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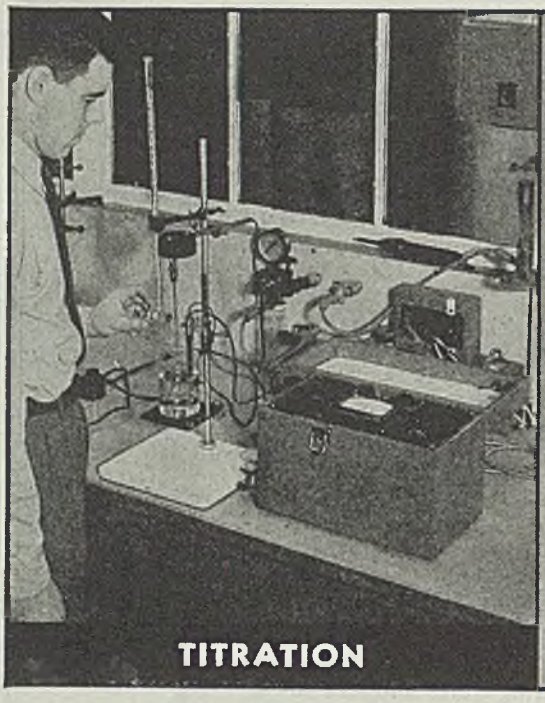

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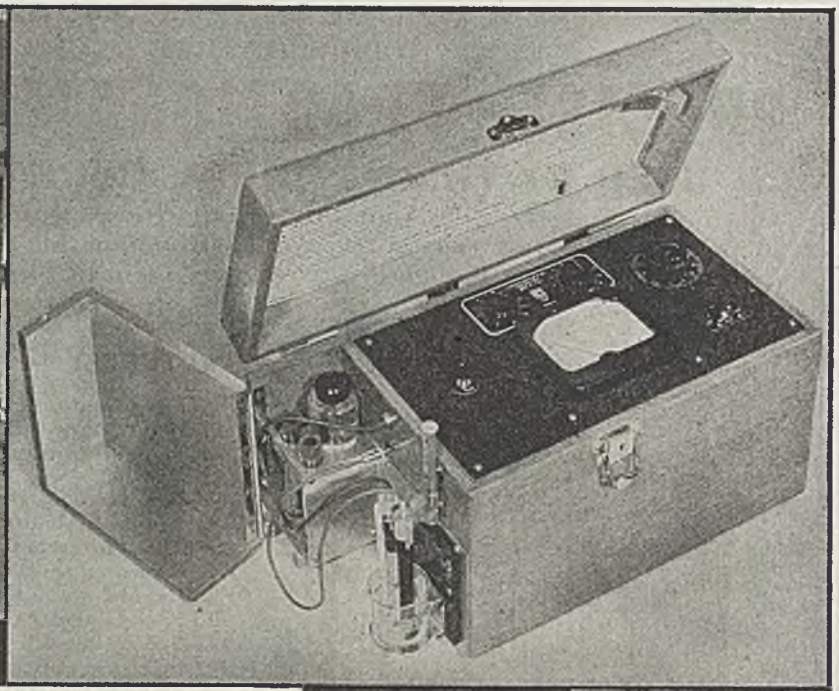

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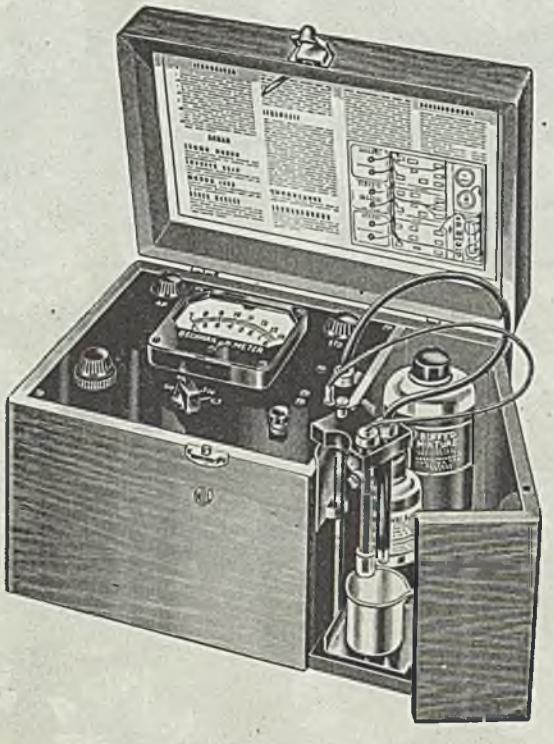

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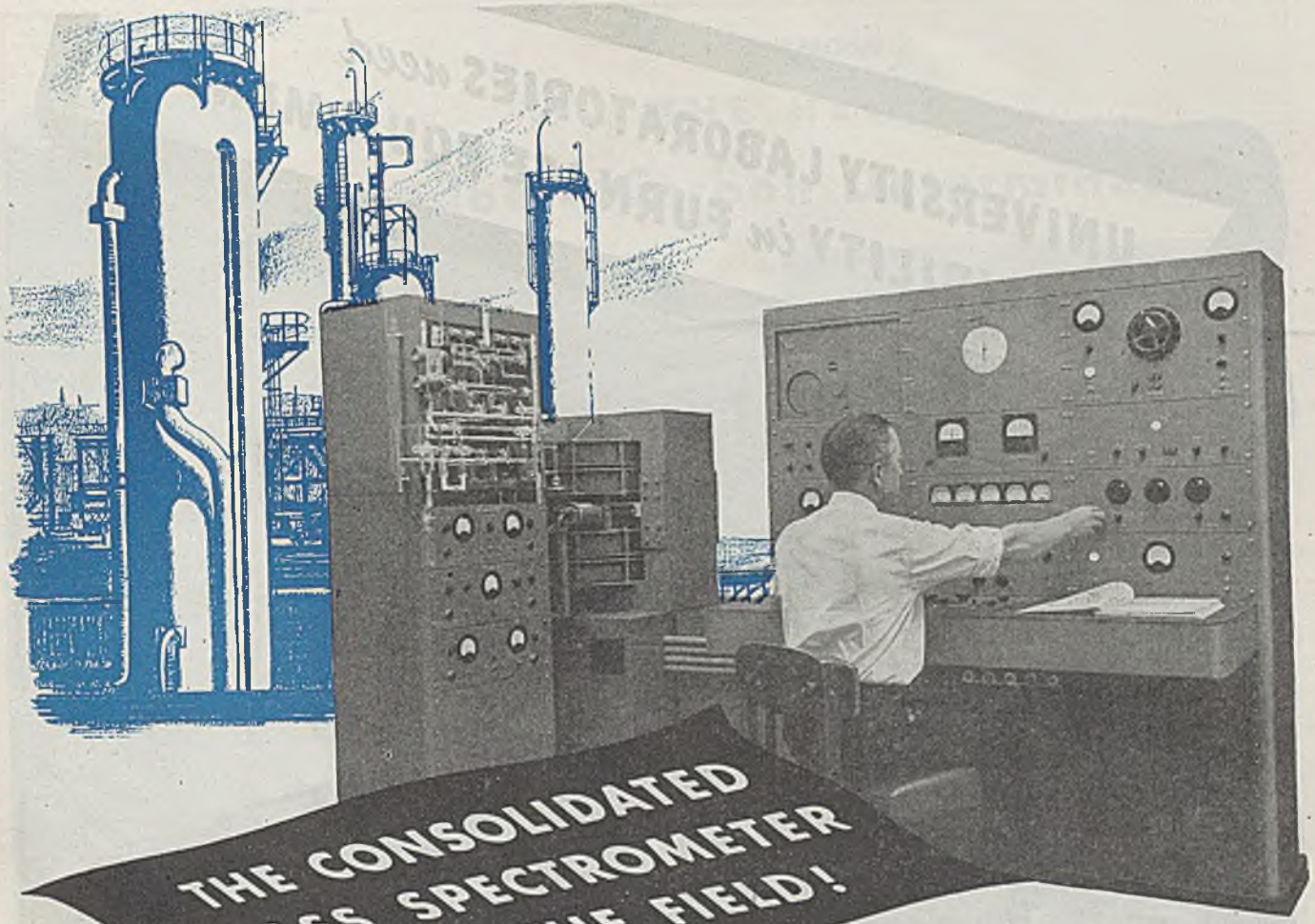

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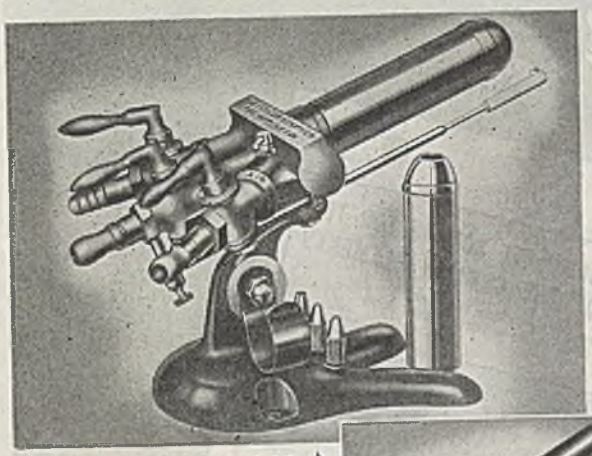

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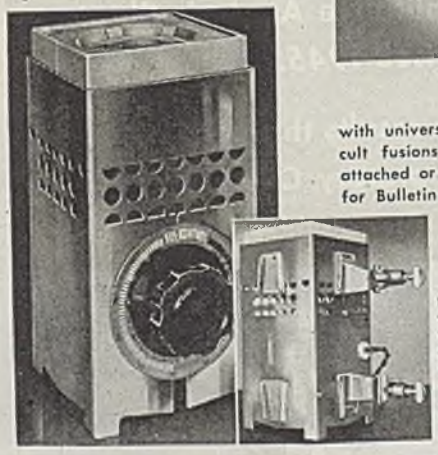

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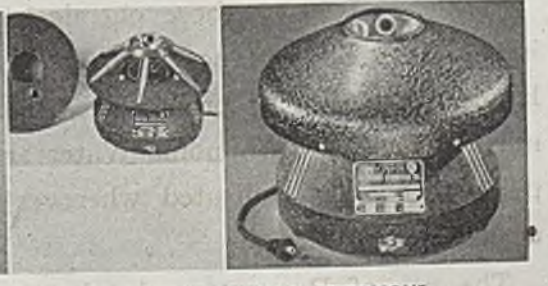

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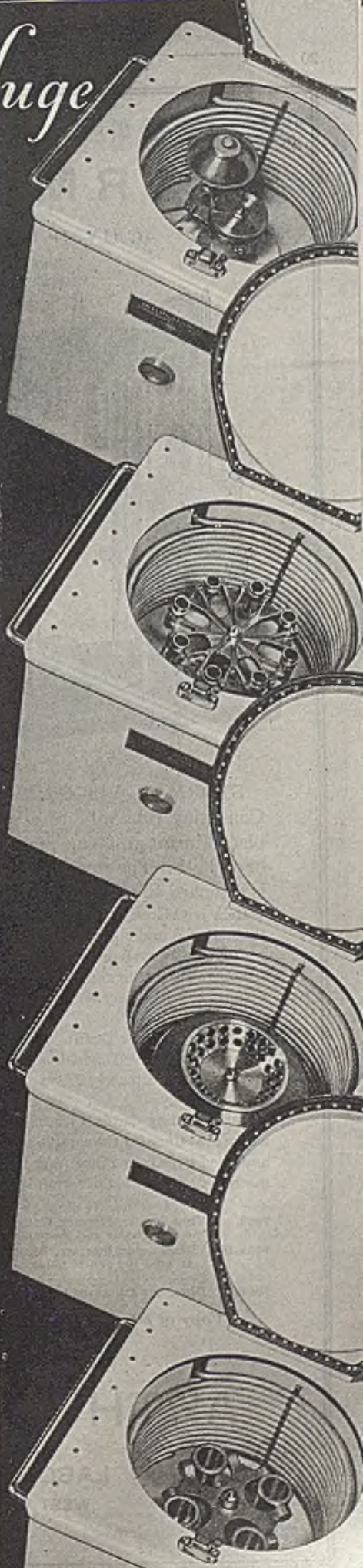

INDUSTRIAL AND ENGINEERING CHEMISTRY
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WALTER J. MURPHY, EDITOR

Editorial Policies

NOTHING is certain in the field of chemistry but change. Certainly the field of analytical chemistry has been no exception during the past decade, although perhaps the changes, revolutionary as they are to the analyst, have not been given the spectacular recognition accorded new developments in industrial chemistry.

The editorial policies of the ANALYTICAL EDITION have been determined solely on the basis of meeting promptly and efficiently the changing requirements of the analytical chemist and analytical chemistry. The innovations that should be introduced have been the subject of many earnest deliberations by the editors and the Advisory Board of the ANALYTICAL EDITION. This is particularly true of the past three years, when the board has been active and has met at regular and frequent intervals.

A statement at this time outlining the present aims and purposes of the journal will serve a most useful function, clarifying for our readers the views and policies of the editors. Your editor, Associate Editor L. T. Hallett, and Assistant Editors G. Gladys Gordon and Stella Anderson have cooperated in the preparation of three statements which we hope will be read, digested, and commented on. The first, discussing the scope of the journal, appears on page 218 of this issue; the second, describing style requirements, will be published in the May issue, and this will be followed by a detailed explanation of the reviewing system which will be printed in the June number.

Walter J. Murphy

Scope of the Analytical Edition

Whenever a paper is written for publication, the author must decide to which journal it may most suitably be sent. Some authors make a conscious effort to see that the type and style of the article conform to the standards or scope of the journal chosen, as they interpret them; others, it would appear, give little thought to this.

When the Analytical Edition was established in 1929, it was intended to serve the analytical chemist by segregating for him worth while contributions in analytical methods and improvements in analytical apparatus in the broad field of industrial and engineering chemistry. While applied analysis has received much attention during succeeding years, the journal has become much broader than its original conception. This, together with the expanded interests of the analytical chemist due to the development of new techniques and instruments, gives point to a short discussion for the guidance of authors on the types of papers which the editors of the Analytical Edition consider most suitable, so that the publication may better serve as the journal for analytical chemistry in all its branches.

1. A paper may present an improved or new procedure for the analysis or testing of some element, compound, or property, details of which must be accurately presented, so that others can duplicate the results. The tools used may be chemical or physical, and the procedures may deal with organic, inorganic, physical, or biological chemistry. The physical chemistry, in many cases, may approach pure physics.

2. A review paper may be invited or submitted. Its purpose should be critical evaluation of work in the given field during a specified period, rather than listing references with a brief abstract, for this is more strictly a bibliography. A bibliography is important if it is complete, and can be invaluable as an aid in research, but, in most cases, is necessarily so specialized as to be of interest to only a limited number of investigators and consequently does not often find a place in the Analytical Edition. Review papers may deal with either methods or instruments.

3. Papers on the evaluation of analytical results or the statistical treatment or interpretation of analytical and test data are becoming increasingly important. Such methods can point out human and method errors and provide a very useful means of eliminating untrustworthy data or methods. Some papers have been published in this field in the Analytical Edition; more are desired.

4. Papers dealing with principles and theory of analytical chemistry are important, in that they form the basis for new approaches to analytical chemistry and the better understanding of old, established methods. They are of interest to all our readers, both academic and practical. Such a paper should be a thoughtful discussion of analysis and does not necessarily include experimental work. Very few papers of this kind have been submitted to the Analytical Edition, and some criticism has come from the more theoretically minded analysts that this is a weakness of the publication. We welcome such papers.

5. General papers on the college training of personnel are important, as well as those which deal with the training programs, organization, and operation of analytical laboratories in industry and in research foundations. The function of the research analyst in a well-integrated research program is now receiving greater attention. A successful attack on some research problems is sometimes impossible unless research on methods is worked out in advance, or carried on simultaneously. The analyst on such problems must be of the highest caliber both as a chemist and as an analyst. While these phases of analytical chemistry present problems to many hundreds of people, few papers on these subjects appear.

6. Efficient physical equipment and layout are as important as properly trained personnel. Many times these subjects are discussed by correspondence between the heads of analytical laboratories, but papers discussing these problems would serve a larger audience. Floor plans of analytical laboratories have been published in the Analytical Edition from time to time, but a critical statement of the reasons for the selection of a particular laboratory setup would enhance their value.

7. The application and development of instruments designed for analyses are likely to receive greater attention from authors. The increase in the importance of this field as envisioned by your editors is indicated by the monthly articles on instrumentation, written by Ralph H. Müller, that began to appear in January of this year.

There may be other subjects which our readers would like to see discussed in the Analytical Edition. We welcome your suggestions.

Optical Methods in Electrophoresis

Principles, Apparatus, Determination of Apparatus Constants, and Application to Refractive Index Measurements

L. G. LONGSWORTH

Laboratories, The Rockefeller Institute for Medical Research, New York, N. Y.

The optical equipment developed at The Rockefeller Institute for the quantitative study of refractive index gradients in solution is described, together with suggestions for its installation and adjustment. This equipment, which is based on the Foucault-Toepler schlieren method, also incorporates the author's scanning modification and the cylindrical lens arrangement of Philpot and Svensson. Although the chief use of the apparatus is to record the moving boundary patterns in the electrophoretic analysis of proteins by the Tiselius method, this application has been adequately discussed elsewhere and is not included here. The precise measurement of refractive index differences in aqueous solutions represents, however, a recent application of the apparatus and is described.

THE optical equipment, as developed at The Rockefeller Institute, for use in the electrophoretic analysis of proteins by the Tiselius moving boundary method (*19*), has been duplicated in other laboratories and has been made available commercially. (The complete apparatus, in essentially the form described here, is supplied by the Klett Mfg. Co., New York, N. Y.) As a result, over forty installations are now in use both here and abroad. Although based on Tiselius' adaptation of the Foucault-Toepler schlieren method, the details of the apparatus have been evolved in this laboratory and an adequate description of them, which is given in this paper for the first time, should prove of value to investigators in the field of electrophoresis. With these same workers in mind a description of precise methods for testing and focusing the equipment and for determining the constants of the apparatus is also included. Not included in this article are descriptions of the electrophoresis process nor of the moving boundary cell and its accessories, since these have been adequately treated elsewhere (*7*, *9*, *10*).

Precise interpretation of the patterns that are obtained in the electrophoresis and the diffusion of proteins has been hindered, however, by the lack of adequate data on the refractive indices of solutions of these materials. This is especially true at 0°, the temperature at which proteins are frequently studied. Since the optical equipment described here is well adapted for such measurements on aqueous solutions in general, this application is discussed.

PRINCIPLE OF THE SCHLIEREN METHOD

The essential features of the schlieren method may be described with the aid of Figure 1.

An image at P of the point source of light, S, is formed by a lens, L. The objective, O, of a camera is placed just to the right of P and is focused on L, thereby forming an image of this lens on the screen at G.

Each portion of L is capable, of course, of forming an image of point S and if this lens is perfect all the images thus formed will superimpose at P. If, however, a portion of the lens is imperfect, the image formed by this area will not coincide with the other images.

In Figure 1 an exaggerated imperfection, idealized as a tiny prism, is indicated at A and the image formed by this portion of the lens as being at Q. Normally this imperfection is not visible in the image of the lens at G, since it is a property of the camera objective that all rays originating in its object plane are brought to focus at the conjugate points in the image plane regardless of the portion of the objective through which they pass. It is assumed, of course, that the intervening regions are optically homogeneous. If, however, the opaque screen, D, is raised so as to intercept the light forming image Q, while allowing that forming image P to pass, the region of imperfection will become visible at A' as a dark area, or shadow, in the otherwise uniformly illuminated image of the lens, L. This will be recognized as a simple example of Foucault's knife-edge test.

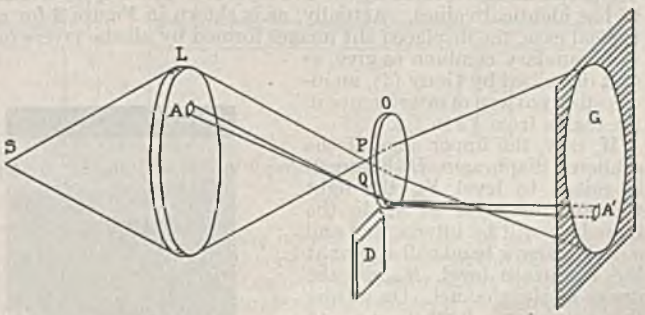

Figure 1. Foucault-Toepler Schlieren Method

Toepler observed that if the lens, L, were free from imperfections the camera could be focused on a plane in front of that lens and variations of refractive index in, or near, that plane could be made visible as shadows, just as the lens imperfections were rendered visible in Foucault's test. The shadows he called schlieren and the method the schlieren method. The lens, L, of Figure 1, is called the schlieren lens, the intercepting screen, D, the schlieren diaphragm, and the camera the schlieren camera. An excellent discussion and bibliography of the schlieren method are given in Schardin's monograph (*13*).

The form that the schlieren method takes for the observation and photography of refractive index gradients that occur in moving boundary and diffusion studies is shown diagrammatically in Figure 2. In this case gravity ensures that the density and, in general, the refractive index are uniform throughout each horizontal layer in the boundary.

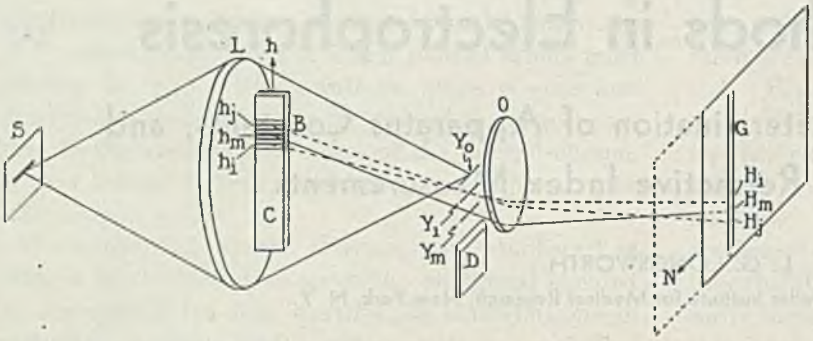

Figure 2. Schlieren Method for Study of Refractive Index Gradients in Solution

The schlieren lens, L, then forms at Y_0 in the plane of the schlieren diaphragm, D, an image of the illuminated, horizontal slit, S. The camera lens, O, is now focused on the cell, C (Figure 2), and forms an image of it on the screen at G. If the fluid in the cell is homogeneous this image will be uniformly illuminated. Suppose, however, that a boundary between, for example, a protein-bearing solution and a buffer solution is present in the channel. Such a boundary will consist of a region, B, of finite thickness, in which the concentration and hence the refractive index, n, vary continuously with the height, h, from its constant value in one homogeneous solution to that in the other solution. The gradient, dn/dh, of refractive index, which may be abbreviated to n', will thus vary from zero to a maximum, and back to zero.

In Figure 2 the variation of the gradient in the boundary is indicated by the density of the shading. Each layer of solution in this boundary acts, therefore, like a prism and deflects the light passing through it. If the denser solution underneath has the higher refractive index, as is usually the case, the deflection is downward. Moreover, if a layer is sufficiently thin the gradient may be considered constant in it and the light passing through this layer forms an image of the slit below the normal one at Y_0. Two such displaced slit images are indicated in Figure 2. The one at Y_m is formed by the layer of solution at h_m having the maximum value of dn/dh—i.e., n_m'. That at Y_1 is the superposition of the two images formed by the two layers at h_i and h_j for which n' has identical values. Actually, as is shown in Figure 3 for a typical case, the displaced slit images formed by all the layers in the boundary combine to give, as first described by Gouy (*4*), an interesting pattern of interference in the region from Y_0 to Y_m.

If, now, the upper edge of the schlieren diaphragm, D (Figure 2) is raised to level Y_m the light through the layer at h_m in the boundary will be intercepted and a dark, narrow band will appear at the conjugate level, H_m, in the image of the channel. On raising this diaphragm further—e.g., to level Y_1—the light from h_i and h_j, together with that from all intervening layers in the boundary, will be intercepted and the dark band in the image of the channel will broaden until the edges of the band are at the conjugate levels H_i and H_j. This, obviously, can be continued until the undeflected rays at Y_0 have been intercepted and the entire image becomes dark.

With a single boundary in the channel a series of fourteen photographs was taken, with the schlieren diaphragm raised a fixed distance between each exposure, and is shown in Figure 4, *a*. The progressive broadening of the schlieren band is apparent. If an exposure is made when the displacement of the diaphragm from the normal slit image is $Y_1 - Y_0$, for example, the edges of the band in that photograph are then conjugate to the levels in the channel at which the refractive index gradient has a value proportional to $Y_1 - Y_0$. The proportionality factor is (*21*), to a very close approximation, the product $a \times b$, in which a is the dimension of the channel parallel to the optic axis and b is the optical distance from the center of the channel to the schlieren diaphragm

$$Y - Y_0 = \Delta Y = abn' \qquad (1)$$

Figure 3. Interference Patterns at Schlieren Diaphragm

SCHLIEREN SCANNING METHOD

In the schlieren scanning procedure (*8*) the stepwise recording shown in Figure 4, *a*, is made continuous.

The image of the cell at G (Figure 2) is masked by a vertical slit of 0.2-mm. width and a photographic plate is moved in the direction, N, of the arrow at a constant rate across this slit. Actuated by the same mechanism, diaphragm D is given a steady movement upward. The resulting photographic positive for the boundary of Figure 4, *a*, is shown in Figure 4, *b*, and is called the boundary pattern. It will be noted that the schlieren bands of Figure 4, *a*, correspond to narrow vertical sections in the complete pattern. For purposes of publication it is the almost universal practice to turn the pattern through 90° as shown in Figure 4, *c*. If more than one boundary is present in the channel each appears as a separate peak in the complete pattern.

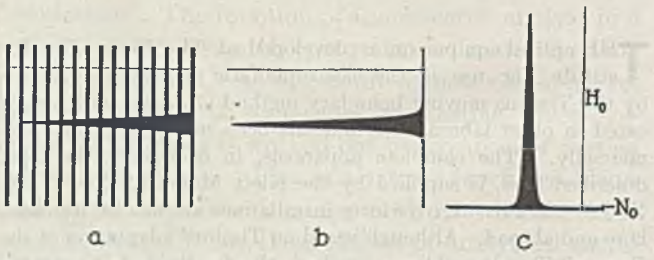

Figure 4. Schlieren Band and Scanning Photographs of a Single Boundary

The outline of the shaded area in Figure 4, *c*, is, therefore, a plot of the refractive index gradient in a thin layer of solution in the channel as ordinate against the height of the layer as abscissa. If E is the enlargement factor for the camera, the scale of abscissas for this plot is Eh, which will be designated as H. The ordinate scale is $abMn'$, designated as N, in which M is the ratio of the rates at which the plate and diaphragm move in the scanning process. The vertical line at H_0 in Figure 4, *c*, is due to a graduation on the cell and is taken as the origin of abscissas. The origin of ordinates is the horizontal line at N_0 joining those portions of the pattern outline that correspond to layers in the channel in which the solution is homogeneous. This is the "base line" of the pattern. In the ideal case this line is straight and horizontal. In practice, however, it is distorted slightly by imperfections in the lenses and in the cell and thermostat windows. Allowance for these distortions is made with the aid of a scanning photograph of the cell when it contains a homogeneous solution.

CYLINDRICAL LENS METHOD

In the scanning method described above the complete pattern is not visible in the focus of the camera but only on the developed photographic plate. It is convenient, however, to be able to observe the pattern directly on the ground glass. The cylindrical lens method (*11, 16, 17*) makes this possible and represents a useful addition to the schlieren camera.

In this method the schlieren diaphragm takes the form of a narrow slit, I (Figure 5), that is inclined to the vertical at an angle of, say, 45°. Moreover, a cylindrical lens, with its axis vertical, is interposed at C and is focused on the inclined slit and on the screen at G. Otherwise, the arrangement is as shown in

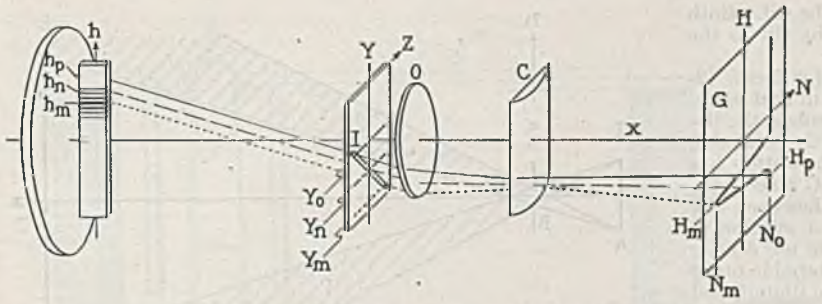

Figure 5. Cylindrical Lens Method

Figure 2. The presence of the cylindrical lens does not alter the vertical coordinate, H, of a point in the image of the cell but, in conjunction with the inclined slit, may displace it along the cross axis, N. As is shown below, this lateral displacement is proportional to the deflection that is suffered by the given pencil of light as it passes through the cell.

As before, if the fluid in the channel is homogeneous, all the light passing through it is concentrated in the normal slit image at Y_0. As can be seen in Figure 5, only the light forming the extreme left-hand portion of this image passes the inclined slit. As the figure is drawn these rays lie to the left of the optic axis, x, and are deflected, therefore, to the right of that axis by the cylindrical lens, thus forming a narrow vertical band of light on the screen at N_0. This is the base line of the pattern. One undeviated pencil of light, that through the layer of homogeneous solution at h_p, is traced in Figure 5 as a full line and forms one element of the base line at the conjugate level, H_p, in the image plane.

With a boundary in the channel, however, the light through the layer having the maximum gradient is deflected to Y_m. As is indicated by the dotted line in Figure 5, only the rays forming the right-hand portion of this image pass the inclined slit. The camera objective, O, brings these to focus at the same level, H_m, as in the absence of the cylindrical lens, but these rays pass the inclined slit to the right of the axis. Consequently they are shifted to the left by the cylindrical lens and form the peak of the pattern at N_m.

The path of the light through another portion of the boundary is indicated by the dashed line in Figure 5 and forms the corresponding element in the pattern. All other elements in the complete pattern are formed similarly. The focusing action of O on the channel ensures that the vertical coordinate, H, of the pattern corresponds to height h while the focusing action of the cylindrical lens, C, on the inclined slit requires that the N coordinate depend upon the lateral position at which the light passes this slit. As in the scanning procedure the factor of proportionality between h and H is the camera enlargement, E. The dependence of the N coordinate upon the refractive index gradient in the cell may be obtained as follows:

As is evident in Figure 5, the displacement, $N - N_0$, of a pencil of light that has been deflected in the boundary depends upon the angle, θ, that the inclined slit makes with the vertical and the lateral magnification, K, of the cylindrical lens. If Y_0 and Z_0 are the vertical and cross-axis coordinates, respectively, of the point of intersection of the inclined slit and the normal slit image, then the equation reads

$$N - N_0 = K(Z - Z_0) = K(Y - Y_0)\tan\theta = Kabn'\tan\theta \quad (2)$$

A pattern obtained with the aid of the cylindrical lens method is shown in Figure 14. Although a slit as diaphragm has been described here in conjunction with the cylindrical lens, one may also use a diagonal straightedge (*11*) or a diagonal bar (*12*). Moreover, it is also possible to use either a slit (*14*), a bar, or a straightedge, in this case in a horizontal position, with the scanning procedure. With either method the straightedge gives the pattern as the outline between a dark and a light field, the slit gives a more or less narrow band of illumination on a dark background, and the bar gives a dark band on a light background. No comparative study of the three types of diaphragm has been made, however. For visual observation of the pattern with the aid of the cylindrical lens method the author prefers a slit. In the permanent photographic record of an experiment it is essential, however, to have a reference line of known orientation, such as that at H_0 in Figure 4, *c*, and this is difficult to obtain with a slit as diaphragm. Consequently a straightedge as diaphragm is used for this purpose, together with the scanning procedure, thereby avoiding the errors accompanying the use of an uncorrected cylindrical lens.

OPTICAL EQUIPMENT

In the diagrams used thus far to illustrate the principles of the schlieren method, no attempt has been made to preserve the proper scale nor to indicate the construction of the various elements of the optical system. Moreover, the constant-temperature water bath in which the cell is immersed has been omitted from Figures 2 and 5. A scale diagram of the complete apparatus, as developed at The Rockefeller Institute, is shown, therefore, in Figure 6. In this modified isometric drawing the dimensions along the cross axis are on a scale one half of that used for the horizontal and vertical distances. In so far as is practicable the letters on this and subsequent diagrams identify the same optical element as in Figures 1, 2, and 5. Proceeding from left to right in Figure 6, for example, S indicates the illuminated horizontal slit, L the schlieren lens, D the schlieren diaphragm, and G the ground glass or photographic plate.

It may be apparent from Figure 6 that the lenses are of rather long focal length. Together with the other elements of the system they must be rigidly mounted on a firm support and provided with sufficient adjustments for their proper alignment. This is the purpose of the optical bench.

The Optical Bench. The optical bench consists of two 5.94-meter (19.5-foot) steel channels, C (Figure 6), bolted together by means of the plates, H. The bench is supported on two concrete blocks, B, and passes through a trough, R, in the block, B', that carries the thermostat, T. In order to minimize vibration the blocks rest on small shock-absorbing Keldur mats placed, at the corners of each block, on a firm foundation, preferably a ground floor. A sensitive spirit level is used in leveling the bench.

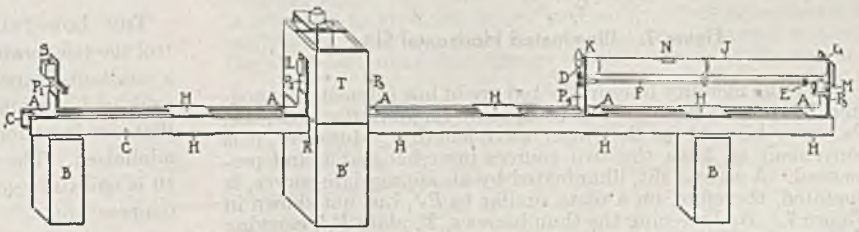

Figure 6. Scale Diagram of Complete Apparatus

Each component of the optical system is carried by a steel plate, P, that is bolted through slotted holes to the vertical faces of the steel angles, A. As shown in more detail in the lower part of Figure 7, the horizontal face of an angle, A, is clamped to the flanges of the channels, C, with the aid of a plate, B. Adjustment along all three axes may thus be made in the alignment and focusing of the components. Once the adjustments have been made it is essential that they be not disturbed by the routine use of the apparatus. First consideration is given, therefore, to the sturdiness of the equipment rather than ease of adjustment.

The Horizontal Slit. The illuminated horizontal slit, S (Figure 6), that serves as the source of light is also shown in detail in Figure 7. This is a view in perspective with the lamp and lamp house removed. The horizontal dimension of the slit shown here is 25 mm., which is about the length of the arc in the 100-watt G.E. mercury lamp, Type H4, used to illuminate the slit. The vertical dimension of the slit is adjustable, both of the leaves, L_1 and L_2, being movable in a T-slot cut across the face of the plate, Q. Since no condensing lens is used, the lamp is placed as close to the slit as its glass bulb permits. Plate Q is mounted therefore, on the steel plate, P_1, but separated from it so that the

socket does not limit the proximity of the lamp to the slit. Both Q and P_1' have openings to pass the light from the slit to the schlieren lens.

Although hidden by P_1' in Figure 7, a holder for 5-cm. (2-inch) square filters is placed in front of the opening in that plate. Either glass or gelatin film filters may be used to isolate the desired lines of the mercury spectrum. A table of the available filters for this purpose is given by Strong (*15*). The author has found a Wratten filter No. 22 (yellow) mounted in C glass satisfactory for work with clear, colorless solutions. If, however, one of the solutions meeting at a boundary is colored, a solution of hemoglobin, for example, it is generally necessary to use a filter with an absorption similar to that of the colored material in order to reduce the shading, at the boundary level, of the illuminated portion of the pattern. A similar shading also occurs in the image at the boundary between a clear and an opalescent solution. In this case the shading is due to the scattering of light by suspended particles and can be reduced, as was shown by Treffers and Moore (*20*), by using red or infrared light.

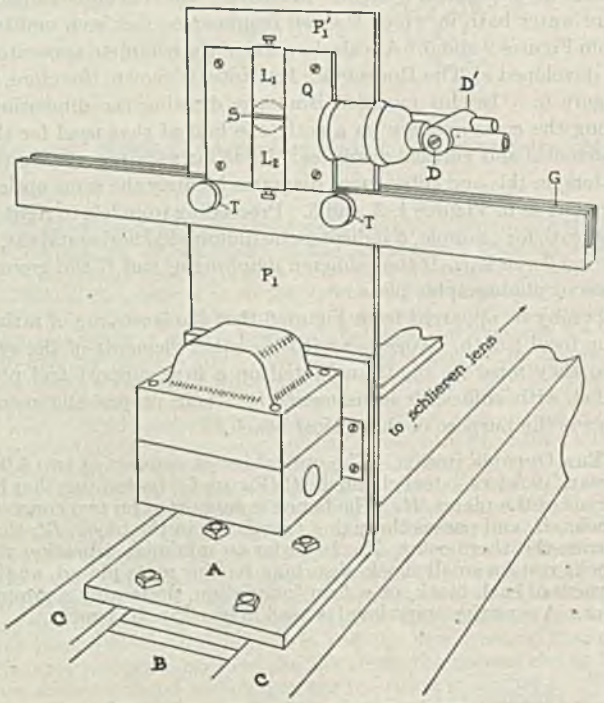

Figure 7. Illuminated Horizontal Slit

Since the mercury lines in the red are of low intensity, a tungsten lamp or Nernst glower (Stupakoff Laboratories, Latrobe, Pa.) may be used for the longer wave lengths. Moreover, it is convenient to have the two sources interchangeable and prefocused. A second slit, illuminated by an appropriate source, is mounted, therefore, on a plate similar to P_1', but not shown in Figure 7. By loosening the thumbscrews, T, plate P_1', carrying the mercury lamp, may be moved out of position along the groove, G, and be replaced by the plate bearing another source of light.

THE SCHLIEREN LENS. The schlieren lens, L (Figures 2 and 5), is undoubtedly the most important single element in the optical system. Upon the performance of this lens depends, in a large measure, the quality of the patterns that one obtains.

In the equipment described here the schlieren lens is an air-spaced achromat with corrections similar to those of a telescope objective, except that it is designed for use at unit magnification and allowance is made for the 30-cm. (12-inch) thickness of water in its image space.

The author's lens has a focal length of 937 mm. and a diameter, d, of 100 mm. Since this diameter is but slightly greater than the height of the channel in the cell, it is essential that this lens be uniformly illuminated. This imposes certain restrictions on the dimensions of the light source and slit that may be derived with the aid of Figure 8.

Let J be the vertical dimension of the source at I (Figure 8) over which the light intensity is constant and w the corresponding dimension of the slit at S. Since the area of the light source exposed by the slit at a given level in the plane of the lens at L is constant for all points between C and D, the illumination between these levels is uniform. In the penumbra above C and below D the light intensity decreases. If the center of the slit is taken as the origin, the coordinates at A are $-x_I$, $-J/2$ while those of B are 0, $-w/2$. The equation of line ABCi s therefore $w = (Jx - 2hx_I)/(x_I + x)$. If the schlieren lens is to be uniformly illuminated the ordinate for C at $x = x_L$ must be $d/2$ or greater. The maximum permissible slit width, w_m, is, therefore, $w_m = (Jx_L - dx_I)/(x_I + x_L)$.

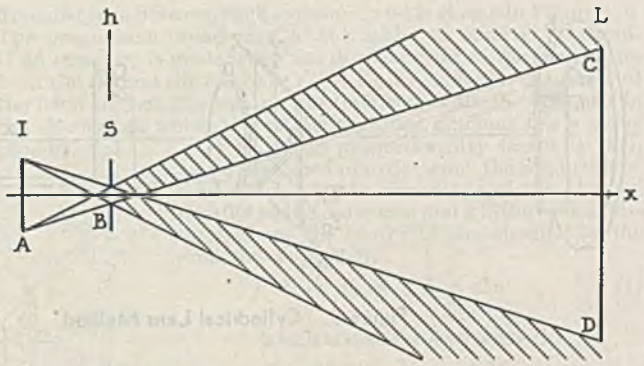

Figure 8. Uniform Illumination of Schlieren Lens

Since the bulb of the mercury lamp has a diameter of 32 mm., $x_I = 16$ mm. The value of J for the arc is about 1.5 mm. Since $d = 100$ mm. and $x_L = 1850$ mm., w_m is about 0.6 mm. Fortunately, this is greater than the optimum slit width of about 0.2 mm. and therefore affords a reserve of illumination for those occasions when the absorption or scattering by a solution is such that the other available exposure controls are inadequate. It is clear, however, that the light source must be accurately aligned with the slit and schlieren lens. This adjustment can be made after loosening the setscrews at D and D' (Figure 7).

Owing to the small horizontal dimension of a channel, 3 mm., no difficulty is experienced in achieving uniform illumination in this direction.

In order to exclude light from the camera that does not pass the channel of the cell the schlieren lens is provided with a mask as shown at M (Figure 9). Moreover, the actual cell consists of a U-shaped channel, in either side of which boundaries may be present. In the case of the cylindrical lens method the patterns formed by the boundaries in the two sides overlap. When using this method it is necessary, therefore, to mask one side when viewing the other. This is done by rotating the plate, A, on the pivots, B and B', to cover one of the slots. The mask swings on the hinges, H and H', to expose the entire lens.

THE LOW-TEMPERATURE THERMOSTAT. In order to control the temperature of the solutions in the cell it is immersed in a constant-temperature water bath, T (Figure 6). Since most work with proteins is done at a temperature of 0.5° it is essential that condensation of moisture on the thermostat windows be minimized. The construction shown in cross section in Figure 10 is effective except when the dew point approaches the room temperature.

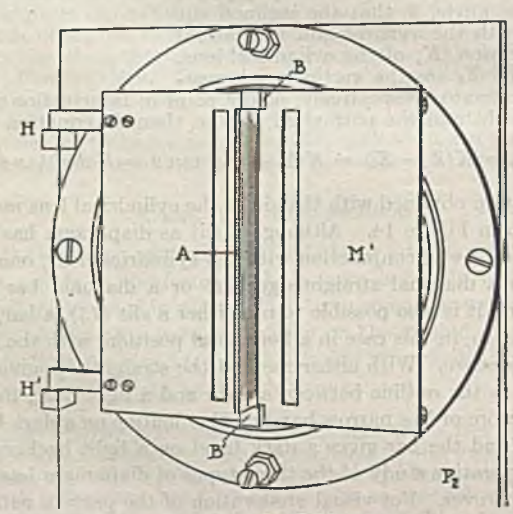

Figure 9. Mask for Schlieren Lens

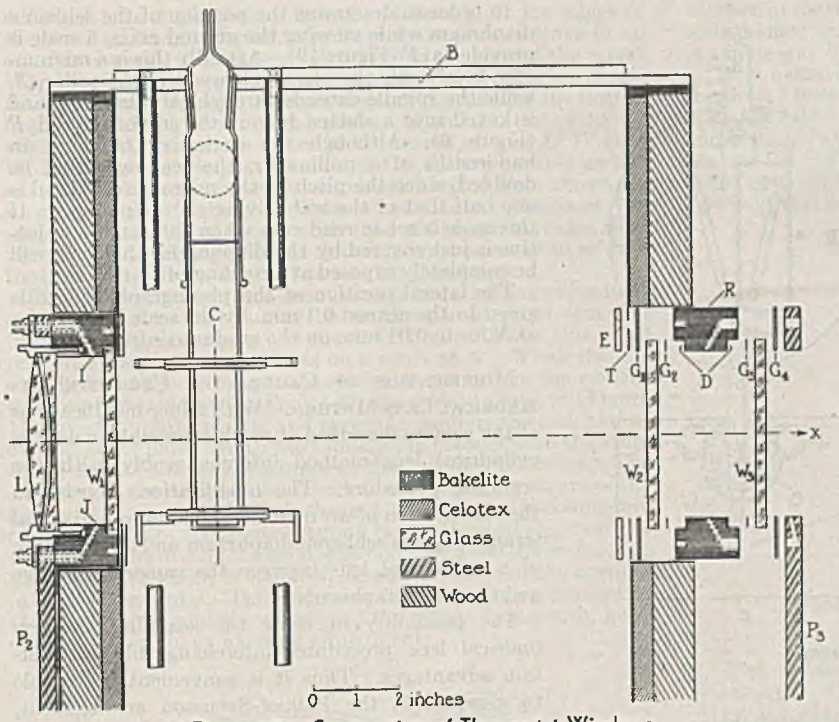

Figure 10. Construction of Thermostat Windows

In this figure the right-hand window is shown unassembled. The glass disks, W_2 and W_3, are seated against the Pliofilm gaskets, G_2 and G_3, in the recesses, D, of the Bakelite ring, R. The rubber gasket, G_1, and the tinned-copper wall of the tank, T, are then clamped between R and the brass ring, E, with the aid of machine screws threaded into the Bakelite ring, thus making the tank watertight at this window. Similarly the space between W_2 and W_3 is made airtight by clamping W_3 and gasket G_4 between R and the steel plate, P_3.

The left-hand window is similar to that on the right except that the schlieren lens, L, replaces one of the glass disks. Provision is made, at I and J for example, for the passage of dry air through the space between the elements of each window.

The Schlieren Scanning Camera. As in the case of an ordinary photographic camera, the schlieren scanning camera is provided with an objective, a means of varying its aperture, a shutter, a bellows, and a plate holder. In addition, provision must be made for a uniform vertical movement of the schlieren diaphragm that is synchronized with the horizontal movement of the photographic plate. Obviously this synchronization must be virtually complete if the ratio, M, of the plate movement to that of the diaphragm is to be a true constant. The components of the camera are described below in the order in which they appear, from left to right, in Figure 6.

The straightedge, D (Figure 6), that serves as schlieren diaphragm in the scanning procedure is shown in more detail at D in Figure 11. It is carried by the rods, R, that slide in the tubes, T. In order to couple the movement of this diaphragm with that of the photographic plate a threaded rod, A, is also attached to D and passes through a threaded bushing in the gear, G. This is driven by the gear, G', attached to the end of the shaft, F, that runs the length of the camera.

Also shown in Figure 11 is the disk, B, that is provided with a series of openings. By rotation of the disk any one of these openings may be placed in front of the objective mounted at O in plate P_4. It fulfills the same function as the iris diaphragm of an ordinary camera. The shapes of the openings are such, however, that they provide a control of the exposure without restricting the lens aperture vertically, thus ensuring that an opening will not act as a schlieren diaphragm.

On the same axis with disk B, but rotating independently of it, is a second disk, C, that is provided, in one quadrant, with a circular hole matching the lens aperture and, in the opposite quadrant, with an adjustable, sector-shaped opening. Driven through spur gears by a small synchronous clock motor, not visible in the figure, this disk serves as the camera shutter. When viewing the pattern on the screen, or photographing it with the scanning device, this shutter is not used, the circular hole in it being left directly in front of the lens. In the photography of schlieren bands and cylindrical lens patterns the sector opening is adjusted to give the proper exposure. (This adjustment is made after loosening, with the aid of a small spanner wrench, the threaded collar that clamps the two leaves of the sector.)

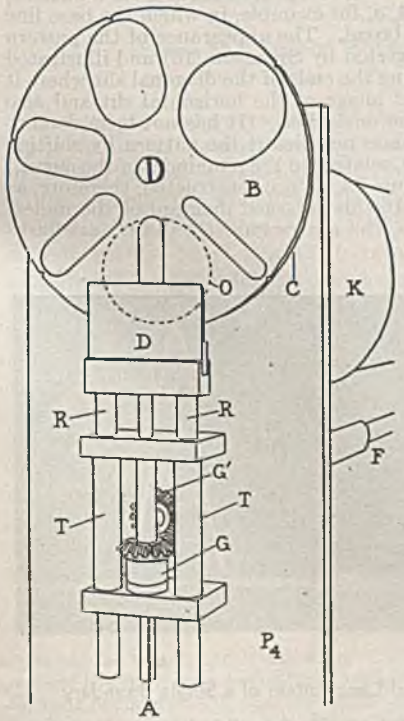

Figure 11. Schlieren Diaphragm

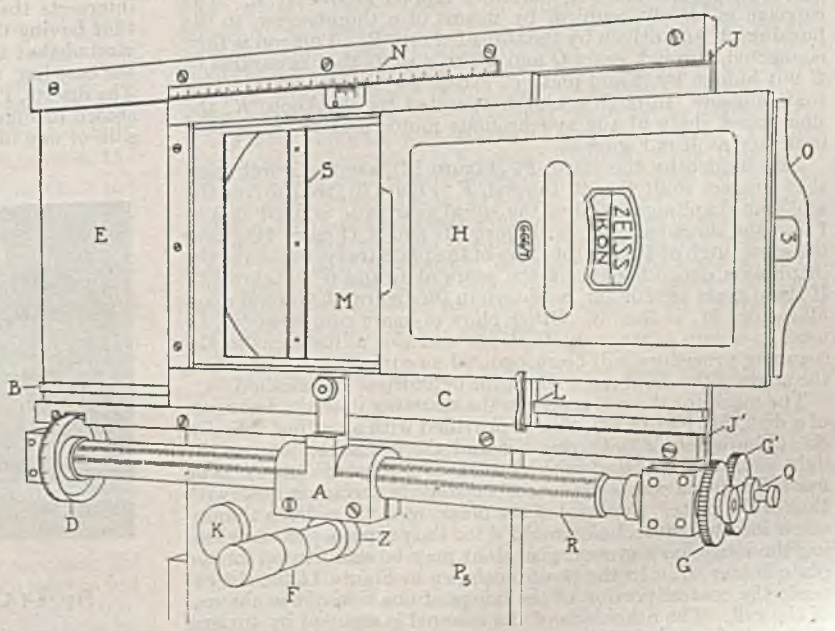

Figure 12. Plate Carriage

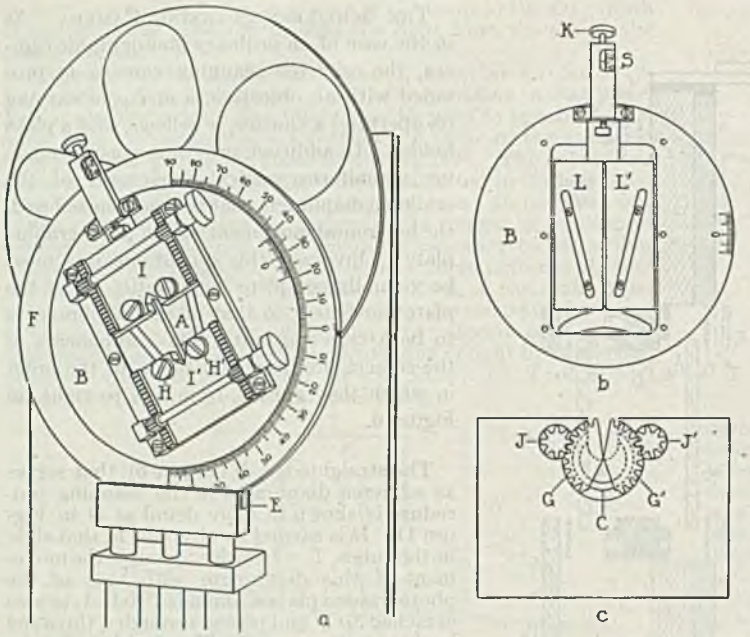

Figure 13. Inclined Slit Diaphragm

The shutter motor is started, and the dark slide of the plate holder removed as an opaque quadrant of the shutter disk moves across the lens. The quadrant containing the open sector then crosses the face of the lens and makes the exposure, after which the dark slide is reinserted as the second opaque quadrant cuts off the light. On completion of the revolution the circular opening is again in front of the lens.

The camera objective, *O*, is a 50-mm. achromat with the same focal length, *f*, and corrections as the schlieren lens. Since it is used at unit magnification the length of the aluminum tube, *J* (Figure 6), that serves as a "bellows" is approximately 2*f*. Since it is not practicable to specify exactly this focal length, some adjustment in the length of the camera is essential. This is provided by the collar *K* (Figure 11), into which one end of the tube slides.

The arrangement for moving the photographic plate horizontally, as the schlieren diaphragm is raised vertically, is shown in Figure 12. The holder, *H*, for 9 × 12 cm. plates is shown partially inserted into the plate carriage, *C*. Although this carriage is provided with flanges that move in slots, *J* and *J'*, these function merely as light baffles, the clearance being sufficient to ensure that the horizontal alignment is maintained solely by the bar, *B*, that fits into the carefully lapped groove at *L*. The carriage is loosely coupled, by means of a thumbscrew, to the bushing, *A*, and driven by the threaded rod, *R*. This rod is then connected, through gears *G* and *G'*, to a shaft that is parallel to *R* but hidden by it and plate P_5 except for the end at *Q*. This shaft engages, through a clutch operated by the knob, *K*, the slow-speed shaft of the synchronous motor and reducing unit indicated at *M* in Figure 6.

Also hidden by the plate, P_5 (Figure 12), are the spiral gears that connect shaft *Q* with the rod, *F* (Figure 6), that drives the schlieren diaphragm. Since the spiral gear ratio is fixed at 3 to 1 and the threaded rods, *R* (Figure 12) and *A* (Figure 11), have the same pitch of 1 mm., the ratio of the plate travel to that of the diaphragm depends only on the gears at *G* and *G'* (Figure 12). If these gears are similar, as shown in the figure, the lateral magnification, *M*, is then 3. Other pairs of gears can, however, be used to obtain other magnifications and this adjustment in the scanning procedure will be recognized as equivalent to changing the angle of the diaphragm slit in the cylindrical lens method.

The mask for the cell image in the scanning procedure consists of a disk, *M* (Figure 12), that is provided with an adjustable slit, *S*. (The width of both this slit and the horizontal one at the light source is adjusted with the aid of a "feeler" gage.) The mask fits into a recess in plate *E*, so that its surface is flush with that of the plate. Removal of the mask, with the aid of a thumbscrew inserted into a hole provided for the purpose, permits viewing the image on a ground glass that may be substituted for the plate holder, *H*. In the position shown in Figure 12 the slit exposes the central portion of the image of one side of the channel of the cell. The other side of the channel is exposed by turning the mask through 180°.

In order to determine the position of the schlieren diaphragm while viewing the ground glass, a scale is provided at *F* (Figure 12). Actually this is a micrometer head with the shank clamped in the collar, *Z*, while the spindle extends through the plate, P_5, and is keyed into a slotted hole in the end of the rod, *F* (Figure 6). Although the scale is graduated in hundredths of a millimeter, the readings must be doubled, since the pitch of the micrometer thread is one half that of the rod driving the diaphragm. If this scale is set to read zero when the camera objective is just covered by the diaphragm, that lens will be completely exposed at a reading of 25 mm.

The lateral position of the photographic plate is given to the nearest 0.1 mm. by the scale and vernier at *N*, or to 0.01 mm. on the graduated drum, *D*.

Modification of Camera for Use with Cylindrical Lens Method. With minor modifications of the camera described above it is possible to use the cylindrical lens method interchangeably with the scanning procedure. The modifications consist in the substitution of an inclined slit for the horizontal straightedge as schlieren diaphragm and the insertion of a cylindrical lens between the camera objective and the photographic plate.

The possibility of using the scanning and cylindrical lens procedures interchangeably has certain advantages. Thus it is convenient to be able to view, as in the Philpot-Svensson arrangement, the pattern directly on the ground glass. The small errors introduced by the uncorrected cylindrical lens are of no consequence in such a visual inspection and may be eliminated by shifting to the scanning process in obtaining the permanent photographic records of an experiment.

In using the cylindrical lens method the straightedge, *D* (Figure 11), is replaced by a diagonal slit as shown at *A* in Figure 13, *a*. Although not visible in this figure, two pins in the groove at *E* aid in fixing the position of the diaphragm.

The angle of the slit is adjusted by rotating the disk, *B*, in the frame, *F*. Adjustment of the length of the slit may be made by moving the inserts, *I* and *I'*, with the aid of the racks and pinions. As shown in Figure 13, *b*, which is a diagram of the disk, *B*, with the inserts removed, the slit width is controlled by turning the knob, *K*, and is given directly on the scale at *S*.

With many boundaries a slit of uniform width gives a pattern, such as that of Figure 14, *a*, for example, in which the base line and top of the peak are broad. The appearance of the pattern can be improved, as suggested by Svensson (*16*) and illustrated in Figure 14, *b*, by tapering the ends of the diagonal slit where it intersects the undeviated image of the horizontal slit and also that having the maximum deviation. (It has not been demonstrated that this device does not distort the pattern by shifting the base line, for example, relative to the remainder of the curve.) The inserts, *I* and *I'* (Figure 13, *a*), are constructed, therefore, as shown in Figure 13, *c*. In this enlarged diagram of the underside of one of the inserts, the gear sectors, *G* and *G'*, are flush-

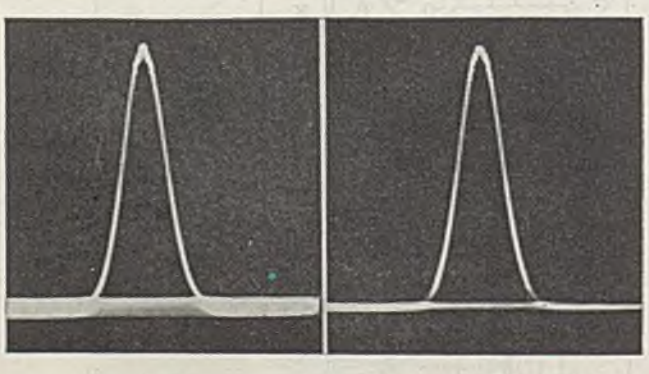

Figure 14. Cylindrical Lens Pattern of a Single Boundary

a. θ = 45°, 1-mm. slit with parallel edges
b. θ = 45°, spindle-shaped slit

mounted. As suggested by Burns and Henke (*2*) the edges of the taper are made co-planar by having the sectors move in an annular channel, *C*, that is concentric with the apex of the taper. When the insert is in position in the assembled slit (Figure 13, *a*), the sectors may be adjusted independently by turning the slotted heads, *H* and *H'*, to which the pinions, *J* and *J'* (Figure 13, *c*), are attached. Since the edges of the leaves, *L* and *L'* (Figure 13, *b*), that form the parallel sides of the slit are beveled underneath while those of the gear sectors are beveled from above, all six edges of the spindle-shaped slit are co-planar. Moreover, the plane of the slit is identical with that of the straightedge with which it is interchangeable, so that it is not necessary to refocus the illuminated horizontal slit on making this change.

Since the cylindrical lens is not used in the scanning procedure, it is mounted so that it may be readily placed in, or removed from, the optical system. As shown in Figure 15 this lens, *C*, is held in a frame, *F*, that pivots on a shaft at *A*. When this assembly is inserted through a rectangular opening in the camera tube, as indicated at *N* (Figure 6), the curved plate, *P'* (Figure 15), overlaps this opening and serves to support the lens and to exclude stray light. With the frame, *F*, in contact with the stop, *S*, the face of the lens is normal to the axis of the camera. The diameter of the camera tube is such that rotation, by means of the knob, *K*, of the cylinder lens through 90° removes it completely from the path of the image-forming rays. Vertical alignment of the lens is afforded by the small clearance between the ends of the bar, *B*, one of which is visible at *E*, and the sides of the opening in the camera tube. The dimensions of this opening parallel to the camera axis and the overlap of the plate, *P*, are such, however, as to provide a focusing adjustment.

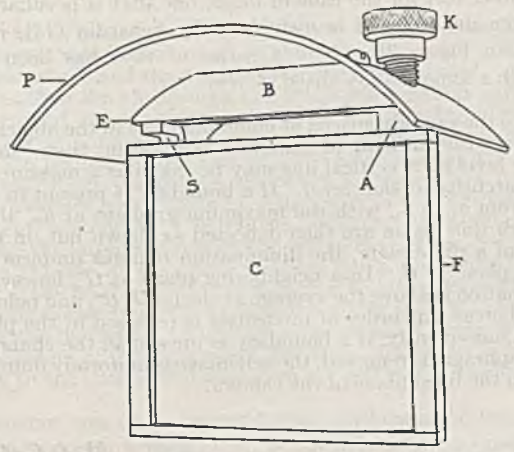

Figure 15. Support for Cylindrical Lens

This completes the description of the optical equipment. Owing to the size of the apparatus it is usually assembled where it is to be used and the responsibility for the alignment and focusing of the components therefore falls upon the investigator. In making these adjustments the chief object is to obtain a sharply defined, vertical base line in the focus of the camera, so that the pattern will be due solely to changes of refractive index in the boundaries and not to aberrations elsewhere in the system. Since imperfect base lines can arise from inadequately corrected or improperly focused lenses, or from schlieren in the windows of the thermostat and moving boundary cell, it is essential to test each of these in turn in order to locate the origin of the distortion, and, if possible, to correct for it. In the tests described below the equipment in the author's laboratory is taken as a typical example.

INSTALLATION OF EQUIPMENT

Testing and Focusing the Schlieren Lens. The schlieren camera is well adapted for testing the schlieren lens.

With the water and windows of the thermostat removed, the axes of the camera and schlieren lens are brought into coincidence and the horizontal slit, provided with an appropriate filter, is centered on their common axis. [The axis of a lens may be found by bringing the images of a light source that are reflected from the surface of the lens, four in the case of an air-spaced achromat, into coincidence with the source itself. Campbell (*3*) has described a simple way to do this.]

It is convenient to make the tests with the cylindrical lens method. Both the horizontal and diagonal slits are nearly closed and the latter is set at an almost horizontal angle. Svensson suggests (*17*) $\theta = 85°$. If the lateral magnification factor, *K*, due to the cylindrical lens is 2.05, as in the equipment described here, a vertical displacement, ΔY, at the diaphragm is converted into a lateral displacement on the screen of

$$\Delta N = K \times \tan\theta \times \Delta Y = 24\ \Delta Y \qquad (3)$$

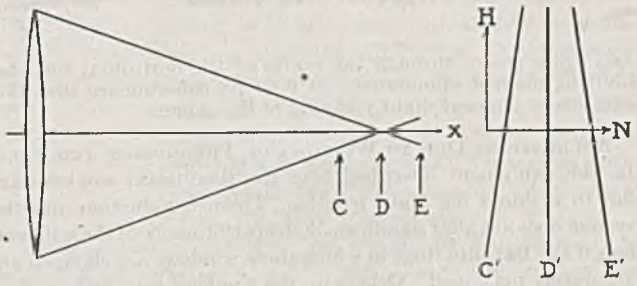

Figure 16. Test for Focus of Illuminated Slit

If the schlieren lens has no spherical aberration, all the (monochromatic) rays come to focus at *D* as indicated in Figure 16. With the diagonal slit at this position a straight vertical line is obtained on the screen as shown at *D'*. Deviations from the vertical become apparent on bringing this line into coincidence with a vertical ruling on the ground glass. Within the limits of error of this test the author's lens is free from spherical aberration. (All the lenses in the electrophoresis equipment at The Rockefeller Institute were made by the Perkin-Elmer Corporation, Glenbrook, Conn.)

A sensitive method for locating the plane, *D*, of the schlieren diaphragm will also be apparent from Figure 16. If, for example, the diaphragm were placed at *C*, the rays from successive horizontal sections of the lens would pass the inclined slit at positions progressively displaced laterally and the line image on the screen, although remaining straight, would now be inclined to the vertical as shown at *C'*. If the diaphragm were placed at *E* the image, *E'*, would have the opposite inclination. In this manner the focal plane of the schlieren lens can be readily located to the nearest millimeter and by shifting the illuminated slit along the axis can be brought into coincidence with the diaphragm.

Figure 16 also serves to illustrate a method for determining the longitudinal chromatism of the lens. Suppose the illuminated slit, provided with a yellow filter, is focused on the diaphragm at position *D*. The yellow line on the ground glass is then straight and vertical. On replacing the yellow by the green filter the straight green line on the screen is now inclined to the vertical as though the diaphragm were at *E* with yellow light. The focus for $\lambda = 5461$ is, therefore, nearer the lens than for $\lambda = 5780$. Since ΔN, the deviation of the green line from the vertical, is 2 mm., $\Delta Y = 0.08$ mm. From similar triangles the displacement of the focus along the axis is $1820\ \Delta Y/100 = 1.5$ mm. The displacement for $\lambda = 4359$ is 3.2 mm. With air in the thermostat the lens is thus undercorrected chromatically. This compensates for the effect of the 30-cm. (12-inch) thickness of water in the image space of the schlieren lens when the thermostat is filled. Under the actual working conditions the chromatic correction of the author's lens is essentially complete. If the windows of the thermostat and cell do not introduce chromatic aberrations, the illuminated slit does not need to be refocused on shifting from one (visible) wave length to another.

In the case of the schlieren lens the only other aberration that needs to be considered is coma (*6*).

The schlieren camera can be used most efficiently if the illuminated horizontal slit, *S* (Figure 2) is placed some 15 to 20 mm. below the camera axis, so that the normal slit image is above that axis. As shown in Figure 17, the peripheral rays then come to focus in the same plane as those through the central portion of the lens but at a different level. The resulting pattern on the screen is shown at *D'* and the deviation from the vertical, ΔN, is a measure of the comatic blurring, ΔY. With the illuminated slit 16 mm. below the axis, ΔN is 2.4 mm., so that $\Delta Y = 0.1$ mm. from Equation 3. By tilting the schlieren lens slightly, so that its

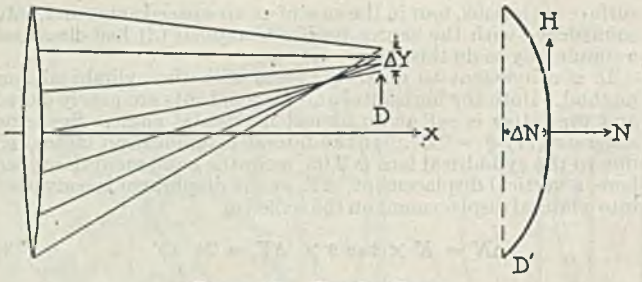

Figure 17. Test for Coma

axis again passes through the center of the horizontal slit, this blurring may be eliminated. It is of no consequence that this axis is now inclined slightly to that of the camera.

Aberrations Due to Windows of Thermostat and Cell. In the equipment described here the thermostat windows are flat to within a few wave lengths. Their introduction into the system does not alter significantly the performance of the schlieren lens if the Bakelite rings in which these windows are clamped are accurately machined. Otherwise the windows may be bowed so as to introduce a form of astigmatism into the system. This is most serious at the windows in contact with the water and is frequently not detectable until the thermostat is filled. If this form of astigmatism is present, the image of the illuminated slit is displaced along the axis as this slit is turned from the horizontal position and is in sharp focus only when the slit is parallel to the axis of the weak cylindrical lens that the bowed window simulates. When viewed, with the aid of the Philpot-Svensson arrangement, on the ground glass of the schlieren camera, astigmatism appears as a blurring of the central portion of the vertical line. If this aberration cannot be eliminated by truing the Bakelite rings, it can be reduced by making the axis of the bowed window horizontal.

Even with vigorous stirring of the thermostat water, localized temperature fluctuations are usually sufficient to be detectable as weak schlieren. The fluctuations are rapid, however, in comparison with the usual exposure interval and thus cancel.

In spite of the progress that has been made in their construction, the windows of the moving boundary cell remain the chief cause for crooked base lines. The optical imperfections in these windows frequently simulate a spherical error in the schlieren lens and may be interpreted with the aid of Figure 18.

Let B, C, and E be the points on the axis at which axial, intermediate, and peripheral rays, respectively, come to focus. With the cylindrical lens method the curves B', C', and E' are the corresponding patterns on the screen. The best position for the diaphragm is at D; in this position the average deviation of the curve from the vertical is a minimum.

In addition to distorting the base line, the imperfections in the cell windows also frequently introduce astigmatism and thus blur this line. The scanning method has an advantage in this regard, since it uses only a narrow vertical section at the center of the channel image where the distortion is usually a minimum.

Focusing the Camera. A 100-mm. precision glass scale (the Zeiss scale No. 126,454 is satisfactory) that may be immersed in water is almost indispensable in the focusing of the camera.

With this scale mounted in the support for the moving boundary cell so that the graduations are in plane C (Figure 10), this support is placed in the thermostat. The approximate position of the object plane for the camera can then be determined by examining the scale image on the ground glass as the support is moved along the optic axis in the thermostat. If, now, a low-power microscope is focused on the ground glass and the image of a scale line examined directly in the microscope after removal of that glass, no difficulty should be experienced in locating the object plane to the nearest 1 or 2 mm. As a check, photographs may be taken with the scale at different positions along the axis in the neighborhood of the object plane and the photographs examined under the microscope. The one with the scale in the object plane will have the lines most sharply defined and, with the aid of a comparator, can be used to determine the enlargement factor, E.

Examination of this scale photograph also indicates that the distortion introduced by the camera objective in The Rockefeller Institute equipment is negligible. Although some comatic blurring of the lines at the edges of the field is apparent, the interval between the lines remains uniform within the limits of error, 5 microns, of the comparator readings. This result might be expected in view of the fact that the camera objective is similar to the schlieren lens and is used at a smaller aperture.

Since it is convenient to have the enlargement factor unity, it is usually necessary to adjust the length of the camera. If the image distance is v' when the enlargement is E and v when $E = 1$, the adjustment is $v' - v = (E - 1)f$, in which f is the focal length of the objective. This follows from the lens formulas, $v/u = E$ and $1/u + 1/v = 1/f$, in which u is the object distance. Since the adjustment is small, f is given with adequate precision by the relation $f = v'/(E + 1)$.

Owing to the convergence of the light after it leaves the schlieren lens, the cell, to be uniformly illuminated, must be placed close to this lens. With the camera adjusted so that $E = 1$ it is moved as a unit along the axis until its object plane coincides with the plane through C (Figure 12). It will also be apparent that these adjustments must precede the focusing of the horizontal slit on the schlieren diaphragm.

Another test for the camera focus, one that is peculiar to the schlieren method and is mentioned by Schardin (*13*), is illustrated in Figure 19, where a series of rays has been traced through a somewhat idealized system.

Since the rays are spaced at equal intervals in the object plane, C, where the incident illumination is uniform, their "density" at any level on a vertical line may be taken as a measure of the light intensity at that level. If a boundary is present in the region from h_1 to h_2, with the maximum gradient at h_m, the rays through this region are then deflected as shown but, in the absence of a diaphragm, the illumination remains uniform in the image plane at G. In a neighboring plane at G', however, the illumination is above the average at the level, H', and below it at H'', whereas this order of intensities is reversed in the plane at G''. Consequently, if a boundary is present in the channel and the diaphragm is removed, the cell image is uniformly illuminated only in the focal plane of the camera.

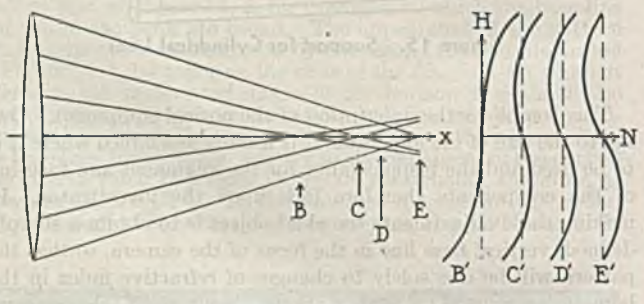

Figure 18. Effect of Spherical Aberration

This test for the camera focus is surprisingly sensitive if the boundary is sharp—i.e., one for which the refractive index difference, Δn, is small and the maximum gradient, n'_m, is large. It is preferable to have n'_m not so large, however, that the lens mount acts as a schlieren diaphragm. With such a boundary in the channel a movement of the ground glass by as little as 1 mm. from the focal plane will produce the effect.

It has already been noted that improper focusing of the horizontal slit on the schlieren diaphragm leads to a pattern with a sloping base line. An improperly focused camera leads, on the other hand, to tilted peaks in the pattern. Thus, if the plate were placed at G' (Figure 19) the maximum gradient in the boundary would appear to be at level H'_m, whereas its true position in the now slightly enlarged image is at H_m. Since the displacement,

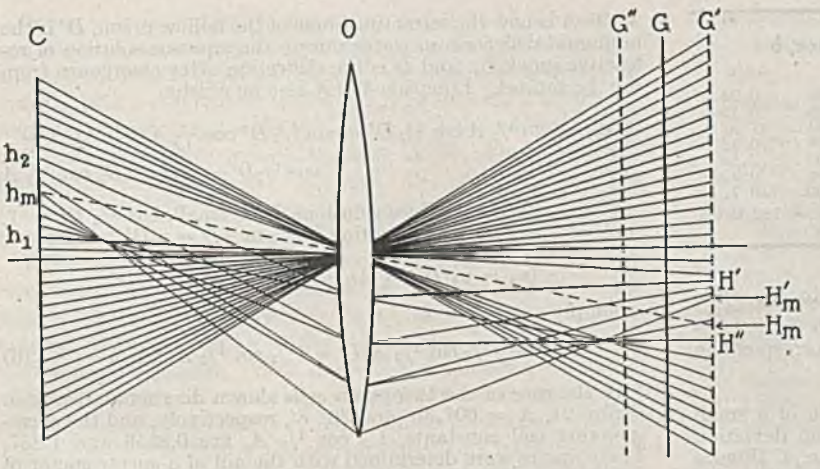

Figure 19. Test for Camera Focus

$H'_m - H_m$, is proportional to the deflection the ray undergoes in the boundary, the resulting peak in the pattern is tilted.

The effects of improper focus of the horizontal slit and of the schlieren camera are illustrated in Figure 20. In this example the boundary was due to a 2% raffinose solution diffusing into water.

In order to demonstrate the symmetry of the gradients in this boundary it was first scanned, as shown in Figure 20, *a*, with the illuminated slit and the camera both properly focused. A white line was then drawn through the maximum gradient parallel to the recorded black reference line. The symmetry about this white line is apparent. Moreover, this symmetry was not lost when the boundary was next scanned after moving the illuminated slit 10 cm. toward the thermostat, Figure 20, *c*. In this figure, however, the sloping base line, due to the improperly focused slit, is evident. Finally, the boundary was photographed with the slit in focus but with the cell moved 10 cm. toward the camera (Figure 20, *b*). Here the base line is horizontal but the tilting of the peak, because the camera is no longer focused on the cell, is apparent from the asymmetry about the white line that is drawn as in *a* and *c*. Although some diffusion occurred during the recording of these patterns, the lower maximum in *b* is due mainly to the shorter distance from the cell to the diaphragm.

Focusing the Cylindrical Lens. Although the use, in the Philpot-Svensson arrangement, of an achromatic cylinder lens would be desirable, only simple lenses have thus far been employed. The one used by the author is a 75 × 75 mm. plano-cylindrical lens with a focal length of 416 mm. Since it is free from a defect sometimes encountered in lenses of this type—namely, the superposition of a spherical component on one of the faces—its introduction into the system does not appreciably shift the image plane of the camera. This lens may be aligned and focused as follows:

If a contact print of the glass scale is made on a photographic plate the rulings will appear in the print as transparent lines on an opaque background. If this print is then placed in the plane of the schlieren diaphragm so that the rulings are vertical, the intersection of each ruling with the image of the illuminated horizontal slit becomes essentially a point source of light in the object plane of the cylindrical lens. Since a point in the object plane of such a lens is brought to focus in the image plane as a line parallel to the axis of the cylinder, a grid of parallel lines will appear on the ground glass of the camera. By rotation of the camera tube the axis of the cylindrical lens, and hence the lines on the screen, can be made vertical. As in the case of the camera objective, this lens may be focused by observing these lines, with the aid of a microscope, as it is shifted along the camera axis. The aberrations introduced by this uncorrected lens will be apparent, however, in that no setting can be found in which all the lines are uniformly sharp.

Blurring due to the lack of correction in the cylindrical lens is not to be confused with that which arises when this lens is not normal to the camera axis. The vertical alignment is not critical but small deviations of the cross-axis of this lens from the normal lead to a blurring that becomes progressively worse from one line to the next. A setscrew in the stop at *S* (Figure 15) is provided for making this adjustment.

The errors introduced by the cylindrical lens are due chiefly to the uncertainty in the position of a blurred line. The magnitude of this uncertainty may be obtained by photographing the grid with the slit image at the axis and 15 mm., say, above and below the axis, respectively. The spacings, at different levels, between the lines on each of the three plates are then determined and are found, in the case of the lens used by the author, to be 10.24 mm. with an average deviation from the mean of 0.01$_6$ mm. The maximum deviations that occur near the edge of the field are as great as 0.06 mm., however, and, until corrected cylindrical lenses become available, render this method unsuitable for precise work. Since the spacing of the points in the real scale is 5 mm., the lateral magnification, *K*, is 2.048.

Determination of Constants *a* and *b* of Equation 1. As is clear from Equation 1, $\Delta Y = abn'$, the dimension, *a*, of the channel parallel to the optic axis and the optical distance, *b*, from the center of that channel to the schlieren diaphragm should be known with a precision equal to that with which the slit image displacement, ΔY, can be determined. Under favorable conditions this displacement can be measured to 0.02 mm. and if it has the maximum value of about 4 cm. permitted by the camera the corresponding error is 0.05%. This precision in the determination of *a* and *b* may be obtained as follows:

The value of *a* near the top and bottom of the channel is determined to 0.01 mm. with the aid of an inside vernier. The following procedure, suggested by D. A. MacInnes, may be used at other levels.

With the center section of the cell in a horizontal position a brass bar whose length, *l*, is slightly less than *a*, and whose ends are flat and parallel, is inserted in the channel with one end resting on what is now the bottom window. With the aid of the micrometer adjustment the difference, $a - l$, is determined by focusing a microscope alternately on the upper end of the bar and on dust particles on the inside wall of the top window. This is repeated as the bar is shifted along the channel. The dimensions of the bar used by the author are 2.475 × 0.95 × 0.27 cm. In this manner the values 2.509, 2.508, 2.507, 2.507, 2.506, 2.506, and 2.507 cm. were obtained at equally spaced intervals along a typical channel. Since the average deviation from the mean value of 2.507 is only 0.03%, *a* may be taken as independent of *h* in this instance. The value of *a* for the other side of the channel in the same cell was also essentially constant and equal to 2.512 cm.

One method for determining the optical distance, *b*, is illustrated in Table I. In the first column is listed each successive

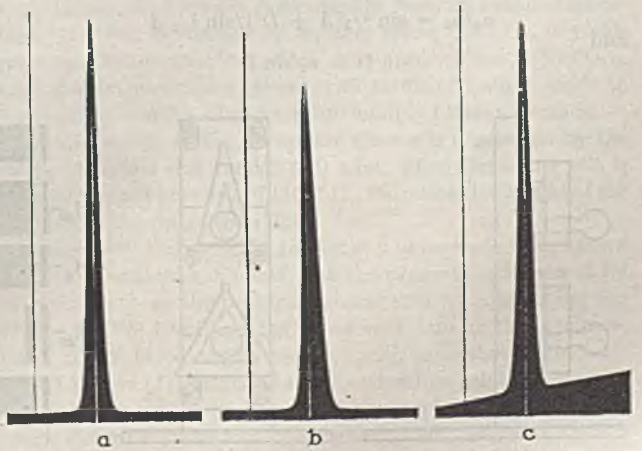

Figure 20. Effect of Improper Focusing of Illuminated Slit and Camera

Table I. Determination of the Optical Distance, b

Element	Δx	n	$\Delta x/n$
$a/2$	1.25	1.334	0.94
Cell window	0.30	1.526	0.20
Cell window to inside thermostat window	23.7$_1$	1.334	17.8$_3$
Inside thermostat window	0.63	1.526	0.42
Air space in double window	2.54	1.000	2.54
Outside thermostat window	0.63	1.526	0.42
Outside thermostat window to diaphragm	159.7$_1$	1.000	159.7$_1$
		b =	182.0$_6$ cm.

element in the path while its dimension, parallel to the optic axis, is given in the second column. The factor, b, is then the sum of these distances after division of each by the corresponding refractive index, column 3, referred to air as unity.

It is also convenient to determine b with the aid of a small glass prism. With the prism oriented for minimum deviation (*5*), it is placed in the object plane of the camera—i.e., C (Figure 10)—and forms at the schlieren diaphragm an image of the illuminated slit that is displaced ΔY cm. below the normal slit image. A crown-glass prism of about 6-diopter power is suitable if the prism angle, A, and the minimum deflection in air, D'', for λ = 5769–5790 are known to within 2″ of arc. (Prisms of this type, acceptable for calibration by the Bureau of Standards, Washington, D. C., may be obtained from the Maberg Optical Co., New York, N. Y., and from the Perkin-Elmer Corporation, Glenbrook, Conn.) The formula $n_G = \sin {}^1/_2 (A + D'')/\sin {}^1/_2 A$, may then be used to compute n_G, the refractive index of the glass. Since the refractive index of water, n_0, is 1.33431 (*18*) at 0.5°, whereas n_G is essentially the same as at room temperature, the deflection in water, D', is $D' = -A + 2\sin^{-1}(n_G \sin {}^1/_2 A/n_0)$. After refraction at the water-air interface the deflection, D, outside of the thermostat is $D = \sin^{-1}(n_0 \sin D')$ and $b = \Delta Y/D$. The value of b obtained in this manner, 182.0$_2$ cm., is in good agreement with that measured directly, 182.0$_6$ cm., Table I, and is probably the more reliable since the prism method tends to compensate for imperfections in the windows.

Measurement of Refractive Index Differences. In order to minimize the disturbing effects of convection in the electrophoresis of protein solutions, and also to reduce the growth of microorganisms, most work on these solutions is done near 0° C. The quantitative interpretation of the patterns has been hampered, however, by the lack of precise refractive index measurements on solutions at this temperature. When used in conjunction with hollow prism cells the optical equipment described here is well adapted for such measurements and this report concludes with a description of this application of the apparatus.

Since the hollow prism is immersed in water no deflection, except that due to imperfections in the prism windows, is observed when it is also filled with water. The deflection that occurs when a dilute aqueous solution is introduced into the prism cell is due to the difference, Δn, between the refractive index of the solution, n_s, and of water, n_0. The method is thus a differential one.

As in the determination of the optical distance, b, with the aid of the glass prism the relations are

$$n_s/n_0 = \sin {}^1/_2(A + D')/\sin {}^1/_2 A \qquad (4)$$

and

$$\sin D = n_0 \sin D' \qquad (5)$$

where A is now the refracting angle of the hollow prism, D' is the minimum deflection in water due to the aqueous solution of refractive index, n_s, and D is the deflection after emergence from the thermostat. Equation 4 may also be written

$$n_s/n_0 = (\sin {}^1/_2 A \cos {}^1/_2 D' + \sin {}^1/_2 D' \cos {}^1/_2 A)/\sin {}^1/_2 A = \cos {}^1/_2 D' + \sin {}^1/_2 D' \cot {}^1/_2 A$$

With sufficiently dilute solutions D' is small, $\cos {}^1/_2 D' = 1$, $\sin {}^1/_2 D' = {}^1/_2 D'$ and Equation 5 becomes $D = n_0 D'$, so that

$$(n_s - n_0)/n_0 = \Delta n/n_0 = ({}^1/_2 \cot {}^1/_2 A)D'$$

or simply

$$\Delta n = ({}^1/_2 \cot {}^1/_2 A)D = ({}^1/_2 \cot {}^1/_2 A)\,\Delta Y/b \qquad (6)$$

In the case of the two prism cells shown diagrammatically in Figure 21, A = 60° 36′ and 30° 8′, respectively, and the corresponding cell constants, ${}^1/_2 \cot {}^1/_2 A$, are 0.8556 and 1.857. These angles were determined with the aid of a spectrometer of ordinary precision after filling the cells with mercury. For an accuracy of 0.05% in the cell constant a 60° angle needs to be known to the nearest 90″ and a 30° angle to the nearest 50″. With the 60° cell an uncertainty of 0.02 mm. in ΔY corresponds to an error of 1×10^{-5} in Δn and the precision of the method thus compares favorably with that of the other available procedures (*1*). It may also be shown that within this limit of error the approximate Equation 6 is valid if ΔY does not exceed 2 cm. With a 60° cell and b = 182 cm. this corresponds to a 5% protein solution. Similarly, if the bisector of the refracting angle is vertical, the error introduced by the difference between the actual deviation and the minimum deviation will not exceed 1×10^{-5} in Δn.

As is shown in Figure 21, *a* and *b*, the two prism cells are supported in such a way as to be interchangeable with the Tiselius cell at C (Figure 10). Although the 60° cell is ordinarily used for dilute solutions and the 30° cell for more concentrated ones, the scanning photograph (Figure 21, *d*) was obtained with the same solution in both cells in order to illustrate not only the experimental procedure but also a test of the method. The solution in this instance was approximately 1 N barium chloride while Figure 21, *c*, was recorded on the same plate with water in the cells. The print (negative) is mounted here so that the height, H, corresponds to that at the cells. The gaps between the exposed areas are due to the opaque cemented joints of a cell.

The right-hand edge of each exposed area in Figure 21, *c* and *d*, is the value of N as a function of H. The displacement of this edge at the level of a cell from that above or below is proportional to the slit image displacement, ΔY, at the schlieren diaphragm, the factor of proportionality being the ratio, M, of the plate movement to that of the diaphragm in the scanning process. If the system were optically perfect and the water in the cells identical with that in the thermostat, the right-hand edges in *c* would all lie on a vertical line. Since this ideal condition is not realized, especially with the 30° cell, correction must be made by subtracting the displacement that occurs with water in a cell from that when solution is present. With the aid of a two-coordinate comparator the value of N in *c* at some level conjugate to the 30° cell, say H_{30}, is determined relative to a value of N outside the cell, say at H_0, as zero. This value of N may be designated N^{30}_0. In Figure 21, *d*, the value of N at the same level, H_{30}, relative to that at H_0, is also determined and is designated N^{30}. The difference, $N^{30} - N^{30}_0$, is then equal to $M\,\Delta Y$ and leads, in the present example, to Δn = 0.01574$_8$. Similar measurements at some level conjugate to the 60° cell, say H_{60}, give the closely agreeing value of Δn = 0.01574$_3$, thus indicating that the effect of varying the refracting angle, A, is given correctly by Equation 4.

Accidental errors in the location of the edge can be reduced by taking the average of readings at values of H conjugate to other levels both inside and outside a cell. Although the water with which a prism cell is filled in determining N_0 should be the same as that used in preparing the solution, the purity of the thermostat water, on the other hand, is not critical.

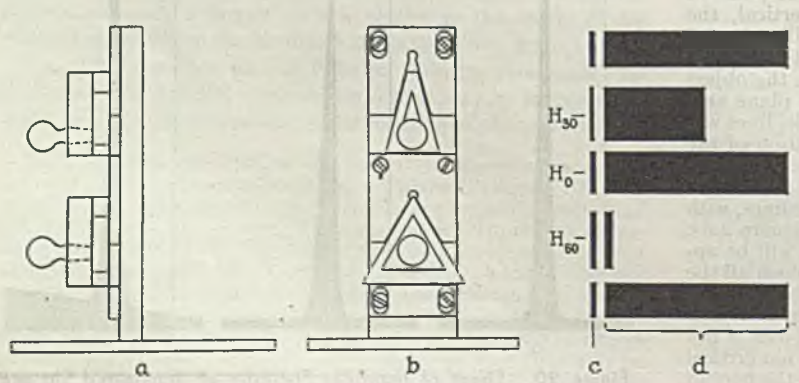

Figure 21. Determination of Differences of Refractive Index

Owing to imperfections in the optical system both the normal and deviated slit images are less sharply defined than the illuminated slit itself. If the slit image displacement were measured directly with a micrometer ocular, say, this blurring represents a source of uncertainty that is eliminated with the schlieren scanning camera since this permits, in effect, the use of images formed by selected, small portions of the field. The prism cell specifications are thus not so exacting as in the conventional spectrometer methods.

While the use of the cylindrical lens method for the direct determination of Δn cannot be recommended, it can be used to advantage to determine the dispersion due to the solute—i.e., the differences $\Delta n(\lambda_1) - \Delta n(\lambda_2)$, in the refractive index increment for different wave lengths. With a solution of sufficient concentration in the prism cell to give a good deflection, the horizontal and inclined slits are both nearly closed, the filter is removed, and the separation of the lines of the mercury spectrum in the focus of the schlieren camera is measured with the aid of a micrometer ocular. If Δn for the yellow lines, say, has been determined independently by the scanning procedure, the values of Δn for the red, green, blue, and violet lines can then be estimated with adequate precision from the respective separations between these and the yellow lines.

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Deproteinization by Metaphosphoric Acid

A Review of Its Value for the Analyst

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A review of the literature on deproteinization by metaphosphoric acid is presented. Metaphosphoric acid is accepted as the most generally reliable deproteinizing agent for determining ascorbic acid in plant and animal materials, and is of value for determining lactose in milk and deproteinizing clinical specimens. It can also be used for isolation and quantitative determination of protein in agricultural and other products.

WHILE there exists today a need for a reliable deproteinizing agent for biological specimens in general which would be readily available, easy to handle, and free from objectionable effects on the constituents to be determined in the filtrate, comparatively little attention has been given to the valuable properties of metaphosphoric acid and its sodium salts, except for ascorbic acid determination.

Although a large number of publications dealing with deproteinization by metaphosphoric acid are to be found in foreign journals, there is a scarcity of coordinated authentic information on metaphosphoric acid and metaphosphates

EARLY WORK

While metaphosphoric acid, HPO_3, can be prepared by combining phosphorus pentoxide with water or with orthophosphoric acid, H_3PO_4, the product is a highly viscous fluid, impractical for use. Where investigators mention metaphosphoric acid without giving particulars, they refer usually to the so-called glacial phosphoric acid which is commercially available in the form of sticks. This is an acid sodium phosphate glass approximating in composition the formula $Na_2O.H_2O.2P_2O_5$. Graham's salt, first obtained in 1833 by fusing and quickly cooling sodium dihydrogen phosphate, is a substantially neutral sodium phosphate glass represented by the formula $Na_2O.P_2O_5$. This glass is commonly though inaccurately designated as "sodium hexametaphosphate". When devitrified by heating below 625° C., it yields crystalline water-soluble sodium trimetaphosphate, $(NaPO_3)_3$. Other sodium phosphate glasses with ratios of sodium oxide to phosphorus pentoxide of up to approximately 1.7 may be made.

The complexity of the phosphate glasses is illustrated by the finding of Karbe and Jander (*24*) that, when Graham's salt is prepared at a temperature of 1160° C., the molecular weight of the anion amounts to more than 7000.

Berzelius (*3*) discovered in 1816 that a phosphoric acid, known today as metaphosphoric acid, has the property, not shared by either the pyro- or the orthophosphoric acid, of coagulating egg white. In 1825 Engelhart (*12*) presented data on the precipitation of dilute blood serum proteins with metaphosphoric acid. In 1880 Lorenz (*31*), experimenting with dilute solutions of gelatin, obtained precipitates by means of metaphosphoric acid or of sodium metaphosphate and hydrochloric acid. Repeated washing with water was found to reduce the phosphorus pentoxide content of the gelatin-metaphosphate compound. No antiseptic

was required during the washing operation, since the compound is resistant to bacterial decomposition.

In 1888 Lieberman (*29*) obtained precipitates of egg albumin with glacial phosphoric acid, which resembled nucleins. A year later Pohl (*43*) treated dilute serum albumin with concentrated sodium metaphosphate and obtained upon acidification with hydrochloric acid a precipitate with a nearly constant phosphorus content (5.54 to 5.69%). Hemialbumoses, prepared from Witte's peptone by means of precipitation with ammonium sulfate, also gave precipitates with metaphosphoric acid, which dissolved on heating and reappeared on cooling.

In 1892 Malfatti (*32*) obtained precipitates by treating blood serum proteins, dissolved in 0.3% hydrochloric acid, with metaphosphoric acid. The precipitates were found to contain 6.1% phosphorus; after repeated (five times) dissolving in ammoniated water and reprecipitation with acetic acid, the phosphorus content was reduced to 1.6%. From the filtrates a product rich in phosphorus was obtained upon acidification with hydrochloric acid.

The following year Deniges (*9*) recommended a 5% solution of sodium metaphosphate with some hydrochloric or sulfuric acid for deproteinization of milk for lactose determination. Five years later Chassaigne (*5*) used sodium metaphosphate solution and hydrochloric acid as deproteinizing agent in his studies on the reducing substances of blood.

Pick (*42*) studied in 1898 the precipitating effect of metaphosphoric acid on aqueous solutions of various albumoses and peptones, and obtained only a trace of turbidity with protalbumose, and with the secondary albumose A a white precipitate, which dissolved on heating and reappeared on cooling. None of the other albumoses, or the peptones, gave any turbidity.

In 1902 Fuld (*16*), who took up Hofmeister's work on protein-metaphosphate compounds, reported earlier (1896) work by Georgiewsky from the same laboratory, who precipitated solutions of serum albumin with an excess of glacial phosphoric acid, as well as with solutions of phosphate glass (Graham's salt) and hydrochloric acid, and obtained products with nearly constant phosphorus content, averaging 3.47% phosphorus, which remained practically the same even after dissolving in ammonia followed by reprecipitation by acid. Fuld also showed the solubility of the protein-phosphate compound in alkalies and neutral salts, a subject which has been investigated in recent studies by Perlmann and Herrmann (*40*), Briggs (*4*), and Horvath (*23*).

In his own experiments, Fuld (*16*) precipitated 12.5% solutions of serum albumin with increasing amounts of 10% glacial phosphoric acid, and obtained products with a nearly constant phosphorus content (average 3.33%). Fuld interprets his lower figure, as compared with Georgiewsky's, as due to more thorough washing of the precipitate, and the prevention of the splitting off of albumoses by the acid by immediately pouring large volumes of water on the precipitate. With crystalline ovalbumin Fuld obtained under similar conditions a product containing 2.43% phosphorus, and with Hamarsten's casein an average of 3% phosphorus. No constant figures were obtained with crystalline hemoglobin (2.83 to 4.06%), owing to the gradual splitting off of hematin. Serum globulins gave unsatisfactory results. Protalbumoses showed turbidity, which dissolved on heating or in excess acid. Peptones gave no turbidity. Fuld also established the fact that the amount of metaphosphoric acid combined by a protein depends on its diaminoacid content, and is suggestive of an amino group link, a fact which has been corroborated in recent times by other investigators (*4, 39, 40*).

In his 1914 study on glucose and lactic acid of blood and other body fluids, Chelle (*6*) used sodium metaphosphate (phosphate glass, 0.6% solution) as deproteinizing agent in the presence of sulfuric acid.

DEPROTEINIZATION OF BLOOD

In 1916 there appeared the substantial paper by Folin and Denis (*13*) on deproteinization of blood and urine by glacial phosphoric acid, which is said to be remarkably effective as a precipitant for the blood proteins. It is better than colloidal iron and fully as good as trichloroacetic acid, and yields filtrates which are as colorless and clear as water, which remain clear, and which boil without foaming.

The recommended procedure is to dilute 5 cc. of blood with an equal volume of water, and to precipitate the proteins by 3 cc. of 25% metaphosphoric acid. The solution should be prepared fresh every several days, as a gradual reversion to orthophosphoric acid takes place. The mixture is diluted to 50 cc. and, after shaking, filtered through a dry filter paper. The technique of nonprotein nitrogen determination in the filtrate is given in detail. For urea determination the urease effect must be completed before the metaphosphoric acid is added. For deproteinization of urine 10 cc. are treated with 1 cc. of 25% metaphosphoric acid. The procedure of ammonia determination as well as of urea in urine by the nesslerization method is fully described.

In the following year, Sjollema and Hetterschy (*46*) used glacial phosphoric acid for deproteinization in the determination of nonprotein nitrogen in dog and ox blood, as well as in horse blood serum, by a modified Bang's technique. The specimens were absorbed on strips of filter paper, weighed, immersed for 1 hour in 3.5% metaphosphoric acid and washed, and the nonprotein nitrogen was determined in the fluid. The authors also conducted parallel deproteinization experiments by phosphomolybdic and trichloroacetic acid. The figures of nonprotein nitrogen in the filtrates were highest for trichloroacetic acid, next for metaphosphoric acid, and lowest for phosphomolybdic acid. Differences disappeared when dialyzed blood was used, where a part of polypeptides, amino acids, etc., were removed. Phosphomolybdic acid gives a precipitate in metaphosphoric acid filtrates.

In 1921 Wolff (*50*), using Bang's technique, found that metaphosphoric acid precipitates only a small part of primary albumoses, leaving 70 to 80% of them in the filtrate. In it are to be found also over 80% of secondary albumoses and all of the peptones. Phosphomolybdic acid, on the other hand, completely precipitates the primary albumoses and leaves only 8% of the secondary albumoses in the filtrate. Peptones are precipitated by it very incompletely.

Wolff conducted parallel precipitation of blood specimens by phosphomolybdic and metaphosphoric acids, and stated that the difference in the nitrogen contents of the filtrates represented approximately the nitrogen of albumoses. By means of such a technique a large number of clinical blood samples were investigated, showing the existence of a distinct hyperalbumosemia (of 90 to 240 mg. % as against 20 to 65 mg. % in normal individuals) in typhoid fever, pneumonia, and pleuritis. In leukemia, the existing blood hyperalbumosemia originates from the white cells which are extremely rich in albumoses. A light hyperalbumosemia was observed often in cancer. Blood hyperalbumosemia was absent in Bright's disease and patients with stomach ulcers and liver disease.

In the same year Guillaumin (*21*) compared the deproteinization of blood plasma by tungstic, metaphosphoric, and trichloroacetic acids, and found that the average minimal required acidity was a pH of 4.8 at a dilution of 1 to 10. With increasing acidity the nonprotein nitrogen of meta- and tungstic acid filtrates declines, while the trichloroacetic acid filtrate is not much affected by it.

Further comparative studies along these lines were conducted in 1922 by Hiller and Van Slyke (*22*) on ox blood, deprived of its urea nitrogen by means of urease and aeration. Results:

	NPN *Mg. %*	Amino N *Mg. %*	Peptide N *Mg. %*
Tungstic acid	28.1	9.2	4.1
Picric acid	...	8.3	4.6
Metaphosphoric acid	28.3	7.9	3.9
Trichloroacetic acid (2.5%)	28.8	7.9	7.0
Colloidal iron	29.4	7.8	...
Trichloroacetic acid (5%)	26.7	7.5	4.9
Trichloroacetic acid (10%)	26.1	7.1	4.6
Alcohol	18.2	4.9	1.4

Investigations by Grigaut (*19*), published during the same year, were conducted on human whole blood, red corpuscles, and serum, using the following technique for deproteinization by metaphosphoric acid:

	Cc.		Cc.
Serum	10	Whole blood (or red cells)	10
Sodium metaphosphate (5%)	8	Sodium metaphosphate (5%)	25
Hydrochloric acid (2 *N*)	2	Hydrochloric acid (2 *N*)	5

In parallel experiments in deproteinization by trichloroacetic acid equal volumes of blood serum and of 20% trichloroacetic acid were used. The blood serum of patients with chronic nephritis and with diabetes showed a twofold and fourfold, respectively, higher nonprotein nitrogen value in metaphosphoric acid filtrates as compared with trichloroacetic acid filtrates, while the uric acid values were practically identical for both precipitants.

In further studies on the deproteinization of blood, spinal fluid, and pathological fluids by metaphosphoric acid, Grigaut and Zizine (*20*) pointed out the importance of proper dilution and acidity. The higher the preliminary dilution of the specimen, the lower is the nonprotein nitrogen of the filtrate. Dilutions of various pathological blood sera 1 to 1 gave much higher nonprotein nitrogen figures in the filtrates than a dilution 1 to 10. As to the proper acidity, there exists for every serum an individual range of complete deproteinization between the so-called minimal and maximal acidity. Expressed in cubic centimeters of hydrochloric acid (2 *N*) per 10 cc. of serum, the ranges are 0.5 to 4.0 cc.; 0.9 to 2.8 cc.; 0.6 to 4.3 cc.; 1.2 to 3.5 cc.; 0.7 to 4.3 cc.; and 0.6 to 3.6 cc. A medium acidity and a dilution of 1 to 1 were used to obtain in the metafiltrate certain substances of the polypeptide type less complex than albumoses and peptones, which are of interest in pathological sera. The techniques used were:

	Cc.		Cc.		Cc.
Serum	10	Whole blood	10	Cerebrospinal fluid	10
Water	6	Water	20	Sodium metaphosphate (20%)	0.5
Sodium metaphosphate (20%, approximately 2 *N*)	2	Sodium metaphosphate	5	Hydrochloric acid (2 *N*)	0.5
Hydrochloric acid (2 *N*)	2	Hydrochloric acid (2 *N*)	5		

In a number of pathological sera metaphosphoric acid (1 to 1) gave a higher nonprotein nitrogen than trichloroacetic acid (1 to 1), which is evidently not due to hydrolysis. The filtrates from metaphosphoric acid were found to contain urobiline, while the trichloroacetic acid filtrates were free of this constituent, a phenomenon which is attributed by the authors to its being bound to protein. Blood filtrates from metaphosphoric acid give urea and uric acid figures similar to the ones from trichloroacetic acid, and are a suitable substitute for tungstic acid filtrates for blood sugar determinations by the Folin-Wu method.

During the same year Cristol (*7*) confirmed Grigaut's data that the metaphosphoric acid filtrates of blood serum contain more nonprotein nitrogen than the trichloroacetic acid filtrate, and established by means of a positive biuret test that this excess nitrogen is of protein nature. In Cristol's opinion this extra nitrogen in the metaphosphoric acid filtrate may be caused by the splitting effect of the metaphosphoric acid itself, and he was able to confirm it experimentally, where the metaphosphoric acid was added either subsequently to the hydrochloric acid or in mixture with the latter (maximal effect), but not where the metaphosphoric acid was added first. The intensity of the biuret reaction in the metaphosphoric acid filtrates was shown also to be dependent upon the duration of the metaphosphoric acid action on the serum, increasing with time. By dialyzing blood serum, Cristol was unable to detect any biuret-positive constituents in the dialyzing fluid.

In 1923, Cristol and Nikolitch (*8*) made a comparative study of deproteinization by metaphosphoric, tungstic, and trichloroacetic acids, using the following technique:

	Cc.	
Plasma (or pathological fluid)	10	
1 *N* Sodium metaphosphate (10.2%)	2	Average pH of filtrate 3.3
Water	36	to 3.6 (strong acidity)
1 *N* Sulfuric acid	2	
Plasma	10	
Sodium metaphosphate (1.5%)	10	Average pH of filtrate 4.5
Water	20	to 4.8 (minimal acidity)
0.1 *N* sulfuric acid	10	
Red blood cells (diluted 1 to 1)	10	
1 *N* sodium metaphosphate (10.2%)	10	Average pH of filtrate 2.0
1 *N* sulfuric acid	10	to 2.2 (strong acidity)
Red blood cells (diluted 1 to 1)	20	
Sodium metaphosphate (1.5%)	30	Average pH of filtrate 4.5
Water	14	to 4.8 (medium acidity)
0.1 *N* sulfuric acid	36	

In deproteinization by tungstic acid the minimal and strong acidities of the plasma filtrates (diluted 1 to 5) were approximately the same as for metaphosphoric acid. The filtrates (diluted 1 to 1) from trichloroacetic acid had as minimal acidity a pH of 2.0, and as strong acidity a pH of below 1. In the metaphosphoric and tungstic acid filtrates higher nonprotein nitrogen results were obtained at higher pH values. At high acidity, the metaphosphoric acid filtrates gave the highest nonprotein nitrogen figures from the three precipitants tested. At weak acidity the results were not constant, but for the red blood cells trichloroacetic acid gave the highest nonprotein nitrogen. Cristol and Nikolitch (*8*) were also able to confirm the findings by previous investigators that in metaphosphoric acid filtrates the nonprotein nitrogen is the smaller the higher the preliminary dilution of the specimen, and that these filtrates give a positive biuret test, indicative of "large polypeptide molecules" (wording of the authors) while the test for albumoses and peptones by the Tanret reagent is negative. These polypeptides, which are precipitated from the metaphosphoric acid filtrates by phosphotungstic acid, are believed by the authors not to be present in the blood in free form, but to be split off by the action of metaphosphoric acid from proteins which have become labile under certain pathological conditions. The authors believe that this increase in nitrogen in metaphosphoric acid filtrates may prove of value from a clinical point of view, and they suggested for such a condition the term "index of lability".

During the same year, Minich (*34*) conducted a comparative study on the deproteinization of oxalated horse blood by various precipitants. The deproteinization by metaphosphoric acid was conducted by the method of Folin and Denis (*13*). Minich obtained the following average figures for nonprotein nitrogen in the filtrates:

	N, Mg. %
Trichloroacetic acid	31.03
Phosphomolybdic acid	27.04
Uranyl acetate	21.85
Phosphotungstic acid	18.58
Metaphosphoric acid	33.25

Metaphosphoric acid gave the highest figures, with trichloroacetic acid following. These results were comparable to the ones obtained by the same author on human blood.

Two years later, Mendel and Goldscheider (*33*) used metaphosphoric acid as a deproteinizing agent in the determination of lactic acid in blood. To 1 cc. of the latter were added 6 cc. of water and 1 cc. of glacial phosphoric acid (5%).

In 1928 Balarev (*2*), experimenting with the precipitation of equal portions of albumin by various metaphosphates acidified with acetic acid, determined the amount of residual metaphosphate in the filtrates.

	Normality of Metaphosphoric Acid Added	Normality of Metaphosphoric Acid in Filtrate
Trimetaphosphate (Tamman's)	$\frac{1}{40}$	$\frac{1}{200}$
Tetrametaphosphate (Tamman's)	$\frac{1}{480}$	$\frac{1}{2400}$
Metaphosphate obtained by heating microcosmic salt	$\frac{1}{2500}$	$\frac{1}{12,500}$
Metaphosphoric acid by dissolving P_2O_5 in water	$\frac{1}{5600}$	$\frac{1}{28,000}$

In 1935 Fujita and Iwatake (*15*) successfully used glacial phosphoric acid (5% solution) for deproteinization of ground organs in the determination of reduced glutathione. Trichloroacetic acid was found unsuitable for this purpose because of the presence of ferric iron in the filtrates, which caused an error by liberating iodine from potassium iodide.

VITAMINS, PROTEINS IN AGRICULTURAL PRODUCTS

The following year there appeared a paper by Musulin and King (*36*) on the use of metaphosphoric acid in the extraction and titration method for vitamin C. It was found that metaphosphoric acid in approximately 2% concentration serves to protect vitamin C in solution against atmospheric oxidation, even in the presence of added copper, and also exerts a protective action against oxidation in the presence of trichloroacetic acid. The rate of reaction with 2,6-dichlorophenol indophenol was not appreciably affected by the presence of metaphosphoric acid. A procedure, including the presence of 2% metaphosphoric acid, was recommended for work with both plant and animal tissues.

The same year Penau and Guilbert (*38*) recommended the use of sodium metaphosphate plus sulfuric acid for deproteinization of blood serum in the determination of the activity of lipase and esterase.

In 1939, Knight, Dutcher, and Guerrant (*25*) successfully used metaphosphoric acid in the determination of vitamin C in milk. To 10 cc. of the latter were added 10 cc. of a 10% solution of sodium metaphosphate, acidified with 0.6 cc. of concentrated hydrochloric acid, with immediate and complete flocculation of protein at a pH of 2.5 to 3.0. The vitamin C was subsequently determined in the filtrate.

In 1939–40 several U. S. patents were granted to Fortune (*14*) on a blood sugar test, urine albumin test, and a test for aminobenzene compounds in body fluids, where the deproteinization is done by "sodium hexametaphosphate" at a pH of approximately 4.5. (While the designation "sodium hexametaphosphate" has frequently been applied to the sodium phosphate glass commercially available under the name of Calgon, this product actually has a molar ratio of Na_2O to P_2O_5 of slightly greater than 1 to 1.)

For blood sugar determination one drop of blood is diluted with about 10 cc. of water, and the protein removed by precipitating capsules consisting of:

"Sodium hexametaphosphate"	1/4 grain
Oxalic acid, anhydrous	1/4 grain
Talc, powdered	2 grains

The albumin test in urine requires no heating, and is conducted on 4-cc. portions of urine, diluted with an equal volume of water, by adding a tablet consisting of:

"Sodium hexametaphosphate"	50 mg.
Glutamic acid hydrochloride	50 mg.
Dextrin	2 mg.

After shaking, the cloudiness is directly compared with a turbidity chart.

In the test for aminobenzene compounds in body fluids the following protein-precipitating and diazotizing agent (in capsules) is used:

Sodium nitrite	2 mg.
"Sodium hexametaphosphate"	50 mg.
Glutamic acid hydrochloride	30 mg.
Talc	120 mg.

For this test approximately 2 drops of blood are required, diluted 30 or 40 times with water.

In 1939 Pfützer and Roth (*41*), working on the determination of proteins in agricultural products, recommended the precipitation of proteins by means of sodium metaphosphate at a pH of 2.5.

This was attained by means of hydrochloric acid and a citrate buffer obtained by dissolving 21 grams of citric acid in 100 cc. of 2 *N* sodium hydroxide, adding 89 cc. of 2 *N* hydrochloric acid, and diluting with water to 1 liter (pH 2.5). An excess of metaphosphate was shown to cause a partial redissolving of the precipitate in such varied products as barley grain, carrots (fresh), and gelatin. The addition of various amino acids, as well as choline, colamine, and urea, did not affect the figures of the precipitated protein. The determinations were conducted on 0.4-gram (dry basis) fresh samples, suspended in 50 cc. of water in a 70- to 100-cc. centrifuge tube; 2 cc. of a 10% solution of sodium metaphosphate were added, followed by 0.2 cc. of 2 *N* hydrochloric acid and 10 cc. of citrate. After thorough mixing, centrifuging, decanting of the supernatant liquid, and washing with the same phosphate mixture, the nitrogen in the residue is determined by the Kjeldahl method.

The following year, Eidelman (*11*) demonstrated that trichloroacetic acid destroys in 24 hours at room temperature 60 to 81% of synthetic dehydroascorbic acid, and 40 to 56% of the latter in cabbage juice, while on addition of metaphosphoric acid 65 to 100% of dehydroascorbic acid can be recovered.

In 1941 Kuether and Roe (*28*) used glacial phosphoric acid for the deproteinization of blood in the determination of ascorbic acid. During the same year, Krautman (*27*) published two papers on deproteinization of blood and cerebrospinal fluid by means of a reagent, consisting mainly of dilute glacial phosphoric acid and tungstic acid. This reagent can also be stored in dry form and diluted before use. For deproteinization 9 parts of this reagent are added to 1 part of specimen. The filtrates are suitable for the determination of glucose, nonprotein nitrogen, urea nitrogen, and uric acid.

The 1941 paper by Vonesch and Zimman (*49*) describes a photometric method for the determination of ascorbic acid in blood, where the deproteinization is conducted by means of metaphosphoric acid, followed by lead acetate. In another photometric method by Morell (*35*) for the determination of ascorbic acid in plant materials, 25 grams of pulped fresh plant tissues are mixed at high speed with 100 cc. of a 3% solution of metaphosphoric acid for about 2 minutes. The filtrate is buffered to pH 3.6, and to it is added a solution of 2,6-dichlorophenol-indophenol (containing 34.4 mg. in 1 liter of water).

In 1942 Loeffler and Ponting (*30*) used dilute metaphosphoric acid in fresh, frozen, and dehydrated fruits and vegetables in the determination of ascorbic acid.

A year later Ponting (*44*) made a special study of the loss of ascorbic acid in 13 acids, and came to the conclusion that only oxalic and metaphosphoric acids were suitable for extracting ascorbic acid from such vegetable materials as cabbage, broccoli, lima beans, peas, and strawberries, some of them frozen, using a Waring Blendor. A concentration of 1% for metaphosphoric acid was found to be satisfactory, using a ratio of 7 volumes of this dilute acid to 1 part of plant material, or higher.

In 1943 Horvath (*23*) treated 2-cc. portions of citrated beef plasma, diluted to 10 cc., with 0.1, 0.2, and 0.3 cc. of a 10% solution of sodium phosphate glass of pH 6.5 (Calgon), and acidified by hydrochloric acid (4%) to a pH of 3.8, which, as well as lower pH values, was found to be suitable for the precipitation of serum proteins. Complete deproteinization was observed where 0.2- and 0.3-cc. portions of the phosphate solutions were used. Deproteinization by acid phosphate glass (mole ratio Na_2O/P_2O_5 = 0.7152; pH of a 1% solution = 2.2), as well as by sodium trimetaphosphate and by sodium tripolyphosphate at a pH of 3.8 was also reported. Further studies were made on the solubility of the protein phosphate compound in neutral salts of mono-, di-, and trivalent metals, as well as in urea. The 1940 work of Briggs (*4*) explains the solubility of protein phosphate in neutral salts by its having many characteristics of a complex coacervate. Briggs found the titration curve of the protein-metaphosphate to be reversible, and confirmed the earlier findings of Perlmann and Herrmann (*40*) that no denaturation of the protein occurs through the action of metaphosphate.

In the same year Doan and Josephson (*10*), while studying the ascorbic acid content of evaporated milk, found that in milk heated to 240° F. for 15 minutes there appear reducing substances which react with the indophenol dye. The interfering substances

were successfully precipitated with the proteins by treating 25 ml. of the reconstituted milk with 26.7 ml. of coagulant consisting of 6% trichloroacetic acid and 4% metaphosphoric acid (the additional 1.7 ml. being used in order to compensate for the volume of the curd). The coagulum was immediately filtered and 20 ml. of the serum (equivalent to 10 ml. of the reconstituted milk) were treated with the indophenol dye.

In 1944 metaphosphoric acid was recommended by the Association of Official Agricultural Chemists (*1*) as a deproteinizing agent for the determination of ascorbic acid. The same year Gawron and Berg (*17*) observed that ferrous iron reduces dichlorophenol-indophenol in the presence of metaphosphoric acid, thus providing a basis for the stepwise determination of vitamin C and ferrous iron in the same aliquot.

In 1945 Kramer (*26*) used metaphosphoric acid in the determination of ascorbic acid content of some army foods. Fresh samples were covered with dilute metaphosphoric acid (3%), macerated in a Waring Blendor, and the vitamin C determined promptly in the filtrates. Vavich, Stern, and Guerrant (*48*), using a similar technique, found a final concentration of 3% metaphosphoric acid to be the optimum for blending unwashed raw peas, preserving the ascorbic acid in the presence of air for one hour, presumably by inactivating the enzymes.

In the same year, a U. S. patent was granted to Gordon (*18*) on the recovery of protein from milk whey or blood serum, where the first step consists in precipitation of the protein by means of metaphosphoric acid, at a pH range of 3.5 to 1.8.

Another U. S. patent, issued to Rushton (*45*), deals with a process for separating protein from aqueous industrial waste material by mixing with it a finely divided solid calcium metaphosphate (300-mesh) and maintaining the mixture in a slightly acid condition (generally in the order of pH 5), and digesting the mixture for a time sufficient to precipitate a substantial proportion of the protein in the form of a protein–phosphate complex, which is said to contain usually about 5% phosphorus pentoxide and 16% nitrogen. The patentee claims his method to be suitable in particular for the removal of protein from milk whey in order to recover subsequently milk sugar or lactic acid.

In 1945, Vavich, Dutcher, Guerrant, and Bechdel (*47*) used metaphosphoric acid in their study of the utilization and excretion of ingested ascorbic acid by dairy cows. Their technique was as follows:

Blood Plasma. Five milliliters of plasma and 10 ml. of 10% metaphosphoric acid were pipetted into a centrifuge tube containing 5 ml. of distilled water. The tube was centrifuged in order to facilitate a sharper separation of the precipitated proteins, and aliquots of the supernatant metaphosphoric acid extract were taken for ascorbic acid determinations.

Milk. An aliquot of milk was pipetted into an equal volume of 10% metaphosphoric acid solution. After shaking to break up aggregates of precipitated protein the mixture was titrated directly with indophenol dye solution to a pink end point which persisted for 30 seconds.

Rumen Fluid. The fluid was deproteinized by a 10% solution of metaphosphoric acid, and the ascorbic acid in the filtrate determined photoelectrically by a variation of the colorimetric method in which the unreacted dye was extracted with xylene.

The application of the indophenol–xylene extraction method to the determination of ascorbic acid in tomatoes and tomato juice was studied by Nelson and Somers (*37*), and preference given to 3% metaphosphoric acid as compared with a 5% sulfuric–2% metaphosphoric acid mixture because ascorbic acid is more stable in 3% metaphosphoric acid, and it is much easier to obtain perfectly clear filtrates using metaphosphoric acid alone than the acid mixture.

CONCLUSIONS

It is evident that deproteinization by means of metaphosphoric acid is already being used for a great variety of purposes. It seems to be generally accepted as the most reliable deproteinizing agent for the determination of ascorbic acid in plant and animal materials alike, mainly because of its stabilizing effect on the ascorbic acid and noninterference with the dichlorophenol-indophenol color reaction. Metaphosphoric acid has found favor in the determination of lactose in milk. It is also of value for deproteinization of clinical specimens, since it permits the determination in the filtrates of most of the constituents of interest to the clinician, giving reliable yields of the constituents without interfering with the standard colorimetric procedures.

The field of application of metaphosphoric acid in analytical chemistry is, however, not limited to deproteinization purposes. It can be used with equal advantage for the isolation of protein from agricultural and other products and the subsequent quantitative determination of the isolated protein. The analyst will undoubtedly find many new applications for the protein-precipitating property of metaphosphoric acid, and develop for it a number of new techniques.

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Application of Platinum Resistance Thermometry to Some Industrial Physicochemical Problems

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Platinum resistance thermometry is discussed in some detail, with mention of new techniques for construction of these thermometers and their convenient size. An automatic recorder facilitates the use of these thermometers. The cryoscopic method of determining the purity of a compound using platinum resistance thermometers is presented, together with the assumptions and limitations of the method. A modified ebulliometer fitted with a platinum resistance thermometer is introduced. A new type of boiling range apparatus incorporating a platinum resistance thermometer is presented and compared with the standard A.S.T.M. method. The increased accuracy resulting from the use of platinum resistance thermometers as well as the automatic recording feature fits these three applications well for industrial use.

THE reasons for the slight use of platinum resistance thermometers in industrial laboratories may be reviewed to advantage. Platinum resistance thermometers have been on the market for some time, but their high cost, calibration, fragility, size, and accompanying electrical measuring equipment have deterred many from using them. It is well, however, periodically to reexamine one's research tools in the light of more recent advances. Since the international temperature scale from −190° to 660° C. is defined in terms of a platinum resistance thermometer (*3*, *28*), it seems appropriate for precision measurements of such physical quantities as freezing point, boiling point, boiling range, vapor pressure, and the like to be made with a platinum resistance thermometer. Recent developments in equipment design, as well as demands for greater accuracy, have brought this admirable method of precise temperature measurement to a point of development such that it deserves to be more widely applied.

PLATINUM RESISTANCE THERMOMETRY

In 1871, Siemens (*18*) suggested that resistances be used as a means of measuring temperature. His materials of construction were faulty, causing many to discredit resistance thermometry. Some years later the monumental work of Callendar (*4*) laid the foundation for the reliability and precision of platinum thermometry as we know it today. In the 50-odd years since, some of those who have labored to perfect the platinum resistance thermometer are Sligh (*22*), Meyers (*12*), Mueller (*13*, *14*), Keesom (*9*, *10*), and Van Dusen (*26*).

The author has employed the methods of Meyers (*12*) for making resistance thermometers.

No. 38 B. and S. wire (approximately 0.1 mm., 0.004 inch, in diameter) of the highest purity platinum obtainable is wound on an iron piano wire No. 00 (approximately 0.2 mm., 0.008 inch, in diameter), so that between turns there is a free space equal to the diameter of the platinum wire. The iron wire is then dissolved out by hydrochloric acid, leaving a helix approximately 0.4 mm. (0.016 inch) in diameter. This platinum helix is wound on a notched mica framework in the form of a cross (see illustration in Meyers' paper). Two leads of pure gold wire size 32 B. and S. (approximately 0.2 mm., 0.008 inch, in diameter) are welded to each end of the coiled platinum helix with a small oxy-gas flame and threaded through the mica. (Where the thermometer is to be read to 0.01° C. this procedure is permissible, but if read to 0.001° a short piece of platinum wire is interposed between the helix and the gold lead to serve as a thermal dam for any possible heat being conducted along the gold leads.)

The four gold leads are kept insulated by mica disks 5 mm. in diameter (spaced by a short length of glass tubing) with four equidistant holes through which the wires pass. The coiled helix, having approximately 25 ohms' resistance at 0° C., occupies a space about 5 mm. in diameter by 20 mm. long. The temperature-sensitive element is thus concentrated within a fairly small space.

The whole assembly is placed in a Pyrex tube of 7-mm. outside diameter. The upper end of each gold lead is welded to a 1-cm. piece of 0.020-inch tungsten wire which is thermally platinized at each end.

Welding gold or platinum wires is facilitated by previously "tinning" the tungsten wire with platinum. A length of the tungsten wire is supported horizontally in a vacuum chamber (bell jar). Strips of 0.001-inch platinum foil 2 × 10 mm. are hung on the wire in the position where the tinning is desired. The bell jar is put in position, the air pumped out to a pressure of 1 micron or less, and an electric current sent through the wire. As the wire glows the oxides of tungsten vaporize and the platinum melts and "wets" the tungsten wire evenly in a homogeneous sleeve.

To the opposite end of the tungsten is welded a short piece of No. 28 B. and S. platinum wire, and the tungsten leads are sealed through the Pyrex wall. The thermometer is then heated to 400° C. for 8 hours, during which time it is continuously evacuated and refilled with dry air every half hour. After sealing off the gas filling neck (located adjacent to the tungsten lead seals at the upper end) with about 0.75 atmosphere of dry air in the thermometer, the four-lead copper cable is soldered to the platinum leads and the protecting head or handle is fastened in place with plaster of Paris. The platinum coil is then annealed by connecting it with a Variac and applying 55 volts across the 25-ohm coil for 40 seconds. This heats the platinum to redness and relieves any strains due to winding, etc., to ensure permanence of calibration. The annealing process is continued as follows:

50 volts for 2 minutes
45 volts for 6 minutes
40 volts for 10 minutes
35 volts for 20 minutes
30 volts for 30 minutes

Figure 1 shows the size of the finished thermometers, which in dimensions and utility compares very favorably with the usual mercury thermometer. It is convenient to construct the thermometer with a ground-glass joint for insertion into various pieces of apparatus.

Platinum metal is admirably suited to serve as the resistor material, because of the very regular and reproducible relation existing between the resistance and temperature.

This relation has often been fitted with a parabolic equation of the form

$$R_t = R_0 (1 + At + Bt^2) \qquad (1)$$

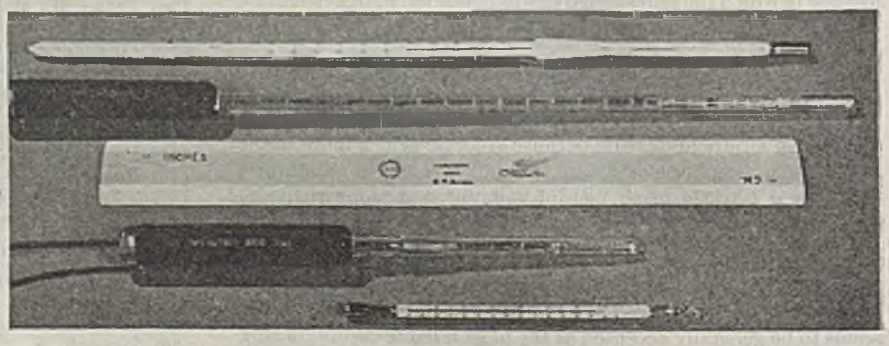


Figure 1. Comparison of Platinum Resistance Thermometers with Conventional Mercury Thermometers

where R_t is the resistance at temperature t, R_0 is the resistance at 0° C., and A and B are constants. To avoid solving Equation 1, it is sometimes put in the form

$$t = \left(\frac{R_t - R_0}{R_{100} - R_0}\right) 100 + \delta \frac{t}{(100 - 1)} \frac{t}{100} \qquad (2)$$

where R_t, R_0, and R_{100} are the measured resistances at $t°$, 0°, and 100°, respectively, and δ is a constant obtained by measuring the resistance at some other fixed temperature, usually the sulfur boiling point. Equation 2 will reproduce the temperature-resistance relationship with sufficient accuracy from −40° to 660° C., but at low temperatures does not reproduce the thermodynamic temperature scale with sufficient accuracy, so in 1925 Van Dusen (*26*) proposed the formula

$$t = \left(\frac{R_t - R_0}{R_{100} - R_0}\right) 100 + \delta \left(\frac{t}{100} - 1\right) \frac{t}{100} + \beta \left(\frac{t}{100} - 1\right) \frac{t^3}{100^3} \qquad (3)$$

where β is another empirical constant obtained by measuring the resistance at a fixed low temperature, usually the boiling point of of oxygen. The form of these equations can be appreciated best by a worker in this field. After constants δ and β are computed, the resistance of each thermometer is calculated for each 10° interval (see Table I).

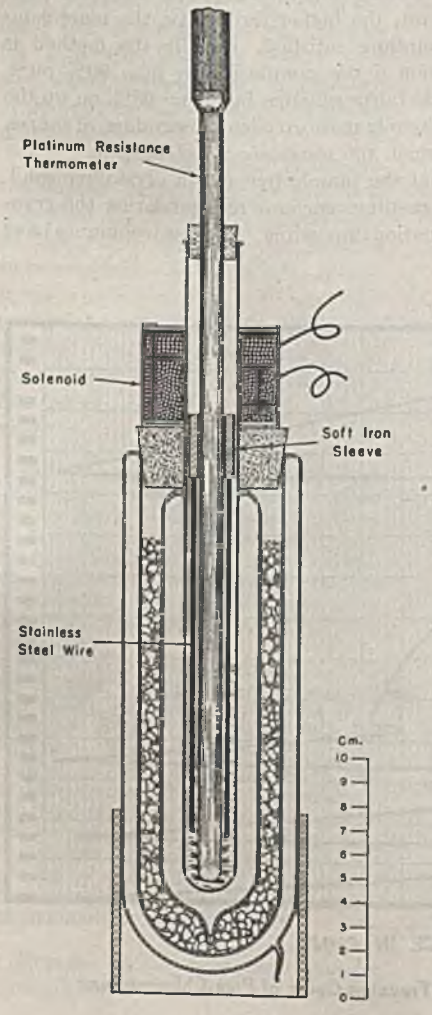
Table I. Platinum Resistance Thermometer, NBS 690

(R_0 = 25.540Ω. R_{100} = 35.560Ω. δ = 1.49$_5$. β = 0.110$_8$)

−T ° C.	$R\Omega$	$\Delta R\Omega$	T ° C.	$R\Omega$	$\Delta R\Omega$
0	25.540		0	25.540	
		1.018			1.016
10	24.522		10	26.556	
		1.022			1.012
20	23.500		20	27.568	
		1.025			1.009
30	22.475		30	28.577	
		1.028			1.007
40	21.447		40	29.584	
		1.031			1.003
50	20.416		50	30.587	
		1.036			1.001
60	19.380		60	31.588	
		1.039			0.997
70	18.341		70	32.585	
		1.043			0.995
80	17.298		80	33.580	
		1.048			0.992
90	16.250		90	34.572	
		1.052			0.988
100	15.198		100	35.560	

Inspection of Table I will disclose the fact that $\Delta R\Omega$ for each 10° interval is substantially 1 ohm, and that it changes slowly enough so that linear extrapolation of each 10° interval is permis-

Figure 2 (Below). Freezing Point Apparatus



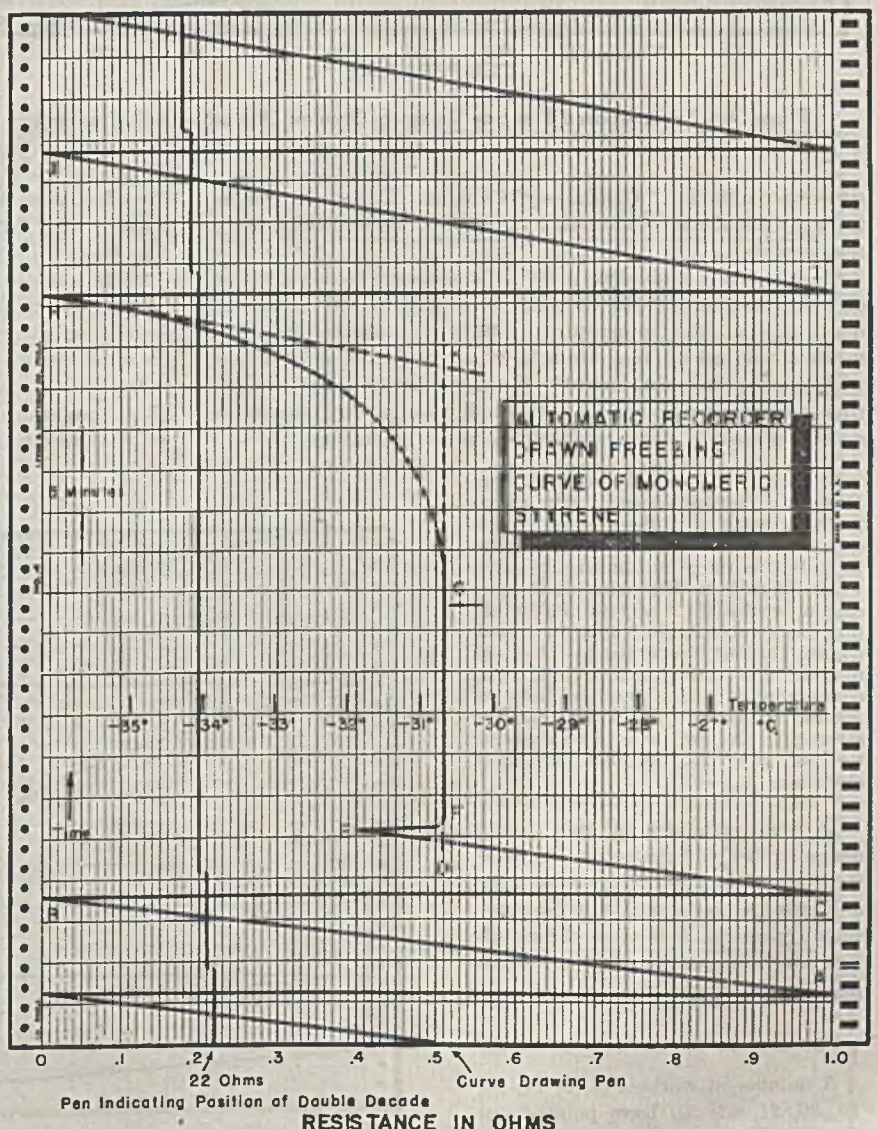


Figure 3. Automatic Recorder-Drawn Freezing Curve of Monomeric Styrene

Because of the necessary reduction of recorder-drawn figures, the original pen traces have been retouched to make them broader

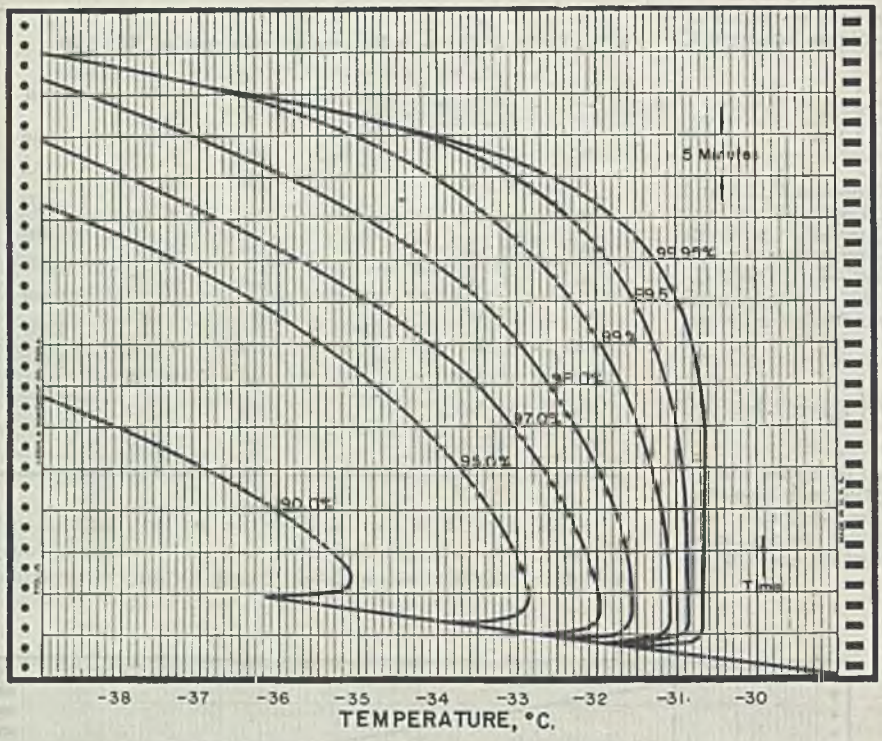


Figure 4. Superimposed Freezing Curves of Styrene Diluted with Ethylbenzene

the true freezing point is known, if the time-temperature curve is observed during the freezing process the true freezing point can be calculated and the method is more cogent. It is true, however, that the method rests on certain assumptions, and the results are proportional to the degree to which the assumptions are fulfilled.

As outlined originally by White (*29*) and more recently by Mair, Glasgow, and Rossini (*11*), the estimation of the amount of solid-insoluble, liquid-soluble impurity from a time-temperature freezing curve involves the following assumptions:

1. The impurity remains entirely in the liquid phase during the time that the temperature lowering is being determined.
2. The rate of crystallization remains constant.
3. Complete thermal equilibrium exists between the solid and liquid phases.
4. That an ideal solution is formed between the liquid phase and the solid-insoluble, liquid-soluble impurity, or, if this system forms a nonideal solution, that it is of sufficient dilution that it may be treated as ideal.

sible when only 0.01° C. accuracy is sought. This greatly facilitates the calculation of measured values of R to the corresponding temperature.

Since 1 ohm is equivalent to 10°, it is necessary to measure the electrical resistance of the platinum coil to within 0.001 ohm in order to calculate the temperature to 0.01° C. Resistance measurements of this order of magnitude have long been adequately made with hand-operated bridges (*13*), but are somewhat tiresome and time-consuming. To eliminate this toil, a completely automatic resistance recorder has been developed in this laboratory.

It consists essentially of a 25-cm. (10-inch), constant-speed, strip chart recorder with a full-scale range of exactly 1.000 ohm, but with the ability to shift this range automatically anywhere from 0 to 100 ohms as the occasion demands. A second pen simultaneously records the range in which the instrument is operating. One can read the chart to within about 0.01 inch, corresponding with a practical limit of ±0.01° C. in the precision of the instrument. A more complete description of this machine has appeared elsewhere (*24*).

As the remainder of this article demonstrates, the perfection and construction of an automatic recorder for a platinum resistance thermometer should considerably increase the usefulness and scope of that instrument in precision thermometry.

CRYOSCOPIC DETERMINATION OF PURITY

A number of workers (*1, 8, 11, 17, 19, 20, 21, 23, 29*) have pointed out the fact that, while the initial freezing point of a compound may be employed to calculate its purity when

Thus the method gets progressively less effective as the concentration of the impurities increases, but what is very important, the higher the purity, the more completely are the assumptions satisfied. Usually the method is open to serious question if the sample is less than 90% pure. At 95% the method is fairly reliable, but from 98% on up the results become progressively more excellent, depending, of course, on the accuracy to which the resistance is determined.

It is paramount that the sample freeze to a crystalline solid. Glass formation is sometimes encountered, rendering the cryoscopic purity determination impossible. Various techniques have

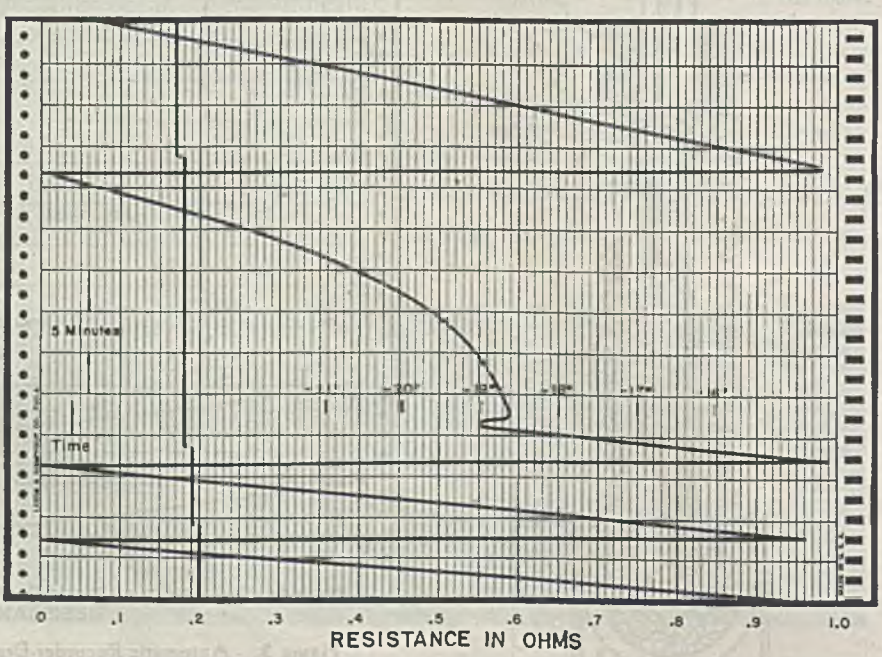


Figure 5. Automatic Recorder-Drawn Freezing Curve of Para-Chlorostyrene

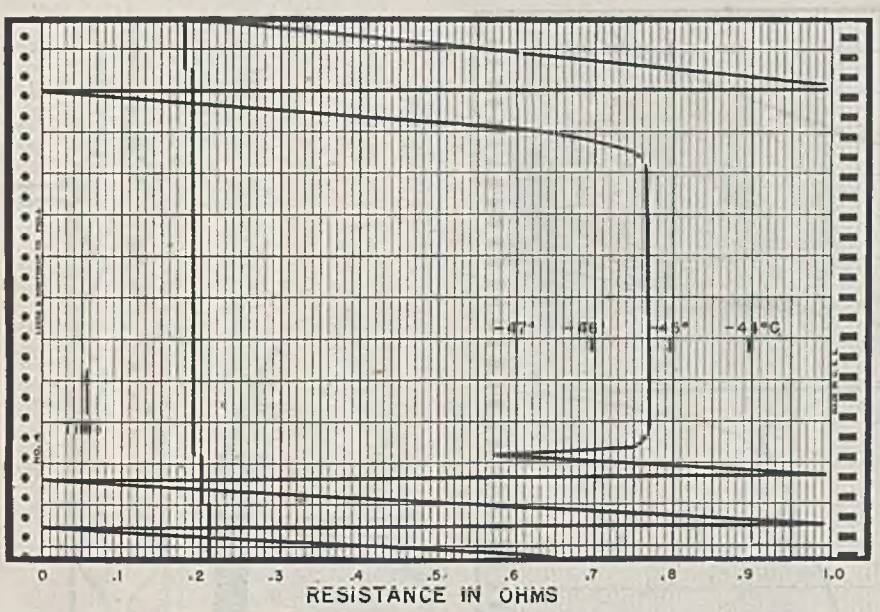


Figure 6. Automatic Recorder-Drawn Freezing Curve of Chlorobenzene

been used to induce crystallization, among which may be mentioned seeding (provided some seed is available), vigorous stirring, scratching the inner wall of the sample tube, plunging into the liquid a wire chilled with liquid nitrogen, and the presence of an inert crystal of the same lattice dimensions.

A number of different cryoscopic techniques have been devised and published (*1*, *8*, *11*, *17*, *19*, *20*, *21*, *23*, *29*); this list does not pretend to be complete. Each has some special merit which best fits it to do some particular job. The procedure described here has been devised to fit general laboratory usage, and has been in use now for some 4 years.

Fifteen milliliters of sample are introduced into a test tube having an unsilvered vacuum jacket around its lower portion (see Figure 2). Some adequate means of stirring the sample at a rate of 40 to 80 strokes per minute is provided, such as a solenoid or a converted windshield wiper reciprocating stirrer.

The test tube is fitted with a rubber stopper which serves to support the test tube in a wide-mouthed thermos bottle containing the refrigerant. Crushed dry ice in a mixture of half and half chloroform and carbon tetrachloride (this mixture is nonflammable) or liquid nitrogen is the refrigerant used, depending upon the degree of cooling required. Supported by a cork stopper at the top of the sample tube is the resistance thermometer.

Because of the poor heat transfer through the vacuum jacket, and the fact that the temperature of the refrigerant is always maintained essentially constant, the cooling rate is constant, thus satisfying the requirements of assumption 2. Several sample containers are available with different degrees of evacuation, permitting proper choice of container, so that near the freezing point the cooling rate will be in the range 0.8° to 1.0° C. per minute. Figure 3 is a time-temperature cooling curve of monomeric styrene automatically recorded by the machine referred to above.

The usual method of plotting time-temperature curves is to plot temperature as the ordinate increasing upward and time as abscissa increasing toward the right. The type of automatic recorder developed for this purpose makes use of a strip-chart machine which plots time increasing upward on the chart as the ordinate, with abscissa recording the resistance increasing

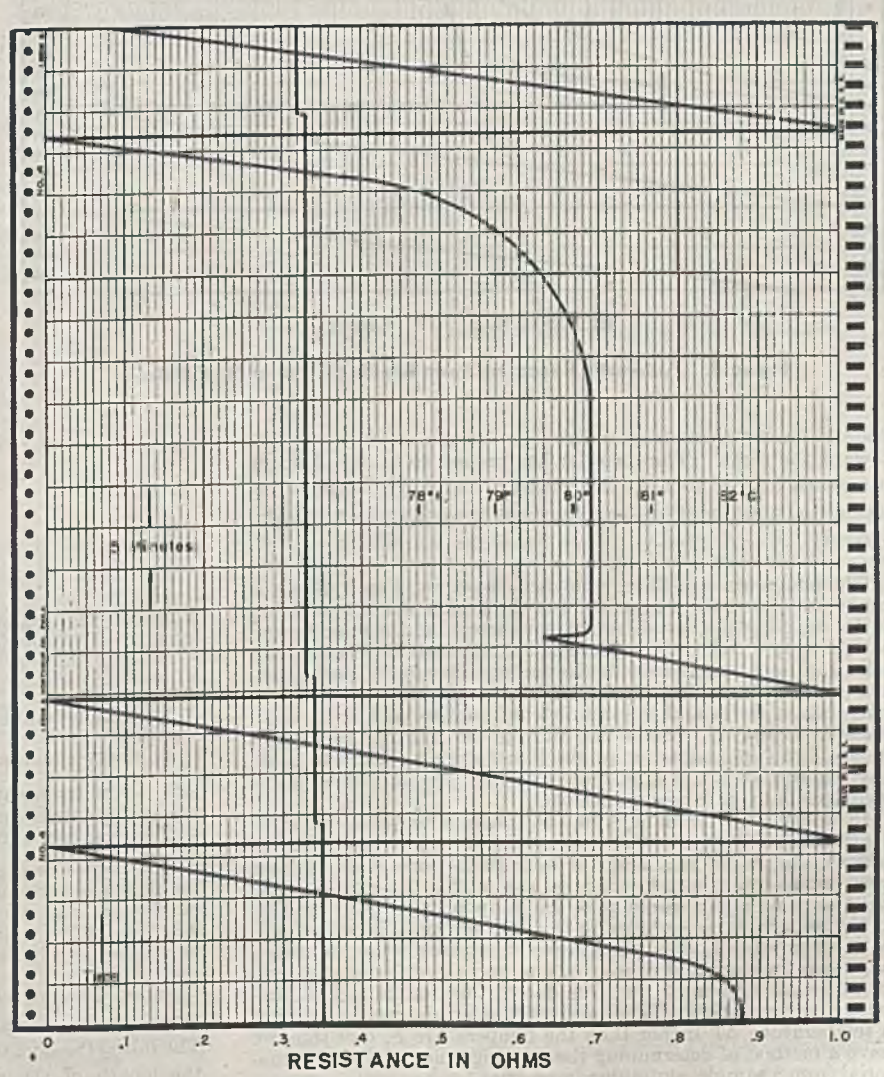


Figure 7. Automatic Recorder-Drawn Freezing Curve of Naphthalene

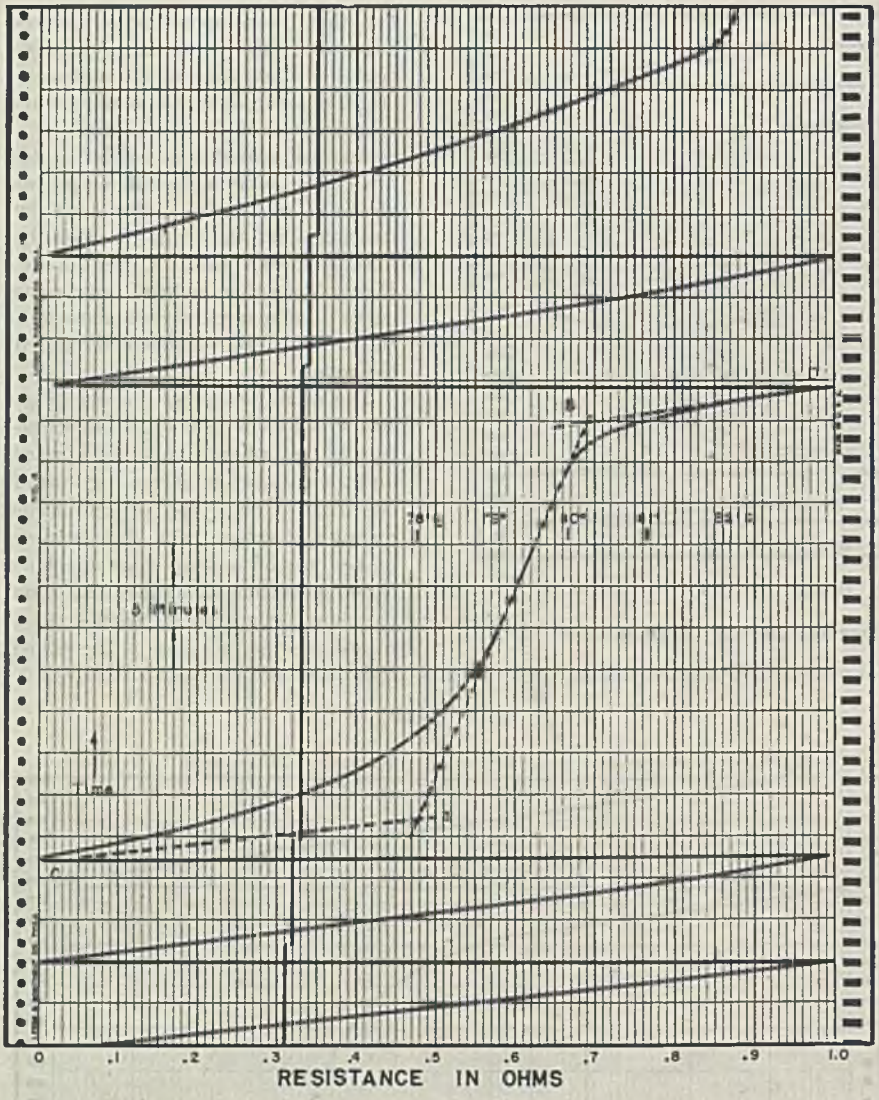


Figure 8. Automatic Recorder-Drawn Melting Curve of Naphthalene

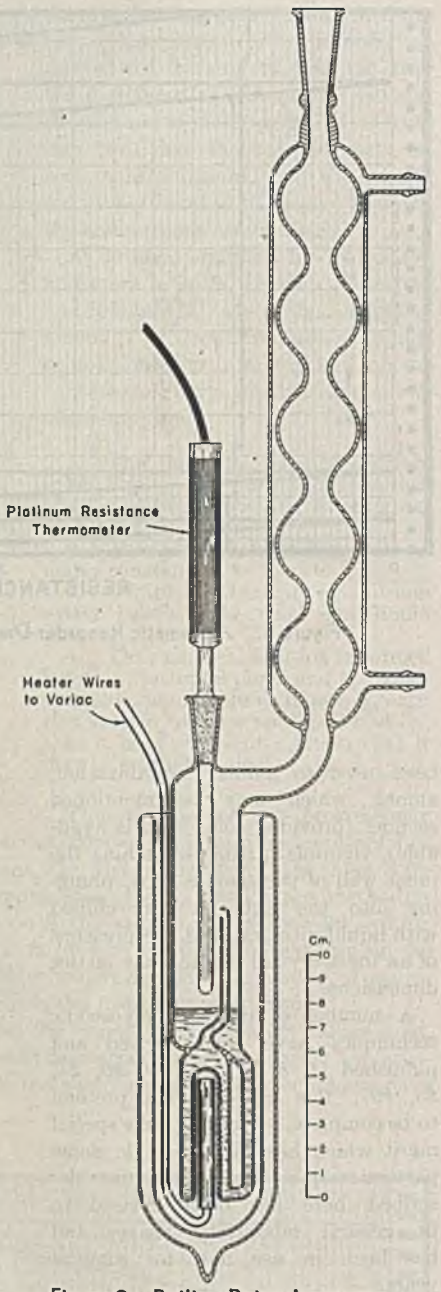


Figure 9. Boiling Point Apparatus

toward the right. The recorder has proved so useful that the users early learned to make the necessary mental geometric translation, and now find it just as convenient to work with as the older system.

The temperature of the sample decreases from A to B, then the range shifts, and the temperature of the sample decreases from C to E. At point E, crystallization is initiated and the temperature of the sample (which is supercooled at E) rises to F where the solid and liquid phases are in thermal equilibrium. The temperature follows from G to H (as the sample crystallizes) where the range again shifts and the sample continues to cool in the solid state from I to J. Sometime during the stirring, the stirrer freezes fast (the magnetic impulse is not strong enough to cause any damage, or if the windshield wiper is used, a flexible link is included) but this causes no trouble.

In working up the data, a line of constant temperature, F, is drawn from F to K, and JI is extrapolated back to K. Now the sample is all liquid at D and if it were 100% pure. it would have been completely frozen in the ideal case at K. Then the sample must have been half frozen at G, the midpoint between D and K. If the impurities concentrate in the mother liquor in accordance with assumption 1, when the sample is half frozen the impurities are twice their original concentration, and the difference in temperature ΔT between F and G is due to this doubling of the impurity concentration. Then pure 100% material would freeze at a temperature ΔT higher than the temperature F, and thus we have a method of determining the freezing point of the pure material from a sample containing impurities.

To a first approximation (neglecting higher terms whose maximum contribution is 2% or less), the purity of the sample is related to ΔT by the following relationship:

$$X = A\ \Delta T \qquad (4)$$

where

X = mole fraction of impurity

A = cryoscopic constant $= \dfrac{\Delta H_f}{RT_f^2}$

ΔH_f = molar latent heat of fusion of material in calories

T_f = absolute temperature of freezing point

R = gas constant per mole = 1.987 calories

In the case of Figure 3 $\Delta T = 0.02°$ C. (hardly observable in the reduced chart) and the initial freezing point of 100.00% pure monomeric styrene should be $-30.63°$ C. Also since $\Delta T = 0.02° \pm 0.01°$ C., our sample contains 0.05 ± 0.02 mole % impurity and thus has a purity of 99.95 ± 0.02 mole %.

Figure 4 shows what happens to the freezing curve as monomeric styrene is diluted with small amounts of ethylbenzene. The initial freezing temperature drops, but even more sensitive is the length of the plateau or straight portion of the cooling curve.

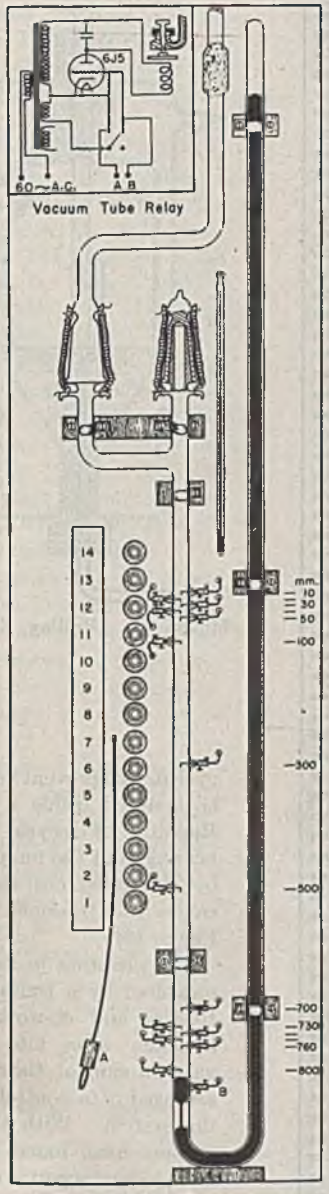


Figure 10. Contacting Barometer

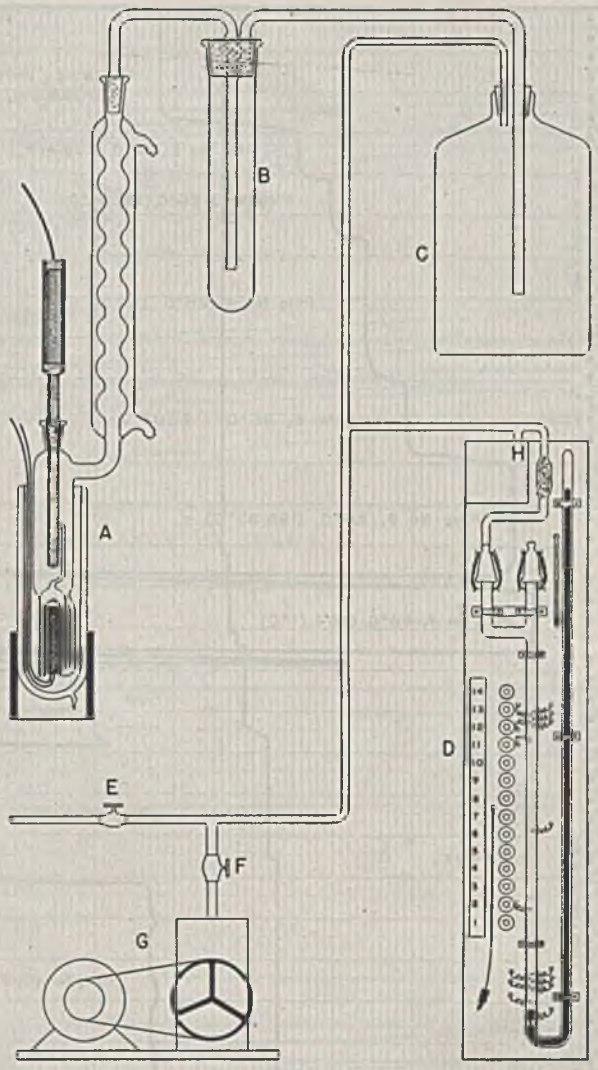


Figure 11. Constant-Pressure Boiling Point Apparatus

Figure 5 shows the freezing curve of a sample of *p*-chlorostyrene which turned out to be about 85 mole % pure. In the light of Figure 4, an experienced operator can tell at once the approximate purity by looking at the shape of the curve and the plateau. The nearest freezing curve to ideality that has been encountered is shown in Figure 6, and probably represents some of the purest chlorobenzene that has been prepared. Figure 7 shows a freezing curve of naphthalene, and is given here in order to compare it with the melting curve shown in Figure 8.

Because the thermal conductivity of organic solids is much less than the liquid, the melting curve does not present as good conditions for realizing thermal equilibrium of the melt. Hence, while the melting point can be determined accurately and checks the initial freezing point very well, measurements of ΔT at a half melted point are often in error. Nevertheless, occasions arise, as, for example, when the sample has supercooled some 20° or 30° before crystallization starts, and so much heat has been lost that the sample never reaches a thermal equilibrium between solid and liquid state, when the initial freezing point is known to be in error. It is then a very convenient thing to let the recorder draw the melting curve (it is necessary to melt the sample to retrieve the resistance thermometer) and thus gain more information about the sample.

Referring to Figure 8, as the sample melts, it attains a thermal melting equilibrium indicated by the dotted line, *AB*. The solid state line is extrapolated at *CA* and the liquid state at *DB*. Point *B* is the melting point of the compound which can be compared with the freezing point obtained in Figure 7 (actually this material was melted in a small furnace as shown in Figure 8, then cooled and the freezing curve in Figure 7 obtained). This sample of naphthalene was about 99.95 mole % pure.

Over the last 4 years, some 125 different organic compounds have been frozen in this laboratory. Frequently in the case of isomeric mixtures, the freezing point has been one of the most reliable analytical tools available. The list includes plastic monomers, hydrocarbons, alcohols, amines, halogenated hydrocarbons, sulfur compounds, acids, aldehydes, ketones, esters, etc., and the freezing temperatures range from −160° to +200° C. This work has served to stress the paucity of heat of fusion data needed to calculate the cryoscopic constant.

EBULLIOMETRY OF LIQUIDS

The problem of accurately measuring the temperature of a liquid boiling under precisely known pressures has received con-

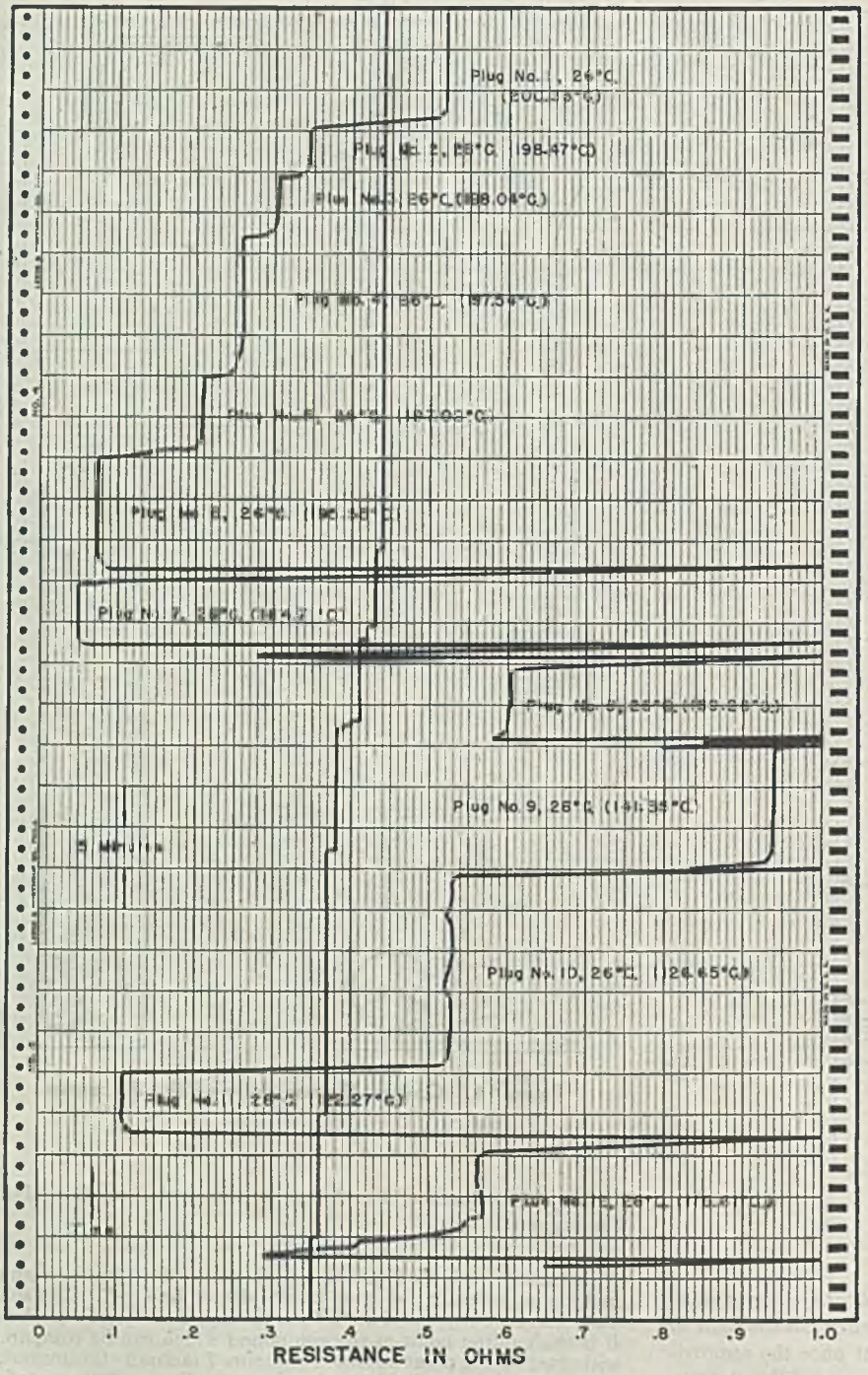


Figure 12. Automatic Recorder-Drawn Boiling Point of Ethylene Glycol under Various Pressures

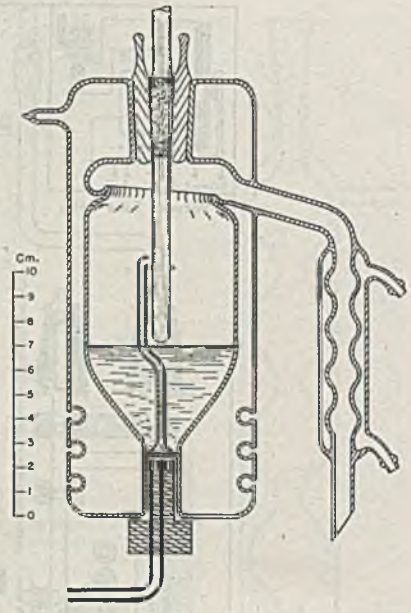


Figure 13. Boiling Range Apparatus

siderable attention (*5*, *6*, *7*, *16*, *25*, *27*, *30*, *31*). Over the last few years a boiling point apparatus which combines accuracy and convenience has been developed in this laboratory. It consists of a boiler and condenser, a special barometer for measuring the pressure, and auxiliary pressure control apparatus.

Figure 9 is a diagram of the boiler, which consists of a small reservoir with a re-entrant well in the bottom. The re-entrant well contains an electric cartridge-type heater (Chromolox C201), and has powdered Pyrex fused to its inner wall to promote smooth boiling. The liquid boils and the vapor pushes hot liquid before it up the Cottrell pump where together they spew out on the platinum resistance thermometer. The vapor is condensed and the liuqid returns to the bottom of the reservoir where it is recycled. To prevent heat loss, the boiler is mounted inside a thermos bottle. Rapidity of recycle prevents superheating, and the temperature recorded by the resistance thermometer soon comes to a thermal equilibrium (see Figure 12).

The pressures in this apparatus are measured by a contacting barometer. Rossini and co-workers (*31*) sealed tungsten wires into the permanent vacuum side of their barometer, and arranged it to control the pressure on the system. With this scheme, the authors have found some slight gas leakage over a period of several months around the tungsten wires. A new model built with the contacts on the open side of the barometer seems to work much better.

Figure 10 shows a diagram of the contacting barometer, which is made of 10-mm. Pyrex tubing with 20-mil tungsten wire sealed into the open arm. By means of a set of binding posts a plug can easily connect any of the contact points with a vacuum tube relay. The armature of the relay is fitted with a rubber pad which can cover or uncover a small hole whose free opening is adjustable by a small needle valve, *H* (Figure 11).

Figure 11 shows the pressure control apparatus. When it is plugged into a pressure less than atmospheric, the vacuum pump, *G*, continually exhausts the system at a rate governed by *F*. When the mercury rises to the appropriate tungsten contact point in the contacting barometer, *D*, the air leak, *H*, opens and admits air until the mercury falls below the contact, thus shutting off the air leak. By careful adjustment of valves *F* and *H* the mercury column (and indirectly the pressure) can be controlled to about ±0.1 mm. on either side of the contact point. These fluctuations in pressure are smoothed out by a 5-gallon surge

tank, *C*, between barostat *D* and boiler *A*, so that the pressure fluctuation on the boiler (as determined by an independent method) is about ±0.02 mm. *B* is a dry ice trap to prevent vapors from the sample from invading the rest of the system. When plugged into a pressure greater than atmospheric, compressed air is fed in at *E*, and *F* is closed. The operation is the same, except that the normal position of the vacuum tube relay is now reversed, because the air leaks out instead of in. By placing pure distilled water in the boiler and measuring the boiling point of the water with a platinum resistance thermometer for each contact point, the pressure value of each contact point was interpolated from the accurate water vapor pressure data of Osborne and Meyers (*15*).

This scheme eliminates considerations of pressure drop between the points where the temperature and pressure are measured. Because the barometer also acts like a thermometer it must always be corrected to 0° C.

Thus by adjustment of the apparatus, the boiling point at some 14 pressure points (see Figure 12) can be measured to ±0.02 mm. of mercury. Examination of Figure 12 will disclose the fact that at lower pressures (plugs 10 and 12) there is difficulty in establishing a steady temperature equilibrium. This difficulty is encountered generally in any dynamic boiling point method.

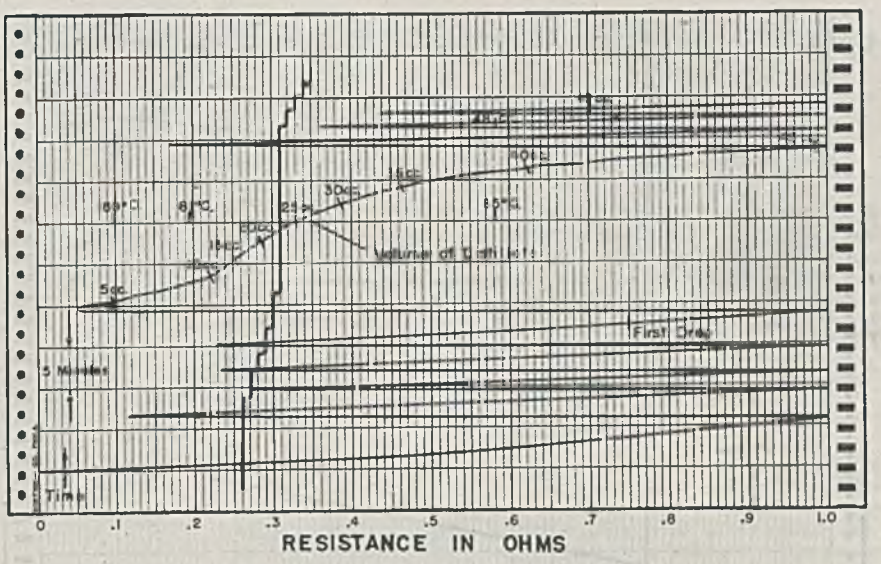


Figure 14. Automatic Recorder-Drawn Boiling Range of Unrefined Benzene

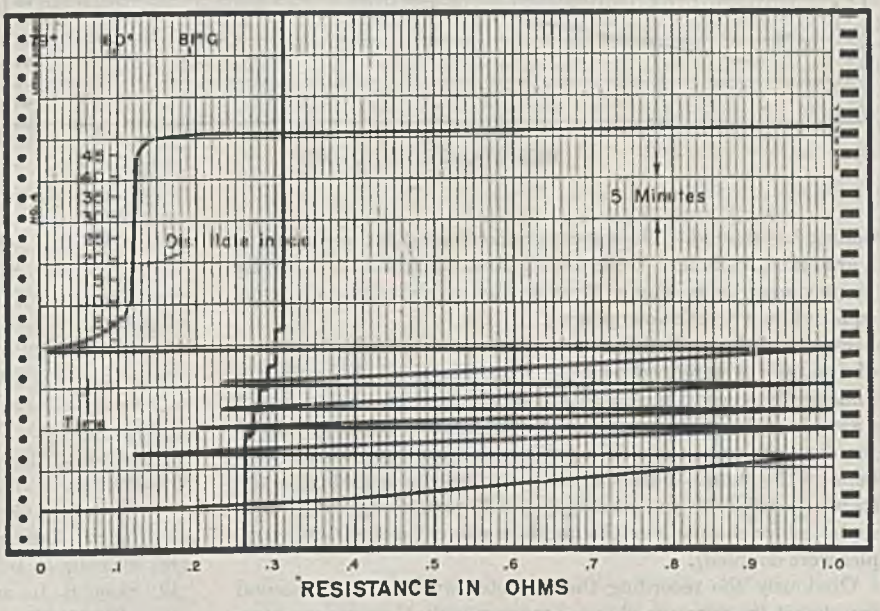


Figure 15. Automatic Recorder-Drawn Boiling Range of A.C.S. Reagent Grade Benzene

BOILING RANGE OF A LIQUID

Much time is spent in determining the boiling range of a particular liquid in order to get some idea of its purity. Standard prescribed methods for accomplishing this have been in use for a number of years (*2*). It would seem that this is an instance in which an automatic recording resistance thermometer could serve to advantage.

Figure 13 gives a diagrammatic sketch of an apparatus designed to be used to determine the boiling range of a liquid.

Fifty milliliters of liquid are placed in the central chamber, and the Cottrell pumping tube is inserted and held in place by the resistance thermometer. The liquid is boiled by means of a heater, which is a flat pancake-type coil of Nichrome ribbon on the upper end of an asbestos plug, and which is inserted in the bottom heater well. The whole apparatus is surrounded by a conventional vacuum jacket. Once the heat is turned on, it is kept at a constant value, so that the boiling rate will always be constant and comparable. The trough at the top of the inner chamber prevents vapor from condensing and running back, once

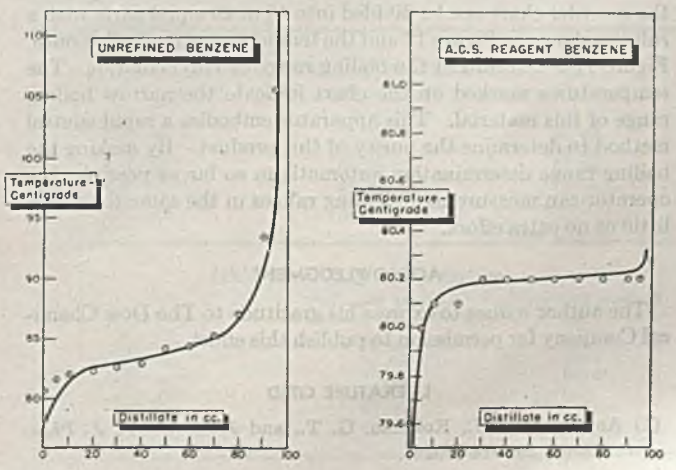


Figure 16. Comparison of This Boiling Range Method with the Standard A.S.T.M. Boiling Range Method

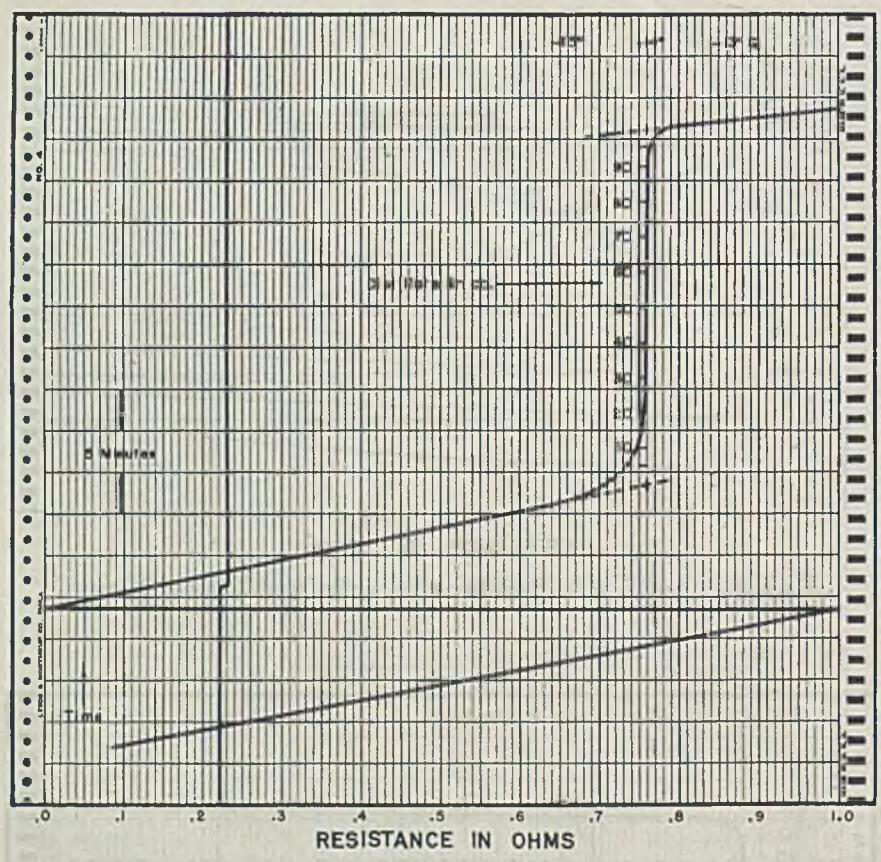


Figure 17. Automatic Recorder-Drawn Boiling Range of Vinyl Chloride

boiling has started. The vapors are condensed and recovered in a graduated cylinder. Once boiling starts, liquid and vapor are pumped over the resistance thermometer which is thus in thermal equilibrium with each component.

Figure 14 shows the time-resistance record of the boiling range of a sample of unrefined benzene, while Figure 15 shows the automatically traced time-resistance record of the boiling range of a sample of A.C.S. reagent grade benzene. In Figures 14 and 15 the distillate was caught in a graduated cylinder and the record kept on the chart. Both samples were also run according to the standard A.S.T.M. procedure (*2*) and Figure 16 shows the data replotted for comparison (the milliliter values for the 50-ml. samples were doubled).

Obviously the recording thermometer working every second can detect temperature changes more quickly than an operator. This is an important factor, particularly on the ends of the curves. Because the rate of heat input is constant, it is not necessary to observe each unit of 5 or 10 ml. of distillate as it comes over, but the recorder chart can be divided into 10 or 20 equal parts with a ruler as shown in Figure 17 and the temperatures read off at once. Figure 17 is a record of the boiling range of vinyl chloride. The temperatures marked on the chart indicate the narrow boiling range of this material. This apparatus embodies a rapid control method to determine the purity of this product. By making the boiling range determination automatic in so far as possible, the operator can measure more boiling ranges in the same time with little or no extra effort.

ACKNOWLEDGMENT

The author wishes to express his gratitude to The Dow Chemical Company for permission to publish this effort.

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Volumetric Quantitative Determination of —SOONa Group in Sodium Benzene Sulfinate

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THE quantitative precipitation of aryl sulfinic acids as ferric salts, reported in 1909 by Thomas (*3*), has been employed in a variety of procedures as the principal reaction in the quantitative determination of the sulfinic acids. Thomas mentioned the formation of sulfonyl chlorides, by reaction with sodium hypochlorite in sodium carbonate solution, but apparently made no attempt to use this reaction as a quantitative tool.

Krishna and Singh (*2*) reported using the ferric salt reaction, employing a solution of ferric chloride of predetermined concentration, filtering off the ferric salt, and determining the excess of ferric chloride. Until recently, Thomas' ferric salt reaction in very concentrated solution has been employed in this laboratory, for want of a better method.

The approximate equivalent of 2.5 grams of sodium benzene sulfinate is dissolved in 25 ml. of distilled water, and made acid to Congo red by dropwise addition of concentrated hydrochloric acid. Fifteen milliliters of 15% ferric chloride solution are added and the resultant slurry is swirled for several minutes until the precipitate turns light yellow. The precipitate is filtered and washed 6 times with 5 ml. of water, and 3 times with 5 ml. of methanol, care being taken to fill in the cracks which form on the filter. The precipitate is then removed to a large crucible (5 cm.) and carefully ignited to Fe_2O_3.

There are disadvantages in using this procedure. The ferric salt is soluble in excess ferric chloride. The precipitate is so voluminous that it is very difficult and time-consuming to wash it free of salts, and the mass on the filter nearly always cracks. Sodium sulfite, known to be present in commercial sodium benzene sulfinate, interferes with the accuracy of the method. Comparative runs on equal samples have also shown that the weight of precipitate becomes less as the initial concentration of sodium benzene sulfinate is reduced.

These objections also apply to the Krishna and Singh method in its application to commercial production.

Krishna and Das (*1*) report a complex gas volumetric analysis involving the liberation of iodine from a mixture of potassium iodide and potassium iodate, and utilization of the free iodine to liberate oxygen from hydrogen peroxide. The success of this method, by admission of the authors, depends on the high purity of the reagents and also on an approximate adjustment of the proportions in which they are used. These prerequisites, in addition to the complexity of the procedure, seem to disqualify it as a simple method.

Thomas' reaction of sodium benzene sulfinate with sodium hypochlorite was investigated and found to give accurate and reproducible results. When sodium benzene sulfinate with only slight excess of sodium carbonate was treated with concentrated (12%) sodium hypochlorite, a small yield of sulfonyl chloride was obtained. However, under the conditions employed in the method described below, the reaction is probably

$$C_6H_5SO_2Na + NaOCl \longrightarrow C_6H_5SO_3Na + NaCl$$

Even if some sulfonyl chloride is formed, the equation

$$C_6H_5SO_2Na + NaOCl + H_2O \longrightarrow C_6H_5SO_2Cl + 2NaOH$$

still gives the same molecular proportions to the reaction as the first equation.

Chlorates present in the sodium hypochlorite solution used presented no problem, since they do not react with the sulfinate and may still be detected after completion of the oxidation.

TEST PAPER. The starch-iodide test paper used in the procedure described here must be very sensitive. The following formula, used in this laboratory, was found satisfactory:

One liter of distilled water is boiled several minutes, and 3 grams of pure cadmium iodide crystals are added to it, followed by 5 grams of potato starch which have been made into a thin paste with water. The mixture is boiled for several minutes. It is allowed to settle for at least 24 hours, and the clear liquor is decanted. Then strips of Reeves-Angel filter paper No. 211, about 12.5 cm. (5 inches) wide, are passed through the solution and dried in a relatively dust-free atmosphere, away from fumes. The strips are cut into thin strips 1.25×12.5 cm. (0.5×5 inches).

PROCEDURE

A sample, *S*, equivalent to approximately 2.5 grams of sodium benzene sulfinate is accurately weighed and transferred to a 250-ml. Erlenmeyer flask, diluted to approximately 150 ml. with distilled water, and made slightly alkaline with dilute sodium hydroxide solution, using a very small spot on brilliant yellow paper as an indicator.

Ten milliliters of 10% barium chloride solution are added and the precipitate of barium sulfite (and barium sulfate) is allowed to digest for 0.5 hour on a steam bath. The solution is then filtered, washed directly into a 250-ml. volumetric flask, and made to the mark at room temperature (Solution A).

Twenty milliliters of about 12% sodium hypochlorite solution are diluted to approximately 1 liter (Solution B). Fifty milliliters of this solution are titrated with standard 0.1 *N* sodium arsenite solution until the blue spot which first appears on starch-cadmium iodide test paper when touched by a drop of the solution ceases entirely to show. (This standardization of solution B must be repeated if the latter is to be used after several hours' standing.) Titration = *b*.

Fifty milliliters of solution B, 50 ml. of 10% sodium carbonate solution, and 100 ml. of distilled water are cooled to about 15° C. Into this solution, solution A is run from a buret until a blue spot ceases entirely to appear, as above. Titration = *s*.

CALCULATION

$$\frac{b \times 0.016416 \times 100}{2 \times \frac{s}{250} \times S} = \frac{205.2 \times b}{s \times S} = \%\ \text{sodium benzene sulfinate}$$

EXPERIMENTAL

A good grade of sodium benzene sulfinate was recrystallized three times from water. The crystals obtained were dried over sulfuric acid in vacuum for 36 hours, then dried at 100° C. in air for 1 hour. The resultant compound was analyzed and found to contain S, 19.51%; C, 44.00%; H, 3.13%. Calculated: S, 19.52%; C, 43.89%; H, 3.07%.

This material was analyzed with the results shown in Table I.

To test further the relative values of the two procedures for commercial application, a mixture of the pure sodium benzene sulfinate with the inorganic salts usually found in the technical product, and sodium benzene sulfonate, a possible impurity, was

Table I. Analysis of Sodium Benzene Sulfinate

	Proposed Method	Ferric Salt Method
	%	%
1st run	100.24	99.66
2nd run	100.10	100.43
3rd run	100.10	95.94
4th run	99.97	....
5th run	99.97	....
6th run	100.11	....

Table II. Analysis of Mixture

	Proposed Method	Ferric Salt Method
	%	%
1st run	80.26	89.3
2nd run	79.89	98.3
3rd run	80.18	94.4
4th run	80.18	..
5th run	80.09	..
6th run	80.18	..

prepared. This combination was thoroughly ground and mixed to ensure homogeneity. Content of the mixture follows:

	Grams		Grams		Grams
NaCl	2.5000	Na_2SO_3	2.5000	$C_6H_5SO_2Na$	40.0000
Na_2SO_4	2.5000	$C_6H_5SO_2Na$	2.5000		

An analysis of the mixture containing 80.00% sodium benzene sulfinate gave the results shown in Table II.

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Determination of 1,2-Propylene Glycol in Ethylene Glycol

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A procedure for the determination of 1,2-propylene glycol in the presence of ethylene glycol consists of a periodate oxidation of 1,2-propylene glycol to acetaldehyde and formaldehyde and of ethylene glycol to formaldehyde, and the separation of the two aldehydes by a blowing-out process, forming the acetaldehyde sulfite addition compound which is titrated with iodine. By this method it is possible to determine 98% of the 1,2-propylene glycol present over a range of 0 to 90% 1,2-propylene glycol in ethylene glycol. A method for determining total glycols by periodate is also given.

A METHOD was desired for the determination of 1,2-propylene glycol in ethylene glycol, in varying amounts and in various types of samples. Fractionation procedures were impractical, since they required too much time and did not easily permit analytical separation because of the small difference in boiling points. Furthermore, this type of method would not be satisfactory when dilute aqueous solutions were encountered.

A search of the literature revealed much work on this problem, but none suitable to the authors' needs.

Hoepe and Treadwell (*4*) developed a method for the quantitative determination of glycerol, ethylene glycol, and 1,2-propylene glycol involving periodate oxidation of the alcohols and depending on accurate determination by the potassium cyanide method of formaldehyde in the presence of acetaldehyde. The authors found this procedure unsuitable, owing to interference of the acetaldehyde. In a method for determining small amounts of lactic acid in blood and urine, developed by Clausen (*1*), the lactic acid was oxidized to acetaldehyde which was removed by an aeration process from the other oxidized materials. Nicolet and Shinn (*6*) determined methyl pentose in the presence of pentoses by a periodate oxidation and separation of aldehydes by aeration. As the basis for the present work Shupe's (*7*) micro application of the Nicolet and Shinn procedure to glycols in cosmetic ingredients was used. Changes in design of equipment and procedure were made to ensure theoretical results in the type samples encountered.

OUTLINE

The oxidation of glycerol by the periodate method of Malaprade (*5*), Fleury and Fatôme (*3*) and adaptation to glycols by Denice (*2*) may be expressed as follows:

$$C_2H_4(OH)_2 + HIO_4 \longrightarrow 2CH_2O + HIO_3 + H_2O$$

$$CH_3CHOHCH_2OH + HIO_4 \longrightarrow CH_2O + CH_3CHO + HIO_3 + H_2O$$

The ethylene glycol yields two molecules of formaldehyde, while the 1,2-propylene glycol reacts to give one molecule each of formaldehyde and acetaldehyde. The aldehydes are separated by blowing them through a saturated solution of sodium bicarbonate containing a definite quantity of glycine. This treatment removes the formaldehyde, and the acetaldehyde is then determined by the sodium bisulfite procedure:

$$HCHO + NH_2CH_2COOH \longrightarrow CH_2NCH_2COOH + H_2O$$

$$CH_3CHO + NaHSO_3 \longrightarrow CH_3C(OH)(H)—SO_3Na$$

$$2NaHSO_3 + 2I_2 + 2H_2O \longrightarrow 4HI + H_2SO_4 + Na_2SO_4$$

The sulfite solution containing the absorbed acetaldehyde is treated with iodine to remove excess sulfite and then made alkaline with saturated sodium bicarbonate which destroys the addition compound. The sulfite thus liberated is titrated with a standard iodine solution with a buffer added just before the end point.

REAGENTS

PERIODIC ACID, 0.1 *M*. Weigh 10.7 grams of sodium metaperiodate into a 500-ml. volumetric flask, and add 200 ml. of water followed by 100 ml. of 1 *N* sulfuric acid. Dilute the solution to volume with distilled water and shake the flask until solution is complete.

IODINE, standard 0.1 *N*.

IODINE, standard 0.02 *N*. Dissolve 30 grams of potassium iodide in 100 ml. of water in a 500-ml. volumetric flask and measure in accurately from a buret 100 ml. of standard 0.1 *N* iodine. Make to volume with distilled water.

IODINE, approximately 0.1 *N*.

SODIUM BISULFITE, 5%. Dissolve 5 grams of sodium bisulfite in 100 ml. of water.

BUFFER (Borax–sodium carbonate mixture). Dissolve 4 grams of borax ($Na_2B_4O_7.10H_2O$) and 5 grams of anhydrous sodium carbonate in 100 ml. of water.

GLYCINE (aminoacetic acid). Dissolve 5 grams of U.S.P. glycine in saturated sodium bicarbonate solution and dilute to 250-ml. volume with saturated sodium bicarbonate solution.

STARCH. Dissolve 0.5 gram of soluble starch in 10 ml. of cold water and add to 90 ml. of boiling water. Boil 5 minutes and cool.

SODIUM BICARBONATE, U.S.P. powder.

SODIUM BICARBONATE, saturated aqueous solution.

SODIUM ARSENITE, standard 0.1 *N*.

POTASSIUM IODIDE, approximately 10%.

APPARATUS

Special equipment used in determining 1,2-propylene glycol consisted of:

CARBON DIOXIDE OR NITROGEN, one cylinder.

FLOWMETER, calibrated for 1.5 liters of carbon dioxide per minute.

REACTION AND ABSORPTION TUBES. The equipment consists of a series of 4 test tubes connected in a manner satisfactory for passing gas through the solutions. The first tube (200 × 29 mm.) is fitted with a 3-hole rubber stopper carrying a small separatory funnel through which the sample aliquot and periodate solutions are added. This funnel also serves as an inlet tube for carbon dioxide. A large-bore glass tube, also inserted in the stopper of the first tube, is divided above the stopper by a piece of rubber tubing. It serves as a reservoir and inlet for the sodium

bicarbonate powder. A pinchclamp on the rubber tubing prevents loss of gas when the sodium bicarbonate is added. The other 3 tubes in the series are 150 × 25 mm.

PROCEDURE

Total Glycols. Pipet an aliquot of not more than 30 ml., containing not less than 15 mg. nor more than 90 mg. of glycols calculated as ethylene glycol, into a 125-ml. Erlenmeyer flask. Pipet in 15.00 ml. of 0.1 *M* periodic acid, mix well, and allow to stand 15 minutes. Add 30 ml. of saturated sodium bicarbonate solution, after which the solution should be approximately neutral. Measure in accurately 50.00 ml. of 0.1 *N* sodium arsenite and finally add 1 ml. of 10% potassium iodide and an excess of solid sodium bicarbonate. Titrate with 0.1 *N* iodine. With a little practice the yellow end point can readily be detected, and the use of this end point without the addition of starch is preferred. The presence of solid sodium bicarbonate improves the quality of the end point by ensuring a saturated solution.

Run a blank determination by placing 15.00 ml. of 0.1 *M* periodic acid in a 125-ml. Erlenmeyer flask, add 30 ml. of saturated sodium bicarbonate solution, 50.00 ml. of 0.1 *N* sodium arsenite, and 1 ml. of 10% potassium iodide, and allow the solution to stand 15 minutes. Add solid sodium bicarbonate in excess and titrate with 0.1 *N* iodine to the yellow end point.

The milliliters of 0.1 *N* iodine used for the determination minus the milliliter used for the blank gives the milliliter of 0.1 *N* iodine equivalent to the periodic acid used to oxidize the ethylene glycol and 1,2-propylene glycol.

$$\frac{\text{Net ml. of 0.1 } N \text{ iodine} \times 0.003102 \times 100}{\text{sample weight}} = \%\text{ by weight of ethylene glycol + 1,2-propylene glycol, as ethylene glycol}$$

The above result (total glycol as ethylene glycol), minus per cent of 1,2-propylene glycol from the following determination times 31/38, equals per cent by weight of ethylene glycol.

Glycerol, if present, must be determined and accounted for, since it is also oxidized by periodate in accordance with the following equation:

$$CH_2OHCHOHCH_2OH + 2KIO_4 \longrightarrow 2CH_2O + HCOOH + 2KIO_3 + H_2O$$

In this case the formic acid may be titrated.

1,2-Propylene Glycol. Accurately weigh a sample of the proper size and dilute to a suitable volume, so that a 10- to 20-ml. aliquot will contain not more than 50 mg. of total glycols and not more than 10 mg. as 1,2-propylene glycol. Pipet an aliquot to the largest test tube of the series described above and stopper it after adding water, if necessary, to make a final volume of 25 ml. Pipet into the second test tube sufficient glycine solution to leave a 10% excess of glycine over the amount required to remove the formaldehyde. Too large an excess will remove some acetaldehyde and cause low results. If the ethylene glycol content is unknown, make a trial determination first and calculate the amount of glycine from the result. Add to the second tube saturated sodium bicarbonate solution sufficient to make the final volume 10 ml. In the third and fourth tubes place 1 ml. of 5% sodium bisulfite and 15 ml. of distilled water.

Place 15 ml. of 0.1 *M* periodic acid in the separatory funnel and connect the carbon dioxide line. Open the stopcock and allow the acid to run into the reaction tube. Mix the solution gently for 15 minutes by passing in a small amount of carbon dioxide. Meanwhile place 4 grams of solid sodium bicarbonate in the large-bore glass tube. After the 15-minute mixing, remove the pinchclamp and tap in the sodium bicarbonate. Replace the clamp and pass in carbon dioxide at the rate of 1.5 liters per minute for 1 hour.

Transfer the contents of tubes 3 and 4 to a 250-ml. Erlenmeyer flask with the aid of a wash bottle. Add 5 ml. of starch indicator and run in the approximately 0.1 *N* iodine from a buret until a blue color persists, shaking the flask continually. Avoid the addition of a large excess at any one time. Discharge the blue color with a drop of 5% sodium bisulfite. After 5 minutes add 0.02 *N* iodine dropwise to the blue starch-iodine end point. Now add 10 ml. of saturated sodium bicarbonate solution and again titrate with 0.02 *N* iodine to the blue color. Before taking the final end point add 10 ml. of the borax-carbonate buffer solution. Record the total volume of 0.02 *N* iodine solution used after the excess sodium bisulfite was removed by the first addition of 0.02 *N* iodine, and calculate the per cent by weight of 1,2-propylene glycol as follows:

Table I. Analytical Data

Sample Weight	Ethylene Glycol Present	1,2-Propylene Glycol Present	1,2-Propylene Glycol Found by Acetaldehyde	Error
Mg.	%	%	%	%
43.98	95.44	4.56	4.59	+0.7
45.98	91.29	8.71	8.61	−1.2
47.98	87.50	12.50	12.50	±0.0
49.98	84.00	16.00	16.22	+1.4
25.85	61.10	38.90	38.16	−1.9
16.90	41.13	58.87	58.24	−1.1
12.61	20.65	79.35	78.22	−1.4
11.06	9.50	90.50	89.56	−2.1

$$\frac{\text{Ml. of 0.02}N \text{ iodine} \times 0.00076 \times \text{aliquot factor} \times 100}{\text{sample weight}} = \%\text{ by weight of 1,2-propylene glycol}$$

DISCUSSION

Various methods were tried to secure better results on the acetaldehyde determination. No noticeable difference was observed when the periodate oxidation was carried out at 0° and 100° C. (In order to keep the contents of the reaction tube at 100° C. without concentration, a condenser was used on the reaction tube.) Following the procedure of Nicolet and Shinn, alanine was used instead of glycine and 0.1 *N* arsenite solution was used to take care of excess periodate, without improving the method.

Best results were obtained by adding the periodate solution to the reaction tube through a separatory funnel and maintaining a closed system by adding the solid sodium bicarbonate through the large-bore glass tube.

That glycine, if present in too high a concentration, will remove some acetaldehyde was demonstrated by placing a glass ampoule containing a known amount of acetaldehyde in the reaction tube and going through the complete procedure. The recovery was 86%. Experimental results showed that formaldehyde was completely removed by the glycine; a 10% excess of glycine over that required to react with the formaldehyde formed proved to be satisfactory. In the lower percentage range of 1,2-propylene glycol very good recovery is obtained. However, in the higher ranges the results tended to run slightly low, since the glycine reacted with a very small amount of acetaldehyde.

The analytical data are given in Table I. The 1,2-propylene glycol used in this work was a regular Dow product and analyzed 100.8% by the periodate method. The ethylene glycol was Eastman Kodak Co.'s No. 133, analyzing 100.1% by the same procedure. Values given in Table I have not been corrected for the fact that the pure materials analyzed more than 100%.

Interfering substances are molecules containing adjacent hydroxyl groups or an amino group adjacent to a hydroxyl group.

An attempt was made to oxidize the formaldehyde to formic acid with hydrogen peroxide: the formic acid could then be determined by reduction of mercuric chloride to the insoluble mercurous chloride. Very high results were obtained, since hydrogen peroxide itself reduces mercuric chloride. However, when silver oxide was tried instead of hydrogen peroxide, the recovery was about 75%.

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Estimation of Salts of Weak Acids by Direct Titration in a Mixed Solvent

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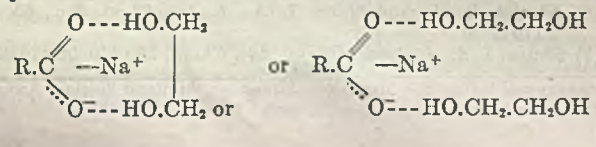
A simple volumetric method for the estimation of alkali metal salts of weak monobasic organic acids (—COONa), and a few weak monobasic inorganic acids, using direct titration in a mixture of cosolvents is described. Weak bases such as aniline, toluidine, and amines can also be titrated with sharp end points in the same media. The method consists of dissolving the salt in a mixture of solvents containing glycol and a higher alcohol or chloroform and titrating with hydrochloric or perchloric acid in the same mix-solvent, the end point being determined either potentiometrically or with the help of methyl red or methyl orange. In such media the order of indicator range is sometimes the reverse of that in an aqueous medium. Further applications of the method have been made or suggested for estimation of soaps, amino acids, weak acid–strong acid mixtures, and equivalent weight of any unknown organic acid.

THERE is no easy and accurate method for the estimation of salts of weak acids such as sodium acetate. Generally it is done (*7, 13*) by distilling the acid liberated by sulfuric acid or phosphoric acid and absorbing it in standard alkali, or by the pyrolytic method. For salts of nonvolatile acids like sodium benzoate the matter is more complicated and may involve extraction from acid with ether. The present paper describes a simple direct method which is rapid and accurate, has some special features and advantages which are not met in aqueous solution, and is remarkably free from all complicating factors due to hydrolysis.

The method depends on the fact that all salts of monobasic weak organic acids containing the group COONa are highly soluble in a glycol or a glycol mixed with some solvent for hydrocarbons. Such mixtures may be referred to as G-H mixtures, where G stands for any glycol type of solvent and H represents any solvent for hydrocarbons, such as hydrocarbons themselves, alcohols, chlorinated hydrocarbons, etc. The solutions thus obtained can be titrated directly with hydrochloric or perchloric acid dissolved in the same solvent mixture, the end point being indicated either potentiometrically or by the color change of indicators. The method likewise can be used with a few weak inorganic acids, the salts of which are soluble in the aforementioned solvents, and can be applied to determine any excess base which might be present in a neutral salt, since no free base is produced by hydrolysis. The excess base which makes the solution alkaline to phenolphthalein or cresol red can be directly determined by titration in the G-H solvent medium. Thus, by a double indicator titration, first with phenolphthalein or cresol red and then with methyl orange or methyl red, both the free base and the combined base can be determined.

Weak acids are far less dissociated in nonaqueous solvents such as alcohol than in water; hence, their sodium salts act as bases towards indicators. However, most are only slightly soluble in such solvents and the end point is not sharp because the color change or the pH change extends over a long interval. The solvent medium here proposed provides high solubility and a sharp end point.

The COONa group in organic salts of the type R.COONa undergoes a specific solvation with glycols, evidently due to hydrogen bond formation as shown below, leading to a high solubility.



Of course, the —COO— group has no double bond in the classical sense as represented in the conventional formula, but both the oxygen atoms are equivalent, owing to a mesomeric shift in the electronic structure. If R is large enough it prevents adequate solvation of the whole molecule by the glycolic group alone, and only by the addition of a hydrocarbon solvent is a high solubility attained.

The solvent properties of G-H mixtures have been extensively studied and the theoretical aspects discussed in separate publications (*10, 12*); here these mixtures will be discussed only in respect to their suitability as solvent media for titration. In conformity with the above idea it has been found that glycols or G-H mixtures are powerful solvents for any organic salt of the type R.COONa. This is illustrated by a few data in Table I, taken from a portion of a forthcoming publication on soap solubility in G-H solvents.

As can be observed, R can stand for both aliphatic and aromatic groups, and hence G-H mixtures might be regarded as an almost universal solvent for alkali metal salts of any monobasic organic acid. They serve as good solvent media for direct titration of the salts with strong acids.

Almost any G-H mixture can be used for these direct titrations, but in making the choice the author has paid attention to the following points: sharp end point, high solvent power, low viscosity, low volatility, freedom from toxicity, and easy availability. Of the three commercially available glycols, ethylene glycol is slightly preferable to propylene and to diethylene glycol in respect to the second and third points, but the advantage is not decisive. From the many solvents for hydrocarbons, isopropyl alcohol has been chosen as the cosolvent with ethylene and propylene glycol as best meeting all the above requirements. In extreme cases like sodium stearate, isopropyl alcohol does not confer sufficient hydrocarbon-dissolving property and hence other more powerful solvents, such as butyl or amyl alcohol, chloroform, dioxane, etc., might be used.

Table I. Solubility of Salts at 25° C. in Glycolic Media

(Grams of anhydrous salt per 100 grams of solvent)

Salt	Ethylene Glycol		Propylene Glycol	
	Alone	With cosolvent	Alone	With cosolvent
Sodium acetate	29.05	22.57[a]	.....	....
Sodium caprylate	29.52	....	22.31	....
Sodium laurate	11.92	....	12.19	....
Sodium oleate	16.43	28.81[b]	11.72	15.33[b]
Sodium benzoate	24.87	21.95[c]	13.13	10.24[a]
Sodium salicylate	Very high	....	Very high	....
Sodium propyl nitronate	30.51	34.89[c]	21.84	18.82[c]
Sodium nitrite	16.78	....	8.47	....
Borax	.....	.....	....	9.05
Sodium metaborate	6.98	....	3.36	....

[a] Chloroform, 20%.
[b] Butyl alcohol, 50%.
[c] *n*-Amyl alcohol, 20%.

The proportion of isopropyl alcohol which can be satisfactorily used as a cosolvent for glycol extends over a wide range, from 15 to 70% by volume being satisfactory. The author has, however, used a mixture 1 to 1 by volume of ethylene or propylene glycol and isopropyl alcohol as a standard solvent medium; although 20 volume % of the latter is slightly better with respect to sensitivity and solubility, the low viscosity of the 1 to 1 composition is advantageous.

The two acids tried, hydrochloric and perchloric, give equally satisfactory results. The latter is preferred, particularly in

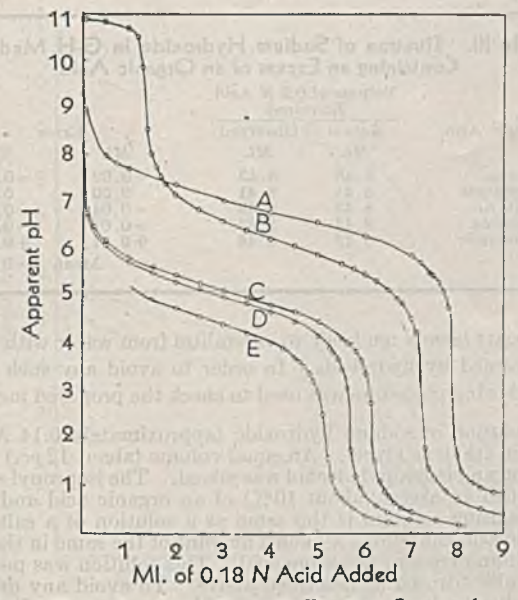


Figure 1. Potentiometric Titration Curves of Organic Acid Salts in G-H Solvents

0.18 *N* perchloric acid, glass electrodes
A. Sodium acetate
B. Sodium cinnamate containing excess alkali
C. Sodium formate
D. Sodium mandelate
E. Sodium salicylate

titrating concentrated solutions, since the perchlorate formed is much more soluble than the chloride and so does not produce any haziness during titration through separation of finely divided salt. This equality in strength, as evidenced by practically identical titration curves, is contrary to the findings of Hall and his co-workers (*5*), who found hydrochloric acid to give a steeper titration curve than perchloric acid in glacial acetic acid medium, and to the observations of others (*2, 3, 6*) on acid strength in nonaqueous solvents. That the intrinsic strength of perchloric acid should be more than that of hydrochloric acid is easily understood, since the coordinate bonds of the chlorine ion to the oxygens are formally equivalent to a transfer of negative charge resulting in a higher protogenic tendency (basic power) of the resulting perchlorate ion. However, this leveling effect on acids of glycolic solvents, which have very weak acidic or protogenic power but possibly no basic or protophilic power, is difficult to understand unless we admit that not only the acidic or basic nature of the medium but also its solvation power is an important factor. This may cause in some cases, as in the case of indicators, a reversal of the normal order of acid strengths.

Potentiometric titrations were conducted satisfactorily using a Beckman glass electrode. The readings on the dial of the pH meter indicate, of course, only apparent pH values, because the scale of acidity has not been thermodynamically established for these solvents, and because of the unknown value for the potential at the liquid junction between the solvent and the saturated potassium chloride solution surrounding the reference electrode. Nevertheless, it is remarkable that the pH changes and the behavior of the indicators are nearly in the same range as in an aqueous medium in weakly acid region. In strongly acid region the apparent pH is much lower; in fact, it is negative in a decinormal acid solution and hence, except as a means of determining the end point, these values have no significance at all.

A few titration curves are shown in Figures 1 and 2. The change at the equivalence point is rapid, and therefore the end point can be ascertained with great accuracy. For comparison, a titration curve of sodium acetate in water is shown in Figure 2. It will be observed that pure glycol could be used as a solvent medium with slight sacrifice of accuracy so far as the steepness of the titration curve is concerned. But two other additional factors stand in its way to be used as such: (1) its viscosity is rather high, which introduces a large "drainage" error in delivering from a buret, and (2) many organic acid salts are insoluble or sparingly soluble in the pure glycol but are sufficiently soluble in suitable G-H mixtures.

The same titration can be done with the help of suitable acid-base indicators. Owing to the uncertainties in the significance of conventional pH measurement in organic solvents, the choice of indicator has to be made by trial and error. Both methyl red and methyl orange can be satisfactorily used. The latter gives a sharper end point, but the former gives a brighter one. In more than 300 titrations by this method in connection with measurements of soap solubility, using both the indicators, methyl red was found more convenient, owing to a greater sensitivity of the eye in judging the methyl red color. The colors of the indicators are somewhat different from those in water and are much brighter and more contrasted. The color on the acid side is bright pink and that on the alkaline side is canary yellow for both indicators.

Of all common solvents, only a few as listed below show bright color and sharp change from alkaline to strong acid with methyl red, whereas in all other solvents a dull brownish orange color with a sluggish change is observed. The suitability of the solvent was judged by a very simple test: Five milliliters of each solvent were taken in each of two separate test tubes and to one was added a drop of glacial acetic acid and to the other a drop of alcoholic 0.2 *N* hydrochloric acid. Obviously, the solvents which show color with acetic acid and/or fail to show sharp change with hydrochloric acid cannot be used. The following solvents more or less qualified in the above test: propylene glycol, diethylene glycol, ethylene glycol, benzyl alcohol, methyl alcohol, phenols, chloroform, methylene chloride, glacial acetic acid, and cyclohexanol. The first two solvents are found best for the purpose, followed very closely by the third. The solvents at the end of the list are rather poor for the purpose. With somewhat less accuracy benzyl alcohol and methyl alcohol probably can be used in place of glycols, if other conditions permit. In fact, with respect to the brightness of the acid color of methyl red, benzyl alcohol (and phenol) is definitely superior to even the glycols, but its low solvent power, less availability, presence of blanks against alkali in even the purest commercial variety, etc., forfeited its chance of being considered for this purpose. If phenols, cresols, benzyl alcohol, etc., become available commercially in purer state they would perhaps be usable in place of the glycols

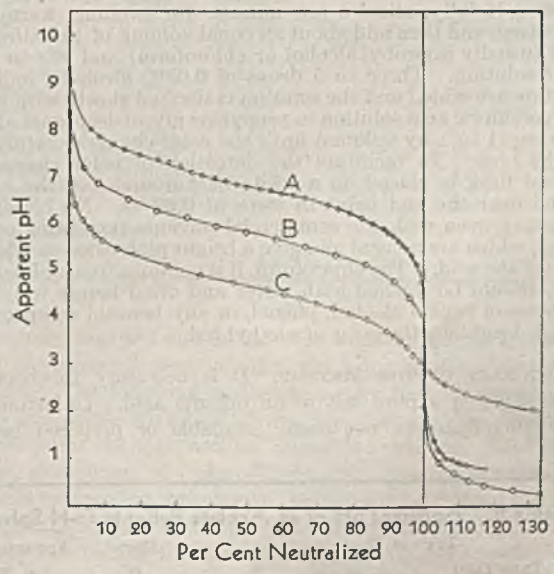


Figure 2. Potentiometric Titration Curve for Sodium Acetate

0.18 *N* perchloric acid, glass electrode
A. G-H solvent *B.* Glycol *C.* Water

for such titrations. As cosolvents ethyl alcohol shows too sluggish a color change to be used, whereas higher alcohols are better.

EXPERIMENTAL

ESTIMATION OF SALTS OF ORGANIC ACIDS. Potentiometric Method. Since the use of the glass electrode under such conditions is a departure from the usual procedure, a rather detailed description of the procedure is given.

All the potentiometric titration curves were obtained by dissolving 0.1 to 0.2 gram of the salt (equivalent to 8 to 10 ml. of 0.18 *N* acid) in 20 ml. of the G-H solvent and titrating with 6 *M* perchloric acid, using a Beckman glass electrode (Type 1190) with a Beckman pH meter. The solution to be titrated was taken in a 50-cc. beaker which was stirred by an electromagnetic device with a small piece of thin glass tubing containing a sealed iron nail. The glass electrode was adjusted with a buffer of pH 7, washed with a spray of distilled water, which was carefully tissued off completely with very soft absorbent paper, and immediately placed in the solution. The type of calomel electrode whose connection was made through a sealed-in thin asbestos fiber was used and was treated in the same way as the glass electrode. In less than 2 minutes very steady readings were obtained and the titration was then carried out as usual with perchloric acid dissolved in the same solvent mixture delivered from a 10-cc. buret. The pH value was checked with the same buffer after titration. It was found that the buffer now gave a value always higher by 0.02 to 0.06 pH unit, which tended to disappear on keeping the electrode immersed for a long time.

In order to determine how far these apparent pH values are reproducible, a solution of sodium acetate–acetic acid (approximately decinormal with respect to both components) in standard G-H mixture was made and its apparent pH was determined from time to time (Table II). The values are fairly reproducible and comparable.

Sodium salts of all the following acids have been found to give satisfactory results: acetic, propionic, butyric, oleic, stearic, benzoic, cinnamic, mandelic, and lactic. It would appear from the titration curve of formic acid that this is about the limit of acid strength suitable to the method with high accuracy, and salts of acids much stronger than formic ($K_\alpha = 2.1 \times 10^{-4}$) cannot be titrated without sacrificing accuracy.

Indicator Method. From 0.2 to 1.0 gram of the salt is dissolved in about 10 to 15 cc. of the solvent mixture in a 50-cc. conical flask or a big test tube (1 × 5 inch) and is titrated with 0.18 *N* perchloric acid, or stronger if necessary, to keep the titer to a convenient volume. In order to effect a quick dissolution of the solid it is advisable first to add 5 cc. of propylene glycol to moisten it fully, allow a few minutes for swelling, warming if necessary, and then add about an equal volume of the other solvent (usually isopropyl alcohol or chloroform) and stir to get a clear solution. Three to 5 drops of 0.05% alcoholic indicator solution are added and the solution is titrated slowly with standard perchloric acid solution in propylene glycol–isopropyl alcohol mixture (1 to 1 by volume) until the color changes sharply to a bright pink. To facilitate the detection of color change the conical flask is placed on a white background and the acid is added near the end point in steps of 0.02 cc. No blanks are necessary even with the commercial solvents (excepting chloroform), which are neutral and give a bright pink color on adding a drop of the acid. The chloroform, if it contains free hydrochloric acid, should be washed with water and dried before use. The presence of benzyl alcohol, phenol, or any benzoid compound, if added, brightens the color of methyl red.

ACCURACY OF THE METHOD. It is necessary to check the method using a pure salt of an organic acid. Unfortunately, such chemicals are not easily available or prepared because these salts have a tendency to crystallize from water with excess acid formed by hydrolysis. In order to avoid any such doubt the following procedure was used to check the proposed method.

Table II. Apparent pH of an Acetate Buffer in G-H Solvent

Type of Electrode	Days	Apparent pH
Type 1190	0	6.33
	1	6.31
	2	6.32
	3	6.30
Type 1190 (a different glass electrode)	3	6.31

Table III. Titration of Sodium Hydroxide in G-H Medium Containing an Excess of an Organic Acid

Organic Acid	Volume of 0.2 *N* Acid Required: Actual	Observed	Error:	
	Ml.	*Ml.*	*Ml.*	%
Acetic	8.45	8.43	−0.02	−0.24
Propionic	8.41	8.41	0.00	0.00
Butyric	8.43	8.39	−0.04	−0.48
Benzoic	8.45	8.43	−0.02	−0.23
Cinnamic	8.45	8.46	+0.01	+0.12
			Mean	−0.17

A solution of sodium hydroxide (approximately 0.14 *N*) was made in ethylene glycol. An equal volume (about 12 cc.) of this solution and isopropyl alcohol was mixed. The isopropyl alcohol contained an excess (about 10%) of an organic acid and hence the resulting solution is the same as a solution of a salt of an organic acid containing a known amount of the same in the presence of an excess of the same acid. This solution was potentiometrically titrated as described above. To avoid any drainage error due to high viscosity of glycol, the amount of glycolic caustic soda used was always determined by weighing. The results obtained are shown in Table III, from which it will be observed that the method is capable of an accuracy of at least 1 part in 500 without any special technique.

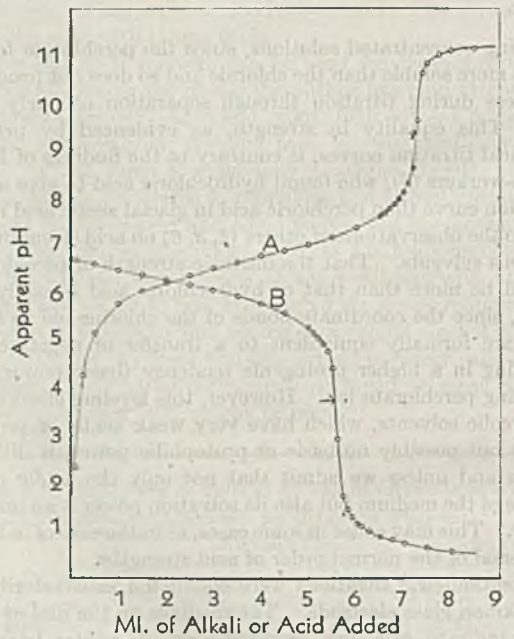


Figure 3. Potentiometric Titration Curves in G-H Solvents, Using Glass Electrode

A. Boric acid by 0.2 *N* alkali
B. Borax by 0.18 *N* perchloric acid

ESTIMATION OF SALTS OF INORGANIC ACIDS. It has been found that not only the COONa group but any sodium salt containing the group *X*OONa, where *X* stands for any negative element, is highly soluble in G-H mixtures and can be titrated accurately in such media. The inorganic salts containing this requisite structure are metaborates, aluminates, nitrites, nitronates, hypophosphites, hyposulfites, sulfinates, chlorites, etc., all of which are highly soluble in glycols and G-H mixtures, and they can be titrated as described above by either the potentiometric or indicator method. The only difficulty is encountered with strongly reducing hyposulfite which tends to decolorize the indicator, but in the 1 to 1 G-H solvent the reducing action is not sufficiently rapid to interfere with the titration. In fact, it has been observed that the G-H solvents, containing higher alcohols,

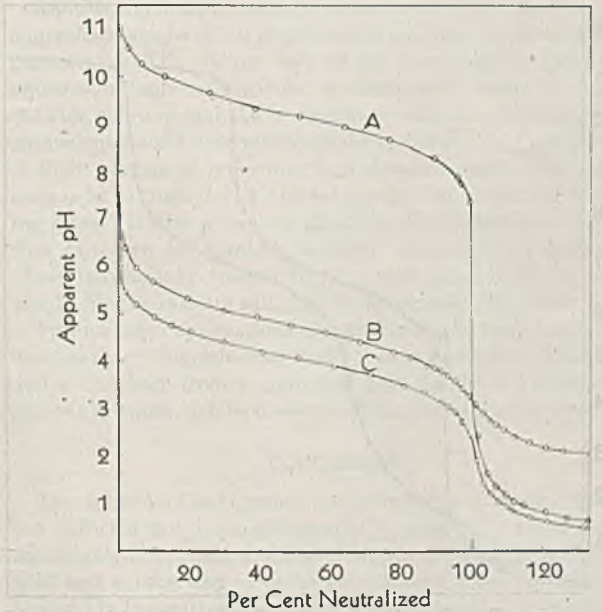


Figure 4. Potentiometric Titration Curves of Weak Bases in G-H Solvents

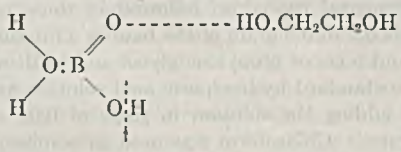
0.18 *N* perchloric acid
A. Ammonia *B.* Aniline in water *C.* Aniline

chloroform, etc., act as a retardant for such reduction processes, whereas in pure glycol the reduction is rapid.

In addition to the above acids there are a fair number of monobasic weak inorganic acids whose salts are sufficiently soluble in G-H mixtures to admit of such a titration. Such compounds are borate, silicate, arsenate, etc., which can be estimated in the same manner.

Estimation of Borates. In glycolic solvents, boric acid behaves as a moderately strong monobasic acid just as it does in aqueous solution in the presence of a large concentration of mannitol or glycerol. Hence, all the alkali-metal borates which have been found to be very highly soluble in glycolic solvents can be easily estimated. The salt is dissolved in glycolic solvent and is titrated with glycolic alkali to the color change of phenolphthalein or cresol red which gives the free acid equivalent. This solution is now titrated with glycolic perchloric acid using methyl red as indicator, which will give the total boric acid equivalent, provided no other weak acid is present in the system. The alkali titration of boric acid or borates gives a very sharp end point, whereas the acid titration is somewhat less accurate, owing to the fairly strong acidity of boric acid in glycolic solvent, making the end point less sharp. This is seen from the potentiometric titration curves of boric acid against alkali and borax against acid (Figure 3). The strong buffering effect of solutions of boric acid in the presence of borates is also worthy of notice.

The explanation of this behavior of boric acid can be given in terms of Lewis' theory. The octet structure of the boron in boric acid is not complete and the former can therefore accommodate two more electrons. Hence it is a monobasic acid (electron acceptor) like boron trichloride. Water forms a coordinate bond by lending a lone pair from its oxygen shell, which is equivalent to giving a formal negative charge to the central atom which inductively holds all the positive hydrogen atoms tighter. Alternatively, we might consider the structure of boric acid as shown below, $HBO_2.H_2O$, where it has a filled octet instead of a sextet as in the other structure, $B(OH)_3$. Glycol solvates the electronegative oxygen atom through hydrogen bond formation, as a result of which the hydroxylic hydrogen of boric acid becomes more prone to dissociation, similar to the stepwise behavior of a dibasic acid. This explanation, however, is different from that of Boesecken and co-workers (*1*), who consider that a coordinate compound is formed with a central boron atom after elimination of water between the hydroxyl groups of boric acid and glycol.



Estimation of Weak Bases. Since glycol is an acidic or protogenic solvent we should expect it, according to the Lowry-Brønsted theory, to augment the apparent basic power of a base, and beyond a certain strength make all bases appear strong. However, the acidic power of glycol will be inferior to water as a titration medium for weak bases. There is, however, one point to consider which makes it decidedly superior to water for such use. Water, owing to its amiphiprotic nature, also shows its protophilic activity on the salt formed during titration and hence obscures the end point. Thus, in titrating aniline in aqueous medium, we have two reactions simultaneously occurring, as shown below:

$$C_6H_5NH_2 + H^+ \longrightarrow C_6H_5NH_3^+$$

$$C_6H_5NH_2 + H_2O \rightleftharpoons C_6H_5NH_2 + H_3O^+$$

whereas in glycolic medium the second reaction, in which the solvent competes for the proton, will be much reduced.

A few potentiometric titration curves for weak bases are shown in Figure 4. It will be observed that aniline ($K_\beta = 4.6 \times 10^{-10}$ in water) gives a curve which is of almost the same type as that of ammonia in water; in other words, its apparent dissociation constant as judged by the steepness at the inflection point has been increased more than 10,000 times that in water. For comparison, its titration curve in water under the same conditions has been shown. It should be noted that the apparent pH of aniline in the G-H solvent titration curve is much lower than the same curve in water, whereas in the case of sodium acetate the apparent pH is higher before neutralization and lower after neutralization (Figure 2). *n*-Butylamine (mono) and ammonia have been found to give practically strong base curves in glycolic solvent.

Table IV. Estimation of Weak Bases by Direct Titration

Base	Taken *Gram*	Found *Gram*	Error %	Sharpness of End Points
Aniline	0.2594	0.2587	Mean error	Fairly sharp
	0.2034	0.2034	−0.16	
	0.1785	0.1776		
	0.2150	0.2154		
Strychnine	0.6525	0.6531	.....	Very sharp
Brucine	0.7821	0.8762[a]	.....	Extremely sharp
		0.7408[b]		
Pyridine	0.2392	0.2095	−12.42	
	0.1993	0.1705	−14.09	Poor
p-Toluidine	0.3040	0.3124	+ 2.7	Fair
Naphthylamine	0.3181	0.3674	+15.8	Poor
Quinoline	0.4309	0.4045	− 6.5	Fair

[a] Calculated as $4H_2O$.
[b] Calculated as anhydrous.

The end point can also be detected with the help of indicators as in the foregoing cases, methyl red being satisfactory for the purpose. The following weak bases were found to give fairly sharp end points with methyl red, the stronger base showing a sharper color change: aniline, quinoline, pyridine, β-naphthylamine, *p*-toluidine, and phenylhydrazine. Phenylhydrazine and to a less extent β-naphthylamine were found to destroy the indicator, probably by a slow chemical reaction, and could be titrated only by conducting the titration very quickly. The actual values could not be checked against titration values, since none of the above bases was obtainable in the analytically pure state, but with fairly pure samples of aniline (Merck, A.C.S. specification) the results agreed within 1% as shown in Table IV. Some of

the bases like toluidine and naphthylamine show high results, perhaps due to the presence of diamines as impurities.

The experimental procedure followed in these cases was to dissolve about 0.2 to 0.5 gram of the base in a mixture of 5 cc. of chloroform and 5 cc. of propylene glycol and to titrate the solution with the standard hydrochloric acid solution as in the previous cases, adding the solution in steps of 0.05 cc. near the equivalent point. Chloroform was used as cosolvent, as it was found that its presence gave a slightly better color change. The dissociation constant of the base in aqueous solution is not a reliable guide as to its suitability in the direct titration, because aniline, though a weaker base than pyridine, was found to give much sharper color changes and a steeper titration curve than the latter. In fact, it is impossible to judge the end point accurately with pyridine by this procedure unless by comparative methods. The pyridine used was an ordinary dry sample which gave about 10 to 12% lower results, which is perhaps due to the presence of its homologs.

Alkaloids like strychnine and brucine gave extremely sharp end points and in the former case, where a pure sample (Merck) was available, the result agreed very well.

Estimation of Salts of Dibasic Acids. Generally speaking, dibasic acids are not amenable to this direct titration for two reasons: (1) their salts are not sufficiently soluble in G-H solvents, and (2) for salts like oxalates and phthalates the free acid liberated during titration is strong enough to effect a color change of the indicators. It might be possible to titrate some of the weaker ones by this method. Strangely enough, hydrazine hydrate, which is dibasic, also could not be titrated in glycolic solvent with accuracy and the potentiometric titration curve shows an almost constant slope over a wide range near the equivalence point.

Choice of Indicators. The suitability of an indicator cannot be judged from these potentiometric curves in nonaqueous solvents and the best indicator for any titration is found only by empirical means. For example, butylamine, which registers an apparent pH of 11.3 in glycolic solvents, shows only a faint alkaline color with phenolphthalein, whereas in water a bright pink color is shown though the pH is only slightly higher (11.45). In fact, phenolphthalein in glycolic solvents is much less bright than in water and methyl red is just the reverse. The author believes that solvation plays a very important role and the color of dyes and indicator is largely influenced by the solvating power of the medium. It has been shown that glycol has a strong solvating power through hydrogen bond formation for $RCOO^-$ ion but has very little power for dibasic acid salts. This might be responsible for the selectivity of the indicators as noted above. Cresol red has been found to have a brighter color than phenolphthalein with the same color change interval and so can be used with advantage in place of phenolphthalein for free alkali titrations.

Even the order of indicator range might change with the nature of the solvent, though usually acids of the same type are nearly equally affected by change of solvent (*4*, *9*). A very striking case observed in G-H medium is the relative range of bromocresol green (pH 3.8 to 5.4) and methyl red (pH 4.4 to 6.2); the figures in parentheses are the color change interval in water. If we prepare a set of buffer solutions in G-H mixtures by partially neutralizing aniline with gradually increasing quantities of perchloric acid it is observed that bromocresol green changes to acid color with about one-third neutralization of aniline, while methyl red requires free acid to be present; though according to the values quoted above for an aqueous medium the reverse is to be expected. Bromophenol blue (pH 3.0 to 4.6) has also been observed to change to acid color at a higher level of pH than methyl red, but to a smaller extent than bromocresol green. That the titration range of sulfonphthalein indicators tends to shift to higher pH values in glycolic solvents is found in a recent work of Masi and Knight (*8*), who found that in 20 to 80 by weight glycol-water mixture the pK of bromothymol blue (dibromothymol sulfonphthalein) is 7.42 and in 40 to 60 glycol-water mixture it becomes 7.69 as against 7.21 in pure water.

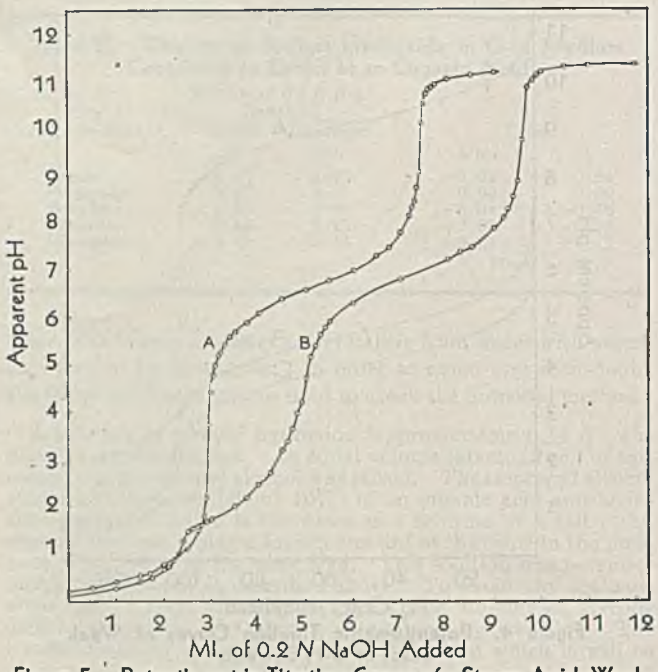


Figure 5. Potentiometric Titration Curves of a Strong Acid–Weak Acid Mixture in G-H Solvents

0.2 N sodium hydroxide
A. Nitric acid–acetic acid mixture
B. Sulfuric acid–acetic acid mixture

APPLICATIONS

Some interesting applications of this method can be made in diverse fields. Soap, as well as the free alkali present in it, can be conveniently measured. A very interesting application is in the estimation of amino acids, where the results are much more complicated because of the dipolar nature of their ions. The results of this method with reference to soaps (*11*) and amino acids and proteins will form the subject of separate publications.

Another suggested application is the estimation of a mixture of a weak acid and a strong acid in a single titration by a double indicator method or a potentiometric method. The possibility of such a method is shown by a typical titration curve of a mixture containing nitric acid and acetic acid by sodium hydroxide in G-H medium (Figure 5, *A*). Two sharp inflections at about apparent pH 4 and 10 are obtained corresponding to the neutralization of the nitric acid and the acetic acid, respectively. Such estimation is frequently necessary in industry with acid mixtures containing sulfuric acid, for example in the manufacture of cellulose acetate. Unfortunately, sulfuric acid is not very well amenable to this method because it does not behave as a very strong acid like the other mineral acids in G-H medium. This is shown by a titration curve of a mixture containing sulfuric acid and acetic acid by sodium hydroxide in G-H medium (Figure 5, *B*). Surprisingly, sulfuric acid, unlike in water, shows two inflections corresponding to its two dissociable hydrogen atoms. The first neutralization occurs around apparent pH 1.5 and is not very sharp, whereas the second or complete neutralization occurs around apparent pH 4 and the latter inflection is only moderately sharp, far less than the other mineral acids. The third inflection occurs as usual around apparent pH 10, corresponding to the complete neutralization of all acids. A better method in such case will be to determine the total acid by neutralization with sodium hydroxide in water or alcohol and then to estimate the sodium salt of the organic acid by direct titration in G-H medium after evaporating off the water or alcohol used for the above total acid titration.

Another application could be made in the determination of the equivalent weight of an organic acid without isolating it in the pure state. The sodium salt of the acid could be treated in aqueous or aqueous-alcoholic medium with excess of calcium chloride solution and the precipitated calcium salt dried and its equivalent weight determined by direct titration in G-H medium. A slight change of procedure is convenient here. The metallic soap is to be treated with chloroform first and after a little swelling is treated with propylene glycol to effect solution. The solution can then be titrated as usual. A sample of lauric acid (Eastman Kodak) treated by this method gave as equivalent weight for the calcium salt, 220.5 (theoretical, 219.23).

Various other applications, depending on the basic fact that we can clearly distinguish analytically a strong acid from a weak acid and a free base from a combined base by direct titration in a glycolic medium, can be easily made in other suitable cases.

CONCLUSIONS

The subject of acidimetry and alkalinity in organic solvents has hitherto not been systematically pursued. Data indicate that highly interesting and useful results are obtainable from this field and a long way is to be gone before these results can be brought in line with the standard data obtained in water, so that a unified theoretical presentation is possible. Two types of study are needed. First, it is necessary to fix up a pH scale in glycolic solvents with the help of a reversible hydrogen electrode and, second, we must have data about the performance of the usual acid-base indicators in the common organic solvents with respect to their sensitivity to all types of acids and bases. The glycolic solvents and their mixtures particularly recommend themselves for our first study, since they behave more closely to water than any other organic solvent, have fairly high solubility for salts, and are expected to be well suited to the functioning of reversible electrodes. The prospect seems definite that such a study will produce many newer and better methods of analysis and a more thorough understanding of the behavior of solvents.

Another fruitful line of study involving organic solvents in analytical chemistry remains practically untouched. The precipitation in gravimetric analysis is often attended with colloid formation and adsorption, to obviate which careful control of pH and other conditions is necessary. It is likely that by addition of suitable organic solvents better and easier control might be achieved, as in some cases of electrode position. It is also imaginable that some easier methods of separation and analysis using some organic solvents as the medium might come out of such studies.

ACKNOWLEDGMENT

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Determining the Moisture Equilibrium Curves of Hygroscopic Materials

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THE wartime packaging of rations and foods for the armed services and civilians has required extensive use of paper, paperboard, and other flexible packaging materials. The protection of moisture-sensitive products by nonhermetically sealed containers has aroused increased interest in the relationship of moisture content to equilibrium relative humidity. In anticipation of such work at the institute, a new procedure for obtaining sorption data was developed, the general principle of which was described by Gane (*2*). The method requires a minimum of uncommon apparatus, conditioning equipment, and technique. Specimens of adequate size may be used and data for complete sorption and desorption isotherms may be obtained simultaneously.

The procedure consists in suspending specimens in small closed vessels in which the relative humidity is controlled by saturated salt solutions. The number of test units is determined by the number of points desired on the sorption isotherms and an appropriate number of salt solutions is used. The specimen is suspended in the vessel by a rod which passes through a hole in the cover of the vessel and can be attached to the pan hook of an analytical balance to permit weighing the specimen without removal from the conditioned atmosphere. The hole in the top of the dish is sealed except during the weighing operation. All the operations are carried on in a room of controlled temperature.

Figure 1 is a photograph of a glass test unit in weighing position in a balance. The left-hand balance pan has been removed and a can with an appropriate slot has been inverted over the pan arrest to act as a platform for the unit.

Figure 2 is a schematic diagram of the glass test unit in normal storage position.

The specimen is normally held in a 93 × 18 mm. Petri dish and is uniformly distributed over the bottom of the dish. This dish is supported by a bracket made from 0.6-cm. (0.025-inch) aluminum sheet. The bracket is 8.1 cm. (3.25 inches) long and 1.25 cm. (0.5 inch) wide; it is bent to fit the dish snugly. This bracket is fastened to the threaded end of a 0.23-cm. (0.092-inch) diameter brass rod by means of two 2-56 brass nuts. The top

Table I. Equilibrium Relative Humidities for Saturated Salt Solutions

Chemical	Formula	Relative Humidity, % 73° F.	86° F.	100° F.
Ammonium phosphate	$NH_4H_2PO_4$	92.9	92.0	91.1
Potassium chromate	K_2CrO_4	86.5	86.3	85.6
Ammonium sulfate	$(NH_4)_2SO_4$	80.1	79.6	79.1
Sodium chloride	NaCl	75.5	75.2	75.1
Sodium acetate	$NaC_2H_3O_2$	74.8	71.4	67.7
Sodium nitrite	$NaNO_2$	64.8	63.3	61.8
Sodium bromide	NaBr	58.5	56.3	53.7
Sodium dichromate	$Na_2Cr_2O_7$	54.1	52.0	50.0
Magnesium nitrate	$Mg(NO_3)_2$	53.5	51.4	49.0
Potassium nitrite	KNO_2	48.6	47.2	45.9
Calcium nitrate	$Ca(NO_3)_2$	51.8	46.6	38.9
Potassium thiocyanate	KCNS	46.6	43.7	41.1
Potassium carbonate	K_2CO_3	43.9	43.5	43.4
Chromium trioxide	CrO_3	39.2	40.0	40.2
Magnesium chloride	$MgCl_2$	32.9	32.4	31.9
Potassium acetate	$KC_2H_3O_2$	22.9	22.0	20.4
Lithium chloride	LiCl	11.1	11.2	11.1

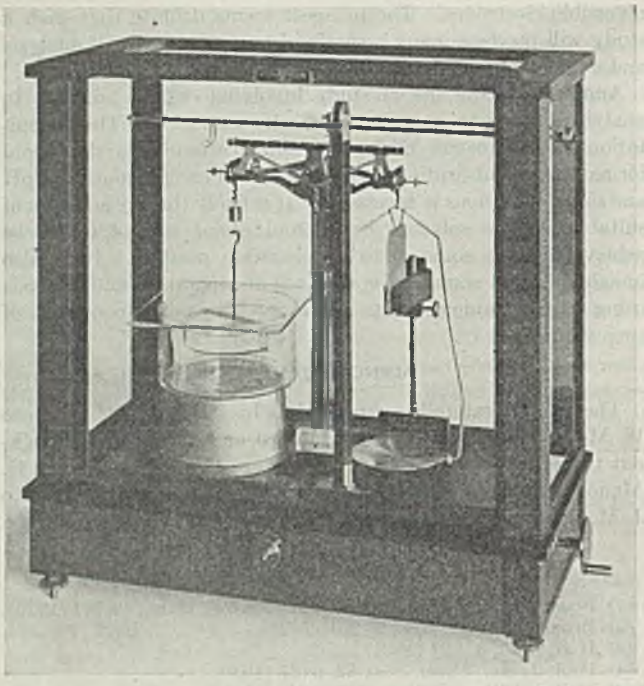

Figure 1. Glass Test Unit in Weighing Position

end of the rod is bent into a flat hook for convenient suspension from the pan hook of a balance or from the counterpoise used with a magnetic damping device, as shown in Figure 1. The over-all length of the rod, 8.1 cm. (3.25 inches), is arbitrary and depends upon the depth of the test unit and the height of the platform upon which the unit rests when in the balance. A brass cone is drilled, slipped on the rod, and soldered in place with the small end down and at a distance of 2.5 cm. (1 inch) from the bottom end of the rod. This cone is cut from a 0.6-cm. (0.25-inch) brass rod and has a half-angle of approximately 30°.

The top of the test unit is a 13.75-cm. (5.5-inch) square of double-strength window glass. A 0.47-cm. ($^3/_{16}$-inch) hole is cut 0.6 cm. (0.25 inch) off center on a line perpendicular to one edge of the plate and passing through its center. This off-center position is necessary because of space limitations in a typical analytical balance. In particular cases, it may be necessary to position this hole farther from the center of the plate. The brass cone is normally seated in the hole of the glass plate to seal the hole against passage of water vapor and to support the suspended system. The top edge of the hole on which the cone rests should be free from serious flaws. If a chipped edge results from the drilling operation, the edge should be ground lightly with Carborundum, using a brass cone of the type described. No sealing grease is employed in the zone of contact between the brass cone and the hole in the glass cover. The slight leak of water vapor through the zone has a negligible effect on the relative humidity in the vessel.

The main body of the test unit is a 125 × 65 mm. crystallizing dish. The top edge of the dish is ground on a flat surface—e.g., plate glass—with Carborundum to remove irregularities. The ground edge is lightly greased with Celvacene (Distillation Products, Inc.). This film of grease effects a good seal between the ground edge of the crystallizing dish and the glass plate when the unit is subsequently assembled.

Approximately 40 cc. of a saturated salt solution, having the desired equilibrium relative humidity, are poured into the bottom of the crystallizing dish. An excess of the salt is transferred to the dish to ensure a saturated condition of the solution while the test is carried out. In obtaining data for a desorption isotherm, where moisture is removed from the specimen and transferred to the saturated solution, it is advisable to have mounds of the excess salt exposed above the level of the solution.

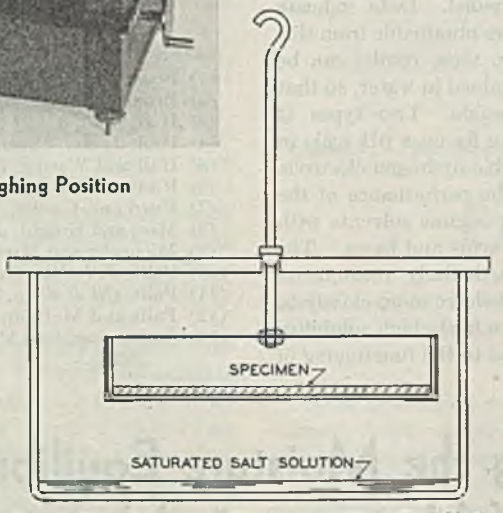

Figure 2. Schematic Diagram of Glass Test Unit

The glass test unit permits visual inspection of the specimen during the test; this is a convenience in testing foods and other materials for which the caking point, or other critical behavior, is of interest.

Table I lists the equilibrium relative humidities for a number of saturated salt solutions at 73°, 86°, and 100° F. (*1*).

PROCEDURE

The number of specimens and their initial conditioning vary with the purpose of the test. If a complete sorption isotherm is contemplated, it is necessary to dry the specimens, before assembling the test units, to a moisture content lower than that corresponding to the first point desired on the curve. The number of specimens depends on the number of points considered desirable in defining the isotherm. For many purposes, six to ten points are sufficient. A corresponding number of saturated salt solutions having equilibrium relative humidities appropriately distributed through the range from 10 or 15% to somewhat over 90% are needed. If a desorption isotherm is wanted, the specimens must be wet or conditioned at a high relative humidity before beginning the tests. On occasion, complete isotherms are not needed; the purpose is served by determining the moisture contents at two or three different relative humidities.

Since the detailed procedure for obtaining sorption data depends largely upon the purpose of the test and the type of material to be tested, it is beyond the scope of this discussion to cover the many phases involved in obtaining such data. The following discussion is therefore limited and presents only the procedure for the assembly and weighing operation of the test units.

A unit is temporarily assembled to obtain its tare. An empty Petri dish is placed in the bracket of the suspending device and the glass-plate cover is placed on a crystallizing dish. The zero of the balance is noted and adjusted if necessary. The left-hand pan is removed from the balance and the platform is placed in position straddling the pan arrest. The unit is placed on the platform and the hooked rod is lifted and gently engaged with the pan hook or with the eye of the counterpoise weight. The unit is then adjusted in position on the platform until the rod hangs freely through the hole in the glass plate. The combined weight of the suspending device and the empty Petri dish is then determined and recorded. The rod is unhooked and the unit is removed from the balance.

To insert the specimen and start the test, the cover of the unit is removed, the Petri dish is taken from the bracket, and a specimen is evenly distributed over the bottom of the dish. For materials which are very hygroscopic it may be desirable to replace the Petri dish with a shallow weighing bottle. Then the moisture content of the specimen can be determined in the same dish which is used to obtain the sorption data. The Petri dish is returned to its bracket, a film of grease is applied to the ground edge of the crystallizing dish, and the saturated salt solution, giving the desired relative humidity, is poured in the bottom of the crystallizing dish. The cover is replaced on the crystallizing dish and pressed lightly into continuous contact with the greased ground edge. The unit is then placed on a storage shelf until time for weighing. The specimens in the test units are weighed in the manner described at approximately 24-hour intervals until the weights become constant.

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Simultaneous Determination of Ethylene and 1,2-Propylene Glycols

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Ethylene and 1,2-propylene glycols can be determined simultaneously by periodate oxidation to formaldehyde and acetaldehyde which are determined polarographically after a simple distillation. The method is applicable to the analysis of mixtures of formaldehyde and acetaldehyde as well as to the determination of other substances which form these aldehydes on treatment. The principal limitation of the method is that there must not be formed any other volatile substances polarographically reducible under the same conditions as the aldehydes.

ALTHOUGH several methods for the quantitative determination of glycols have been described in the literature, little attention has been given to their determination in mixtures of each other. Those methods that have been used involve tedious and time-consuming procedures. It seemed desirable, therefore, to obtain a simple and rapid method for quantitatively determining the amounts of ethylene and 1,2-propylene glycols in the presence of one another.

The method described in this paper consists essentially of oxidizing the glycol mixture with periodic acid to give formaldehyde and acetaldehyde according to the following equations:

$$CH_2OH.CH_2OH + HIO_4 \longrightarrow 2HCHO + HIO_3 + H_2O$$

$$CH_3.CHOH.CH_2OH + HIO_4 \longrightarrow HCHO + CH_3.CHO + HIO_3 + H_2O$$

The resulting aldehydes are then determined polarographically.

From an examination of the equations of the reaction, it is seen that the acetaldehyde content gives a measure of the propylene glycol concentration. The ethylene glycol can be estimated by deducting the formaldehyde produced by the oxidation of propylene glycol from the total amount of formaldehyde found in the mixture.

Johnson (*5*) determined 2,3-butylene glycol by oxidizing it with potassium periodate in an acid medium to give acetaldehyde. This was distilled into a standard bisulfite solution to form the bisulfite addition product, which was subsequently determined by titration of the bound bisulfite with standard iodine solution. Hoepe and Treadwell (*3*) described a method utilizing the Malaprade reaction for the determination of glycerol, ethylene glycol, and 1,2-propylene glycol in mixtures. The resulting formic acid, total aldehyde, and formaldehyde were determined titrimetrically on aliquots of the oxidized solution. Mixtures of formaldehyde and acetaldehyde were determined by Ionescu and Slusanschi (*4*) using dimedon (dimethyldihydroresorcinol) as a precipitant, and noting the length of time required for the crystals to appear.

Boyd and Bambach (*1*) described a polarographic method for the determination of formaldehyde as a step in the procedure for the determination of serine in protein hydrolyzates. The method consisted of treating the hydrolyzate with periodic acid, quantitatively separating the resulting formaldehyde by distillation, and determining it polarographically using 0.05 *N* potassium hydroxide in 0.1 *N* potassium chloride as the supporting electrolyte solution. The effect of the presence of other aldehydes such as acrolein, acetaldehyde, and propionaldehyde on the determination of formaldehyde was investigated by Whitnack and Moshier (*6*). They found that the most satisfactory results are obtained in 0.1 *N* lithium hydroxide containing 0.01 *N* lithium chloride at constant temperature and constant pH without the removal of dissolved oxygen.

EXPERIMENTAL

The procedure for the polarographic determination of formaldehyde in the presence of acetaldehyde as previously described by Whitnack and Moshier was followed in this study. A solution consisting of 0.1 *N* lithium hydroxide in 0.01 *N* lithium chloride was used as the supporting electrolyte medium. It was not necessary to add a maximum suppressor, inasmuch as no maximum was found to occur under the conditions used.

Preliminary studies revealed that the formaldehyde wave appeared at −1.45 to −1.58 volts (dropping mercury electrode *vs.* pool) and the acetaldehyde wave appeared at −1.65 to −1.85 volts under the conditions of pH, concentration range, and capillary used. In the actual determination, it was found necessary only to determine the galvanometer readings at a point before the appearance of the formaldehyde wave, −1.400 volts, at the height of the formaldehyde wave, −1.610 volts, and at the height of the acetaldehyde wave, −1.890 volts. These are the only points required for the quantitative determination of the aldehyde content of the mixture resulting from the oxidation of the glycols.

It was found that the height of the acetaldehyde wave decreased markedly (approximately 15% after 1 hour) when allowed to stand in contact with the alkaline electrolyte solution. The formaldehyde on the other hand was only slightly affected (approximately 5% decrease after 1 hour). For this reason, and also because of the volatilization of the acetaldehyde while in the polarographic cell, all determinations were made after standing in contact with the base solution in the cell for the same length of time (5 to 10 minutes).

MATERIALS AND APPARATUS

GLYCOLS. Pure ethylene glycol (Coleman and Bell Co.) and redistilled 1,2-propylene glycol (Eastman Kodak Co.) were used. The refractive indices and the specific gravities of the glycols checked the values reported in the literature.

ALDEHYDES. A stock standard solution of formaldehyde (0.795 mg. per ml.) was prepared from commercial formalin and standardized by the hydrogen peroxide method. A stock standard solution of acetaldehyde (0.470 mg. per ml.) was prepared by weighing a sample of pure acetaldehyde in an ampoule and diluting to a definite volume with distilled water; the acetaldehyde was purified by distillation through an efficient distillation column.

PERIODIC ACID. An approximately 0.5 *N* solution was prepared by dissolving 11 grams of periodic acid (The G. Frederick Smith Chemical Co.) in distilled water and diluting to 100 ml. The solution was stored in a dark glass-stoppered bottle.

BASE SOLUTION. This consisted of a 1 *N* lithium hydroxide solution in 0.1 *N* lithium chloride.

POLAROGRAPH. The Fisher Elecdropode was used in this work and all measurements were made at one tenth of the galvanometer sensitivity. The polarographic cell was a water-jacketed cell of about 35-ml. capacity connected in series through a small circulating pump with a constant-temperature bath thermostatically controlled at ±0.2° C.

DISTILLING APPARATUS. A simple distilling apparatus was used consisting of a semimicro 100-ml. Kjeldahl flask, a spray trap, a vertical condenser about 30 cm. in length, and a water bath for cooling the receiver.

PROCEDURE

PERIODIC ACID OXIDATION. An aqueous solution of the glycol mixture containing approximately 5 to 20 mg. of each of the glycols is pipetted into a 100-ml. Kjeldahl flask, and approximately 3 ml. of the periodic acid solution are added, with sufficient water to bring the total volume to about 60 ml. A few glass beads are introduced into the flask to prevent bumping.

The Kjeldahl flask is placed in an upright position and connected to the distilling unit. A deep receiver flask—e.g., a Pyrex test tube, 45 × 190 mm.—containing 75 ml. of distilled water is used to absorb the aldehydes and to cover the end of the condenser to a height of several centimeters. In order to minimize any loss of acetaldehyde due to volatilization, the receiver

is placed in an ice-water bath and kept cold throughout the course of the distillation.

The contents of the reaction flask are heated, gently at first, and the solution is distilled over at a rate of 3 to 4 ml. per minute until about 5 ml. remain in the Kjeldahl flask. Near the end of the distillation, the receiver flask is lowered so that the end of the condenser no longer extends below the surface of the liquid in the receiver. After the distillation is complete, the end of the condenser is rinsed with distilled water. The distillate is then quantitatively transferred to a 250-ml. volumetric flask, care being taken not to exceed a total volume of 225 ml. The aldehyde solution can be kept in this manner until ready for the polarographic measurements.

POLAROGRAPHIC ANALYSIS. Immediately prior to the polarographic determination of the aldehydes, 25 ml. of the 1 *N* lithium hydroxide solution in 0.1 *N* lithium chloride are added to the distillate in the volumetric flask and the contents are diluted to the mark. In this way, the resulting solution has a concentration of 0.1 *N* lithium hydroxide in 0.01 *N* lithium chloride.

This practice seemed most advisable, since condensation of aldehydes is rapid in the presence of strong alkalies. By adding the base solution to the aldehyde mixture in the manner proposed, the time of contact of the aldehydes with the lithium hydroxide is reduced to a minimum.

The polarographic cell and electrodes are rinsed several times with the solution to be analyzed. A sample of the solution is then placed in the cell and allowed to come to constant temperature. After a definite length of time has elapsed from the moment of the addition of the supporting electrolyte solution, the galvanometer deflections at applied voltages of −1.890, −1.610, and −1.400 volts are read. The height of the acetaldehyde wave is the difference in the galvanometer reading between the first and second points; the height of the formaldehyde wave is the difference between the second and third points.

These wave heights are then compared to the values obtained from a 250-ml. solution prepared from the standard aldehyde solutions. The concentrations of formaldehyde and acetaldehyde in the latter solution should be approximately the same as those in the sample solution polarographed, though in the latter case it is not necessary to submit the aldehydes to a periodic acid oxidation or distillation, since there is no change in concentration as a result of such treatment.

CALCULATIONS

1,2-PROPYLENE GLYCOL.

$$\text{Weight in sample, mg.} = W_p = \frac{H_a}{H_s} \times V \times C_s \times \frac{76}{44}$$

$$= \frac{1.727 \times V \times H_a \times C_s}{H_s}$$

where C_s = mg. of acetaldehyde per ml. of stock standard solution

V = volume in ml. of stock standard acetaldehyde solution taken

H_a = acetaldehyde wave height from periodic acid oxidation of glycol mixture

H_s = acetaldehyde wave height of standard

$$\text{Per cent by weight} = \frac{100\,W_p}{S}$$

where S = weight of sample in mg.

ETHYLENE GLYCOL.

$$\text{Weight in sample, mg.} = W_e = \frac{1.033 \times V \times H_f \times C_s'}{H_s'} - 0.408\,W_p$$

where W_p = mg. of 1,2-propylene glycol in sample

C_s' = mg. of formaldehyde per ml. of stock standard solution

V = volume in ml. of stock standard formaldehyde solution taken

H_f = formaldehyde wave height from periodic acid oxidation of glycol mixture

H_s' = formaldehyde wave length of standard

$$\text{Per cent by weight} = \frac{100\,W_e}{S}$$

where S = weight of sample in mg.

DISCUSSION

The values obtained at three different temperatures indicates that, within the range of 20° to 30° C., the height of the formaldehyde wave increases approximately 6.5% for each 1° C. rise in temperature. This is in agreement with the observation of Boyd and Bambach (*1*). The acetaldehyde wave height, however, increases only approximately 1.5 to 2.0% per degree rise in temperature, which is the same as that found by Elving and Rutner (*2*).

The data obtained in these studies indicate that, under the conditions used, there is a linear relationship between the height of the wave and the corresponding aldehyde content of the solution resulting from the action of periodic acid on the glycol mixture.

Using synthetic mixtures of the pure glycols an average precision and accuracy of better than 1.5% was obtained for propylene glycol and 2.2% for the ethylene glycol.

It is important that any material capable of being oxidized by periodic acid to give formaldehyde or acetaldehyde be absent. This includes such substances as α-amino alcohols, hydroxyamino acids (serine and threonine), and polyalcohols (glucose and other sugars). Monohydroxy alcohols, in general, such as methanol or ethanol do not affect the determination.

The polarographic method described need not necessarily be limited to the determination of mixtures of glycols but can also be successfully applied to the determination of formaldehyde and acetaldehyde in mixtures and to the determination of substances which form these aldehydes on oxidation—e.g., a mixture containing 1,2-propylene glycol and glycerol. The real limitation of the technique described—oxidation to formaldehyde and acetaldehyde, separation of the aldehydes, and polarographic measurement of the aldehyde content—is that there be absent and not produced any volatile substances polarographically reducible in the same potential range as the aldehydes under the conditions used.

Representative data on the analysis of mixtures of ethylene glycol and 1,2-propylene glycol in the manner described are given in Table I.

Table I. Simultaneous Determination of Ethylene Glycol and 1,2-Propylene Glycol in Synthetic Mixtures

Ethylene Glycol			Propylene Glycol		
Present	Found	Error	Present	Found	Error
Mg.	*Mg.*	%	*Mg.*	*Mg.*	%
4.31	4.28	−0.7	8.26	8.13	−1.6
4.31	4.53	+5.1	16.52	16.50	−0.1
4.31	4.34	+0.7	16.52	16.72	+1.3
8.62	8.95	+3.8	16.52	16.43	−0.2
8.62	8.55	−0.8	16.52	16.71	+1.1
8.62	8.25	−4.3	16.52	17.02	+2.8
10.94	11.02	+0.7	(15.16)	...	...
10.94	10.66	−2.5	15.16	15.38	+1.4
10.94	10.91	−0.3	15.16	15.52	+2.4
10.94	10.98	+0.4	15.16	15.26	+0.7
10.94	10.98	+0.4	15.16	15.18	+0.1
10.94	10.60	−3.1	22.74	22.55	−1.1
16.41	15.93	−2.9	15.16	14.91	−1.6
16.41	17.34	+5.7	22.74	23.02	+1.1
Av.		±2.2			±1.3

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Colorimetric Determination of Iron

In the Presence of Large Concentrations of Copper and Nickel

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The usefulness of disodium-1,2-dihydroxybenzene-3,5-disulfonate as a chromogenic reagent for the determination of iron has been extended. A technique has been applied which allows estimation of iron in the presence of large quantities of copper and nickel. As little as 0.025% iron in blue vitriol can be estimated without prior separations.

YOE and Jones (*2*) proposed the use of disodium-1,2-dihydroxybenzene-3,5-disulfonate (tiferron) as a colorimetric reagent for the estimation of ferric iron. They found that comparatively large concentrations of a number of diverse ions offer no interference, but that, in the presence of 1.0 p.p.m. of iron, the copper concentration must be less than 25.0 p.p.m.

In the writer's laboratory, iron determinations are made on a considerable number of liquors incident to the electrolytic refining of copper. These liquors contain comparatively small amounts of iron in the presence of sulfuric acid, arsenic, antimony, up to 18% copper, and up to 8% nickel. In addition, small percentages of iron are determined in blue vitriol samples.

A method which would permit iron estimations without prior separations of the copper and nickel would have obvious advantages. Silverthorn and Curtis (*1*) determined chromium in the presence of manganese which interferes at the wave length employed. They measured the total absorption caused by the manganese and chromium together and then corrected for the light absorbed by the manganese alone. Standard curves were used for this purpose.

It was the purpose of this investigation to attempt to devise a method for estimating iron in the presence of arsenic, antimony, nickel, and copper, using the technique of Silverthorn and Curtis as a basis and tiferron as the chromogenic reagent.

APPARATUS

All the optical measurements were made with a Coleman model 11 Universal spectrophotometer having a band width of 35 mμ. Cylindrical glass cells having an inside diameter of 16 mm. were employed.

SOLUTIONS

TIFERRON. This reagent was obtained from LaMotte Chemical Products Co. An aqueous solution containing 25 grams per liter was used.

BUFFER. This solution contained 20 grams of sodium hydrogen carbonate and 10 grams of sodium carbonate per liter of solution, twice the strength of the buffer used by Yoe and Jones (*2*). The stronger solution was used in order to obtain the proper pH with test aliquots containing sulfuric acid.

STANDARD IRON. Analytical grade iron wire was dissolved in dilute hydrochloric acid. The solution was oxidized with bromine water and the excess expelled by boiling. The solution was made up to 1 liter and used as a stock solution.

STANDARD NICKEL. A stock solution was made from reagent grade nickel sulfate crystals having an iron concentration of 0.0005%. Its exact strength was determined electrolytically.

STANDARD COPPER. A stock solution was made from reagent grade copper sulfate pentahydrate crystals. Exact strength was determined electrolytically. Crystals contained 0.00035% iron.

GENERAL PROCEDURE

Transfer a suitable aliquot to a 50-ml. volumetric flask and add enough tiferron solution to take care of all the copper, nickel, and iron present. Mix, dilute to the calibration mark with the buffer solution, and mix thoroughly. Transfer a portion of the solution to an absorption cell and measure the light transmittancy at 500 mμ, using water as the reference solution. Readings should be taken within 4 minutes following the addition of the buffer solution. The per cent iron is read from tables, prepared as described below.

The procedure presupposes some knowledge as to the approximate concentrations of copper and nickel in the sample. If the nickel content is not more than sixty times that of the iron, its effect may be neglected.

The minimum quantity of tiferron required for the test is determined by the amounts of iron, copper, and nickel present. Experiments have shown that in terms of dry tiferron, the respective requirements for 1 part of iron, 1 part of copper, and 1 part of nickel are 40, 12, and 1.25 parts. These quantities are somewhat in excess of minimum requirements. It has been found that an excess of the reagent has no effect in the presence of copper and iron but does influence the results to a negligible extent when nickel is present. A large excess of the reagent should be avoided because of its cost.

WAVE LENGTH–TRANSMITTANCY RELATIONS

The wave length–transmittancy characteristics for iron, copper, and nickel are shown in Figure 1. The respective concentrations used for these tests were 0.050, 15.0, and 49.0 mg. per 50 ml.

It will be noted that the shapes of the nickel and copper curves are similar and differ from that of the iron curve. The maximum absorption for iron corresponds to a wave length of about 490 mμ, which is close to the 500 established for the testing work. The absorption minima for copper and nickel are in the neighborhood of 550 mμ.

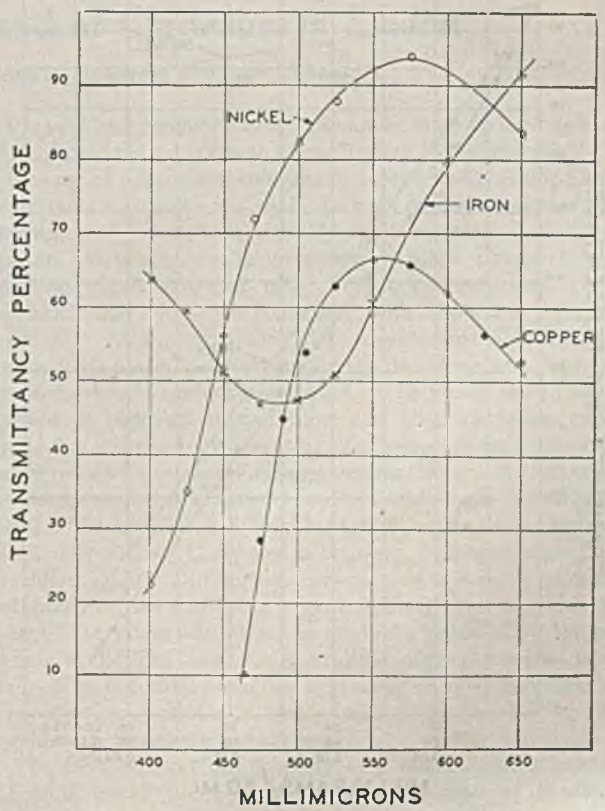

Figure 1. Transmittancy Curves for Nickel, Iron, and Copper

Table I. Aliquot Schedule

Type of Material	Initial Sample Weight	Dilution Volume	Volume of Aliquot	Volume of Tiferron	Use Schedule
	Grams	*Ml.*	*Ml.*	*Ml.*	
A liquor	5.0	200	2.0	12	A
B liquor	5.0	200	1.0	2	B
C liquor	5.0	200	1.0	2	B
D liquor	5.0	200	2.0	8	A
E liquor	5.0	200	1.0	2	B
Blue vitriol	25.0	500	2.0	14	C

Table II. Iron Schedule

% Transmittancy	% Iron		
	4% Cu	6% Cu	8% Cu
	Schedule A, A and D liquors		
50	0.148	0.136	0.122
49	0.154	0.142	0.128
48	0.160	0.148	0.134
47	0.165	0.153	0.139
	Schedule C, Blue Vitriol		
	24% Cu	25% Cu	26% Cu
25	0.045	0.040	0.035
24	0.050	0.045	0.040
23	0.056	0.051	0.046
22	0.062	0.057	0.052
21	0.069	0.064	0.059

CONCENTRATION-TRANSMITTANCY RELATIONS

The concentration–transmittancy relations were investigated for copper, nickel, and iron at 500 mμ (Figure 2). The procedure outlined above was followed, using varying quantities of the standard solutions.

The curves cut the vertical axis at a point representing 96.5% transmittancy instead of the expected 100%, because water, rather than a blank containing the buffer and tiferron, was used as the reference solution.

It will be seen that Beer's law holds for nickel and iron, while there is a considerable deviation corresponding to the lower concentrations of copper.

The absorption by the nickel complex is comparatively low and its effect may be neglected with respect to lower concentrations.

At higher nickel concentrations, corrections should be applied for the nickel present. The method outlined below for copper can be used. Because of the increase in light absorption with time, readings should be taken within 4 minutes after addition of the buffer as recommended in the general procedure.

The colors developed by copper and iron are relatively stable, no material change in transmittancy taking place over the first half hour.

Comparatively high concentrations of arsenic and antimony, both trivalent and quinquevalent, showed no light absorption at 500 mμ. No further consideration has, therefore, been given these elements.

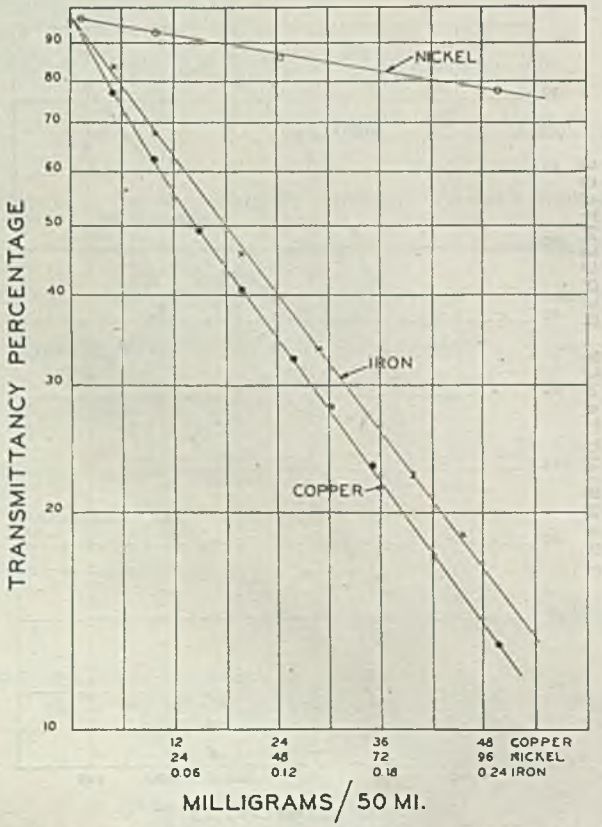

Figure 2. Transmittancy-Concentration Curves for Nickel, Iron, and Copper

CORRECTION FOR COPPER PRESENT

The presence of copper in a sample will manifest itself by an increase in the light absorbed, resulting in an apparent increase in the concentration of iron. Readings must therefore be corrected for the copper present.

This may be done by referring to the copper curve in Figure 2 and noting the transmittancy corresponding to the known copper concentration. Reference is then made to the iron curve, and the iron concentration corresponding to this transmittancy is the correction for the copper present. This correction is subtracted from the total apparent iron equivalent to the actual instrument reading. The difference is equal to the true iron concentration.

An example will make this clear. Assume that an aliquot contains 12 mg. of copper and that the instrument reading is 40. Reference to the copper curve in Figure 2 shows that the percentage transmittancy corresponding to 12 mg. of copper is 56. Reference to the iron curve shows that 56% transmittancy is equivalent to 0.074 mg. of iron. The apparent iron equivalent to the actual instrument reading of 40% is 0.12 mg. The true iron content is then 0.12 − 0.074 or 0.046 mg.

As an aid in routine work, a table has been arranged to cover some of the samples tested in the author's laboratory showing the dilution, size of aliquot to be taken, and volume of tiferron to be used. In addition, tables have been assembled to show the concentration of iron corresponding to various instrument readings and corrected for a number of different copper concentrations. Abbreviated forms are represented by Tables I and II.

CHECKS ON KNOWN MIXTURES

Some determinations were made on "synthetic" mixtures containing varying quantities of copper, nickel, and iron (Table III).

The results of determinations made on some production materials whose iron contents had previously been determined gravimetrically are shown in Table IV.

In making these determinations, a quantity of the sample was transferred to a beaker, diluted with water, and 5 ml. of 1 to 4

Table III. Iron Checks on Synthetic Mixtures

Composition of Mixture			Fe Found
Cu	Ni	Fe	
	Mg./50 ml.		*Mg./50 ml.*
6	1.44	0.060	0.058
8	1.44	0.030	0.031
10	0.028	0.010	0.018
10	0.028	0.030	0.040
15	2.80	0.050	0.052
20	0.056	0.020	0.025
20	0.056	0.030	0.034
20	0.056	0.060	0.063
30	0.112	0.010	0.018
30	0.112	0.030	0.040
30	0.140	0.070	0.068
30	0.140	0.090	0.090
30	0.140	0.100	0.100
30	1.40	0.070	0.071
30[a]	14.00	0.070	0.075
30[a]	49.00	0.070	0.070
30[a]	98.00	0.070	0.070

[a] Corrected for nickel present.

Table IV. Iron Checks on Materials in Process

Type of Material	Approximate Percentage				Percentage of Fe	
	As	Sb	Cu	Ni	Gravimetric	Colorimetric
H-3 liquor	0.2	0.03	2.70	0.70	0.024	0.023
H-3 liquor	0.2	0.03	2.67	0.74	0.025	0.025
H-3 liquor	0.2	0.03	2.70	0.72	0.028	0.031
H-4 liquor	0.2	0.03	1.75	0.82	0.025	0.025
H-4 liquor	0.2	0.03	1.71	0.85	0.028	0.027
H-4 liquor	0.2	0.03	1.95	0.82	0.038	0.034
H-5 liquor	0.2	0.03	2.64	0.65	0.021	0.019
H-5 liquor	0.2	0.03	2.92	0.67	0.022	0.020
O liquor	0.6	...	15.20	0.40	0.054	0.050
A liquor	1.2	..	15.70	1.70	0.230	0.215
E liquor	2.8	..	3.80	4.50	0.416	0.365
G liquor	0.3	0.08	3.00	..	0.024	0.023
Blue vitriol	....	..	25.1	..	0.032	0.025
	...	..	25.1	..	0.025	0.020
	...	..	25.1	..	0.026	0.024
	...	..	25.1	..	0.026	0.027
	...	..	25.1	..	0.070	0.063
	...	..	25.1	..	0.086	0.080

sulfuric acid were added to prevent the precipitation of arsenic and antimony. A few milliliters of saturated bromine water were then added and the excess was expelled by boiling. The solution was cooled, and if the acidity was initially high it was neutralized by means of ammonium hydroxide, and a slight excess of 1 to 4 sulfuric acid added. The test was again cooled, transferred to a volumetric flask, diluted to the mark, and thoroughly mixed. An aliquot was transferred to a 50-ml. volumetric flask and the general procedure outlined above was followed.

DISCUSSION

Iron may be determined in the presence of large quantities of copper and nickel with excellent accuracy, provided the iron concentration is not less than 0.05 mg. per 50 ml. Below this concentration, the expected error is somewhat greater but will still be sufficiently small for many purposes.

The iron was accurately determined in a "synthetic" mixture containing 30 mg. of copper, 98 mg. of nickel, and 0.070 mg. of iron per 50 ml. In this case it was necessary to correct for both the copper and nickel present.

The maximum concentration of nickel which may be present in a mixture is probably much higher than the maximum reported on in this investigation. In such cases, it would be necessary to take transmittancy readings very quickly following addition of the buffer solution.

It is believed that the utility of many of the published spectrophotometric analytical methods can be extended materially by the use of corrected tables similar to those described in this paper. The author is now applying this technique to the estimation of small concentrations of nickel in samples of the type shown in Table IV and expects to publish the findings at a later date.

ACKNOWLEDGMENT

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Destruction of Organic Matter in Blood Fibrin and Chromacized Medical Catgut by Wet Oxidation

Determination of Iron in Blood and of Chromium in Sutures

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THE determination of the mineral components of blood and the evaluation of chromium in chromacized medical catgut sutures are generally carried out starting with a dry-ashing procedure. This operation is time-consuming. Subsequent to dry-ashing at elevated temperatures the residue may require a fusion process to redissolve the ash. For these reasons a wet-ashing procedure is recommended and herein described.

The literature on the use of mixtures of nitric and perchloric acids in the wet-oxidation of organic matter preparatory to the determination of sulfur, phosphorus, and arsenic as well as the metallic components iron, chromium, aluminum, calcium, magnesium, sodium, and potassium has been previously surveyed (*4*, *7*). The important applications are the determination of sulfur in coal (*9*), chromium in leather (*3*), iron in leather (*6*), calcium, phosphorus, and the alkali metals in plant materials (*1*), sulfur in organic compounds (*4*), and iron in wine or beer (*7*).

CHEMICAL ASPECTS OF WET-OXIDATION ACID MIXTURES CONTAINING PERCHLORIC ACID

Because perchloric acid is known, when hot and concentrated, to exert a powerful oxidizing effect in contact with organic matter, its use for wet-ashing purposes is retarded by unwarranted apprehension of violent reactions. When properly used for wet-mineralization of samples composed preponderantly of organic matter, perchloric acid is entirely dependable. Samples of heterocyclic ring nitrogen compounds such as occur in coal which are oxidized with great difficulty by hot concentrated sulfuric acid, as in the Kjeldahl reaction, are readily and rapidly oxidized by mixtures of nitric acid and perchloric acid; vanadium must be used as catalyst to speed the oxidation to a time limit of 15 minutes (*9*).

The use of mixtures of concentrated nitric and perchloric acids as oxidants for organic materials of a wide variety of sources gives smooth, easily controlled, suitably rapid reactions because the mixture exerts a gradually increasing oxidation potential. The addition of concentrated nitric acid to concentrated (72%) perchloric acid dilutes the latter acid, which lowers its oxidation potential. At the same time the concentration of nitric acid, owing to its partial dehydration by the perchloric acid, increases its strength and oxidation potential. The mixed acids may be applied at ordinary temperatures and their oxidation effects gradually increased by elevating the temperature. The more easily oxidized portions of the organic matter are first attacked. As the temperature is raised the nitric acid excess is finally distilled off and the diluted perchloric acid begins to concentrate, and finally at 200° C. attains a constant boiling mixture (72% perchloric acid). During the course of this process its oxidation potential increases and the organic matter present which has escaped the nitric acid attack is gradually oxidized by the perchloric acid. The more easily oxidized organic material is destroyed by hot 60% perchloric acid and the most difficultly destroyed organic matter is finally oxidized by the boiling 72% perchloric acid, quietly and efficiently.

The most outstanding property of a mineralization reaction following mixed nitric-perchloric acid digestion at gradually elevated temperature is the fact that the organic matter is destroyed without accompanying carbonization, which is so pre-

dominant in such reactions as a Kjeldahl digestion. Most, if not all of the carbonaceous material is volatilized as gaseous products. With some types of organic material the reacting solution remains clear throughout the entire oxidation reaction. In other cases the solution acquires a chocolate-brown coloration due to degradation products of the decomposition of organic matter. In case any carbonaceous material is formed, the conditions are indicated as unsatisfactory and the proportion of mixed acids employed should be altered, the temperature relations changed, or additional reagent added to the reaction mixture—for example, sulfuric acid.

INFLUENCE OF SULFURIC ACID ADDITIONS TO OXIDATION MIXTURE

If the organic matter present affords smooth oxidation with extreme difficulty, the oxidation potential of perchloric acid may be very markedly increased by the addition of sulfuric acid. In this case the final state of oxidation is brought about through the the dehydration of perchloric acid by the sulfuric acid added and by the higher temperature produced in the latter stages of the reaction. The effect of the nitric acid is accomplished and the excess is boiled off at 140° to 150° C. The oxidation by perchloric acid begins at 150° to 160° C. as a concentration of the perchloric acid, due to the presence of sulfuric acid, does not stop at 72% strength, but may increase to 85% or even higher, depending upon the proportion of sulfuric acid present. Under these conditions the oxidation potential of the perchloric acid due to dehydration is gradually increased to a degree favorable to oxidations of the most resistant type. Use of sulfuric acid as a reaction component precludes, of course, the determination of sulfur and causes complications in presence of calcium salts.

INFLUENCE OF CATALYSTS

Just as in the Kjeldahl digestion, the oxidation time of which is much diminished in the presence of copper, mercury, selenium, etc., the presence of catalysts likewise shortens the reaction time with perchloric acid oxidation. Two such catalysts are chromium and vanadium. Here the mechanism of the reaction depends upon the fact that hot concentrated perchloric acid oxidizes chromium and vanadium to chromic and vanadic acids. These in turn are reduced by any traces of organic matter persisting and the process is repeated. When all organic matter is destroyed the perchloric acid oxidizes the chromium and vanadium to chromic and vanadic acids, which color the hot acid orange, and the chromic acid forms reddish orange crystals in the cooled 72% perchloric in which it is quantitatively insoluble. A few milligrams of either chromium or vanadium are sufficient for considerable diminution of the time required for the mineralization and the oxidation of a given sample of organic matter. Often their use causes a too energetic effect.

An estimate only can be made of the oxidation potential acquired by the gradual elevation of the temperature of the reaction mixture of nitric, perchloric, and sulfuric acids together, especially with the added influence of catalysts such as chromium and vanadium, or in some cases cerium. The temperature range covered is from room temperature, with gradually increasing increments, through the boiling range of nitric acid, perchloric acid, and finally sulfuric acid, at approximately 360° C. The estimated oxidation potential thus attained covers the range of 1 to 2 volts, as indicated by the ease with which chromium and cerium are oxidized to their highest stages of valence by such mixtures. The gradual increase in potential in ever-increasing increments is analogous to the playing of a chromatic scale on the piano keyboard from the lower pitched notes of the bass to the higher pitched notes of the soprano. With studied application of such oxidizing conditions to a given type of organic matter, no violent reactions are probable and the operation as a whole becomes perfectly reliable. Thousands of such reactions for the destruction of organic matter, in accordance with the literature references previously cited, are daily routine control procedures. For new applications, the analyst who prefers dry-ashing procedures, because of apprehension concerning hazardous application of the nitric-perchloric-sulfuric acid wet-ashing procedure, is depriving himself of one of the most reliable, time-saving, and precise procedures. Conditions, once established for the proper application of this procedure to a specific case, bring into play easily recognized superior advantages. The present discussion demonstrates these facts as applied to the mineralization of blood fibrin and chromacized catgut sutures.

APPARATUS EMPLOYED

An electrically heated hot plate with inset rheostat for variable heat control, such as is always available from reagent supply houses, or a modified (*8*) Rogers ring burner and Nichrome wire gauze can be used, and intermediate heat intensities other than those provided by the device itself can be arranged by the use of heavy asbestos paper in one-, two-, or three-layer portions. The actual heat attained under given conditions should be determined by the use of the acids employed without sample present. In general, a beaker containing concentrated sulfuric acid and inserted thermometer serves as an adequate test. A maximum temperature of 210° to 225° C. was found suitable for the two types of samples under discussion. The use of the Rogers ring burner as a substitute for electrical heating provides very suitable variable heat adjustment.

Vycor glass Erlenmeyer flasks, 500-ml. capacity, should be employed for safety from cracking or fracture due to sudden heat gradients. Their use avoids all danger that a flask containing hot oxidizing acids may crack or open at the bottom, causing damage through fire to wooden table tops or fume hood floors. These flasks entail some additional expense in first costs, but they are more than justified by the increased facility with which they may be rapidly cooled and heated. For the determination of chromium in sutures they are practically indispensable, since the flask and contents must be chilled instantly from 200° C. to ice bath temperatures to prevent the hydrogen peroxide error (*4*).

An efficient fume hood with forced draft ventilation is essential if a large number of operations are to be carried out at one time. The excess acids must be volatilized. The organic degradation products resulting from the oxidation by this "cracking" process are acrid and disagreeable. If such a fume hood is not available, a perfect substitute has been described (*5*).

DESTRUCTION OF ORGANIC MATTER IN BLOOD FIBRIN

The oxidation is complete, rapid, and smooth running if perchloric acid alone is employed. The reaction is begun after addition of 60% perchloric acid by placing the acid-treated sample directly on the hot plate capable of elevating the temperature eventually to 210° to 225° C. The blood fibrin is at the start dissolved in the perchloric acid, giving a deep purple product. After 3 minutes the color becomes chocolate brown, accompanied by noticeable carbonization. With 10 minutes' treatment there is a generous foaming reaction, as evolved gases

Table I. Destruction of Organic Matter in Blood Fibrin Using Nitric and Perchloric Acids

Determination	Blood Fibrin	72% $HClO_4$	67% HNO_3	Time Completion, HNO_3 Reaction	Total Reaction Time
	Grams	*Ml.*	*Ml.*	*Min.*	*Min.*
1	3	10	10	7	13
2	4	10	10	5	13
3	5	12.5	17.5	7	14
4	6	15	20	5	20
5	7	15	20	6	20
6	8	15	25	7	33
7	9	15	25	7	16
8	10	17.5	30	8	15
		With Addition of 25 Mg. of CrO_3			
9	5	15	15	5	28
		With Addition of 0.001 Gram of Ammonium Vanadate			
10	10	17.5	30	6	13
		60% $HClO_4$			
11	1	15	Absent	..	18
12	2	15	Absent	..	15
13	3	21	Absent	..	13
14	4	21	Absent	..	18
15	4	25	Absent	..	21
16	5	30	Absent	..	19
			2 mg. $(NH_4)_4VO_4$		

are given off together with fumes of perchloric acid and the reaction mixture rapidly turns light brown. In 5 additional minutes' heating time the reaction is complete, as shown by the absence of any color except that from a little chlorine resulting from a slight decomposition of the hot concentrated acid. The addition of 2 mg. of ammonium vanadate as catalyst was of no appreciable advantage. The volume of 60% perchloric acid used should be 5 to 6 ml. per gram of blood fibrin with a minimum of 10 to 15 ml. to provide enough material to cover the bottom of the 500-ml. Vycor flask. Results obtained are given in Table I, determinations 11 to 16.

The time rate of oxidation of blood fibrin is increased by the use of 67% nitric and 72% perchloric acids. No carbonization of the organic matter occurs. Reaction results show a vigorous oxidizing action due to nitric acid accompanied by a rapid evolution of nitric oxide and gaseous decomposition products. During this stage the frothing may amount to a 2.5- to 5-cm. (1- to 2-inch) thick layer. After 3 to 4 minutes the nitric acid reaction is complete and after an additional 4 to 5 minutes the excess nitric acid has been boiled off. The solution then rapidly turns chocolate brown in color, a 1.25-cm. (0.5-inch) foam coating appears, and fumes of the decomposition products of perchloric acid form. The color of the solution rapidly becomes lighter, carbon dioxide is evolved, and the reaction is complete in 15 to 18 minutes if generous portions of nitric acid are employed. Chromic acid as catalyst brings no time advantage and as little as 0.5 mg. of vanadium causes the reaction mixture to catch fire (Table I, determinations 10 and 9). Osmic acid is of no advantage in speeding up the reaction.

DETERMINATION OF IRON IN BLOOD FIBRIN

For determining the effective recovery of iron in blood, samples of 5 grams each are oxidized, as previously described, and the acid-soluble residue containing excess perchloric acid is transferred from the 500-ml. Vycor flask to a 150-ml. Pyrex beaker. The samples are then evaporated on a hot plate with the beakers covered and the cover glass elevated, using twin watch glass supports (*2*), until the perchloric acid is completely removed. If a trace of brown appears in the otherwise white residue in spots, 0.5 ml. more of 72% perchloric acid is added and reheated until the excess is volatilized and the last trace of oxidizable material is evolved. Finally the contents of the beaker are cooled, dissolved in water, and transferred to a 250-ml. graduated flask. It is not as a rule necessary to filter because of the presence of a little dehydrated silica.

Now 1 ml. of a 10% hydroxylamine hydrochloride solution is added, and a small piece of Congo red paper which will turn dark blue because of the acid present. A solution of dilute aqueous ammonia is added, a few drops at a time, until the Congo red paper just turns pink (pH 4 to 4.5), followed by addition of 5 ml. of a saturated aqueous solution of 1,10-phenanthroline. The red color of 1,10-phenanthroline ferrous iron immediately appears. The solution is then diluted to the mark in the graduated flask, and mixed. The color intensity is determined using a Cenco Photelometer or its equivalent and a 1-cm. cell with a green filter. From a calibration chart plotted with log transmittancy on the vertical axis and milligrams of iron on the horizontal, the amount of iron present can be rapidly determined. If the samples are not filtered before color production, the color comparisons must be made immediately after color formation.

To determine the accuracy with which iron is retained by the mixed acid mineralization of the product, samples of blood fibrin were made ready and known amounts of iron added before oxidation. As a result of a group of analyses of the unfortified blood fibrin the iron present was found to be 70 parts per million: 200 micrograms of iron added to fortify the blood samples could be then recovered within an accuracy of 5 to 6 micrograms.

Since sulfur is present in blood fibrin, the treatment described is capable of oxidizing it to sulfate. Because of the presence of sulfur, special treatment is required if sodium and potassium are to be determined in the residue from oxidation of organic matter.

DESTRUCTION OF ORGANIC MATTER IN CHROMACIZED CATGUT

Catgut sutures of varying degrees of chromacization were cut into 6-mm. lengths and dried for 8 to 12 hours in an electric oven at 80° C. and stored in glass weighing bottles in preparation for mineralization and determination of chromium.

Weighed samples of 1 to 2 grams were transferred to 500-ml. Vycor Erlenmeyer flasks and treated with varying proportions of 72% perchloric acid and 67% nitric acid. The most satisfactory mixture was found to be 20 ml. of perchloric to 6.5 to 7.0 ml. of nitric acid.

The sample, in a short time after heating begins, is completely dissolved. In 5 or 6 minutes the nitric acid reaction accompanied by the production of brown fumes is over and the excess nitric acid is volatilized. During this process it would appear that nitration products of the organic matter are formed. During and at the end of the nitric acid reaction the solution is green in color. As the reaction mixture rises from 140° through 160° to 180° a vigorous reaction takes place, with the copious formation of gaseous products of reaction, but little or no foaming. The solution may or may not turn light brown in color, depending upon the rate of temperature elevation. In 8 to 13 minutes the decomposition of organic matter is complete, as shown by the fact that the color of chromic acid appears.

The sample is kept on the hot plate for 2 to 3 minutes after the appearance of the chromic acid color at not more than 200° C. and is quickly removed from the hot plate and plunged into an ice bath with a swirling motion during 5 seconds. It is then quickly diluted by the addition of 60 to 70 ml. of cold water, and the refluxing still head is rinsed into the flask and removed. A boiling chip of Filtercel is added and the solution boiled 2 to 3 minutes to remove the small amount of chlorine formed during the oxidation reaction. The solution is then diluted to 200 ml. with cold distilled water, a drop of 0.025 *M* ferroin indicator is added, and the solution is titrated using 0.033 *N* ferrous sulfate to the first production of a pink cast to the solution from the first drop excess of reducing agent. One ml. of 0.033 *N* ferrous sulfate solution corresponds to 1.1112 mg. of CrO_3.

The results from a series of analyses are given in Table II.

Table II. Mineralization of Chromacized Catgut and Determination of Chromic Oxide

(20 ml. of 72% $HClO_4$ and 6.5 ml. of 67% HNO_3 per sample. Time for HNO_3 reaction, 5 to 6 minutes. Hot plate temperature, 210° to 225° C. during oxidation of sample and 200° C. during oxidation of chromium)

Sample No.	Weight	Reaction Time	0.033 *N* Fe^{++} Required	CrO_3 Present
	Grams	*Min.*	*Ml.*	%
1	2	10	25.15	1.40
	2	13	25.30	1.41
	2	9	25.04	1.39
				Av. 1.40 ± 0.01
2	2	11	31.55	1.75
	2	9	31.42	1.75
				Av. 1.75
3	2	11	31.90	1.77
	2	10	30.60	1.70
				Av. 1.74 ± 0.04
4	2	11	31.70	1.76
	2	13	31.60	1.76
				Av. 1.76
5	2	10	41.32	2.30
	2	10	41.60	2.31
	2	9	41.87	2.33
	2	10	41.08	2.28
				Av. 2.30 ± 0.03
6	1.002	15	14.65	1.62
	1.006	15	14.72	1.63
	1.012	13	14.51	1.61
				Av. 1.61 ± 0.01
7	1.0037	15	16.03	1.78
	1.0017	14	16.19	1.80
				Av. 1.79 ± 0.01

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Analysis of Mixtures of Meta- and Para-Cresols and Their Butylated Products

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Data are presented for the analysis, by freezing point and cloud point determinations, of binary cresol and butylated cresol mixtures such as are encountered in the butylation-fractionation-debutylation method of separating *m*- and *p*-cresols. The procedures are simple and rapid, and yield results which are readily duplicated. A cloud point method for determining small amounts of water in glycols and glycerol is suggested.

FROM their mixtures *m*- and *p*-cresols are separated by a method involving alkylation with isobutylene, separation by fractional distillation of the tertiary butyl derivatives so produced, and dealkylation of the individual di-*tert*-butylcresols to give the pure parent cresols (*3*). In the commercial operation (*4*) it is important that reliable and quick methods be available for analyzing the various distillates handled. The determination of freezing points is a convenient method of analysis. A measurement of the critical solution temperature of the phenolic mixture in diethylene glycol and other solvents offers another rapid and accurate test for this purpose.

MATERIALS

The *m*- and *p*-cresols employed in securing the data reported herein were Eastman practical grade chemicals which were subjected to a purification sequence comprising fractional distillation under reduced pressure and at about 15 to 1 reflux ratio through a 20-plate packed glass column, collecting only the middle portion which came over within a 1° C. temperature range. This material was melted, allowed to cool, and seeded. When the crystallization was about half complete, the crystals formed were separated from the mother liquor and distilled through the 15-plate column, again collecting only a heart cut. The tertiary butylated derivatives of these cresols were prepared by alkylation with isobutylene (*3*) and purified by fractionation-recrystallization-fractionation treatments. Triisobutylene was made by the action of 63% sulfuric acid on Eastman *tert*-butyl alcohol and purified by means of an alkali wash, a rough distillation through a Hempel column, and two fractional distillations through the 20-plate column. The cresols, their butylated derivatives, and the triisobutylene were believed to be of at least 99.5% purity. Their properties are listed in Table I.

FREEZING POINTS

METHOD. A sample of 5 to 10 grams of the cresol mixture was placed in an unsilvered evacuated Dewar test tube (16×150 mm., inside dimensions) equipped with a glass loop stirrer and a calibrated thermometer, readable to 0.1° C., held in place by means of a cork stopper (an Anschütz thermometer was found very suitable for this work). When the material was a solid, the tube was first heated to melt the sample. After a preliminary trial, the Dewar test tube was placed in a water bath held at a temperature about 2° below the indicated freezing point. The

Table I. Properties

Name of Compound	Boiling Point at 20 Mm. °C.	Freezing Point °C.
m-Cresol	...	11.5[a]
p-Cresol	...	34.7[a]
Mono-*tert*-butyl-*m*-cresol	...	21.3
2-*tert*-Butyl-4-methylphenol	126.5	51.7
4,6-Di-*tert*-butyl-3-methylphenol	167.0	62.1
2,6-Di-*tert*-butyl-4-methylphenol	147.0	69.7[b]
Triisobutylene	174.9–5.3[c]	...

[a] W. Seaman, Laboratory for Analytical Research, Calco Chemical Division, American Cyanamid Co., Bound Brook, N. J. (private communication), reports highly purified *m*- and *p*-cresols to freeze at 11.65° and 34.63° C., respectively.
[b] Melting point.
[c] At 745.7 mm.

Table II. Freezing Points of Mixtures of *m*- and *p*-Cresols[a]

p-Cresol %	*m*-Cresol %	Freezing Point ° C.	*p*-Cresol %	*m*-Cresol %	Freezing Point ° C.
0.0	100.0	11.5	44.5	55.5	5.5
2.6	97.4	9.6	52.7	47.3	2.1
6.5	93.5	6.6	59.0	41.0	−0.5
9.9	90.1	3.9	60.9	39.1	−0.4
12.2	87.8	2.1	65.2	34.8	4.0
13.1	86.9	2.5	72.1	27.9	10.7
18.2	81.8	5.5	80.1	19.9	18.6
23.8	76.2	8.0	88.2	11.8	25.5
30.1	69.9	8.1	95.1	4.9	31.2
31.5	68.5	8.4	100.0	0.0	34.7
35.1	64.9	7.6			

[a] Freezing point data on *m*- and *p*-cresol mixtures have been reported by Desseigne (*1*), who used cresols freezing at 10.1° and 34.5° C., respectively.

Table III. Freezing Points of Mixtures of *m*-Cresol and Mono-*tert*-butyl-*m*-cresol

m-Cresol in Mono-*tert*-butyl-*m*-cresol			Mono-*tert*-butyl-*m*-cresol in *m*-Cresol		
m-Cresol %	Mono-*tert*-butyl-*m*-cresol %	Freezing point ° C.	Mono-*tert*-butyl-*m*-cresol %	*m*-Cresol %	Freezing point ° C.
0.0	100.0	21.3	0.0	100.0	11.5
1.0	99.0	20.4	2.4	97.6	10.2
3.0	97.0	18.8	4.4	95.6	9.2
5.4	94.6	16.9	7.0	93.0	7.9
7.9	92.1	14.9	10.0	90.0	6.4
10.7	89.3	12.7	13.1	86.9	4.7
13.8	86.2	10.2	16.1	83.9	3.1
17.1	82.9	7.6	19.0	81.0	1.3
20.0	80.0	5.2	22.2	77.8	−0.3
21.4	78.6	3.6			

Table IV. Freezing Points of Mixtures of Mono-*tert*-butyl-*m*-cresol and 2-*tert*-Butyl-4-methylphenol

Mono-*tert*-butyl-*m*-cresol in 2-*tert*-Butyl-4-methylphenol			2-*tert*-Butyl-4-methylphenol in Mono-*tert*-butyl-*m*-cresol		
Mono-*tert*-butyl-*m*-cresol %	2-*tert*-Butyl-4-methyl-phenol %	Freezing point ° C.	2-*tert*-Butyl-4-methyl-phenol %	Mono-*tert*-butyl-*m*-cresol %	Freezing point ° C.
0.0	100.0	51.7	0.0	100.0	21.3
2.2	97.8	50.5	2.0	98.0	20.2
4.9	95.1	48.9	5.0	95.0	18.5
10.5	89.5	45.8	10.0	90.0	15.5
16.5	83.5	42.0	16.0	84.0	11.9
22.7	77.3	38.3	21.7	78.3	6.4
25.7	74.3	36.0	27.3	72.7	0.0
32.6	67.4	31.0	32.4	67.6	−3.2
36.0	64.0	29.2	34.8	65.2	−4.8
39.3	60.7	27.3			

Table V. Freezing Points of Mixtures of Mono-*tert*-butyl-*m*-cresol and 4,6-Di-*tert*-butyl-3-methylphenol

Mono-*tert*-butyl-*m*-cresol in 4,6-Di-*tert*-butyl-3-methylphenol			4,6-Di-*tert*-butyl-3-methylphenol in Mono-*tert*-butyl-*m*-cresol		
Mono-*tert*-butyl-*m*-cresol %	4,6-Di-*tert*-butyl-3-methyl-phenol %	Freezing point ° C.	4,6-Di-*tert*-butyl-3-methyl-phenol %	Mono-*tert*-butyl-*m*-cresol %	Freezing point ° C.
0.0	100.0	62.1	0.0	100.0	21.3
1.3	98.7	61.0	1.2	98.8	20.6
3.4	96.6	59.0	2.4	97.6	20.0
5.4	94.6	56.9	4.4	95.6	16.8
7.3	92.7	54.7	6.9	93.1	17.4
9.4	90.6	52.2	9.2	90.8	16.0
11.7	88.3	49.7	10.7	89.3	15.0
15.0	85.0	44.9	12.2	87.8	14.1
18.5	81.5	40.4	14.8	85.2	12.4

Table VI. Freezing Points of Mixtures of Triisobutylene and *m*-Cresol

Triisobutylene in *m*-cresol			Triisobutylene in *m*-cresol		
Triisobutylene %	*m*-Cresol %	Freezing Point ° C.	Triisobutylene %	*m*-Cresol %	Freezing Point ° C.
0.0	100.0	11.5	14.1	85.9	6.8
2.6	97.4	10.3	18.1	81.9	6.0
5.2	94.8	9.2	22.4	77.6	5.6
8.7	91.3	8.1	24.4	75.6	5.4
10.7	89.3	7.6			

Table VII. Critical Solution Temperatures

Weight Percentage of 2,6-Di-*tert*-butyl-4-methylphenol	Weight Percentage of Diethylene Glycol	Cloud Point, ° C.
75.0	25.0	181.2
58.2	41.8	190.0
49.1	50.9	190.2
47.4	52.6	190.1
37.5	62.5	189.0
27.0	73.0	181.7

Table VIII. Critical Solution Temperatures

Weight Percentage of 4,6-Di-*tert*-butyl-3-methylphenol in 2,6-Di-*tert*-butyl-4-methylphenol	Cloud Point, ° C. (Weight Ratio of Phenolic Mixture to Diethylene Glycol = 1.0 to 1.0)
0.00	190.2
1.45	188.5
3.42	185.4
5.76	181.5
8.79	176.0

Table IX. Critical Solution Temperatures

Weight Percentage of Mono-*tert*-butyl-*m*-cresol in 2,6-Di-*tert*-butyl-4-methylphenol	Cloud Point, ° C. (Weight Ratio of Phenolic Mixture to Diethylene Glycol = 1.0 to 1.0)
0.00	190.2
3.50	184.0
6.80	179.3
8.75	173.5

sample was then stirred until it was supercooled about 1° C. and, if necessary, seeded to bring about crystallization. After crystallization had started, the stirring was intermittent, about 0.5 to 1 minute between sets of 2 or 3 strokes of the stirrer. Under these conditions the temperature was found to rise to a maximum, where it remained until the greater part of the material froze. The maximum value was taken as the freezing point temperature of the sample.

The data obtained, given in Tables II to VI, are for the full range of *m*- and *p*-cresol mixtures, and for the particular distillate compositions handled in practice.

CRITICAL SOLUTION TEMPERATURES

The observation that mono- and di-*tert*-butyl-*m*-cresols and mono-*tert*-butyl-*p*-cresol effect a pronounced lowering of the critical solution temperature of 2,6-di-*tert*-butyl-4-methylphenol with diethylene glycol, and that 2,6-di-*tert*-butyl-4-methylphenol appreciably raises that of 4,6-di-*tert*-butyl-3-methylphenol with a 20–80 volume mixture of glycerol–ethylene glycol was used as a basis for a cloud point method of analysis for mixtures of the butylated cresols. The cloud points can be determined rapidly with ease and are duplicatable to within 0.1° C. (*2*).

For the two systems investigated, the consolute temperatures occurred very near the 50–50 weight ratio of butylated cresol and solvent (see Tables VII and XI). It is advantageous to use this particular proportion, for small errors in making up the mixtures are not serious because of the small change in temperature with composition at this point.

The determinations were made by weighing into the Dewar test tube (the same as used for freezing point determinations) equal amounts of butylated cresol mixtures and the solvent, immersing in an electrically heated bath of clear oil, and stirring until complete solution was effected. Then, on slow cooling and with continued stirring, the cloud point was readily discernible. The data collected for binary mixtures of 4,6-di-*tert*-butyl-3-methylphenol, mono-*tert*-butyl-*m*-cresol, and mono-*tert*-butyl-*p*-cresol in 2,6-di-*tert*-butyl-4-methylphenol, with diethylene glycol as the solvent, are listed in Tables VIII, IX, and X. Determinations made with binary mixtures of 2,6-di-*tert*-butyl-4-methylphenol in 4,6-di-*tert*-butyl-3-methylphenol, with a 20–80 volume mixture of glycerol–ethylene glycol as the solvent, resulted in the data given in Table XII. Because 2,6-di-*tert*-butyl-4-methylphenol is considerably less soluble in such a mixed solvent, its effect is to raise the cloud point. Diethylene glycol could not be employed as the solvent in this instance on account of its high solubility for 4,6-di-*tert*-butyl-3-methylphenol.

Table X. Critical Solution Temperatures

Weight Percentage of 2-*tert*-Buyl-4-methylphenol in 2,6-Di-*tert*-butyl-4-methylphenol	Cloud Point, ° C. (Weight Ratio of Phenolic Mixture to Diethylene Glycol = 1.0 to 1.0)
0.00	190.2
2.42	185.3
5.24	179.3
6.87	175.7
8.69	171.0
9.10	169.6

Table XI. Critical Solution Temperatures

Weight Percentage of 4,6-Di-*tert*-butyl-3-methylphenol	Weight Percentage of Blend (20% Glycerol and 80% Ethylene Glycol, by Volume)	Cloud Point ° C.
32.8	67.2	174.4
39.2	60.8	176.0
49.0	51.0	179.2
50.0	50.0	178.9
52.6	47.4	179.2
57.0	43.0	179.0
65.7	34.3	173.5

Table XII. Critical Solution Temperatures

Weight Percentage of 2,6-Di-*tert*-butyl-4-methylphenol in 4,6-Di-*tert*-butyl-3-methylphenol	Cloud Point, ° C. (Weight Ratio of Phenolic Mixture to Glycerol–Ethylene Glycol Blend = 1.0 to 1.0)
0.00	178.7
1.08	181.9
3.98	189.3
8.68	199.2

Table XIII. Effect of Water on Cloud Points of Alkylphenol Mixtures in Diethylene Glycol or in a Glycerol–Ethylene Glycol Blend

Water Added to Solvent	Cloud Point Rise: Equiweight mixture of (*i*) in (*j*)	Cloud Point Rise: Equiweight mixture of (*k*) in (*l*)
%	°C.	°C.
0.0	0.0	0.0
0.5	2.7	2.9
1.0	5.2	4.0[a]
1.5	6.9[a]	...

[a] Erratic results obtained caused by loss of water from solvent by vaporization.
(*i*) = mixture of 4% 4,6-di-*tert*-butyl-3-methylphenol and 96% 2,6-di-*tert*-butyl-4-methylphenol.
(*j*) = diethylene glycol (sp. gr. 1.1135/80° F.).
(*k*) = mixture of 4% 2,6-di-*tert*-butyl-4-methylphenol and 96% 4,6-di-*tert*-butyl-3-methylphenol.
(*l*) = blend comprising 20–80 volume mixture of glycerol and ethylene glycol.

In the determination of these data, Eastman quality glycerol, ethylene glycol, and diethylene glycol were employed. The ethylene glycol was heated to 190° C. to drive off water before use. The solvents were characterized by the specific gravities:

Glycerol	1.260 at 80°/60° F.
Ethylene glycol	1.110 at 80°/60° F.
20% glycerol–80% ethylene glycol (by volume)	1.142 at 80°/60° F.
Diethylene glycol	1.1135 at 80°/60° F.

Small amounts of water in the solvents have considerable effect on the cloud points, and for this reason it should be emphasized that the data presented are intended to serve only as a guide. A new set of curves can be constructed readily for each batch of solvent by determination of a few key points, or the solvents might be standardized by matching definite cloud point values when mixed with selected pure tertiary butylated cresols. Table XIII exemplifies the influence of water. This pronounced effect suggests the use of cloud points, with alkylated phenols or other suitable compounds, as a method for determining accurately low percentages of water in glycols and glycerols.

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Determination of Cobalt in High-Cobalt Products

Separation from Iron by Phosphate

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A method is given in which cobalt is separated from iron in high-cobalt products by one precipitation with tribasic sodium phosphate. Since all the conditions for the reaction can be precisely controlled, the method is extremely accurate and can be used for research or referee work. Cobalt can be determined electrolytically directly following the removal of iron and aluminum by this procedure, chromium, nickel, and vanadium being the only common interfering elements. This is particularly advantageous for mining and metallurgical products, where high percentages of cobalt are frequently encountered and the resulting bulky precipitate formed with α-nitroso-β-naphthol is inconvenient to handle. The method of separating iron with zinc oxide in a volumetric flask and determining cobalt on an aliquot portion of this supernatant liquid is inaccurate, but a similar aliquoting procedure may be employed if sodium phosphate is used to remove the iron.

THE major problem confronting chemists in the analysis of cobalt is the separation of this element from iron. This is particularly important in the mining and metallurgical industry, where a high concentration of iron is usually encountered in cobalt-containing samples. Frequently, as in high-cobalt alloys, mattes, and slags, both cobalt and iron are present in large amounts.

The various methods for this separation have been reviewed and it was considered that the procedure described by North and Wells (*3*) for the precipitation of iron by sodium phosphate offered the greatest possibilities. This determination has been thoroughly investigated and various alterations in the procedure have been introduced. It is now employed in this laboratory for all research samples and for all other products where the amount of cobalt present is over 2.5 to 3.0%.

PREVIOUS WORK

In the precipitation of iron with ammonia some cobalt is always occluded in the gelatinous precipitate. Noyes and Bray (*4*) have shown that 2 mg. of cobalt are almost wholly retained in the hydroxide precipitate by 100 mg. of aluminum, iron, titanium, and zirconium, and the addition of larger quantities of cobalt leads to further occlusion. The use of potassium cyanide before precipitation of iron with ammonia produces a clean separation of the iron from cobalt, but the presence of potassium cyanide is objectionable in the subsequent determination of the cobalt.

The method developed by Fairchild (*2*), in which the trivalent metals are hydrolyzed into basic alums, also takes a considerable time and does not remove the last traces of iron from the cobalt. In the zinc oxide precipitation of iron some cobalt will always be retained in the iron precipitate unless three precipitations are carried out. It is also very difficult to be certain that the last traces of iron have been precipitated by the zinc oxide. The method for the precipitation of iron by zinc oxide does not lend itself to precise control and depends too much on judgment by the eye alone. Very accurate results can be obtained by experienced workers with the zinc oxide method, but these are largely the result of a compensation of errors in which the amount of cobalt lost is balanced by the amount of iron left in solution. It is also, of course, impossible to determine cobalt directly by electrolysis after a zinc oxide precipitation.

North and Wells (*3*) described a method in which iron is separated from cobalt by precipitation with dibasic sodium phosphate. This method permits very precise control of all the conditions during the precipitation and forms the basis of the procedure at present employed in this laboratory.

PRESENT PROCEDURE

REAGENTS. Tribasic Sodium Phosphate Solution. Dissolve 34.05 grams of reagent quality tribasic sodium phosphate dodecahydrate, $Na_3PO_4.12H_2O$, in 1 liter of water.

Cobalt Precipitating Solution. Dissolve 10 grams of α-nitroso-β-naphthol in 500 ml. of acetic acid and heat just to boiling. Add 500 ml. of water and filter.

Acetic Acid Wash Solution. Add 25 ml. of acetic acid to 1 liter of water.

Cobalt Indicator Solutions. (1) Dissolve 0.05 gram of nitroso-R-salt in 100 ml. of water. (2) Dissolve 0.5 gram of phenylthiohydantoic acid in 100 ml. of hot alcohol. Either indicator solution may be used.

SEPARATION OF COBALT

Decompose the cobalt samples in the usual way with concentrated hydrochloric and nitric acids and 1 to 1 sulfuric acid, adding a few drops of bromine or hydrofluoric acid if necessary. In the case of arsenical cobalt ores Scott and Furman (*5*) advise removing the arsenic in the decomposition by repeated treatments with hydrochloric and sulfuric acids and the addition of 1.0 gram of sodium thiosulfate.

SEPARATION FROM HYDROGEN SULFIDE GROUP OF ELEMENTS. Separate cobalt from copper, bismuth, cadmium, mercury, lead, tin, arsenic, antimony, molybdenum, etc., by gassing the acid solutions of the samples with hydrogen sulfide and filtering.

SEPARATION FROM IRON. Boil off the excess hydrogen sulfide, cool the solutions a little, and make up to approximately 350 ml. For each 0.1 gram of iron present add 25 ml. of the sodium phosphate solution plus 5 to 10 ml. in excess. For samples containing appreciable quantities of aluminum and other elements precipitated as phosphates, more sodium phosphate must be added to ensure complete precipitation. Add ammonia to the solution until it can be seen that purple cobalt phosphate is precipitating or until litmus paper turns blue. A pH of approximately 5.6 will now have been reached. Add 10 ml. of acetic acid and reduce the pH from 3.5 to 3.0. Oxidize the solution by adding 5 to 20 ml. of a 3% solution of hydrogen peroxide. Add sufficient hydrogen peroxide to make sure that all the iron is oxidized. If the amount of iron present in the sample is unknown, then add 20 ml. of the phosphate solution at first. After oxidation add further quantities of sodium phosphate solution in amounts of 5 to 10 ml. at a time until it can be seen that a small excess is present. The precipitate of ferric phosphate should come down creamy white in color.

If the precipitate of ferric phosphate is small in bulk, boil the samples for a few minutes to ensure coagulation of the precipitate, but if there is a large amount of ferric phosphate present heat the samples for only a short time; otherwise severe bumping will take place. Filter the samples through No. 31 Whatman filter papers to which a little paper pulp has been added. Wash the precipitates seven or eight times with hot acetic acid wash solution. Boil the samples to expel the excess hydrogen peroxide.

For the rapid analysis of samples with a high iron and cobalt content, filter off the ferric phosphate precipitate on a Büchner funnel or precipitate the iron in a standard liter flask. In the latter case, after precipitation make the samples up to the liter mark and mix well. When the precipitate has settled decant more than 500 ml. Filter this portion through a dry filter paper and take exactly 500 ml. for determination of the cobalt. The amount of cobalt retained in the precipitate will be very small.

DETERMINATION OF COBALT

SEPARATION BY PRECIPITATION WITH α-NITROSO-β-NAPHTHOL. Before precipitating the cobalt add 5 to 10 ml. of the sodium phosphate solution and a few drops of hydrogen peroxide to make sure that all the iron has come down. If there is any residual ferric phosphate filter the samples again. Add 4 ml. of hydrochloric acid to every 100 ml. of solution, followed by 25 ml. of the

cobalt precipitating solution for every 10 mg. of cobalt present. Boil the samples gently for about 10 minutes and allow them to stand until the precipitate has settled. Filter off the precipitate through No. 42 Whatman paper, wash well with hot 5% hydrochloric acid and finally with hot water, and ignite. Weigh the cobalt as Co_3O_4. If desired, the precipitate may be dissolved in acids, evaporated to dryness with sulfuric acid, and weighed as cobalt sulfate (*1*).

Not more than 50 mg. of cobalt can be readily precipitated by this method. Beyond that amount it appears to be difficult to ensure complete precipitation by the reagent without contamination from occlusion.

Separation by Electrodeposition. Test the cobalt solutions for the presence of any residual iron by adding 5 to 10 ml. of phosphate solution and a few drops of hydrogen peroxide. Reduce to small bulk by evaporation and filter if necessary. Add 5 grams of sodium tetraborate, $Na_2B_4O_7.10H_2O$, 10 ml. of 1 to 1 sulfuric acid, and 40 to 50 ml. of ammonia.

Heat the solutions to 90° C. and electrolyze at 3 amperes and 6 volts, using a platinum gauze cathode and a rotating anode. It is better if possible to electrolyze at 4 or 5 amperes and in no case should the electrolysis be carried out at less than 3 amperes. Better results will always be obtained if the cobalt is plated out as rapidly as possible.

Towards the end of the electrolysis test the solutions for the presence of cobalt by either of the two indicator solutions, nitroso-R-salt or phenylthiohydantoic acid. When a drop of the solution has failed to give a red coloration with a drop of the indicator solution on a spot plate, continue the electrolysis for a further 15 to 20 minutes. Wash the cathodes in water with the current running, then remove from the cabinet and wash in alcohol, dry, and weigh.

The electrolytic method is preferred in this laboratory, owing to the fact that much larger amounts of cobalt can be determined. All results quoted were obtained by this method.

RESULTS

Table I shows the results obtained on various mixtures of a cobalt and an iron solution. The amount of iron solution added in each case was equivalent to 0.5 gram of iron, and this was separated from the cobalt by one precipitation with sodium phosphate.

Table I. Recovery of Cobalt from Synthetic Solutions after Separating Iron with Phosphate

Solution No.	Iron Added	Cobalt Added	Cobalt Found	Loss
	Gram	*Mg.*	*Mg.*	*Mg.*
1	0.5	40.9	40.9	Nil
2	0.5	61.3	61.1	0.2
3	0.5	81.8	81.8	Nil
4	0.5	102.3	102.3	Nil

Table II contains results on various typical Nkana samples. The results obtained by the phosphate method were checked carefully throughout for any loss of cobalt. The iron precipitates were dissolved and tested for cobalt colorimetrically and none was found. The results are compared with those given by other workers using the zinc oxide method. The latter results were obtained only after much careful work and time had been spent on the samples.

DISCUSSION

Ferric iron is precipitated from a solution of ferric sulfate by tribasic sodium phosphate according to the reaction:

$$2Na_3PO_4 + Fe_2(SO_4)_3 \longrightarrow 2FePO_4 + 3Na_2SO_4$$

This means that 1.0 gram of Fe^{+++} requires 6.81 grams of tribasic sodium phosphate dodecahydrate. This reaction takes place at a pH of approximately 1.6. The sodium phosphate solution is made up to contain 34.05 grams of sodium phosphate in 1 liter so that 100 ml. of this solution will precipitate 0.5 gram of Fe^{+++}. In the absence of other elements precipitated by

Table II. Separation of Cobalt from Iron in Metallurgical Products with Sodium Phosphate and Zinc Oxide

Sample	Iron	Cobalt: Sodium phosphate precipitation of iron	Cobalt: Zinc oxide precipitation of iron
	%	%	%
Mill feed	2.38	0.14	0.14
Cobalt oxide ore	2.98	0.55	0.55
Cobalt concentrate	7.77	2.40	2.40
Cobalt reverberatory matte	13.62	3.78	3.79
Copper converter slag	40.87	4.95	4.95
Cobalt converter slag	38.10	9.44	9.45
Cobalt alloy	48.37	36.80	36.80

phosphate, very little excess of the phosphate solution need be added. A necessary condition for the precipitation of iron by the phosphate in stoichiometric proportions is the complete oxidation of the iron. In any case a large excess of phosphate should be avoided; otherwise some of the cobalt is precipitated as the phosphate on the addition of ammonia before electrolysis.

The ferric phosphate precipitate is somewhat bulky and the maximum amount of precipitate which can be conveniently handled is that given by 0.5 gram of iron. For rapid analysis it is best to select a weight of sample such that not more than 0.3 gram of iron is present. For routine work large quantities of ferric phosphate may be conveniently filtered and washed on a Büchner funnel. Samples which contain a large amount of iron (60% and over) and a small amount of cobalt (2.5% and under) are analyzed in this laboratory by an ammonium thiocyanate colorimetric method shortly to be published.

It has been found by experiment that the method of adjustment of the pH for the precipitation of iron as given above gives the best results. No cobalt is retained by the iron under these conditions. It is also better to oxidize the iron after addition of the phosphate and adjustment of the pH rather than before.

The ferric phosphate precipitate should be washed with a dilute acetic acid wash solution. If water alone is used and excess phosphate is present there is a danger of precipitating some cobalt as the phosphate in the pores of the flocculent precipitate. Since ferric phosphate is insoluble in acetic acid, the iron precipitate is not redissolved by the wash solution.

INTERFERING IONS

It is beyond the scope of this paper to discuss all the ions which may have to be separated in the determination of cobalt. Ores carrying the rarer elements would have to be treated with special methods. The separation of the more common elements may be outlined here.

The separation of iron as phosphate follows prior removal of silica, together with barium and lead, by dehydration with sulfuric acid. Tungsten will also be removed in the preliminary decomposition by the usual procedures. A number of elements, such as copper, bismuth, arsenic, antimony, and molybdenum, will be eliminated by treatment with hydrogen sulfide in acid solution.

Aluminum is completely precipitated with the iron at a pH of 3.5. It must be remembered that 1 gram of aluminum requires 14.1 gram of sodium phosphate dodecahydrate for its precipitation, so that if much of this element is present a large quantity of phosphate must be added and it is also advisable to allow the samples to stand for a short time after precipitation. Zirconium and titanium will be completely precipitated with the ferric phosphate. If much titanium is present, the phosphate precipitate will be yellow in color after the addition of hydrogen peroxide. Manganese, vanadium, and chromium are not precipitated as phosphate under these conditions. Uranium is precipitated quantitatively with iron by sodium phosphate. Uranium phosphate appears to have a slightly greater occluding power towards cobalt than does ferric phosphate, and for accurate cobalt determinations in the presence of much uranium it would be necessary to dissolve and reprecipitate the uranium phosphate. Zinc may be separated from cobalt as the phosphate by carefully adjusting the pH to 4.5 to 4.8 with acetic acid by a pH meter. Nickel can-

Table III. Adsorption of Cobalt by Iron Precipitates

Precipitant for Iron	Cobalt Present	Cobalt Found	Loss
	Mg.	*Mg.*	*% of total cobalt*
Ammonia	184.0	61.2	66.74
Zinc oxide	47.2	40.8	13.56
Sodium phosphate	184.0	182.2	0.98

not be separated as the phosphate, since the pH of the precipitation, 6.3, is higher than that of cobalt, 5.3.

Few elements interfere with the determination of cobalt by α-nitroso-β-naphthol after the phosphate separation. Copper, palladium, and ferric salts are precipitated completely with the cobalt, while silver, chromium, vanadium, uranium, and bismuth are precipitated but not quantitatively. The more common elements which are likely to interfere are either removed with the hydrogen sulfide group or as phosphate with the iron, except chromium and vanadium. These two elements may be eliminated by a sodium hydroxide separation. Zinc, manganese, and nickel do not interfere. If there are large quantities of other elements present, however, the voluminous precipitate of cobalt is apt to adsorb other ions, and it is then advisable to carry out this precipitation twice.

The ions of some metals will interfere with the electrolysis of cobalt either by depositing with it or by precipitating as hydroxides or phosphates which retain cobalt. Unless zinc has previously been completely separated it will form complex cations in excess ammonia and be deposited with the cobalt. Nickel will also be deposited with the cobalt and may be determined by dissolving the cathode deposit in nitric acid and separating with dimethylglyoxime in the usual manner. If a large excess of phosphate has been used in precipitating iron, calcium, strontium, and magnesium will be precipitated in the ammoniacal electrolytic solution and may occlude some cobalt. If a large quantity of calcium is present it may be precipitated as the oxalate after the phosphate separation. If much magnesium is present an excess of phosphate must be rigorously avoided. Chromium will be precipitated as the hydroxide in the ammoniacal electrolytic solution. Since this precipitate may occlude some cobalt, the chromium should be previously separated with sodium hydroxide and sodium peroxide. If large quantities of manganese are present they may be removed by initial treatment with nitric acid and potassium chlorate, or the separation of cobalt and manganese phosphates can be carried out in the presence of ammonium citrate (*1*). When small quantities of manganese are fully oxidized and the electrolysis is not unduly prolonged after deposition of all the cobalt, manganese will not deposit on the cathode, provided also that the concentration of ammonia in the electrolytic solution is kept high. Vanadium forms a complex with cobalt in the ammoniacal electrolytic solution and it is then impossible to deposit any of the cobalt on the cathode. Vanadium should be previously separated with sodium hydroxide and sodium peroxide.

COMPARATIVE RETENTIVE POWERS OF IRON PRECIPITATES

Iron was separated from cobalt in various samples by the method of precipitation in a liter flask and decantation. The precipitants used were ammonia, zinc oxide, and sodium phosphate solution. The amount of iron present varied from 0.4 to 0.5 gram. The results are given in Table III.

Many analytical texts recommend the separation of iron from cobalt by the addition of zinc oxide to a volumetric flask containing the solution of the sample, followed by the withdrawal of an aliquot portion of the supernatant liquid for the determination of cobalt. Table III indicates that this practice may be unsatisfactory even for routine work. On the other hand, sodium phosphate can be used for all routine work in this manner, since the adsorptive power of ferric phosphate for cobalt is shown in Table III to be very low.

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Colorimetric Determination of Cobalt with Ammonium Thiocyanate

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A rapid and accurate colorimetric method is given whereby cobalt may be determined in the presence of other common ions. This method can be extremely valuable in the laboratories of mining and metallurgical industries where routine determinations of cobalt are carried out. The useful range for this procedure lies within the limits 0.01 to 4.0% cobalt.

THE alkali thiocyanates have been used by many workers to detect the presence of small quantities of cobalt. Potassium and ammonium thiocyanates form complexes with cobalt which are represented by the formulas $K_2Co(SCN)_4$ and $(NH_4)_2Co(SCN)_4$. These complexes may be extracted from aqueous solutions by organic solvents. A rapid and accurate method for the determination of cobalt in ores and concentrates has been developed in the Nkana laboratory by comparing the blue color of these extracts of the cobalt complex by either visual or photoelectric means. The method has been investigated in great detail and applied successfully to the determination of cobalt in various mining and metallurgical samples in Northern Rhodesia for the past three years.

The alkali thiocyanate complexes of cobalt were first described by Skey (*6*), Morrell (*4*), and Vogel (*9*) in the years 1868 to 1879. Since that time a number of papers have appeared on this subject, but the most notable contributions have been presented by Treadwell (*8*), Powell (*5*), Feigl and Stern (*1*), Tomula (*7*), and Gorski (*2*).

Treadwell treated a solution of a cobalt salt with concentrated ammonium thiocyanate and extracted the complex with amyl alcohol or with a mixture of one part of amyl alcohol to one part of ether. A pure extract of the complex shows a characteristic absorption spectrum. If ferric iron is present in the cobalt solution, ferric thiocyanate is formed which colors the alcohol extract red. The iron may be removed by treatment with sodium hydroxide. Treadwell quotes Vogt's analysis of the ammonium thiocyanate complex which gives Co = 18.01%, S = 39.15%, and NH_4 = 10.42%. This is calculated to the formula $(NH_4)_2Co(SCN)_4$. This complex is easily decomposed even by damp air to give cobalt and ammonium thiocyanates.

Powell considered the minimum concentration of ammonium thiocyanate necessary for the formation of the cobalt complex was 25% and preferred to use 30%. Varying results were obtained according to the acidity or alkalinity of the cobalt solution. Sodium pyrophosphate may be used to suppress the color due to iron.

Feigl preferred acetone as a solution medium for the complex in drop reactions for the detection of cobalt. The color of the complex is stronger if saturated solutions of ammonium thiocyanate are used. The fixation of iron may be brought about by the addition of phosphate, but all the methods for the suppression

of the color due to iron are effective only if the latter is present in much less amount than the cobalt.

Tomula carried out experiments to determine the maximum concentration of thiocyanate which was necessary to produce the greatest intensity of color of the complex. For a cobalt concentration of 1.192×10^{-4} mole of cobalt chloride per liter a 5% concentration of thiocyanate was sufficient. Acetone may be preferred as a solution medium for the complex. Nickel salts form a green complex with the thiocyanate which is not soluble in acetone, but the presence of this green complex necessitates the use of yellow filters in the colorimeter. Tomula quotes the work of Ditz as showing that the capacity of a solvent for preventing decomposition of a complex varies inversely as its dielectric constant.

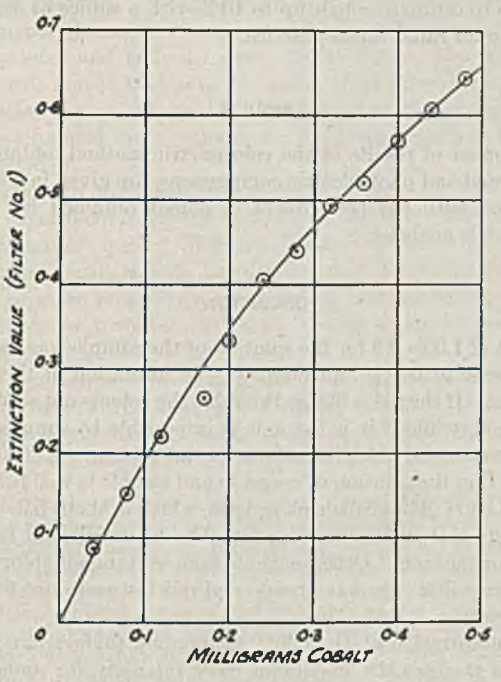

Figure 1. Calibration Curve for Ammonium Cobaltothiocyanate

In the method given by Gorski for the detection of small amounts of cobalt 15 ml. of ethyl alcohol and 5 ml. of a 5 *N* solution of potassium or ammonium thiocyanate are added to 5 ml. of the test solution. The concentration of thiocyanate in the mixture is now 1 *N*. By this method 0.003 mg. of cobalt may be determined.

REAGENTS

Ammonium Thiocyanate Solution. Dissolve 600 grams of ammonium thiocyanate, NH_4CNS, in 1 liter of water.

Sodium Phosphate Solution. Dissolve 83.3 grams of tribasic sodium phosphate dodecahydrate, $Na_3PO_4.12H_2O$, in 1 liter of water.

Sodium Thiosulfate Solution. Dissolve 200 grams of sodium thiosulfate, $Na_2S_2O_3.5H_2O$, in 1 liter of water.

Combined Sodium Phosphate and Thiosulfate Solution. To increase the speed of the analysis the sodium phosphate and thiosulfate solutions may be combined in one. It is not advisable to include the ammonium thiocyanate in this also. Dissolve 125 grams of $Na_2S_2O_3.5H_2O$ and 31.25 grams of $Na_3PO_4.12H_2O$ in 1 liter of water.

Ammonium Acetate Solution. Dissolve 700 grams of ammonium acetate, $NH_4C_2H_3O_2$, in 1 liter of water.

Tartaric Acid Solution. Dissolve 50 grams of tartaric acid, $C_4H_6O_6$, in 100 ml. of water.

Amyl Alcohol–Ether Mixture. Mix 3 parts by volume of amyl alcohol with 1 part of ethyl ether.

METHODS OF COMPARISON

Visual. The color of the cobalt thiocyanate complex fades after a short time and therefore a permanent set of standards cannot be made up from organic solutions of the complex. Solutions of copper sulfate may be used as permanent standards, since the blue color of the copper sulfate solutions matches almost identically the blue of the cobalt complex. Copper sulfate solutions are made up to match varying amounts of cobalt and are kept in sealed test tubes. The color of these will last for a considerable period, but the standards should be checked against known amounts of cobalt from time to time.

A solution of copper sulfate in water containing 8 grams of cupric sulfate pentahydrate per liter will match an extract containing 0.02 mg. of cobalt per 10 ml. By progressively increasing the strength of the standard solutions by 8 grams of cupric sulfate pentahydrate, a range of standards is obtained which will match extracts of the cobalt complex at intervals of 0.02 mg. of cobalt. It has not been found practicable to take this set of standards beyond 0.40 mg. of cobalt. This means that the final standard will contain 160 grams of cupric sulfate pentahydrate per liter.

In Table I are given the weights of samples taken and the dilutions according to the percentage of cobalt present in the sample.

The number of milligrams of cobalt present in an extract is ascertained by matching the color with that of the standard solutions. To obtain the percentage of cobalt present in the sample this number of milligrams is taken multiplied by one of the factors given in the final column of Table I according to the weight of sample taken and the dilution. For example, let us assume that a 0.5-gram sample is taken, and its solution is diluted to 50 ml. From this solution 5 ml. are taken to give the cobalt complex. The extract of this complex matches a standard corresponding to 0.24 mg. of cobalt. By multiplying 0.24 mg. by 2 a percentage of 0.48 for cobalt is obtained. Since 0.24 mg. of cobalt were present in the aliquot the percentage of cobalt in the sample is seen to be

$$0.00024 \times 10 \times \frac{100}{0.5} = 0.48\%.$$

Photoelectric. A calibration curve for the color intensities of the extracts of the cobalt complex may be drawn up by means of an absorptiometer.

For this work a Spekker photoelectric absorptiometer was used with 1-cm. glass cells and Spekker red filter No. 1. The absorption of light by the complex was in each case compared with the absorption of a blank mixture of amyl alcohol and ether extracted from the reagents. In the construction of the curve 10-ml. extracts of the cobalt complex ranging from 0.02 to 0.50 mg. of cobalt were used. In this case the increment of cobalt content was again 0.02 mg. Only the intermediate points of the calibration curve are shown in Figure 1.

Table I. Weights and Dilutions of Samples According to Percentage of Cobalt Present

Cobalt in Sample %	Sample Taken for Analysis *Grams*	Dilution *Ml.*	Aliquot Taken for Analysis *Ml.*	Factor for % Cobalt
0.01 to 0.20	2.0	50	5	0.5
0.16 to 0.80	0.5	50	5	2
0.80 to 3.20	0.5	200	5	8

PROCEDURE

Decompose the cobalt samples with 10 ml. of nitric acid and 20 ml. of hydrochloric acid, adding a few drops of bromine or hydrofluoric acid if necessary. Decompose samples having a high iron content with a solution of nitric acid and potassium chlorate. Take the samples down to dryness but do not bake. Traces of nitric acid have no effect on the formation of the thiocyanate complex.

Take up the samples in approximately 25 ml. of water, and add exactly 1 ml. of hydrochloric acid for every 50 ml. of subsequent dilution of the sample. To effect solution boil the samples gently for a few minutes. Cool the solutions and wash out into appropriate measuring cylinders or calibrated flasks. The amount of dilution of the samples depends on the amount of cobalt present and can be read off from Table I.

After dilution the pH of the sample solutions is 1.0 to 0.9. This is one of the most important steps in the analysis. In samples where the percentage of cobalt is entirely unknown and the first dilution attempted has proved insufficient, it is of no use to try further dilution unless the pH is adjusted by the careful

addition of more acid with the help of a pH meter. It is better otherwise to weigh out another and smaller amount of sample.

COBALT SAMPLES WITH LESS THAN 40% IRON. Measure out 5 ml. of sodium thiosulfate solution, 3 ml. of sodium phosphate solution (or 8 ml. of the combined sodium thiosulfate and phosphate solution), and 10 ml. of ammonium thiocyanate solution. Add with vigorous agitation 5 ml. of the solution of the sample. The pH is now 3.5 to 4.0 and the concentration of ammonium thiocyanate is 26%.

Table II. Gravimetric and Colorimetric Determinations of Cobalt in Metallurgical Products

	Per Cent Cobalt		
	Gravimetric	Colorimetric	Difference
Mill tails	0.06	0.06	0.00
Mill feed	0.14	0.14	0.00
Cobalt oxide ore	0.55	0.56	+0.01
Primary cobalt concentrate	0.70	0.68	−0.02
Secondary cobalt concentrate	3.04	3.04	0.00
Cobalt concentrate	2.40	2.40	0.00
Copper concentrate	0.64	0.64	0.00
Cobalt gravity concentrate	10.15	10.00	−0.15
Cobalt gravity tails	0.21	0.20	−0.01
Copper reverberatory slag	1.47	1.44	−0.03
Cobalt reverberatory slag	0.63	0.64	+0.01
Cobalt reverberatory matte	3.79	3.84	+0.05
Electric furnace slag	2.21	2.24	+0.03
U. S. Bureau of Standards No. 115	0.08	0.08	0.00

Add 10 ml. of the amyl alcohol–ether mixture and shake the whole thoroughly again. Transfer to a separatory funnel, run off the lower aqueous layer, and discard. Transfer the solution of the cobalt complex to a test tube or a 1-cm. absorptiometer cell. For visual comparison match the intensity of color of the test solution with the standard copper sulfate solutions. The comparison may be carried out in a LaMotte comparator for hydrogen-ion determinations with a source of artificial light, or the tubes may simply be held against a white background out of direct sunlight. It will be found possible to take a reading halfway between any two standards if the color of the test solutions ies between them.

In the photoelectric comparison, absorption of the test solution is compared with an amyl alcohol–ether blank. The amount of cobalt present in the test solution is then read off from the calibration curve.

By either means of comparison the amount in milligrams of cobalt present in the extract is obtained and the percentage of cobalt in the sample is given by multiplying by the appropriate factor shown in the last column of Table I.

COBALT SAMPLES WITH MORE THAN 40% IRON. Carry out the analysis of these samples in exactly the same way as for samples with less iron, but in this case add 2 ml. of ammonium acetate solution and 3 drops of tartaric acid solution. The pH is still 3.5 to 4.0 and the concentration of ammonium thiocyanate is 24%.

INTERFERING IONS

Copper does not interfere with the production of the blue color of the cobalt complex even when it is present in amount equaling 60% of the sample.

Iron, if present in amount greater than 40% of the sample, will interfere unless ammonium acetate and tartaric acid are used.

Chromium, manganese, nickel, zinc, titanium, molybdenum, and uranium do not give colored complexes which are soluble in amyl alcohol and ether. Other common elements such as silica, aluminum, calcium, magnesium, phosphorus, bismuth, arsenic, lead, and the alkalies are without effect.

Vanadium under these conditions also forms a blue complex which is extracted by the amyl alcohol–ether solution. If, however, ammonium acetate and tartaric acid are added to the reagents this blue complex is not formed, and vanadium will not interfere with the determination of cobalt.

SENSITIVITY

The smallest amount of cobalt which can be conveniently determined by either visual or photoelectric comparison is 0.02 mg. It can be seen from the calibration graph obtained from the absorptiometer readings that the relationship between the color intensity of the cobalt complex and the cobalt content of the extracts is not linear but follows a curve of wide radius. The last three standards employed in the visual comparison are slightly more intense in color than corresponding extracts of cobalt, so that accurate determinations cannot be carried beyond 0.40 mg. of cobalt at the most. By use of an absorptiometer accurate determinations can be obtained up to 0.50 mg. of cobalt.

Using the range 0.02 to 0.50 mg. of cobalt, accurate determinations have been carried out on Nkana samples between 0.01 and 4.00% cobalt. By taking smaller weights of sample, it has been possible to estimate cobalt up to 10% with a sufficient degree of accuracy for rapid works practice.

RESULTS

A number of results of the colorimetric method, obtained by both visual and photoelectric comparisons, are given in Table II, compared with the percentages of cobalt obtained by careful gravimetric analyses.

DISCUSSION

A pH of 1.0 to 0.9 for the solution of the sample was found by experiment to be the optimum for the formation of the cobalt complex. If the pH is higher than this the color is not so strongly developed, while if it is lower it is impossible to suppress with small amounts of phosphate the color due to iron. A final pH of 3.5 to 4.0 in the solution of reagents and sample is well below the precipitation pH of cobalt phosphate, which is about 5.3.

Sodium thiosulfate has been found to be an efficient reducing agent for the iron. Other reagents such as stannous chloride and hydrogen sulfide gas have been tried out but were not found so satisfactory.

A concentration of 24 to 26% ammonium thiocyanate is sufficient to produce the maximum color intensity for amounts of cobalt ranging from 0.02 to 0.40 mg. For quantities of cobalt up to approximately 0.14 mg. a concentration of 17% ammonium thiocyanate is sufficient, but beyond this 26% concentration must be used. It is not so convenient in routine analyses to alter the concentration of a reagent according to the samples and therefore a standard concentration of 24 to 26% ammonium thiocyanate has been given in the procedure. Increasing the amount of thiocyanate beyond 26% did not further intensify the color.

The dielectric constant of amyl alcohol at 0° C. and infinite wave length is 17.4 (*3*). The dielectric constant of ethyl ether is 4.68. A mixture of these two organic liquids will then have a lower dielectric constant and greater capacity for preventing decomposition of the complex than methyl alcohol, ethyl alcohol, or acetone which have dielectric constants of 35.0, 28.4, and 26.6, respectively. A mixture of 3 parts by volume of amyl alcohol to 1 part of ether has been found to give a maximum intensity of color.

The use of ammonium acetate and tartaric acid solution makes it possible to determine cobalt on samples high in iron and vanadium. By this method small amounts of cobalt may be determined in steels and in the iron precipitates in a gravimetric analysis of cobalt.

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Most Economic Sampling for Chemical Analysis

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IN AN expository article (*3*), Mandel has outlined the application of modern statistical methods to chemical experiments. It is the purpose of this paper to acquaint the chemist with a recently developed technique of analysis of data, known as "sequential analysis", that has important application to chemical analyses. The new method is designed to enable the experimenter to reach a decision with the minimum number of observations, and is based upon (*6*) and (*7*). The interested reader will find it profitable to obtain these papers, since their application is more general than the subject matter of this paper; a more extended memorandum on the present subject has also been issued (*2*).

The problem of interest here is the following:

The experimenter, by his qualitative and elementary analysis and molecular weight determinations, together with certain theoretical considerations, has decided that the compound is one of two or more possibilities; how many determinations shall he make in order to obtain a certain degree of confidence in his analysis?

This problem is of wide application, as can be seen from the following notes of H. Gisser, Frankford Arsenal Ordnance Laboratory, who suggested the need for an exact mathematical procedure in these matters:

In the study of Diels' hydrocarbon (from selenium dehydrogenation of cholesterol) the first suggested formula was $C_{18}H_{16}$. From available analyses, Ruzicka (*5*) suggested it might be $C_{17}H_{14}$. Later work proved $C_{18}H_{16}$ correct. Elucidation of the structure of retene ($C_{18}H_{18}$) was delayed because of poor analytical results. Fieser reports that although derivatives and degradation products of retene had been prepared in pure condition, the relationship between them remained obscure until the analytical investigations of Bamberger and Hooker (*1*) cleared up the empirical formulas. The formulas previously assigned required revision. Concerning the structure of morphine, the following estimates are found in the literature: Liebig (1831) $C_{34}H_{36}O_6N_2$; Regnault (1838) $C_{35}H_{40}O_5N_2$; Laurent (1847) $C_{34}H_{38}O_6N_2$, the last corresponding to the present accepted formula $C_{17}H_{19}O_3N$. On the structure of a papaverine Merck gave (1848) $C_{70}H_{21}O_4N$; Hesse gave (1870) $C_{21}H_{21}O_4N$, and his results were confirmed by other laboratories; Merck's formula was later shown to be correct.

PRELIMINARY REMARKS

In the application of statistical methods to the problem of determining the formulas of organic compounds, the following is presupposed:

1. The formula is one of two possibilities. From these two possibilities, the chemist can calculate the expected percentage for each element (or for some derivative compound).
2. The variance has been calculated for each set of readings. As in (*1*), the estimate of the variance is given by the formula:

$$S^2 = \frac{\sum_{\alpha=1}^{N} (x_\alpha - \bar{x})^2}{N-1}$$

where Σ stands for a summation, x_α for any of the series of observations, N for the total number of observations, and $\bar{x}$ for the arithmetic mean. It is further presupposed that there has been gathered a considerable amount of data, and that these data plus the underlying chemical theory are sufficient to establish with some degree of confidence the following:

3. That the observations obey a specific normal, or Gaussian law of error.
4. That the estimate of the variance is "very nearly" exact.

No estimate can be exact at any finite stage of scientific progress; it is therefore a matter of some difficulty to specify the degree of precision that must be obtained. In general, the accuracy one should obtain before applying the procedures of this paper depends upon the fineness of the distinction that the experimenter wishes to determine. As a practical suggestion, the variances should be computed on the basis of 200 or more observations, all made essentially under the same conditions—i.e., all presupposed to have the same error. It is to be noted that the variances obtained from different experiments may be averaged, provided the errors of observation are assumed to be the same. There are to be found in the statistical literature certain tests for the homogeneity of a set of variances, to be applied when some doubt exists concerning the advisability of averaging.

These remarks might lead the chemist to disregard all exact procedures, on the grounds that he can never reach the kind of precision the mathematical statistician demands. This is an all too common mistake in the experimenter's attitude towards statistical procedures, a mistake that is sometimes encouraged by the statistician himself. One would be foolish indeed to expect that all observations are governed by exactly the same law of error, and that the errors of determination are really fixed for all observers. The conditions under which the statistician's procedures are outlined are ideal or formal, and the conditions under which the chemist operates are practical or nonformal. If the experimenter waits for the ideal to show up before using statistical procedures, he will have to wait forever. Rather, the experimenter must use his own judgment, based on his and others' extensive experience, together with the cooperation of the statistician, to decide whether or not a given procedure is feasible. To fail to apply the best procedures available, because their conditions cannot be met exactly, is as absurd as failing to use a certain machine because its cogs do not operate exactly as designed. In the sequel, we will symbolize known variance by σ^2.

PROCEDURES

In the procedure, we shall consider two cases, one in which all the information is to be obtained from one type of analysis, the other where at least two different types of analysis are made.

CASE 1. Suppose then, that the experimenter decides that the compound is one of two possibilities, X and Y, and that he selects for study, say, one of the elements A (or else the molecular weight). The two possibilities will determine values for this element A—that is, granting X as the true formula, one can calculate the expected percentage of A, say α_1, and granting Y as the true formula, one can calculate the expected percentage of A, say α_2. Let σ_α^2 be the variance of percentage determinations of A. In general, the element A which should be selected for study is that one which makes the value $(\alpha_2 - \alpha_1)^2/\sigma_\alpha^2$ the largest, for the larger this value, the fewer the expected number of determinations necessary to reach a decision. However, if A is difficult to determine, it may be desirable to sacrifice economy of sample size for economy of time and expenditure, and some other element will be selected.

The procedure for this case follows:

Let $\overset{n}{\Sigma}\alpha$ represent the sum of the α determinations up to and including the nth determination. Thus if the percentages for hydrogen were 5.02, 5.10, and 5.06 for the first three observations, we would have $\overset{1}{\Sigma}\mathrm{H} = 5.02$, $\overset{2}{\Sigma}\mathrm{H} = 10.12$, $\overset{3}{\Sigma}\mathrm{H} = 15.18$. Calculate

$$h = \frac{4.595}{\alpha_2 - \alpha_1}\sigma_\alpha^2$$

and

$$s = \frac{\alpha_2 + \alpha_1}{2}$$

where $\alpha_2 > \alpha_1$.

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Accept the hypothesis that the true percentage, α, is α_1 as soon as

$$\sum^{n} \alpha \leq sn - h \ (n = \text{number of samples})$$

Accept the hypothesis that the true percentage, α, is α_2 as soon as

$$\sum^{n} \alpha \geq sn + h$$

If $\sum^{n} \alpha$ lies between $sn - h$ and $sn + h$, take an additional observation and repeat the procedure until enough readings have been taken.

CASE 2. Consider now the case where the experimenter decides to choose more than one element to investigate. Suppose that there are two possibilities open, and independent tests have been run on A and B; possibility X requires a value α_1 for A and β_1 for B; possibility Y requires a value α_2 for A, and β_2 for B. Let n be the number of determinations on α and m the number on β. As before, let σ_α^2 be the variance for determinations of α, σ_β^2 be the variance for β determinations. The function

$$\left[\frac{\alpha_2 - \alpha_1}{\sigma_\alpha^2}\right]\sum^{n}\alpha + \left[\frac{\beta_2 - \beta_1}{\sigma_\beta^2}\right]\sum^{m}\beta - n\left[\frac{\alpha_2^2 - \alpha_1^2}{2\sigma_\alpha^2}\right] - m\left[\frac{\beta_2^2 - \beta_1^2}{2\sigma_\beta^2}\right]$$

is "critical" here; if it is less than or equal to -4.595, then accept possibility X, and if it is greater than or equal to $+4.595$, accept possibility Y. If it lies between -4.595 and $+4.595$, make another determination on α or β or both. The extension of this function to cases where more than two elements are independently investigated is obvious. Also note that the molecular weight can be included. It is important to emphasize that the values of α and β in the procedure must have been determined independently—for example, β should not be determined by subtraction of α from 100.

If the above procedures are followed, it can be shown that the expected number of determinations necessary to reach a decision is less than that of any other test procedure involving the same risks of error. It has been assumed throughout that a confidence of 0.99 is sufficient for these determinations—i.e., an error would be committed no more frequently than one time in a hundred in the long run; if greater or less confidence is desired, the procedure is only modified by replacing the number 4.595 throughout by another figure; the following table may be convenient in this connection:

Confidence Level	Value to Replace 4.595
0.999	6.907
0.98	3.892
0.95	2.944
0.90	2.197
0.80	1.386

The significance of this table is explained as follows: Every experiment is designed to enable us to choose some hypothesis about the natural world, and to reject alternative hypotheses. In the case at hand, the hypotheses are concerned with the correct chemical formula of a given compound. Now no experiment is "ideal" in the sense that one hypothesis can be selected without any possibility of error. Rather, any method we may use for choosing one hypothesis over the others on the basis of the data at hand will in the long run lead to mistaken choices. The advantage of statistical procedures in general lies in the fact that they enable us to estimate the frequency of wrong decisions for a given method, and that they propose methods that in some sense "minimize" this frequency of wrong choices. If the above procedure is used, and the value of 4.595 is employed, then the chance of accepting possibility X when possibility Y is actually true is 0.01—i.e., we go wrong in this way, in the long run, one time in a hundred. Similarly, the chance of accepting possibility Y when possibility X is actually true is 0.01. If 6.907 is used, the chances in each case are 0.001; if 3.892 is used, the chances are 0.02, etc. Now these estimates of the chance of a wrong decision depend upon the presuppositions we make, particularly the presupposition of normality of distribution, and the accuracy of the variance. This is the manner in which these initial assumptions are important; as mentioned before, we cannot ever be sure that the assumptions are true, and hence the estimates of the chances of making a mistake are only approximations, the best approximations that can be made for a given state of our knowledge.

EXAMPLE

The following is taken from (4). Two possibilities were open here:

X $C_{19}H_{12}O_6Br_4S$ (Carbinol sulfonic acid)

Y $C_{19}H_{10}O_5Br_4S$ (Lactoid)

Bromine and sulfur were selected for analysis. Hence this example falls under Case 2. The two hypotheses were

$$X\ Br_2 = 46.47;\ S_2 = 4.66$$

$$Y\ Br_1 = 47.72;\ S_1 = 4.79$$

Two determinations were made independently for each element with the following results:

Bromine 47.75, 47.70
Sulfur 4.76, 4.88

Were enough determinations made? The errors of estimate are not given, but to complete the example we suppose that the standard deviations are about 0.500%, in each case. This gives a variance of $(0.5)^2 = 0.25$. (Note. This value of the error of a single determination is supposed only for the sake of illustration; actually, the errors are probably smaller.)

We treat the problem sequentially, by considering the results of the first determinations, Br = 47.75, S = 4.76. We have

$$\sum^{1}\text{Br} = 47.75$$

$$\sum^{1}\text{S} = 4.76$$

$$n = m = 1$$

$$Br_2 - Br_1 = 1.25$$

$$(Br_2 - Br_1)/\sigma_{Br}^2 = (1.25)/0.25 = 5.0000$$

$$S_2 - S_1 = 0.13$$

$$(S_2 - S_1)/\sigma_S^2 = (0.13)/0.25 = 0.5200$$

$$Br_2^2 - Br_1^2 = 117.7375$$

$$(Br_2^2 - Br_1^2)/2\sigma_{Br}^2 = 117.7375/0.5 = 235.4750$$

$$(S_2^2 - S_1^2)/2\sigma_S^2 = 1.2285 = 2.4570$$

We have, then, as the critical function

$$5\sum^{n}\text{Br} + 0.52\sum^{m}\text{S} - 235.4750\,n - 2.4570\,m$$

(Note that in the subsequent determinations, only $\Sigma\alpha$ and $\Sigma\beta$ have to be calculated, since the values 5, 0.52, 235.475, and 2.4570 remain the same throughout.) For $n = m = 1$, the critical function is

$$5(47.75) + 0.52(4.76) - 235.4750 - 2.4570 = 3.2932$$

The value 3.2548 lies between -4.595 and $+4.595$, and hence the first pair of determinations was not sufficient, and additional determinations must be taken. For $n = m = 2$, we have

$$\sum^{2}\text{Br} = 95.45$$

$$\sum^{2}\text{S} = 9.64$$

$$n = m = 2$$

Hence the critical function is

$$5(95.45) + 0.52(9.64) - 2(235.4750) - 2(2.4570) = 6.3988$$

The value 6.3988 is greater than 4.595, and hence possibility Y (lactoid) should be accepted.

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Drill Sampling Device for Fish Livers

Precision and Accuracy

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Because of the unhomogeneous nature of fish livers, errors in vitamin A assays due to sampling may be large unless an adequate number of cores are taken. In developmental work on a drill sampling device in which over 2000 cans of soupfin shark and grayfish livers were tested, 100 cores per ordinary commercial lot were sufficient to give vitamin A assays that were reproducible within 5%. An investigation of the accuracy of the sampling showed that any bias, if present, was of small magnitude. Essentially the same analytical results were obtained whether the livers were fresh or frozen when sampled. The sampling was rapid. A hundred cores could be taken in 15 minutes or less, and the rate at which the livers were cored could be varied within wide limits without appreciably affecting precision.

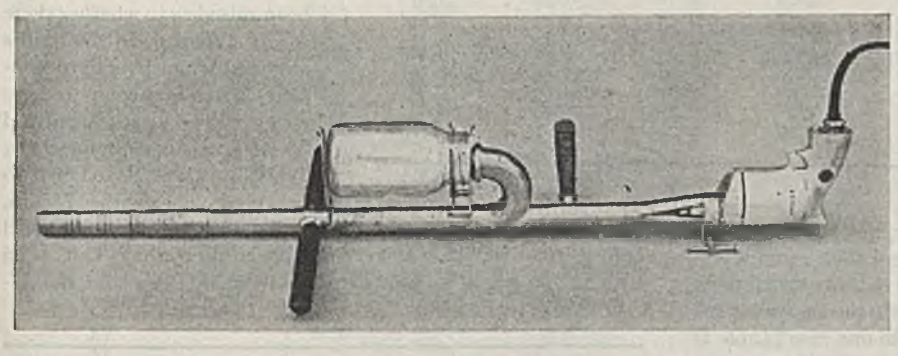

Figure 1. Motor-Driven Core Sampler

A SURPRISING feature of the fish liver industry has been the practice of "blind buying". Livers are bought from the fishermen without the benefit of a test for vitamin content. The buyer may examine the livers visually (dark-colored livers usually contain more vitamin A than light-colored livers), but since the variation in the appearance of the livers is not commensurate with the variation in their potency, visual inspection will detect only differences of large magnitude. This practice has persisted even though livers for some of the species, such as male soupfin shark, may vary in value per pound from as low as 25 cents to as high as $12 or $13. Obviously, liver trading on the basis of "blind buying" is extremely hazardous.

A primary difficulty interfering with sales on a potency basis has been the problem of sampling. While hard-frozen livers can be sampled with conventional core samplers, a method has been needed whereby the "fresh" livers could be sampled just as they came from the fishing vessel. (If the livers are not frozen, salted, or otherwise preserved, they are called "fresh" by the trade, even though they may actually be stale.) The fishermen are ordinarily at sea for several days, and trips of 3 or 4 weeks' duration are not uncommon. When fishing conditions are favorable, the fishermen are anxious to put out to sea again; understandably, they are not willing to delay long in settling their business. For this reason, the freezing of livers in preparation for sampling, while practiced extensively in sales between liver buyers and processors, has never been popular in primary sales, since a delay of several days often occurs while the livers are being frozen.

To meet the need for a rapid method of sampling "fresh" livers, this laboratory has developed a motor-driven core sampler (Figure 1) consisting of a tube within which a tightly fitting, rotating auger elevates the liver material into a sample bottle (*1*, *3*). Figure 2 shows shark livers that had just been landed in the process of being sampled by a commercial processor with a sampler fabricated in his own shop. In the course of the development of the sampler over 2000 cans of soupfin shark (*Galeorhinus zyopterus*) and grayfish (*Squalus sucklii*) livers were tested. The present paper reports the results of this work.

A previous study (*2*) of a somewhat theoretical nature showed that taking a large number of cores is required for proper sampling and that, in general, it is necessary to take at least 100 cores from an ordinary commercial lot if the precision is to be within +5%. However, conditions vary greatly. Not only are there differences of texture in the livers of various species such as soupfin shark, grayfish, ling cod, halibut, and tuna, but the texture also varies within even a single species. Some livers will be fresh and firm; others will be stale, soft, and perhaps partially decomposed. In addition, there may be other differences in the livers, attributable to seasonal or geographic causes, so that the number of variables involved in sampling is too large to allow for a rigorous determination of precision under all circumstances. Consequently, in order to test the conclusions of the earlier theoretical studies, it has been necessary to adopt an empirical procedure in the study of liver sampling and to limit these experiments to livers landed in the Pacific Northwest.

Figure 2. Motor-Driven Core Sampler in Use
Some shark livers are exposed to show nature of material

Table I. Reproducibility of Samples of Fresh Grayfish (Dogfish) Livers

(One core taken per can)

Total Cans in Lot	Sample No.	Oil Content, %	Deviation of Oil Content from Average, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency from Average, Relative %	Liver Potency, Millions of Units Vitamin A per Lb.	Deviation of Liver Potency from Average, Relative %
16	1	72.5	0.96	13,600	0.74	4.48	0.00
	2	73.1	0.14	12,600	6.67	4.18	6.70
	3	73.9	0.96	13,500	0.00	4.53	1.12
	4	73.3	0.14	14,800	9.63	4.92	9.82
	5	73.0	0.28	12,900	4.44	4.28	4.46
	Av.	73.2	0.50	13,500	4.30	4.48	4.42
27	1	74.6	1.91	17,400	6.97	5.90	5.15
	2	73.3	0.14	18,600	0.54	6.20	0.32
	3	73.1	0.14	19,100	2.14	6.34	1.93
	4	71.6	2.19	19,400	3.75	6.30	1.29
	5	73.5	0.41	19,000	1.61	6.34	1.93
	Av.	73.2	0.96	18,700	3.00	6.22	2.12
40	1	66.6	1.04	7,710	0.39	2.33	1.70
	2	68.0	1.04	7,620	1.55	2.35	0.84
	3	67.6	0.45	7,620	1.55	2.34	1.27
	4	66.9	0.60	8,110	4.78	2.46	3.80
	5	67.5	0.30	7,660	1.03	2.35	0.84
	Av.	67.3	0.69	7,740	1.86	2.37	1.69
56	1	69.4	0.73	5,220	2.97	1.65	1.78
	2	69.6	1.02	5,420	0.74	1.71	1.78
	3	67.9	1.45	5,640	4.83	1.73	2.98
	4	68.7	0.29	5,220	2.97	1.62	3.57
	Av.	68.9	0.87	5,380	2.88	1.68	2.53
135	1	70.9	0.28	14,200	0.70	4.57	0.87
	2	70.6	0.70	13,900	2.80	4.46	3.25
	3	71.8	0.98	14,300	0.00	4.66	1.08
	4	70.9	0.28	14,800	3.50	4.76	3.25
	Av.	71.1	0.56	14,300	1.75	4.61	2.11

In forming a sample for purposes of study, one core was taken from each can in the lot, and the cores were then composited. Additional samples from the lot were formed in the same manner. Where the number of cans in the lot was small, the assays tended to be somewhat divergent. However, even in one case (Table I) where the lot contained only 16 cans, giving only 16 cores per sample, the greatest deviation from the average of 5 samples from this lot did not exceed 10%. In general, as can be seen from Tables I and II, when lots containing a fairly large number of cans were sampled, assays of both "fresh" grayfish and soupfin shark livers were reproducible to within 5%.

Livers and viscera are received in so many different conditions that it is beyond the scope of this laboratory to test them under all circumstances. However, a few experiments have been performed with livers from halibut, ling cod, rock cod, and sole, as well as with halibut viscera. These lots were all soft-frozen. While it was not possible to make an extensive test of precision, the samples appeared to be representative, and no difficulties were encountered. In Table III are reported the results of sampling 35 cans of soft-frozen tuna (*Germo alalunga*) livers. These data show that the sampler, at least with frozen livers, will work well with species other than the grayfish or soupfin shark.

A question has sometimes been raised as to whether there is any difference in the results obtained when livers are sampled frozen as compared to when they are sampled "fresh". Using soupfin shark livers, this was tested in two instances: once with 17 cans, and once with 84 cans. The livers were first sampled before freezing and then resampled after they had been frozen. As can be seen in Table IV in neither case did the dif-

Table II. Precision in Sampling 100 Cans of Fresh Soupfin Shark Livers

(One core taken per can)

Sample No.	Oil Content, %	Deviation of Oil Content from Average, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency from Average, Relative %	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	Deviation of Liver Potency from Average, Relative %
1	66.4	0.91	57,100	2.56	17.3	1.71
2	67.0	1.82	56,500	3.58	17.2	2.27
3	65.5	0.46	60,800	3.75	18.1	2.84
4	64.5	1.97	59,900	2.22	17.6	0.00
Av.	65.8	1.29	58,600	3.03	17.6	1.71

Table III. Precision of Samples of 35 Cans of Soft-Frozen Tuna Livers

(One core taken per can)

Sample No.	Oil Content, %	Deviation of Oil Content from Average, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency from Average, Relative %	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	Deviation of Liver Potency from Average, Relative %
1	26.0	0.38	6,500	4.33	0.77	4.05
2	26.5	1.53	6,230	0.00	0.75	1.35
3	26.3	0.77	6,360	2.09	0.76	2.70
4	25.8	1.15	6,070	2.57	0.71	4.05
5	26.1	0.00	6,010	3.53	0.71	4.05
Av.	26.1	0.77	6,230	2.50	0.74	3.25

Table IV. Results Obtained in Sampling Fresh and Frozen Soupfin Shark Livers

Total Cans in Lot	Oil Content, %		Oil Potency, U.S.P. Units Vitamin A per Gram		Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	
	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
17	62.3	64.0	116,000	114,000	32.8	33.1
84	61.2	61.8	97,000	97,600	27.0	27.4

Table V. Accuracy of Samples of 100 Cans of Fresh Soupfin Shark Livers

(One core taken per can)

Sample No.	Oil Content, %	Deviation Oil Content of Core Sample from Ground Sample, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency of Core Sample from Ground Sample, Relative %	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	Deviation of Liver Potency of Core Sample from Ground Sample, Relative %
1	66.4	3.4	57,100	4.8	17.3	1.1
2	67.0	4.4	56,500	5.8	17.2	1.7
3	65.5	2.0	60,800	1.3	18.1	3.4
4	64.5	0.5	59,900	0.2	17.6	0.6
Ground sample	64.2		60,000		17.5	

Table VI. Accuracy of a Sample of 16 Cans of Fresh Grayfish Livers

(5 cores taken per can)

	Oil Content, %	Oil Potency, U.S.P. Units Vitamin A per Gram	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.
Ground sample	72.7	14,200	4.67
Core sample	73.2	13,500	4.48
Relative % deviation of core from ground sample	0.69	4.92	4.07

Table VII. Precision of Samples of 55 Cans of Fresh Soupfin Shark Livers Taken with Sampler

(3/8-inch and 9/16-inch augers)

Sample No.	Diameter of Auger, Inches	Cores per Can	Wt. of Sample, Lb.	Sampling Time, Min.	Oil Content, %	Deviation of Oil Content from Average, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency from Average, Relative %	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	Deviation of Liver Potency from Average, Relative %
1	3/8	1	0.79	10	63.7	0.31	74,500	3.76	21.6	3.85
2	3/8	5	3.66	43	63.1	1.25	71,400	0.55	20.5	1.44
3	9/16	1	2.95	10	64.4	0.78	70,700	1.53	20.7	0.48
4	9/16	5	7.86	36	64.2	0.47	70,500	1.81	20.5	1.44
Av.					63.9	0.70	71,800	1.91	20.8	1.80

Table VIII. Precision of Samples of Grayfish Livers

(One core per can taken by sampler with 9/16-inch auger)

Total Cans in Lot	Sample No.	Sampling Time, Min.	Oil Content, %	Deviation of Oil Content from Average, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency from Average, Relative %	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	Deviation of Liver Potency from Average, Relative %
78	1	8	70.4	0.14	12,800	0.79	4.09	1.24
	2	8	70.0	0.43	13,000	2.36	4.13	2.23
	3	10	70.6	0.43	12,300	3.15	3.94	2.48
	4	11	70.0	0.43	12,400	2.36	3.94	2.48
	5	13	70.4	0.14	12,800	0.79	4.09	1.24
Av.			70.3	0.31	12,700	1.89	4.04	1.93
105[a]	1	..	71.1	1.57	15,100	2.58	4.88	0.81
	2	..	70.2	0.29	15,400	0.65	4.91	0.20
	3	..	69.1	1.29	15,700	1.29	4.92	0.00
	4	..	69.4	0.85	15,800	1.94	4.98	1.22
Av.			70.0	1.00	15,500	1.62	4.92	0.56
255	1	6	71.0	2.16	8,350	1.53	2.70	1.12
	2	8	70.2	1.00	8,000	1.42	2.74	2.62
	3	15	68.1	2.01	8,800	3.77	2.72	1.87
	4	20	68.7	1.15	8,500	0.24	2.64	1.12
	5	23	69.3	0.29	8,150	3.89	2.57	3.75
Av.			69.5	1.32	8,480	2.17	2.67	2.10
256	1	20	61.3	0.49	9,140	2.01	2.54	1.20
	2	34	62.3	1.14	8,850	1.23	2.50	0.40
	3	45	61.3	0.49	8,890	0.78	2.48	1.20
Av.			61.6	0.71	8,960	1.34	2.51	0.93
Av.				0.84		1.75		1.38

[a] These livers were soft-frozen. Other values in table are for fresh livers.

ference in the average results obtained by sampling the livers in the two states exceed 2%.

In the study of liver sampling, the primary object had been to develop a method that would give reproducible assays of the vitamin A content. If it were later found that the absolute vitamin content was not given by the analytical method, and that the method was uniformly biased in one direction, a correction factor could be applied. In order to determine whether or not such a bias existed, the accuracy of the sampler was tested twice, once with soupfin shark and once with grayfish livers. In these tests, samples were first taken with the corer, and then the remains of the livers were ground. Three separate ground samples were taken in each case to test their homogeneity. The three ground samples from each lot gave equivalent assays well within the limits (2%) of the method of analysis. Therefore, any deviation of the assay of the core sample from that of the ground sample can be regarded as evidence of a defect in the core-sampling method.

The results of these two tests, recorded in Tables V and VI, indicate that the core samples may be slightly high as regards oil content and slightly low as regards oil potency. Further tests would have to be made to establish this point. Fortunately, these two discrepancies tend to neutralize each other in the calculation of the liver potencies, and if any bias exists, it is of small magnitude.

In many cases, considerable free oil is to be found in the containers, especially if the livers have been long removed from the fish or have been frozen and subsequently thawed. Free oil was encountered in the case of the 84 cans of soupfin shark livers recorded in Table IV. These had been frozen and thawed, and the livers were buoyed up with oil. Nevertheless, the average results given by sampling the thawed livers were the same as those obtained after they had been refrozen. The analyses reported in Table V also were made on a lot containing appreciable free oil, yet the results obtained by core sampling were not greatly different from those obtained with ground samples.

Although the experience with frozen livers is limited, sufficient work has been done to show that the sampler will be a welcome addition in this field also. Under present-day conditions, freezing facilities are difficult to obtain, and the livers are often held at temperatures too high for hard-freezing. Consequently, there has been a real need for a device which will sample livers in the soft-frozen condition. The electric drills that are equipped for holding drill shanks up to 0.25 inch in diameter generally do not have sufficient power for use in sampling soft-frozen livers. However, those electric drills that are equipped for taking 0.5-inch shanks are able to handle the greater torque encountered with the frozen material. A skilled machinist can without difficulty make this change-over from the setup recommended for sampling fresh livers.

The standard sampler, employing a 1.125-inch auger, as described by McKee, Sanford, and Bucher (*1*), was used in the above tests. Smaller augers of 0.375-inch and 0.563-inch diameter had been tried in earlier experiments (*4*), and, for the information of those who might consider use of such modified equipment, results of the trials are presented in Tables VII and VIII. Precision was satisfactory, although clogging of the tube made frequent cleaning of the auger necessary.

The results summarized in Table VIII demonstrate that wide variations in the rate of sampling do not appreciably affect precision. These data also show the extreme rapidity with which the sampling can be carried out. Ordinarily, 100 cores can be taken within 15 minutes, so that more time is used for the incidental operations of removing and replacing the can lids than for the actual sampling. The ease of use and the satisfactory results obtained have led to the adoption of the device by many firms engaged in the fish liver trade.

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Presented at the Northwest Regional Meeting, American Chemical Society, October 20, 1945.

Colorimetric Determination of *p,p'*-DDT in Technical DDT

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A colorimetric procedure for the determination of *p,p'*-DDT is based on the color produced when this compound is heated in a mixture of glacial acetic and concentrated sulfuric acids. The method is applicable to the estimation of *p,p'*-DDT in mixtures with the *o,p'*-isomer and in technical DDT when a small empirical correction is made.

OF THE several analytical procedures described for DDT (*1*) only three (*2*, *3*, *4*) are suited for analysis of *p,p'*-DDT in technical DDT and these are macroprocedures. The present method, based on the formation of a yellow color when *p,p'*-DDT is heated in a mixture of concentrated sulfuric and glacial acetic acids, provides a simple, rapid means of determining 0.05 to 0.5 mg. of this compound with an accuracy of ±1%. The procedure described has been applied to the analysis of synthetic mixtures of *p,p'*-DDT and *o,p'*-DDT (1-trichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane) and to several commercial samples of technical DDT.

It is believed that the colored product formed in this test occurs by oxidation of the DDT to the carbinol by the hot sulfuric acid. Aromatic carbinols are known to give colored solutions in sulfuric acid (*5*).

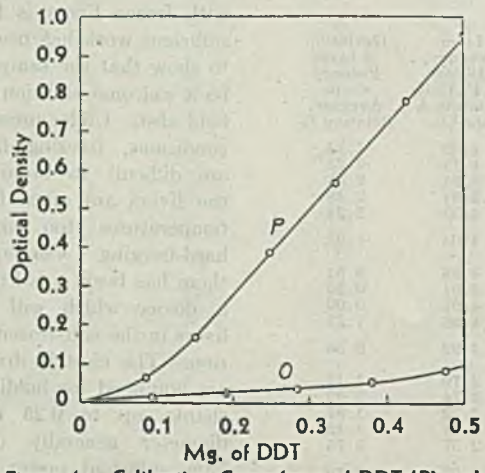

Figure 1. Calibration Curve for *p,p'*-DDT (*P*) and *o,p'*-DDT (*O*) at 435 mμ

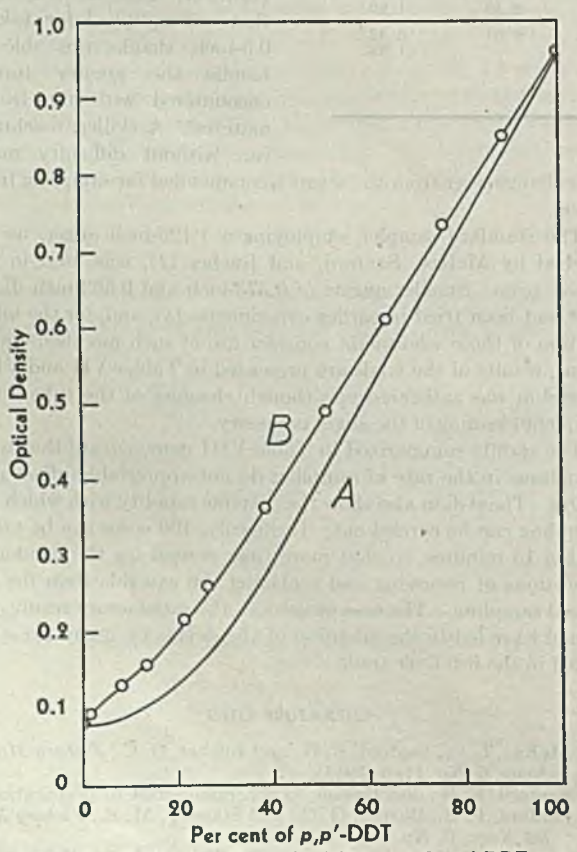

Figure 2. Calibration Curve for Mixtures of *p,p'*-DDT and *o,p'*-DDT at 435 mμ

A. Theoretical curve calculated by addition from absorption of separate solutions of *p,p'*-DDT and *o,p'*-DDT
B. Observed values

APPARATUS

The Beckman photoelectric quartz spectrophotometer, Model DU, was used with absorption cells of 1.00-cm. optical depth. Unless otherwise indicated, the measurements were made at 435 mμ using a slit width of 0.04 mm. The instrument was calibrated against colored solutions of known maximum absorption.

PROCEDURE

Weigh 0.06 gram of sample into a 250-ml. volumetric flask, dissolve, and dilute to 250 ml. with glacial acetic acid (1 ml. = ca. 0.25 mg. of sample). Using a pipet, transfer 2 ml. to a 25 ×

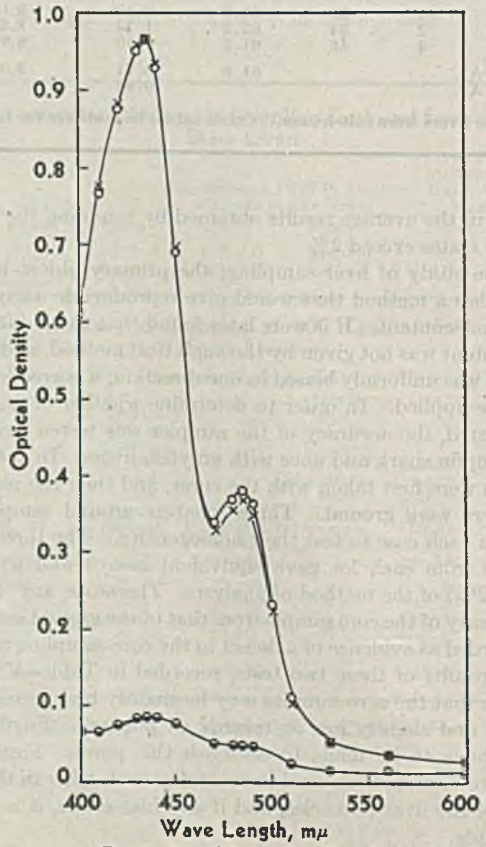

Figure 3. Absorption Spectra

O. *p,p'*-DDT, 0.500 mg. per 2 ml. of HOAc and 10 ml. of H_2SO_4
●. *o,p'*-DDT, 0.477 mg. per 2 ml. of HOAc and 10 ml. of H_2SO_4
×. Technical DDT, 0.500 mg. per 2 ml. of HOAc and 10 ml. of H_2SO_4
Density values for technical DDT multiplied by a constant

Table I. Analysis of Samples of Technical DDT

Sample No.	Melting Range	Analyzed	Optical Density Observed	Optical Density Corrected	p,p'-DDT Found
	° C.	Mg.			%
1	81–99	0.499	0.715		
		0.499	0.710		
		0.499	0.720	0.716	74.1
			Av. 0.715		
2	80–97	0.500	0.720		
		0.500	0.715		
		0.500	0.720	0.718	74.5
			Av. 0.718		
3	78–94	0.519	0.720		
		0.519	0.730		
		0.519	0.720	0.698	72.3
			Av. 0.723		

200 mm. test tube. Add 10 ml. of concentrated sulfuric acid, C.P. Mix the contents of the tube, cool to room temperature, then immerse in a vigorously boiling water bath for 10 minutes. Cool, transfer sufficient solution to a spectrophotometer cell, and determine the optical density at 435 mμ.

The concentration of glacial acetic acid is critical; 1 ml. resulted in the development of 20% more color, 3 ml. in the development of 80% less color. A stable and maximal color was obtained with p,p'-DDT when the mixture was heated for from 9 to 14 minutes. After cooling the sample this color is stable for at least one hour.

Calibration curves for p,p'-DDT and o,p'-DDT and for synthetic mixtures of the two were obtained using aliquots of standard solutions made up to 2 ml. with glacial acetic acid and carried through the procedure described above. The melting ranges of the samples of isomers used were: p,p'-DDT, 107–107.5°; o,p'-DDT, 73.5–74°.

RESULTS AND DISCUSSION

The intensity of the color produced by p,p'-DDT is much greater than with o,p'-DDT (Figure 1). For analytical purposes the influence of o,p'-DDT is, however, not negligible, since mixtures of the two isomers show greater absorption than the sum of the absorption of the separate components (Figure 2). In the analysis of mixtures it is necessary, therefore, to use the experimentally determined calibration curve.

The absorption curve of the colored solution produced by this reaction from technical DDT is similar to that of p,p'-DDT (Figure 3). In fact, it may be brought into near coincidence by multiplication by a factor throughout. This factor is not necessarily the reciprocal of p,p'-DDT concentration because of the enhancement of color by o,p'-DDT as referred to above. More accurate results are obtainable by taking this effect into account.

The procedure for estimating the p,p'-DDT content in technical DDT is, then, the following: The optical density measured by analysis of a sample of technical DDT is multiplied by the factor $\frac{0.50}{\text{mg. analyzed}}$, which gives the optical density that would be obtained if the weight of the sample analyzed were 0.50 mg. The per cent p,p'-DDT is then read from Figure 2, *B*. The error incurred in the calculation by neglecting the impurities (assumed inert) other than o,p'-DDT is well within the experimental error.

In Table I are given the results of analyses of three commercial samples of technical DDT.

ACKNOWLEDGMENT

The sample of o,p'-DDT and one sample of technical DDT were kindly supplied by Weldon G. Brown.

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THIS work was carried out under contract with the Medical Division of the Chemical Warfare Service.

Semimicrodetermination of Saponification Equivalent by Rieman's Double-Indicator Method

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A method is described for the determination of the saponification equivalent with 5- to 100-mg. samples for saponification equivalents from 100 to 1000.

WITH some modifications, Rieman's double-indicator macromethod (*4*) for the determination of saponification equivalent has been adapted to a semimicroprocedure in accordance with his suggestion that it should be more easily adapted to a microprocedure than the standard method.

The procedure of Marcali and Rieman (*3*) is limited to compounds which are readily soluble in alcohol and which can easily be saponified. From 3- to 8-mg. samples, with saponification equivalents of about 200 to 300, are saponified in 2 drops of alcoholic potassium hydroxide; in the modified method 5 ml. of 0.5 *N* potassium hydroxide are used.

The modified method is especially useful on compounds that are difficult to dissolve in ethyl alcohol. Its accuracy is within 5% of the theoretical on pure samples of esters as well as ketones that will completely open the ring in the presence of alkali (Table I).

REAGENTS

0.5 *N* potassium hydroxide (approximate) in absolute or 95% ethyl alcohol (*2*).
Ethyl alcohol.
Distilled water.
Phenolphthalein in ethyl alcohol, 1%.
0.5 *N* hydrochloric acid (approximate).
0.025 *N* hydrochloric acid (standardized).
0.010 *M* bromophenol blue [1.3 grams of bromophenol blue and 2.0 millimoles (80 mg.) of sodium hydroxide, diluted with distilled water to 200 ml.].

APPARATUS

The saponification flask may be made from a 20 × 150 mm. Pyrex test tube. The flask, graduated at 5.0 and 10.0 ml., has a 19/38 standard taper female joint.

A Liebig condenser, 15 to 20 cm. in length, is connected to the saponification flask by a 19/38 standard taper male joint.

Table I. Comparison of Results by Macro- and Semimicromethods

Compound Analyzed	Saponification Equivalent: Macro	Calculated	Semimicro (modified method)
Ethyl iodophenyl undecylate	412[a]	416	408
Linseed oil	284[a]	285 (approx.)	280
Soybean oil	275[a]	285 (approx.)	278
	275[b]		
3a,4,7,7a - Tetrahydro - 2,3,-5,6 - tetraphenyl - 4,7-methanoindene-1,8-dione[c]	460[d]	464	455
α - 1,2,4,5,6 - Pentaphenyldicyclo - (2,2,1) 5 - heptene-7-one[c]	490[d]	506	500
Ethyl acetoxy propionate[e]	160[a]	160	104
β-Acetoxy ethyl lactate[e]	179[a]	179	120

[a] Standard method.
[b] Double-indicator method (macro).
[c] Ketones.
[d] Potentiometric method.
[e] Acetylated esters.

An Ascarite tube is connected to the end of the condenser by means of a U-tube and rubber tubing.

A 25 × 150 mm. hydrometer cylinder (or test tube) is used as the titrating vessel.

A 10-ml. buret graduated in 0.05 ml. is used for the titrations.

PROCEDURE

By means of a 12-cm. weighing tube the sample is weighed by difference into the saponification flask, ±0.3 ml. of 0.5 *N* alcoholic potassium hydroxide is added up to the 5-ml. graduation mark (10 ml. may be used if the sample is too difficult to dissolve). The flask is immediately connected to the condenser, which has the Ascarite or Caroxite tube attached. The mixture is refluxed from 0.5 to 3 hours.

After saponification, the condenser is rinsed with 1 to 2 ml. of alcohol and the flask disconnected. Five drops of phenolphthalein are immediately added and the solution is slightly acidified with 0.5 *N* hydrochloric acid. The mixture, which has about 0.25 ml. of 0.5 *N* hydrochloric acid in excess, is rinsed with ethyl alcohol into a 25 × 150 mm. hydrometer cylinder (or test tube). The total volume should be about 10 to 15 ml.; 0.5 *N* potassium hydroxide is added until the solution is slightly pink. The solution is then made just colorless with an unmeasured amount of 0.025 *N* hydrochloric acid.

Three drops (0.3 ml.) of bromophenol blue are added and the resulting blue solution is titrated with the standardized 0.025 *N* hydrochloric acid until a green color appears. The first color change is taken as the end point, which is blue-green. With a little practice, this end point can be reproduced to within 0.02 ml. of 0.025 *N* hydrochloric acid.

A blank of from 0.2 to 1 ml. of 0.025 *N* hydrochloric acid should be run under identical conditions. This variation is due to the impurities in the reagents, the volume of potassium hydroxide, and the length of the reflux period. If the volume of potassium hydroxide is measured within 0.5 ml., the blanks will be constant within 0.05 ml. of 0.025 *N* hydrochloric acid. The blank is the amount of 0.025 *N* hydrochloric acid added to change the bromophenol blue solution from blue to blue-green when no soap is present.

PRECAUTIONS. The saponified solution is transferred (after acidifying) to a 25 × 150 mm. cylinder because small amounts of 0.5 *N* potassium hydroxide cling to the etched walls of the flask. If the solution were not transferred, this small amount of 0.5 *N* potassium hydroxide in the etched walls of the flask would only slowly be neutralized by the acid and would interfere in the titration of the soap with 0.025 *N* hydrochloric acid.

DISCUSSION

According to Rieman (*4*) the sharpness of the end point is increased with a decrease in the volume of the solution to be titrated. Because several organic compounds are difficult to dissolve in ethyl alcohol, the titrating volume of the modified method cannot be reduced. However, in most cases, the accuracy is sufficient to identify the compound.

Table II. Precision of Double-Indicator Semimicromethod

Compound Analyzed	Saponification Equivalent Found	Length of Reflux Period, Hours
Ethyl iodophenyl undecylate	404	1
	405	1
	410	1
	416	3
	405	3
Ethyl acetoxy propionate	100	1
	105	1
	103	3
α-1,2,4,5,6-Pentaphenyldicyclo-(2,2,1,)-5-heptene-7-one	495	3
	497	3
	508	2
	505	2
	495	3

In this laboratory, no trouble was encountered with the formation of fatty acids, probably because of the small amount of sample and the relatively large volume. The semimicromodification gives low results on acetylated esters (Table I). The precision of the method is given in Table II.

The fourth and fifth samples in Table I will be explained in a paper by C. F. H. Allen in *Journal of the American Chemical Society*.

CALCULATIONS (*1*)

Saponification equivalent = *SE*

$$SE = \frac{\text{mg. of sample}}{(\text{ml. of } 0.025\ N\ \text{HCl} - \text{ml. of HCl blank})\ (\text{exact normality of HCl})}$$

$$SE = \frac{\text{mg. of sample}}{\text{ml. of } 1\ N\ \text{KOH required to saponify}}$$

The volume of hydrochloric acid used in the calculation is the amount added between the phenolphthalein and bromophenol blue end points. It is equivalent to the volume of potassium hydroxide required to saponify the sample.

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COMMUNICATION 1058 from Kodak Research Laboratories.

NOTES ON ANALYTICAL PROCEDURES

Isopropyl Alcohol in Cotton Wax Determination

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THE Conrad technique (*2*) for determining wax in cotton fiber was found more efficient and complete, within a reasonable extraction period, than any other technique tried. This method, long used at the Southern Regional Research Laboratory employs ethyl alcohol as the extracting solvent. The substitution of isopropyl alcohol as the extracting solvent is the subject of the present report.

Isopropyl alcohol, which has many of the properties of ethyl alcohol, is plentiful, readily available, cheaper than even denatured ethyl alcohol (*1*), and free from federal tax and accompanying restrictions. Moreover, it yields identical results when substituted for ethyl alcohol in the Conrad technique. Proof that this substitution can be made without sacrificing accuracy should, therefore, increase the usefulness of this excellent analytical procedure.

EXPERIMENTAL

In the application of the Conrad technique in the following experiments, the alcohols used throughout were the purified azeotropic water-alcohol mixtures, 95% ethyl alcohol distilled from potassium hydroxide, and 91% isopropyl alcohol decanted from sodium hydroxide and then distilled. To facilitate the phase separations, 100 ml. of water were used instead of the 75 ml. recommended in the original Conrad procedure.

Wax determinations were made on a series of cotton bagging materials using 95% ethyl alcohol and then repeated, substituting 91% isopropyl alcohol as the extracting medium. The procedures were identical in all other respects.

The results (Table I) show that isopropyl alcohol extracted practically the same percentages of the waxy substances as the ethyl alcohol, the small differences observed being within the usual limits of experimental error.

Experiments in which the quantities of alcoholic extract from identical cotton fabrics were given as a function of time (Table II) showed that no difference existed between ethyl and isopropyl alcohols in extraction time. Isopropyl alcohol extracts as rapidly as ethyl alcohol and, when the same techniques are used, yields the same quantities of wax. Moreover, the general appearance of the two extracts was identical, and no differences were detected in the properties of the extracted fabrics.

Table I. Wax Values by Conrad Method

Series No.	Treatments	Wax Values: Ethyl alcohol solvent	Wax Values: Isopropyl alcohol solvent
		%	%
439	Gray goods, control	0.72	0.71
440	Kier boiled and soap washed	0.74	0.74
444	Kier boiled	0.60	0.64
445	Kier boiled, soured	0.53	0.54
446	Kiered, soured, H_2O_2 bleach	0.36	0.33
447	Kiered, soured, chlorine bleach antichlor	0.40	0.40
448	Kiered, soured, chlorine bleach, sour	0.41	0.41

Table II. Speed of Extraction of Wax from Cotton by Isopropyl and Ethyl Alcohol

Extraction Time	Wax Values: Isopropyl alcohol	Wax Values: Ethyl alcohol
Hours	%	%
2	0.83	0.79
4	0.81	0.87
6	0.85	0.84
7	0.87	0.85
8	0.86	0.85

Table III. Sugars Transferred to Chloroform by Ethyl and Isopropyl Alcohol by Distribution from Solutions Containing 1 Gram of Sugar

Sugar Taken	Isopropyl Alcohol	Ethyl Alcohol
	Mg.	*Mg.*
Sucrose	0.0	0.8
	0.1	0.8
Dextrose	0.0	0.0
	0.0	0.0
Levulose	0.0	2.4
	0.1	1.6
Blank determination	0.3	0.0
	0.0	0.0

Conrad (*2*) has pointed out that no appreciable quantities of the sugars occurring in cotton fibers, such as perhaps sucrose, dextrose, or levulose, are transferred from ethyl alcohol to the chloroform with the wax. To compare the relative quantities of sugars transferred to chloroform by ethyl alcohol and by isopropyl alcohol, duplicate 1-gram samples of each sugar were dissolved in 100-ml. quantities of isopropyl alcohol and in like quantities of ethyl alcohol in the separatory funnels, chloroform and water were added, and the determination was completed in the same way as for wax. Table III shows that isopropyl alcohol, probably because it is a better solvent for sugars than ethyl alcohol, carried all the sugars into the watery layer, allowing none to be transferred to the chloroform. In this respect, isopropyl alcohol seemed superior to ethyl alcohol as a solvent in wax determinations. The differences, however, are not significant.

Soap, when present, is determined as wax by the Conrad technique. This characteristic is not changed by substitution of isopropyl for ethyl alcohol.

CONCLUSIONS

The substitution of isopropyl for ethyl alcohol as the extracting solvent in the Conrad method for the determination of wax in cotton fiber does not alter the values obtained. Extraction with isopropyl alcohol is accomplished as rapidly and completely as with ethyl alcohol, with no apparent damage to the resulting fabrics. The general characteristics of the extracts obtained with both solvents appear to be identical; and both solvents eliminate the transfer of sugars to the isolated wax.

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Appreciation is expressed to Carl M. Conrad for helpful criticisms and advice on the work here presented.

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Preparation of Silica Gel for Chromatography

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DURING the past 2 years the authors have prepared for the chromatography of penicillin considerable quantities of silica gel. This method of preparation is a modification of that reported by Gordon, Martin, and Synge (*1*) and described in the experimental section of their paper, "Partition Chromatography in the Study of Protein Constituents".

Silica gel which is satisfactory for partition chromatography is difficult to prepare under the best of conditions. Since it is likely to be more widely used in the future, the method of preparation is reported in detail.

PROCEDURE. In a 115-liter, glass-lined tank, 34 liters of sodium silicate (Merck's sodium silicate solution, sp. gr. 1.38 to 1.40, Bé. 40° to 42°) and 10 liters of water are mixed with a high-speed stirrer (Lightnin air mixer, Type AR-25, 1750 r.p.m., 0.25 h.p., two 7.6-cm. propellers). Hydrochloric acid (10 *N*) is added very slowly (17 liters in 2.5 to 3 hours) with vigorous stirring until the mixture is permanently acid to thymol blue (pH 2.0 to 2.8). A large separatory funnel is convenient for the addition of the acid.

When about 4 liters of the hydrochloric acid have been added, a very thick, gummy mass is formed. At this point the addition of acid is stopped, and the mass is thoroughly broken up with a heavy wooden paddle. More acid is added dropwise with stirring until a thin suspension results. The rate of the addition of acid can then be increased until the desired pH is reached.

The suspension is allowed to digest at 25° C. with continuous stirring for 2 hours, then filtered by suction on a large stoneware filter (66-cm. diameter). Sharkskin paper (Carl Schleicher and Schull Co., 116 West 14th St., New York, N. Y.) plus a thick filter pad has been used for this filtration. A hard cake of very fine silica gel forms on top of the filtering material. This must be broken up in order to speed the filtering process which usually requires about 14 hours. At this point the silica gel can be aged in 0.2 *N* hydrochloric acid as described by Synge (*1*). This requires 2 days and increases the buffer-adsorbing titer of the product by approximately 20 to 40%.

In either case the silica gel is suspended in tap water, filtered, and washed until free of acid. The washed silica gel is dried at 200° C. for 12 hours and ground to a particle size of 50- to 150-mesh, using a Fitzpatrick Model D hammer mill. The ground silica gel is washed with distilled water until free of chloride (silver nitrate test) and dried in shallow pans at 250° C. for 24 to 48 hours. This time and temperature are necessary in order to obtain a product having a maximum buffer-adsorbing titer. Silica gel prepared without aging in 0.2 *N* hydrochloric acid adsorbs 85 to 100% of its weight of water without becoming lumpy or moist. For purifying penicillin the less expensive unaged product has been found satisfactory.

The final product is transferred to dry bottles while still hot and sealed to avoid moisture uptake. From 34 liters of sodium silicate solution approximately 15 kg. of 50- to 150-mesh silica gel are obtained. This is enough for the chromatography of approximately 100,000,000 units of Penicillin G.

The isolation of pure penicillins using silica gel chromatography will be reported in a separate publication.

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Determination of Small Amounts of 4-Vinyl-1-cyclohexene (Butadiene Dimer) in Tetrahydrophthalic Anhydride

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TETRAHYDROPHTHALIC anhydride (4-cyclohexene-1,2-dicarboxylic acid anhydride) is usually prepared by the Diels-Alder reaction (*1*) of 1,3-butadiene with maleic anhydride. The product, which is obtained in practically quantitative yield, is pure except for the presence of minute amounts of the dimer of butadiene, 4-vinyl-1-cyclohexene, which is formed at the temperature needed to keep the maleic anhydride liquid for ready reaction—e.g., 80° to 100° C. From the viewpoint of control and constancy of product, it was desirable to have a rapid, simple method for determining fractions of a per cent of the butadiene dimer in the tetrahydrophthalic anhydride. A solution was found in the selective extraction of the vinylcyclohexene from aqueous alkaline solution by cyclohexane and in the determination of the vinylcyclohexene in the cyclohexane extract by the determination of the extent of unsaturation.

Subsequent to the work described in this report a method for the determination of vinylcyclohexene in recycle styrene was described by Laitinen, O'Brien, and Wawzonek (*2*). The sample is dissolved in dioxane and the styrene polymerized on refluxing, using sodium as catalyst; the vinylcyclohexene left in solution is determined by the iodine chloride method. The method is sensitive to 0.2% vinylcyclohexene and is accurate to ±0.2% (absolute) over a range of 1 to 20% vinylcyclohexene. Since this method involves removal of the principal constituent by selective polymerization, it could not be applied to the determination of vinylcyclohexene in tetrahydrophthalic anhydride; in addition, the sensitivity was insufficient for the range of concentration covered.

PROCEDURE. The sample size used is governed by the amount of vinylcyclohexene believed present. For the range of 0.01 to 0.10% a 10-gram sample, weighed to the second decimal place, of product tetrahydrophthalic anhydride is added to a 250-ml. separatory funnel containing 40 ml. of aqueous 10% sodium hydroxide solution, followed by the addition of 50 ml. of cyclohexane (free of unsaturated material). After agitation and separation into layers, the lower aqueous layer is removed and the extraction of the cyclohexane fraction is repeated. Three extractions are sufficient. The extent of unsaturation of the cyclohexane extract can be satisfactorily determined by the bromide-bromate titration method of Mulliken and Wakeman (*3*). The percentage of vinylcyclohexene in the sample is readily calculated from the bromine number found, 296 being the bromine number for pure vinylcyclohexene, or from the milliequivalents of bromine consumed, 27.05 being the equivalent weight of vinylcyclohexene.

When the procedure was applied to samples of vinylcyclohexene-free tetrahydrophthalic anhydride (bromine number = 105), the cyclohexane extract had a bromine number of 0.00. Application of the procedure to small samples of vinylcyclohexene showed quantitative recovery within 10 relative per cent. Analysis of synthetic mixtures of vinylcyclohexene and tetrahydrophthalic anhydride containing 0.02 to 2.0% of the former showed similar recovery. The vinylcyclohexene used in these experiments was obtained by careful fractionation of the dimer prepared by the liquid-phase thermal dimerization of butadiene. The physical constants of the material used checked the values reported in the literature. Analysis of a typical preparation of tetrahydrophthalic anhydride indicated 0.038 ± 0.0035% vinylcyclohexene to be present.

The determination of vinylcyclohexene by halogen addition other than by the method of Mulliken and Wakeman (*3*) is discussed by Laitinen and co-workers (*2*), who found that 93 to 97% of the theoretical amount of halogen was added. The bromate method used in this report was found to give 94 ± 2% of the calculated halogen addition, which is sufficiently accurate for the low concentration range covered. Application of a correction of the type used by Laitinen and co-workers (*2*) would improve the accuracy of the results; for high concentrations of vinylcyclohexane it would be preferable to use such a correction.

It is apparent that the procedure described can be applied to the determination of vinylcyclohexene in any saturated or unsaturated substance which is not extracted by cyclohexane from alkaline or neutral aqueous solution.

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Distilling Apparatus for Production of Pure Water

FREDERIC E. HOLMES, 6515 Blueridge Ave., Cincinnati 13, Ohio

THE apparatus shown in Figure 1 was developed and used in the Clinical Laboratory at Bushnell General Hospital (Army Service Forces, Ninth Service Command, Brigham, Utah) to supply water of considerably higher purity than that ordinarily available, for preparation of gold sols, and for other special serological and chemical preparations and uses. It was not necessary to obtain water of the extreme purity required for certain physical measurements or for determining extremely minute amounts of various substances. The assembly and operation of the still had to be of utmost simplicity, for use by personnel whose interest and training were primarily in clinical methods, without diverting attention from their primary work to the still during operation to avoid entrainment, flooding, or other faults in performance or to make control tests of the distillate. The apparatus was designed to meet severe limitations of bench space and head room and of material available for construction.

The present still is a modification of one used at Wright Field (*1*).

The output of batch operation (approximately 2 liters daily) was adequate, making it possible to eliminate the reservoir and accessories for maintaining constant level of raw water in the boiling flask used at Wright Field. More elaborate accessories for control of flow of cooling water were eliminated by the "mixer", in which relatively large changes in rate of flow of cold water, obtainable by a crude valve such as a sink faucet, induce small changes in rate of flow of convection currents between mixer and condenser.

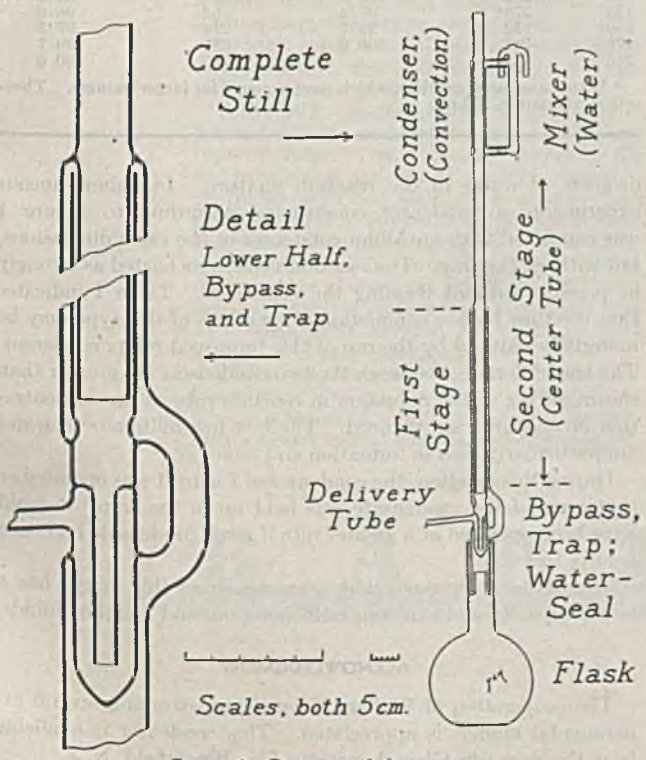

Figure 1. Diagram of Apparatus

The column is held upright by a single clamp, with its weight resting on a ring of clean cotton in the bottom of the water seal. (This type of joint was used to reduce the danger of breakage because ball-and-socket ground joints were not available.) A Meker burner is adequate for heating. Rate of boiling and condensation are adjusted to waste about one third of the water vapor through the vent at the top.

A sample submitted to the Bureau of Standards was reported to contain nonvolatile electrolyte equivalent to 0.28 p.p.m. of sodium chloride by conductivity measurement, part of which may have been dissolved from the container in transit.

The same distillation may be performed in two steps in a column assembled with standard parts. The suggested column consists of a boiling flask, Hempel distilling column packed with short pieces of glass tubing in the lower half, take-off adapter with stopcock, and Liebig reflux condenser (Ace Glass, Inc., Catalog Nos. 5910, 5245, 6615, 6902, and 5030), all connected by ball-and-socket joints. Each batch of water is first refluxed with the stopcock closed to drive off gases and other volatile solutes. The stopcock is then opened for collection of the distillate. In this column less glass surface is exposed to action of hot water, and much of the soluble material from the surface of the glass which would otherwise contaminate the distillate is extracted during the first boiling and remains with the residue in the boiling flask during the distillation.

When the trace of dissolved material from the glass can be considered negligible, the choice of columns becomes a matter of convenience and personal preference. The column used at Bushnell has some advantage in simplicity of operation. No packing is required to prevent entrainment. It is almost impossible for the apparatus to function improperly. No attention is required for change from a first to second stage of distillation, and, since both stages proceed simultaneously, only half the time is required for the same duration of reflux in the entire distillation. Such considerations may be of considerable importance in a busy laboratory where the still is easily forgotten.

The dimensions of the column are not critical, and can be estimated with sufficient accuracy by reference to Figure 1; 33-, 24-, and 19-mm. (outside diameter) tubing is satisfactory for outer (first-stage), middle (return), and inner (second-stage) tubes. A tube approximately 12 × 40 mm. is fused into the neck of a 2-liter flask to form the water seal.

ACKNOWLEDGMENT

The author wishes to thank George F. Liebig, Jr., for calling his attention to the advantages of the standard column, which is undoubtedly in common use by many chemists but not familiar to others.

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Regeneration of the Walden Silver Reductor

EUGENE H. HUFFMAN, Radiation Laboratory, University of California, Berkeley, Calif.

THE usual method for regeneration of the silver reductor is that given by the originators of the reductor (*1*). This method consists of filling the column with about 0.1 *M* sulfuric acid and allowing a zinc rod to remain in contact with the silver until the dark silver chloride has disappeared. Although effective, this method sometimes requires as much as 48 hours. When such reductors are subjected to considerable use, it may be necessary to have several of them undergoing reduction for each one in use. To avoid the necessity of having at least 12 reductors at hand, the following directions for regenerating the silver have been used in this laboratory:

Approximately 15 grams of crystalline chromic chloride are dissolved in 50 ml. of water in a 250-ml. Erlenmeyer flask and about 8 grams of granular zinc added. Concentrated hydrochloric acid is then added slowly until there is a brisk evolution of hydrogen, and more acid is added as the reaction subsides. When the reduction to chromous chloride is complete, as indicated by the blue color, the solution is poured into the reductor and allowed to percolate through the silver–silver chloride column. The solution is green for a while as it leaves the reductor. The reaction is complete when the solution comes out with a blue color. The chromous chloride solution is then rinsed from the reductor with 0.1 *M* sulfuric acid and the reductor is ready for use.

The total time required for the regeneration of a reductor which has been darkened for three quarters of the length of the silver column is 20 to 30 minutes, including the preparation of the chromous chloride solution. The reductors have the same characteristics after this treatment as after regeneration by zinc.

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New Type of Reflux Condenser

WILLARD T. SOMERVILLE, Elmhurst, N. Y.

WHEN a reflux condenser of conventional design is operated at or near its capacity, the friction between the ascending stream of vapor and the descending condensed film on the glass causes a reduction in the amount of return condensate and a holdup on the condensing surfaces. The condensed film is thickened and in a short time enough fluid is held back so that the space between the condensing walls is bridged with liquid. This results in "slugging" or "flooding", which becomes worse on the continued entrance of vapor into the condensing chamber. It is then necessary to stop the operation to permit the fluid to drain back into the reaction vessel. Often it is not desirable or possible to interrupt the refluxing, especially when exothermic reactions are encountered, to allow the trapped condensate to return. The present paper relates to a reflux condenser with increased capacity and a nonflooding feature.

The apparatus shown in Figure 1 (patent applied for) consists of a trap integrally constructed with an Allihn condenser; the upper part of the trap is cooled via the common wall. The trap has three or more openings in the floor, permitting the condensate to drain back to the reaction vessel. The path of the vapor, for the most part, is free of contact with returning condensate. When this apparatus is operated at or near capacity, the retained liquid is held in the trap and does not impair the efficiency of the condenser. If any fluid is held in the bottom of the trap, it will return to the vessel when the violence of the ebullition subsides. This condenser has been found very useful in controlling bumping and foaming liquids, since they are thrown against the side wall and can drain back.

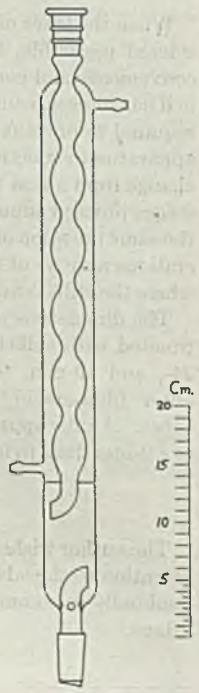

Figure 1

In Table I, data are given on an esterification using mineral acid as catalyst, and benzene as the azeotropic water-entraining substance. This reaction proceeded at a rate faster than the water could be removed, resulting in the formation of numerous large droplets of water in the reaction mixture. In a simultaneous experiment, a condenser constructed according to Figure 1 was compared with an Allihn condenser of the same dimensions, but without the trap. The reaction vessel was heated as strongly as possible without flooding the condenser. Table I indicates that the time for the completion of reactions of this type may be materially reduced by the use of this improved reflux condenser. The true differences between the two condensers are greater than shown, owing to the reduction in reaction rate as the concentration of reactants are reduced. The last few milliliters of water are particularly slow in formation and removal.

Table I. Esterification of Phenylacetic Acid with *n*-Pentanol

(Reaction mixture, 15 moles of acid, 20 moles of alcohol, 500 ml. of benzene, 5 ml. of H_2SO_4)

	Condenser of Figure 1		Allihn Condenser of Same Dimensions	
Time	Water in moisture trap used	Reaction water collected	Water in moisture trap used	Reaction water collected
Min.	*Ml.*	%	*Ml.*	%
0	0	0.0	0	0.0
15	35	12.4	32	10.8
25	48	17.0	42	14.9
45	96	33.9	84	29.8
55	124	44.8	108	38.3
70	151	53.3	131	46.4
90	214	75.6	186	66.9
105	259	91.5	229	81.3
135	276[a]	97.4	268	95.0
150	282	99.7	274[a]	97.2
175	283	100.0	281	99.7
215	...	...	282	100.0

[a] Water measured was hot, which may account for larger volume. Theoretical amount is 270 ml.

During this reaction, the condenser of Figure 1 was operated so that none of the condensate was held up in the trap; it could have been operated at a greater rate if some condensate had been held in this trap.

The above data show that a condenser of this design has a larger capacity and can cope with bumping and foaming liquids.

ACKNOWLEDGMENT

The cooperation of William Geyer in constructing several experimental models is appreciated. This condenser is available from the Scientific Glass Apparatus Co., Bloomfield, N. J.

Current Developments in

INSTRUMENTATION IN ANALYSIS

Discussed by Ralph H. Müller

The use of manometers, McLeod gages, Pirani gages, and ionization manometers is a familiar story. The ionization gage is among the most sensitive with practically no lower limit of sensitivity. It has been subject to the possibility of burnout, or the diminution of electron emission in the presence of reactive gases.

The recent development of the Alphatron vacuum gage by the Vacuum Engineering Division of the National Research Corp. of Boston supplies a new means of measuring partial vacua over the range of 1 micron to 10 mm., with special provisions for extending this to atmospheric pressure for leak tests and the like. The advantages of the Alphatron have been more evident in vacuum dehydration, distillation, electronic and refrigeration operations, but its principle of operation and the interesting phenomena which are involved seem to hold much of interest for the analyst.

The complete Alphatron gage is shown in Figure 1. The control box contains the power supply, amplifier, and meter calibrated directly in dry air pressure. The controls include an off-warm up-on switch, a three-step range selector, a zero adjust control, and a zero set button. The gage proper is shown at the right of Figure 1. Its lower end is fitted with a 0.75-inch I.P.S. vacuum connection. Above this is the ionization chamber. The top section of the unit contains the first amplifier stage. A 10-foot, 10-conductor shielded cable connects the gage to the control box.

The construction of the gage and its method of operation are evident from Figure 2. The ionizing source, *A*, consists of not more than 275 micrograms of radium sealed by a special foil to prevent the loss of radon. Alpha-particles from this source produce extensive ionization of the gas within the gage and the positive gas ions are collected by the grid, *B*. A potential of less than 50 volts is maintained between the grid and collector plate, *C*, and careful measurements have shown that this is sufficient to produce saturation ionization currents without introducing secondary ionization by electron impact. The inner dimensions of the gage are such that all alpha-particles reach the collector plate; consequently, the ionization produced by the alpha-particles is confined to that portion of the Bragg curve preceding the maximum.

The ionization currents are of the order of 2×10^{-10} ampere per mm. of air pressure. The amplifier is thus required to measure currents between 2×10^{-13} and 2×10^{-9} ampere. The pre-amplifier is shown in Figure 3; the large terminal pillars connect it to the ionization gage when it is inserted in the gage. A detailed description of the amplifier is given by Mellen in *Electronics* for April, 1946. It is a D.C. amplifier with a high degree of inverse feedback, so that the gain is largely independent of variation in tube characteristics or supply voltages.

Figure 1

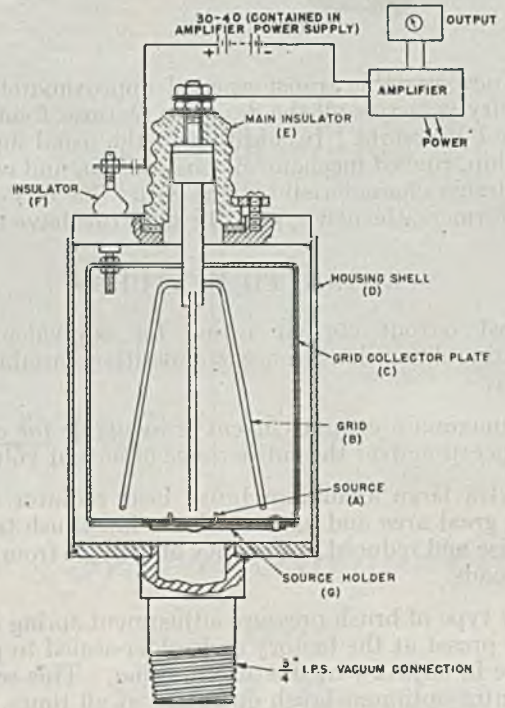

Figure 2

The response of the gage is linear over more than four orders of magnitude in pressure. Typical calibration curves are shown in Figure 4.

The relative response to various gases, taking dry air as unity, is given as: helium 0.23, hydrogen 0.26, neon 0.50, water vapor 0.88, air 1.00, argon 1.2, carbon dioxide 1.5, acetone vapor 2.8. The uncertainty in these factors is given as ± 0.02 with the exception of argon (± 0.1) and acetone (± 0.2). The manufacturers have submitted a paper to the *R.S.I.* describing the general properties of these gages, including a discussion of the computation of relative response factors.

The Alphatron may be used for the measurement of simple binary mixtures of known gases as shown in Figure 4. If the total pressure is known from an independent measurement, the composition may be determined. From its general ruggedness and freedom from the danger of burnout this gage would seem to be very useful in the analytical laboratory.

Nuclear Physics and Analytical Chemistry

In a previous column we predicted that the applications of nuclear physics to analysis would not be restricted to the familiar tracer techniques. While there is, no doubt, much of interest already known in this connection and we await governmental whim or edict for its release, there does seem to be a means for investigating some interesting possibilities. The Alphatron is a readily available means of undertaking one of those studies, aside from its more specific purpose of measuring pressure. Careful examination of the literature reveals that there is still much to be

Instrumentation

Figure 3

learned about the mechanism of ionization in gases by alpha-particles. The phenomenon is extremely complex, although some general relationships have been established.

The relative stopping power can be computed from Bragg's rule—i.e., that it is proportional to the square root of the atomic weight, and for molecules it may be estimated in the form of: $S = N_1\sqrt{A_1} + N_2\sqrt{A_2} + \ldots$, where the N's and A's represent number of atoms and respective atomic weights. The total ionization can be measured over the entire range of the alpha-particle and if this quantity is multiplied by the stopping power, one then has a value for the molecular specific ionization. One is not restricted to the use of a computed value of S; it can be measured very accurately. Nevertheless, the results indicate the presence of other complicating factors. For one thing, a large part of the ionization is due to secondary electrons, the so-called delta-rays. These secondary acts of ionization are easily seen in cloud-track photographs, especially at low pressures. The individual values of ionization potentials undoubtedly control the relative amounts of ionization which are produced

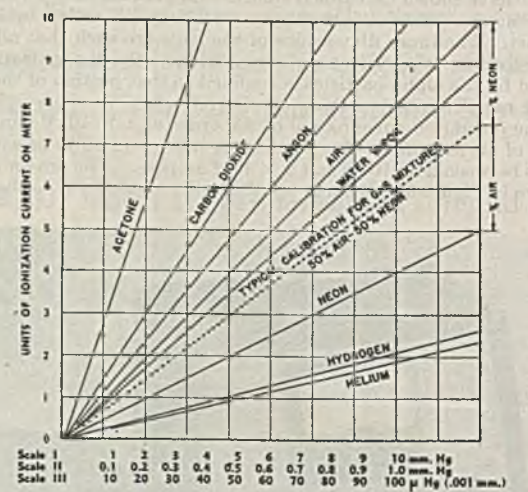

Figure 4

To all these factors must be added dissociation energies, for there is good evidence to indicate that some of the energy is expended in disruption of the molecule. We have found that the data given for the Alphatron are fairly well accounted for if the ionization is assumed to be proportional to the stopping power, computed from the Bragg rule, and inversely proportional to the ionization potential. For those cases in which the latter quantity is not certain, the reverse calculation leads to reasonable values for the ionization potential.

We hope to see a revival of interest in this problem. New materials and techniques are available. The phenomena are highly interesting and may have some practical uses in analysis.

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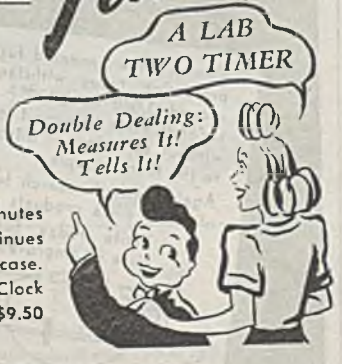

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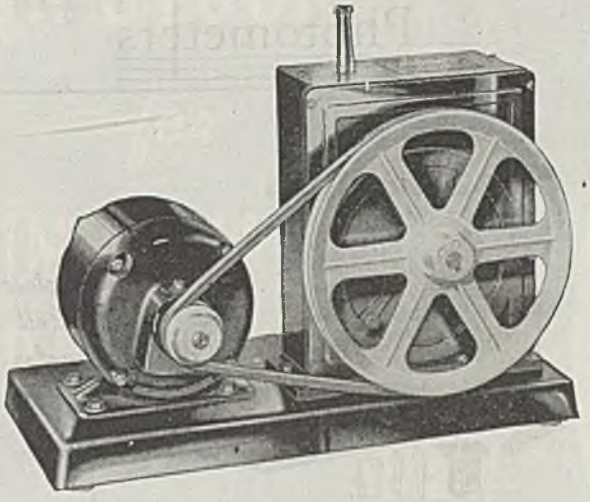

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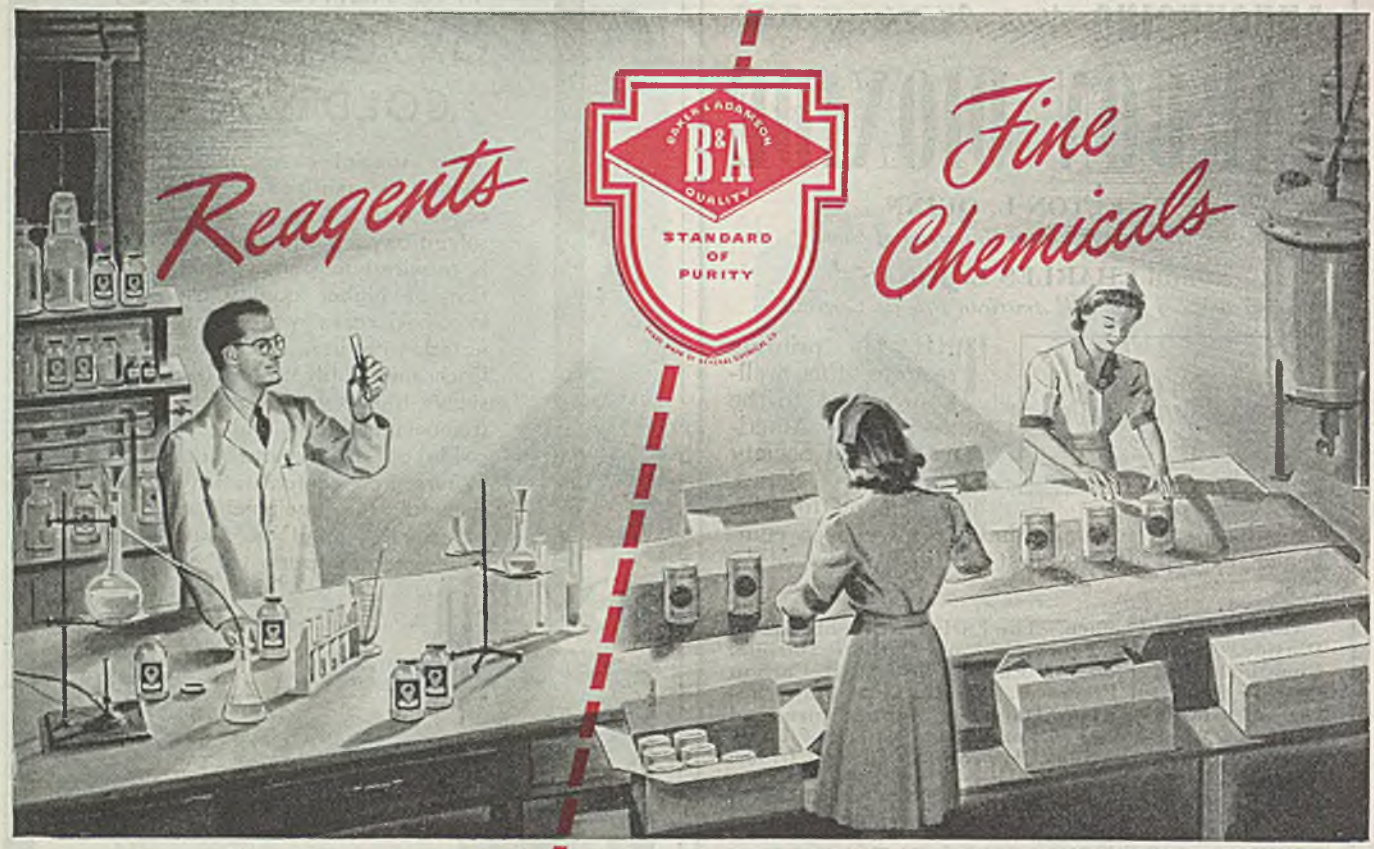

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