	1.113	1385	0.540
5.52	9.75	1.688	8.99	0.922	1215	0.448
6.64	11.70	1.745	9.45	0.807	1100	0.392
7.60	13.38	1.780	9.65	0.722	1004	0.351
Run 8, Corn Sirup (ρ = 77.85, μ = 0.00268, H = 0.0575)						
0.52	1.025	0.660	1.273	1.242	238	0.645
1.49	2.62	1.032	3.49	1.332	400	0.692
2.47	5.42	1.375	5.09	0.939	375	0.487
4.59	9.12	1.623	7.16	0.785	370	0.408
5.69	11.03	1.697	7.92	0.718	354	0.373
6.62	12.65	1.742	8.54	0.675	342	0.351
7.01	13.33	1.760	8.82	0.661	338	0.343
Run 9, Corn Sirup (ρ = 81.3, μ = 0.01235, H = 0.053)						
0.52	1.025	0.660	0.858	0.837	56.4	0.452
1.54	2.80	1.150	2.255	0.805	61.0	0.435
2.54	4.45	1.387	3.61	0.810	73.8	0.437
4.05	7.17	1.578	5.69	0.810	84.0	0.437
5.54	9.77	1.690	7.76	0.795	88.5	0.429
6.50	11.45	1.737	9.10	0.795	90.8	0.429
7.54	13.26	1.777	10.54	0.795	92.8	0.429

Table III. Calibration Data

Meter V-1, Spherical Float
d_t = 0.8125 inch d_f = 0.7418 inch
Max. x = 9.375 inches ρ_f = 151.5
Max. w = 0.4375 inch

x (Inches)	$\frac{S_0}{1000}$	$\frac{D}{100}$	$\frac{q}{1000}$	V	Re	C
Run 10, Water (ρ = 62.4, μ = 0.000815, H = 0.0603)						
0.6	0.717	0.474	1.34	1.869	677	0.946
1.2	1.067	0.535	1.81	1.695	694	0.859
2.4	2.47	0.832	3.38	1.370	872	0.695
3.6	4.80	1.216	6.32	1.317	1225	0.667
4.8	8.07	1.640	7.92	0.981	1155	0.497
6.0	12.29	2.08	8.67	0.706	1125	0.358
7.2	17.42	2.53	9.18	0.527	1021	0.267
8.4	23.5	2.99	9.67	0.411	941	0.208

Meter V-2, Spherical Float
d_t = 0.8125 inch d_f = 0.7690 inch
Max. x = 9.375 inches ρ_f = 151.5
Max. w = 0.4375 inch

Run 11, Water (ρ = 62.4, μ = 0.000815, H = 0.0611)						
0.6	0.492	0.310	1.01	2.05	486	1.030
1.2	0.842	0.415	1.41	1.675	532	0.843
2.4	2.25	0.745	3.12	1.388	790	0.698
3.6	4.58	1.141	5.65	1.233	1078	0.620
4.8	7.85	1.570	8.22	1.047	1259	0.527
6.0	12.07	2.02	8.97	0.743	1150	0.374
7.2	17.20	2.47	9.52	0.553	1045	0.278
8.4	23.3	2.94	10.04	0.431	969	0.217

Meter V-3, Spherical Float
d_t = 0.8125 inch
Max. x = 9.375 inches d_f = 0.7500 inch
Max. w = 0.4375 inch ρ_f = 636

Run 12, Water (ρ = 62.4, μ = 0.000815, H = 0.383)						
0.6	0.650	0.427	3.30	5.08	1660	1.020
1.2	1.000	0.495	4.21	4.21	1590	0.846
1.8	1.583	0.629	5.98	3.78	1820	0.760
2.4	2.40	0.795	8.28	3.45	2095	0.694
3.0	3.45	0.980	10.95	3.17	2380	0.637

Table IV. Calibration Data

Meter V-4, Cylindrical Float
d_t = 0.816 inch d_f = 0.780 inch
Max. x = 9.375 inches ρ_f = 168.7
Max. w = 0.4375 inch v_f = 0.314 cu. in.
d_h = 0.073 inch S_f = 0.478 sq. in.
d_r = 0.0682 inch

x (Inches)	$\frac{S_0}{1000}$	$\frac{D}{100}$	$\frac{q}{1000}$	V	Re	C
Run 13, Water (ρ = 62.3, μ = 0.000678, H = 0.0935)						
1	0.652	0.335	1.35	2.07	637	0.845
2	1.619	0.585	3.12	1.93	1004	0.787
3	3.24	0.905	5.25	1.62	1350	0.661
4	5.50	1.253	7.81	1.42	1635	0.579
5	8.43	1.620	9.83	1.166	1735	0.475
6	11.97	1.990	10.80	0.902	1650	0.368
7	16.19	2.37	11.52	0.712	1555	0.291
8	21.1	2.76	11.81	0.560	1420	0.229
9	26.6	3.14	12.05	0.453	1310	0.185
Run 14, Salt Solution (ρ = 68.05, μ = 0.000968, H = 0.0810)						
1	0.652	0.335	1.07	1.64	386	0.719
2	1.619	0.585	2.55	1.576	647	0.690
3	3.24	0.905	4.65	1.435	913	0.630
4	5.50	1.253	6.58	1.198	1054	0.525
5	8.43	1.620	8.33	0.988	1125	0.433
6	11.97	1.990	9.56	0.799	1118	0.351
7	16.19	2.37	10.38	0.641	1068	0.281
8	21.1	2.76	10.94	0.518	1005	0.227
9	26.6	3.14	11.28	0.424	934	0.186
Run 15, Salt Solution, (ρ = 70.9, μ = 0.001345, H = 0.0756)						
1	0.652	0.335	1.20	1.84	325	0.836
2	1.619	0.585	2.45	1.515	467	0.689
3	3.24	0.905	4.65	1.435	685	0.652
4	5.50	1.253	7.52	1.367	903	0.621
5	8.43	1.620	9.13	1.084	925	0.493
6	11.97	1.990	9.82	0.820	859	0.372
7	16.19	2.37	10.32	0.638	794	0.290
8	21.1	2.76	10.75	0.509	740	0.231
9	26.6	3.14	11.03	0.415	638	0.189
Run 16, Corn Sirup (ρ = 78.0, μ = 0.00370, H = 0.0636)						
1	0.652	0.335	0.60	0.920	69	0.455
2	1.619	0.585	1.45	0.895	111	0.443
3	3.24	0.905	2.90	0.895	171	0.443
4	5.50	1.253	4.80	0.873	231	0.432
5	8.43	1.620	7.15	0.848	290	0.419
6	11.97	1.990	10.15	0.847	355	0.419
7	16.19	2.37	13.70	0.846	424	0.418
8	21.1	2.76	18.0	0.852	496	0.421
9	26.6	3.14	22.7	0.853	565	0.422

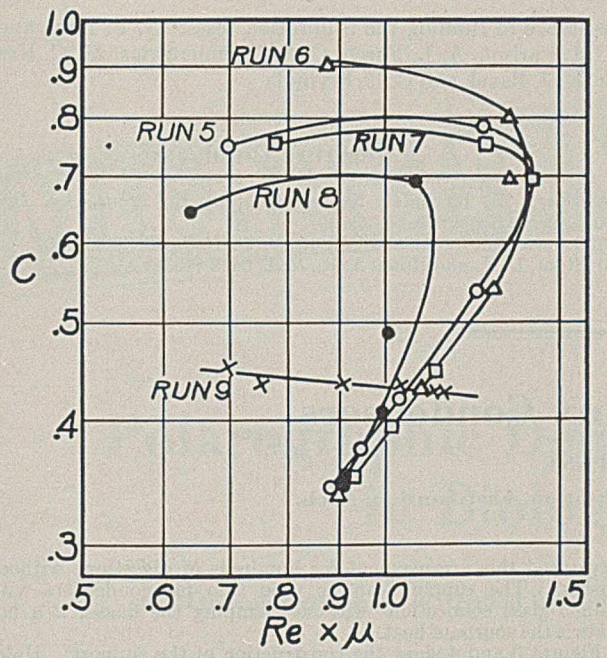

FIGURE 4. PLOT OF C *vs.* $Re \times \mu$ FOR METER P-2

quirements for even a poor weir and the meter should therefore give very unreliable results. Furthermore, a plot of log q *vs.* log x does not even approximate a straight line. This conception was therefore rejected.

The work of Schoenborn and Colburn (*2*) in which the rotameter was considered as a variable size orifice, offers a convenient method of correlation as well as a means of comparison of these meters with the rotameter. The equation developed by them

$$C = \frac{V}{(2gH)^{1/2}} = \frac{q}{S_0\left[\frac{2gv_f(\rho_f - \rho)}{S_f\rho}\right]^{1/2}} \quad (1)$$

was used. The constant, C, involves the properties of the instrument and when plotted against some characteristic of the flowing fluid, such as Reynolds number, gives a smooth curve. Schoenborn and Colburn found that a nearly universal curve could be drawn from data on various rotameters. Since the flow rate appears in both ordinate and abscissa, they plotted C *vs.* Re/C to eliminate this undesirable feature.

Such a plot for the present meters showed a series of curves, very similar in shape but spaced at different values of Re. Several of the runs are plotted in Figure 4 as C *vs.* $Re \times \mu$, the abscissa having been modified in order to bring the curves together, so that several curves could be shown in one illustration. At very high viscosity C was nearly constant.

Several definitions are necessary in order to calculate the quantities involved. The available area for flow is considered as the total area of both slots and the annular areas within the pipe. For Type P meter with spherical floats this becomes

$$S_0 = 2wx + \frac{\pi}{4}(d_t - d_f)^2 \quad (2)$$

In the case of the cylindrical floats, the additional area $\pi/4 \times (d_h - d_r)^2$ must be added to the right-hand side of Equation 2. The wetted perimeter, p_w, is considered as the entire series of lines outlining S_0. For the spherical float this becomes

$$p_w = 4x + \pi(d_t + d_f) \quad (3)$$

and for the cylindrical float

$$p_w = 4x + \pi(d_t + d_f + d_h + d_r) \quad (4)$$

The equivalent diameter, D, used in the Reynolds number then becomes

$$D = \frac{4S_0}{p_w} \quad (5)$$

In the case of the Type V meter, the angle is so small that the sides of the V-shaped slot were considered equal to the reading. The $2wx$ term in Equation 2 must be replaced by wx for this type of slot and twice the width of the slot at reading x must be subtracted from the right-hand side of Equations 3 and 4.

Discussion

In all cases the meters were accurate to 3 per cent of the quantity flowing and those in which the clearance between float and tube was smallest were accurate within 1 per cent. The ranges of the meters were from a minimum flow of 150 to about 2400 pounds per hour of water. The range can be varied by varying the dimensions of the slots, the tube, and the float as well as the material of the float. The clearance between the metal tube and glass jacket must be at least 0.5 inch for a 0.75-inch pipe with slots up to 0.125 inch. For wider slots, the clearance should be greater. The minimum flow rate is determined by the clearance between metal float and pipe. The cylindrical float was tried with the object of eliminating the effect of viscosity as set forth by Fisher *et al.* (*1*). No such result was shown, the constant, C, varying as much as with the spherical float. Although only two slots were tried, a greater number could be distributed on corresponding axes around the tube.

These meters, operating on the principle of variable available area for flow, as does the rotameter, are easy to construct and are inexpensive. One Type P installation in which two floats of molybdenum were used simultaneously in a 0.75-inch tube to measure the cooling water in a commercial heat transfer investigation gave satisfactory service for several months and was doing well when the investigation was ended. The meters may be made of various metals to meet existing corrosion conditions.

The curves of Figure 4 serve as a measure of comparison of this meter with the rotameter. While no universal curve was obtained, a single type of hooked curve resulted in all but the two cases of very high viscosity. Two values of C were obtained for each value of Re, the higher value of C being applicable at low meter readings and the lower values of C corresponding to the higher meter readings. The higher values of C at the low meter readings are not deemed reliable.

A logarithmic plot of C *vs.* $DV\rho$ for the upper three fourths of the readings for any single meter operating on liquids of ordinary viscosity (0.0006 to 0.0025 pound per foot second) can be drawn as a straight line from which the experimental points for that particular meter will vary but little.

Nomenclature

C = meter coefficient
d_f = diameter of float
d_t = diameter of tube
d_h = diameter of hole through float
d_r = diameter of guide rod
D = equivalent diameter of flow area
g = acceleration of gravity
H = difference in fluid head
p_w = wetted perimeter
q = quantity of flow, volume
Re = Reynolds number
S_0 = area available for flow
S_f = area of float at largest diameter
V = velocity of flow at constriction
v_f = volume of float
w = width of slot
x = meter reading; height of float above bottom of slots

ρ = density of fluid
ρ_f = density of float
μ = viscosity

All values are in terms of feet, pounds, and seconds unless otherwise designated in the text.

Acknowledgment

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A Support for Reflux Condensers

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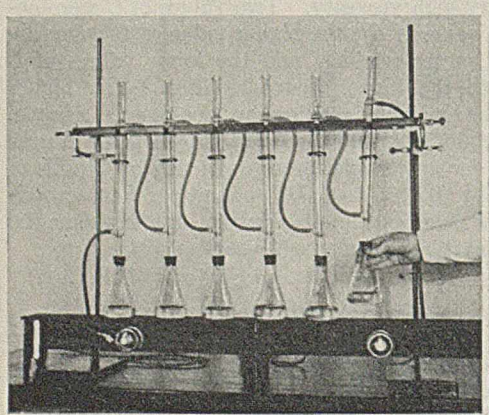
FOR extraction procedures and other operations involving refluxing, it is frequently necessary to arrange a battery of condensers in such a manner that the attached flasks can be rotated individually in order to mix their contents. Since the condensers must usually be clamped rigidly to ensure their safety, it is often difficult to obtain sufficient elasticity in the mounting to permit vigorous rotation of the attached flasks without having to loosen clamps.

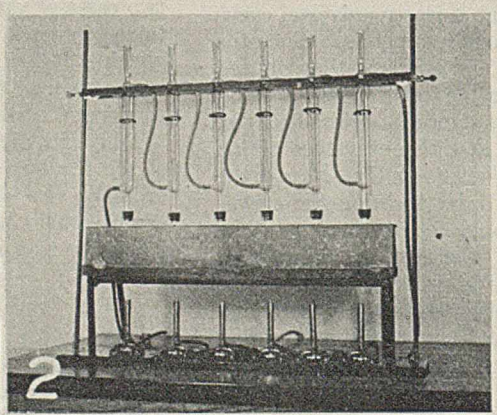
The author was confronted with the task of digesting a large number of samples of plant material, and in order to minimize this difficulty, executed the support shown in the accompanying figures. Such a support is easy to construct and has proved convenient and effective.

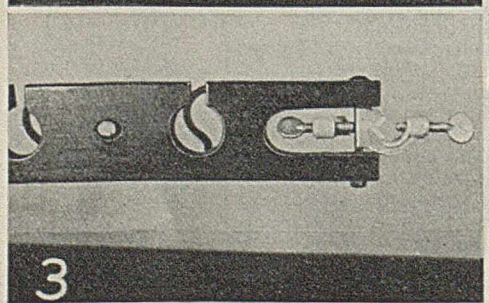
Figures 1 and 2 show a support for a battery of six condensers in use with an electric hot plate and water bath, respectively. Each flask can be raised and rotated freely without interfering with the others or having to loosen clamps. With pliable connecting stoppers, such a support can be used with a water bath without having to clamp the flasks, since the buoyant force of the water supports them from below. The author and associates have used this arrangement for hundreds of digestions without mishap. The support can be used also for condensers with ground-glass connections without clamping the flasks, if a hot plate is the source of heat.

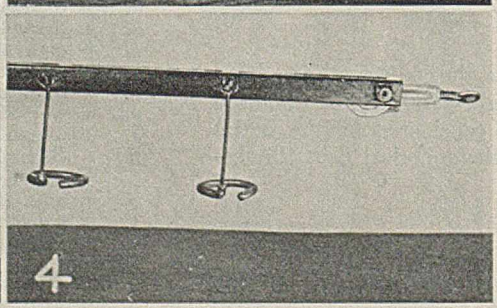
Figures 3 and 4 show the construction of the support. Holes large enough to accommodate the condensers to be used are bored in a piece of board of appropriate dimensions, depending upon the number of reflux units desired, and notches large enough to permit the upper nipple of the condenser to pass through are cut from one side of the board to the holes. Other holes of correct size to permit the condenser tubing to pass through freely without being constricted are bored between the large ones at a slight angle with respect to the upper side of the board. A notch should be made in each end of the board, and a 0.375-inch bolt passed through in such a way that an ordinary clamp holder can be used to attach the support to ringstands. A small ring for holding a screw is bent on one end of a piece of moderately stiff wire of appropriate length and a ring that will almost encircle the jackets of the condensers to be used is bent on the other. The large ring should not be closed completely but should have a gap at the back big enough for the upper nipple of the condenser to pass through. Small-bore rubber tubing should be passed around the large ring to act as a cushion against the condenser jacket. One of these holders should be screwed onto the back of the support in line with the center of each large hole.

To place the condenser in position, the upper end is passed through the large hole from below and the condenser is turned in such a way that the upper nipple will pass through the gaps in the wire ring and in the board as it is raised upward. When it has passed through the upper notch, the nipple is placed on the board so that it points in the direction the water is to flow. After all the condensers are in place and equipped with rubber tubing, they should be aligned by adjusting the wire holders.









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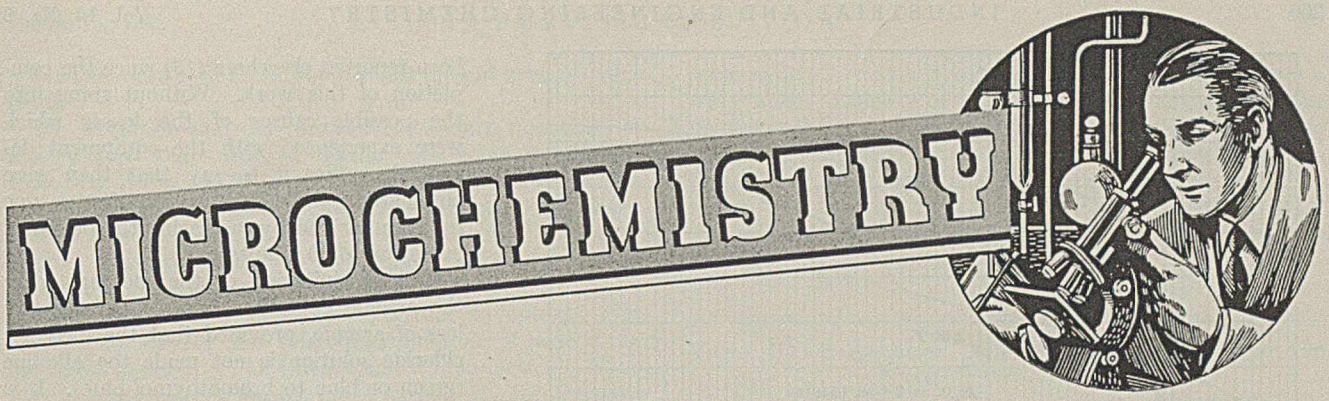

Polarographic Determination of Arsenic in Biological Material

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THE uncertainties and inconveniences attending the use of the Gutzeit method (*1*) have resulted in the introduction of a number of improved procedures (*3, 4, 7, 8, 11, 12*) for the microdetermination of arsenic in biological material. Although these methods are much better suited for general work than the Gutzeit procedure, they possess certain disadvantages which prompted the investigation of polarographic methods.

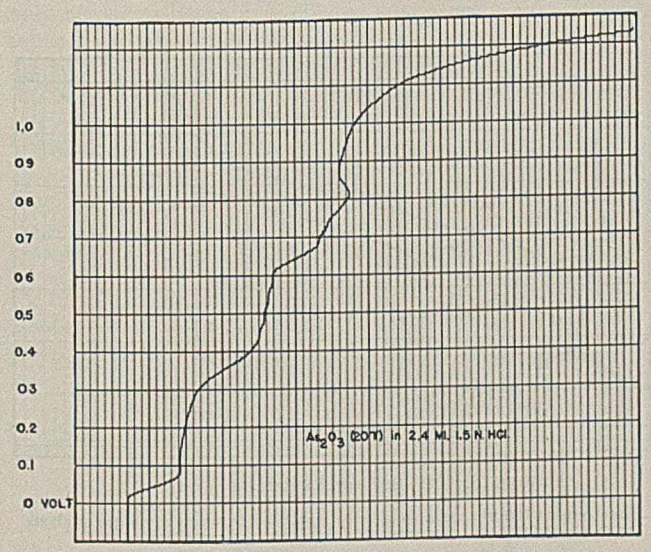

FIGURE 1. POLAROGRAPHIC CURVE GIVEN BY REDUCTION OF ARSENIC

20 micrograms as As_2O_3 in 2.4 ml. of 1.5 *N* hydrochloric acid

The literature contains a few references to polarographic studies on arsenic. Bayerle (*2*) worked with sodium arsenite in alkaline solutions but gave no definite information regarding the possibility of obtaining quantitative results with the procedure, while Kacirkova (*9*) reported that arsenic in relatively high concentrations gives a number of steps that are difficult to separate quantitatively. Preliminary work confirmed this report, but the author found that when the quantities of arsenic (trivalent) were kept below 100 micrograms per milliliter, well-defined steps were obtained, one of which was suitable for use in quantitative analysis (Figures 1 and 2).

Arsenate ion apparently is not reduced by the polarograph (*10*), although it is possible that reduction could be accomplished if a suitable electrolytic base were found. No definite steps due to quantitative reduction were observed with sodium arsenate in neutral aqueous solution, 5 per cent acid, 10 per cent tartaric acid, 1 *N* hydrochloric acid, 0.125 *N* hydrochloric acid, 25 per cent sulfuric acid, or alkaline solutions varying from slight alkalinity to about 0.2 *N* sodium hydroxide.

The polarographic method depends upon the isolation and concentration of the arsenic by the evolution of arsine and its absorption in mercuric chloride solution in a manner similar to that described by Cassil (*3, 4*). The mercury arsenides are changed to arsenious oxide by heating with the excess mercuric chloride, the mercury is precipitated by reduction with hydroxylamine, and the solution is then ready for the polarographic determination.

Procedure

PREPARATION OF SAMPLE. The sample of biological material is prepared by the wet-ashing procedure described by Hubbard (*8*). Nitrogen oxides should be removed from the sample solution by treatment with saturated ammonium oxalate solution, as in the Gutzeit method. An aliquot or the entire prepared sample may be used in the determination; the method cited ends with the sample dissolved in 50 ml. of approximately 40 per cent sulfuric acid, a solution satisfactory for use in the arsenic isolation step.

ISOLATION OF ARSENIC. The prepared sample is mixed with 10 to 15 ml. of hydrochloric acid in a 125-ml. Erlenmeyer flask and diluted with water to about 70 ml., after which 5 ml. of potassium iodide solution (*4*), 1 ml. of stannous chloride solution (*4*), and 10 to 12 grams of stick zinc are added. A Kjeldahl trap fitted with a glass delivery tube, which dips to the bottom of an ordinary 15-ml. graduated centrifuge tube containing 2 ml. of mercuric chloride solution (1.6 grams per 100 ml.), is immediately attached to the flask, which is then placed on a hot plate or in a water bath. It is advisable to provide some means of removing hydrogen sulfide from the evolved gas, although small quantities do not interfere. The simplest method is to put a gas-washing bottle with a fritted-glass disk, containing about 100 ml. of lead acetate solution (10 grams per 100 ml.), between the trap and the delivery tube. No arsine is retained in this bottle, so that it need not be cleaned frequently.

The evolution of hydrogen is allowed to proceed for about 30 minutes; then the delivery tube is disconnected and the centrifuge tube, containing both the mercuric chloride and the delivery

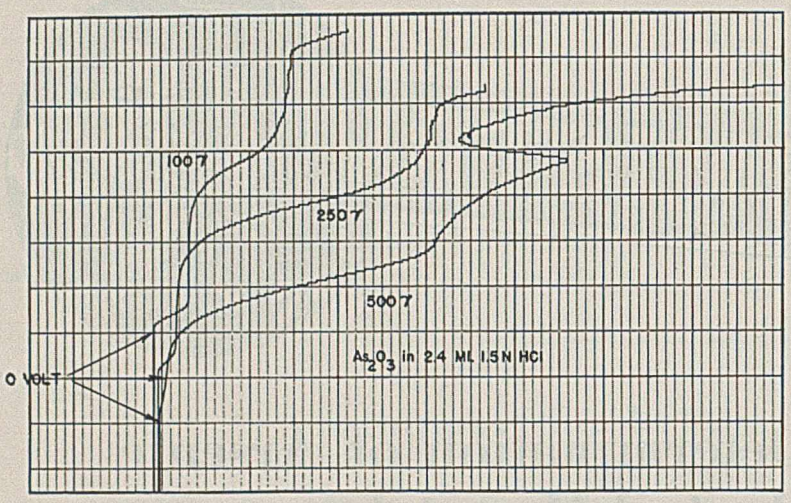

FIGURE 2. STEPS GIVEN BY ARSENIC

Quantities indicated as As_2O_3 in 2.4 ml. of 1.5 *N* hydrochloric acid

tube, is placed in a water or steam bath. The centrifuge tube is heated for about 5 minutes in order to change the mercury arsenides to arsenious oxide and mercurous chloride, and the delivery tube is rinsed off with hot water and removed. The solution is concentrated to about 2 ml., 1 drop of bromothymol blue solution (0.2 gram per 100 ml.) and 0.2 ml. of hydroxylamine sulfate solution (40 grams per 100 ml.) are added, and ammonium hydroxide is added to the hot solution carefully, in small drops, until the mercury precipitates. Upon addition of the first portion of ammonium hydroxide a flocculent precipitate appears; when more alkali is added it causes the mercury to be precipitated in a finely divided black form. At this step arsenic may be lost if the solution becomes too alkaline; it should not turn blue but should show the yellow-green color indicative of about pH 6. It is then heated until evolution of nitrogen ceases, it is cooled, and the volume is noted.

DETERMINATION OF ARSENIC. Two milliliters of the clear supernatant liquid are placed in the electrolysis cell, 0.4 ml. of 9 *N* hydrochloric acid is added, nitrogen is bubbled through the solution for no longer than 5 minutes, and the mixture is polarized under nitrogen from 0 to −0.7 volt. [If the quantity of arsenic is expected to be low (less than 20 micrograms), as judged from the appearance of the mercury arsenides in the centrifuge tube in the previous step, 3 *N* hydrochloric acid is used instead of the 9 *N* acid.] The half-wave of the arsenic step occurs at −0.35 volt (standard calomel electrode) in 1.5 *N* hydrochloric acid and at −0.5 volt in 0.5 *N* acid. The step height is measured and compared with a calibration curve made by taking known quantities of standard arsenious oxide solution, adding them to 2-ml. portions of the mercuric chloride solution contained in centrifuge tubes, and following the procedure described above, beginning with "The solution is concentrated.....". These determinations should all be made at the same temperature, with the same capillary and drop rate. Such precautions would not be necessary if it were possible to use an internal standard (*6*), but the complexity of the arsenic curve (Figure 1) prevents this.

Discussion

The arsenic isolation procedure has been shown by Cassil and others (*3*, *4*) to be quantitative, but the author was not able to confirm Cassil's recovery of quantities of arsenic above 30 micrograms with his method. Losses of arsenic of about 10 per cent usually occurred when granulated zinc and the 5-minute evolution were employed. Similar low results have been reported elsewhere (*13*) since the completion of this work. Without going into the possible causes of the losses which were experienced with the equipment described, suffice it to say that they were eliminated by the substitution of stick zinc for granular and by increasing the period of evolution to 30 minutes.

The precipitation of the mercury after the arsenic evolution does not cause any loss of arsenic, provided that the mercuric chloride solution is not made too alkaline (green or blue to bromothymol blue). It is necessary to remove mercuric ions from the solution before polarizing. Various methods were tried, including precipitation as the iodide, phosphate, oxide, and hydroxide, but all either caused loss of arsenic or failed to remove the mercuric ions completely. Reduction by hydroxylamine was the only method found which removed the mercury without resulting in loss of arsenic.

The arsenic solutions are polarized in acid of two different strengths: 0.5 *N* and 1.5 *N*. The weaker acid is used when it is necessary to employ high recorder sensitivities (with small quantities of arsenic); this gives a polarographic curve which is much smoother than that obtained with strong acid. With larger quantities of arsenic it is necessary to use 1 *N* or stronger acid, in order to bring the half-wave potential of the quantitative arsenic step as low as possible, so that it does not fuse with the next step. Even under these conditions, the steps fuse when the quantity of arsenic in the cell is greater than 100 micrograms per ml. (Figure 2).

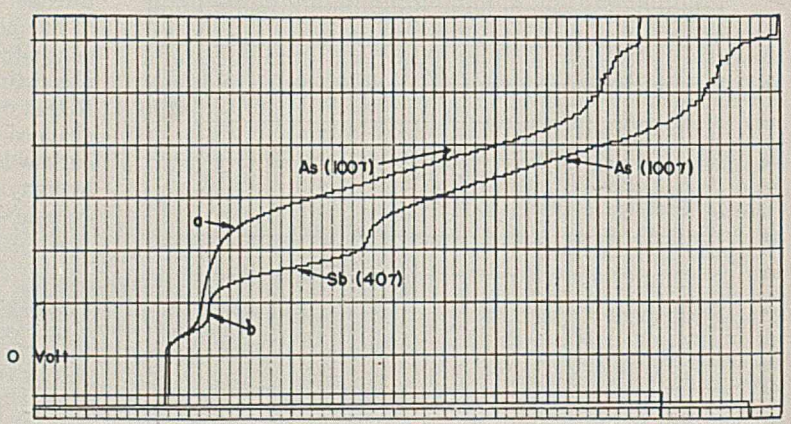

FIGURE 3. EFFECT OF ANTIMONY

a. 5 mg. of antimony plus 100 micrograms of arsenic carried through complete analysis
b. Same solution in electrolysis cell as *a* plus 40 micrograms of antimony

Antimony is an interfering element in most methods that depend upon the isolation of arsenic as arsine. It does not interfere in the polarographic method, however. Quantities of antimony as high as 5 mg. (antimony sulfate in sulfuric acid) were added to the flask before evolution of arsine; the arsenic step was not affected and no step due to antimony appeared (curve *a*, Figure 3). When, however, antimony sulfate was added directly to the electrolysis cell, an antimony step occurred at −0.16 volt in 1.5 *N* hydrochloric acid and at −0.22 volt in 0.5 *N* acid (curve *b*, Figure 3). Therefore, even if antimony were introduced into the solution being polarized, it would always be detected by its characteristic step and would

not interfere with the arsenic step unless present in large quantities.

The evolution and determination of known quantities of arsenic and comparison of results with some results obtained by Hubbard (*8*) with his procedure, indicate that the error of the polarographic method lies between 3 and 5 per cent in the case of quantities of arsenic above 10 micrograms; quantities below 10 micrograms can be determined within 1 microgram (see Tables I to III).

The polarographic method has certain advantages over other methods for the microdetermination of arsenic in biological material. The Gutzeit and Marsh methods, while sensitive, are neither flexible nor accurate; the former gives results accurate to 10 per cent only under the best conditions, while greater errors are often encountered. Also, it is frequently necessary to repeat analyses because the range of ascertainable quantities is quite small. Colorimetric methods employing the molybdenum blue color are as accurate as the polarographic procedure but usually are considerably more time-consuming. Total elapsed time between the completion of sample preparation and the final arsenic determination is usually about 24 hours; this time is consumed mainly by the evaporation of the arsenic trichloride distillate and preparation for the color development. During this manipulation all dust contamination must be avoided, since phosphate and silica, as well as arsenic, give the blue color. The total elapsed time with the polarographic procedure is less than an hour, and with the proper equipment a trained operator could run over twenty analyses per day. The titrimetric method used by Cassil (*3, 4*) is probably the most rapid when a large number of analyses must be run daily and it compares favorably in accuracy with the polarographic and colorimetric procedures, provided antimony is absent. However, the polarographic technique has a distinct advantage when samples need be run only occasionally, as in a clinical laboratory. No standard solutions are necessary, so that time is not used in standardizing volumetric solutions for one or two analyses.

The calibration curves, once made, are valid indefinitely, so long as the same capillary is used in the polarograph. In addition, as little as 1 microgram of arsenic can be determined by the polarographic method, while the titrimetric procedure is not useful for quantities of arsenic below 5 micrograms. With the polarograph, determinations on samples with high arsenic content need be repeated only if the quantity of arsenic evolved is over 1 mg., so that the method covers a wide range. Dangers of contamination are slight, since the unique specificity of polarographic procedures eliminates interference from all common substances, including antimony. Considering these many factors, the polarographic procedure seems to be the method of choice for clinical work.

The Leeds & Northrup Electro-Chemograph was used in this work.

Table I. Recoveries of Known Quantities of Arsenic

Arsenic Taken *Micrograms*	Arsenic Found *Micrograms*
5	5.6
5	4.5
10	10.0
10	9.6
20	19.5
20	19.5
30	30.0
30	30.5
100	99
100	100
300	296
300	285

Table II. Recoveries of Known Quantities of Arsenic

Arsenic Added to Synthetic Urine Salts (*5*) Equivalent to 50 Ml. of Urine *Micrograms*	Arsenic Found *Micrograms*
0	Nil
2	2.1
5	4.7
10	10.3
50	50.0
100	99
200	196

Table III. Comparison of Results by Photometric and Polarographic Methods

Sample	Quantity *Grams*	Arsenic Found: Photometric Method (*8*) *Micrograms*	Arsenic Found: Polarographic Method *Micrograms*
Rabbit tissues:			
Colon	5.2 (not dried)	2.5	2.5
Heart	4.2	2.7	1.3
Kidney	5.1	33	31
Liver	5.5	18	18.5
Lungs	4.1	3.5	4.0
Lungs	6.4	10.5	11.3
Uterus	4.8	2.4	2.1
Uterus	4.9	12.4	11.5
Blood (human)	8.2	2.8	2.6
	6.3	9.6	7.5
Feces (rabbit)	5 (not dried)	60	59
Feces (human)	12.5	137	134
Feces (human)	4	312	309
	Ml.		
Urine (rabbit)	25	1.8	1.2
	25	2.7	3.9
	25	3.6	4.2
	25	89	88
	25	166	163
Vomitus (human)	25	52	52
	25	60	58
	19	100	101
	10	154	142
	Grams		
Spinal fluid (human)	2.7	1.6	2.0

Summary

A polarographic method is described for the microdetermination of arsenic in biological material. The arsenic is evolved from the prepared sample as arsine, which is absorbed in mercuric chloride solution; the mercury arsenides are changed to arsenious oxide and mercurous chloride by heating with the excess mercuric chloride; the mercury is precipitated by reduction with hydroxylamine; and the resulting solution is polarized in hydrochloric acid. The method is rapid and covers a range from 1 microgram to 1 mg. of arsenic. With quantities of arsenic below 10 micrograms, the error is 1 microgram; above 10 micrograms, the error is 3 to 5 per cent. No interfering substances have been encountered.

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Apparatus for Precision Calibration of Pipets, Volumetric Flasks, and Burets

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THE apparatus described here was designed for rapid calibration of pipets and flasks well within usual tolerances—e. g., one part per thousand for volumes of 10 ml. or more, and within 0.01 ml. for those less than 10 ml. In practice it appears to be subject to errors usually less than one tenth as great. A pipet of 0.5-ml. capacity may be measured to about 0.001-ml. approximation of calibration by other accepted means (*3*).

Apparatus

Figure 1 gives a schematic diagram of the essential elements of the calibration apparatus. Convenient dimensions are given below.

CONTROL VESSEL. A double Y-tube is rigidly constructed from six standard-taper stopcocks (preferably Pyrex) of about 2-mm. bore, with connecting tubes of approximately 4-mm. bore and 7-mm. outside diameter. The Y-angles are about 60°. Vertically ascending from a 7-cm. delivery tip are stopcocks *D*, *C*, and *A* with intervals of 6 and 10 cm., respectively. The stem above *A* extends 5 cm. to a neatly fire-polished butt at *Q*. Midway between *A* and *C* is joined the branch ascending at 30° from horizontal. About 6 cm. along this is stopcock *E* and 8 cm. farther is *F* with another butt end after about 3 or 4 cm. Midway between *E* and *F* is a vertically ascending branch about 14 cm. in length with stopcock *B* about 4 or 5 cm. above the Y-joint. The top of this branch is a butt at *G* like that above *A*.

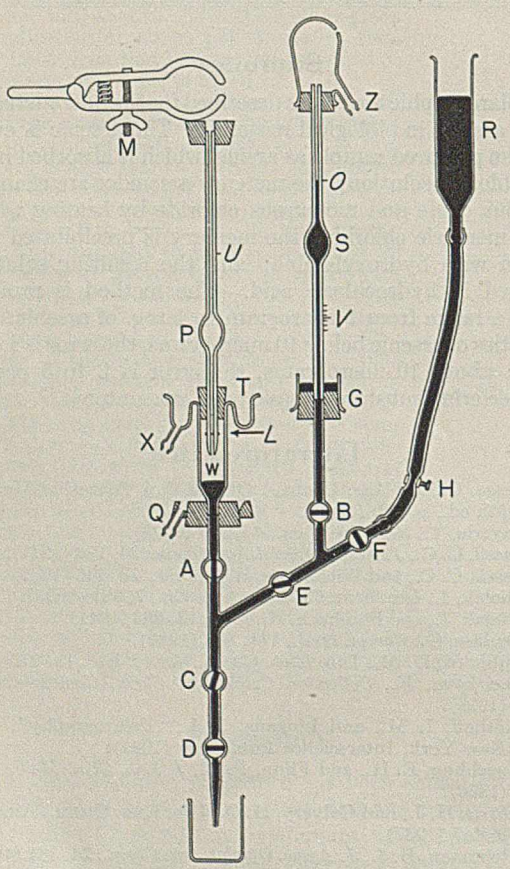

FIGURE 1. APPARATUS FOR PIPET CALIBRATION BY REFILLING OR MERCURY WEIGHING

Each of these is fitted with an inverted No. 8 stopper bored with a slightly smaller hole for tight fitting around the 7-mm. glass tubing, on which it is mounted with the glass butts halfway through in each case (*Q* and *G*). The other halves of the borings supply joints to apparatus placed vertically above. The stopper at *G* is surrounded by a short glass cylinder, about 4 cm. high and 4 cm. in outside diameter, to form a well. The stopper at *Q* is surrounded by a moat with a drain to a waste bottle. The moat may be made by use of a rubber ring with chamfered inner edge and the drain supplied by a boring fitted with a small glass tube. The apparatus is mounted in clamps on a firm upright rod—e. g., a ring stand clamped to the bench with a C-clamp. Between clamp jaws and apparatus are placed rubber stoppers suitably bored and slit on one side, one between *C* and the Y-joint and another 3 or 4 cm. above *B*.

MERCURY CHARGE AND RESERVOIR. To the butt beyond *F* is attached rubber tubing, leading to reservoir *R* at suitable height, with a Hoffman clamp, *H* (or another stopcock), just beyond the butt near *F*. Either through this lead, or a temporary substitute, vacuum is applied at *F* and also at *B* and *A* as required to fill the apparatus with mercury from the tip below *D* to and through stopcocks *A*, *B*, and *F* with careful exclusion of air. Then reservoir *R* is filled with mercury and placed in position, and air is expelled from the connections to *F* by slightly whirling part of the rubber tube.

STANDARD PIPET. *A* and *D* are left closed and *C* and *E* open (partly, at least) while mercury is allowed to run through *F* and *B* partly to fill well *G*. Through this mercury, the surface of which is cleaned by aid of a small glass vacuum attachment, if necessary, a standard pipet, *S*, is inserted and joined (glass to glass) through the rubber stopper at *G*. The pipet is provided with capillary arms above and below its bulb, adapted, if necessary, at the lower end to make a good fit (about 7 mm. in outside diameter).

For convenience of reading, the arm capillary volume has been made about 0.005 ml. per cm. for a 0.5-ml. standard pipet and 0.03 ml. per cm. for 3- or 5-ml. standards. The volume of mercury that is delivered from an initial mark, *O*, above the bulb to a point below is read on a scale, *V*, calibrated by weight of mercury delivered from the tip through open *E*, *C*, and *D* with *A* and *F* closed and controlled at *B*. The tip is touched to a level mercury surface at the beginning, the discharged mercury caught in a tared vessel, and the tip touched to the level surface of mercury in it at the end of delivery. At the top of pipet *S* may be attached an overflow safety vessel, *Z* (of well-known type), leading to a catch bottle. However, careful adjustment of clamp *H* and the level of *R* make this unnecessary. *S* is clamped lightly at the bulb to the main support rod.

WATER CHAMBER. A vessel, *W*, on the main vertical stem above *A* is attached through the rubber stopper at *Q*, previously flooded with a few drops of water to cover the hole in the stopper so as to exclude air from the joint. *W* is adapted to fit this joint below, about 7 or 8 mm. in outside diameter at the base, to allow formation of a mercury-water interface in a large-diameter tube (25 mm. or more in inside diameter); and to admit attachment of pipet *P* through a rubber stopper at the top of *W*. *W* is provided with a surrounding moat, *T*, at the top, conveniently made all of glass with a drain, *X*, leading to a waste bottle. Alternatively the moat and drain might be made with a rubber ring as at *Q*, or with a rubber stopper around *W* and a glass attachment like that at *Z* but without the dome top.

ATTACHMENTS FOR PIPET TO BE CALIBRATED. The tip of pipet *P* must be above the mercury-water interface throughout all calibration operations on it. Any parts required to be visible for reading or observation of flow should be well above or below the rubber stopper at *T*. The bulb of a small pipet, if near the tip, may be placed below *T*. Air is carefully excluded by flooding *W* with water just before insertion of stopper *T*, the overflow passing away through *X*. In calibration between marks, *P* will have an upper mark, *U*, and a lower mark, *L*. *L* may be placed, as convenient, above or below *T*. In calibration to drain and touch, *L* may be a temporary mark—e. g., a fine rubber ring cut from tub-

ing—placed where the meniscus stands after completing drainage with the tip against the wet wall of a receiving vessel (*3*), or touching the tip of the drained pipet to a level water surface and then slowly withdrawing it, as prescribed by convention. The former is usually preferred, but the latter was used in experiments described below.

Vessel *W* is clamped firmly to the main support rod (with care not to interfere with readings). It is convenient to place a rubber stopper at the top of pipet *P* to be calibrated, and adjust a clamp from the main support so that its movable jaw presses gently upon the top. This furnishes a micrometer screw type of adjustment, *M*, acting on the remote stopper at *T* as a tambour to effect minor adjustment of the air-water interface in the pipet at the upper mark, *U*, and later at the lower mark, *L*, or at the actual tip as required in blow-out or to-contain types of calibration. Slight adjustments at the lower points are usually admissible in calibrations, if the pipet is simply refilled with a definite volume of water after drainage, approximately as in use.

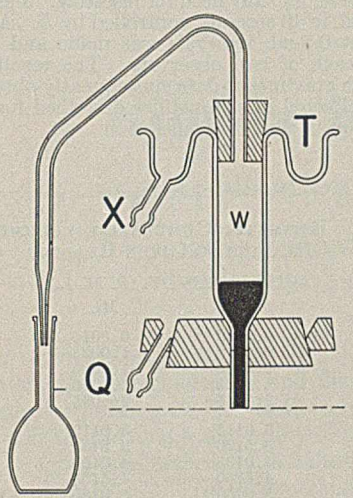

FIGURE 2. ADAPTATION WITH DELIVERY TUBE FOR CALIBRATION OF VOLUMETRIC FLASKS OR OTHER VESSELS

Stopcock *C* is adjusted in preliminary trial to control flow rate conventionally, so as to approximate conditions of drainage of pipet *P* expected in actual use. *E* is used chiefly to control flow rate from *S* to *P*. The other stopcocks, *F*, *B*, *A*, and *D* are usually either closed or wide open in use. Accordingly, it is convenient to distinguish *C* and *E* from the others by a red rubber cover, made from tubing with a little hole cut in the side and slipped over the stopcock handle.

Procedure

Have in place the required standard pipet, *S*, and suitable vessel, *W*. Take care to eliminate any entrapped air. Usually keep *C* and *E* partly open, and never have *A*, *B*, *F*, and *D* all closed longer than necessary to deliberate operation. Have *H* adjusted to give steady flow so as not to entrap air in the standard pipet when refilling (from *V* to *O*) with *B* and *F* wide open and *A* and *D* closed.

Then, with *B* closed and *A* open, adjust mercury in *W* through *F* or *D*, so that it will extend up to the wider cylindrical portion but not high enough to reach pipet *P* in later operations—i. e., the water in *W* between the top of the mercury level and the pipet bottom should have a volume slightly exceeding that to be contained in the pipet. The rubber stopper for use with *M* may be attached at the pipet top before inserting the pipet into *W*. If the pipet has a stopcock, keep it open throughout.

Flood *W* with water. Immediately insert *P* and the stopper at *T*; or insert the stopper only, run water up to flood the hole, and insert the pipet tip through the drop of water and the stopper, if only the tip is to enter *W*. The latter is convenient when several pipets may be calibrated without removal of the stopper at *T;* the former is required when the bulb is to be placed within *W* or when a temporary marker (rubber ring) is used near the tip as in drain and touch calibration. Adjust the clamp at *M* to give a slight tension.

Operate *F* to fill pipet *P* up to *U* (approximately). Close *F*, open *D*, and adjust *C* to give the required outflow rate. Repeat the filling and drainage till the adjustment is satisfactory. If air has been admitted to *W*, remove the pipet and stopper at *T* and replace, after flooding *W* as previously. A small amount of air in *W* might not interfere appreciably with preliminary adjustment of *C*, but might affect later operations considerably.

Close *A*, open *B*, and fill standard pipet *S* to *O*. To facilitate this, *E* may be set in advance so as to restrict subsequent downward flow and then mercury run from *F* into *S* till above *O*, *F* closed, and *D* opened to run mercury slowly down to *O*. Close *B*, then *D*, and open *A*. Adjust water level in *P* to *U* (by *F*, *D*, and *M* as necessary). Open *D* wide, and drain pipet *P* to the lower mark as required. Adjust by *M*, if necessary. Open *B*, control flow rate by *E*, and run back to *U* (or down to the prescribed *V* in the standard, or both in succession) using *B* as shut-off. Read *V* on *S*, or set new mark *U* on *P* if the previous mark is found in repeated trial to yield a delivered volume outside tolerable limits.

If required as a final check calibration, the weight and temperature of mercury delivered from the tip below *D* may be used, provided that the drainage is made by control at *D* alone, with *M* not used except at *U*.

In calibration of volumetric flasks or tubes, the procedure is essentially the same except that an inverted U-delivery tube with a fine tip (illustrated in Figure 2) is used at *T* instead of *P;* and, after delivery, water is added to the system, as required, by dipping the tip of this tube in water and running mercury out through *D*. If air bubbles form in the delivery tube, they may be removed by running more air into it (opening *D*) to contact the entrapped air and following by reversal of flow (closing *D*, opening *F*) to run the air out. Just before the start and at the finish of the measured delivery, the tip is touched to a water surface, first in the beaker used for filling and, finally, within the vessel being calibrated.

Experimental Results

A standard 0.5-ml. pipet was made with marks in close approximation on the *V*-scale to 0.5 ml. and 0.005 and 0.010 ml. above and below. Recalibration at the 0.5-ml. mark by weight of mercury delivered (as described above) gave the *V* estimates, 0.49924, 0.49974, 0.49921; mean $V \cong 0.4994$. The difference of 0.01 ml. in volume delivered corresponded approximately to 20 mm. on the *V*-scale.

As an illustrative application, a 0.5-ml. Ostwald pipet was calibrated in various ways. Its natural outflow time was between 6 and 7 seconds. In drain and touch delivery (touching as previously described after 5 seconds more) calibration from the original mark by weight of water delivered gave the volume estimates, 0.4915, 0.4913, 0.4917, 0.4903; and $\overline{V} \cong 0.4912$. The position of the meniscus in the tip after touching was noted (about 29 mm. from the end) and a fine red rubber ring set as lower mark *L* in that position. In the volumetric apparatus against the standard pipet with the indicated outflow times in seconds (*D. T.*), the following values of *V* were obtained: $V = 0.4915, 0.4915, 0.4915$, *D. T.* = 5.8 to 6.6; $V = 0.4930, 0.4930, 0.4935$, *D. T.* = 21.0 to 21.4; $V = 0.4940, 0.4940, 0.4940$, *D. T.* = 55.4 to 55.8. Thus, at natural drainage rate $V \cong 0.4915$ volumetrically and $V \cong 0.4912$ gravimetrically, a difference of 0.0003 ml. This is remarkable in view of the rapid outflow rate. Volumetric readings were made to the estimated nearest 0.0005 ml. (tenth of smallest scale division on *S*—i. e., to the nearest millimeter). Thus, it would seem that even greater precision might be attainable with closer reading, but the precision was already far greater than sought for the purpose.

The same pipet was calibrated also to deliver from another initial mark, *U*, to the tip, simulating blow-out and to-contain calibration according as the drainage time was approximately that of free delivery or relatively very great. The results are given in Table I. For drainage time (*D. T.*) of 134 seconds or more, there appears close approximation in the 13 observations to their mean value, 0.49885 ml. Recalibration of the dry pipet with mercury, using the meniscus correction (*3*), gave the volume estimates, 0.4983, 0.4987, and 0.4993 ml. with a mean *V* of 0.49877 ml. Not only are the volumetric

TABLE I. VOLUMETRIC MEASURE OF DELIVERY TO TIP IN GIVEN DRAINAGE TIMES

Drainage Time Range, Seconds						
1.6–1.8	7.4–8.0	22–23	65–66	134–138	420–445	500–552
Ml. × 10^{-4}						
4885	4950	4975	4990	4985	4985	5000
4895	4965	4970		4985	4985	4995
	4960	4980		4985	4990	
	4955			4985	4990	
	4960			4985		
				4990		
				4990		
Mean 4890	4958	4975	4990	4986	4988	4998

TABLE II. VOLUMETRIC AND GRAVIMETRIC CALIBRATIONS OF 3-ML. PIPET

	Observer W. R. T.		Observer C. E. F.		Observer P. M. L.	
	V	*V'*	*V*	*V'*	*V*	*V'*
	Ml.	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
	3.003	3.0010	3.001	3.0000	3.002	3.0014
	3.002	3.0014	3.003	3.0020	3.002	3.0018
	3.003	3.0019	3.002	3.0017	3.002	3.0014
Mean	3.0027	3.0014	3.0020	3.0012	3.0020	3.0015

and gravimetric means in close approximation to each other, but only 2 of 13 individual observed volumetric values differ from 0.4988 ml. by more than 0.0005 ml. and the greatest deviation is only 0.0012 ml.

Gravimetric calibration to blow-out after free drainage gave the volume estimates, 0.4928, 0.4932, 0.4918, 0.4930, with a mean of 0.4927. This differs by 0.0031 ml. from the volumetric mean for *D. T.* between 7.4 and 8.0 seconds (Table I). Obviously, with such short drainage time, considerably more variation in delivery is to be expected than in the cases approximating complete drainage more closely, and such an influence is evident by comparison with the first column of Table I where *D. T.* is approximately 2 seconds.

Although the same size of capillary tubing could be used for larger standard pipets, actually 0.03 ml. per cm. was the volume difference on the scales of the 3- and 5-ml. *S*-pipets used. Accordingly, a comparison of results by different observers was made to indicate reproducibility of calibration of a stopcock pipet for delivery of 3 ml. between marks. As a control on the volumetric estimates (*V*), the mercury delivered each time was caught and weighed in a tared vessel to give a corresponding gravimetric estimate (*V'*). The results are given in Table II.

The apparatus may readily be adapted, as indicated in Figure 3, by use of a modified vessel, *W*, so that flow from the vessel to be calibrated goes directly to the standard pipet, *S*. This is useful in calibration of instruments such as burets and Van Slyke-Neill chambers for manometric gas analysis.

CALIBRATION OF MICROBURET. As illustrated in Figure 3, a modified vessel, *W*, was made as follows:

From a 7-mm. outside diameter adapted tip to fit *Q*, a 15-mm. outside diameter tube rose to 34 cm. above, joined to a bulb about 50-mm. outside diamter and 4-cm. length with tapered ends about 3 or 4 cm. above and below, joining an 8-mm. outside diameter delivery tube, arched at the top and turned down leading to a level approximating that of stopcock *D*, then turned upward again and adapted to a cup, *T*, similar to that in the vessel described above although a trough was not actually used. The modified vessel, *W*, was joined at *Q* as previously described, clamped just below the bulb and around the cup below *T* about 15 cm. to the left of *C*. Mercury was run up almost to its top. Then water was added at cup *T*, and run into the bulb by withdrawing mercury through *D*. At first some air was trapped in the bulb and top of the delivery tube, but this was removed by repeating the raising and lowering of the mercury level, and supplying water at *T* as required.

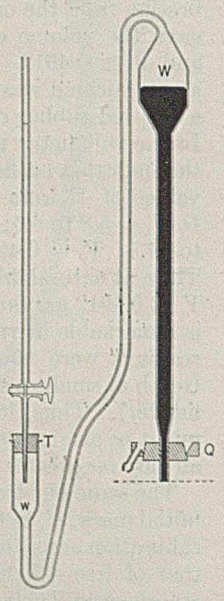

FIGURE 3. MODIFIED VESSEL *W* FOR CALIBRATION BY DIRECT FLOW, ALL ONE PIECE FROM *Q* TO *T*

In the manner previously employed, the tip of a 10-ml. microburet was inserted through a stopper at *T*, and the buret clamped near the top in a vertical position. Using a standard pipet, *S*, but with *O* below and *V*-scale above, successive withdrawals were made from the microburet to readings as given in Table III with the estimated cumulative volumes (sum of successive readings on the standard). Just before each withdrawal, the buret setting was carefully checked to guard against any drift, such as might result from a temperature change during an interval of delay. However, there was seldom any need for resetting. The delivery speed was about 1 ml. in 40 seconds, controlled by *E*. A set of observations at about 0.5-ml. intervals was made and at about 5-ml. intervals by each of two observers. The results are given in Table III, with gravimetric data subsequently obtained by weight of mercury delivered in the manner described for check calibrations in the case of the 3-ml. pipet (*V'*).

TABLE III. ESTIMATED CUMULATIVE VOLUME FROM ZERO READING TO GIVEN READING

Reading	V_1 (W. R. T.)	V_2 (P. M. L.)	V_1/V_2
Ml.	*Ml.*	*Ml.*	*Ml.*
0.5	0.5020	0.5015	1.0010
1.0	0.9990	1.0025	0.9965
1.5	1.5000	1.5035	0.9977
2.0	1.9985	2.0015	0.9985
2.5	2.5055	2.5065	0.9996
3.0	3.0115	3.0110	1.0002
3.5	3.5120	3.5135	0.9996
4.0	4.0135	4.0105	1.0007
4.5	4.5200	4.5155	1.0010
5.0	5.0250	5.0185	1.0013
5.5	5.5250	5.5195	1.0010
6.0	6.0280	6.0180	1.0017
6.5	6.5245	6.5150	1.0015
7.0	7.0300	7.0190	1.0016
7.5	7.5335	7.5190	1.0019
8.0	8.0345	8.0235	1.0014
8.5	8.5410	8.5270	1.0016
9.0	9.0435	9.0290	1.0016
9.5	9.5465	9.5325	1.0015
10.0	10.0490	10.0350	1.0014
5.0	5.019	5.017	1.0004
10.0	10.040	10.033	1.0007
5.0	5.015[a]	5.016[a]	
10.0	10.032[a]	10.034[a]	

[a] Gravimetric observations, *V'*, made subsequently by W. R. T.

Discussion

The present system of calibration by equal volume displacement differs primarily from others previously developed (*1, 2, 4*) in that the measurement is made in the standard pipet with a liquid (mercury) which does not wet the glass. Thus is avoided a source of drainage variation which otherwise may become progressively worse with use. Obviously, another liquid forming a similar layer above mercury may be used if required instead of water. The volume range employed thus far has been from 0.5 to 10 ml. Great variations in pressure on the glass by the mercury might interfere with precision in large volume calibration; and, perhaps, use of volumes exceeding 100 ml. might give unsatisfactory results. In the case of the apparatus constructed by the author, addition of a gas pressure equivalent to about 48 cm. of mercury in the standard pipet, with *B* closed, caused a depression of about 0.002 ml. on the *V*-scale. Slight influences of this nature are compensated automatically by the suggested

method of calibration of the standard pipet system, and a check upon variability of such influence is covered by replicate observations.

Only those variations in temperature that occur during the usually short period required for actual measured volume displacements affect the precision. If the volume coefficient of thermal expansion is the same for the glass of the standard pipet as for that of the apparatus being calibrated, and their temperatures are equal, correction to a prescribed standard temperature is automatically made. Otherwise, a correction for a difference in thermal expansion coefficient may be introduced in the usual manner.

The system should be most useful where many pipets, small flasks, or tubes are to be calibrated. Manufacturers of such apparatus should thus be aided in meeting tolerances prescribed by the National Bureau of Standards for certification, with a consequent saving by a lower frequency of rejection. Furthermore, improvement in precision of setting initial marks on apparatus may permit actual reduction of some such tolerances without increase in cost.

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Riboflavin Analysis of Cereals

Application of the Microbiological Method

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AMONG the rapid methods proposed for the estimation of riboflavin, the microbiological and fluorometric appear to be the most promising (*2, 7*). Both have been shown to give comparable results on a wide variety of biological materials (*6*) and to agree satisfactorily with values obtained by animal assays (*1, 3, 8, 10, 11*). Neither method, however, is universally applicable without modifications designed to remove interfering impurities. The microbiological procedure may be adversely affected by extraneous solids (*8*) or other nonflavin growth-influencing factors (*4*), while the fluorometric procedure is accurate only if pigments and nonflavin fluorescent substances are compensated for or removed (*5, 6, 9*).

For the past two years the authors have used the microbiological method for the examination of cereals and cereal products, and more recently have applied the fluorometric technique to this same study. Numerous instances have been found where the results from the two methods did not agree and these findings have led to a study of the factors which are responsible for this lack of agreement.

Microbiological Studies

While in the microbiological method the direct assay of the solid, finely ground cereals has an advantage in the possibility that incomplete extraction may thereby be eliminated, the presence of the suspended solids can lead to unreliable results (*8*). When different levels are used in the microbiological assay there is frequently a marked tendency toward appreciably lower values as the weight of the sample is increased. This introduces uncertainties as to the accuracy of the average of the several levels (Table I).

In extracts prepared by autoclaving with water and centrifuging, this tendency is decreased although not entirely removed. If uniformity is taken as a criterion of reliability, the values for the aqueous extracts would be more acceptable. However, the assays thus obtained are materially lower than those from the direct assay and at once the question of extraction efficiency is introduced. Either extraction is incomplete or some nonflavin growth factor is removed. But if the latter is present and is responsible for the higher values in the direct assay, why is there a falling off in the assay values as the weight of the sample is increased?

If one attempts to improve on the extraction conditions by treating the sample with takadiastase prior to removing the undissolved solids, there is a further change in the assay results. The absence of uniformity at the different levels is almost entirely removed, but the riboflavin values are still lower! For this reason it is believed that completeness of extraction is a minor problem compared to the behavior of the organism under the different assay conditions.

In order to determine whether the residue remaining after extraction contained any appreciable quantity of riboflavin, it was subjected to assay. The solid had to be used, since further extraction yields extracts very dilute in riboflavin. The results indicated that refined cereal residues contained 6 to 8 per cent of their original flavin, while cereal grains contained up to 20 per cent. However, these assays were as erratic as the direct assays on the original solid samples and no decision could be made as to whether the results were due to riboflavin in the residues or to the presence of extraneous solids in the assay medium. The latter can cause fictitiously high values which could readily account for the "apparent" riboflavin content of the residues.

A partial answer to these problems was found in the examination of cereals to which pure riboflavin had been added.

Table I. Effect of Weight of Sample on Direct Microbiological Assay

Cereal Product	Weight of Sample	Riboflavin
	Gram	*μg./g.*
Whole wheat flour	0.053	2.33
	0.068	2.20
	0.083	2.13
	0.098	1.86
		Av. 2.13
Patent flour	0.21	0.49
	0.27	0.41
	0.33	0.36
	0.39	0.32
		Av. 0.37

Table II. Recovery of Pure Riboflavin Added to Patent Flour

(Microbiological assay of aqueous extracts)

Sample	Riboflavin Found	Recovery
	μg./g.	%
Patent flour	0.50	..
Patent flour + 0.55 μg. per gram	1.15	118
Patent flour + 1.1 μg. per gram	1.95	132
Patent flour + 2.2 μg. per gram	3.55	138
Patent flour + 4.4 μg. per gram	6.50	137

Aqueous extracts of patent flours containing various quantities of flavin were assayed. The results are shown in Table II.

It is obvious that some factor other than flavin is influencing the growth of the organism. Strangely enough, this influence is greater as the actual amount of flour in the assay is decreased. Higher recoveries are obtained with the larger quantities of added riboflavin where the actual sample weights are less.

The fact that recovery values decrease toward the theoretical 100 per cent as the weight of the sample increases leads to the suspicion that the flour contributes some inhibitor which prevents the true evaluation of the riboflavin content. The observation that below a certain sample weight recoveries become constant, suggests that this inhibitor effect can be diluted until it is no longer measurable. This reasoning indicates that the true flavin content of the flour alone may be appreciably higher than the 0.5 microgram per gram actually observed.

However, when corrections for this inhibitor effect are made in order to estimate the flavin content of the flour, the values are widely divergent, ranging from 0.7 to 2.1 micrograms per gram. Such a simple accounting for interfering factors cannot be applied.

Table III. Apparent Riboflavin Content of Flour Derived by Deducting Added Riboflavin

Amount Added	Total Found	Apparent in Flour	Difference
μg./g.	μg./g.	μg./g.	μg./g.
0.55	1.15	0.60	..
1.10	1.95	0.85	0.25
2.20	3.55	1.35	0.50
4.40	6.50	2.10	0.75

Another approach to the problem can be made by assuming that the added riboflavin is quantitatively recovered and that the values representing the difference between the total flavin found and that added is a measure of the growth factors or "apparent flavin" in the flour. Such calculations are shown in Table III.

The regular increases in the amounts of added riboflavin are accompanied by uniform differences in the apparent flavin contents. If it is assumed that the difference of 0.25 microgram per gram prevails between the flour alone and that containing the lowest amount of added riboflavin, a value of 0.35 microgram per gram would be approximately the true value for the flour. As will be shown, this figure closely agrees with analyses carried out by other procedures. Thus, rather than being too low the microbiological values are about 30 per cent too high for patent flour when aqueous extracts are assayed. This suggests that the flour contains a nonflavin growth-stimulating factor and leads to the postulate that both inhibiting and stimulating factors are involved. Relative concentrations of these materials may decide the total influence on the assay and thus account for the variable results obtained at different assay levels—i. e., high values which decrease with increasing sample weights.

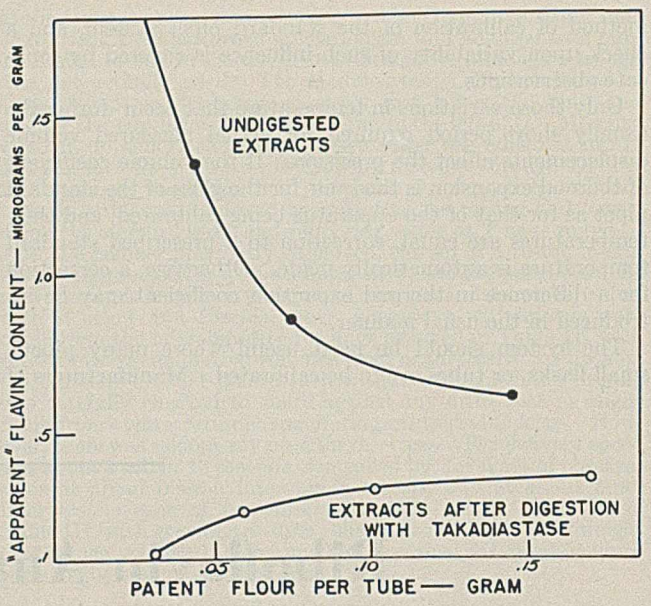

Figure 1. Riboflavin Assays of Aqueous Extracts before and after Digestion with Takadiastase (Patent Flour)

Since more uniform values are obtained when extracts are digested with takadiastase, the same flour blends were examined by treating aqueous extracts with this enzyme. The entirely different picture which resulted is shown in Table IV.

The effectiveness of the takadiastase is at once apparent. Recoveries no longer exceed the theoretical but are relatively constant and quantitative within limits of experimental error.

If the amount of added riboflavin is deducted from the total, the apparent values for the flour are now approximately the same for each recovery level. While the relative difference between 0.35 and 0.12 is considerable, at the highest levels of added flavin it is actually only about 5 per cent of the total. The contrast between the assays of aqueous and digested extracts is shown graphically in Figure 1.

The inhibiting effect which accompanies the increasing amounts of flour in the undigested extracts is absent in those which have been digested with takadiastase. The stimulating effect which produces high values for the apparent flavin content of the undigested extracts, particularly at the lower concentrations, is also shown.

Similar recovery experiments have been carried out on whole wheat flour and similar observations have been made. Table V compares the microbiological values obtained on aqueous and digested extracts.

As in the case of the patent flours, the apparent values obtained from the aqueous extracts are much higher than those from extracts which have been digested with takadiastase, and the apparent values for the aqueous extracts are less constant than those obtained after enzymatic digestion.

Table IV. Recovery of Pure Riboflavin Added to Patent Flour

(Microbiological assay of aqueous extracts treated with takadiastase)

Added	Total Found	Apparent in Flour	Recovery[a]
μg./g.	μg./g.	μg./g.	%
0.55	0.90	0.35	100
1.1	1.42	0.32	97
2.2	2.44	0.25	95
4.4	4.52	0.12	93

[a] Assumed that flour alone contains 0.35 microgram per gram.

TABLE V. MICROBIOLOGICAL ASSAYS OF AQUEOUS AND TAKADIASTASE-DIGESTED EXTRACTS OF WHOLE WHEAT FLOURS CONTAINING ADDED RIBOFLAVIN

Sample	Aqueous Extracts	Apparent in Flour	Digested Extracts	Apparent in Flour
	μg./g.	μg./g.	μg./g.	μg./g.
Whole wheat flour	1.9	1.9	1.05	1.05
Whole wheat + 0.5 μg. per g.	2.7	2.2	1.55	1.05
Whole wheat + 1.0 μg. per g.	3.3	2.3	2.20	1.20
Whole wheat + 1.5 μg. per g.	3.9	2.4	2.65	1.15
Whole wheat + 2.0 μg. per g.	4.5	2.5	3.15	1.15
		Av. 2.26		1.12

TABLE VI. RECOVERY OF RIBOFLAVIN ADDED TO WHEAT STARCH

(Determined microbiologically)

Sample	Aqueous Extracts	Takadiastase-Treated Extracts
	μg./g.	μg./g.
Wheat starch	Trace	Trace
Wheat starch + 2 μg. per g.	4.05	2.5

They show a regular increase as did the patent flours but the magnitude is less since the actual quantities of flour assayed cover a smaller range. These relations are shown in Figure 2.

Just what mechanism is involved in these observations is not easily deduced from the facts now known. Diastasis of the starch appears to be one factor, since studies with purified starch prepared from wheat flour gave similar results. The data in Table VI show a recovery experiment on such material.

While the digestion did not bring the found and calculated values into entire agreement, it did markedly lower the stimulating effect of the undigested starch.

The other major component of flour, the gluten protein, was also examined for its effect on riboflavin recovery. Treatment with takadiastase did not change the results, and recoveries with and without digestion were theoretical within the limits of experimental error. Thus, the discrepancies observed in the microbiological assay of aqueous extracts of cereal products appear to be due at least in part to the starchy constituents or closely associated substances.

The actual nature of these is not known. Their effect on the growth of the organism may be either chemical or physical, or both. That colloidal phenomena may be involved is suggested by the physical appearance of the assay media. Cloudiness decreases as we go from direct assay to aqueous extracts and finally to extracts digested with the enzyme. Supporting this idea is the observation that the cloudier extracts prepared by centrifuging gave higher values than those clarified by filtration. While the possibility is not ruled out that this difference may be due to adsorption of flavin on the filter paper, the magnitude of the difference is much greater than any adsorption which has been observed with pure solutions.

When fuller's earth is added to solutions of pure riboflavin and then removed by centrifugation and assayed as the solid, the microbiological method quantitatively measures the flavin content of the original solution. When aqueous extracts of cereals are similarly treated the values are much lower than those found by assay of the extracts themselves. It was at first believed that these observations indicated incomplete adsorption of the riboflavin, but in view of later observations it is probable that the lower values resulted from separation of the flavin from nonflavin growth factors.

Experiments have also been tried with the synthetic adsorbent, Florisil, or Supersorb as it was formerly termed. Passage of flavin solutions through columns of this material has been shown to remove the vitamin quantitatively (*3*, *5*). Extracts of cereals were passed over Florisil and the unadsorbed fraction was examined microbiologically. The results are shown in Table VII.

The fact that the 5-ml. quantity of extract gave no growth of the organism in the absence of added riboflavin is evidence that adsorption from the aqueous extract has been complete. On the other hand, in the presence of added riboflavin, a definite growth stimulation is observed. This must be partly due to some nonflavin factor, since the found riboflavin considerably exceeds the amount which was added. That this may be a colloidal response is not excluded, since no essential clarification of the extract is effected by the treatment with Florisil.

The same experiments were carried out on whole wheat flour with entirely similar results.

TABLE VII. MICROBIOLOGICAL ASSAY OF AQUEOUS EXTRACTS OF PATENT FLOUR FOLLOWING ADSORPTION ON FLORISIL

Volume of Extract	Riboflavin Added	Riboflavin Found
Ml.	*μg./tube*	*μg./tube*
1.3	0.15	0.253
1.6	0.15	0.248
2.0	0.15	0.255
2.4	0.15	0.258
5.0	None	0.000

Fluorometric Studies

The recent development of improved fluorometric procedures for riboflavin has enabled us better to evaluate the observations made by the microbiological studies. Mention has been made of the efficiency of Florisil for adsorbing flavin from cereal extracts in confirmation of the report by Conner and Straub (*3*). Accordingly, the aqueous and digested extracts examined by the microbiological method were treated with this adsorbent and the eluates examined fluorometrically. Table VIII shows the results.

The rather good agreement between the values for the two types of extracts is in sharp contrast to the results obtained microbiologically. Instead of finding much higher values by aqueous extraction, we actually observe values slightly below those obtained by takadiastase digestion. This lends confirmation to the belief that the microbiological assay of aqueous extracts gives excessively high results due to the presence of nonflavin growth factors and that enzymatic digestion removes most of this interference. The apparent values for the

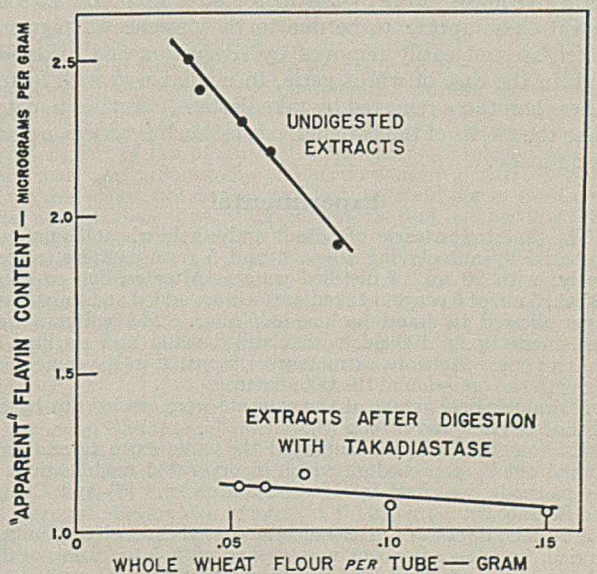

FIGURE 4. RIBOFLAVIN ASSAYS OF AQUEOUS EXTRACTS BEFORE AND AFTER DIGESTION WITH TAKADIASTASE (WHOLE WHEAT FLOUR)

TABLE VIII. RECOVERY OF RIBOFLAVIN ADDED TO PATENT FLOUR

(Determined fluorometrically)

Sample	Aqueous Extracts	Digested Extracts	Apparent in Flour
	μg./g.	μg./g.	μg./g.
Flour + 0.55 μg. per g.	0.80	0.85	0.30
Flour + 1.1 μg. per g.	1.35	1.40	0.30
Flour + 2.2 μg. per g.	2.40	2.60	0.40
Flour + 4.4 μg. per g.	4.35	4.75	0.35

flour are constant and in good agreement with the 0.35 microgram per gram obtained by "correcting" the microbiological assays of aqueous extracts. This value is also practically the same as that obtained microbiologically from extracts prepared by the diastatic treatment and is believed best to represent the actual riboflavin content of the flour sample.

The same study has also been applied to whole wheat flour (Table IX).

The large difference between the two methods is seen in the values obtained for the aqueous extracts, where the microbiological values were nearly twice those shown in Table IX. The fluorometric examination of the digested extracts gave values somewhat higher than those for the aqueous extracts, indicating that the enzymatic hydrolysis slightly improved the extraction. From these values the apparent riboflavin content of the flour alone has been calculated. The average of 1.03 is in excellent agreement with the 1.05 found by microbiological assay of the digested extract of the same flour, but much below the 1.9 found when the aqueous extract was assayed.

On the basis of the observations reported in this paper it is apparent that the method used in preparing extracts of cereals or cereal products is vital to the success of the microbiological assay. Digestion of the samples with takadiastase is one means of eliminating factors which adversely affect the results. It also improves the extraction of riboflavin and in the case of patent and whole wheat flours it brings into close agreement the results obtained by the microbiological and fluorometric procedures.

There are, however, other cereal products which give somewhat different values by the two methods. Here the discrepancies are apparently due to other factors than those encountered in refined and whole wheat flours. Bran and wheat germ give lower values by the fluorometric method. To some extent these appear to be due to the presence of pigments which are not easily removed by adsorption and oxidation, and, in the case of wheat germ, to nonflavin growth factors other than those removed by takadiastase. Studies to determine the merits of the two methods for such products are now being made.

Experimental

The digested extracts of patent and whole wheat flours were prepared by autoclaving 10-gram and 5-gram samples, respectively, with 90 ml. of distilled water. After cooling to about 50° C., 5 ml. of 6 per cent takadiastase were added and the suspensions allowed to stand for one-half hour. The volumes were then made up to 100 ml. with distilled water and clarified by centrifuging. Aqueous extracts were prepared in the same manner with the omission of the takadiastase.

Microbiological assays of the extracts were carried out by the method of Snell and Strong (*8*).

Fluorometric analyses employed the same extracts and were carried out by a procedure which incorporated modifications of the methods described by Hodson and Norris (*6*) and Conner and Straub (*3*).

A 20-ml. aliquot of the extract was passed through a column of Florisil, and the adsorbent was washed with 5 to 10 ml. of distilled water, and then dried in a current of air. The riboflavin was eluted with a solution of 20 per cent pyridine in 2 per cent acetic acid until the eluate, collected in a graduated cylinder, amounted to 20 ml.

Fourteen milliliters of the well-mixed eluate were pipetted into the cuvette of a Pfaltz & Bauer fluorophotometer and the fluorescence was determined (*A*). Prior to this measurement the intensity of the fluorophotometer light source was adjusted by means of the iris diaphragm to give a 25-scale division deflection of the galvanometer when the cuvette contained an 0.1μg. per ml. solution of sodium fluorescein. Under this condition a solution of pure riboflavin (0.2μg. per ml.) gave a galvanometer deflection of 27 scale divisions. Filters used were a 511–038 combination for the incident light and No. 351 for the fluorescent light.

After determining the fluorescence of the eluate (*A*), 1 ml. of solution containing 1.5μg. of pure riboflavin in 20 per cent pyridine–2 per cent acetic acid was added, and the resulting fluorescence was measured (*B*). A 10- to 20-mg. quantity of solid sodium hydrosulfite was then stirred in to reduce the riboflavin and the fluorescence again observed (*C*).

The riboflavin content of the sample was calculated from the equation

$$\frac{A - 1.07\,C}{B - 0.934\,A} \times 0.1 \times \frac{100}{\text{wt. of sample}}$$

where *A*, *B*, and *C* are the galvanometer deflections in scale divisions, and the numerical values 1.07 and 0.934 are correction factors which compensate for dilution with the added solution of pure riboflavin.

NOTES. All the analytical operations were carried out in a darkened room to avoid losses of riboflavin from excessive light.

The sodium fluorescein used was the standard product supplied by the Eastman Kodak Company. Different preparations of this compound may vary in their fluorescence properties, thus requiring concentrations other than that of the solution noted above. The actual concentration should be adjusted to yield a fluorescence intensity similar to that of the pure riboflavin solution.

The takadiastase should not contain sufficient amounts of riboflavin to effect the assay results appreciably. The Parke-Davis product employed in these studies did not contribute significant quantities.

The autoclaving of the cereal with distilled water may result in riboflavin losses if the hydrogen-ion concentration is too low. Under such conditions extraction with dilute acid may be necessary. In the instance of the flours used in the present studies, the pH ranged between 5.5 and 6.0. The quantitative recovery of added riboflavin demonstrated that no measurable extraction losses occurred.

TABLE IX. RECOVERY OF RIBOFLAVIN ADDED TO WHOLE WHEAT FLOUR

(Determined fluorometrically)

Sample	Aqueous Extracts	Digested Extracts	Apparent in Flour
	μg./g.	μg./g.	μg./g.
Whole wheat flour	0.80	0.95	0.95
Whole wheat + 0.5 μg. per g.	1.35	1.60	1.10
Whole wheat + 1.0 μg. per g.	1.85	2.00	1.00
Whole wheat + 1.5 μg. per g.	2.30	2.55	1.05
Whole wheat + 2.0 μg. per g.	2.80	3.05	1.05
			Av. 1.03

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Extraction of Metals from Aqueous Solutions with Dithizone

LEAD

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The results of a series of studies on the effect of pH, diverse anions, and amount of reagent used in lead determinations with dithizone are reported. It was found that favorable pH ranges for extraction vary considerably with different masking anions, whereas only limited change occurs with variation in amount of excess reagent.

DITHIZONE (diphenylthiocarbazone) is an important reagent for the separation and determination of traces of metals. It has become established in the A. O. A. C. methods for the determination of lead, mercury, and zinc (*1*), while other metals reported as being adaptable to dithizone analysis include bismuth, copper, cadmium, silver, tin, cobalt, nickel, palladium, and thallium. Fischer has published a wealth of data concerning the use of the reagent from both qualitative and quantitative viewpoints. His review articles (*6*, *7*) cover an extensive survey of the progress made with dithizone, and include references to much of the important literature on the subject.

The principal disadvantage of dithizone is its nonspecificity. This problem has been solved partially for particular cases by a number of investigators, but has yet to receive a more thorough and comprehensive examination from a fundamental standpoint. A more selective extraction of metals in the presence of interfering cations has been accomplished in some instances by controlling the pH of the aqueous solution, using masking reagents, or varying the solvents for extracting the dithizonates. Wichmann, in a recent review article (*11*), has given an excellent discussion of these practices; furthermore, he has established a plea for a more critical study of the dithizone system with respect to the equilibrium curves for the various metals, obtained by plotting percentage of extraction against pH value. Clifford and Wichmann (*5*) have initiated this task with a preliminary study of the distribution of lead and its complex in solutions of varying pH.

It is the purpose of this work to repeat the experiments of these men, adhering to a more strict pH measurement, and to augment their results by studying the extractability of lead under varying experimental conditions, including pH, diverse anions, and amount of reagent used. It is hoped that through these and similar investigations, the selectivity of dithizone may be materially increased.

Apparatus

GLASSWARE. Pyrex glassware was used throughout. Since dithizone is a very sensitive reagent, extreme precautions were taken when cleaning the equipment. Two 50-ml. Pyrex burets, employed for measuring the volume of dithizone, were calibrated by weighing.

STILLS. It was necessary to redistill many of the C. P. chemicals used in the lead determinations. For this purpose an all-Pyrex still, adapted from a 1-liter distilling flask, was used. For redistilling water, an elaborate all-Pyrex still, built especially for the purpose, was employed. For effecting a preliminary rectification of chloroform in the recovery process, a 90-cm. (3-foot) Pyrex Vigreux column was found useful.

pH METER. For measuring pH values a glass electrode (quinhydrone in 0.1 *M* hydrochloric acid) was used. The reference electrode was the calomel-saturated potassium chloride type. A "universal" potentiometer assembly described by Mellon (*10*) was utilized.

PHOTOMETER. An Aminco neutral wedge filter photometer was found to give highly satisfactory results in the final determinations. The instrument is designed to give scale readings as a linear function of concentration for solutions following Beer's law. Aminco, style D absorption cells, 0.9995-cm. and 1.9920-cm., respectively, with parallel fused ends, were used. An all-glass, "Pb", color filter (Aminco), having an effective wave length of 510 millimicrons was used in all cases.

COMPARATOR. A Bausch & Lomb Duboscq color comparator was employed in a trial of the balancing method of colorimetry.

SHAKER. In the lead determinations a mechanical shaking apparatus was found to be thoroughly satisfactory as a means of establishing a standard shaking procedure, as well as a time saver. The machine allows direct addition of reagents to the funnels without removing the funnels. It is so constructed as to give rotary, end-over-end motion to the funnels. A 3-minute shaking period was allowed in each case, and was found sufficient.

Reagents

ACETIC ACID, C. P., redistilled, diluted to 0.50 *M*.

AMMONIA SOLUTION (CONCENTRATED), C. P., redistilled into cold redistilled water.

AMMONIA SOLUTION (DILUTE). Concentrated solution diluted to 0.50 *M*.

AMMONIA-CYANIDE SOLUTION. Equivalent of 150 ml. of 15 *M* ammonia solution and 200 ml. of 10 per cent potassium cyanide solution diluted to 1 liter.

AMMONIUM ACETATE SOLUTION, 10 per cent weight by volume from purified salt. Solution was purified by extraction with dithizone solution.

AMMONIUM TARTRATE SOLUTION. A 0.50 *M* solution of reagent grade salt.

CHLOROFORM, U. S. P., redistilled, stabilized with 1 per cent, by volume, absolute ethanol (latter redistilled over solid potassium hydroxide). Chloroform was recovered by method of Biddle (*2*). Considerable difficulty was experienced with chloroform that had been recovered a number of times. Solutions of dithizonates containing this multi-recovered chloroform became cloudy on standing a few minutes after extraction. This cloudiness, which was probably due to an emulsion formation, necessarily vitiated any subsequent photometer readings; however, it was found possible to effect a temporary clarification of the solution by filtering through paper. Apparently the new phase appeared as part of the ternary system, chloroform-alcohol-water. The cause was undoubtedly the accumulation of a fairly high percentage of alcohol during purification, resulting in increased water absorbability. Since the chloroform that had become thus contaminated was rectified with difficulty, it was found expedient to introduce little or no additional alcohol as preservative after the first reclamation process.

Furthermore, in the purification process, it is advisable to reflux chloroform that hints of phosgene formation, for 2 to 3 hours prior to the final distillation. In such case, when phosgene has formed, alcohol addition is recommended to prevent further oxidation.

CITRIC ACID SOLUTION, prepared from C. P. crystals according to method of Clifford and Wichmann (*5*).

DITHIZONE. The solid was obtained from the Eastman Kodak Company. It was found to be of high purity, but it was repurified by the A. O. A. C. method (*1*) as an added precaution.

DITHIZONE STOCK SOLUTION. A 0.1250-gram portion of the purified solid was dissolved in redistilled chloroform and diluted to 500.0 ml.

DITHIZONE STANDARD SOLUTION. The stock was diluted to give 12.5 micrograms of dithizone per ml.

HYDROCHLORIC ACID. C. P. acid was redistilled and diluted to 1 *M*.

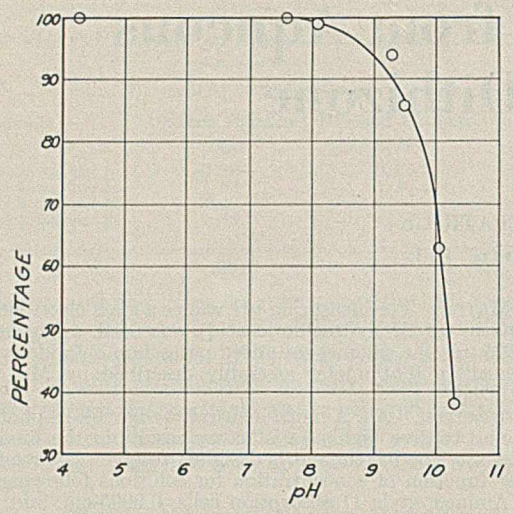

FIGURE 1. EFFECT OF pH UPON RETENTION OF DITHIZONE BY CHLOROFORM LAYER

LEAD NITRATE STOCK SOLUTION. C. P. lead nitrate which had been recrystallized five times and analyzed for lead was used to prepare 500 ml. of solution containing 0.500 gram of lead. A few drops of nitric acid were included.

LEAD NITRATE STANDARD SOLUTION. A 10.00-ml. portion of the stock solution was diluted to 1000 ml. to contain 10 micrograms of lead per ml. The solution strength was checked by the titrimetric and mixed-color dithizone methods of analysis.

NITRIC ACID (CONCENTRATED). C. P. reagent distilled.

NITRIC ACID (DILUTE). Concentrated nitric acid diluted to 0.1 *M*.

POTASSIUM CYANIDE SOLUTION. Reagent grade salt used to make a solution 10 per cent weight by volume.

TARTARIC ACID SOLUTION. A 0.10 *M* solution prepared from analytical reagent solid.

WATER. Redistilled over sulfuric acid from an all-Pyrex still. Stored in an all-Pyrex delivery apparatus.

Preliminary Study in Developing Procedure

SELECTION OF METHOD. A rather extensive study of three general methods of final lead determination was undertaken in order to select the most appropriate procedure for this type of work. Of the methods attempted—titrimetric; "one color", using a Duboscq comparator; and "mixed color", using a filter photometer—the last named was found to be greatly superior to the others. The one-color procedure was abandoned because of the low order of reproducibility attainable and because it proved to be time-consuming; certain other disadvantages were also prominent. The titrimetric method, although of value in certain standardized dithizone determinations, was poorly adaptable to this study.

DITHIZONE PARTITION. Figure 1 shows, in a semiquantitative manner, the partition of dithizone between the chloroform and aqueous layers for varying pH. It is entirely evident that suitable correction must be made for transfer of dithizone to the aqueous layer at pH values above 8 when the mixed-color technique is employed. At values above 10 the partition coefficient rapidly approaches values increasingly in favor of the aqueous phase. Evidently dithizone extractions at higher pH values than 10 are subject to considerable modification before becoming valuable in quantitative determination.

DETERMINATION OF VOLUME OF DITHIZONE NECESSARY. Some trouble was encountered in preparing dithizone solutions of exactly known concentration for use as standards. In the first place, chloroform that had been recovered frequently seemed to cause fading of a solution, evidently through oxidation by phosgene (cf. *4*). Also, since no statement of analysis could be obtained for the dithizone solid used, there was no assurance that the material was sufficiently pure for direct preparation of standards by weight; nevertheless, standard tests (*5*) failed to reveal more than a trace of impurity. In spite of this fact, it was deemed advisable to subject the dithizone to the A. O. A. C. purification method (*1*). A stock solution was prepared from the resulting product by dissolving a carefully weighed amount in fresh, redistilled chloroform. A standard solution, made up immediately thereafter from the stock, gave a reading of 16.4 on the photometer. This value was then assumed to be correct for a solution containing 0.0125 mg. of dithizone per ml. After standing for several days, the standard dithizone slowly lost strength, as evidenced by a gradual decline to a constant photometer reading of 15.4. Since the solution follows Beer's law (*9*), and since the absorption attributive to a small amount of dithizone oxidation product is negligible, it was thought reasonably accurate to use 16.4/15.4 times the volume of dithizone originally calculated to be a 25 or 50 per cent excess. Thus, in the results recorded herewith, the volumes used were all determined on this basis.

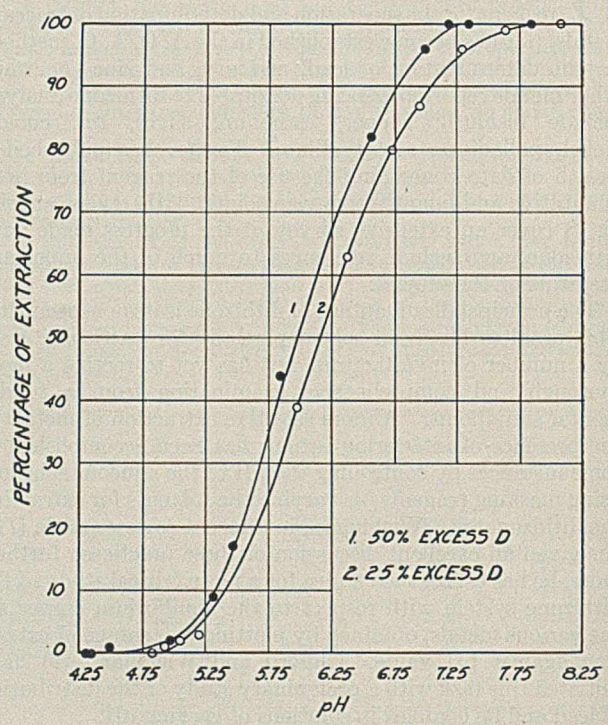

FIGURE 2. EFFECT OF pH AND EXCESS DITHIZONE UPON LEAD EXTRACTION FROM ACETATE SOLUTIONS

STANDARDIZATION. It was found advisable to construct calibration curves for each new dithizone standard prepared, using a constant volume of dithizone solution for amounts of lead ranging from zero concentration to 100 micrograms in 50-ml. samples. These solutions were extracted at previously determined optimum pH values, and in necessary cases, appropriate corrections were applied for distribution of dithizone to the aqueous layer. The resulting photometer readings were plotted against the amount of lead introduced. This plot is a straight-line function. Since the reciprocal of the slope of the straight line has the units $\frac{\gamma\ \text{Pb}}{\text{photometer unit}}$ it is desirable to use this reciprocal as a "lead factor". When the lead factor is multiplied by the photometer reading ob-

tained in the final measurement, the number of micrograms of lead extracted is obtained. In the case of 100-microgram samples, as used in these studies, percentage of extraction is obtained directly.

Blank Determinations. Since the reagent is sensitive to extremely small amounts of constituent, blank determinations are essential in all cases, even though meticulous care may have been used in purification of reagents. It was found experimentally that the best way to determine blank corrections for this type of determination was to run a series of blank analyses for each set of determinations at various pH intervals over the applicable range. The blanks did not reach zero with suitably low pH values, as might have been expected, but the variation was very nearly a linear function of pH value in all cases. Thus, from a few blank determinations it was possible to interpolate for any desired pH value in making corrections in photometer readings.

Correction of Photometer Readings. The percentage of lead extracted from a solution is given by the product of the standardization factor and a corrected photometer reading. This corrected reading was obtained by adding the correction for loss of dithizone in aqueous solution to the original reading on the extracted chloroform solution, and subtracting the appropriate blank.

Determination of pH. The measurements of pH in this work were made with a glass electrode, corrected for temperature variations. The order of reproducibility was ±0.02 pH unit. Clark and Lubs buffers (*3*), prepared from recrystallized potassium dihydrogen phosphate, boric acid, potassium chloride, and C. P. sodium hydroxide, were used in calibrating the instrument.

Lead Extraction

Cyanide-Ammonia-Citrate Curves. The procedure for determining percentage of extraction of lead from cyanide-ammonia-citrate solution was adapted from that of Clifford and Wichmann (*3*). A 10-ml. portion of the ammonia-cyanide solution, 10.00 ml. of standard lead nitrate, and sufficient citric acid solution to bring the pH to the desired value were measured into a 250-ml. separatory funnel by means of burets. The mixture was diluted to 50 ml. with water. A definite volume of standard dithizone solution was added and the funnels were shaken. After allowing the funnels to stand for a few minutes, separation was effected, and 10 ml. of the aqueous layer were drawn off for pH determination. The 4/5 aliquot remaining was acidified with about 15 ml. of 1 *N* hydrochloric acid, and re-extracted with pure chloroform for the purpose of correcting for dithizone that may have been lost to the aqueous layer at higher pH values. At pH values of 8 and lower this step was proved to be superfluous.

A curve determined experimentally by plotting pH values obtained against volume of citric acid solution used, in 10 ml. of ammonia-cyanide solution, proved helpful in deciding the volume of the former solution necessary to approximate desired pH values.

Cyanide-Ammonia–Hydrochloric Acid Curves. The curves for these solutions were obtained by the same technique as the above, 1 *N* hydrochloric acid merely being substituted for the citric acid solution. Difficulties in adjusting pH for certain ranges were encountered, due to the poor buffering action of this solution. Below pH values of about 7.5, only an extremely small amount of acid caused a pronounced drop in pH. It was found advisable to use an approximate volume of acid, shake the mixture, and then note the color of the chloroform phase. By subsequently adding ammonia-cyanide solution or hydrochloric acid solution dropwise, followed by shaking, it was possible to adjust the solutions to obtain a fairly good range of pH values, using the dithizone complex itself as an indicator.

Acetate-Ammonia Curves. The acetate solutions adapted themselves readily to this type of determination. Ten milliliters of the ammonium acetate solution, 10.00 ml. of standard lead solution, and a calculated volume of either ammonia or acetic acid solution were added to separatory funnels and diluted to a total volume of 50 ml.

A theoretical curve of pH plotted against volume of acetic acid or ammonia, as the case might be, was constructed to predetermine amounts of the latter to use. This curve was adaptable with very good success and no difficulty was had in approximating very closely desired pH values. The rate of reaction of the lead with dithizone was considerably slower in acetate solutions, but aside from this fact no peculiarities were noted.

Tartrate-Ammonia Curves. The procedure for tartrate solutions was identical to that for acetate, except that tartaric acid was employed for lowering the pH; 10 ml. of the ammonium tartrate solution were used in each case.

Discussion of Results

A practically negligible difference was obtained for the curves showing extraction with 50 per cent excess dithizone as compared to those representing extraction with 25 per cent excess, for both tartrate solutions and cyanide-ammonia–hydrochloric acid solutions. On the other hand, a shifting of the upper portion of the curve is observed for the acetate solutions, but almost no change for lower pH values (see Figure 2).

By reference to Figure 3 it is observed that for cyanide-ammonia-citrate solutions, the curve for 82 per cent excess dithizone assumes a position nearly 0.5 pH unit to the left of the curve for 37 per cent excess. This is in accord with the expectations of Clifford and Wichmann, but only a limited change can be effected by this procedure, and even then it is not generally applicable to all solutions. The curve for 13 per cent excess dithizone demonstrates the necessity for using considerably more than an equivalent amount of dithizone in a quantitative determination. Between 25 and 50 per cent is advised. Less than 25 per cent may result in incomplete extraction unless more than one extraction is made. More than 50 per cent excess tends to decrease precision through a shorter range in photometer readings.

Figure 4 demonstrates the effect of various complexing anions on the extraction of lead. The function of citric or tartaric acid in the solution to be extracted is to prevent the metals from precipitating as the hydroxide or phosphate (*5*, *8*). It is advisable, therefore, not to use citric acid if some other dodge may be employed. Tartrates, provided they

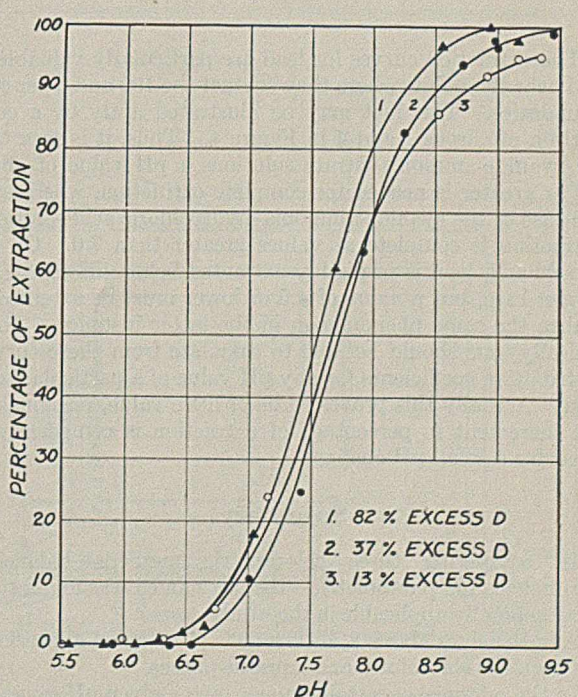

Figure 3. Effect of pH and Excess Dithizone upon Lead Extraction from Cyanide-Citrate Solutions

serve the purpose of citrates, are satisfactory as regards extraction at favorable pH.

Ammonia-cyanide solutions acidified with hydrochloric acid are readily extracted and show the greatest pH range for complete extraction; however, on account of the toxic nature of the hydrogen cyanide formed in overacidification, this latter procedure is not generally recommended.

The use of acetate ion as a complex former is seen not to interfere markedly in the dithizone extraction. Furthermore, it has a decided advantage in that pH is very easily regulated to nearly any desired value. It is suggested that the pH of ammonia-cyanide solutions might be regulated by the use of acetic acid, although these mixtures have not been tried in this laboratory.

High pH values are to be avoided whenever possible in lead determinations because of the partitioning of dithizone to the aqueous phase. In special cases, however, when particular interferences occur, recourse to higher pH may become obligatory.

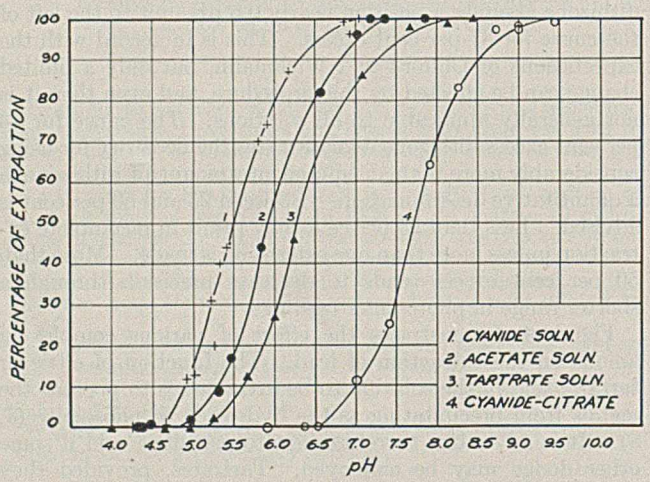

FIGURE 4. EFFECT OF ANIONS UPON LEAD EXTRACTIONS, USING 50 PER CENT EXCESS DITHIZONE

The extraction curves for lead are particularly valuable at and near the points where they contact the 100 and 0 per cent boundaries. This fact may be illustrated aptly by a comparison of curves 1 and 4 in Figure 4. Thus, it is seen that for cyanide-ammonia-citrate solutions a pH value of about 9.5 or greater is needed for complete extraction, whereas, in the case of the cyanide-ammonia–hydrochloric acid solutions, extraction is complete at values greater than 7.0. On the other hand, lead is negligibly extracted below pH 6.5 in the former case, but a value of 4.5 or lower must be attained to realize the same phenomenon in the latter instance. Theoretically, one should be able to calculate from these curves, distribution coefficients for any pH value of a particular solution. Actually this proves to be of little value, inasmuch as the increment in percentage of extraction is comparatively great for a small pH change.

Summary

In comparing three colorimetric methods—balancing, titrimetric, and photometric—the last named was found to be most generally applicable in this study.

At pH values above 8, dithizone is partitioned appreciably between the chloroform and aqueous phases.

In the presence of certain anions, over a given pH range, an increase in excess of dithizone causes a limited increase in percentage of lead extracted.

The anions, cyanide, citrate, acetate, and tartrate, were studied with regard to their effect on the extractability of lead by dithizone.

The use of citrates in extraction solutions is found to be inadvisable.

Improvements in the method of reclaiming chloroform residues are recommended.

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PRESENTED before the Division of Analytical and Micro Chemistry at the 102nd Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Application of Infrared Radiation to Spot-Testing

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THE spot plate is a very useful device in microqualitative analysis, in that it often eliminates special, more expensive apparatus and requires no great skill in microtechnique. Nevertheless, many micro tests have not been considered applicable to direct spot-plate work, because the methods depend upon warming, evaporation, or fusion. Thus, many of the tests given by Feigl (*1*) involve the use of microcrucibles, microburners, microcentrifuge cones, and similar apparatus, as well as appropriate steam baths or water baths.

The author has found, however, that an appreciable number of these tests may be performed directly on the spot plate by using an infrared lamp as a source of heat.

The infrared lamp, preferably with a reflector, is mounted on a ring stand several inches from the base. Heat may then be applied to a mixture on a spot plate by placing the spot plate on the base, directly under the lamp. The heat may be controlled by either of two methods:

1. Regulation of distance between lamp and spot plate. This is accomplished either by mounting the lamp in a fixed position and placing the spot plate on an adjustable ring, or by mounting the lamp so that its height is adjustable and placing the spot plate on the base of the stand. In either case, the adjustable part may be moved into different positions and the approximate maximum temperature noted. Positions for several temperatures may thus be empirically found and indicated on the stand. The heat, of course, will vary inversely as the square of the distance between the spot plate and the lamp.
2. Regulation of voltage.

Among the many micro tests that have been adapted as spot tests by the author are tests for chlorates, ketohexoses, malates, aluminum, Fehling's test, Millon's test, Benedict's test, etc.

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A Simple Method for B_1 Determination

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OWING to the high cost of equipment available for B_1 determinations, the author has developed a comparatively simple and rapid method which involves the use of the catox apparatus (*2*). The principle is the stimulation of yeast by B_1 as described by Schultz, Atkin, and Frey (*3*). The method gives reliable results with very small amounts of material; 0.01 microgram of B_1 can be determined with fair accuracy.

Description of Apparatus

The reaction is carried out in an apparatus similar to the one used for oxidase determinations (*1*), and differs from the other apparatus mainly in two respects: It is used for measurements of increased pressure and the calibration of the manometer indicates directly the weight of carbon dioxide produced.

The apparatus is shown in Figure 1. Compartment 1 contains 1 cc. of nutrient solution (*3*) and 1 cc. of the vitamin-containing extract or thiamin solution of known concentration. Compartment 3 contains 1 cc. of 0.4 per cent yeast suspension (free from added vitamins). Compartment 2 is used only when the effect of other substances on the reaction is to be studied. 4 fulfills a function in catalase determinations and is eliminated for the purpose here described. 5, the manometer, is graduated so that every division is equal to 100 micrograms of carbon dioxide, when apparatus is charged as indicated. 6 is a ground joint provided with corresponding vent holes, 7, to allow for equalization of pressure when manometer is at right angles to the body of the apparatus. When manometer is filled with mercury, the apparatus can be closed by rotating the manometer through 90°. It is then as shown in illustration. 8,8′ is a rubber band which, with the aid of the glass hooks shown, ensures tightness of the apparatus during the reaction.

Method

Six experiments are usually run concurrently. In some of these standard thiamin solutions and in some the unknown, with and without sulfite correction, were used (Table II).

The apparatus is clamped on a shaking machine mounted in a constant-temperature chamber maintained at 30° C. The air vents are adjusted to allow for equalization of internal and external pressure.

After temperature has been maintained at 30° C. for 25 minutes, the apparatus is closed as described, making use of a special trap door in the constant-temperature chamber. The shaking machine is set in motion and readings are made at the end of 90, 120, 150, and 180 minutes. The readings of the unknowns are interpreted in terms of B_1 concentration by interpolation from the readings obtained with the standards. The author's shaking machine has a stroke of 6 cm. and a period of two excursions per second.

A check sample of flour furnished by the American Association of Cereal Chemists was reported as having a B_1 content of 2.9 I. U. per gram (average of three other laboratories). One vitamin-testing laboratory in New York City obtained 2.76 I. U. per gram (average of 3) for the same sample. Results obtained by method here described were 2.75 and 2.83 I. U. per gram.

Table I shows results of a test of hard candy.

Constant-Temperature Chamber

While the temperature should be in the vicinity of 30° C., a constant temperature chamber is not essential if all experi-

TABLE I. HARD CANDY

(Laboratory No. 13,046)

Apparatus	1	2	3
Contents of compartment A	0.5 cc. of 0.25% soln. of sample 1 cc. of nutrient solution	0.20 cc. of thiamin soln. (0.1γ per cc.) 0.30 cc. of H_2O 1 cc. of nutrient soln.	0.40 cc. of thiamin soln. (0.1 γ per cc.) 0.10 cc. of H_2O 1 cc. of nutrient soln.
Contents of compartment B	1 cc. of 0.4% yeast suspension	1 cc. of 0.4% yeast suspension	1 cc. of 0.4% yeast suspension
Temperature, ° C.	31.0	31.0	31.0
Reading at end of 180 minutes	17.9	16.5	19.4

B_1 in 0.5 cc. of 0.25% solution (0.00125 gram of sample) $= 0.02 + \frac{0.020}{19.4 - 16.5} \times (17.9 - 16.5) = 0.0297$ microgram

B_1 in 1 gram of sample = 23.76 micrograms

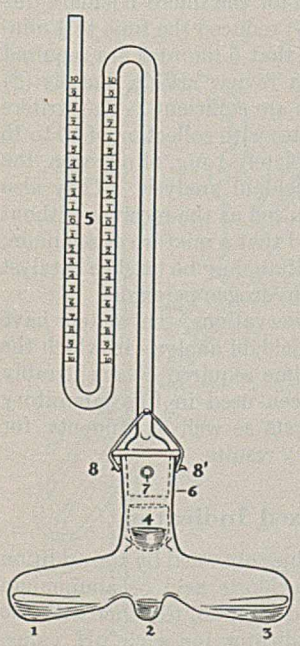

FIGURE 1

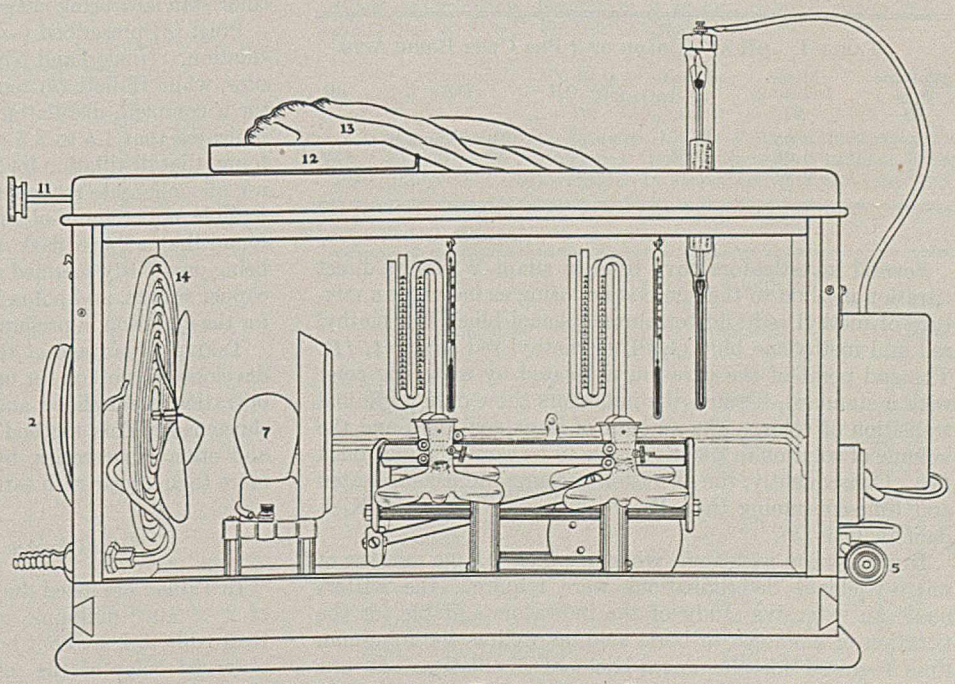

FIGURE 2. CONSTANT-TEMPERATURE CHAMBER

TABLE II. READINGS AT THE END OF 3 HOURS

No Thiamin	1 cc. of Unknown Extract (0.1%)	0.005 γ B_1	0.015 γ B_1	0.025 γ B_1	0.035 γ B_1
4.7	8.5	6.4	7.3	8.4	9.2

Accordingly, the 0.1 per cent extract contains 0.026 microgram per cc., or 26 micrograms per gram of material.

ments are carried on simultaneously under identical conditions.

The constant-temperature chamber used is very convenient for this purpose and is shown in Figure 2. In general, its operation is obvious from the illustration. 12 is a trap door which, when opened, exposes sleeve 13. After arm is slipped into this sleeve, a slide also closing opening and operated by lever 11 admits the arm to turn the manometers. The front is a removable panel with double glass and a black slide to eliminate light. Where light effect is suspected, a black lamp, 7, is used. The motor is mounted in the rear and its shaft operates the fan through pulley 2. It also operates the shaking machine through a reducing gear. Shaking is started and stopped through a clutch arrangement operated by knob 5. 14 is a cooling coil making maintenance of box at 30° C. possible at higher outside temperatures.

The author will be glad to assist investigators in securing the equipment required in connection with the method.

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Micro-Kjeldahl Determination of Nitrogen

A New Indicator and an Improved Rapid Method

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WINKLER (*12*) proposed in 1913 the direct titration of ammonia, absorbed in boric acid solution, by mineral acid for the Kjeldahl determination of nitrogen. A thorough study was later made by Markley and Hann (*3*), who recommended it as a standard method. It has three advantages over the regular back titration method: only one standard solution is needed, there is no danger of spoiling a determination because of insufficient standard acid for the absorption of the ammonia, and in case the content of the receiving flask is sucked back to the distilling flask, through an error of operation, distillation can be resumed without bad effect.

TABLE I. pH AND COLOR OF 2 PER CENT BORIC ACID

2% Boric Acid	Mixed Indicator	0.01 *N* HCl	0.01 *N* NH_4OH	Color	pH
Ml.	*Ml.*	*Ml.*	*Ml.*		
5	0.05	...	...	Bluish purple	4.52
5	0.05	0.02	...	Pink	4.26
5	0.05	...	0.02	Bluish green	4.90

Several investigators have tried to adapt Winkler's direct titration method to the micro scale, using as indicator a mixture of methyl red and tetrabromophenol blue (*10*), methyl red and methylene blue (*1*, *9*), or methyl red alone (*4*, *11*). The end point of the titration is located by matching color with a standard, because the indicators show only a gradual transition of color. The amount of boric acid used and the volume of solution in titration have to be carefully controlled (*4*). Consequently, the operation becomes more complicated and time-consuming than Pregl's procedure for micro-Kjeldahl analysis (*8*).

In connection with some work in which a large number of micro-Kjeldahl determinations were required, the writers made an extensive study of the indicators suitable for the titration of ammonia in boric acid, as well as the minimum time required for the micro-Kjeldahl distillation. It was found that bromocresol green (tetrabromo-*m*-cresolsulfonphthalein) compares favorably with the indicators mentioned above. This indicator changes from blue color in ammonia solution to greenish yellow at the end point. While the two colors, blue and greenish yellow, are not easily distinguishable, the transition is rather sharp. A still better indicator has been found, however, in a mixture composed of 5 parts of bromocresol green and 1 part of methyl red, which gives a bluish-purple color in 2 per cent boric acid solution, changing to bluish green in the presence of a trace of ammonia and to pink with a trace of mineral acid. The intensity of color does not vary appreciably with the amount of indicator added. The transition at the end point is very sharp and distinct, a color standard being entirely superfluous.

Pregl (*8*) prescribed 6 minutes for the micro-Kjeldahl distillation. Niederl and Niederl (*5*) reduced the time to 4 minutes, while Hallett (*2*) remarked that 5 minutes are required for a complete distillation. In a review article, Parnas (*6*) indicated that 1.5 to 2.5 minutes are sufficient. The writers found that distillation for 2 minutes with collection of 10 to 15 ml. of condensate recovered completely 1 mg. of nitrogen, the feasible upper limit of micro-Kjeldahl analysis. They also found that a Pyrex flask may be used as the receiver without being previously steamed out, and that a mixture of selenium, copper sulfate, and potassium sulfate may be used as catalyst for the digestion in preference to hydrogen peroxide.

Taking advantage of these observations, the writers have developed a method for micro-Kjeldahl analysis in which the operation is simplified and the time required is considerably shortened. This method has been used in this laboratory and other laboratories, by analysts as well as students, for more than a year with satisfactory results.

Sensitivity of Mixed Indicator

In Table I are listed the pH values obtained by the addition of 2×10^{-4} millimole of hydrochloric acid or ammonium hydroxide to 2 per cent boric acid solution, together with the observed color of the mixed indicator for each pH value, determined by means of a glass electrode.

Distillation Experiments

A solution of ammonium sulfate containing an equivalent of 0.125 mg. of nitrogen per ml. was prepared by dissolving C. P. ammonium sulfate in distilled water. The concentration of the solution was determined by both nitrogen and sulfur analysis.

Measured volumes of the ammonium sulfate solution were delivered from a microburet into a series of micro-Kjeldahl digestion flasks. To each flask was added 0.5 ml. of concentrated sulfuric acid. The content was diluted or concentrated, as the case may be, to about 2 ml. Ammonia was then recovered by distillation in the micro-Kjeldahl distilling apparatus under different conditions as outlined in Table II, which gives the complete group of test distillations. No results have been omitted because of inaccuracy. In the first series (*a*), Pregl's procedure (*8*) was followed, using methyl red as indicator. In the other series (*b* to *f*), 0.05 ml. of the mixed indicator was added to the Pyrex receiving flask, no matter whether it contained 5 ml. of 4 per cent or 2 per cent boric acid.

Table II. Recovery of Ammonia

		Nitrogen Found					
			Direct-Titration Method				
			With 5 ml. of 4% boric acid			With 5 ml. of 2% boric acid	
		(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)
$(NH_4)_2SO_4$	Nitrogen Present	Pregl's back-titration method	Steamed out, distilled 3 min., drained 3 min.	Not steamed out, distilled 3 min., drained 3 min.	Steamed out, distilled 1 min., drained 1 min.	Steamed out, distilled 3 min., drained 3 min.	Not steamed out, distilled 1 min., drained 1 min.
Ml.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
8.00	1.000	0.998	1.004	....	....	....	1.000
4.00	0.500	0.497	0.500	0.500	0.500	0.498	0.505
3.00	0.375	0.379	0.378	0.377	0.375	0.379	0.375
2.00	0.250	0.255	0.251	0.246	0.249	0.252	0.246
1.00	0.125	0.124	0.125	0.129	0.124	0.129	0.126
0.50	0.062	0.062	0.063	0.059	0.063	0.062	0.059

These experiments seem to indicate that ammonia recovery is not affected by the method of titration, whether back (*a*) or direct (*b* to *f*); concentration of boric acid (2 per cent or 4 per cent); time of distillation between 2 (*d*, *f*) and 6 minutes (*b*, *c*, *e*); nor by steaming out the receiving flask. The most simple and rapid procedure (*f*) was therefore chosen as the standard method for micro-Kjeldahl analysis.

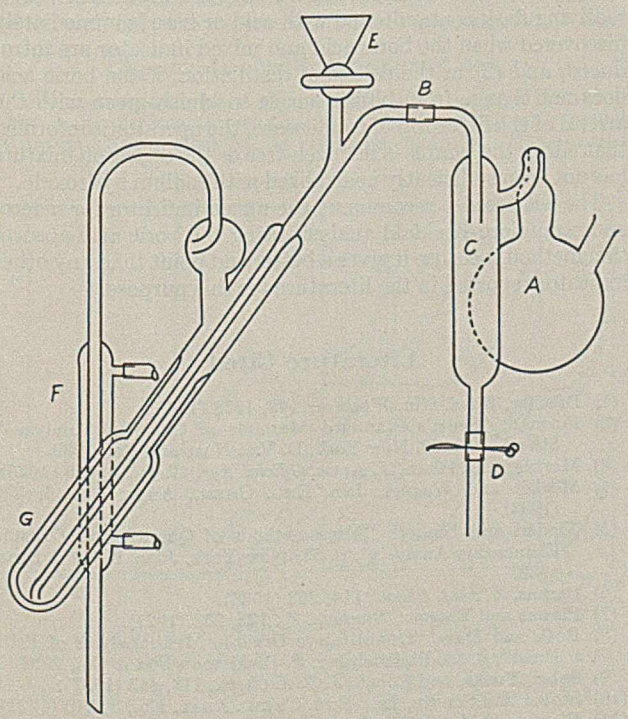

Figure 1. Distillation Apparatus

Analysis of Known Compounds

In order to check the accuracy of the new method, a number of known compounds were analyzed with the procedure described in detail below. The results are shown in Table III.

Apparatus

The apparatus used in the microanalytical laboratory of the University of Chicago is described because it is simple, compact, and easy to make. Other types of apparatus for micro-Kjeldahl analysis may be used without changing the procedure.

Digestion Flasks. The digestion flasks are made from ordinary 15-cm. (6-inch) Pyrex test tubes with the bottom blown out to form a bulb of about 25-mm. diameter and 6-ml. capacity.

Digestion Stand (Figure 1). A digestion stand for 6 flasks is made from a Transite board, 10 × 35 cm., with 6 holes of 22-mm. diameter drilled in it. The board is set on a metal frame with four legs 10 cm. high. A heavy copper wire supports the necks of the digestion flasks. The burner is made from a Bunsen burner with its tube replaced by a copper tubing 35 cm. long. Six tiny holes, about 1 mm. in diameter, are drilled along the copper tubing, 2.5 cm. below the holes of the Transite. The first hole should be about 8 cm. away from the air screw of the burner. A fume duct (*5*) is not necessary when the digestion is carried out in the hood.

Distillation Apparatus (Figure 2). This is a modification of the Parnas-Wagner apparatus (*7*) with the rubber connections eliminated. It is made of Pyrex glass and is built in two compact units joined glass-to-glass with short rubber tubing, *B*. This renders the apparatus less rigid, and reduces the danger of breakage due to bumping when water boils in the steam generator, *A*. The whole apparatus is conveniently clamped onto an iron stand and occupies a desk space of 30 × 40 cm. The steam generator is made from a 1-liter round-bottomed flask to which a side arm is attached for refilling. When it is two-thirds filled with distilled water before distillation is begun, enough steam will be generated for 8 to 12 determinations.

Reagents

Mixed Indicator. Prepare 0.1 per cent bromocresol green and 0.1 per cent methyl red (both indicators purchased from Eastman Kodak Co.) solutions in 95 per cent alcohol separately.

Table III. Analysis Results

Compound	Nitrogen Calculated	Nitrogen Found
	%	%
Benzamide	11.56	11.49
Phthalimide	9.53	9.59
Acetanilide	10.38	10.23
Urea	46.65	46.54
Urotropin	39.99	40.28
Oxamide	31.82	32.08
p-Chloroaniline	10.98	11.01
Tetramethyldiaminobenzophenone	10.45	10.52
p-Bromoacetanilide	6.55	6.63
Benzanilide	7.11	7.07
Glycine	18.66	18.74
Alanine	15.73	15.75
p-Toluidine	13.08	12.91
p-Tolylurea	18.66	18.65
p-Bromoaniline	8.15	8.21
Uric acid	33.34	33.53
$C_6H_5.NH.SO_2.(CH_2)_2CO_2CH_3$	5.76	5.72
p-Anisidine	11.38	11.45

Mix 10 ml. of the bromocresol green with 2 ml. of the methyl red solution in a bottle provided with a dropper drawn out into a fine capillary. The dropper delivers about 0.05 ml. per 4 drops.

Boric Acid 2 Per Cent. Dissolve 10 grams of boric acid (crystal) in 500 ml. of boiling distilled water. After cooling, transfer the solution into a glass-stoppered bottle. It keeps indefinitely.

Hydrochloric Acid 0.01 *N*. Dilute standard 0.1 *N* hydrochloric acid quantitatively. Check the concentration of the final solution against pure sodium carbonate or by chlorine analysis.

Sodium Hydroxide 30 Per Cent. Dissolve 150 grams of sodium hydroxide pellets in 350 ml. of distilled water. Store the solution in a bottle closed with a rubber stopper.

Catalysts. Powdered selenium; pulverized mixture of potassium sulfate (1 part) and copper sulfate pentahydrate (3 parts). Only a small quantity is needed.

Procedure

Digestion. Weigh out a 2- to 5-mg. sample, containing 0.2 to 1 mg. of nitrogen, and transfer it into the bottom of the digestion flask. Use a long-handled charging tube for solid samples. For semisolids and heavy oil, use a porcelain microboat (Coors, size 00000) which is slid into the digestion flask with the sample. Add about 3 mg. of powdered selenium and 5 mg. of copper sulfate–potassium sulfate mixture, followed by 1 ml. of concentrated sulfuric acid. When a large volume of the sample is taken, as in the case of biological fluids and dilute solutions, it is better to acidify the sample (after introduction into the digestion flask) with a drop of sulfuric acid, and concentrate the volume to less than 1 ml. before adding the catalyst and 1 ml. of concentrated sulfuric acid. Place the digestion flask on the digestion stand and boil the digestion mixture gently with a flame about 2 cm. high. The reaction is usually complete in 10 minutes. After cooling, add 2 ml. of distilled water, mix, and again cool.

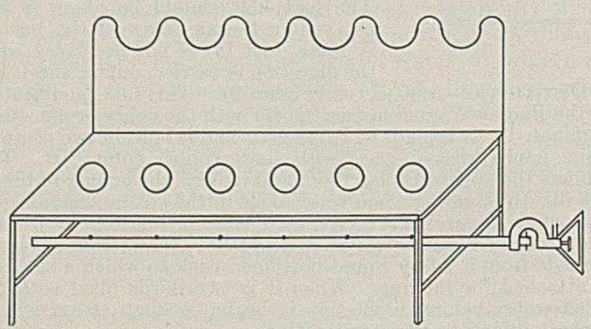

Figure 2. Digestion Rack

Distillation and Titration. A 25-ml. graduated cylinder for 30 per cent sodium hydroxide, a 5-ml. pipet for boric acid, and several 50-ml. Pyrex Erlenmeyer flasks are needed. Place a beaker under the condenser (*F*, Figure 2). Boil the distilled water in the steam generator, *A*, with a flame from the Bunsen burner about 10 cm. high, stopcock *E* and pinch clamp *D* being closed. Cold water is running in the condenser, from which about 5 ml. of distillate should collect per minute. Remove the burner, whereupon the condensate in the distilling flask, *G*, is sucked back into the steam trap, *C*. Fill funnel *E* with distilled water and open the stopcock momentarily to drain the water into *G*. Replace the burner under the steam generator for about 20 seconds and remove it again. Meanwhile rinse a 50-ml. Pyrex Erlenmeyer flask with distilled water. Introduce 5 ml. (need not be accurately measured) of 2 per cent boric acid and 4 drops of the mixed indicator into the Erlenmeyer flask. Fill the microburet with 0.01 *N* hydrochloric acid to the zero mark. By this time the distilling flask, *G*, is empty.

Replace burner under the steam generator, and open pinch clamp *D* to remove liquid from the steam trap, *C*. Leave the pinch clamp on the glass tubing through which the steam escapes. Replace the beaker under the condenser with the Erlenmeyer flask containing boric acid, and support the flask in an oblique position, so that the tip of the condenser is completely immersed in the liquid. Smear a trace of vaseline on the lip of the digestion flask to prevent the liquid from dribbling down outside. Hold the digestion flask in one hand, open the stopcock *E* with the other hand, and pour the digestion mixture through the introduction funnel into *G*. Rinse the digestion flask twice with about 2 ml. of distilled water, then introduce 8 ml. of 30 per cent sodium hydroxide and close stopcock *E*. Replace the pinch clamp *D* on the rubber tubing, whereupon steam enters *G*, stirs up the digestion mixture and sodium hydroxide, and liberates ammonia which escapes with steam through the condenser into the boric acid solution.

The boric acid changes from bluish purple to bluish green as soon as it comes into contact with ammonia. The change, which is very sharp, takes place between 20 to 60 seconds after the pinch clamp is closed and usually coincides with the time when the first drop of condensate reaches the Erlenmeyer flask. One minute after the boric acid has changed color, lower the Erlenmeyer flask so that the condenser tip is 1 cm. above the liquid. Wash the end of the condenser with a little distilled water. Continue distillation for another minute, then remove the burner. Bring the Erlenmeyer flask to the titration stand and titrate until the blue color disappears. (If preferred, the titration may be continued until a faint pink tinge appears; 0.02 ml. is then subtracted from the buret reading. There is no danger of missing the end point, because after the pink tinge appears, the intensity of pink color increases tremendously with a trace more 0.01 *N* hydrochloric acid. The titration may be done in daylight or artificial light.) The distilling flask *G* is now again empty. *E* is washed with distilled water as described above, and distillation of the next sample follows. Distillation and titration require from 5 to 8 minutes.

Conclusion

This paper presents a simple and rapid method for micro-Kjeldahl analysis. The sample is digested with sulfuric acid in the presence of selenium, copper sulfate, and potassium sulfate. The ammonia is distilled into 2 per cent boric acid solution and titrated directly with standard 0.01 *N* hydrochloric acid, using a mixed indicator which consists of 1 part of methyl red and 5 parts of bromocresol green. After digestion of the samples, which is usually complete in about 10 minutes, an operator can easily run 8 to 10 distillations and titrations in an hour.

The present method eliminates the standard alkali and conserves the standard acid. The mixed indicator gives a sharp and clear-cut end point. Its characteristic of giving distinctly different colors to 2 per cent boric acid containing a trace of ammonia or mineral acid has certain merits: (1) a receiving flask contaminated with acid or base is immediately discovered when the boric acid and mixed indicator are introduced, and (2) in the course of distillation, if the boric acid does not change from bluish purple to bluish green with the arrival of the first drop of condensate, the operator is informed that either the sample is nitrogen-free or the digestion mixture has not been sufficiently neutralized with sodium hydroxide.

The writers also recommend the mixed indicator for macro- and semimicro-Kjeldahl analysis using the boric acid absorption method, because it gives a better end point than any other indicator reported in the literature for this purpose.

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Amperometric Titration of Copper with Benzoinoxime

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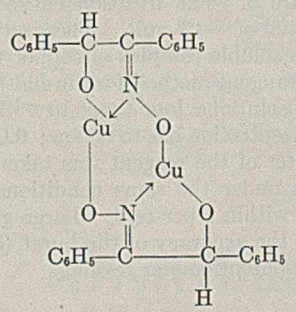
ALPHA-BENZOINOXIME (cupron) was first recommended as a specific organic reagent for copper in an ammoniacal solution by Feigl (*2*) in 1923. Since then the reaction with copper has been studied, not only in regard to its analytical use, but also from the viewpoint of the chemistry of complex compounds. The reason for the latter investigations was the surprising stability of the salt in ammoniacal solutions, although the copper in the established formulas does not seem to have its normal coordination number of four. Feigl (*3*) explains this behavior by assuming chelation between the copper and the benzene rings. Investigations done jointly with Dubský (*1*) suggested the possibility of the formation of a basic "diol" salt; addition of ammonia was also observed. But before any other explanation is proposed, molecular weight determinations or x-ray diffraction work should be used to exclude the possibility of a dimeric structure:



With copper, the reaction is specific only in an alkaline (ammoniacal) solution. In acid solutions other metals are also precipitated; thus benzoinoxime was used for the determination of molybdenum by Knowles (*4*). In the work reported here only the copper reaction was investigated.

Apparatus and Reagents Used

A compensating type of polarometer with a Leeds & Northrup Type K potentiometer was used, the cell having an external saturated calomel electrode as reference electrode. Oxygen was expelled from the supporting solutions by purified nitrogen. The general procedure of titration was that described in previous papers (*5*).

The 0.01 *M* copper sulfate stock solution was prepared from recrystallized C. P. copper sulfate, and to prevent basic salt formation a drop of 0.1 *M* sulfuric acid was added to 250 ml. The 0.01 *M* stock solution of benzoinoxime was prepared from the Eastman Kodak chemical by dissolving in 50 per cent ethyl alcohol. The supporting solutions were made up from Baker's C. P. chemicals in redistilled water.

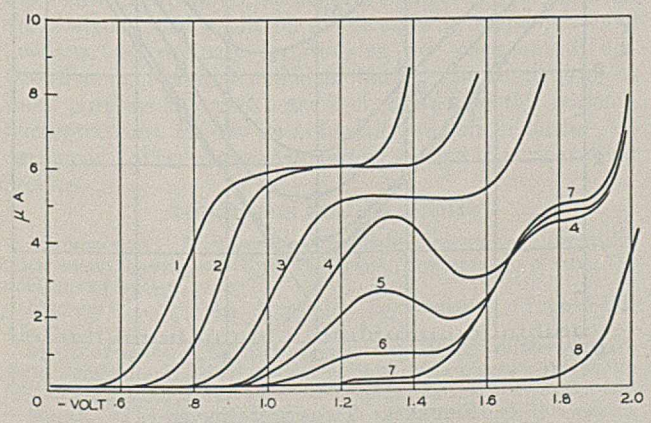


FIGURE 1. CURRENT-VOLTAGE CURVES

Approximately 5 × 10^{-4} *M* α-benzoinoxime in buffer solutions of indicated pH

1. pH = 0.8 3. pH = 4.8 5. pH = 6.5 7. pH = 8.2
2. pH = 2.7 4. pH = 5.6 6. pH = 7.0

Temperature 22–24° C., Saturated Calomel Electrode t = 2.9 seconds, m = 1.64 mg. sec.$^{-1}$ Hg

Current-Voltage Curves of Copper and Benzoinoxime

It was found, in accordance with the observations of Stackelberg and Freyhold (*7*), that the cupritetrammino ion is reduced in a supporting solution of 0.1 *M* ammonium chloride and ammonium hydroxide in a double wave with a half-wave potential of approximately −0.17 volt for the reaction $Cu^{++} \rightarrow Cu^{+}$ and −0.40 volt for the reaction $Cu^{+} \rightarrow Cu$.

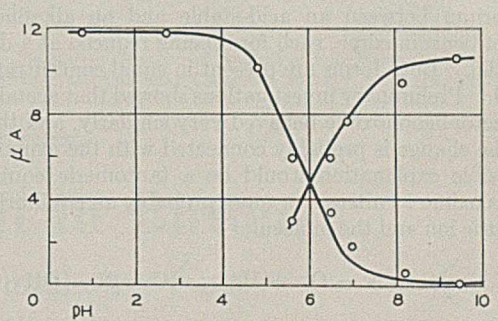


FIGURE 2. WAVE HEIGHT CHANGE FOR APPROXIMATELY 10^{-3} *M* BENZOINOXIME WITH pH OF SUPPORTING SOLUTION

Both half-wave potentials are shifted with increasing or decreasing ammonia concentration to more negative or positive values—about 0.1 volt per pNH_4OH. The waves were without maxima if a few drops of a 10 per cent gelatin solution were added.

The current-voltage curves of benzoinoxime were taken in buffer solutions of different pH—acetic acid–sodium acetate, boric acid–sodium hydroxide, and ammonium chloride–ammonium hydroxide. The pH was measured with a Beckman glass electrode pH meter. For a given concentration of the oxime in the acid region, a wave was observed having a half-wave potential varying with the pH of the solution as $E_{1/2} = -(0.71 + 0.6\ pH)$ volt. There was also found to be a pronounced dependence of the wave height on the pH, as indicated in Figure 1. If we plot the diffusion current per millimole per liter for different values of the pH, we obtain a sigmoid type of curve (Figure 2). From Figures 1 and 2 we can also see that when the first wave is disappearing, another wave at a more negative potential appears. The second wave can be observed only after pH 5 is exceeded, because of the interference with the hydrogen wave. When the first wave is diminishing (between pH 4 and 8) maxima are formed which make the determination of the diffusion current of the first wave uncertain. The figures also indicate that the sum of the two waves is almost constant. The half-wave potential of

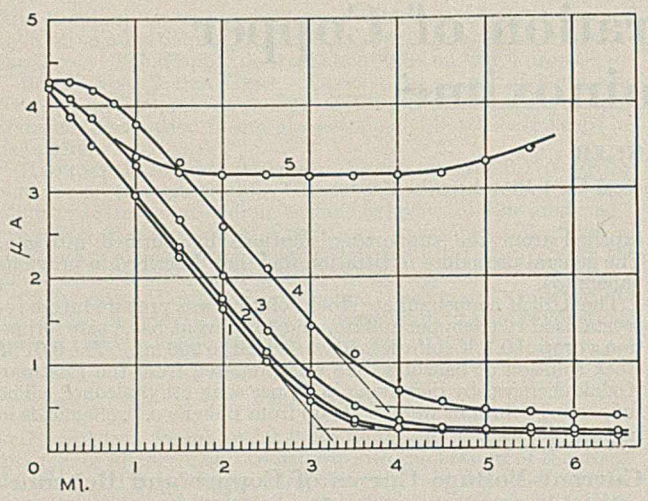


FIGURE 3. TITRATION CURVES
As in Figure 4 at an applied potential of −1.0 volt
Concentration of ammonia:
1. 0.01 *M* 3. 0.1 *M* 5. None
2. 0.05 *M* 4. 0.5 *M*

the second wave is around −1.63 volt and is not markedly influenced by the pH of the solution.

This result leads to the conclusion that we have a mobile equilibrium between an acid-stable and an alkaline-stable form of benzoinoxime, each form being reduced at a different potential. Both forms are present in equal concentrations at pH 6.0. Preliminary investigations showed that acetaldoxime and acetophenonoxime behaved very similarly, and therefore that the change is probably connected with the oxime group. A possible explanation would be a tautomeric equilibrium such as oxime ⇌ nitroso, or more probably an equilibrium between the ion and the molecule:

$$\rangle C = N - O^- + H^+ \rightleftharpoons \rangle C = N - OH$$

The neutral molecule would be favored by the increasing acidity of the solution and reduced at more positive potentials. The latter reaction gives

$$\frac{\left[\rangle C = N - O^-\right]\left[H^+\right]}{\left[\rangle C = NOH\right]} = Ka$$

If A is the total amount of oxime present, and x the amount ionized at any pH, we obtain

$$x = \frac{A}{1 + \frac{[H^+]}{Ka}}$$

At $x = \frac{A}{2}$ we have the condition that $[H^+] = Ka$, and we find $Ka \doteq 10^{-6}$ as the dissociation constant of benzoinoxime.

Titration of Copper with Cupron

From the current-voltage curves of copper and the reagent in an ammoniacal solution, it can be deduced that in the presence of a maximum suppressor at applied potentials between −0.8 and −1.4 volt, L-shaped titration curves would be obtained, whereas at potentials higher (to about −1.9 volt) the V-type titration curves should result. This was actually observed.

Titrations made in supporting solutions of 0.1 *M* ammonium chloride with variable amounts of ammonia from 0.001 to 0.5 *M* are shown for an applied potential of −1.0 volt in Figure 3 and for an applied potential of −1.7 volt in Figure 4. With increasing ammonia concentration the titration curves deviate strongly from straight lines, so that a determination of the end point by the tangent method is made with considerable difficulty and large errors result. This result led to a theoretical investigation with Stevenson (*6*), where the shapes of the ideal titration curves were calculated for substances with different solubilities. It was shown that the curves in a precipitation reaction $A^{n+} + B^{n-} \rightleftharpoons AB$ should consist of a straight line and a hyperbola, depending on $\frac{S}{A_0}$ where S = solubility product and A_0 the initial amount of substance present. From the shapes of the curves in the copper-cupron titration, we can conclude that the solubility of the copper salt increases strongly with increasing concentration of ammonia. The same behavior was found when the titration was carried out in a supporting solution of 0.1 *M* potassium chloride with different concentrations of ammonia. During the titrations it was observed that in 0.5 *M* ammonia the precipitate formed was light green and crystalline, as contrasted with the much darker green precipitates in lower concentrations of ammonia. If the titration was carried out in ammonium chloride or potassium chloride alone, no precipitate was formed and the titration curve was very flat, as shown in curve 5, Figure 3. The titration curve was similar at an applied potential of −1.7 volt. These curves might indicate formation of a soluble complex of copper with the reagent.

To use the tangent method for the end-point determination of copper in solutions less than 10^{-3} *M*, the ammonium hydroxide concentration has to be low; 0.02 *M* solutions were used. The titer of the reagent was taken as the average of five titrations under the same conditions. The values obtained agreed within 1 per cent. Large graphs were used to correspond to the accuracy of the buret (5 ml. with 0.01-ml. divisions) and potentiometer readings.

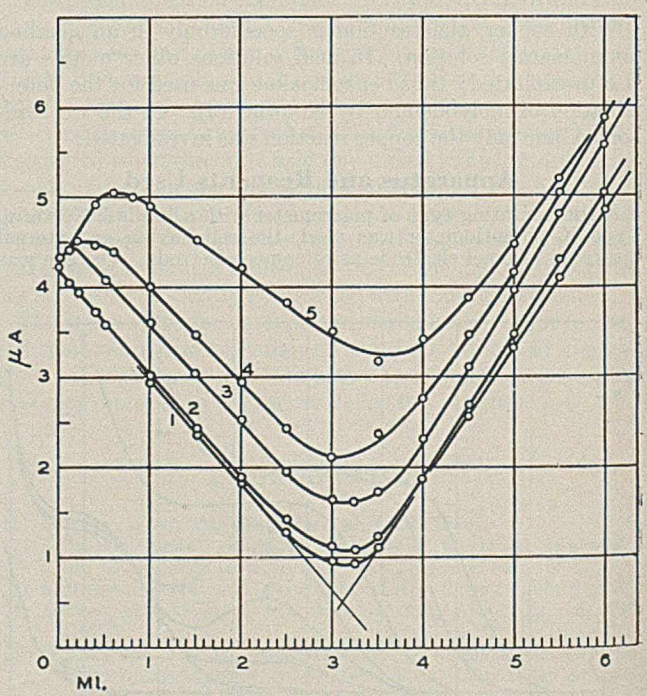


FIGURE 4. TITRATION CURVES
1.907 mg. of copper in 45 ml. of supporting solution of 0.1 *M* ammonium chloride with varying concentration of ammonia in presence of 4 drops of 10% gelatin with approximately 0.01 *M* benzoinoxime solution in 50% alcohol. Applied potential −1.7 volt
Concentration of ammonia:
1. 0.01 *M* 3. 0.1 *M* 5. 0.5 *M*
2. 0.05 *M* 4. 0.2 *M*
Temperature, 23° C., t = 3.0 seconds, m = 1.64 mg. sec.$^{-1}$ Hg

In Table I are given a few results obtained with different amounts of copper in 40 ml. of 0.1 *M* ammonium chloride and 0.02 *M* ammonia with 5 drops of 10 per cent gelatin, obtained at an applied potential of −1.7 volt.

TABLE I. DETERMINATION OF COPPER

Used	Found	Error
Mg.	*Mg.*	*Mg.*
0.636	0.646	+0.010
1.271	1.290	+0.019
1.907	1.910	+0.003
2.543	2.538	−0.005

The possibility of using 0.001 *M* benzoinoxime for titration of 0.05 to 0.5 mg. of copper in 20 ml. of 0.1 *M* ammonium chloride and 0.01 *M* ammonia was studied, but the points obtained were so scattered that only approximate lines could be drawn. The errors were sometimes more than 10 per cent.

Influence of Other Ions

The interference of other metal ions on the copper titration was investigated briefly. Ferric ions present in about double the amount of copper and precipitated as hydroxide gave results a few per cent too low in 0.02 *M* ammonia, but when the concentration of ammonia was increased to 0.1 *M*, the results were only about 1 per cent too low. In the presence of precipitated lead, the results were somewhat higher. Zinc present in five times the concentration of copper gave values 6 per cent too high in 0.1 *M* ammonia, but if present in smaller concentration than the copper, the results were only 0.5 to 2 per cent too high. With small amounts of zinc, the V shape of titration curve could also be used. Nickel interferes strongly.

Summary

α-Benzoinoxime was investigated as a possible reagent for amperometric (polarometric) titration of copper.

It was found that the reagent is reduced at the mercury dropping electrode in waves, with half-wave potentials and wave heights which depend on the pH of the supporting solution. It is suggested that the wave height dependence is a result of a mobile ionization equilibrium. It was also observed that the shapes of the titration curves depend strongly on the concentration of ammonia, because of the increasing solubility of the precipitate. For 10^{-3} *M* copper solutions, concentrations of ammonia less than 5×10^{-2} are favorable. The results obtained are accurate to within about ±1 per cent. The influence of a few other metals was studied.

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Distribution of Salt in Butter

A Volumetric Micromethod

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THE macromethods for the determination of salt in butter are adequate for obtaining the average salt content of a churning, but from the bacteriological standpoint such general information is inadequate. Since butter is not a homogeneous mass and organisms are not uniformly distributed through it, the bacteriological changes in a lot of butter are the combined result of the changes in numerous small portions. Accordingly, in studying the effect of salt distribution on bacterial action in butter, salt contents of as small portions as possible are desired, and for this reason a microprocedure for the determination of salt in butter was developed. The method can also be applied to various other products.

Apparatus and Reagents

MICROSCOPE. A Spencer, wide-field, low-power, binocular microscope, having a ×6 magnification, was used in picking the portions of butter.

MICROBALANCE. An Ainsworth microbalance (Type FDJ) was employed.

MICROSPOONS. The microspoons in which the butter samples were weighed and ashed were made by fusing pieces of platinum foil about 7 mm. in diameter to 24-gage platinum wires 2.5 cm. in length. The centers of the spoons were made slightly concave, and the wires were bent so that the spoons were level when hanging.

SPOT PLATE. The titrations were made in a white porcelain spot plate with depressions 20 mm. in diameter and 5 mm. deep.

CAPILLARY BURETS. The burets needed for this work are shown in Figure 1. They were made from pieces of capillary tubing, which was tested for uniformity of bore by introducing enough mercury to form a column about 2 cm. long and moving this mercury along the tube while measuring its length. Uniform pieces of tubing about 30 cm. long and 1 mm. in bore were selected. One end was drawn out to a fine tip. A piece of rubber tubing was attached to the upper end and, equipped with a plug and screw clamp, served to fill and discharge the buret. A celluloid ruler served as a scale.

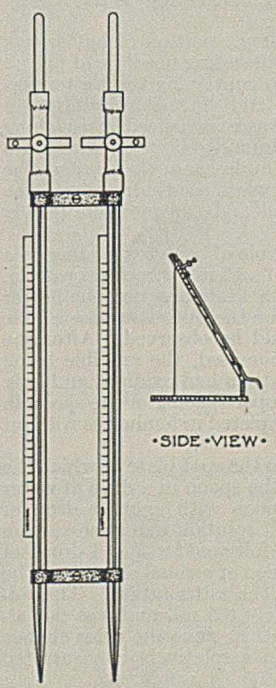


FIGURE 1. BURETS

Calibrating the burets was done by weighing various amounts of mercury on an ordinary analytical balance. The bores of the capillaries were sufficiently uniform so that a single calibration factor was determined for each buret, and used to convert the length of column of solution discharged to cubic centimeters.

For the titrations, a special technique must be used. The buret is filled by compressing the screw clamp, immersing the tip in a small tube of reagent, and unscrewing the clamp. It is filled a little above the top mark on the scale. The tip is wiped with a cloth or piece of filter paper and is then just touched to the surface of distilled water in another tube and the screw clamp is

TABLE I. MICROTITRATIONS ON 0.01 *N* SODIUM CHLORIDE SOLUTION WITH APPROXIMATELY 0.01 *N* SILVER NITRATE

Trial No.	Cm. Divisions on Buret Scale Corresponding to Volume of Solution: NaCl solution	$AgNO_3$ solution
1	14.0	14.1
2	14.0	14.2
3	14.0	14.1
4	14.0	14.1
5	14.0	14.1
6	14.0	14.2
7	13.0	13.1
8	13.0	13.1

TABLE II. SALT DISTRIBUTION IN NORMAL COMMERCIAL BUTTER

Churning No.	Incorporation of H_2O in Butter	NaCl, Macro-method	NaCl in Microsample: No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No .8	No. 9	No. 10
		%	%	%	%	%	%	%	%	%	%	%
1	Very good	2.53	2.40	2.21	2.27	2.48	2.53	2.26	2.48	2.46	2.65	2.68
2	Good	2.35	2.50	1.77	2.18	1.83	2.14	2.17	2.09	1.90	1.92	1.98
3	Fair	2.21	2.46	2.26	2.36	2.36	2.17	2.19	3.26	2.05	2.06	2.28
4	Very poor	1.74	1.24	1.48	2.16	1.69	4.32	1.86	2.82	1.99	1.82	2.16

compressed until the level in the buret is brought exactly to the mark. The movement of the liquid in the capillary is smooth and precise only when the tip is immersed.

SODIUM CHLORIDE SOLUTION. A 0.01 *N* sodium chloride solution was prepared from reagent quality sodium chloride.

SILVER NITRATE SOLUTION. An approximately 0.01 *N* silver nitrate solution was prepared and standardized against the standard sodium chloride solution, using the microtitration that was later employed for the analyses. The silver nitrate solution was kept in a dark bottle covered with aluminum foil and the concentration was checked at frequent intervals.

DICHLOROFLUORESCEIN SOLUTION. One-tenth gram of dichlorofluorescein was dissolved in 60 ml. of ethyl alcohol (95 per cent) and the volume made up to 100 ml. with distilled water. One milliliter of this solution was then diluted with 9 ml. of ethyl alcohol.

Procedure

The butter to be studied was tempered and held at approximately 13° C. for sampling. This temperature was low enough so that the original body and texture of the butter were not changed and yet high enough to avoid any great tendency for moisture from the air to condense on the sample. To minimize the chances of weighing condensate from the air, a freshly exposed surface was used for each microsample, and the butter was broken instead of cut, so that the original texture at the freshly exposed surface was not changed.

An approximately 0.2-mg. (±0.05-mg.) portion of butter was picked under the microscope with a dissecting needle and placed in a weighed microspoon. The spoon containing the butter was hung on the pan of the microbalance and the weight determined to within 0.002 mg. No difficulty was experienced in weighing the microsamples except on warm, humid days; on such days moisture condensed on the cool sample, and then when the sample was placed on a relatively warm platinum spoon the temperature rather quickly increased and the moisture slowly evaporated.

The microspoon containing the sample of butter was hung on a Nichrome wire drawn across the top of a 15-ml. porcelain crucible. As the crucible was heated slowly with a microburner, the butter melted and spread in a thin film over the entire surface of the spoon; no spattering of the fat could be observed. After the butter had charred, the heat was increased, the crucible being heated just to redness until all the carbon had oxidized and only the ash and salt remained. With proper spacing of the spoon in the crucible, the heating could be completed in 5 minutes without loss of sodium chloride.

The ash and salt were transferred to the spot plate in which the titration was to be made by placing the spoon in a drop of water in a depression and covering the spoon with another drop of water. After several minutes the salt solution was washed from the spoon with 3 or 4 drops of water followed by 3 or 4 drops of 95 per cent ethyl alcohol. The addition of alcohol sharpened the end point and facilitated removal of the salt solution. The volume of the solution was approximately 0.5 ml. and was not allowed to become much greater than this, since the color change in the titration was more distinct in a relatively concentrated solution.

The color change at the equivalence point is due to the adsorption of the indicator on the surface of the precipitate. Accordingly, increasing the surface of the precipitate also increases the visibility of the color. The surface of the silver chloride precipitate was increased in the determination by adding from a capillary buret a volume of 0.01 *N* sodium chloride solution equivalent to 10 to 12 cm. on the buret scale. The addition of sodium chloride also corrected any error due to the color change at the end point

A small drop of dichlorofluorescein was added with a capillary pipet, and the solution was titrated with silver nitrate. The solution was stirred continuously during the titration, and the first permanent color change was taken as the end point. The titrations were made in a dark room under a fluorescent light.

The amount of silver nitrate in excess of the amount necessary to react with the added sodium chloride was equivalent to the sodium chloride from the butter. From the results of the titration and the weight of the sample, the percentage of salt in the sample was calculated.

In some samples of butter large water droplets were present on freshly cut surfaces. The salt contents of these individual droplets were determined as follows: A portion of each droplet was removed with a very small, weighed capillary tube, the ends of the tube were sealed with a small flame, and the tube was reweighed. The tube was crushed in a few drops of water in the depression of a spot plate and the chloride titrated. The salt concentration of the solution was high enough so that no additional sodium chloride was necessary.

Accuracy of Titration

The accuracy of the titration could not be determined by analyses on duplicate samples, since butter is not a homogeneous mass and duplicate microsamples could not be obtained. However, the accuracy was checked by titrating a 0.01 *N* sodium chloride solution, using the microprocedure, and expressing the volumes of the solutions as centimeter divisions on the buret scale.

Table I gives the results of eight titrations of the 0.01 *N* sodium chloride solution. The titration values checked to within 0.1 cm. on the buret scale; this represents a volume of 0.0004355 ml. of an approximately 0.01 *N* silver nitrate solution. Since the water-alcohol solution of the ash and salt was clear, it is probable that the titrations made on the butter samples were as accurate as those made on the salt solution.

Analyses of Butter

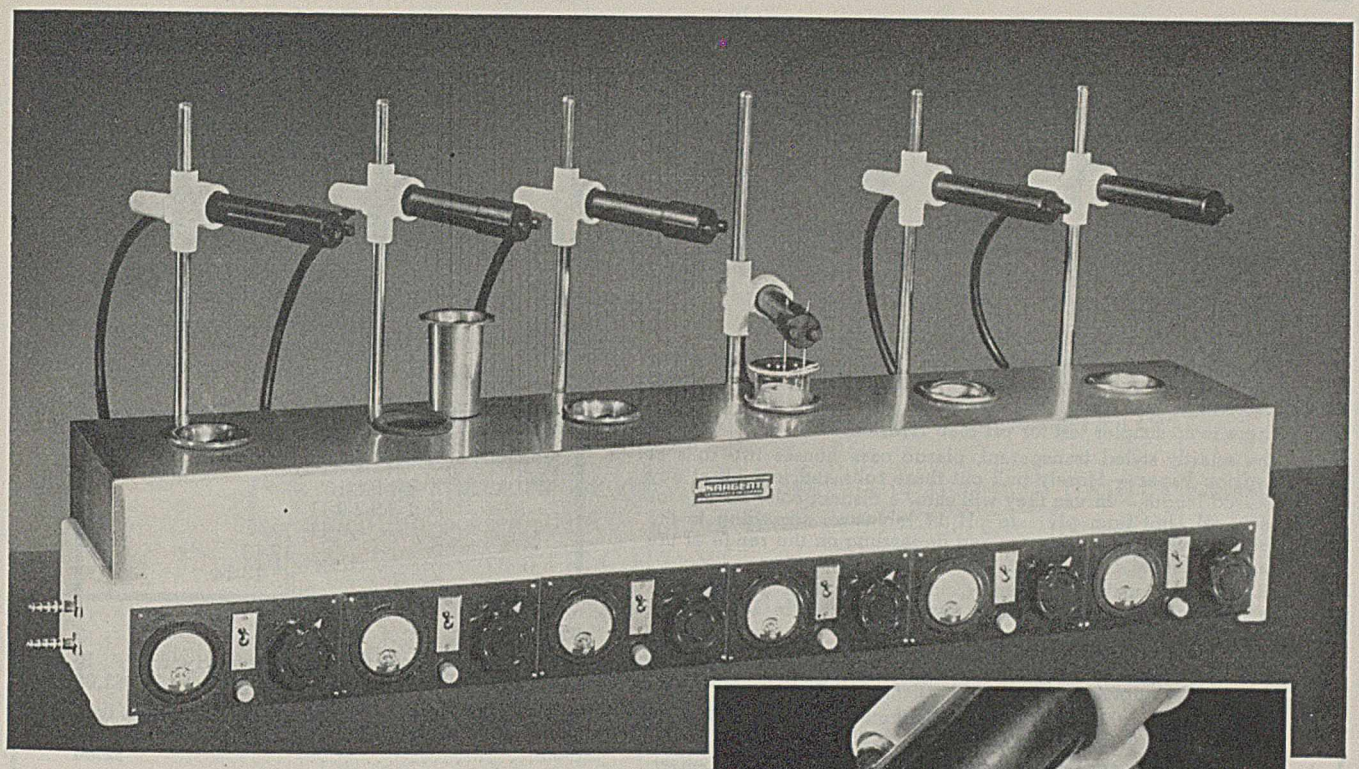
The distribution of salt was studied in normal commercial butter from a number of churnings. With each churning ten microsamples were picked from a 15-gram portion of butter. The data on four representative churnings are given in Table II. These churnings ranged from thoroughly worked to very poorly worked butter, as judged by the size and number of moisture droplets appearing on freshly cut surfaces of the cold butter. The analyses show that the salt contents of microportions of butter vary considerably, even in thoroughly worked butter. In poorly worked butter the variations are much greater.

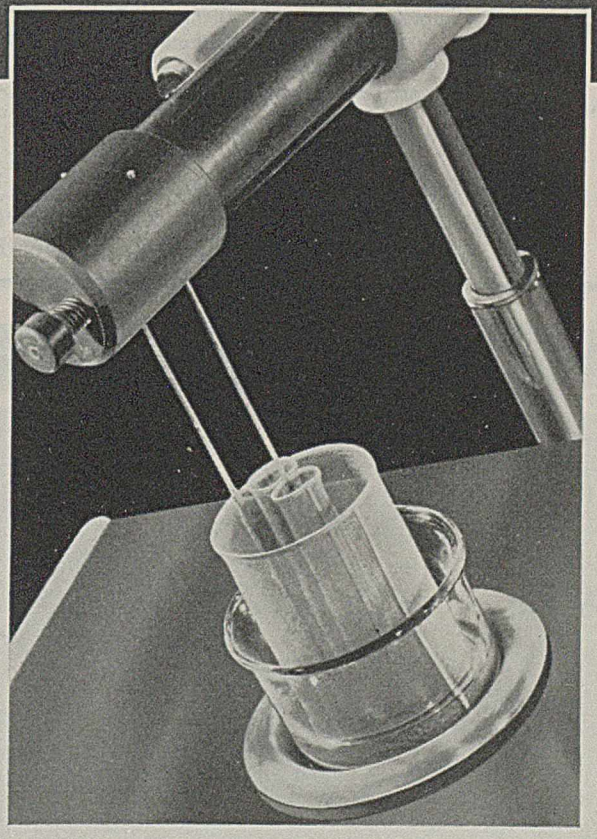
Summary

A reasonable degree of accuracy undoubtedly can be obtained in determining the sodium chloride content of an approximately 0.2-mg. sample of butter. However, the salt content of such a microsample is not representative of a churning of butter. The value of the method lies in the study of the salt distribution in butter, and it is particularly useful in the study of the effect of salt distribution on bacterial action.

The method should find application in the study of nonuniformity in other products. For homogeneous materials it is a rapid and a precise method for chloride analysis.

JOURNAL Paper J-906 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 119.





SARGENT ANALYZER DESIGNED FOR HIGH SPEED ELECTROLYTIC ANALYSES

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Separation and Determination of Lead

REAGENT—Salicylaldoxime
METHOD—Gravimetric
REFERENCE—Ligett and Biefeld, *Ind. Eng. Chem., Anal. Ed., 13,* 813 (1941)

Lead is precipitated quantitatively as lead salicylaldoximate, if the reaction is carried out above pH 8.9. Under these conditions, the precipitate is of constant composition ($PbC_7H_5O_2N$) and may be dried and weighed. The separation and gravimetric determination of the lead are rapid as well as accurate; in strong ammoniacal solution, complete separation is obtained from silver, cadmium, and zinc. The reagent is available as *Eastman 2956 Salicylaldoxime, MP 57-59°—10 grams, $1.45.*

Write for an abstract of the article in which the separation and determination of lead, with salicylaldoxime, are described. . . . Eastman Kodak Company, Chemical Sales Division, Rochester, N. Y.