THE ANALYST

A Monthly Publication dealing with all branches of Analytical Chemistry: the Journal of the Society of Public Analysts and Other Analytical Chemists

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Published for the Society by
W. HEFFER & SONS, LTD., CAMBRIDGE, ENGLAND

Volume 75
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No. 897, Pages 633-694
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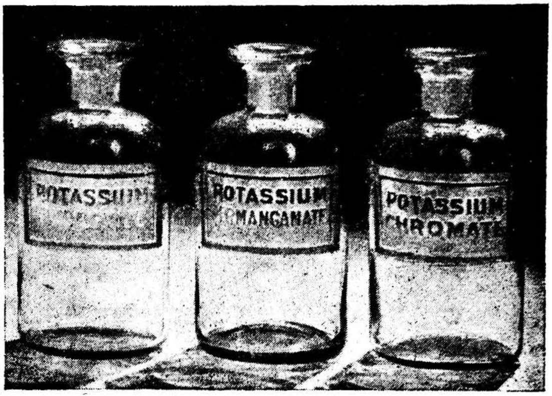
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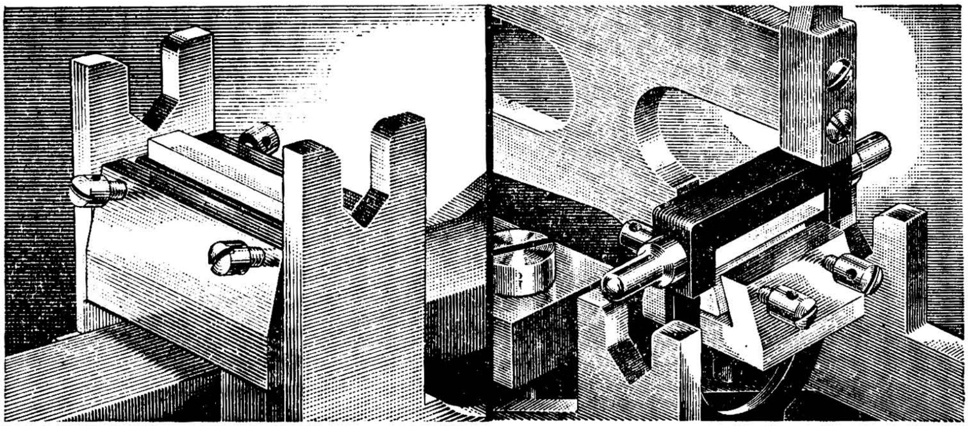

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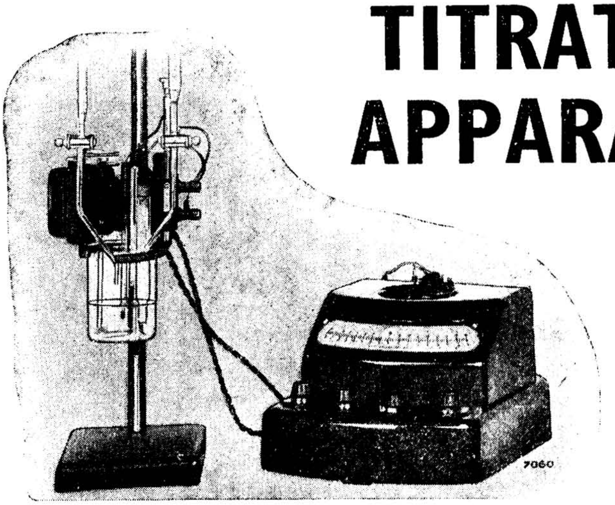

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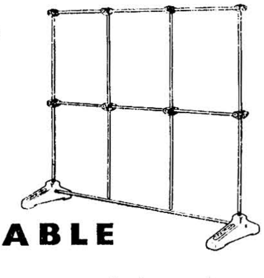

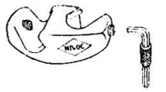

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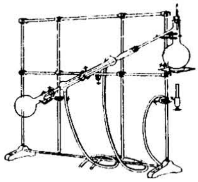

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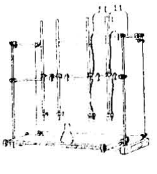

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, October 4th, 1950, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The chair was taken by the President, Mr. George Taylor, O.B.E., F.R.I.C.

The following papers were presented and discussed: "The Evaluation of Liming Materials for Agricultural Purposes," by A. M. Smith, Ph.D., D.Sc., F.R.I.C., A. Comrie, B.Sc., A.R.I.C., and K. Simpson, B.Sc., A.R.I.C.; "The Accurate Determination of 'Phosphoric Anhydride' by Means of Quinoline Phosphomolybdate," by H. N. Wilson, F.R.I.C.; "The Determination of Potassium in Fertilisers by Flame Photometry," by L. Brealey, B.Sc.

NEW MEMBERS

Alec Harold Adams, B.Sc., M.Sc. (Lond.), A.R.I.C.; James Bernard Attrill, B.A. (Cantab.); Douglas Bryan, B.Sc. (Notts.); William Edwin John Field, B.Sc. (Lond.), F.R.I.C.; Albert William Harrington; Gordon Hopkins, B.Sc., M.Sc. (Lond.), A.R.I.C.; Roy James MacWalter, B.Sc., Ph.D. (Lond.), F.R.I.C., A.M.I.Chem.E.; Peter Morries, B.Sc. (Birm.); Donald Pickles, B.Sc. (Lond.), A.R.I.C.; Kenneth Albert Proctor, B.Sc. (Notts.); K. R. Srinivasan, M.A., F.R.I.C.; Robert Guy Stuart, B.Sc. (Lond.), F.R.I.C.; Robert Arthur Sutton, B.Sc. (Liv.), A.R.I.C.; William Leon David Diaper, B.Sc.Tech. (Manc.); James Robert Embleton, A.R.I.C.; John Brian Dudley Robinson, B.Sc. (Reading).

DEATHS

We regret to record the deaths of

George Mason Hills
Charles Arthur Hallas.

Theoretical and Practical Considerations in the Determination of Aneurine (Vitamin B_1) with Special Reference to the Recovery Factor

By H. N. RIDYARD

SYNOPSIS—An analysis is made of the factors affecting the "recovery" of aneurine added to extracts in the determination of this substance. The effects of variation of sample and errors in volume measurements are shown to be considerable. The combination of aneurine during digestion, the possibility of effects at the oxidation stage, and interference with the emission of fluorescent light in the fluorimeter are considered. Results obtained in practice are given to illustrate the arguments and an investigation of the effects in the fluorimeter cell is included in some detail. The losses that take place in base-exchange methods are mentioned. The remarkable stability of the thiochrome method is shown both by the expressions developed theoretically and by the practical results.

Some of the considerations raised may have application to other estimations that involve similar raw materials and methods.

THE addition of pure aneurine to extracts prepared for the fluorimetric determination of this substance has frequently been used as a criterion of the efficiency of the analytical procedure.[1,2,3,4,5]

For this purpose, two similar extracts of the same sample, to one of which a known amount of aneurine has been added, are submitted to the same analytical procedure. The amount of aneurine found in the extract to which no addition has been made is then subtracted from the amount found in the other portion and the difference, "aneurine recovered," is divided by the amount of aneurine added, to give the "recovery factor."

The added aneurine has commonly been assumed to be subject to the same losses as that already present in the material under examination, and it is this assumption and the accuracy of the method in relation to the physical and chemical factors involved that form the subject of the present investigation.

Recovery experiments of this kind are commonly used for checking various analytical procedures and are often of great value, but examination of their theoretical bases appears to have been neglected, although their complete adequacy has been questioned.[6]

When recovery experiments are applied to a complex system such as we have in the fluorimetric estimation of aneurine they have definite limitations and conceal sources of error, which, if overlooked, can lead to fallacious conclusions. Some of the considerations brought forward in this paper are applicable to a wide variety of recovery experiments, others are specific to the estimation of aneurine. Furthermore, although additions are commonly used as a check on analytical procedure, in the estimation of aneurine the "recovery factor" has sometimes been used to correct the amount found—a procedure much more open to question.

It appeared therefore that an analysis of the various factors involved would be of value to workers in this and similar fields, especially as the remarkable stability of the thiochrome method becomes more understandable in the course of the investigation.

The fluorimetric determination of aneurine may be considered in four successive stages—

I. Extraction of the aneurine from the raw material either by steeping in acid water or by heating with dilute acid to boiling-point, adjusting to pH 4·5 and digesting with takadiastase or other source of phosphatase according as aneurine pyrophosphate (cocarboxylase) is absent or present. The extract is sometimes passed over base-exchange materials.

II. Oxidation of the aneurine to thiochrome with alkaline ferricyanide, a process said to be only about 70 per cent. efficient.[7]

III. Extraction of the thiochrome by means of *iso*butyl alcohol.

IV. Fluorimetric examination of this extract.

I. FACTORS INVOLVED AT THE EXTRACTION STAGE

I *a*. THE MODE OF ADDITION AND THE ERRORS CONCERNED IN THIS—

Aneurine can be added to extracts in two ways—

I *a* 1. A definite weight of material can be extracted with acid water or digested in a buffer solution as is necessary, and a similar weight extracted in the same manner with a precisely similar fluid containing a definite amount of aneurine.

I *a* 2. An extract can be prepared, centrifuged, filtered or otherwise clarified, and divided into two or more portions, to one of which is added a solution of aneurine.

It will be seen that any factor, chance or otherwise, which affects the two extracts or portions of extracts in a different manner may appear as a considerably increased error in the recovery factor owing to the method of calculation.

I *a* 1. In the first method, the homogeneity of the sample is obviously a factor in the type of result obtained, and many materials remain heterogeneous in spite of careful mixing, or may indeed segregate in the process of mixing. In extracts of samples of imported flour, enriched by synthetic aneurine, a small proportion showed deviations from the mean B_1 content which were as high as 0·05 μg. per ml. or even more, and this condition was not improved by further mixing of the flour. If only one extract without aneurine and one with were taken, such deviations would frequently act in opposition. If the mean quantity of aneurine in the flour extract should be 0·6 μg. per ml. (a normal level) the level without addition could be 0·55 or 0·65 and that with (say) 0·5 μg. per ml. of added aneurine could be 1·15 or 1·05 μg. per ml. The first pair of results would give a recovery of 0·6/0·5 = 120 per cent. and the second pair 0·4/0·5 = 80 per cent. For samples less homogeneous, the difficulties of obtaining similar fractions and exact weights of material increase, and the chance of error is increased accordingly.[8]

The question of volumes is of less importance than it is in method I *a* 2, for the added aneurine is measured as a standard solution and no error in concentration is involved; even a cylinder should measure 50 ml. to within ±0·25 ml. Treated as above this could give recoveries of 101 or 99 per cent.

The remaining error to be considered at this point is that of analysis. In good work the deviation from the mean value is less than 0·02 μg. per ml. For the great majority of samples it rarely reaches 0·03 μg. per ml. and is of the same order in solutions of any concentration between 0 and 1·0 μg. per ml. If with a solution containing 0·6 μg. per ml., the greatest error given above operated in opposite ways at the levels with and without an addition of 0·5 μg. per ml., the results could be 0·57 or 0·63 without addition, and 1·13 or 1·07 with addition, which lead to recoveries of 0·56/0·50 = 112 per cent. and 0·44/0·50 = 88 per cent. Both percentage errors will increase rapidly as the quantity of added aneurine decreases.

In rare and extreme cases these factors could all operate the same way to give recoveries of 120 × 101 × 112 = 136 per cent. or 80 × 99 × 88 = 70 per cent.

These considerations show that to rely upon two extracts only (one with and one without addition) is useless. Nevertheless, because of the accuracy of the volume factor the method has commonly been used in this laboratory when recovery data were desired. The precaution has always been taken of using at least five extracts in all: two without additions, one with a moderate addition and two with larger additions, the results being plotted on squared paper.[8] If any point diverged seriously from a straight line drawn through the points, the matter was further investigated.

I *a* 2. In the second procedure an extract is prepared, divided into two or more portions and aneurine added to one or more of these. This method is at once subject to the criticism that the extraction stage remains unchecked, although valuable information can obviously be obtained. The mode of addition will be the critical factor. The aneurine must be dissolved in the same buffer solution or dilute acid as is used in the extraction (see Table VI below). The manner of volume measurement is important. One mode of addition known to have been used was to add very small volumes of a strong aneurine solution from a graduated pipette and to neglect the increase in volume of the extract. Two different 1-ml. graduated pipettes were found to deliver 0·1 ml. with a standard deviation of 0·002 ml. for six successive deliveries from each, and of 0·008 ml. for six separate deliveries each; the maximum error being 0·011 ml. and the first deliveries always high. A 10 per cent. error in the recovery factor can thus easily be made, and the errors due to determination will still be present to affect the result. As the volume error decreases with better technique or larger volumes, it is usually preferable to add several ml. at least of aneurine solution, with care to add the same volume of acid water or buffer to the unfortified levels.

The question of blank determination (fluorescence of a solution treated with caustic soda but not ferricyanide) must also be considered. This is advisedly determined in extracts with and without additions, and is, subject to the usual error of determination, normally the same in both. But if their independent blanks are subtracted before calculating the recovery factor, the blank errors could be added to those given above; hence in the determination of the recovery factor the aneurine values obtained should be subtracted from one another before subtraction of the blank for determination of the aneurine level in the solution with no addition. The blank may of course be partly oxidised when ferricyanide is added.[9,10]

I *b*. COMBINATION OF ADDED ANEURINE DURING DIGESTION—

One other factor must be considered at this stage when digestion processes are carried out during extraction of the raw material. Enzymic digestion sets up a state of equilibrium between free and combined forms of aneurine. Aneurine is considered to be phosphorylated by adenosine triphosphate[11]; the aneurine pyrophosphate so formed combining with a protein to form carboxylase. In the following treatment no particular mechanism is assumed, but the equilibrium states of one reaction involving aneurine and of three successive reactions, are considered in respect of the effect of adding aneurine to the system.

I *b* 1. ONE EQUILIBRIUM—

$$\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{C}$$

where A and C are free and combined forms of aneurine respectively. If now a is the total concentration of A free and combined, b the concentration of B free and combined and y the amount of C formed, we have at equilibrium

$$\frac{(a-y)(b-y)}{y} = \mathrm{K}' \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (\mathrm{I}\, b\, 1.1)$$

Hence, neglecting the term y^2 as very small indeed (since a is of the order 10^{-6} g. mol. per litre)

$$y = \frac{ab}{\mathrm{K}' + a + b} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (\mathrm{I}\, b\, 1.2)$$

From (I *b* 1.2) we see that for y to be just detectable (*i.e.*, $= 10^{-8}$ g. mol. per litre), if $a = 10^{-6}$ g. mol. per litre then $\frac{b}{\mathrm{K}'} \nless 10^{-2}$. The free aneurine measured by the thiochrome method will be $a - y = a\left(1 - \frac{b}{\mathrm{K}' + a + b}\right)$. If now we add aneurine of a concentration a' to the solution, the free aneurine measured will be $(a + a')\left(1 - \frac{b}{\mathrm{K}' + a + a' + b}\right)$. The recovery factor determined will be the difference between these two observed values divided by a', *i.e.*,

$$\text{Recovery factor} = 1 - \frac{(a + a')b}{a'(\mathrm{K}' + a + a' + b)} + \frac{ab}{a'(\mathrm{K}' + a + b)} \quad (\mathrm{I}\, b\, 1.3)$$

Since a and a' are of the order 10^{-6} the denominators of the two fractions in (I *b* 1.3) are virtually the same unless both K′ and b are less than 10^{-4}, and the expression becomes $1 - \frac{b}{\mathrm{K}' + b}$ and is independent of aneurine concentration.

I *b* 2. THREE EQUILIBRIA—

$$\mathrm{D} + \mathrm{E} \rightleftharpoons \mathrm{B} \qquad \mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{C} + \mathrm{F} \qquad \mathrm{C} + \mathrm{H} \rightleftharpoons \mathrm{M}$$

Let d, e, a, f, h, be the initial total concentrations of D, E, A, F, H, and at equilibrium let x of D and E be transferred, y of A, and z of H; K_1, K_2, K_3, being the (reciprocal) equilibrium constants of the three reactions. Solving for x and z in the equilibrium equations of the first and third reactions (rejecting terms involving second powers of x, y or z) and substituting in the second equation, we find that—

$$y = \frac{ade(K_3 + h)}{K_3K_2f(K_1 + d + e) + (K_3 + h)(ad + ae + de) - ade} \quad \ldots \quad \ldots \quad (\text{I } b\ 2.1)$$

The first and third reactions have a buffering effect, and from considerations similar to those advanced above in the case of one equilibrium it seems unlikely that the recovery factor will be noticeably affected by aneurine concentrations, although the possibility must be borne in mind.

Although these theoretical aspects have been considered for several years, it is only recently that practical results have been obtained which indicate that added aneurine may be combined to a marked extent at the digestion stage in the case of two or three materials. As these observations were made during investigations with a new adaptation of the sand absorption method,[9] which will be described in a forthcoming paper, and very little work has yet been done on the phenomenon, further comment is not possible here.

I *c*. BASE-EXCHANGE PURIFICATION AND RECOVERY—

The use of zeolitic materials to purify extracts needs special mention. These usually lessen the optical interferences (see Table VII, Eluates), but may lead to losses by irreversible adsorption of aneurine, displacement by other basic material (organic or inorganic) in the extracts used, or inadequate adsorptive capacity. Irreversible adsorption, always found with Decalso,[9] might be proportional to the weight of zeolite used, the concentration of aneurine, the time of contact, or to all three. Hence it cannot be assumed that recovery is the same at the levels of addition and no addition. One example has been met with in which a 96 to 100 per cent. recovery was obtained with a lower analysis value than that obtained by another method giving a similar recovery. This might have been due to a chance combination of factors mentioned above, or to a genuine separation of aneurine from another substance fluorescing after oxidation. Safeguards lie in recovery experiments at more than one level of extract concentration and total aneurine - zeolite ratio and by comparison with other methods (see also III and IV, wheats, below).

II. ERRORS AT THE OXIDATION STAGE

Little special information has been obtained concerning any factor affecting recovery at the oxidation stage, and, with the possible exception of a few materials which have given serious and complicated difficulties in other ways, there is no experimental reason to suspect differences in the proportion of aneurine oxidised to thiochrome in solutions with and without addition, or indeed that the recovery at this stage is less than with pure aneurine solutions.

The oxidation stage with pure aneurine solutions probably includes not only the main reaction, aneurine → thiochrome, but also a side reaction.

If complex extracts introduce a substance that can combine with the aneurine during the oxidation process, the three reactions might be represented thus—

$A \xrightarrow{K_1} B$	$A \xrightarrow{K_2} C$	$A + D \xrightarrow{K_3} E$
aneurine → thiochrome	side reaction	

Let the initial concentrations of A and D be a and d respectively, and let the amounts of B, C and E formed at time t be p, q and r respectively, and let $p + q = y$.

Then

$$\frac{dp}{dt} + \frac{dq}{dt} = \frac{dy}{dt} = (K_1 + K_2)(a - y - r) \quad \ldots \quad \ldots \quad \ldots \quad (\text{II } 1)$$

$$\frac{dr}{dt} = K_3(a - y - r)(d - r) \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (\text{II } 2)$$

Hence $\dfrac{dr}{dy} = \dfrac{K_3}{K_1 + K_2}(d - r)$ and $r = d\left(1 - e^{-\frac{K_3}{K_1 + K_2}y}\right)$ (compare R. Wegescheider[12]).

This value of r is expanded in series, terms involving y to powers greater than 1 being discarded (since y is of the order 10^{-6}), and substituted in (II 1). Hence, on integration, transferring to the exponential form and expanding, we find that—

$$y = p + q = a\left\{(\mathrm{K}_1 + \mathrm{K}_2)t - \tfrac{1}{2}(\mathrm{K}_1 + \mathrm{K}_2)^2 t^2\left(1 + d\,\frac{\mathrm{K}_3}{\mathrm{K}_1 + \mathrm{K}_2}\right)\right.$$
$$\left. + \tfrac{1}{6}(\mathrm{K}_1 + \mathrm{K}_2)^3 t^3\left(1 + d\,\frac{\mathrm{K}_3}{\mathrm{K}_1 + \mathrm{K}_2}\right)^2 - \ldots\right\} \quad .. \qquad \text{(II 3)}$$

The stability of this oxidation reaction, in spite of all kinds of foreign substances in the various extracts used, is well expressed by this equation, since p and q will not vary appreciably from their values with pure solutions unless $d\mathrm{K}_3/(\mathrm{K}_1 + \mathrm{K}_2)$ is comparable with 1, *i.e.*, either d must be of the order $a \times 10^4$, or, if d is of the same order as a, K_3 must be of the order $(\mathrm{K}_1 + \mathrm{K}_2)10^4$.

III. THE *iso*BUTANOL EXTRACTION STAGE

In *iso*butanol extraction, interferences are less likely, with the important exception of the effect of high concentrations of dissolved materials in the aqueous solution changing the proportion of aneurine passing to the *iso*butanol layer. This is most likely to occur when using base-exchange methods with eluates containing high concentrations of a salt, for changes in the concentration of the salt may lead to serious errors.[9,13]

IV. FACTORS INVOLVED IN THE FLUORIMETRIC MEASUREMENTS

Although it has been upon this stage that attention has been concentrated in published references to recovery, the somewhat complicated process has not been adequately treated.

Fluorescence is considered to be due to a molecule of the fluorescing substance absorbing a quantum of light of short wavelength and correspondingly high energy content, and subsequently emitting a quantum of longer wavelength and lower energy content; the difference in energy due to the change in wavelength being dissipated as heat.

Three factors can interfere with this simple relationship between absorbed and emitted light—

IV *a*. Short wavelength light may be absorbed by some substance other than that fluorescing.

IV *b*. The molecule of fluorescent substance may lose its acquired energy by collision with other molecules, either of the same substance, or other substances. The latter process is termed "quenching," but in the literature concerned with aneurine estimation the term appears to be used loosely to cover all forms of loss of output of fluorescent light.

IV *c*. The fluorescent light may be absorbed before it leaves the solution.

IV *a*. ABSORPTION OF SHORT WAVELENGTH LIGHT IN THE CELL BY SUBSTANCES OTHER THAN THIOCHROME—

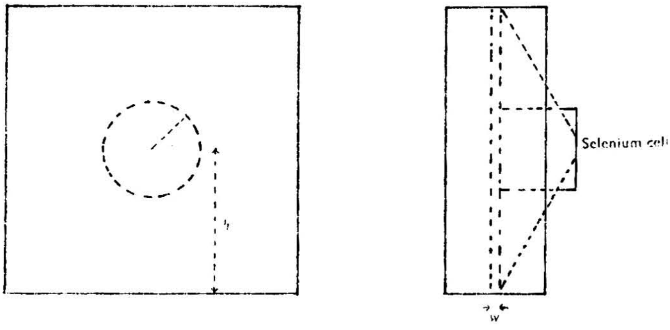
In view of the complex nature of the extracts used for aneurine estimation, it is obvious that this effect is likely to be present in the fluorimeter.[3] Now the addition of aneurine to an extract will ultimately involve a change in the total light absorbed in the cell, of which the light absorbed by the thiochrome is only a part. As the light absorbed varies logarithmically as compared with the concentrations of the absorbing substances and since, further, in dilute solution the fluorescence is proportional to the light absorbed by the thiochrome and is measured at some distance up the cell, it can be seen that the results of changing the concentration of the aneurine are not simple. A detailed examination of the light absorption in the cell, and a determination of the extinction coefficients concerned, was made in order to investigate the matter.

In the Spekker fluorimeter a narrow band of light passes vertically through a rectangular cuvette, the fluorescent light emitted being collected by a circular selenium cell from a circular patch of indefinite extent; an area in the centre of the cuvette having the greatest effect (Fig. 1). Let a beam of light incident on the base of the cuvette have a width w and an intensity I_o; and the circle from which light falls on the photo-cell a radius r and centre situated at a height h above the base of the cuvette.

From the Beer - Lambert law, the light absorbed in this circular region by the thiochrome in a solution containing other absorbing substances will be—

$$\frac{K_aC_a}{K_aC_a+K_bC_b} \times wI_o \int_{-r}^{r}\left\{e^{-(h-\sqrt{r^2-x^2})(K_aC_a+K_bC_b)} - e^{-(h+\sqrt{r^2-x^2})(K_aC_a+K_bC_b)}\right\}dx \qquad \text{(IV } a \text{ 1)}$$

where K_aC_a are the absorption coefficient and concentration of thiochrome in the cuvette, and K_bC_b the absorption coefficient and concentration of another absorbing substance. Other factors, K_cC_c, etc., could be added if necessary without changing the argument.



Front view of cuvette Side view of cuvette

Fig. 1. Optical effects in fluorimeter cuvette

This expression, rearranged, integrated in series, and simplified (terms beyond the second being discarded as extremely small), reduces to

$$K_aC_a\,\pi r^2 wI_o e^{-h(K_aC_a+K_bC_b)}\left\{1+\tfrac{1}{8}[r(K_aC_a+K_bC_b)]^2\right\} \quad \ldots \quad \ldots \quad \text{(IV } a \text{ 2)}$$

The light absorbed by a corresponding thiochrome solution free from other absorbing agents will be given by (IV *a* 2) with the omission of K_bC_b wherever it occurs .. .. (IV *a* 3)

The relative amount of light absorbed by the thiochrome in the two will be given by (IV *a* 2) divided by (IV *a* 3), *i.e.*, will be

$$e^{-h(K_bC_b)}\left\{\frac{1+\frac{1}{8}[r(K_aC_a+K_bC_b)]^2}{1+\frac{1}{8}[r(K_aC_a)]^2}\right\} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{(IV } a \text{ 4)}$$

In a particular solution, the actual aneurine concentration, multiplied by this factor, gives the apparent aneurine concentration that would be obtained in the analysis if light absorption by substances other than thiochrome were the only disturbing condition. The factor (IV *a* 4) would then be the "recovery factor"; it is denoted by "R" in the remainder of this discussion.

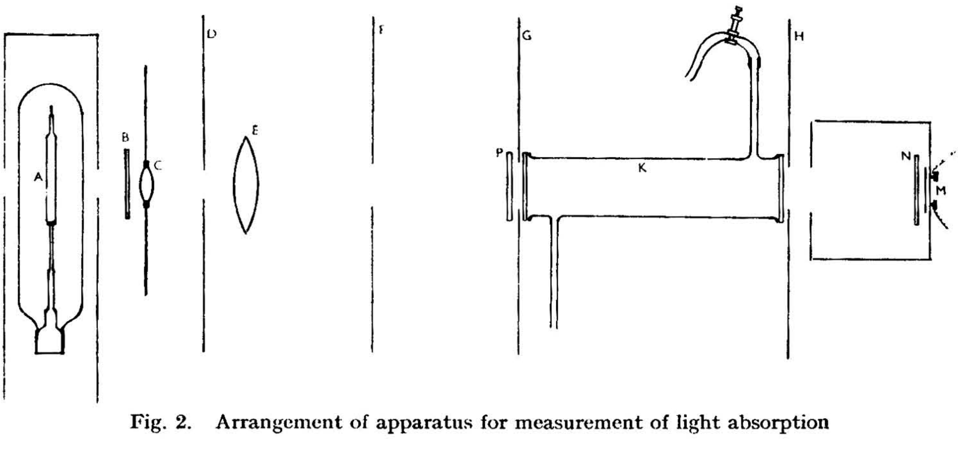


Fig. 2. Arrangement of apparatus for measurement of light absorption

Experimental determination of absorption coefficients—The determination of the absorption coefficients was undertaken with the simple apparatus shown in Fig. 2. The right-hand side block of the Spekker fluorimeter was removed, and the light from the mercury discharge lamp A, after passing a filter B (Chance's OX1, UV), the lens C normally mounted on the fluorimeter, and an 8 mm. cardboard diaphragm D, was further concentrated by another lens E, on to a series of cardboard diaphragms F, G, H, with holes 16 mm. in diameter, extended over a distance of about 1 metre. By this means a reasonably parallel beam was obtained, which passed through either of the cells used without touching the side. The cells K (9·2 and 29·2 cm. long, and 26·0 mm. diameter) were glass tubes with flanged ends and side tubes. The end plates were squares of photographic plate sealed on with Seccotine. The light, after passing through the cell fell on a selenium cell M, immediately in front of which was placed another Chance filter, OX1, N. A further filter P (Chance OV1, purple 8), was interposed in the beam before the cell. The two glasses together give a narrow transmission band in the 365 mμ. region, as shown in Fig. 3 (manufacturers' curves).

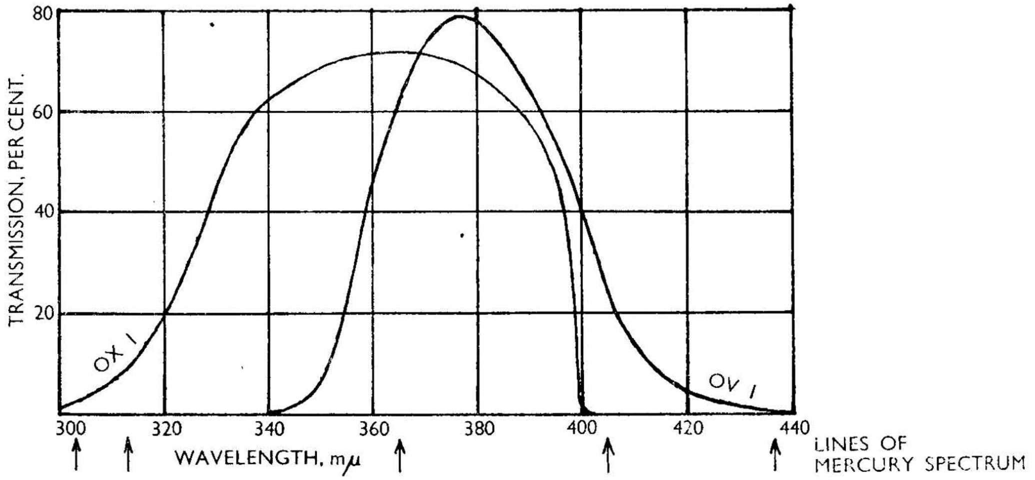


Fig. 3. Manufacturers' curves for transmission through Chance Glass Filters OX1 (ultra-violet) and OV1 (purple)

The solutions were prepared by precisely the same oxidation method as is used in normal procedure, but larger tubes were used, and all quantities multiplied by ten. The *iso*butanol extract (250 ml.) was syphoned off from the aqueous layer by means of a syphon bent upwards at the tip to prevent contamination. The first attempts to determine the transmission of these solutions failed, as cloudiness caused a larger loss of light than the true absorption. The normal, or increased, amounts of ethyl alcohol added delayed the formation of cloud, but did not prevent it. The formation of minute crystals of sodium carbonate was found to be the main cause. Finally, a technique was adopted of syphoning the *iso*butanol extract into 350-ml. stoppered bottles and storing these in the refrigerator overnight. This threw out of solution a certain amount of water with dissolved sodium hydroxide. The next day the bottles were transferred to the instrument room (in darkness) and allowed to warm up without undue disturbance. Immediately before the determination of its transmission the *iso*butanol extract was transferred to a beaker containing 10 ml. of absolute alcohol and drawn up into the absorption cell. By this means clear solutions that gave remarkably reproducible results were obtained. The extracts were later found to have fluorescences indistinguishable from those of corresponding extracts prepared in the normal manner immediately before making the comparison. Two or more blanks, made by oxidising acid water only, and one or two standards made by oxidising solutions of pure aneurine only, were used in each run, and all determinations, including the extraction of any raw material, were repeated on several different days.

The absorption coefficient of solutions derived from pure aneurine—From the results obtained with the *iso*butanol extracts prepared from standard solutions of pure aneurine, used in each day's work, the absorption coefficient was calculated using the formula

$$K_a = \frac{\log_e \frac{I_o}{I}}{C_a \times l}$$

where the intensities I_o and I were given by the galvanometer deflections obtained when the cell was filled with *iso*butanol extracts obtained by the oxidation of acid water and an aneurine solution respectively, C_a was the concentration of the aneurine solution used *in µg. per ml.*, and l was the length of the cell. The results obtained after the adoption of the technique described are shown in Table I. It was the agreement of the values of this coefficient which was used as the final check on the clarity of the solutions and the correctness of other dispositions.

It should be mentioned here that the aneurine used throughout this work, and in terms of which all aneurine concentrations are expressed, was a good commercial crystalline sample kept in a screw-topped bottle in the open laboratory. At a later date and for another purpose this was repeatedly and very carefully compared with the international standard aneurine by the thiochrome method and found to be equal to the international standard × 0·91. K_a for the international standard would thus be 0·0087 per µg. per ml. per cm.

TABLE I

LIGHT ABSORPTION COEFFICIENTS OF SOLUTIONS DERIVED FROM PURE ANEURINE

29·2 cm. cell			9·2 cm. cell		
Date	Concentration, µg./ml.	K_a	Date	Concentration, µg./ml.	K_a
22.11.45	0·8	0·00814	30.11.45	4·0	0·00760
,,	2·0	0·00807	,,	2·0	0·00793
13.12.45	1·0	0·00745	2. 2.46	1·2	0·00807
28.12.45	2·0	0·00837	,,	2·0	0·00797
22. 1.46	1·0	0·00755	8. 2.46	2·0	0·00797
,,	2·0	0·00780	15. 2.46	1·6	0·00790
25. 1.46	0·8	0·00794	15. 3.46	2·0	0·00826
	Mean	0·00790		Mean	0·00796

Mean of all results 0·00793. Standard deviation 0·00026.

Absorption in solutions derived from extracts of bran—Turning to solutions in which light was likely to be absorbed by substances other than thiochrome, bran was taken as a material with a low recovery which varies with the concentration of bran in the extract. The first bran chosen, B.1045, was made up in concentrations of 2, 3, 4, 5, 6, 7, 8 g. in 50 ml., with five

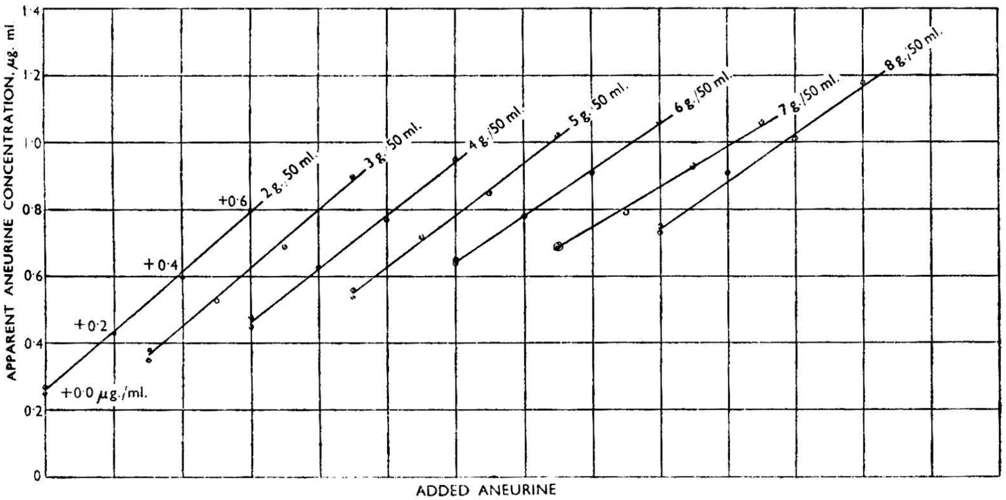


Fig. 4. Bran B.1045. Observed aneurine concentration

separate extracts at each concentration. Two of these contained no added aneurine, the others 0·2, 0·4, 0·6 µg. per ml. respectively. On analysis these yielded the values shown in Fig. 4. The highest value at 8 g. bran concentrations with 0·6 µg. per ml. addition was off the normal scale of readings and hence was somewhat uncertain. Blank values were neglected as largely destroyed on oxidation.[9]

Absorption measurements were carried out on *iso*butanol extracts prepared as described above. These extracts were prepared twice for work on different days and with a different arrangement of the apparatus, two different cells employed and by different disposition of the diaphragms, etc., the light intensities were changed. A provisional value for the aneurine concentration was obtained from the 3 g. bran concentration as the most reliable, the mean value of 0·365 µg. per ml. at no addition divided by recovery interpreted from the graph as 87·5 per cent. giving a level of 0·417 µg. per ml. as the true value, or 0·139 µg. per ml. per g. per ml. It was assumed for this purpose that the "recovery" at this bran concentration was the same for the "no addition" level as for that with additions; the justification for this assumption lying only in the reasonableness of the subsequent results as a whole. It was provisionally assumed also that the concentration of the absorbing substance would be proportional to the bran concentration, which was used for the term C_b in the expression $I = I_o e^{-l(K_aC_a + K_bC_b)}$, where I_o is the incident intensity and I the intensity of light leaving the cell, which has the length l. K_a is the absorption coefficient considered previously, C_a the concentration of aneurine, and K_bC_b the corresponding values relating to the component derived from bran. From this it follows that

$$K_b = \frac{\log_e \frac{I_o}{I} - K_aC_a \times l}{C_b \times l}$$

and values of this are given in Table II.

TABLE II

LIGHT ABSORPTION COEFFICIENTS, BRAN B.1045

Concentrations			Cell length, cm.			
			29·2*		9·2†	
Bran in extract (g./50 ml.)	Added aneurine (µg./ml.)	Total aneurine in extract (µg./ml.), C_a	Galvanometer deflection	Absorption coefficient K_b	Galvanometer deflection	Absorption coefficient K_b
0	0	0	35·1		38·0	
2	0·0	0·278			24·7	0·0222
3	0·0	0·417	5·0	0·0212	20·7	0·0208
3	0·6	1·017	4·6	0·0205	19·9	0·0206
4	0·0	0·556			16·2	0·0219
5	0·0	0·695	1·5	0·0204	13·6	0·0211
5	0·6	1·295	1·5	0·0200		
6	0·0	0·834			11·1	0·0210
7	0·0	0·973	0·5	0·0197	9·0	0·0211
7	0·6	1·573	0·4	0·0201	10·3	0·0160
8	0·0	0·813			7·5	0·0208
			Means ..	0·0203		0·0206

* Date of determination, 22.11.45. † Date of determination, 30.11.45.

The remarkably good agreement of these figures was encouraging, especially in view of the very small deflections obtained with the long cell. Hence notice was taken of the general decline in the absorption coefficient with increase in concentration of bran, and therefore of aneurine, and particularly the very low level recorded with 7 g. + 0·6, which actually gave a larger deflection than 7 g. + 0. To test if this were real, two pairs of solutions were prepared, 6 g. per 50 ml. + 0·0 and + 0·6, and 8 g. per 50 ml. + 0·0 and + 0·6. No more of the original bran was available, so some bran from a fresh milling of the same wheat was used, and in view of this the results agreed well with those obtained earlier. As the apparent fall in absorption might be caused by blue fluorescent light passing the OX1 filter in front of the selenium cell, a second filter of the same type was interposed as an extra precaution. In spite of this the results were more definite than before (Table III).

TABLE III

LIGHT ABSORPTION COEFFICIENTS, BRAN SIMILAR TO B.1045 EFFECT OF ANEURINE CONCENTRATION

Concentrations		13.12.45	
Bran, g./50 ml.	Added aneurine, µg./ml.	Cell length, 9·2 cm. Galvanometer deflection	Absorption coefficient
6	0·0	13·7	0·0208
6	0·6	16·1	0·0190
8	0·0	10·3	0·0197
8	0·6	13·4	0·0163

The probable value for the absorption coefficient K_b at each concentration was obtained by plotting the values obtained at no addition and at 0·6 µg. per ml. addition on a graph, drawing lines passing evenly between the points, and reading from these the values required at each bran concentration. The values for 0·2 and 0·4 µg. per ml. addition were obtained by symmetrical interpolation between the values for 0·0 and 0·6 µg. per ml. obtained from the curves. From these, the values of R given in column 4, Table IV, are derived (formula (IV *a* 4) above, *r* taken as 1·5 cm., see Table XI). A calculation was then made of the apparent aneurine concentration C_aR that would be given by each of the bran extracts if this light absorption were the only interference; and these values are given in column 5,

TABLE IV

EFFECT OF LIGHT ABSORPTION ON APPARENT ANEURINE CONCENTRATION IN BRAN EXTRACTS, AND OF LIGHT ABSORPTION + QUENCHING

Concentrations				Effect of light absorption only,			Effect of light absorption + quenching	
Bran extract, g./50 ml. C_b	Aneurine added, µg./ml.	Total aneurine, µg./ml. C_a	Factor, R	C_aR µg./ml.	Aneurine content observed, µg./ml.		(IV *b* 4) $K_\delta = 0·0098$	(IV *b* 6) $K_\zeta = 0·11$
2·0	0·0	0·278	0·9175	0·255	0·25	0·27	0·250	0·254
	0·2	0·478	0·9186	0·440	0·43		0·431	0·438
	0·4	0·678	0·9193	0·623	0·60		0·611	0·619
	0·6	0·878	0·9201	0·810	0·80		0·794	0·804
3·0	0·0	0·417	0·8804	0·364	0·38	0·35	0·353	0·361
	0·2	0·617	0·8820	0·546	0·53		0·530	0·538
	0·4	0·817	0·8837	0·721	0·69		0·700	0·710
	0·6	1·017	0·8857	0·900	0·90		0·871	0·884
4·0	0·0	0·556	0·8457	0·471	0·48	0·45	0·453	0·463
	0·2	0·756	0·8485	0·642	0·63		0·617	0·627
	0·4	0·956	0·8519	0·814	0·77		0·783	0·790
	0·6	1·156	0·8545	0·987	0·95		0·949	0·952
5·0	0·0	0·695	0·8140	0·566	0·56	0·54	0·539	0·548
	0·2	0·895	0·8181	0·733	0·72		0·700	0·703
	0·4	1·095	0·8221	0·900	0·85		0·857	0·854
	0·6	1·295	0·8262	1·070	1·02		1·019	1·005
6·0	0·0	0·834	0·7838	0·653	0·65	0·64	0·616	0·618
	0·2	1·034	0·7894	0·816	0·78		0·770	0·762
	0·4	1·234	0·7951	0·982	0·91		0·926	0·904
	0·6	1·434	0·8008	1·150	1·06		1·085	1·043
7·0	0·0	0·973	0·7564	0·736	0·69	0·69	0·688	0·676
	0·2	1·173	0·7626	0·896	0·79		0·837	0·807
	0·4	1·373	0·7697	1·057	0·93		0·988	0·934
	0·6	1·573	0·7768	1·222	1·06		1·142	1·057
8·0	0·0	1·112	0·7305	0·814	0·75	0·73	0·754	0·738
	0·2	1·312	0·7388	0·970	0·91		0·898	0·866
	0·4	1·512	0·7466	1·130	1·01		1·046	0·990
	0·6	1·712	0·7557	1·297	1·18?		1·200	1·114

Table IV. For comparison the values actually observed are given in column 6. Each observed aneurine concentration is the mean of three or four determinations on one extract. The duplicate values given are such means from two separate extracts.

It will be seen from these figures that the observed values are in almost every result lower than those calculated. Hence, as would be expected, there is another form of interference, possibly quenching.

IV *b*. THE EFFECT OF QUENCHING IN ADDITION TO LIGHT ABSORPTION—

It was assumed in the first instance that one molecule of thiochrome, activated by absorption of one quantum of ultra-violet light, is de-activated by collision with one molecule of some substance derived from the bran whose concentration would be proportional to that of the bran itself.

A stationary state will be reached when the rate of formation of activated thiochrome molecules will be equal to their rate of destruction. Now let [T] be the actual thiochrome concentration, $[T]_B$ the apparent thiochrome concentration in presence of substances derived from bran, $[T^*]_o$, $[T^*]_B$ the stationary state concentration of activated thiochrome molecules in absence and presence of bran respectively; $[I_{abs}]_o$ and $[I_{abs}]_B$ the corresponding rates of absorption of light by the thiochrome. Then $T + h\nu \xrightarrow{K_\alpha} T^*$, $T^* \xrightarrow{K_\beta} T + h\nu'$. In addition, when bran is present $T^* + B \xrightarrow{K_\gamma} T + B$, where B is a molecule of substance derived from bran. Then

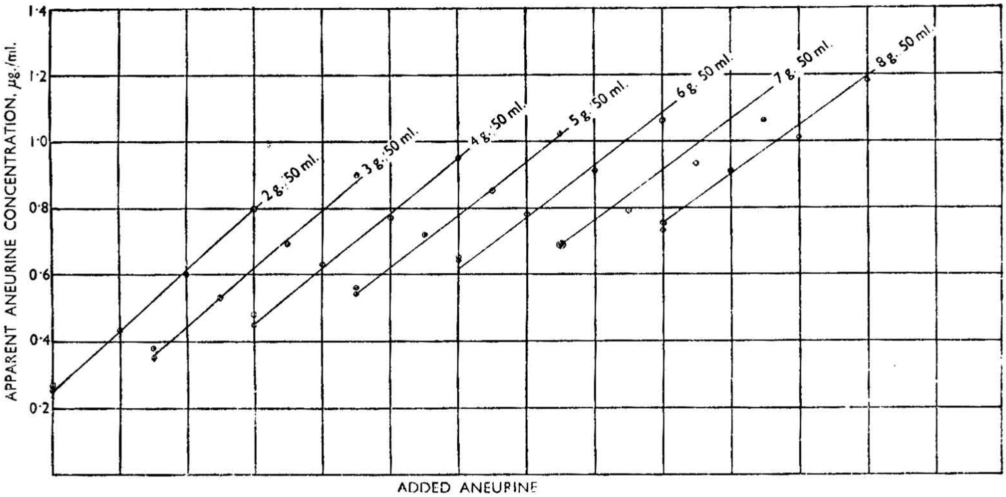


Fig. 5. Bran B.1045. Effect of light absorption and quenching proportional to bran concentration. Curves of the expression $\dfrac{C_a R}{K_\delta C_b + 1}$. Observed values in circles.

$$K_\alpha[I_{abs}]_o = K_\beta[T^*]_o \quad \ldots \quad \text{(IV } b\text{ 1)}$$

$$K_\alpha[I_{abs}]_B = K_\gamma[T^*]_B[B] + K_\beta[T^*]_B \quad \ldots \quad \text{(IV } b\text{ 2)}$$

Hence

$$\frac{C_a \text{ observed}}{C_a} = \frac{[T]_B}{[T]} = \frac{[T^*]_B}{[T^*]_o} = \frac{[I_{abs}]_B}{[I_{abs}]_o} \times \frac{K_\beta}{K_\gamma[B] + K_\beta} = \frac{R \times K_\beta}{K_\gamma[B] + K_\beta} \quad \text{(IV } b\text{ 3)}$$

Since it has been assumed that [B] is proportional to C_b, this may be transformed to give

$$C_a \text{ observed} = \frac{C_a R}{K_\delta C_b + 1} \quad \ldots \quad \text{(IV } b\text{ 4)}$$

and

$$K_\delta = \frac{K_\gamma}{K_\beta} = \frac{C_a R - C_a \text{ observed}}{C_a \text{ observed} \times C_b} \quad \ldots \quad \text{(IV } b\text{ 5)}$$

The value of $K\delta$ will best be obtained from a high bran and aneurine concentration, and 5 g. per 50 ml. + 0·6 μg. per ml. was chosen as the most reliable high value. Substitution of the appropriate values for C_aR and C_a observed gave $K\delta = 0{\cdot}0098$. This value was used for the calculation of the apparent aneurine concentrations given in column 7, Table IV, and plotted as the curves in Fig. 5, on which the observed values are indicated by the circles.

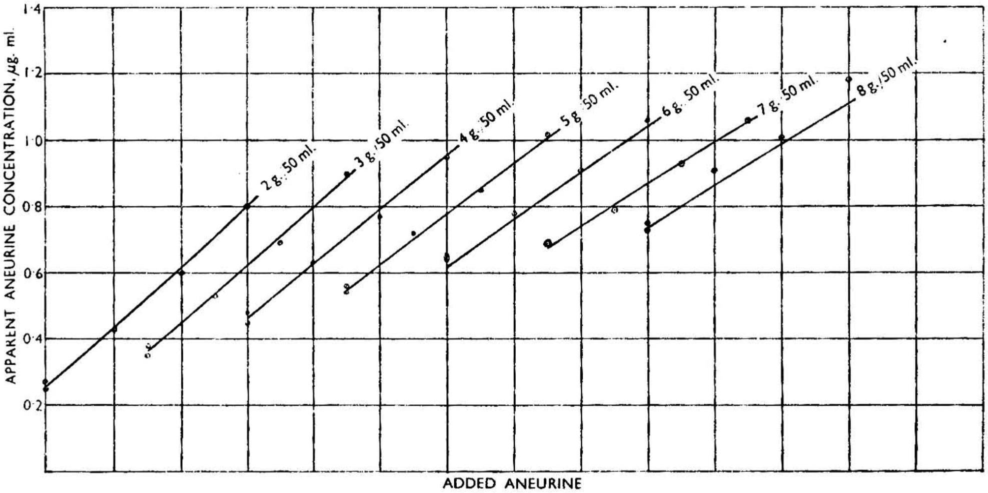


Fig. 6. Bran B.1045. Curves of the expression $C_aR - 0{\cdot}11 \left(\frac{C_b}{7} \times C_aR\right)^2; \frac{C_b}{7} \not> 1$.

Observed values in circles

The differences between the observed and calculated results show means of +0·014 (16) and −0·019 (19). Although this could be regarded as satisfactory, the fact that all the results at 7 g. concentration are low is disturbing. This peculiar change in slope of the curves of observed aneurine concentration when passing from 6 to 7 g. of bran per 50 ml. was too great to be due to normal experimental error, and could be a real effect—*e.g.*, the concentration of some component could reach a maximum at 7 g. of bran, or all the molecules of thiochrome in the fluorimeter cell could be brought into some kind of association with molecules of a disturbing substance. Only a deviation proportional to the square of the aneurine concentration would account for the changes in slope. Various provisional hypotheses were considered, and as a result of examining these it was found that the expression

$$C_aR - K_\zeta \left(\frac{C_b}{7} \times C_aR\right)^2 \qquad \frac{C_b}{7} \not> 1 \quad .. \quad .. \quad (\text{IV } b\ 6)$$

gave values in excellent agreement with the experimental results. K_ζ was determined from the concentration 7 g. per 50 ml. + 0·6 μg. per ml., and found to be 0·11. Values for the

TABLE V

LIGHT ABSORPTION COEFFICIENTS, BRAN B.1388

Concentrations			8.2.46
Bran, g./50 ml. (C_b)	Added aneurine, μg./ml.	Total aneurine, μg./ml. (C_a)	9·2 cm. cell Absorption coefficient
2	0·0	0·54	0·0242
2	0·6	1·14	0·0252
4	0·0	1·08	0·0233
6	0·0	1·62	0·0246
6	0·6	2·22	0·0238
7	0·0	1·89	0·0242
8	0·0	2·16	0·0247
8	0·6	2·76	0·0245
		Mean	0·0243

above expression are given in column 8, Table IV, and are plotted as curves in Fig. 6, the observed values being indicated by circles.

A second bran, B.1388, gave absorption coefficients very similar to those obtained from B.1045 (Table V).

In this material the aneurine content was greater than in B.1045, which indicates the presence of more germ, whereas the drop in absorption coefficient with increase in aneurine concentration was not noticeable, possibly owing to the high initial aneurine level. The losses due to light absorption were again found to be insufficient to account for the low recoveries, and an additional factor of the same form as before was found to do this with great precision.

$$C_a \text{ apparent} = C_aR - 0{\cdot}055\left(\frac{C_b}{4} \times C_aR\right)^2 \qquad \frac{C_b}{4} \not> 1$$

as is shown in the accompanying graph (Fig. 7). It will be noticed that the bran appears to exert its maximum effect on the solution at a concentration of 4 g. per 50 ml., while the constant K_ζ is one half of the value found with bran B.1045. An expression of this form is not easily accounted for in view of the very low aneurine concentrations involved, and further data are obviously required. The formation of "dimers" has been suggested as a cause of quenching at higher concentrations.[14]

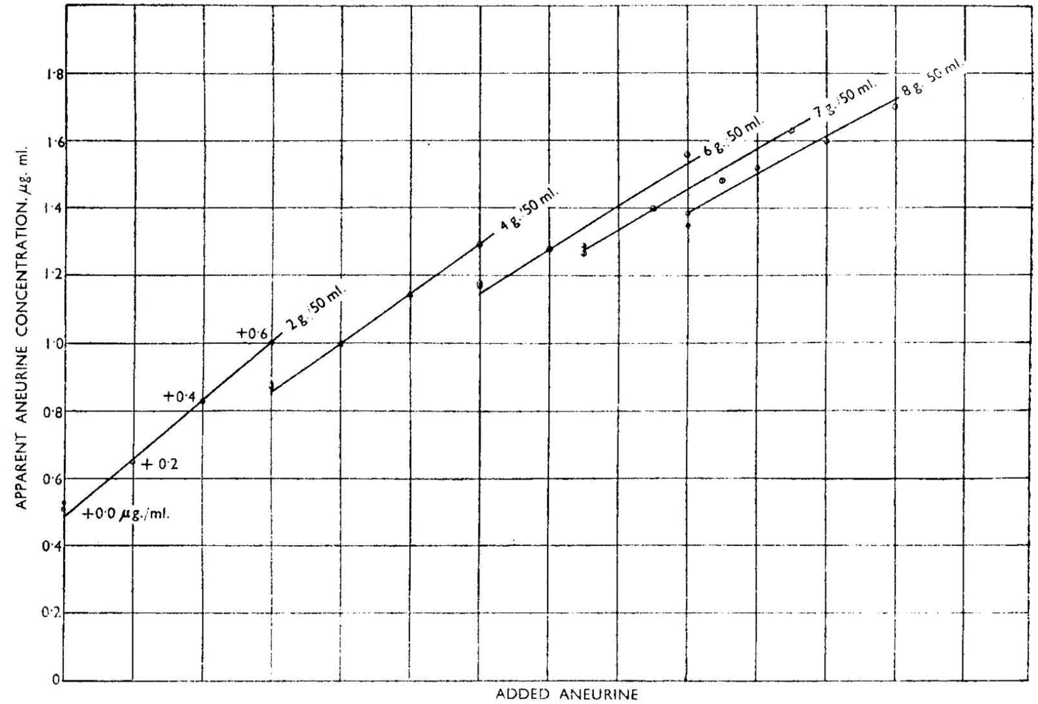


Fig. 7. Bran 1388. Curves of expression $C_aR - 0{\cdot}055\left(\frac{C_b}{4} \times C_aR\right)^2 ; \frac{C_b}{4} \not> 1.$

Effect of acid concentration on absorption coefficient—The effect of the concentration of the acid used in extraction of the bran was also examined, with the following results—

TABLE VI

EFFECT OF ACID CONCENTRATION ON ABSORPTION COEFFICIENT
BRAN CONCENTRATION, 7 G. PER 50 ML.

Absorption coefficient uncorrected for aneurine concentration

Hydrochloric acid	Absorption coefficient
0·12 *N*	0·0184
0·24 *N*	0·0220
0·36 *N*	0·0255

Wheats—The absorption coefficients,

$$K_w = \frac{\log_e \frac{I_o}{I} - K_a C_a \times l}{C_w \times l},$$

of a number of wheats were determined, and found to be surprisingly similar to one another (Table VII). Together with these, the eluates derived from sand adsorption[9] were examined, and the results illustrated the remarkable degree of purification effected by this method.

TABLE VII

LIGHT ABSORPTION OF EXTRACTS DERIVED FROM WHEATS AND ELUATES OF WHEAT

(Where the variety is mentioned more than once it was grown at a different location)
Corrected for aneurine concentration

		Concentrations					
Lab. No.	Variety	Wheat, C_w	Added aneurine, μg./ml.	Total aneurine, C_a μg./ml.	Cell length, l	Absorption coefficient, K_w	
B.1054	Little Joss	5 g./50 ml.	0·0	0·35	29·2	0·00742	
1060	"	"	"	0·35	"	0·00600	
B.1073	Juliana	"	"	0·35	"	0·00548	
1076	"	"	"	0·35	"	0·00455	
1079	"	"	"	0·39	"	0·00573	
1303	Despres 80	"	"	0·38	"	0·00615	
1308	"	"	"	0·44	"	0·00564	
1400	Steadfast	"	"	0·40	"	0·00540	
1421	Conditioned Manitoba	"	"	0·43	"	0·00534	
1441	"	"	"	0·42	"	0·00483	
	Manitoba	"	"	0·46	"	0·00556	15.2.46
	"	"	0·4	0·86	"	0·00555	
	"	10 g./50 ml.	0·0	0·88	"	0·00548	
	"	5 g./50 ml.	"	0·47	9·2	0·0056	27.2.46
	"	"	0·6	1·07	"	0·0059	
B.1320	Mixed Grist	"	0·0	0·375	29·2	0·00559	
"	(70% Manitoba)	10 g./50 ml.	"	0·75	"	0·00558	
"	"	"	"	0·75	"	0·00610	
"	"	"	"	0·375	"	0·00617	
			Eluates				
	Manitoba	5 g./50 ml.	0·0	0·305*	"	0·00058	15.2.46
	"	10 g./50 ml.	"	0·565*	"	0·00043	
B.1320	Mixed Grist	5 g./50 ml.	"	0·278*	"	0·000138	25.1.46
	"	10 g./50 ml.	"	0·555*	"	0·000154	

* In the eluate.

When it is considered that more than half of the total absorption of these eluates was due to the thiochrome, and that it had to be calculated from the separately determined aneurine absorption coefficient and concentrations and deducted before calculation of the absorption coefficient of the eluate, the agreement is quite remarkable and leads to greatly increased confidence in the methods employed. The difference between the eluates from the two wheats is interesting in view of the similarity of the coefficients for the plain extracts. The difference is much too great to be error in galvanometer reading ($I_o/I = 44{\cdot}7/35{\cdot}6$ for 10 g. per 50 ml. of Manitoba; 43·0/36·6 for 10 g. per 50 ml. of mixed grist), is too great to be attributed to an error in the estimation of thiochrome concentration, and is unlikely to be caused by cloudiness in the solutions in view of the excellent agreement of the pairs of results, and of all other results after the first few days' work early in the investigation. The values for the thiochrome absorption coefficient were particularly good (0·00794 and 0·00790) on the two days on which these eluates were examined.

The factor R for the Manitoba wheat was calculated and as with bran found to be inadequate to account for the low recovery found. The difference between C_aR and observed aneurine concentration was accounted for well by a linear correction plus a square law correction to allow for 15 per cent. of bran. Blank values have again been neglected. Results are given in Fig. 8.

IV *c*. ABSORPTION OF BLUE LIGHT—

The absorption coefficients for blue light of *iso*butanol extracts derived from Manitoba wheats and from pure aneurine were determined, and are given in Table VIII. Since the fluorescent light passes only 0·7 cm. of the solution when leaving the cell, this factor has been neglected.

TABLE VIII

ABSORPTION COEFFICIENTS FOR BLUE LIGHT

*iso*Butanol extract	Absorption coefficient
Aneurine (1·0 μg./ml.)	0·00037/μg./ml.
Manitoba wheat, 10 g./50 ml. + 0·0	0·000278/g./50 ml.
Manitoba wheat, 10 g./50 ml. + 0·4	0·000219/g./50 ml.

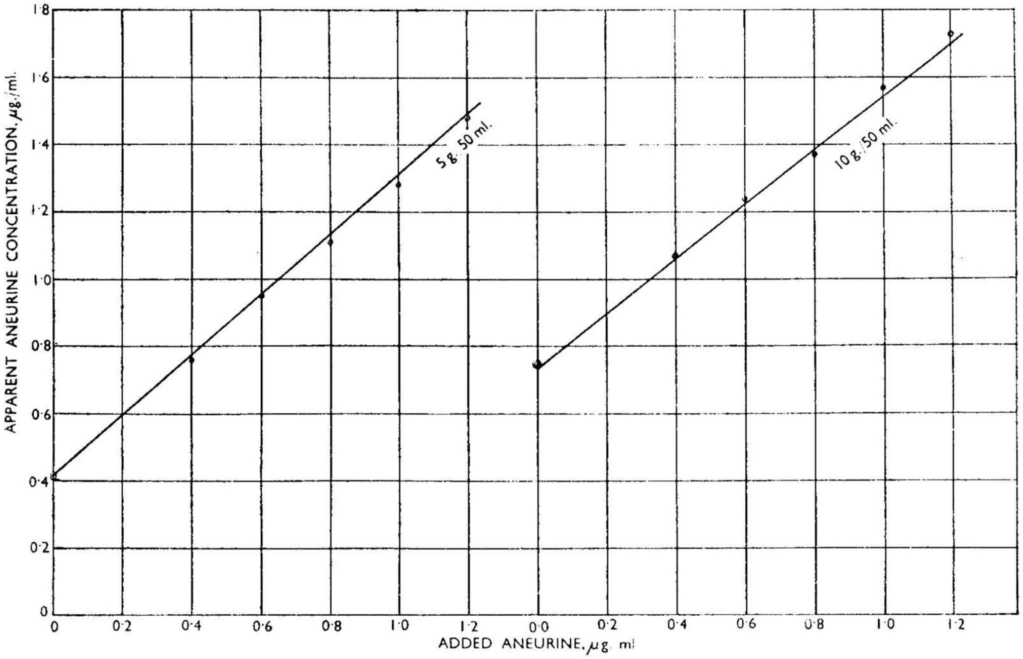


Fig. 8. Manitoba wheat. Curves of the expression $C_aR - 0{\cdot}055\left(C_aR \times \frac{C_w \times 15}{4 \times 100}\right)^2 - 0{\cdot}01\ C_w \times C_aR$. Observed values in circles

Yeast—The ultra-violet absorption of *iso*butanol extracts prepared from digests of bakers' yeast was also examined, with the results shown in Table IX.

TABLE IX

ABSORPTION COEFFICIENTS OF *iso*BUTANOL EXTRACTS DERIVED FROM YEAST, (FOR ULTRA-VIOLET LIGHT)

Concentrations				
Yeast, g./50 ml.	Added aneurine, μg./ml.	Total aneurine, μg./ml.	Buffer (pH 4·5)	Absorption coefficient, per g./50 ml.
1	0·0	0·22	Acetate	0·0153
1	0·5	0·72	,,	0·0145
2	0·0	0·44	,,	0·0117
1	0·0	0·22	Phosphate	0·0141
1	0·5	0·72	,,	0·0133
2	0·0	0·44	,,	0·0138

The fluorescence of solutions derived from pure aneurine—The fluorescence of solutions derived from pure aneurine was compared with the light absorbed, which was calculated from the absorption coefficient relating to pure aneurine (0·00793 per µg. per ml.).

Solutions of pure aneurine of various concentrations were oxidised and extracted with *iso*butanol in the usual manner, and the extract placed in the fluorimeter cuvette. The selenium cell receiving the fluorescent light was connected directly to the galvanometer with the same series - shunt arrangement as was used with the absorption measurements to diminish sensitivity, and the deflections given by the various solutions noted. The relative amount of light absorbed was calculated from the formula given above (IV *a* 3), *r* being the radius of a circle which includes about 100 per cent. of the light collected (1·5 cm., see Table XI). The factor relating deflection to light absorbed was calculated from the deflection at one concentration. The results are given in Table X, and it will be seen that there is good agreement between the observed and calculated figures at concentrations much above those used elsewhere in this work. As was assumed before this study was undertaken, quenching due to interaction of activated thiochrome molecules can be neglected where C_a is of the order 10^{-6}.

TABLE X

LIGHT ABSORBED RELATIVE TO FLUORESCENCE

For solutions derived from pure aneurine

Concentration of aneurine, µg./ml.	Relative light absorbed	Galvanometer deflection (cm.)			
		14.2.49		22.2.49	
		Calculated	Observed	Calculated	Observed
0·2	0·00158	0·379	0·44	0·346	0·36
0·4	0·00315	0·755	0·88	0·690	0·69
0·6	0·00472	1·130	1·17	1·032	1·03
0·8	0·00626	1·502	1·45	1·372	1·35
1·0	0·00771	1·85	1·85	1·69	1·73
2·0	0·01538	3·54	3·62	3·37	3·35
4·0	0·02978	7·13	7·08	6·52	6·49
6·0	0·04328	10·39	10·15	9·48	9·38
8·0	0·05594	13·40	13·32	12·25	12·15
10·0	0·06779	16·30	16·33	14·84	14·84
20·0	0·11630			25·44	24·95
		(correlation factor at 1·0 µg.)		(correlation factor at 10·0 µg.)	

Radius of circle from which is derived the light falling on the selenium cell in the Spekker fluorimeter—To gain some idea of the radius of the circle from which the fluorescent light measured in the Spekker fluorimeter is derived, tinfoil diaphragms were placed in turn *behind* the fluorimeter cuvette, and the deflection of the galvanometer noted, the left-hand (comparison) cell of the instrument being cut off from the light source by a screen. The following results were obtained (Table XI).

TABLE XI

LIGHT PASSING DIAPHRAGMS PLACED IMMEDIATELY BEHIND THE FLUORIMETER CUVETTE

Diaphragm radius, cm.	Deflection, cm.	Percentage passed
0·500	0·5	22
0·625	0·8	35
0·750	1·0	43
0·900	1·4	61
0·975	1·8	78
1·500	2·3	100
No diaphragm	2·3	

DISCUSSION OF RESULTS

It will be seen from the foregoing considerations that the determination of aneurine involves an exceedingly complex series of chemical reactions and physical processes. The addition of pure aneurine to extracts under examination is a valuable method of investigation if carried out with meticulous care, but neither recovery of added aneurine nor consistency of results is an adequate measure of the agreement of a result with the amount of aneurine actually present in the sample. Nor is agreement with biological estimation a fully satisfactory criterion, since utilisation may very well be affected by other substances present (*cf.* vitamins A and E[15,16]), and important biochemical effects may easily be obscured. The fullest possible theoretical understanding of the determination is a great safeguard. It will be seen from the present study that factors affecting recovery of added aneurine are not, strictly speaking, linearly related to the total concentration of aneurine present in an extract, but that the deviations from linearity may be very small owing to the very low concentrations under consideration. Nevertheless, in bran and probably other materials, part of each deviation appears to be proportional to the square of the aneurine concentration, but this needs confirmation, and if confirmed, satisfactory theoretical explanation. Some support for this is given by the common experience in this laboratory that the quotient—

$$\frac{\text{apparent aneurine concentration}}{\text{recovery factor}}$$

increases with the factor and therefore with concentration of starting material. Base-exchange methods of purifying extracts do not completely remove the need for this kind of study, for, although as a rule they greatly diminish optical interferences, they also add some new complications.

The writer's thanks are due to Mr. G. G. Grindley for assistance in the absorption measurements and aneurine determinations.

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THE RESEARCH ASSOCIATION OF BRITISH FLOUR MILLERS
CEREALS RESEARCH STATION, ST. ALBANS

July, 1949

Analysis of Penicillin Mixtures by Paper Chromatography of the Hydroxamic Acid Derivatives

BY P. B. BAKER, F. DOBSON AND A. J. P. MARTIN*

(Read at the meeting of the Society on Wednesday, April 6th, 1949)

SYNOPSIS—A rapid method that is independent of biological assays and suitable as a routine procedure is described for estimating one or more species of penicillin in a mixture.

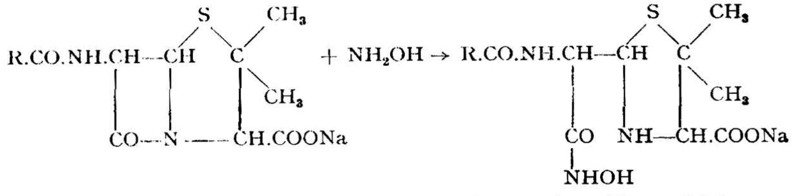
This method requires less than 8 hours for completion; it is based on the fact that the relatively stable hydroxamic acid derivatives of the various penicillins show different partition coefficients between *iso*propyl ether - *iso*propyl alcohol and phthalate buffer, at a given pH, and can therefore be separated by paper chromatography. A novel apparatus for use with volatile solvents and heavily buffered papers, is described.

A direct, qualitative result is obtained by developing the chromatograms with dilute ferric chloride solution. A quantitative result is attained by extracting the iron complexes of the various hydroxamic acids with butyl alcohol, measuring the degree of extinction in a colorimeter and reading the penicillin concentration from standard curves.

GOODALL and Levi[1] have described a method of analysis of penicillin based on partition chromatography on buffered paper strips and using bacteria-sown plates to detect and estimate the various penicillins. When the present work was carried out that was the only published method capable of routine application to give a reasonably complete analysis of a mixture of several different penicillins. The accuracy of the method is essentially that of the biological assay, which is sufficient for most purposes when the content of one species of penicillin is high, but barely adequate when more than one penicillin is present in considerable amount. A further disadvantage of the method is the length of time, three days, that it takes. Although we have had but little experience of the Goodall and Levi method, we believe that considerable experience is required before results comparable with those obtained by its originators can be obtained.

For these reasons it seemed worth while to reinvestigate the possibilities of other methods that use paper chromatography. Little progress was made, however, until the colorimetric method of Ford[2] was adapted to our requirements.

Ford made hydroxamic acids by mixing penicillin and hydroxylamine—



and showed that the Fe··· complexes with the hydroxamic acids could be used for colorimetric estimations.

These hydroxamic acids are relatively stable substances that can be separated on paper chromatograms at room temperature without serious loss. After separation the paper is sprayed with ferric chloride to give reddish-brown spots on a buff background at the positions of the individual hydroxamic acids. These spots can be extracted from the paper and the various penicillins estimated colorimetrically in the extracts.

The colorimetric method is far less sensitive than the biological. It is suitable for crude or pure salts of penicillin, but not for culture fluids without a preliminary extraction. A

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strongly coloured spot is given by 100 μg. of penicillin. A convenient amount for quantitative estimation is 1 mg.

With buffer-loaded paper chromatograms it is difficult to keep control of the humidity of the paper during development, and this difficulty is accentuated when a volatile solvent is used. Goodall and Levi, by rigorous attention to the routine of handling the paper, succeeded in reproducing their conditions, even though their paper was loaded with strong phosphate solution and their chamber had walls covered with cloth that was wet with water. It is evident that no equilibrium can be attained in such a system, and it is to be expected that the chromatographic behaviour will vary with every change in the previous history of the paper, with the shape and size of the vessels and with the temperature and time of running.

An attempt was made to arrange conditions so that the chromatograms could run under equilibrium conditions. The air within the chamber was kept saturated with respect to both phases on the paper. To do this was found to be difficult, and not until both phases were pumped continuously over the walls of the chamber were reproducible results obtained. No method of simply stirring the air or the liquid, or both, was adequate.

With the apparatus described below, however, it was found that the paper came to substantial equilibrium in half an hour and that the temperature was comparatively unimportant, a variation of as much as 10° C. during the run being without any deleterious effect. It is believed that this type of apparatus will be of general utility when volatile solvents (*e.g.*, ethyl ether) and heavily buffered papers must be used.

If the stationary phase is an aqueous solution of a non-volatile substance, it is of course not necessary that it should be circulated round the vessel, but it can be replaced by any other solution with the same vapour pressure, and this may be advantageous when expensive or corrosive solutions are concerned.

The role of the buffer with which the paper is loaded is worthy of some discussion. Satisfactory chromatograms are obtained with either citrate or phthalate buffers, but not with oxalate or phosphate of the same pH value. With oxalate or phosphate the spots of the hydroxamic acid complex are elongated and the R_F values are small. With citrate or phthalate the spots are compact and the R_F values much greater. This may be explained by the assumption that the hydroxamic acids exist as dimers in the mobile phase (a mixture of *iso*propyl ether and *iso*propyl alcohol) when phosphate or oxalate buffers, which are insoluble in the mobile phase, are used. When citrate or phthalate buffers are used, citric or phthalic acid dissolves in the mobile phase to an extent that is large compared with the amount of hydroxamic acid present and chelation between the hydroxamic acid and the buffer acid occurs. Since the buffer is in excess, the amount of chelated product is proportional to the concentration of hydroxamic acid and not, as with the dimer, to the square of the concentration. As a result the partition coefficient is more in favour of the mobile phase and is comparatively insensitive to the concentration of the hydroxamic acid. Hence the spots on the chromatograms are faster running and more compact with phthalate or citrate than with oxalate or phosphate buffers.

METHOD

Paper—Whatman No. 4 paper is used. It is dipped in 0·10 *M* potassium hydrogen phthalate solution and air-dried. The paper comes finally into equilibrium with 0·50 *M* buffer solution and should be dry enough to gain rather than lose water when it is placed in the chromatogram box. To retain heptyl penicillin hydroxamic acid on a reasonable length of paper while the other penicillins are adequately developed, the lower third of the paper should be dipped in phthalate buffer of pH 6·2.

Whatman No. 1 paper will give satisfactory chromatograms but a much longer time of development is then required.

Preparation of hydroxamic acids—In 1 ml. of a mixture of equal volumes of 4 *N* hydroxylamine hydrochloride and 3 *N* sodium hydroxide are dissolved 10 to 40 mg. of penicillin salt. Ten μl. of this solution are applied as a spot to the chromatogram and air-dried. Ten such spots can be accommodated on a sheet 15 cm. wide. A single spot suffices for a qualitative analysis, ten for a quantitative analysis.

Mobile phase—The mobile phase is *iso*propyl ether containing 15 per cent. v/v of *iso*propyl alcohol. To each 100 ml. of the mixture are added 2·4 ml. of 0·10 *M* potassium hydrogen phthalate to give approximate saturation with respect to the stationary phase. The mixture

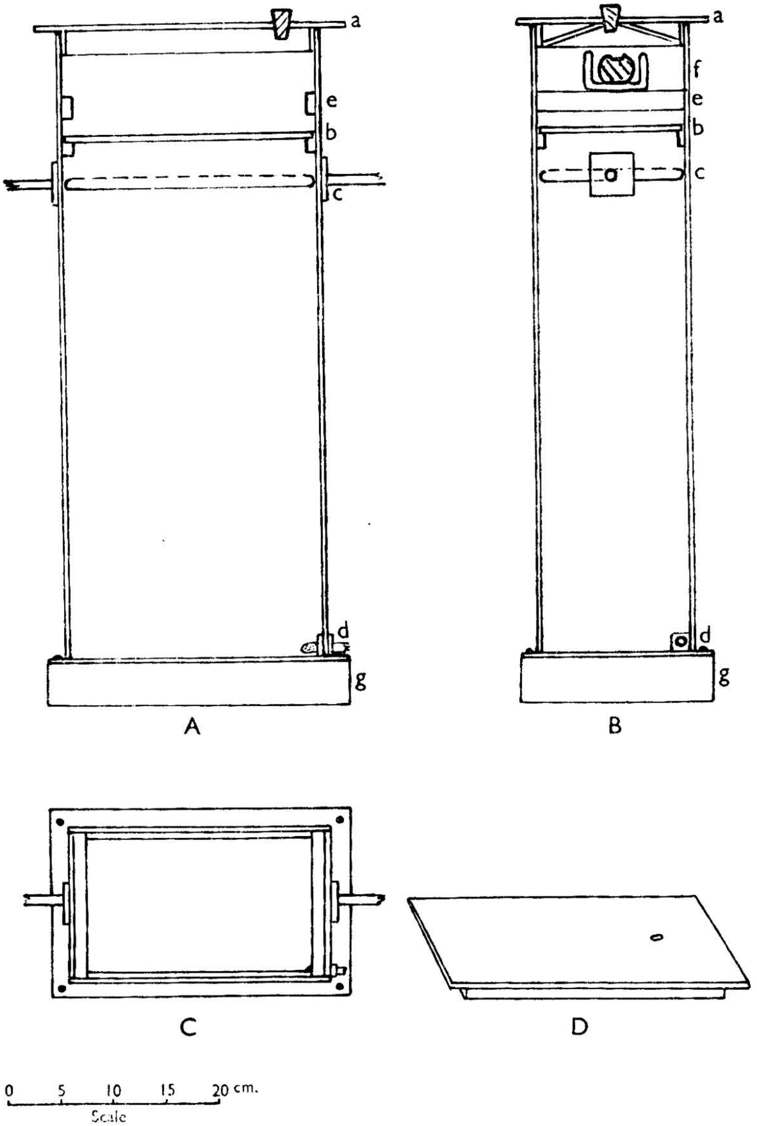

Fig. 1. Diagram of chromatogram box for use with volatile solvents

A. Front elevation (trough absent)
B. Side elevation (trough present)
C. Plan
D. Isometric view of lid.

The box is constructed of 3/16-inch Perspex.

(*a*) Flanged, gabled lid, with stoppered opening for filling trough; (*b*) rods for supporting cloth lining; (*c*) perforated tube delivering pumped liquid to cloth lining; (*d*) outlet (with gauze filter) for return of liquid to pump; (*e*) support for trough; (*f*) stainless steel trough (end view alone shown); (*g*) wooden base should be free from aldehydes or peroxides: distillation of the solvents from saturated bisulphite or 5 *N* sodium hydroxide ensures this.

Apparatus—The apparatus used, Fig. 1, consists of a Perspex box 15 × 25 × 60 cm. with a gabled lid, so that liquid condensing on the top does not drip on to the chromatograms. The walls of the box are lined with cotton cloth, over which both phases are pumped continuously by a diaphragm pump delivering 1500 ml. per minute. The liquids are drawn from the bottom of the box, and, in setting up the apparatus, special attention is paid to ensuring that *both phases* are circulated.

The papers, already spotted with hydroxamic acids and hanging from an empty trough, are placed in the chromatogram box and the pump is started. After thirty minutes the trough is filled with 50 ml. of the mobile phase. Six hours later the paper is removed, air-dried and sprayed with 2 per cent. ferric chloride solution in 0·01 *N* hydrochloric acid. Inspection of the sheet now permits a qualitative analysis.

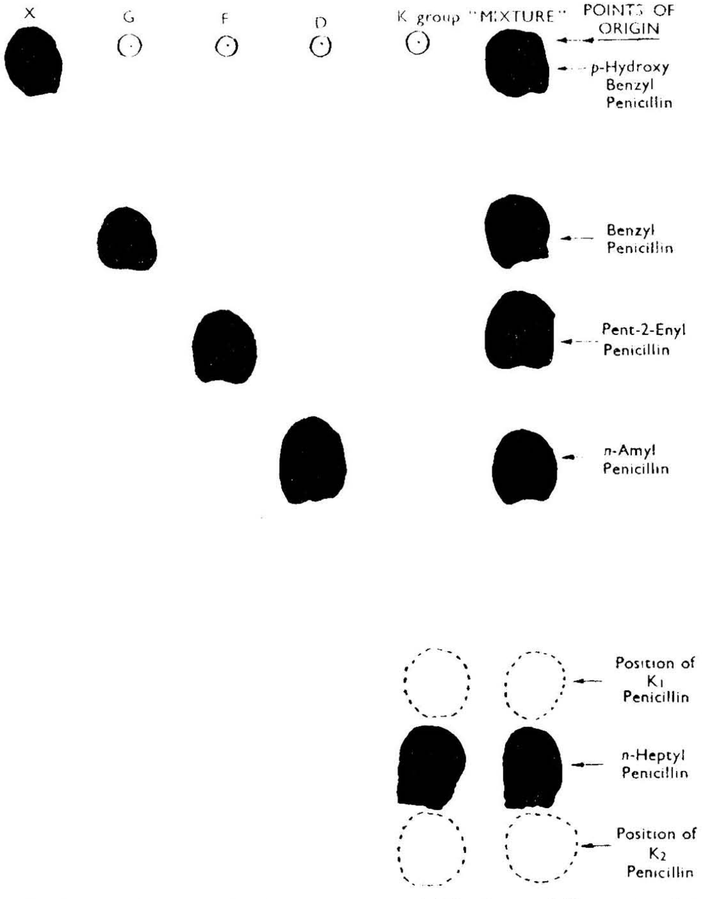

Fig. 2. Chromatogram showing various penicillin types, 100 μg. per "spot"

Extraction technique—The chromatograms are cut across into strips, each containing the 10 spots from one species of penicillin, which will have an average area of 75 sq. cm. Each strip is macerated with 1 ml. of 20 per cent. ferric chloride in 0·1 *N* hydrochloric acid, 10 ml. of *n*-butanol, 2 g. of anhydrous sodium sulphate and 0·6 g. of sodium chloride. The tubes are held in a water-bath at 20° C. until measured. The paper and salt can be packed at the bottom of the tube with a glass rod so that 7 to 8 ml. of the butanol can be poured off for the colorimetric measurement. The measurements were made in a photo-electric colorimeter (Evans Electroselenium Ltd.) with a "tricolour" green filter, No. 404. A more elaborate instrument would no doubt give greater accuracy.

The extinction of the butanol solution prepared in this way increases with temperature and the extraction and measurement should be made at a constant temperature. The colour appears to be stable for many hours and the solution obeys Beer's law within the error of measurement.

The reagent mixture was arrived at in the following way. Satisfactory extraction of the hydroxamic acid from the paper required a somewhat polar solvent, which unfortunately also dissolved enough ferric phthalate to make a large and variable blank. To displace the hydroxamic acid from an aqueous solution into, say, butanol, without multiple extractions it was necessary to "salt out" with sodium sulphate or some other salt. The depth of colour of a given butanol solution of hydroxamic acid is dependent upon the amount of ferric chloride in it. Since the amount of ferric chloride sprayed on the paper is liable to variation, a large excess of ferric chloride was added to the extraction mixture. In the presence of saturated sodium sulphate, the ferric chloride is almost completely converted to ferric sulphate, which is not extracted by the butanol; hence no colour is obtained. By saturating the solution with sodium chloride also, the ferric chloride activity is raised and good colour development of the hydroxamic acid is obtained with a small and constant ferric chloride blank. The high concentration of chloride and sulphate in the aqueous phase, by competing with the relatively small amount of phthalate, so reduces the amount of ferric phthalate dissolved in the butanol layer that the blank remains independent of the area of paper taken. By this procedure no dilution or making up to known volume is required and practically the whole of the hydroxamic acid is dissolved in the 10 ml. of butanol, of which a large proportion is available for use in the colorimeter.

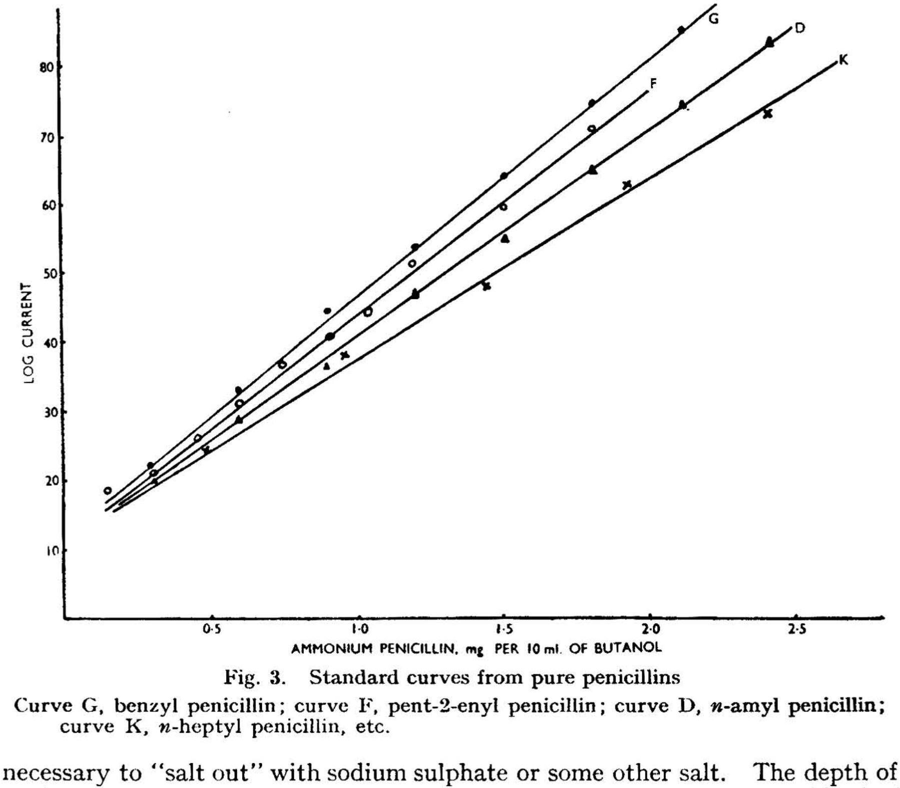

Fig. 3. Standard curves from pure penicillins

Curve G, benzyl penicillin; curve F, pent-2-enyl penicillin; curve D, *n*-amyl penicillin; curve K, *n*-heptyl penicillin, etc.

RESULTS

A typical chromatogram is shown in Fig. 2. The R_F values[3] of the hydroxamic acids from different penicillins are shown in Table I.

Standard curves from pure penicillins are given in Fig. 3, showing the relation between log current and amounts of penicillins of different kinds. If the gradient for penicillin G is taken as 100, the gradients of the other curves are F 95·5, D 86·3, K 75·8. Pure penicillin

X has not been available and it is uncertain whether the K penicillin is wholly *n*-heptyl. Other K varieties have not been available.

TABLE I

R_F VALUES OF HYDROXAMIC ACIDS FROM VARIOUS PENICILLINS

Parent penicillin	R_F value
n-Heptyl (K)	0·57
n-Amyl (D)	0·27
Pent-2-enyl (F)	0·20
Benzyl (G)	0·13
p-Hydroxy benzyl (X)	0·01

Tables II and III show analyses of control mixtures of pure penicillins, Table IV analyses of an unknown mixture.

TABLE II

ANALYSIS OF A CONTROL MIXTURE CONTAINING 75 PER CENT. OF BENZYL PENICILLIN AND 25 PER CENT. OF *n*-AMYL PENICILLIN

Benzyl, %	*n*-Amyl, %
73·2	26·8
72·4	27·6
76·3	23·7
76·6	23·4
75·2	24·8

TABLE III

ANALYSIS OF CONTROL MIXTURE CONTAINING 50 PER CENT. OF BENZYL, 8 PER CENT. OF PENT-2-ENYL, 22 PER CENT. OF *n*-AMYL AND 20 PER CENT. OF *n*-HEPTYL PENICILLINS

Benzyl, %	Pent-2-enyl, %	*n*-Amyl, %	*n*-Heptyl, %
48·8	9·3	14·7	27·2
49·1	7·7	19·6	23·6
46·8	7·7	22·1	23·2
47·9	7·2	23·0	21·7

TABLE IV

SAMPLE 316 AMMONIUM SALT

Benzyl, %	Pent-2-enyl, %	*n*-Amyl, %	K-group, %
43·7	14·6	24·4	17·3
44·8	14·0	24·9	16·3
46·0	17·0	27·3	9·7
47·0	16·0	25·0	12·0
41·0	15·5	24·7	18·8
44·0	17·8	25·1	13·1
43·8	15·3	26·0	14·9
44·0	16·4	25·3	14·3
47·5	13·5	25·5	13·5
44·1	15·8	26·6	13·5

The authors wish to thank Sir Jack Drummond, F.R.S., Director of Research, Boots Pure Drug Co., for his encouragement and for permission to publish this work.

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First submitted, *May*, 1949
Amended, *June*, 1950

Paper Chromatography in Penicillin Production Control

By J. W. ALBANS and P. B. BAKER

(*Read at the meeting of the Society on Wednesday, April 6th, 1949*)

SYNOPSIS—The application of a method for estimating penicillin species as described by Baker, Dobson and Martin in the previous paper, to culture filtrates and similar process samples, is discussed. The changes in composition that take place during fermentation and the use of the technique for examining such fermentation variables as the composition of the medium, the rate of aeration, the effect of precursors and of the fermentation period are also discussed. Some confirmation has been obtained of the existence of substances previously indicated, but not yet characterised, by the microbiological chromatographic technique.

The construction of a large vessel for multiple analyses is described. Examples of the degree of reproducibility of the results are shown.

As knowledge of penicillin therapy has increased and as methods of fermentation and processing penicillin have developed, there has been a demand for a rapid and accurate method of estimating the different penicillin species in fermentation liquors and in the fractions obtained during extraction and purification. Of the several methods proposed, the differential assays of Schmidt, Ward and Coghill[1] and of Higuchi and Peterson[2] are of limited application unless a wide range of suitable organisms is available for the tests. The inherent error in biological assays of this kind is then multiplied and the method becomes cumbersome. The Craig[3] counter-current distribution technique has been found suitable for a small number of samples, but it is slow and laborious. A paper chromatographic method developed by Goodall and Levi[4,5] gives a direct qualitative estimate of the various penicillins in mixtures, but the quantitative treatment proposed is open to criticism. Moreover, the delay in obtaining the results, 72 hours, renders the method of limited use for production control when the results are required with a minimum of delay.

The foregoing paper by Baker, Dobson and Martin,[6] which describes a method for the separation and estimation of the penicillin hydroxamic acids, refers mainly to work on the crystalline penicillins that were obtained from fermentation liquors by various methods of extraction and purification. The method is claimed to be suitable for the differential estimation of penicillin species in mixtures; and with those penicillins that have been isolated and for which a standard curve can be constructed it is claimed that the results for specific penicillins are reliable. The present paper deals with applications of this basic technique to the estimation of the penicillins in culture fluids and similar process samples obtained during the extraction of penicillin.

Any method of analysis that is to be useful as a control test for a production plant must fulfil certain conditions. When the processing of a large quantity of material is dependent on the results of a control test, the test should be speedy. The method must be one that can easily be carried out by unqualified assistants and should require the minimum of special apparatus. The technique to be described has been employed successfully by two assistants for more than a year. Throughout this period, supervision has been reduced to a minimum. The results obtained have been satisfactory and periodical checks on known mixtures of penicillins have shown that accuracy has been maintained. The only special apparatus used is the tank made of Perspex and this will last indefinitely. The diaphragm pump, mentioned in the previous paper,[6] has been replaced by a cheap centrifugal pump readily available from stock. The other apparatus consists of items normally used in a biochemical laboratory. On only one point does the method fail to fulfil the conditions mentioned above, *viz.*, that the minimum period for dealing with a sample of liquor is about 12 hours and for a solid, 8 hours, but this rather long delay has not caused any trouble. Moreover, it often happens that the constitution of a sample is known before the normal bio-assay is available. Further, the method of calculating the results is very simple.

THE ESTIMATION OF PENICILLIN SPECIES IN FERMENTATION LIQUORS AND PROCESS SAMPLES—

Owing to the low concentration of penicillin compared with other solids, the normal method of separating and estimating the hydroxamic acids of the penicillins[6] cannot be applied directly to culture filtrates and similar process samples. Thus, for a culture fluid of 500 units per ml., in order to achieve the desired loading of 1 mg. of each penicillin species, at least 300 mg. of inactive material would be applied at each spot. To overcome this difficulty the penicillins are extracted into ether at about pH 2 and then converted into the hydroxamic acids. Removal of ether and subsequent concentration of the aqueous residue are carried out under such conditions that inactivation is reduced to a minimum.

METHOD

Extract 200 ml. of culture filtrate (or the equivalent of any other sample), previously clarified with the aid of kieselguhr, with two 100-ml. portions of ether at pH 1·5 to 2·5, adjusting with 20 per cent. phosphoric acid. Break the emulsion by centrifuging. Transfer the ether extract to a 6-in. crystallising dish containing 0·5 ml. of 4 *M* hydroxylamine hydrochloride and 0·7 ml. of 3 *M* sodium hydroxide. Mix well and see that the aqueous layer does not become too acid; adjust it to pH 6·2 with *N* sodium hydroxide, if necessary. Remove the ether by means of a gentle stream of air over the surface. Concentrate the aqueous residue (4 to 6 ml.) to about 0·5 to 1·0 ml. by means of a blast of warm air from a hair-drier fixed about 12 in. above the dish. Remove any insoluble matter by means of a filter-stick and apply the clear filtrate to buffered paper as previously described.[6] (A check on the concentration achieved can be obtained by spotting samples of the concentrate and a control of known strength on to a filter-paper soaked in 2 per cent. ferric chloride.)

In the earlier stages of the work the results were rather erratic, owing in the main to insufficient control of the extraction and conversion stages. Attempts to avoid emulsification led to incomplete extraction, *e.g.*, only 25 per cent. of added penicillin could be extracted from solution in a corn-steep-liquor medium, whereas with an aqueous solution, where no emulsification occurred, the recovery was more than 90 per cent. A centrifuge had been used quite successfully to break the emulsions, but it was thought that an alternative method that involved no fire risk was desirable. The use of surface-active compounds showed some promise, but to work out the exact conditions would probably have required a considerable amount of study and this idea has not yet been pursued further. If the fermentation liquor, free from mycelium, is heated to 60° C. and rapidly cooled before clarification, emulsification is less troublesome. The precipitation of proteins by tannic acid is unsatisfactory as the blue stain produced when the papers are sprayed with ferric chloride masks the bands due to penicillin.

The acid extraction should, of course, be carried out rapidly, and it is of more importance to separate the ether extract and add it to the hydroxylamine reagent quickly than to attempt to make fine adjustments in the degree of acidity. In experiments with solutions of penicillin salts, it has been shown that, under the conditions recommended, there is no differential extraction of any of the penicillin species. It is vital that traces of emulsion or of aqueous layer be not added to the dish or the mixture will be too acid for conversion to hydroxamic acids. For the concentration of the hydroxamic acid solutions, lyophilic drying has been used with success, but the stability of these compounds has made it possible to use the quicker method described earlier as a routine.

LARGE APPARATUS FOR MULTIPLE ANALYSES—

As the development of this method of analysis progressed it became necessary to construct a larger vessel. In view of the critical humidity conditions necessary for good separation, a prototype (10 × 16 × 24 in. high), consisting essentially of three of the smaller vessels, was built. The spargers and curtains are shown in Fig. 1.

However, it was eventually found that spargers and curtains round the outer walls of the vessel were sufficient to achieve the desired degree of humidification in this size of vessel. With the arrangement indicated in Fig. 1, movement of the zones on paper 3*b* was rather slower than on the other papers and, in fact, fairly wide variations in the distances travelled were shown on the various papers (see Table I).

TABLE I

DISTANCES OF TRAVEL, MM., OF ZONES IN $6\frac{1}{2}$ HOURS

	Papers					
	1*a*	1*b*	2*a*	2*b*	**3*a***	**3*b***
G	56	55	69	64	**74**	**49**
F	78	79	97	93	**100**	**75**
D	102	107	128	124	**132**	**99**
K	240	240	300	289	**285**	**254**

This difference in the distance of travel is attributed to striation in the spargers. In order to obtain similar separation on the different papers in the tank, it is advisable to have the spray evenly distributed round the walls of the tank. This refinement is not absolutely essential, however, for, although the absolute distances of travel vary, the relative movements are constant, provided that the conditions specified[6] are satisfied. This constancy of relative movement is shown by Tables II and III.

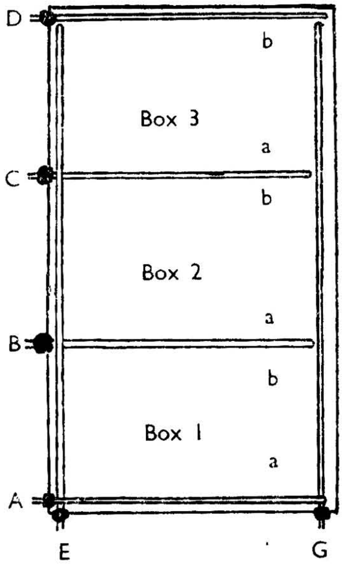

Fig. 1. Large apparatus for multiple analyses

TABLE II

RATIOS OF DISTANCE OF TRAVEL

	Papers						
	1*a*	1*b*	2*a*	2*b*	3*a*	**3*b***	**Mean**
G	1·00	1·00	1·00	1·00	1·00	**1·00**	**1·00**
F	1·39	1·44	1·41	1·44	1·35	**1·53**	**1·43**
D	1·83	1·94	1·85	1·92	1·78	**2·05**	**1·89**
K	4·28	4·36	4·34	4·46	3·85	**5·17**	**4·41**

TABLE III

MEAN VALUES OF RATIOS UNDER VARYING HUMIDITY CONDITIONS

	Spargers			
	All in	B out	B, C out	**B, C, D out**
G	1·00	1·00	1·00	**1·00**
F	1·43	1·40	1·46	**1·42**
D	1·89	1·91	1·95	**1·91**
K	4·41	4·90	5·30	**4·56**

This constancy of relative movement for penicillins G, F and D is of very great importance in determining the identity of the bands, as will be seen later. The variation shown by penicillin K is due to the fact that this band is in the pH 6·2 region of the paper, and the relative movements of bands in the two regions are not comparable.

The degree of reproducibility attained with this vessel is indicated by the two examples given in Table IV.

TABLE IV

REPLICATE ANALYSES OF A MIXTURE

Operator A

	G	F	D	K
	per cent. w/w			
	47·5	13·5	25·5	13·5
	44·1	15·8	26·6	13·5
	46·0	14·2	28·0	11·8
	44·0	14·5	27·5	14·0
	47·3	11·7	27·8	13·2
	45·4	14·8	27·4	12·4
Mean ..	45·7	14·1	27·15	13·07

Operator B

	G	F	D	K
	per cent. w/w			
	48·7	14·6	23·6	13·1
	49·4	15·7	22·7	12·2
	45·0	15·2	25·3	14·5
	43·2	13·1	23·9	19·8
	47·5	15·8	24·8	11·9
	45·4	15·1	26·2	13·4
Mean ..	46·5	14·9	24·4	14·1

For the vessel the following details are regarded as essential—

(i) The return line to the circulating pump should not be of greater diameter than ¼ in.; otherwise, with a centrifugal pump, there is a tendency for the lighter ether layer to be circulated in preference to the salt solution, and this will lead to streaky and indistinct zones.

(ii) To prevent warping of the longer sides of the vessel it is advisable to fix metal strips along the top. In addition, to prevent warping and leakage of vapour, flanges should be *screwed* to the lid.

(iii) The spraying system should form a continuous ring round the vessel, with the pump feeding at two diametrically opposite points. In this way the sprays deliver at an even pressure and the two phases remain emulsified.

(iv) If the pump or any part of the apparatus is made of brass or other material attacked by the phthalate solution, the latter can be replaced by a 12 per cent. w/v solution of anhydrous sodium sulphate, which has approximately the same vapour pressure as 0·5 *M* phthalate.

(v) With the increased surface area of the lid condensation occasionally occurs. If the droplets fall on the paper strips the strips become waterlogged and separation does not take place. A piece of filter-paper fastened to the lid to absorb the moisture, or a gabled lid to induce the drops to run to the side walls, overcomes this difficulty.

APPLICATIONS TO FERMENTATION STUDIES—

The simplicity of this technique has led to its use in problems of fermentation. It is possible to investigate how the production of the different penicillins is affected by such factors as precursors, composition of the medium, aeration and culture variation. For example, a high rate of aeration favours the production of penicillin K, as is shown in Table V, where the proportion of penicillin K increases as the volume of medium in "shaken flask" cultures decreases.

TABLE V

Volume	100 ml.	80 ml.	60 ml.	40 ml.
K, per cent.	14·4	49·3	63·3	90·2
G, per cent.	85·6	50·7	36·7	9·8

The changing picture during fermentation has been followed on a small scale by the use of cultures that produce a mixture of penicillins predominantly G, F, D and K. Under the conditions used, the G content rose rapidly to a maximum and then decreased. This latter fall in G content was associated with an increasing production of penicillin K and an increasing biological titre. In fermentations of this kind, in which a mixture of penicillins is produced, the biological assay is of little value and can, in fact, be misleading.

Detection of "new" penicillins—During some work recently carried out on fermentation liquors, samples taken during the early stages of fermentation have shown a number of bands not associated with any of the major penicillins, X, G, F, D, K, but corresponding in position on the chromatogram to the minor constituents detected by the Goodall and Levi technique.[5] The entities responsible for these bands have not been isolated nor has their biological activity been determined; but, since they are decomposed by penicillinase one is led to suppose that they are similar in structure to the known penicillins.

TABLE VI

POSITIONS OF UNIDENTIFIED ZONES

	(*a*)	(*b*)
X	0·08	—
?	—	0·55
G	1·00	1·00
F	1·50	1·49
D	2·13	2·05

One species has been detected between the bands due to X and G, but, as this was in a sample from a single shaken flask culture, it has not been possible to follow its production or ultimate fate. A second "unknown" species, lying between D and K on the chromatogram, has been produced in the early stages of some stirred fermentations. The proportion rises to a maximum and eventually the band disappears as the fermentation is prolonged. Tables VI and VII indicate the positions of these two fractions, and the figures show the relative distances travelled by the various penicillins. In Table VI, the values in column (*a*) are the means of 64 determinations. In column (*b*) the existence of a compound producing a band between those due to penicillins X and G is indicated.

In Table VII the presence of a penicillin that produces bands between those due to penicillins D and K is shown. As the fermentation proceeds this band disappears.

TABLE VII

	60 hours	72 hours	84 hours
G	1·00	1·00	1·00
F	1·47	1·49	1·46
D	1·92	1·99	1·97
?	2·25	2·24	—
K	5·50	5·61	5·94

Thirdly, in many instances the K zone has been separated into its three components. In this connection a recent paper[7] on the estimation of the penicillins as their "R-group acids," indicates the heterogeneity of penicillin K, but the side chains of the individual compounds have not been established. A fourth mauve band appearing at the lower end of the pH 4 paper has been identified as due to phenylacetic acid; it also disappears as fermentation is prolonged.

These points indicate the complexity of the problems confronting those investigating fermentation. At this stage we offer no theories about the constitution of compounds that appear as minor constituents nor on their role or fate during fermentation, but it seems likely that further investigation of them would give useful information about the mode of production of the various penicillins.

Our thanks are due to numerous colleagues for providing samples for this investigation and to Boots Pure Drug Company Limited for permission to publish this paper.

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First submitted, *May*, **1949**
Amended, *June*, **1950**

The Isolation of the Lines of the Mercury Arc by Filters

With Especial Reference to Photo-electric Absorptiometry

BY J. W. NICHOLAS AND F. F. POLLAK

SYNOPSIS—The light filters normally used or recommended for use in England with the mercury vapour lamp photo-electric absorptiometer do not isolate monochromatic light, with the exception of the 577/9 mμ. lines. An improved series is described, which give monochromatic light in the more frequently used regions of the mercury spectrum, and an approximation to it in some regions which have not hitherto been used, *i.e.*, infra-red, red, orange and blue-green.

THE use of filter photometers in place of spectrophotometers for absorptiometric determinations has certain advantages such as lower cost, greater speed and greater suitability for routine use by relatively unskilled operators, but normally there are several disadvantages. Filter photometers are not suitable for measurements of more than one unknown in the same coloured solution and no specific extinctions can be determined and published for use by other workers on other instruments. Sensitivity and accuracy are also reduced by the use of mixed light, and apparent deviations from Beer's law occur when dealing with substances having sharp absorption bands (*cf.* Müller[1]).

States and Anderson[2] find that with a substance obeying Beer's law a curved calibration graph is to be expected when there is stray light, with the exception of the special case where the solution has equal densities for the stray light and the desired wavelength. Lothian[3] has shown that when a finite waveband is used instead of monochromatic light the effective wavelength varies with the absorption curve of the solution being examined, and that the measured density bears no easily predictable relationship to the true density.

Some of these difficulties can be obviated by the use of a mercury vapour lamp as light source, and this was available in the Zeiss Pulfrich photometer (Heilmeyer[4]) with filters said to give isolation of the 436, 546 and 577 mμ. lines.[1] Vaughan[5] used the mercury arc in conjunction with the Hilger Spekker photo-electric absorptiometer. Müller[1] points out that with a mercury arc and a highly selective filter, a filter photometer need not give results any less accurate than a spectrophotometer. In applying a photo-electric absorptiometer to spectrophotometric procedures we found certain discrepancies which led us to believe that, with the exception of Ilford No. 606 for the 577/579 mμ. lines, truly monochromatic light was not obtained with the filters normally advised or supplied for the purpose.

It has been suggested (*e.g.*, Stross[6]) that interference filters might have an application in absorptiometry because of their greater transmission. It should be noted, however, that although their transmission maxima are sharp, the positions of these maxima are subject to a tolerance of ±10 mμ., both between different filters and between different places on the same filter (Müller[7]). This tolerance makes it impossible for them to be used for spectrophotometric procedures with a continuous source, and may well lead to considerable difficulties with a line source. At their present stage of development it would appear that they present no advantages for the 365, 436, 546 and 577 mμ. lines. Staats[8,9] has described the design of filters for the isolation of all the main lines of the mercury arc. She employed an exclusively mathematical treatment based on published data for light source, filters and receivers. Her figures take no account of background spectrum immediately adjacent to the required line, and the region 532 to 561·5 mμ. is considered as identical with the 546 mμ. line, and 561·5 to 595 mμ. with the 577/9 mμ. lines. She also fails to mention the 3906·4 A. line, which would be passed by both her 365 and 405 mμ. filters. Unfortunately, Corning and Jena glasses only are used, and neither of these is available to the English analyst.

In the course of his development of absorptiometric methods for metallurgical analysis, Vaughan[5] has also examined a small number of filters spectrographically with the mercury arc. He gives a plate in which only Ilford 606 and Wratten 74 appear to transmit an adequate quantity of monochromatic light, but in the text gives Wratten 62 and Ilford 604 (omitted from the plate) as suitable alternatives to Wratten 74, and also suggests the use of Ilford 601 in the violet region, although his own plate shows this to be far from monochromatic. He mentions a suggested use of Wratten 36 + Wratten 2A for the isolation of the 436 mμ. line, but does not record any experiments with this combination. Unfortunately his plate does not include the red end of the spectrum, although he states that red and orange filters can also be used. He gives no details of spectrographic or photographic technique, but it appears from the absence of background and the suppression of the line at 4960·3 A. that his spectra are under exposed and that his filters have therefore been tested too leniently.

PRESENT PRACTICE FOR THE ISOLATION OF LINES

For convenience, the groups of closely adjacent lines that would not normally be resolved by a monochromator are considered as single lines and given a single nominal wavelength. Thus we shall refer to the 3650, 3654, 3662 and 3663 A. group as the 365 mμ. line.

365 mμ.—

Messrs. Hilger and Watts Ltd.[10,11,12] and Haywood and Wood[13] recommend Wood's glass (supplied by Hilger as H556) without any supplementary filter. This glass is apparently identical with Chance OX1, as is Ilford 828; the material appears to vary considerably from melt to melt. Our spectrogram, Fig. 1*b*, shows that it is not monochromatic in the ultraviolet when used alone, and in addition it transmits red and infra-red.

405 mμ.—

Hilger and also Haywood and Wood advise Chance 8 (OV1) + Wratten 2 for isolating the 405 mμ. line. Haywood and Wood describe OV1 as a heat absorbing filter, which it certainly is not, having a transmission at 750 mμ. of 46 per cent.[14] Gentry and Sherrington,[15,16] by using this combination, obtained curved calibration graphs for tungsten and therefore substituted Ilford 601 for Wratten 2. Although this combination is a great improvement, we found appreciable transmission of the 365, 391 and 436 mμ. lines.

436 mμ.—

For 436 mμ., Hilger and also Haywood and Wood recommend Chance 6 (OB2) and Wratten 50. Hadley[17] used Calorex (ON3) and Wratten 50, but found fluctuations due to heat effects on the gelatin filter. For this reason he preferred Calorex and Chance OB1.* As an alternative he suggests modifying the Spekker so that the right-hand filter is on the right-hand side of the drum. This difficulty has been overcome in the new model H760 of the Spekker (*cf.* Isbell[18]). Rogers,[19] Harrison,[20] de Lippa[21] and Lennard[22] used Ilford 601, as did Davis,[23] who obtained a curved calibration graph with the silicomolybdate colour (*cf.* Fig. 11).

546 mμ.—

Calorex and Ilford 605, as advised by Hilger and by Haywood and Wood, were used by Rogers,[19] British Iron and Steel Research Association[24] and Parker.[25] Calorex and Ilford 604, recommended by Vaughan, have been used by Edwards and Gailer,[26] Edwards and Robinson[27]

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* Manufacture now discontinued—Chance Bros. (personal communication).

KEY TO SPECTOGRAMS

		Relative* exposure, sec.
Fig. 1.	365 mμ.	
	(*a*) Unfiltered light source (control)	1
	(*b*) Wood's glass, 2 mm. (Hilger, H556)	3
	(*c*) Chance OB2, 1 mm. + Wratten 17 + Chance OX1, 3 mm. (recommended combination)	24
Fig. 2.	405 mμ.	
	(*a*) Control	1
	(*b*) Chance OV1, 2 mm. + Wratten 2	11
	(*c*) Chance OV1, 2 mm. + Ilford 601	48
	(*d*) Chance OV1, 4 mm. + Wratten 86C + Chance OB2, 1 mm. (the recommended combination (see Table II) gives an identical spectrogram, but is preferred because of lower red and infra-red transmission)	252
Fig. 3.	436 mμ.	
	(*a*) Control	1
	(*b*) Chance OB2, 2 mm. + Wratten 50	19
	(*c*) Chance ON13, 2 mm. + Ilford 601	8
	(*d*) Chance OB2, 2 mm. + Wratten 36 + Wratten 2A (two thicknesses)	21
	(*e*) Chance OB2, 2 mm. + Wratten 2A (three thicknesses) + Wratten 36 (recommended combination)	28
Fig. 4.	492 mμ.	
	(*a*) Control	16
	(*b*) Ilford 603 + 804	216
	(*c*) Ilford 603 + Wratten 5 + Ilford 302 + Ilford 804 (recommended combination)	684
Fig. 5.	546 mμ.	
	(*a*) Control	8
	(*b*) Chance ON13, 2 mm. + Ilford 605	256
	(*c*) Chance ON13 + Ilford 604	1420
	(*d*) Wratten 16 + 74 + Ilford 804 (recommended combination)	260

* Exposures are calculated for a slit width of 0·04 mm., although for the longer exposures a slit of 0·08 mm. and half the exposure time was actually used. It should be noted that with intense lines necessitating a control exposure of 1 sec. there was some suppression of the background and the 4960·3 A. line. Spectrograms have also been taken with ten times the exposure and they do not show any foreign line.

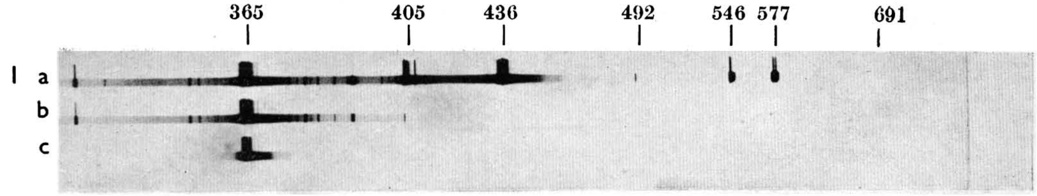

Fig. 1

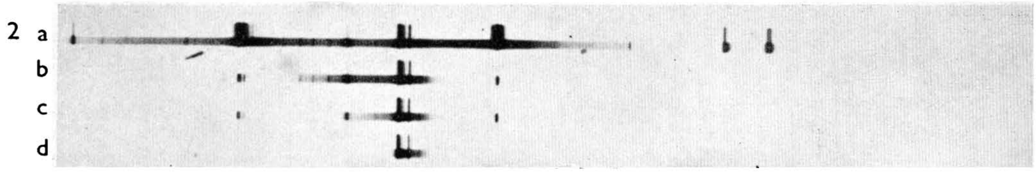

Fig. 2

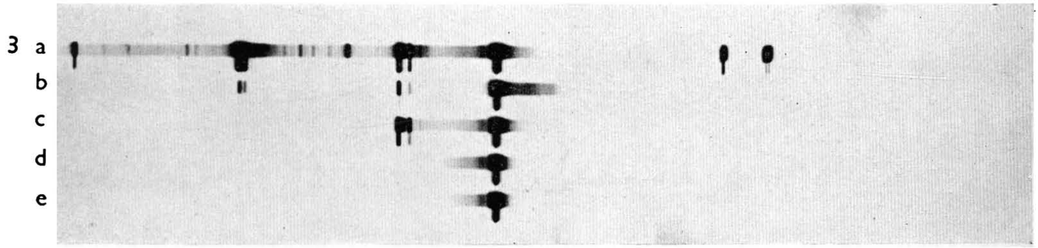

Fig. 3

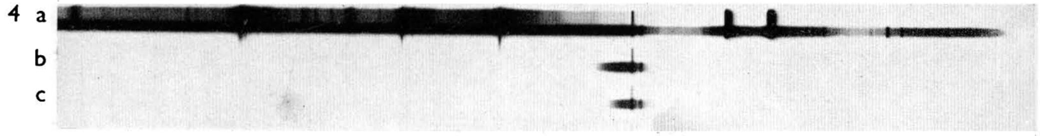

Fig. 4

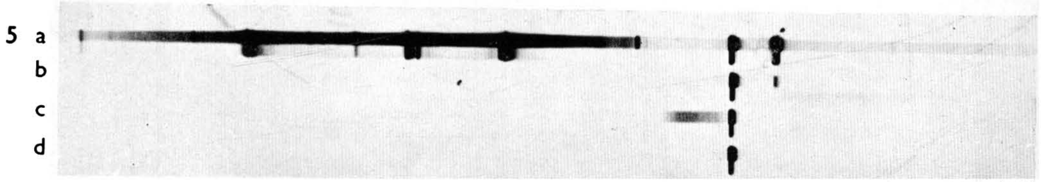

Fig. 5

Figs. 1–5. Spectrograms showing the isolating properties of various filter combinations (see Table II, p. 669, and Key on p. 664)

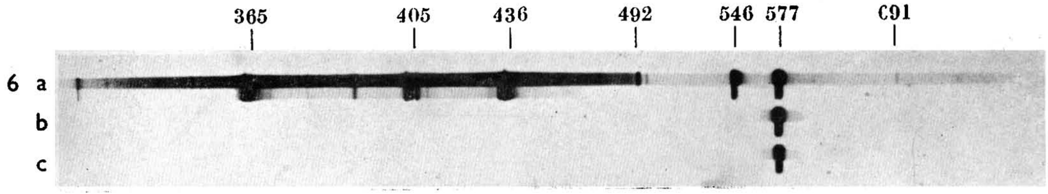

Fig. 6

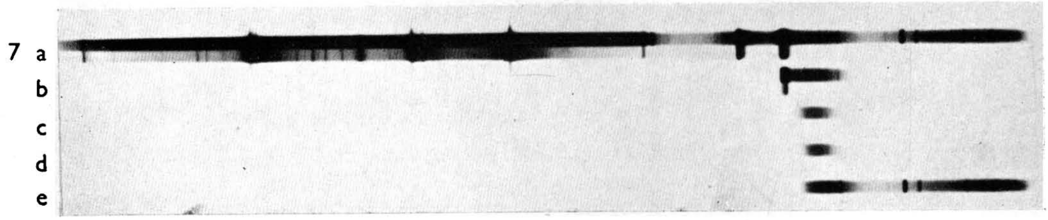

Fig. 7

Fig. 8

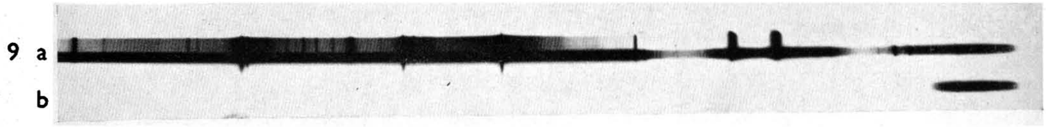

Fig. 9

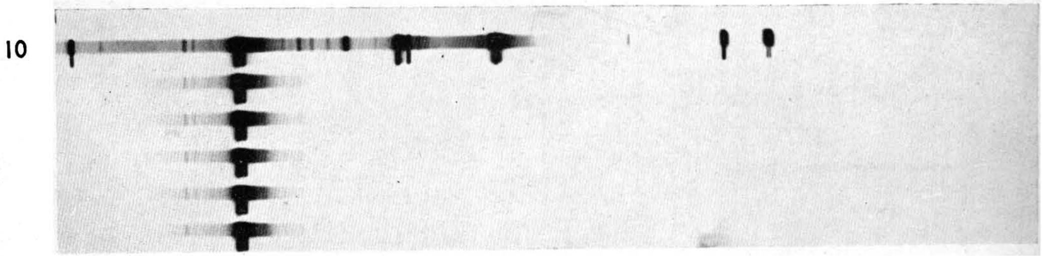

Fig. 10

Figs. 6–10. Spectrograms showing the isolating properties of various filter combinations (see Table II, p. 669, and Key on p. 665)

KEY TO SPECTOGRAMS—*continued.*

		Relative* exposure, sec.
Fig. 6.	577 mμ.	
	(*a*) Control	8
	(*b*) Chance ON13, 2 mm. + Ilford 606	182
	(*c*) Ilford 812 + 804 (recommended combination)	270
Fig. 7.	607 mμ.	
	(*a*) Control	30
	(*b*) Chance ON13, 2 mm. + Ilford 607	288
	(*c*) Ilford 202 + Wratten 25 + 804	1494
	(*d*) Wratten 26 + Ilford 804 (recommended combination)	1614
	(*e*) Wratten 25 + Ilford 801 ("orange-red" combination)	66
Fig. 8.	691 mμ.	
	(*a*) Control	30
	(*b*) Chance ON13, 2 mm. + Ilford 206 + Chance ON13, 2·5 mm. (recommended combination)	514
Fig. 9.	735 + mμ.	
	(*a*) Control	30
	(*b*) Wratten 88A (recommended filter)	48

Fig. 10. Uniformity of filters.

Spectrograms of a number of finished filters intended to isolate the 365 mμ. line.

Control exposure, 1 sec.

Relative exposure for filters, 25 to 28 sec.

* Exposures are calculated for a slit width of 0·04 mm., although for the longer exposures a slit of 0·08 mm. and half the exposure time was actually used. It should be noted that with intense lines necessitating a control exposure of 1 sec. there was some suppression of the background and the 4960·3 A. line. Spectrograms have also been taken with ten times the exposure and they do not show any foreign line.

and Lennard.[22] Harrison[28] abandoned 604 in favour of 605 because the latter was not sensitive to a yellow interfering colour. This suggests that some light of shorter wavelength than desired was passed by 604 (*cf.* Fig. 5). The possibility of using Wratten 74 has been mentioned by Vaughan and by Haywood and Wood. Fig. 5*b* shows the considerable yellow transmission of Ilford 605, and Fig. 5*c* shows the high transmission of blue-green background by Ilford 604.

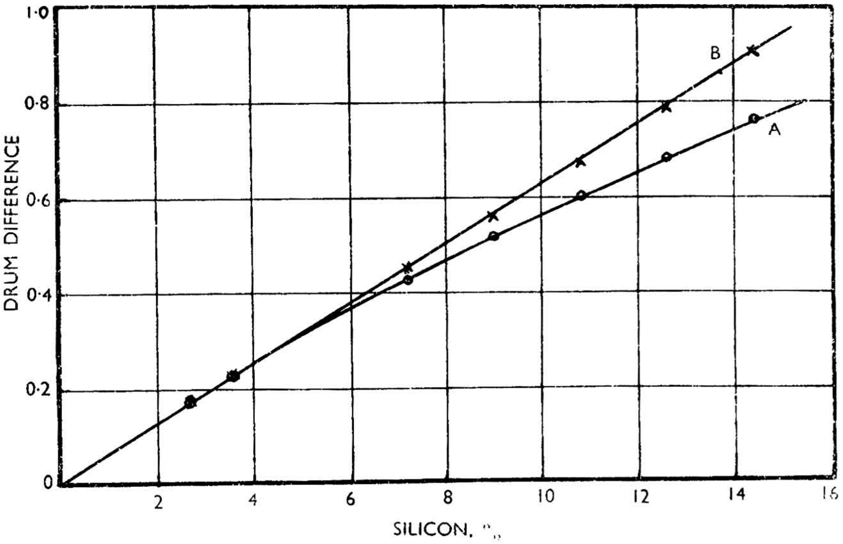

Fig. 11. Calibration curves for the estimation of silicon in aluminium as silicomolybdate. Curve A, with traditional combination of Chance 6 and Wratten 50; curve B, with proposed 436 mμ. combination. Final concentrations were: aluminium (containing 0 to 14·4 per cent. of silicon added as sodium silicate), 0·32 g. per litre; nitric acid, 0·128 *N*; ammonium molybdate, 10 g. per litre

577 mμ.—

For this line, workers have been almost unanimous in using Ilford 606, usually with Calorex as recommended by Hilger, by Vaughan and by Haywood and Wood, *e.g.*, Harrison,[20] British Aluminium Co.,[29] Bairstow, Francis and Wyatt.[30] Most workers report straight line graphs. Payne[31] has used Ilford 607 with Calorex. It is difficult to see the advantage of using such a combination (see Fig. 7*b*).

GENERAL CONSIDERATIONS

It has been suggested that glass filters are more satisfactory than gelatin. The usual cause of failure of filters appears to be heat by conduction and convection, and with careful use the effect of radiation is small. Filters are not exposed to heat in the H760 Spekker absorptiometer, and we assume that any user of the H560 instrument will have modified it as recommended by Hadley.[17] Some gelatin filters are markedly fluorescent, and if placed immediately in front of the photo-cell large errors occur. When placed in front of the solution under test the error will be very small, but we have nevertheless tried to eliminate the effects of fluorescence (*e.g.*, by absorbing the exciting or fluorescent radiation, or both, by non-fluorescent filters) and the filters recommended will give consistent results irrespective of their distance from the photo-cell. Fluorescent elements are marked (f) in Table II. It has been usual to place a heat-absorbing filter between the lamp house and the gelatin filter to protect the latter from radiant heat. Since a filter can only be heated by radiation being absorbed, the dyed gelatin films will scarcely be heated at all by the infra-red, although they will be heated by the strong visible lines. We have therefore allowed other considerations to determine the position of the heat absorber, and our filter combinations are intended for use in one direction only. The components in Table II are listed in the order in which light passes through them.

EXPERIMENTAL METHODS

Probable filter combinations were selected from the publications of Messrs. Kodak,[32] Ilford[33] and Chance[14] to ensure that filters commercially available in England were used, The following filters were examined in various combinations—

Wratten: 2, 2A, 4, 5, 8, 9, 16, 17, 21, 22, 25, 26, 29, 32A, 35, 36, 44, 44A, 45, 50, 62, 70. 73, 74, 75, 86C, 87, 88, 88A and 89.

Ilford: 201, 202, 206, 302, 601, 603, 604, 605, 606, 607, 608, 801, 802, 803, 804, 805, 806, 807, 808, 809 and 812.

Chance: OB2, OB10, ON12, ON13, OX1 and OV1.

Densities of individual filters were measured with a double-cell photo-electric absorptiometer that will be described elsewhere.[34] The filter combinations used on this instrument were improved as we went along, and for the final measurements were those we recommend later. The exposure required for any filter combination was determined from the sum of the densities of its elements at the required line, the aim being to make the photographic density the same as in a spectrum of the unfiltered light source in which the region of the required line showed considerable background. Spectrograms were taken of the mercury arc (Siemens lamp MB/D, 125 watt, as used in the Spekker) in the Hilger medium quartz spectrograph E498. The light source at a distance of 38 cm. from the slit was focussed on the collimating lens by the Hilger quartz condensing lens F1026. Slit widths of 0·04 and 0·08 mm. were used. A two-step sputtered metal filter (Hilger F1219), having densities at 450 mμ. of 0·0 and 0·98, was placed over the slit. Spectrograms were taken on Kodak III-L plates, which have a fairly uniform sensitisation extending as far as 900 mμ. Development was in Kodak D 19b developer, undiluted.

Messrs. Kodak's statement[32] that "all dyed gelatine film filters* transmit freely in the infra-red" indicated that this region required full investigation. In view of the difference between the spectral sensitivity of the plate and photo-cell, and in view also of the relatively wide range over which an integrated value was required, measurements were made on the absorptiometer already mentioned rather than on the plate. The absorptiometer was used as a single-cell instrument to measure the fraction of the light transmitted by the filter that would pass through Ilford 608, this value being corrected for the density of Ilford 608 itself. This method takes into account the spectral response curve of the photo-cell and the result is referred to as the *effective* red and infra-red transmission.

The effect of this transmission can best be shown by considering a hypothetical extreme case of a substance with a density of 0·801 (15·812 per cent. transmission) at the required wavelength and a density of zero in the red and infra-red. Let us assume that a density reading of 0·800 is actually obtained, corresponding to a transmission of 15·849 per cent. Let the effective percentage of red and infra-red light be R and let the intensity of the required line on the photo-cell at the zero setting be I; then at the zero setting the red and infra-red intensity will be $IR/100$, and the total intensity will be $I(1 + R/100)$. During the reading the intensity of the line on the photo-cell will be $\left(\frac{15{\cdot}812}{15{\cdot}849}\right) I$, and the intensity of red light will be $\left(\frac{RI}{15{\cdot}849}\right)$. As the totals at the zero setting and during the reading will be equal,

$$\left(\frac{15{\cdot}812}{15{\cdot}849}\right) I + \left(\frac{RI}{15{\cdot}849}\right) = I.\ \left(1 + \frac{R}{100}\right),$$

and therefore $R = 0{\cdot}044$ per cent. We have therefore tried to reduce R below this level by the incorporation of one or other of the following heat-absorbing elements: Chance ON13, OB2, Ilford 801, 802, 803 or 804. In the H760 model of the Spekker absorptiometer there is a built-in heat absorbing filter. This will normally be unnecessary, and in the case of the 691 mμ. and infra-red filters will interfere.

As an example of the effect of this stray radiation we show in Table I the density readings of a 1 in 100 dilution of blood as oxyhaemoglobin with various filter combinations intended

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* Ilford Ltd. manufacture a series of gelatin filters absorbing in the near infra-red (Nos. 801 to 804). These contain a copper salt, not an organic dye.

to isolate the 577 mμ. line. The solution has a sharp absorption band near 577 mμ. and very low red and infra-red absorption.

TABLE I

EFFECT OF VARIOUS FILTER COMBINATIONS INTENDED TO ISOLATE THE 577 Mμ. LINE

	Optical density	
	Ilford 812	Ilford 808
Alone	0·950	0·727
+ Chance ON13, 2 mm.	0·960	0·763
+ Chance OB2, 2 mm.	0·980	0·917
+ Chance OB2, 4 mm.	0·980	0·964
+ Ilford 801	0·959	0·785
+ Ilford 802	0·971	0·865
+ Ilford 803	0·972	0·884
+ Ilford 804	0·980	0·937

A water-cell only absorbs radiation of wavelength greater than 1·4 μ. and its effect on the *effective* infra-red transmission was found to be negligible. It was also found that a thermometer placed in a position corresponding to the left-hand photo-cell of the Spekker absorptiometer showed no significant rise in temperature. Moreover, Messrs. Evans Electroselenium Ltd. (the makers) state that infra-red is not harmful to their photo-cells. The left-hand water-cell is placed very near the lamphouse and its rise in temperature owing to conduction and convection causes the formation of air-bubbles with consequent drift in the zero setting. We have therefore abandoned the use of this cell, and where difference methods are employed the right-hand water-cell has also been dispensed with.

In the estimation of the sensitivity of the Spekker absorptiometer with our recommended filters, the left-hand aperture was completely closed by a shutter and the drum was opened to give a full-scale deflection (about 0·5 μ-amp.) on the Cambridge spot galvanometer at maximum sensitivity. Following Vaughan's nomenclature this gives the air-to-air setting that should give full sensitivity.

RESULTS—

In general, we used one basic filter to absorb as many foreign lines as possible, and one or more supplementary filters to obtain complete isolation. In addition, heat and red-absorbing filters were incorporated. The Cambridge spot galvanometer normally used with the Spekker absorptiometer has a sensitivity of 170 mm. per μ-amp., and will not give a satisfactory response with some combinations. For these the substitution of a more sensitive galvanometer, *e.g.*, Tinsley type VS6/45, is recommended. In view of the importance of the red region, which has not previously been used, an alternative filter combination of higher transmission but wider waveband is given. In all the red combinations the lines are much less intense in relation to the background and the filters do not give such strictly monochromatic light as the others. Following the practice of many workers with the tungsten lamp we shall refer to each region by its dominant wavelength, which in both cases is a line (607 and 691 mμ). There is no commercially available filter that will cut off the infra-red on the long wave side, so it is necessary to rely on the falling off in sensitivity of the photo-cell with increasing wavelength. Unfortunately different photo-cells vary in their response to infra-red, but no doubt the makers would be able to select cells having the desired characteristics. It should be noted that the response in the infra-red is more sluggish than in the visible and ultra-violet regions, but not enough to interfere unduly with the convenience of taking readings.

PREPARATION OF FILTERS—

For some wavelengths as many as five constituent filters were used. For convenience and to reduce the density by the avoidance of surface reflections we made compound filters containing all the gelatin films between glass plates, which were clear glass or glass filters according to requirements. Filters are usually cemented with Canada Balsam.[32] The yellowing of this with age may interfere with the transmission of wavelengths below 492 mμ., and it is possible that other media such as dammar or plastics may be preferable here (see Nicholas and Pollak[35]). It is not essential for colourless glass to be optically worked, as the light already has to traverse the optically imperfect silica and glass walls of the lamp, and we have found the glass of photographic plates perfectly satisfactory. Glass filters must be plane-parallel to avoid the introduction of density gradients.

TABLE II

RECOMMENDED FILTER COMBINATIONS

Wave-length, $m\mu$.	Recommended combination	Individual densities*	Over-all density (cemented)	Effective red and infra-red transmission, %	Air-to-air setting on Spekker for full deflection	Remarks
365	OB2, 1 mm.	0·627				
	Wratten 17 (f)	0·607				
	OX1, 3 mm.	0·229	1·411	<0·03	0·985	
405	OV1, 4 mm.	1·180				Requires sensitive galvanometer
	Wratten 86C†	0·902				
	OB10, 1·43 mm.	0·242	2·272	0·2		Combination amended November, 1950; spectrogram of this new combination is identical with Fig. *2d*.
436	OB2, 2 mm.	0·369				
	Wratten 2A (treble thickness) (f)	0·354				
	Wratten 36	0·852	1·410	<0·03	1·28	See Fig. 11
492	Ilford 603	1·030				
	Wratten 5	0·343				Requires sensitive galvanometer
	Ilford 302	0·110				
	Ilford 804	0·122	1·496	0·1		
546	Wratten 16	0·170				
	Wratten 74	1·085				
	Ilford 804	0·239	1·411	<0·03	1·52	
577	Ilford 812	0·967				Satisfactory results also obtained with Ilford 606, 812 or Wratten 73 with any heat absorber
	Ilford 804	0·627	1·527	<0·03	1·30	
"607"	Wratten 26 (f)	0·290				Includes 607 to 623 $m\mu$. lines.
	Ilford 804	1·119	1·374	—		Requires sensitive galvanometer
"691"	ON13, 2 mm.	0·333				Transmits 672 to 709 $m\mu$.
	Ilford 206	0·365				Requires sensitive galvanometer
	ON13, 2·5 mm.	0·499	1·239			
735 +	Wratten 88A		0·197	—		Requires sensitive galvanometer
"Orange red"	Wratten 25 (f)					Density measured at 607 $m\mu$. Not monochromatic; intended for use when only Cambridge spot galvanometer available
	Ilford 801		0·357	—	1·46	

NOTES—Filters marked (f) are fluorescent.
Filters not marked Wratten or Ilford are made by Chance Bros.

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* The density of many glass filters differs from melt to melt and the thickness is not therefore a complete specification. We have therefore given the density of elements in proved satisfactory combinations in addition.

† Messrs. Kodak have recently changed the composition of Wratten 86C without changing its designation or giving any other indication to the user. It is therefore probable that recent batches will not give results identical with ours. Suitable batches should correspond with the published absorption curve,[32] and have a density at 405 $m\mu$. of about 0·9. This combination is recorded in the hope that the original filter will again be made available.

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SILVER END
WITHAM, ESSEX

First submitted, *February*, 1950
Amended, *June*, 1950

The Use of Polarographic Methods for the Analysis of Fine Chemicals

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(*Read at the meeting of the Physical Methods Group on Friday, October 7th, 1949*)

SYNOPSIS—The function of polarographic methods in a laboratory dealing with the analysis of fine chemicals is discussed at length and followed by outlines of the methods at present in use. Detailed procedures are given for new methods.

THE analyst in charge of a laboratory controlling the production of thousands of different fine chemicals is faced with what appears to be an almost insuperable task. The vast variety of the samples to be analysed, together with all the various possible impurities in them and the ever-growing demand by customers for very pure chemicals of known analysis, sets the analyst a very difficult problem. It is only by the use of every possible modern tool that he is able to attempt to cope with the problem, and the polarograph is one of the most essential of these tools.

It must first be said that the application of the polarograph to any problem in such a laboratory is only justified if there exists no simpler or more rapid method by other techniques. Each particular application of the polarograph calls for very careful investigation before the method can be put into routine use, and it will be obvious, therefore, that the amount of research required would render its wholesale application to the analysis of thousands of different chemicals out of the question. The polarograph is, therefore, only used in our laboratory where it is justified on the grounds of accuracy, speed or simplicity, or where no other method is known to exist.

There are many hundreds of polarographic methods recorded in literature, but this paper deals mainly with those found to be useful in one particular fine chemical laboratory. We find the polarograph to be of most use for the estimation of trace amounts of elements or groupings, either organic or inorganic; it is seldom used for the determination of the major constituents in a chemical.

In the determination of trace elements or groupings in fine chemicals, it may be stated as a general rule that, if the half-wave potential of the trace to be determined precedes the half-wave potential of the compound under test, then the determination of the trace is usually a simple matter. Solution of the compound in water, in which the compound itself acts as a supporting electrolyte, followed by taking a polarogram should theoretically give the desired result. If, however, the half-wave potential of the compound under test precedes that of the trace being determined, then interfering elements or groupings must either be complexed or removed by some method such as electrolysis.

We find the most expedient method of measurement to be that using internal standards.[1] This greatly decreases the time required for a determination and avoids the use of thermostats. We find it advisable to make two separate additions of known amounts of the substance being determined and to take a polarogram after each addition to ensure that the increases in wave heights are linear.

INORGANIC TRACES

ZINC—

The polarograph is especially valuable for the estimation of zinc owing to the difficulty of estimating traces of this element by other methods. Zinc gives very well-defined waves both in acid and in alkaline solutions and can readily be estimated, *e.g.*, in ferrous sulphate,[1] cadmium salts,[2] aluminium salts[3] and copper salts. In the determination of zinc in ferrous sulphate it is not necessary to remove the iron, and this transforms what was a very difficult problem into a very simple one. For the determination of zinc in cadmium, it is necessary to remove the cadmium by electrolysis and we have found that in doing so there is no loss of zinc. This electrolysis must be from a solution made just acid with either sulphuric or perchloric acid, and the current should be made 3 to 4 amps., copper-plated platinum electrodes[4] being used. Similarly, in copper salts, the copper is removed by electrolysis prior to the polarographic estimation. The determination of zinc in organic compounds falls into two classes, (*a*) those compounds, such as insulin and thiouracil, which must first be subjected to wet oxidation because they yield interfering waves, and (*b*) those substances that do not give interfering waves. Zinc may be determined directly in phenol after treatment with excess of caustic soda. If an organic sample is wet oxidised with sulphuric and nitric acids before the polarographic estimation of zinc, care must be taken to remove all traces of residual nitrate by treatment with ammonium oxalate because the nitrate group has a half-wave potential in alkaline solutions close to that of zinc and can cause serious interference. It is better to use sulphuric and perchloric acids whenever possible. Kolthoff[5] states that the nitrate group gives no interference in alkaline solution, but this is not our experience.

Another interesting procedure recently developed is for zinc in thorium salts, when the thorium is complexed with sulphosalicylate at pH 8·5 in the presence of a small amount of gelatin. The method is excellent for the range of 20 p.p.m. to 1·0 per cent. of zinc in thorium.[6]

NICKEL—

There are a number of elegant methods for the determination of nickel in the presence of many elements, but most of these are unsuitable for the determination of small quantities of nickel in the presence of large amounts of cobalt. Traces of nickel can be determined in cobalt salts by the simple expedient of neutralising and then complexing the cobalt with ammonium thiocyanate.[7] This effectively separates the cobalt and nickel waves. When dealing with complex amino-cobalt compounds or organic salts of cobalt it is preferable to convert them to cobalt sulphate by wet oxidation with sulphuric acid before neutralisation. The colorimetric determination of nickel in the presence of large amounts of cobalt was extremely tedious, even by those methods that were accurate, whereas by aid of the polarograph it is simple and rapid.

COBALT—

The problem of determinating small traces of cobalt in nickel has been for a long time unsolved, although polarographic methods were known by which it was possible to separate large traces of cobalt from nickel, *e.g.*, those involving the use of ammonium oxalate as a supporting electrolyte.[8] None of the methods proposed, however, was found to be satisfactory for the determination of small traces of cobalt in nickel. A method has been proposed lately involving the use of Trilon B (ethylene diamine tetra-acetic acid) as a base solution.[9] We have tried the method suggested, but quite apart from the fact that the sensitivity claimed by the authors is poor, not indicating less than 0·4 per cent. of cobalt in nickel, we have not succeeded in obtaining good waves for low concentrations of cobalt. We feel that the application of Trilon B to this particular problem is far more likely to be solved on a photometric basis. We are, however, proceeding with further polarographic experiments with this reagent.

LEAD—

Lead is not normally determined polarographically in our laboratories as other methods exist, but in certain cases where it is tedious to estimate by these other methods, such as in ferrous sulphate,[1] it is rapidly determined polarographically, using the salt as the ground solution. Reliable results are obtained. Lead can be determined directly in nickel salts using the nickel salt as supporting electrolyte.[10] Another useful application is the determination of traces of lead in zinc salts simply by dissolving the salt in dilute hydrochloric acid and taking a polarogram.[11] Lead is also determined in cadmium salts by using a cyanide supporting electrolyte when the lead wave appears about −0·4 volt before the cadmium wave. We have also found that we can determine lead directly in phenol by adding excess of sodium hydroxide and then taking the polarogram. Lead may be determined in the presence of large amounts of tin, aluminium and iron since at pH 6·5 to 7·0 these elements are not reduced, although lead gives a wave at about −0·5 volt. Nitrates and free hydrochloric acid must be eliminated as they give drawn-out waves.[12] The supporting electrolyte is a 30 per cent. solution of calcium chloride. Lead can be determined in aluminium salts in a sodium carbonate ground solution. Interference from tin is prevented by oxidation, from iron by reduction in alkaline solution and from copper by precipitation with potassium thiocyanate.[13]

COPPER—

Trace amounts of copper are not normally determined on the polarograph since the colorimetric method using sodium diethyldithiocarbamate is so rapid and simple. The polarographic method is, therefore, only used where it is difficult to apply other methods. For example, with ferrous salts we have found it advantageous to use the polarographic method which does not involve the removal of the iron.[1] As in the case of lead, copper can be determined directly in nickel salts by using the nickel salt as supporting electrolyte.[10] Copper may also be determined polarographically in the presence of cadmium, nickel, zinc and manganese in lead and its salts.[14] Davies and Key[15] have described a method in which copper is determined in the presence of iron with *N* potassium fluoride or sodium potassium tartrate as supporting electrolyte.

CADMIUM—

The polarographic estimation of this element is most usefully applied in our laboratories to the determination of traces in zinc salts. Since the cadmium wave precedes the zinc wave by about 0·4 volt it is only necessary to dissolve the zinc salt in water and take a polarogram; the salt itself serves as supporting electrolyte.[11]

IRON—

Iron is not normally determined polarographically as so many other methods exist, but we find it useful to determine this element in cobalt salts by dissolving the salt in alkaline tartrate solution when, if all the iron is present in the ferric state, its wave appears well before the cobalt wave. We have not found this method much use below 0·1 per cent. of iron, although at and above this figure good waves are obtained. This procedure has also been recommended for the determination of iron in manganese salts.[16]

A method has recently been proposed whereby iron may be determined in an acidic oxalate supporting electrolyte at pH 5 provided the iron is first reduced to the ferrous state. With the exception of copper, which must be removed, no other ion soluble in dilute acid solution containing sulphur dioxide gives an interfering wave. It is claimed that for small amounts of iron this procedure is more accurate than either volumetric or gravimetric procedures.[17]

VANADIUM—

Vanadium is sometimes determined polarographically in organic materials after wet oxidation,[18] although it is not usually determined polarographically in fine chemicals.

TELLURIUM—

We find it convenient to determine small amounts of tellurite in the presence of a large amount of selenite[19] by addition of a slight excess of ammonia, de-oxygenation with sodium sulphite and polarography over the range −0·5 to −1·0 volt. It is advisable to add a little gelatin to improve the wave shape.

TIN—

A useful method for the determination of tin in phenol has been published,[20] but normally we do not determine this element polarographically.

ANTIMONY AND BISMUTH—

Page and Robinson[21] have described methods for the micro-estimation of antimony and bismuth in inorganic and organic compounds. Tervalent antimony may be directly determined; quinquevalent antimony must first be reduced. In *N* sulphuric acid the half-wave potential of antimony and bismuth are respectively −0·34 and −0·02 volt and in *N* nitric acid they are −0·17 and −0·03 volt. Thus it is possible to estimate traces of both in sulphuric acid; the wave, however, coalesces in hydrochloric or nitric acid. Bismuth can be determined in copper salts by polarography in tartrate and citrate media.[22] In acid tartrate of pH 4·5, bismuth can be determined in the presence of large amounts of lead and cadmium.

ALKALINE EARTHS—

The alkaline earths are reduced at large negative potentials. Thus their trace estimation in the presence of most other elements is impossible without some preliminary chemical separation, so that a polarographic method has no advantage over the standard technique. Zlotowski and Kolthoff,[23] however, claim that barium, strontium and calcium can be determined in the presence of each other in alcohol - water media. When present in approximately the same amounts, the barium wave appears first followed by the strontium wave and finally the calcium wave. Magnesium interferes with the calcium determination. In our laboratories, however, we have found the flame photometer to be the ideal instrument for determination of trace amounts of alkalies and alkaline earths, and we do not, therefore, see any reason for application of the polarograph.

NITRATE—

The determination of nitrate in sodium nitrite can be readily accomplished by means of the polarograph. Haslam and Cross[24] have described a method in which the nitrite is first decomposed with sodium azide in hydrochloric acid solution and, after concentration, the solution is polarographed in a lanthanum base solution over the voltage range −1·2 to −2·1 volts. This method is satisfactory over the range 0·04 to 1 per cent. of sodium nitrate.

NITRITE IN NITRATE—

In the presence of uranyl ions and dilute hydrochloric acid, nitrite and nitrate ions are both reduced at approximately —0·9 volt (*versus* the saturated calomel electrode). The diffusion current is proportional to the nitrite concentration when the ratio of uranyl ion to nitrite is greater than unity. Under these conditions, the reduction of nitrite involves three electrons, indicating a reduction to nitrogen. Analysis of the wave shows that the reduction is irreversible. A solution can be analysed for both nitrate and nitrite ions in two polarographic experiments. The diffusion current due to the two constituents in the original solution is first measured. The nitrite in a second sample is oxidised to nitrate by hydrogen peroxide in acid solution, the excess of peroxide is destroyed catalytically by manganese dioxide in alkaline solution, and the diffusion current is measured.[25]

IODATES IN IODIDE—

We have found it very convenient to determine iodates in iodides when both are water-soluble, *e.g.*, the potassium salts. For potassium iodide, the solution is made slightly alkaline with sodium hydroxide, de-oxygenated with hydrogen and a polarogram is taken, when the iodate wave appears at about −1·2 volts.

BROMATE IN BROMIDE—

Bromate in bromide may easily be determined in the same way as iodate in iodide. It is, unfortunately, not possible to determine chlorate in chloride in a similar manner owing to the fact that the reduction potential of the chlorate is higher than that of the supporting electrolyte.

ORGANIC APPLICATIONS

In the field of fine organic chemical analysis, the use of the polarograph has been less well developed than in the inorganic field. Quantitative organic polarography in general has from the beginning lagged behind inorganic developments. However, among other applications of polarographic methods to organic analysis the following are valuable methods in the analysis of fine chemicals.

ALDEHYDES IN ALCOHOL—

The rapid and accurate determination of acetaldehyde in ethyl alcohol has become possible by means of the following procedure, which is an application of the method by Adkins and Cox[26] for the polarographic measurement of aldehyde.

The alcohol is mixed in equal proportions with a 1 *M* solution of ammonium chloride and, after de-oxygenation of the solution, a polarogram is taken, when the acetaldehyde wave occurs in the range −1·0 to −3·0 volts. Internal standards are then added and measurement made as usual. We have found this method to have great advantages over the usual method with Schiff's reagent.

FURFURALDEHYDE IN FORMALDEHYDE—

Reduction of furfuraldehyde occurs in acid, neutral and alkaline media giving a single wave in acid and alkaline solutions and two waves in solutions of pH 4·0 to 7·0. Within this range, with increasing acidity the first wave gets smaller and the second larger, but the total wave-height remains unchanged. With pH 7, the reduction potential is 0·25 to 0·30 volt more positive than that of hydrogen, and under these conditions formaldehyde does not interfere.[27]

NITROBENZENE IN ANILINE—

Small quantities of nitrobenzene in aniline may very conveniently be determined by the method of Haslam and Cross.[28] To a known amount of the sample is added a little concentrated hydrochloric acid containing nigrosine and a polarogram is taken. From 0·01 to 0·05 per cent. of nitrobenzene in aniline can be determined with an accuracy closer than 4 per cent.

GAMMA ISOMER IN GAMMEXANE (BENZENE HEXACHLORIDE)—

A useful method of assay as distinct from the trace determinations so far discussed is the determination of the gamma isomer of benzene hexachloride as described by Dragt.[29] The gamma isomer is the only one of the five isomers reduced at the dropping mercury electrode under the conditions described. The method consists of taking a solution of the isomers in acetone, alcohol and water, buffered with a potassium chloride - sodium acetate buffer and, after de-oxygenating, taking a polarogram through the range −0·5 to −2·0 volts. Other methods exist for the determination of this isomer, but this is by far the most rapid with the possible exception of the infra-red method.

MALEIC - FUMARIC ACID MIXTURES—

It is claimed that there exists no satisfactory method for the estimation of these acids in the presence of each other, except by a recent polarographic method.[30] This method enables the analyst to solve the problem in a simple manner by polarography in an ammonium hydroxide - ammonium chloride buffer solution of pH 8·2 as supporting electrolyte. The maleate wave precedes the fumarate wave. If interfering substances are present, maleic and fumaric acids must be precipitated as their barium salts in alcoholic solution. The precipitate is soluble in the base solution.

Our experience with this method has not been particularly fortunate. We find that the method works quite well when maleic and fumaric acids are present in roughly equal quantities. It fails entirely when there is an overwhelming proportion of one or other constituent. Thus we were quite unable to detect 6 per cent. of fumaric acid added to maleic acid. The authors of this method refer throughout their work to roughly equal quantities of both and make no reference to unequal proportions. We find that 25 per cent. of fumaric acid had to be present before we could detect the slightest sign of a second wave.

PEROXIDES AND ALDEHYDES IN ETHER—

The method normally employed in our laboratories for the determination of peroxides in ether is based on the oxidation of ferrous thiocyanate and subsequent measurement of colour. Recently, however, we have been trying out the polarographic method of Gosman.[31] Peroxide and aldehyde are extracted from the ether by shaking with an equal volume of 0·01 *N* lithium hydroxide and determined by polarography of the aqueous layer. The peroxide wave occurs at −1·3 volts and the acetaldehyde wave at −1·8 volts. It is claimed that this method shows waves where chemical methods have failed to detect any peroxide. The method has the disadvantage that not all the peroxide or aldehyde is extracted in one shaking with the lithium hydroxide. From the partition coefficients, which are 0·45 for peroxide and **0·63** for aldehyde, it is possible to calculate the amount originally present in the ether. The waves are measured by comparison with the increases in wave height obtained by the addition of dilute solutions of hydrogen peroxide and acetaldehyde. In spite of its apparent disadvantage, it appears that the method is a promising one.

PEROXIDE IN DIOXAN (DIETHYLENE DIOXIDE)—

Peroxide may be determined in dioxan very easily by the following technique. The dioxan is mixed with an equal volume of *M* lithium hydroxide solution and, after de-oxygenation with hydrogen, a polarogram is taken over the range −0·5 to 2·0 volts. To estimate the peroxide content a known volume of a standard diluted hydrogen peroxide solution is added and polarography repeated.[32]

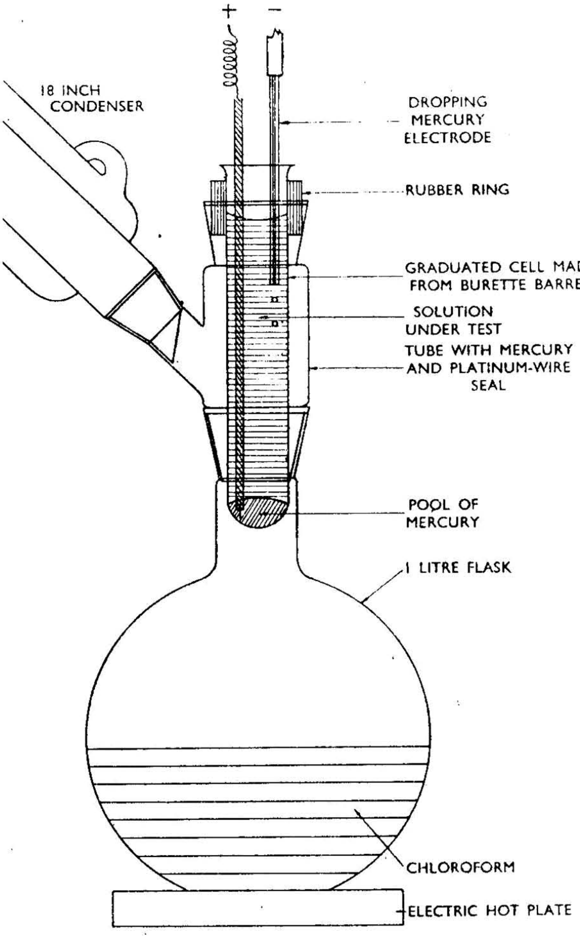

Fig. 1

HYDROGEN PEROXIDE—

The polarographic determination of hydrogen peroxide can be carried out by making use of the reduction step at −1·0 volt *versus* the saturated calomel electrode. The method is limited to peroxide concentrations of less than 0·15 per cent., owing to the oxidation of mercury by hydrogen peroxide at higher concentrations. If a stationary platinum micro-electrode is used in place of the dropping mercury electrode, current - voltage curves in the range from 0 to −0·6 volt *versus* the saturated calomel electrode show a reduction step whose height is proportional to the hydrogen peroxide concentration over a much wider range, and determinations can be carried out simply by measuring the limiting current at −0·6 volt. The upper limit of peroxide concentrations that can be measured depends on the concentration of the supporting electrolyte, and if saturated potassium chloride solution is used, concentrations up to 0·9 per cent. can be determined.[33]

THIOMERSALATE—

Page and Waller[34] have recently described an interesting polarographic method for the estimation of thiomersalate. A well-defined wave at about −0·5 volt is obtained in *N* hydrochloric acid. This method is very useful for the determination of this antiseptic in vaccines and pharmaceutical preparations.

THE DETERMINATION OF BENZANTHRONE AND ANTHRAQUINONE IN THE PRESENCE OF EACH OTHER—

An interesting application has been suggested[35] for these two chemicals since both give a good wave in 70 per cent. methyl alcohol containing 0·1 *N* sulphuric acid. Benzanthrone has a half-wave potential at 0·96 volt while anthraquinone has a half-wave potential at −0·36 volt. The wave height is proportional to the concentration and this simplifies the determination of small quantities of one in the presence of large amounts of the other.

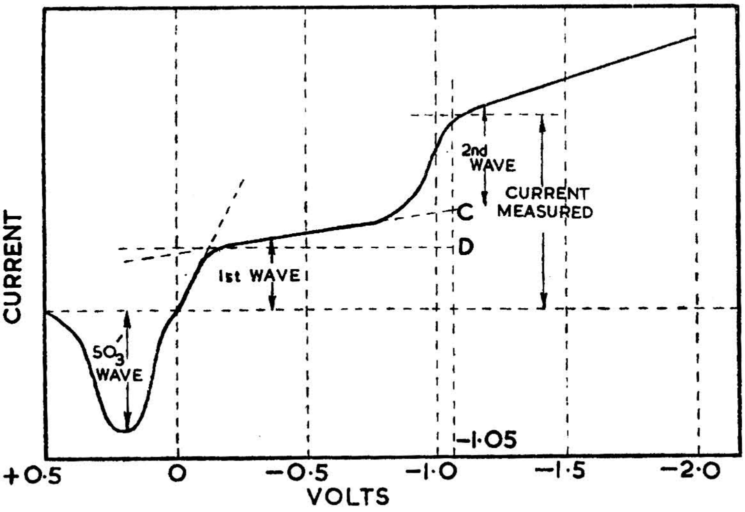

Fig. 2

DETERMINATION OF PHENYL MERCURIC ACETATE IN A GELATIN BASE—

Some recent interesting experimental work in the B.D.H. laboratories has led to the development of a method by L. J. J. Hillman for the determination of small quantities of phenyl mercuric acetate in a gelatine base. Page[34] had reported two waves for this compound in dilute hydrochloric acid base solution and this has been confirmed by us.

Our problem was complicated by the presence of the large amount of gelatin, which made the solution very viscous at room temperature, and consequently it gave an erratic polarogram owing to the mercury drop not falling at a steady rate.

This difficulty was overcome by conducting the experiment at an elevated temperature in a specially constructed polarographic cell. Details of this cell are shown in Fig. 1. It consists of a graduated cell made from a burette barrel by sealing one end. The cell is heated by immersion in the refluxing vapour of an organic liquid. The solvent used to produce a constant temperature was chloroform (AnalaR) and the working temperature was found to be 58° ± 0·2° C. It is essential that the temperature be kept constant.

Preparation of calibration curve—A standard solution of phenyl mercuric acetate was made up as follows. Take 6 g. of glycerol and dissolve in it 0·15 g. of phenyl mercuric acetate, add 2·25 g. of triethanolamine and make up to 100 ml. with water. Take various known amounts of this solution and to each add 0·5 ml. of 0·01 per cent. methyl red solution, 0·4 g. of gelatin and 2 crystals of sodium sulphite (AnalaR). Make the volume of each portion to 10 ml. with distilled water. Place each solution in turn in the polarographic cell and heat in the vapour of the refluxing chloroform for about a quarter of an hour so as to allow a uniform temperature to be achieved. Then set the potentiometer at −1·05 volts, adjust the zero current and condenser current to give a good deflection, and take the reading on the galvanometer scale. Disconnect the lead to the polarographic cell so that it is no longer in circuit and take another reading; the difference between these two readings gives the diffusion current from the cell at −1·05 volts, which is proportional to the phenyl mercuric acetate concentration.

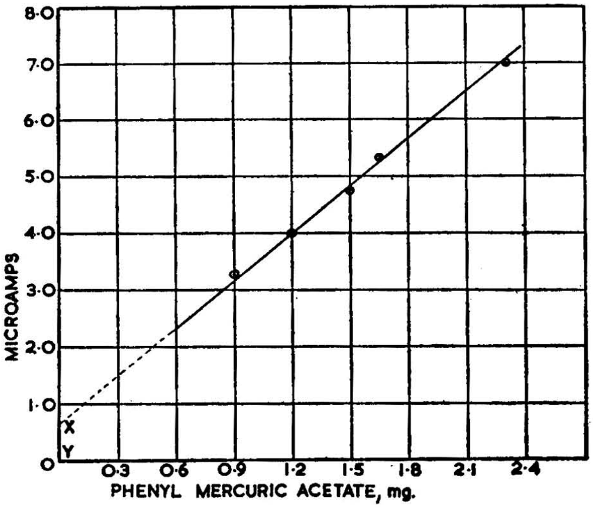

Fig. 3

It is necessary to adopt this procedure since an anodic wave due to sulphite just before the first phenyl mercuric acetate wave makes it impossible to estimate the residual current (see Fig. 2). Plot the diffusion current obtained from the above solutions against the respective concentrations of phenyl mercuric acetate when a straight line graph (Fig. 3) is obtained. Sodium sulphite was used as it was not possible to obtain a satisfactory degree of de-oxygenation in a reasonable time by the use of hydrogen.

The graph does not pass through the origin since the current measured by this method includes the residual plus the diffusion current at −1·05 volts. It will be found that the current in microamps represented by XY in Fig. 3 corresponds to that represented by CD in Fig. 2, and their values correspond to the residual current at −1·05 volts.

Accuracy—By means of the calibration graph so constructed, a series of twenty samples containing known amounts of phenyl mercuric acetate were estimated by an operator who did not know the true contents. All the results obtained were found to be within ±5·0 per cent. of the true value.

Thanks are due to Mr. A. Jewsbury, B.Sc., A.R.I.C., and to Mr. L. J. J. Hillman of these laboratories for assistance and constructive advice in the preparation of this review, and to the Directors of the British Drug Houses, Ltd., for permission to publish.

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First submitted, *November*, 1949
Amended, *April*, 1950

Attempts at the Potentiometric Determination of Chloride in Biological Fluids with the Silver - Silver Chloride Electrode

BY McD. DUXBURY

SYNOPSIS—Unsuccessful attempts to use the silver - silver chloride electrode for the potentiometric determination of chloride in biological fluids are described.

It is shown that this electrode can be used for the rapid and accurate determination of chloride in simple non-biological fluids. The construction of a simple calomel half-cell suitable for this purpose is described.

The poisoning effect on the silver - silver chloride electrode of various substances has been investigated. The effect of glycine on the electrode has also been investigated.

THE method of using the silver - silver chloride electrode for the determination of chloride in biological fluids was suggested in 1947 by Dr. W. R. Domingo, of Kampen (Netherlands), who had been using the method for the determination of chlorides in soil. The writer was asked by Prof. E. J. King to see whether the method could be used for the determination of chloride in routine biochemical analysis.

In the past, nearly all the work done on the silver - silver chloride electrode has been by physical chemists studying ionic activities. The electrode has also been widely used for potentiometric titration. In the present work the electrode has been placed in a chloride solution of unknown concentration and the potential measured. From this potential the concentration of chloride ion that controls the electrode potential has been determined from a potential - concentration graph prepared by calibration of the electrode with standard sodium chloride solutions.

METHOD

PREPARATION OF THE SILVER - SILVER CHLORIDE ELECTRODES—

The electrodes were prepared by two methods, (*a*) after Dr. Domingo, and (*b*) after A. S. Brown.[1] In method (*a*) free cyanide is present during the deposition of silver, and in method (*b*) all traces of free cyanide are excluded. The platinum electrodes used were of the plate type with a total surface area of about 0·5 sq. cm.

(*a*) *After Dr. Domingo*—The electrodes consisted of squares of platinum of surface area 0·5 sq. cm. These were silver-plated in a solution containing 1·6 g. of silver nitrate and 1·9 g. of potassium cyanide per 100 ml. (C.D. 5 mA. per sq. cm. for 1 hour) and then "chloridised" in a 2 per cent. potassium chloride solution (C.D. 1 mA. per sq. cm. for 30 minutes).

(*b*) *After A. S. Brown* (*modified*)—The electrodes consisted of circular plates of platinum of surface area 0·5 sq. cm., the surface being highly polished and the edges smooth so as not to produce focal points during electrolysis. The silver-plating solution was prepared by adding a solution of silver nitrate (2 g. in 50 ml. of water) to a solution of potassium cyanide (2 g. in 50 ml. of water) until there was a small amount of undissolved silver cyanide to ensure that there was no free cyanide present. The electrodes were silver-plated in this solution by using a platinum foil anode, and to prevent the liberated hydrocyanic acid from contaminating the cathode, the electrodes were in separate containers joined by an inverted U-tube acting as a liquid junction. The electrodes were silver-plated for 6 hours at a C.D. of 0·5 mA. per sq. cm. and then, after having been washed in running water for 2 days, they were "chloridised" in a 1 per cent. sodium chloride solution for 1 hour at a C.D. of 0·5 mA. per sq. cm.

The author found that the characteristics of both types of electrodes were approximately the same.

PREPARATION OF SATURATED CALOMEL ELECTRODE—

This was prepared as described by Britton[2] with the following modifications. A potassium nitrate - sodium nitrate - agar gel bridge was used in place of the usual saturated potassium chloride bridge so as not to contaminate with chloride the solution under test. In order to reduce errors due to liquid-junction potentials to a minimum, a solution was used in which the mobilities of the ions were balanced. This solution consisted of the two salts in the ratio $KNO_3 : NaNO_3 = 6{\cdot}3 : 1$, the solutions being saturated in respect of potassium nitrate. The apparatus, which is shown in Fig. 1, has proved to be satisfactory in the determination of chloride in non-biological fluids, and is simpler than the lead sulphate electrode described by Scott.[3]

POTENTIOMETER—

A simple slide wire type potentiometer was used—Unicam H.1 Portable Potentiometer. Since polarisation took place when the electrodes were made to supply a current, the following null method was used in taking potentiometer readings. The potentiometer was set to give a reading below the expected reading. The potentiometer key was momentarily depressed and the deflection of the galvanometer needle observed. The potentiometer readings were then increased by successively smaller increments (5 to 1 mV.) and after each increment the key was again momentarily depressed and the deflection observed. This process was carried out until there was no movement of the galvanometer needle upon depressing the key. To obtain an accurate reading, the potentiometer was set to 1 mV. below the last reading obtained and then the setting was increased by 0·25 mV. at a time until there was just no movement on momentarily depressing the potentiometer key. The observation of small movements of the needle was facilitated by means of a black hair line and a small hand lens fixed over the null-point. By this means, readings within 0·25 mV. could be taken.

Using this type of potentiometer, which caused the silver - silver chloride electrode to give a current while a reading was being taken, it was found that the usual wire type electrode, owing to its small surface area, tended to polarise more rapidly than the plate type of electrode. The plate type electrodes were more sensitive to small changes in potentiometer settings than the wire type electrodes.

VARIABLE POTENTIOMETER READINGS—

The plate electrode was tested in standard sodium chloride solutions from 100 mg. of sodium chloride per 100 ml. to 1 mg. of sodium chloride per 100 ml. It was found that the potential readings of successive series gradually increased. As an example, readings for a 10 mg. of sodium chloride per 100 ml. solution in successive series were 122·7, 123·2, 124·5, 125·0, 126·8, 128·2, 129·2 and 131·8 mV.

Various possible causes for these variable readings were considered and investigated. They were: (1) contamination by diffusion of chloride through the agar gel bridge, (2) temperature changes, (3) irregular concentration of silver chloride attained when the silver - silver chloride electrode was placed in the solution under test, (4) potentiometer inaccuracies and (5) "ageing effect" caused by concentration polarisation as described by Smith and Taylor.[4] All causes except the last were eliminated.

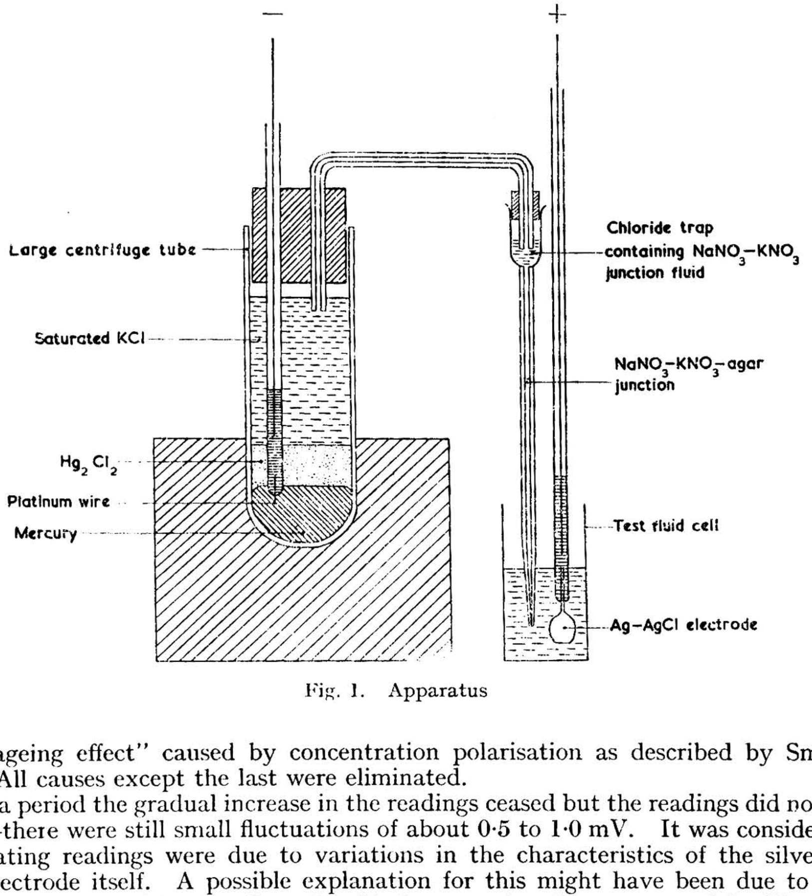

Fig. 1. Apparatus

After a period the gradual increase in the readings ceased but the readings did not remain constant—there were still small fluctuations of about 0·5 to 1·0 mV. It was considered that the fluctuating readings were due to variations in the characteristics of the silver - silver chloride electrode itself. A possible explanation for this might have been due to the uncovering of "hot spots" in the silver chloride layer, as has been suggested by Hornibrook.[5]

The electrode was tested, after it had aged, against a series of standard sodium chloride solutions ranging from 100 to 1·0 mg. of sodium chloride per 100 ml. and the resulting figures (Table I) were plotted logarithmically. Comparison of the figures in columns 2 and 3 shows that the observed values for the e.m.f. of the cell (E) fall very close to those calculated from the straight line drawn through the experimental points for 50 mg. per 100 ml. and 1 mg. per 100 ml. on the graph of E against the logarithm of the chloride ion concentration (log C)

$$E\ (\text{calc.}) = 179{\cdot}2 - 51{\cdot}4 \log C$$

where 179·2 (mV.) is the point at which the straight line intercepts the X axis, and where $51{\cdot}4 = (179{\cdot}2 - E)/\log C = \cot (180 - \phi)$, ϕ being the angle which the straight line makes with the X axis.

RESULTS

ATTEMPTS AT THE POTENTIOMETRIC DETERMINATION OF PLASMA AND CEREBRO-SPINAL FLUID CHLORIDE—

An attempt was then made to determine the chloride in plasma after initially calibrating the electrode against four standard NaCl solutions. The potentiometric determinations of chloride were carried out on 1 in 100 dilutions of plasma, the chloride content of which had previously been determined by the iodometric method of Haslewood and King.[6,7] The potentiometric determinations were carried out with (*a*) proteins present, and (*b*) proteins precipitated by the zinc method of Somogyi.[8]

TABLE I

POTENTIOMETER READINGS FOR STANDARD SODIUM CHLORIDE SOLUTIONS

NaCl concn., mg./100 ml. C	Potential difference observed (mV.) E	Potential difference calculated (mV.) E (calc.)	Difference (mV.) E − E (calc.)
100	76·8	76·4	+ 0·4
90	79·0	78·8	+ 0·2
80	81·0	81·4	− 0·4
70	84·0	84·4	− 0·4
60	87·5	87·8	− 0·3
50	91·8	91·8	0·0
40	96·5	96·9	− 0·4
30	102·2	103·4	− 1·2
20	112·8	112·3	+ 0·5
10	127·2	127·8	− 0·6
9	130·2	130·2	0·0
8	133·2	132·8	+ 0·4
7	136·2	135·8	+ 0·4
6	139·5	139·2	+ 0·3
5	143·2	143·3	− 0·1
4	147·8	148·3	− 0·5
3	154·8	154·7	+ 0·1
2	163·5	163·7	− 0·2
1	179·2	179·2	0·0

The calculated values in the third column are obtained from the equation for the straight line drawn through the experimental points on the graph of E against log C.

$$E\ (\text{calc.}) = 179{\cdot}2 - 51{\cdot}5 \log C.$$

The potentiometric determinations were erroneous, the results with proteins present were too high, and with proteins precipitated too low (Table II). While taking the readings, it was noticed that the potential tended to drift. It was thought that this was due to poisoning effect.[9] The filtrate after the precipitation of proteins by Somogyi's method was found to contain zinc ions.

TABLE II

DETERMINATION OF PLASMA CHLORIDE BY CHEMICAL AND POTENTIOMETRIC METHODS

		Potentiometric method			
		Proteins present		Proteins precipitated	
Specimen	Chloride determined by iodometric method of Haslewood and King, NaCl mg./100 ml.	mV., 1 in 100 diln.	NaCl, mg./100 ml.	mV., 1 in 100 diln.	NaCl, mg./100 ml.
A	632	135·0*	620	139·2*	520
B	563	130·2	760	142·8	440
C	573	129·5	790	142·2	450
D	563	125·0	960	141·8	460
E	561	132·2	700	141·2	475

* During the taking of the potentiometer readings it was noticed that the potential tended to drift, becoming lower in the presence of proteins, and higher after proteins had been precipitated by the zinc method of Somogyi. This potential drift was of the order of 0·25 mV. per minute.

An attempt was then made to determine chloride in cerebro-spinal fluid (C.S.F.), but even with the small amounts of protein present in C.S.F. potential drift was observed.

POISONING EFFECT—

Potential readings were taken in chloride solutions containing increasing concentrations of (*a*) zinc sulphate, (*b*) potassium hydroxide, (*c*) sodium sulphate and (*d*) sulphuric acid. Each of these substances had a poisoning effect on the electrode and potential drift was observed. The observed changes in potential were not proportional to the concentration of the foreign substance.

No poisoning effect was observed in the presence of the crystalloids normally found in the blood, *i.e.*, glucose, urea, sodium carbonate, potassium and phosphorus as dihydrogen potassium phosphate, and calcium as calcium gluconate, in concentrations normal in plasma.

PLASMA ULTRA-FILTRATES—

A 1 in 10 dilution of plasma was filtered through cellophane in a Seitz pressure filter. The plasma ultra-filtrate was shown to be free from protein by means of the biuret reaction. A 1 in 100 dilution of plasma was prepared from this filtrate and the potentiometric determination of chloride attempted. Again potential drift was observed. This potential drift could not be put down to the presence of protein. It therefore seems that the substance having the poisoning effect on the electrode is neither a protein nor any of the usual blood crystalloids. It might possibly be due to amino-acids, although these are present in small quantities only.

EFFECT OF GLYCINE—

As it was thought that the potential drift observed might be caused by the presence of amino-acids, it was decided to repeat the experiments of Katsu[10] on the effect of glycine on the silver - silver chloride electrode.

It was found that with glycine in a concentration of 1 *M* (7500 mg. per 100 ml.) there was an immediate rise in potential of approximately 3 mV. but no potential drift, which thus confirms Katsu's results. In the presence of glycine in a concentration of 5 mg. and 10 mg. per 100 ml. there was no significant effect upon the potential readings. Katsu did not examine the effect of glycine in low concentrations.

Katsu suggested that in the presence of glycine the chloride ion activity of chloride solutions was reduced and considered that this reduction was caused by the adsorption of the chloride ions by glycine because his measurements could be made to fit Freundlich's adsorption isotherm. But since the idea of adsorption on a small molecule like glycine has no physical meaning, all that can safely be claimed is that in the presence of a large excess of glycine, the chloride ion activity of chloride solutions is diminished.

ATTEMPT AT THE DETERMINATION OF PLASMA CHLORIDE BY CALIBRATING AFTER EACH READING—

Since it had been impossible to determine plasma chloride potentiometrically in an ultra-filtrate, it was decided to attempt the determination in the presence of protein and to allow for drift by taking a reading with a standard sodium chloride solution after each plasma reading and to apply the necessary correction to the initial calibration curve of the electrode. Readings were taken after the electrode had been immersed in the test fluid for a period of 5 minutes. By this method results varying by 1 to 5 per cent. from chloride determinations by the iodometric method of Haslewood and King[6] were attained.

DISCUSSION OF RESULTS

Neuhausen and Marshall[11] stated that their potentiometric determinations of chloride in serum and blood were in agreement with those found by chemical methods. In view of the findings of the present author, those of Neuhausen and Marshall are surprising. They obtained their chloride ion concentration by direct calculation from the Nernst equation on the assumption that their electrodes were perfect, *i.e.*, gave a change of 59 mV. for a tenfold change in chloride ion concentration. In their paper they gave no experimental proof that their electrodes were perfect. The electrodes prepared by the writer, using a modified Brown[1] technique, gave a difference of only 52 mV. between concentrations of sodium chloride of 50 mg. and 5 mg. per 100 ml., and 50 mV. between concentrations of sodium chloride of 10 mg. and 1 mg. per 100 ml. The earlier electrodes prepared gave similar figures. The author therefore believes that the results of Neuhausen and Marshall should be treated with reserve.

CONCLUSIONS

While it is possible to determine the chloride concentration within 1 per cent. in pure chloride solutions by means of the silver - silver chloride electrode, the determination in biological fluids is unsatisfactory owing to the poisoning effect causing potential drift. If correction is made for this potential drift, a determination correct to about 5 per cent. can be carried out in biological material. It would be possible to determine the chloride in very small amounts of material, *e.g.*, on 0·1 ml. or even 0·01 ml., with one hundredfold dilution; but in view of the simple and more accurate microchemical methods available, the potentiometric determination of chloride in biological fluids cannot be considered satisfactory. The silver - silver chloride electrode can however be used for the rapid detection and accurate determination of chloride in tap water or other simple chloride solutions (*cf.* Scott[3]).

A full account of this work is deposited in the Department of Chemical Pathology, Post-graduate Medical School, London, W.12.

ACKNOWLEDGMENTS

The author is indebted to Professor E. J. King for the opportunity of carrying out the investigation in his department, and to Dr. W. Klyne for helpful criticism.

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May, 1950

Phthalic Acid as a Selective Reagent for Zirconium

BY A. PURUSHOTTAM AND BH. S. V. RAGHAVA RAO

SYNOPSIS—Phthalic acid precipitates zirconium quantitatively from solutions 0·35 *N* with respect to free hydrochloric acid. From solutions 0·3 *N* in hydrochloric acid, most elements, such as thorium, iron, aluminium, beryllium, uranium, manganese, nickel and ceria earths can be separated in a single precipitation, but tin, titanium, vanadium and chromium require a second precipitation. The reagent is thus selective for zirconium.

SCHOELLER[1] recommended tannic acid as a selective reagent for zirconium. This reagent gives an easy separation from most other elements, although two stages are required, but does not prove so satisfactory in separations from thorium. This paper describes the use of phthalic acid, which reacts more advantageously than tannic acid to thorium, titanium and tin.

EXPERIMENTAL

ESTIMATION OF ZIRCONIUM—

A stock solution of pure zirconyl chloride in 0·1 *N* hydrochloric acid was standardised by precipitation with *m*-nitrobenzoic acid[2] and mandelic acid.[3] Two 20-ml. portions were found to contain 0·0550 and 0·0551 g. of ZrO_2. The precipitation with phthalic acid was carried out by diluting 20-ml. portions to 100 ml., boiling, and adding 100 ml. of a boiling

4 per cent. solution of phthalic acid. The boiling was continued for 2 minutes after the addition of the reagent. The white gelatinous precipitate was collected, after an hour, on an 11-cm. Whatman No. 42 filter-paper, washed with hot 0·1 per cent. solution of the reagent and ignited to ZrO_2. The ZrO_2 found was 0·0550 g., which was in excellent agreement with values obtained by the *m*-nitrobenzoic acid and mandelic acid precipitations.

SEPARATIONS—

As the separation of other elements that give precipitates with phthalic acid depends on the acid concentration, the solubility of the zirconium precipitate was investigated by carrying out the precipitation under various conditions of acidity. The results are shown in Table I. With increase in acid concentration the nature of the precipitate alters, becoming more gelatinous and taking longer to settle. When ammonium nitrate is added in sufficient quantity, the flocculence returns and the precipitate settles more readily.

TABLE I

EFFECT OF FREE ACID

Hydrochloric acid concentration	ZrO_2 taken, g.	ZrO_2 found, g.	Difference, g.
0·01 *N*	0·0275	0·0275	0·0000
0·01 *N*	0·0550	0·0550	0·0000
0·1 *N*	0·0275	0·0275	0·0000
0·1 *N*	0·0550	0·0551	+ 0·0001
0·1 *N*	0·0275	0·0274	− 0·0001
0·2 *N*	0·0550	0·0550	0·0000
0·3 *N*	0·0028	0·0029	+ 0·0001
0·3 *N*	0·0275	0·0274	− 0·0001
0·3 *N*	0·0275	0·0276	+ 0·0001
0·3 *N*	0·0550	0·0550	0·0000
0·3 *N*	0·0550	0·0551	+ 0·0001
0·35 *N*	0·0550	0·0549	− 0·0001
0·4 *N*	0·0550	0·0535	− 0·0015
0·4 *N*	0·0275	0·0265	− 0·0010

The precipitation of zirconium is thus quantitative in solutions containing free hydrochloric acid up to a concentration of 0·35 *N*, but for the best results an acid concentration of 0·3 *N* is recommended.

PROCEDURE—

To the chloride solution, add 30 ml. of saturated ammonium nitrate solution. Add sufficient 2 *N* hydrochloric acid to give a solution that will be 0·3 *N* in acid when the solution is diluted to 200 ml. by the subsequent operations. Dilute to 100 ml., and boil. Add, with continuous stirring, 100 ml. of a boiling 4 per cent. solution of phthalic acid and gently boil for 2 minutes. Keep hot over a boiling water-bath for 2 hours and then set aside to cool for an hour. Collect the precipitate on an 11-cm. Whatman No. 42 filter-paper, wash once with a hot 0·1 per cent. solution of phthalic acid in 0·3 *N* hydrochloric acid and then with 0·1 per cent. of phthalic acid in 2 per cent. ammonium nitrate solution. Ignite and weigh as ZrO_2.

TEST SEPARATIONS—

Results obtained with the above procedure are given in Table II; the weight of the metallic chloride or nitrate added has been calculated to the oxide. The concentration of hydrochloric acid was adjusted to 0·3 *N* in the final solution.

OBSERVATIONS ON THE TEST SEPARATIONS—

Iron—Iron is incompletely precipitated by phthalic acid in neutral solution. In 0·3 *N* hydrochloric acid no precipitation occurs.

Thorium—No precipitation takes place on the addition of phthalic acid to a cold neutral solution of thorium chloride or nitrate. On long and continuous boiling, however, a white precipitate forms, but dissolves on the addition of a few drops of hydrochloric acid. From 0·3 *N* hydrochloric acid solution thorium is not precipitated under any conditions.

Nickel—Nickel is not precipitated by phthalic acid even from neutral solutions. This is very convenient in the determination of zirconium in ores that have been subjected to peroxide fusion in a nickel crucible.

Tin and titanium—Tin in the form of stannous chloride is not precipitated by the reagent from 0·3 *N* hydrochloric acid solutions, but in the presence of zirconium small quantities are carried down. Titanium (as titanium tetrachloride) behaves similarly. For both metals, the first precipitate is dissolved in hydrochloric acid (1 + 1), diluted and neutralised with ammonia (Congo red indicator), and the calculated quantity of 2 *N* hydrochloric acid is added to give a 0·3 *N* solution at a dilution of 200 ml. A slight precipitate that appears during neutralisation will dissolve at this stage. Precipitation with phthalic acid is now repeated.

TABLE II

RESULTS OF TEST SEPARATIONS BY THE RECOMMENDED PROCEDURE

ZrO_2 taken, g.	Added substance	Added g.	ZrO_2 found, g.	Difference, g.
0·0550	ThO_2	0·0870	0·0550	0·0000
0·0550	ThO_2	0·0870	0·0551	+ 0·0001
0·0550	ThO_2	0·1740	0·0551	+ 0·0001
0·0550	ThO_2	0·1740	0·0549	− 0·0001
0·0550	Fe_2O_3	0·2014	0·0548	− 0·0002
0·0275	ThO_2	0·0870	0·0275	0·0000
0·0550	Al_2O_3	0·4102	0·0548	− 0·0002
0·0550	MnO	0·1894	0·0550	0·0000
0·0550	R_2O_3*	0·1732	0·0548	− 0·0002
0·0550	BeO	0·1648	0·0549	− 0·0001
0·0550	U_3O_8	0·1920	0·0550	0·0000
0·0550	NiO	0·5216	0·0548	− 0·0002
0·0550	V_2O_4	0·2648	0·0549(*a*)	− 0·0001†
0·0550	V_2O_4	0·2648	0·0548(*b*)	− 0·0002
0·0550	TiO_2	0·1143	0·0577(*a*)	+ 0·0027
0·0550	TiO_2	0·1143	0·0546(*b*)	− 0·0004
0·0550	SnO_2	0·1840	0·0563(*a*)	+ 0·0013
0·0550	SnO_2	0·1840	0·0547(*b*)	− 0·0003
0·0550	Cr_2O_3	0·3050	0·0550(*a*)	0·0000†
0·0550	Cr_2O_3	0·3050	0·0549(*b*)	− 0·0001

* Ceria earths; composition not determined.
† Slightly coloured.
(*a*) Single precipitation. (*b*) Double precipitation.

Other elements—Aluminium, beryllium, uranium, manganese, ceria earths, vanadium (V_2O_4) and chromium are not precipitated even in neutral solution, but the precipitate of zirconium in admixture with vanadium or chromium is slightly coloured although the weight of ZrO_2 does not show an excess. After a double precipitation, the ZrO_2 is pure white. It is possible that the vanadium and chromium ions are adsorbed and that traces resist washing; even these traces are removed in a second precipitation. Thus in the absence of iron, thorium, tin and titanium, most other separations can be affected in neutral solution. Tantalum, niobium and tungsten are not usually present in chloride solution; other elements have not been investigated, but they rarely occur in association with zirconium.

REFERENCES

1. Schoeller, W. R., *Analyst*, 1944, **69**, 259.
2. Osborn, G. H., *Ibid.*, 1948, **73**, 381.
3. Kumins, C. A., *Anal. Chem.*, 1947, **19**, 376.

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April, **1950**

ERRATA: September (1950) issue, p. 459. Third line of "Procedure," *for* "30 ml." *read* "300 ml."

November (1950) issue, p. 569. Line 20, *for* "is more efficient" *read* "is no more efficient."

Notes

PAPER TEST FOR CITRUS JUICES

WHEN lemon, orange and grapefruit juices are titrated with dilute caustic soda for the determination of acidity, the solution develops a yellow colour when the pH exceeds 7·0. It has now been found that if the reaction is carried out on filter-paper under standard conditions, the colour appears to be characteristic of citrus juices. The reaction is not given by mixtures which might reasonably be termed "citrus substitutes" and it can be used to estimate approximately the proportion of citrus juice present in a liquid.

The test conditions adopted are as follows—cut filter-paper (Ford's white blotting 408 mill, or Postlip 633DL have been found suitable) into strips 2·5 cm. wide and 20 to 22 cm. long. Support the strips above a sheet of plate glass so that they are vertical, with the lower end just touching the glass. Run 0·1 ml. of juice on to the plate glass by the end of the filter-paper strip so that it is absorbed by the paper. As soon as absorption is complete, run 0·1 ml. of distilled water on to the plate, and when this in turn has been absorbed, run on 0·1 ml. of 0·1 *N* sodium hydroxide. When absorption of the sodium hydroxide is complete, a yellow band is visible, the top edge of which is about half-way between the lower end of the paper strip and the upper edge of advance of the solutions (R_F approximately 0·5).

It has been our practice to dry the test strips as soon as possible at a temperature of 55° C. and to inspect them closely when dry. When this is done soon after preparation, the coloured band becomes more intense and is seen to consist of two bands diffusing into one another; the forward edge is of a deeper, orange, colour.

The following juices have all given strong positive reactions to this test—

Lemon juice	South African *var.* Eureka (two samples)
	Italian » Messina
Orange juice	South African *var.* Valencia (three samples)
	» » » Navel
Grapefruit juice	» » » Marsh
	» » » Triumph

Lime juice also gave a strong yellow band, but it was distinguishable from the citrus reaction by its much greater width.

The upper edge of penetration of the juice into the filter-paper was marked by a translucent line. This has always been found in tests on citrus juices and is caused by citrus oil. It affords a very delicate test for the presence of oil, 5×10^{-6} ml. of oil giving a definite line, and 2×10^{-6} ml. a line readily detected. Davis[1] has described a similar colour reaction in diethylene glycol solution and has used it to determine naringin and hesperidin in citrus fruits. As the colour reaction now described might be expected to occur with extracts from a wide variety of fruits, etc., extracts were prepared from various materials by boiling under reflux with dilute sulphuric acid and chloroform. The two layers were finally separated, the chloroform layer being evaporated and the residue taken up in dilute sulphuric acid. The two fractions were then tested for colour production by the method described. It was first ascertained that lemon juice when extracted still gave a strong positive reaction.

Extracts were prepared from rose hips (*Rosa canina*), privet berries (*Ligustrum vulgare*), dandelion (*Taraxacum dens leonis*) leaves and flowers, onion (*Allium cepa*) skins, and walnut (*Juglans regia*(?)) twigs and leaves. None of these in acidified aqueous extract gave a positive reaction and hence it appears that the paper test is more characteristic of citrus juices than is that described by Davis.

A wide range of citrus squashes known to be genuine and of various ages has been tested and all have given a positive result. Boiling such a solution, or boiling with hydrogen peroxide before testing, did not impair the development of the yellow colour. The presence of much oil, as in orange squash, interfered with the reaction on Postlip paper and a less clearly defined band was obtained, but with a very wide oil band. Thus, this paper provides a very delicate test for oil, but is correspondingly less sensitive for citrus juice in the presence of much oil (about 0·03 per cent.).

The presence of tartrazine in amounts such as are sometimes used for the colouring of lemon or grapefruit squashes did not interfere with the test, nor did the artificial colouring matters added to orange squashes. When tartrazine was present, the quantity of colour was too small to be detectable, and the orange colours formed one or more separate bands, according to the colours present. These bands, however, were faint, separate from the reaction band and usually of a different colour.

Of other fruit juices tested by this method, none gave a distinct yellow band. Juices of granadilla, rhubarb and golden plums gave faintly discoloured zones extending about half-way up the zone of penetration; cherry and apple juices gave two fairly distinct bands, the upper being the most marked and of a purple-brown colour, followed closely by a yellow band; gooseberry and plum syrups gave dark brown bands. Grape juice gave hardly any colour, but a distinct oil line. It thus appears that the double reaction—the orange-yellow band (R_F 0·5) and the translucent oil band—may be characteristic of citrus juices.

The quantitative application of the test to the estimation of the proportion of citrus juice present in a liquid has not been fully explored. Colour "standards" have, however, been prepared by dilution of fresh juice, and "unknowns" have been compared with these "standards." Solutions containing 30 per cent. by volume of citrus juice have on all occasions given a definite reaction. With but 10 or 15 per cent. of juice present, however, the reaction is uncertain and even with the help of "standards" it is difficult to distinguish between 10 and 20 per cent. by volume of juice.

REFERENCE

1. Davis, W. B., *Anal. Chem.*, 1947, **19**, 476, abstracted in *Analyst*, 1948, **73**, 283.

RESEARCH DEPARTMENT
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TODDINGTON, GLOS.

D. DICKINSON
F. J. T. HARRIS

May, 1950

THE SEPARATION OF MIXTURES OF ADIPIC AND SEBACIC ACIDS

WISE[1] in a Note on the Separation of Mixtures of Adipic and Sebacic Acids has reached certain conclusions about the operations involved in obtaining fairly pure specimens of adipic and sebacic acids from a mixture of the two acids by the method of Clasper and Haslam,[2] in which an aqueous solution of the two acids containing alkali equivalent to the adipic acid is extracted with ether in a continuous extractor. Fresh ether is supplied continuously to the system and it is very doubtful whether Wise's simple treatment is applicable to this procedure.

Careful experiments on purified samples of adipic and sebacic acid have been carried out in the following way. Known amounts of adipic and sebacic acids were weighed into a ground-glass stoppered flask and a volume of 0·1 *N* sodium hydroxide equivalent to the adipic acid was added. This volume was made up to 50 ml. with water and 35 ml. of ether were added. The whole was shaken thoroughly before placing it in a thermostat for half an hour at 20° C. The contents of the flask were then transferred to a continuous liquid - liquid extractor and extracted for 4 hours. The ether extract was then removed, evaporated to dryness and finally dried to constant weight at 120° C. The aqueous solution was acidified with 10 ml. of 20 per cent. v/v hydrochloric acid and re-extracted with ether for 4 hours. The ether extract was again evaporated to dryness and dried to constant weight at 120° C. The weights of the recovered acids together with the determined equivalents are shown in Table I.

TABLE I

		Mixture I	Mixture II
Adipic acid	Weight taken, g.	0·2503	0·2508
	Equivalent	72·8	72·8
	Weight found, g.	0·2597	0·2604
	Equivalent	74·3	74·2
Sebacic acid	Weight taken, g.	0·2511	0·2515
	Equivalent	101·6	101·6
	Weight found, g.	0·2401	0·2389
	Equivalent	101·7	101·2

It is unlikely that these results can be explained on the basis of solvent extraction alone as has been suggested by Wise. It does seem probable, however, that so-called stoicheiometric extraction plays some part in the separation.

REFERENCES

1. Wise, W. S., *Analyst*, 1950, **75**, 219.
2. Clasper, M., and Haslam, J., *Ibid.*, 1949, **74**, 224.

IMPERIAL CHEMICAL INDUSTRIES LIMITED
PLASTICS DIVISION
WELWYN GARDEN CITY, HERTS.

J. HASLAM
M. CLASPER

July, 1950

APPLICATION OF RADIO-FREQUENCIES TO CONDUCTIMETRIC ANALYSIS

IN a recent paper[1] the author showed that by the application of a "negative set-back" a zero-shunt circuit could be employed to increase sensitivity when titrating by rectified radio-frequency current.[2,3] Fig. 1 illustrates how, by a series of adjustments of the coupling between the R.-F. oscillator and the conductimetric tube, a solution concentration - conductivity graph was plotted over a range of concentrations from *N*/10 to *N*/81920. The microammeter used had a range extending only to 60 μ-amp. By this technique the actual current passed through the solution did not at any time exceed 50 μ-amp., so that no appreciable temperature rise was observable.

The procedure was to fill the conductimetric tube with *N*/10 hydrochloric acid and then adjust the coupling to give a deflection of 50 μ-amp. After noting the reduction of the meter reading that occurred when the solution was diluted by addition of an equal volume of distilled water, the coupling was further increased to restore the deflection to 50 μ-amp. before repeating the process.

The curves, Fig. 1, show the relationship between solution concentration and the sum of the successive reductions of current from 50 μ-amp. Curve A is parabolic and shows the relationship as far as *N*/1280. For curve B the ordinates are the logarithms of the concentrations shown on the right-hand side of the figure, and this curve goes to the extreme limit where the solution is no longer distinguishable from pure distilled water. The electrodes on the conductimetric cell were 1 mm. apart.

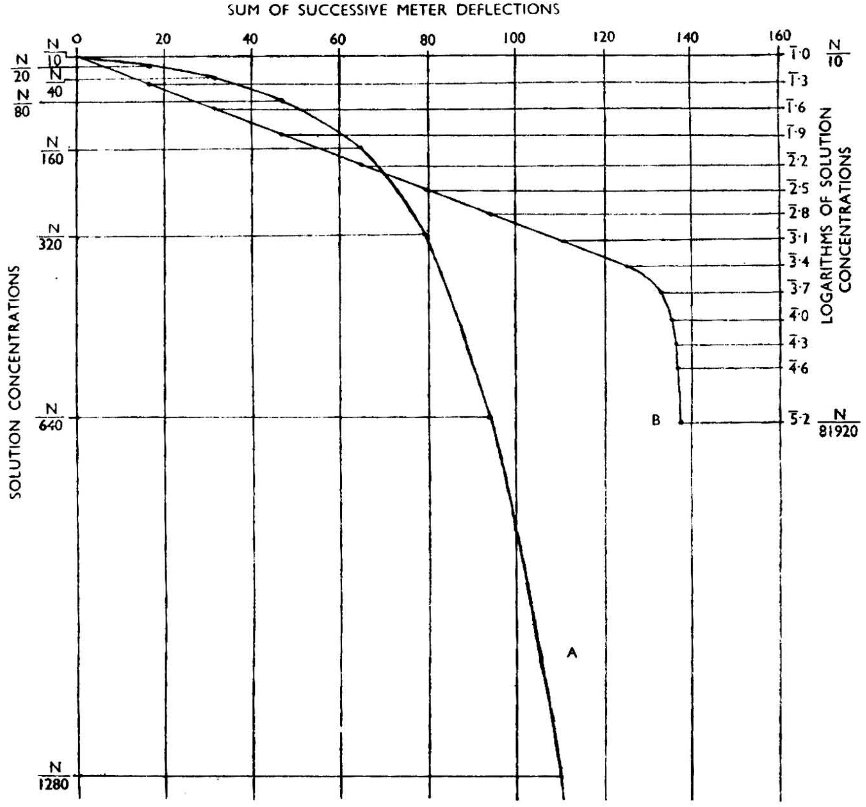

Fig. 1

REFERENCES

1. Blake, G. G., *Analyst*, 1950, **75**, 32.
2. ——, *J. Sci. Inst.*, 1947, **24**, 101.
3. ——, *Ibid.*, 1945, **22**, 174.

DEPARTMENT OF PHYSICS
UNIVERSITY OF SYDNEY
AUSTRALIA

G. G. BLAKE

July, 1950

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1950, **75**, 564).

Public Analyst	*Appointments*
CAHILL, Terence John (Deputy)	County of Northumberland.
CAHILL, Terence John (Deputy)	County of Cumberland.
HARRAL, James Charles (Deputy)	Urban District of Castleford.
HARRAL, James Charles (Deputy)	Borough of Batley.
HARRAL, James Charles	Borough of Keighley.
LEES, Arnold (Additional)	Borough of Lancaster.
*TURNER, Mervyn Edward Dennant	Borough of Swindon.
TURNER, Mervyn Edward Dennant (Deputy)	County of Herefordshire.
WALKER, George Hugh (Additional)	Borough of Lancaster.
WOOD, Eric Charles	County of West Suffolk.

* Non-member.

NOTIFICATION of the following appointments has been received from the Department of Health for Scotland since the list of appointments as at January 1st, 1947, appeared in *The Analyst* (1947, **72**, 64).

DAVIES, David Richard Aylmer (Additional)	County of Wigtown.
DAVIES, David Richard Aylmer (Additional)	County and Burgh of Dumfries.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1950, **75**, 564).

Agricultural Analyst	*Appointments*
HARRAL, James Charles (Deputy)	County Borough of Dewsbury.
WOOD, Eric Charles	County Borough of Norwich.

British Standards Institution

NEW SPECIFICATIONS*

B.S. 1583 : 1950. One-mark bulb pipettes. Price 2s.
B.S. 1669 : 1950. Industrial Perforated Plates. Price 2s.

The British Standards Institution inform us that they have opened a branch office at 12, Hilton Street, Manchester, 1, Telephone: Central 4856, where a complete set of British Standards may be consulted and copies may be purchased.

* Obtainable from the British Standards Institution, Sales Department, 24, Victoria Street, London, S.W.1.

DRAFT SPECIFICATION

A FEW copies of the following draft specification, issued for comment only, are available to interested members of the Society, and may be obtained on application to the Secretary, Miss D. V. Wilson, 7–8, Idol Lane, London, E.C.3.

Draft Specification prepared by Technical Committee LBC/6—Standard Distillation Apparatus.
CM(LBC) 6119—Draft Revision of B.S. 658, Distillation Apparatus.

Reviews

PHYSICO-CHEMICAL CONSTANTS OF PURE ORGANIC COMPOUNDS. By J. TIMMERMANS. Pp. viii + 693. New York and Amsterdam: Elsevier Publishing Company, Inc. London: Cleaver-Hume Press, Ltd. 1950. Price 95s.

Chemical handbooks contain tables of physical properties that serve a purpose, but it is not generally realised until comparisons are drawn from the original literature how true is the author's remark that most of the numerical data accumulated during the last hundred years must be excluded if "precision worthy of contemporary science" is desired. It is, for example, startling to read (p. 419) that there are no reliable data for *n*-amyl acetate. To separate the wheat from the chaff is no light undertaking where the bulk of the latter is so great. Chemists therefore owe Professor Timmermans a great debt for performing this operation, for which his association with the International Bureau of Physico-Chemical Standards renders him particularly well fitted.

The properties quoted are critical constants, density of saturated vapour pressure, boiling-point, freezing- or melting-point, critical solution-point, density, surface tension, refractive index, viscosity, dielectric constant, specific rotatory power, specific heat and heats of vaporisation, fusion, transition and combustion. It must at once be stated that this whole range of properties is not available for each compound quoted; extensive lists of melting-points *per se* are not given. In addition to the results, published or unpublished, of the Bureau at Brussels, the literature has been covered to the beginning of this year.

The appeal of this book to the analyst as such is limited; nevertheless, should the occasion arise, it is useful to know where thoroughly reliable data, if available, may readily be found.

B. A. ELLIS

PHYSICAL METHODS IN CHEMICAL ANALYSIS. Vol. I. Edited by WALTER G. BERLE. Pp. viii + 664. New York, Academic Press Inc. 1950. Price $12.00.

This is the first of two volumes to be published under the general title "Physical Methods in Chemical Analysis." It is stated that "the subject-matter has been divided in such a way that all methods dealing with the interaction of radiation with matter (in addition to mass spectrometry) appear together in Volume I. Electrical, magnetic and miscellaneous techniques and the methods of separation will appear in Volume II."

Volume I comprises 12 sections, each written by a specialist in his particular field. These are: "Absorption Phenomenon of X-rays and γ-rays," by George L. Clark; "X-ray Diffraction Methods as Applied to Powders and Metals," by William L. Davidson; "X-ray Diffraction as Applied to Fibres," by John A. Howsmon; "Electron Diffraction," by L. O. Brockway; "Spectrophotometry and Colorimetry," by W. R. Brode; "Emission Spectrography," by J. Sherman; "Infra-red Spectroscopy," by H. H. Nielsen and Robert A. Oetjen; "Raman Spectra," by J. H. Hibben; "Polariscopic and Polarimetric Examination of Materials by Transmitted Light," by C. D. West; "Refractive Index Measurement," by L. W. Tilton and J. K. Taylor; "Electron Microscopy," by R. D. Heidenreich; "Mass Spectrometry," by H. W. Washburn.

The declared aim of the book is to describe those physical methods that have either proved themselves of considerable value in quantitative work or are destined to play an important role in the future. The plan has been to deal with each technique adequately enough "to minimise the need for consulting more specialised texts or the original literature." It should perhaps be stated at once that the main aim has been attained—the description of those physical methods that have proved of value. The more extravagant claim, which implies a knowledge of the future importance of this or that technique, can only be put to the test of time.

Of the plan itself, this too may be said to come near succeeding. Each section does in fact present a very complete and clear account of all the important aspects of apparatus and techniques, including their limitations. The reader who is unfamiliar with these techniques will realise at once not only that most of them call for specialist operators, but that the reference concerning the minimising of literature searches and the like is somewhat superfluous.

It will also be obvious that here is a composite picture not only of new techniques but of a new and wider conception of what is to be considered the analytical domain. There is a breadth and grandeur about this newly adopted picture that transcends conventional views of chemical analysis and also, for that matter, micro-chemical analysis.

The first four sections have a common link in that they are all concerned with the information that may be deduced from techniques involving the absorption or diffraction of X- or γ-rays. The lead content of petrol, for example, has been determined with a precision of 1 per cent. by absorption methods using an X-ray photometer and by means of micro-radiography it has been possible to analyse multi-component alloy systems in terms of phase structures, micro-defects and porosity, and strain.

In the space of 100 pages there is an excellent account of the application of X-ray diffraction methods to powders and metals, including a section on the elements of crystal structure. Besides an account of the powder method of identification and analysis the author gives useful and clear explanations of the other possibilities of the X-ray method, including the measurement of crystallite size and alloy compositions, and the study of structure, precipitation hardening and magnetic properties. The section ends with clear illustrations of the special value of the methods in studying metal working processes—deformation, preferred orientation and annealing.

An equally fascinating account is given in the next section of the use of diffraction methods in the study of fibres. The difficulties of examining systems in which there is a gradual blending of all possible degrees of perfection from perfect crystalline forms to totally random arrangement of molecules in space are well brought out.

Electron diffraction technique is equally well described. Here at last we have a tool for analysis of surface films; the composition and structure of oxide films on metals have been determined and variations of composition within the film for film thicknesses of 100 to 300 A. have been demonstrated. This indeed is micro-analysis.

The ground covered in the section on "Spectrophotometry and Colorimetry," by Wallace R. Brode of the Bureau of Standards, is best illustrated by the headings: Nomenclature, Theory of light absorption, Instrumentation, Experimental procedure, Application and interpretation of data, Colour description. The treatment of this important subject reaches a high standard.

In the sections on emission and absorption spectroscopy and on Raman spectra there are again very full accounts of the theoretical background to the techniques. Indeed, this is throughout a characteristic feature of the book. It is particularly true of the section on polariscopic and polarimetric examination and refractive index measurement, but sufficient examples of practical application are given to show the importance and value of these methods in analysis.

The final section on mass spectrometry maintains the high standard and in the face of the results that are quoted, for example, of light hydrocarbon mixtures, it will be admitted that this technique has come to stay. The promise of simpler and more compact instruments, even at the expense of some versatility, is good news.

It will be realised that this book has much more to offer the reader than earlier and more superficial books setting out to cover similar ground. It will serve to emphasise the revolution that has taken place, is taking place and, with some, has yet to take place in analytical thinking.

Nobody should be deceived into believing that one has only to read about one of these specialised techniques to become a competent practitioner. The book makes no such promises. No specialist will expect his reading to be confined to one of these sections. Few, if any, of the special techniques can be really valuable until their practitioner has acquired sufficient skill and experience to merit the name of specialist. They are not likely to become dilettante side lines of the average analyst.

For the analyst, such a book is educative and probably revealing. While analytical practitioners are at present undoubtedly scarce, there is perhaps an even greater scarcity of those who can clearly formulate the "analytical problem." The present renaissance of analysis springs largely from the needs of industrial research; for the problems that arise, simple arithmetical statements of composition seldom suffice, too narrow a conception of what constitutes analysis fails to give answers and the use of any or all of these newer tools and techniques is fully justified and is comprehended in the word "analysis."

It remains only to add that the covers and binding are worthy of the book.

R. C. CHIRNSIDE

Publications Received

REPORT OF THE PROCEEDINGS OF THE TENTH SESSION OF THE INTERNATIONAL COMMISSION FOR UNIFORM METHODS OF SUGAR ANALYSIS, BRUSSELS, 1949. Pp. ii + 54. London: The International Commission for Uniform Methods of Sugar Analysis. 1950. Price 5s.

COMPTES RENDUS DE LA QUINZIEME CONFERENCE, AMSTERDAM, 1949. Pp. 191. International Union of Pure and Applied Chemistry, Paris.

THE NATIONAL FORMULARY. Ninth Edition. Pp. xl + 877. Washington: American Pharmaceutical Association. 1950. Price $8.00 (in U.S.A.); $8.75 (elsewhere).

DDT AND NEWER PERSISTENT INSECTICIDES. Second Edition. By T. F. WEST, D.Sc., Ph.D., F.R.I.C., and G. A. CAMPBELL, M.Sc., F.R.I.C. Pp. xiv + 632. London: Messrs. Chapman & Hall. 1950. Price 50s.

TEXTBOOK OF QUANTITATIVE INORGANIC ANALYSIS. Revised Edition. By Professor I. M. KOLTHOFF, Ph.D., and E. B. SANDELL, Ph.D. Pp. vii + 794. London: Macmillan & Co., Ltd. 1950. Price 30s.

ORGANIC REAGENTS AND ORGANIC ANALYSIS. Second Edition. By Staff of the Research Laboratory of Hopkin and Williams, Ltd. Pp. 263. London: Hopkin & Williams, Ltd. 1950.

THE IDENTIFICATION OF MOLECULAR SPECTRA. Second Edition. By R. W. B. PEARSE, D.Sc., F.R.A.S., and A. G. GAYDON, D.Sc. Pp. vii + 276. London: Chapman & Hall, Ltd. 1950. Price 50s.

GERMAN-ENGLISH DICTIONARY FOR CHEMISTS. Third Edition. By A. M. PATTERSON. Pp. vii + 541. New York: John Wiley & Sons Inc. London: Chapman & Hall, Ltd. 1950. Price 40s.

BIOLOGICAL METHODS GROUP

THE Sixth Annual General Meeting of the Group will be held in the rooms of the Chemical Society, Burlington House, Piccadilly, London, W.1, on Tuesday, December 19th, 1950, at 6.15 p.m.

The Annual General Meeting will be followed at 6.30 p.m. by an Ordinary Meeting at which the following papers will be read and discussed: "Assessment of ACTH Activity," by I. D. K. Halkerston and M. Reiss; "*Euglena gracilis* as an Assay Organism for Vitamin B_{12}," by G. E. Shaw; "The Effect of Interfering Agents on the Vitamin B_{12} Plate Assay (*E. coli* Mutant Method)," by W. F. J. Cuthbertson, Valerie Herbert, H. F. Pegler and C. Quadling.

The Annual General Meeting is confined to members of the Group, but visitors will be welcome at the Ordinary Meeting.

MICROCHEMISTRY GROUP

THE following meetings of the Group will be held during 1951—

Annual General Meeting—This will be held on Friday, January 26th, at the Sir John Cass College, Jewry Street, Aldgate, London, E.C.; it will include an exhibition of microchemical apparatus and a symposium on "Radiochemical Techniques in Microchemistry."

Spring Meeting—This has been tentatively arranged for Edinburgh at the beginning of April. It is hoped that this will be a joint meeting with the Edinburgh and East of Scotland Section of the Royal Institute of Chemistry and that it will include a symposium on "Microbiochemical Methods."

Autumn Meeting—This has been tentatively arranged for Liverpool at the end of September, in conjunction with the Liverpool and North-Western Section of the Royal Institute of Chemistry. It is intended that this meeting shall include a symposium on "Chemical Microscopy," with special reference to Fluorescence Methods.

THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

FOUNDED 1874. INCORPORATED 1907.

THE objects of the Society are to encourage, assist and extend the knowledge and study of analytical chemistry by holding periodical meetings, by promoting lectures, discussions and conferences, and by the publication of a journal devoted to analytical chemistry; to study questions relating to the adulteration of food, drugs and commercial articles generally, and its detection; and to promote the efficiency and proper administration of the laws concerned with the repression of adulteration.

Every candidate for membership of the Society must be not less than twenty-one years of age and be or have been engaged in analytical, consulting or professional chemistry. Each candidate for election must be proposed by three members of the Society, who must provide written testimony of their personal knowledge of his or her scientific and professional fitness. If the Council of the Society in their discretion think fit, such testimony may be dispensed with for a candidate not residing in the United Kingdom. Every application is placed before the Council and the Council have the power in their absolute discretion to suspend or reject any application, or to elect the candidate to membership.

The Entrance Fee is £1 1s. The Annual Subscription is £2 2s. The Society's official year runs from March to March, but the financial year begins on January 1st and subscriptions are due on that date.

Ordinary Meetings of the Society are held in London, usually on the first Wednesday in October, November, December, February, April and May; the Annual General Meeting is usually held on the first Friday in March. Notices of all meetings are sent to members by post.

The Analyst, the official organ of the Society, is issued monthly to members, and contains reports of the proceedings of the Society, original papers and notes, information about analytical methods, Government reports and reviews of books. All members receive in addition *Abstracts C*, the analytical section of British Abstracts, providing a reliable index to the analytical literature of the world.

Forms of application for membership may be obtained from the Secretary, 7/8, Idol Lane, London, E.C.3.

LOCAL SECTIONS AND SUBJECT GROUPS

THE North of England Section and the Scottish Section were formed to promote the aims and interests of the Society among the members in those areas. Members of the Society residing in England or Wales north of Birmingham may become members of the North of England Section and those resident in Scotland members of the Scottish Section.

The Microchemistry Group, the Physical Methods Group and the Biological Methods Group have been formed within the Saciety to further the study of the application of microchemical, physical and biological methods of analysis. All members of the Society are eligible for membership of the Groups.

There is no extra subscription for membership of a Section or Group. Application for registration as a member should be made to the Secretary.

The Sections and Groups hold their own meetings from time to time in different places.

THE DISTILLERS COMPANY LIMITED has a vacancy for an experienced analyst, aged 27–30 years, to take charge of an analytical team concerned with antibiotics research in the Central Research and Development Department, Great Burgh, Epsom, Surrey. This post offers exceptional opportunities for an analyst with experience of biochemical analysis wishing to enter the antibiotics research field. Applicants must possess a 1st or 2nd class honours B.Sc. degree, or equivalent. The commencing salary will depend on qualifications and experience. Apply to the Controller of Research and Development.

ALLEN & HANBURYS, Ware, Herts., require an Analyst for special investigations concerned with process research. Wide experience in the analysis of drugs essential and manufacturing experience an advantage. Apply Personnel Manager.

IMPERIAL CHEMICAL INDUSTRIES LIMITED, Dyestuffs Division, require ANALYST for post in Manchester district, preferably with experience of synthetic medicinals and packed pharmaceutical products. Applicants should be graduates in chemistry and/or pharmacy. Salary according to training and experience. Applications to Staff Dept., Hexagon House, Blackley, Manchester, 9. Ref. Analyst.

AN additional senior Analytical Chemist is required by the Nelson Research Laboratories, English Electric Co., Ltd., Stafford. Applicants should possess a degree and/or be an A.R.I.C., and should have had at least three years' industrial experience of inorganic analysis. Apply giving full details mentioning reference 415C to Central Personnel Services, English Electric Co., Ltd., 24–30, Gillingham Street, London, S.W.1.

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ASSISTANT ANALYSTS (MALE). Applications are invited for appointments as assistant chemists in the Department of the Corporation Chemist and City Analyst. Applicants should possess a Degree in Applied Chemistry and/or the Associateship of the Royal Institute of Chemistry. Previous experience in the laboratory of a Public Analyst would be an advantage. Salary will be in accordance with A.P.T. Grades I, III, and V (£390 to £570). The initial salary will depend on the qualifications and experience of the applicant. The appointments are subject to the provisions of the Corporation Superannuation Scheme, and the successful applicants will be required to pass a medical examination. Applications, stating age, qualifications, and experience, accompanied by copies of not more than three recent testimonials, should be sent to the Corporation Chemist and City Analyst, 20, Trongate, Glasgow, C.1, not later than three weeks after the publication of this advertisement.

ASSISTANT CHEMIST required by Food Factory. A.R.I.C. or equivalent qualification. Young man with some experience in the analysis of foodstuffs, fertilisers, waters, etc. Must be a reliable analyst. Salary £600 + £25 to £750 per annum. Applications at once, together with *fullest* particulars, which will be treated in confidence, to The Secretary, Messrs. Marsh & Baxter Limited, Brierly Hill, Staffs.

ASSISTANT CHEMIST, male, Inter.B.Sc., with experience of inorganic analysis and/or process control required by Electrical Company in Boreham Wood, Herts. Write giving age and details of experience to Box 3760, THE ANALYST, 47, Gresham Street, London, E.C.2.

EXPERIENCED responsible METALLURGICAL CHEMIST required for ferrous and non-ferrous analysis. Wider metallurgical experience an advantage. Please apply in writing stating age, qualifications and experience to Messrs. Sandberg, 40, Grosvenor Gardens. London, S.W.1.

LABORATORY and Office Premises in North London to let, equipped benches, fume cupboards, total floor area 4,000 sq. ft. Write Box No. 3[illegible]59, THE ANALYST, 47, Gresham Street, London, E.C.2.

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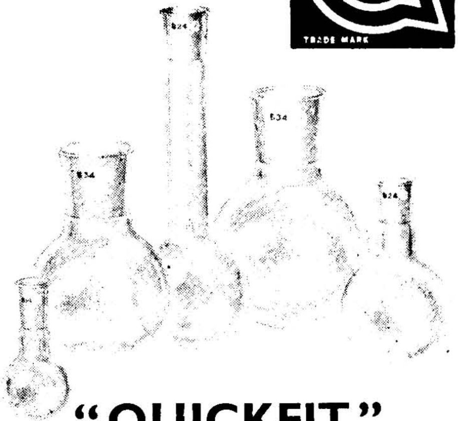

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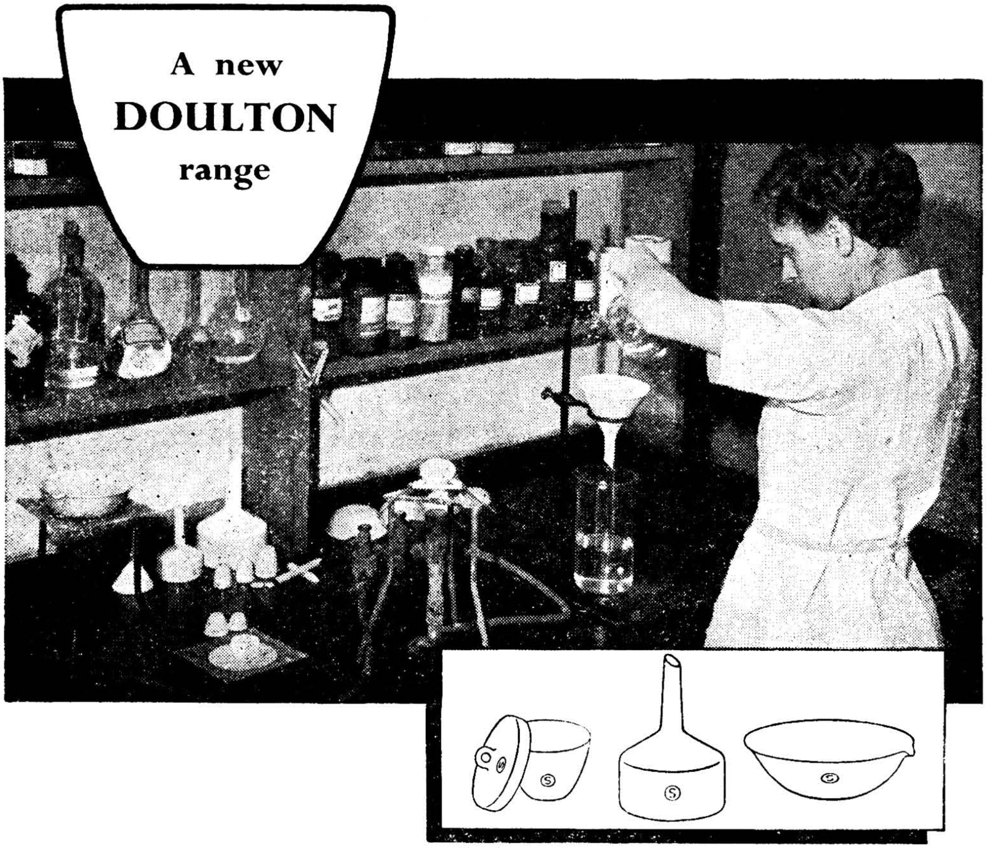
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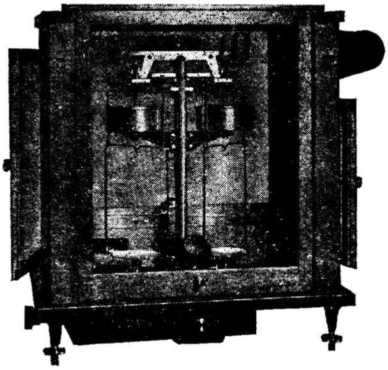

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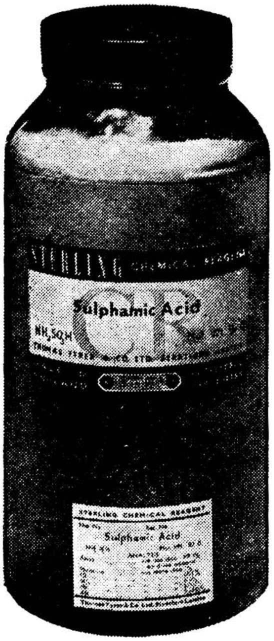
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THE ANALYST

THE JOURNAL OF
THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

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Printed and Published for the Society of Public Analysts and Other Analytical Chemists by W. Heffer & Sons Ltd., Cambridge, England. Communications to be addressed to the Editor, J. H. Lane, 7–8, Idol Lane, London, E.C.3. Enquiries about advertisements should be addressed to Walter Judd Ltd., 47, Gresham Street, London, E.C.2.

Entered as Second Class at New York, U.S.A., Post Office

THE ANALYST

THE JOURNAL OF THE

Society of Public Analysts and Other Analytical Chemists

A MONTHLY JOURNAL DEVOTED TO THE ADVANCEMENT OF ANALYTICAL CHEMISTRY

VOL. 75

1950

PUBLISHED FOR THE SOCIETY BY
W. HEFFER & SONS LTD.
4, PETTY CURY, CAMBRIDGE, ENGLAND

ERRATA

Vol. 75, 1950:

pp. 71 to 73. *For* "phenodoxone," *read* "phenadoxone" throughout.

p. 111, line 2 of Public Appointments, *for* "County Borough of Southampton," *read* "County of Southampton."

p. 206. Below Fig. 3, *for* "g.," *read* "μg."

p. 251. Paper by Sutton and Markland. The date of reading should be *November 26th*, **1949.**

p. 445. Line 6 (Equation 3), *for* "1·2A − 0·069 B.I.," *read* "1·2A + 0·069 B.I."

p. 459. Third line of "Procedure," *for* "30 ml.," *read* "300 ml."

p. 569. Line 20, *for* "is more efficient," *read* "is no more efficient."

p. 663. Under the heading "436mμ," *for* "Calorex (ON 3)" *read* "Calorex (ON 13)."

p. 669, Table II. In column 5, opposite 405mμ, *for* "0·2" *read* "0·1."

INDEX TO VOLUME 75

INDEX TO NAMES

* Denotes authors of original papers and notes that have been published in *The Analyst.*

INDEX TO SUBJECTS

* Denotes original papers and notes that have been published in *The Analyst.*