THE ANALYST

The Journal of The Society of Public Analysts and Other Analytical Chemists

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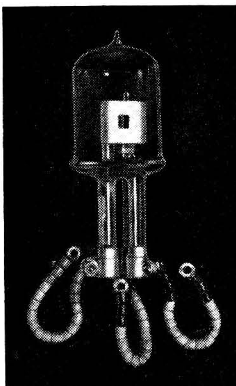

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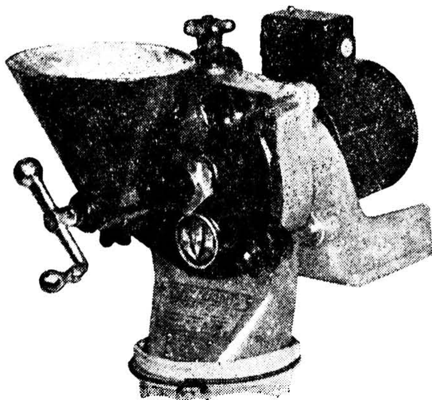

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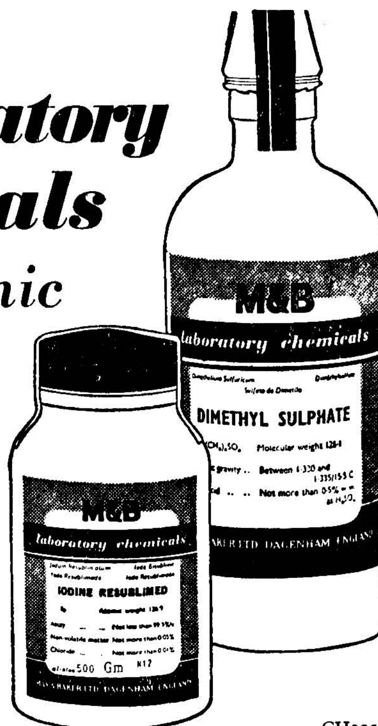

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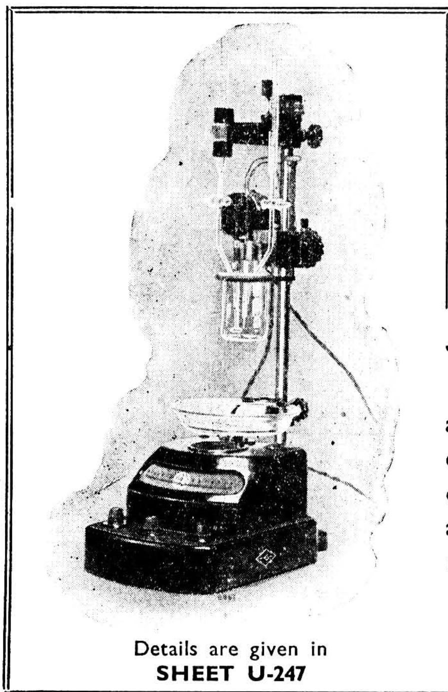

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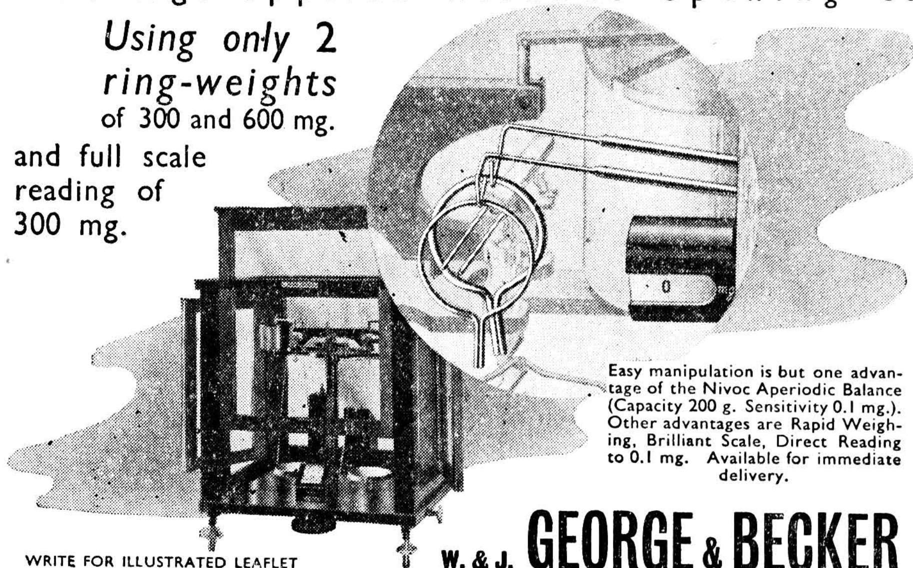

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Extraordinary General Meeting of the Society was held at 6 p.m. on Wednesday, August 24th, 1949, in the Hall of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.2. The President occupied the chair. The following Special Resolution, which had been recommended by the Council, was proposed by the President, seconded and carried unanimously.

"That the Articles of Association of the Society be altered as follows:—

"Article 29.—By deleting the article and side note and substituting therefor the following:—

29. Votes may be given either personally or by proxy, except in the case of voting by ballot under Article 36 (A) hereof, in which case votes may be given personally or through the post as provided by that Article. No person shall act as proxy unless he is a member of the Society." Proxies

DEATHS

WE regret to record the deaths of

Sir Robert Robertson (Honorary Member).
Narenda Nath Dutta.
John William Hawley.
Clifford Hanks Robinson.
Herbert Frederick Stephenson.

The Statistical Use of Several Analytical Constituents for Calculating Proportions of Ingredients in Certain Food Products

BY E. H. STEINER

THE application of statistics in calculating the proportion of an ingredient in a food product has already been considered in the case where only one analytical constituent is available for the determination.[1] It was shown how limits can be assigned within which the true proportion of the ingredient is likely to lie, and that these limits depend primarily on the amount of permissible risk of rejecting a product as deficient in the ingredient when it has in fact the correct proportion. For any pre-assigned risk, however, the values of the limits depend on the natural variation encountered among the values of the proportion of the constituent in samples of the ingredient. No improvement in analytical accuracy can overcome this source of variation (and consequent error in the final estimation), but there remains the possibility of lessening this error by the use of two or more constituents in the determination of the ingredient. For example, in the analysis of fruit pulps, determinations of both soluble solids and insoluble solids might be made. It is the purpose of the present paper to show how several such measurements may best be combined to obtain the most accurate assessment of the proportion of ingredient present.

The combination of two constituents

Various ways of combining data from two different determinations are possible. For example, the apparent percentages of an ingredient calculated from different constituents might simply be averaged in the usual way. Suppose a strawberry pulp shows 7·0 per cent. of soluble solids (determined by specific gravity) and 0·80 per cent. of free acid (as hydrated citric acid). Average figures for these two constituents in strawberries are 9·01 and 0·96 per cent. (these and other figures quoted below are based on data collected over a large number of years at the laboratories of the British Food Manufacturing Industries Research Association.) Separate estimates of the fruit content of the pulp would then be 77·7 per cent. from the soluble solids and 83·3 per cent. from the acidity.

Averaging these two results would give 80·5 per cent. as an improved estimate of the proportion of fruit, but this is not necessarily the best that can be made. It ignores, for instance, differences in variability shown by the respective constituents in the fruit itself. Actually, soluble solids show only about half the variability of free acid in samples of strawberries (the coefficients of variation are 10·3 and 24·2 respectively), so that relatively more weight should be attached to estimates based on the former. Further, no account is taken of any correlation between the constituent values. If those samples of strawberries with high soluble solids contents tend to have high free acid values, and *vice versa*, there is obviously little to be gained from averaging the two estimates.

The possibility of improving the estimate by the use of two constituents is governed partly by the relative extent to which the constituents vary in the fruit, and also by the degree of correlation between them. If the soluble solids and free acids contents show an association or correlation as above to a high degree, then the combination "soluble solids minus free acid" would be expected to show less variation than either of them singly. Such a combination could be used as a *single* analytical figure with which to divide the corresponding quantity observed in the fruit pulp. Similarly, if the two constituents are negatively correlated, the combination "soluble solids plus free acid" should be more constant. In the example quoted above, these two combinations would lead to apparent percentages of strawberries in the pulp of $\frac{6{\cdot}2}{8{\cdot}05} \times 100 = 77{\cdot}0$ per cent. and $\frac{7{\cdot}8}{9{\cdot}97} \times 100 = 78{\cdot}2$ per cent. respectively.

The optimum combination

The practice of averaging the two apparent percentages calculated from each constituent separately is precisely equivalent to weighting constituents in inverse proportion to their mean values and using the combination as a single figure. Viewed in this way, the normal procedure and the other two suggested above are particular instances of using a certain linear combination of the constituents. Now the variation of any such linear combination can be expressed exactly in terms of the standard deviations and correlation coefficients of the components, hence it becomes possible to calculate multiplying factors for weighting the coefficients, so that the variation of the resulting combination is less than that of any other linear combination. This will be called the optimum combination.

It will be observed that the apparent proportion of fruit varies according to the particular combination of the constituents used. Clearly all three values calculated above for the percentage of strawberries cannot be correct and, in the absence of information on the precise soluble solids and free acid contents of the strawberries used for the pulp, we cannot assert that any value calculated represents the true value. We can, however, select that percentage resulting from the use of the combination that shows least variation in samples of strawberries. By the consistent use of this combination with all samples of strawberry pulp, we shall, *on average*, be nearer the truth than by the use of any other. It is in this sense that the optimum combination arrived at will be considered the best, and the resulting estimate of the apparent proportion of ingredient the most accurate one.

In considering the calculation of the proportion of an ingredient in a foodstuff from the amount of a single constituent characteristic of the ingredient,[1] it was shown that where the amount of the constituent in the foodstuff is determinable exactly, *i.e.*, without significant analytical error, the accuracy of the estimate of the amount of the ingredient depends solely on the coefficient of variation of the constituent in samples of the ingredient. This condition applies to fruit pulps, where the whole of the value of the constituent determined in the pulp is contributed by the ingredient and the experimental error is small compared with the

natural variation shown by the ingredient. In such cases the maximum improvement in accuracy is obtained when the coefficient of variation is minimised, by the combined use of two or more constituents. In the more general type of analysis, as in meat products or where the analytical error is appreciable, the proportion of ingredient is calculated from the ratio of *two* variable quantities. The problem of combining measurements to provide the most accurate final analysis is then more complex, and will not be considered here, in view of the fact that suitable data for treatment appear to be lacking.

Calculation of the optimum combination of two constituents

Let x_1 and x_2 denote two different constituents characteristic of the ingredient (*e.g.*, soluble solids and free acid in strawberries). Also let their mean values and standard deviations be $\bar{x}_1$, σ_1 and $\bar{x}_2$, σ_2 respectively and r be the correlation coefficient.

Consider a combination of the two constituents given by $x_c = x_1 + kx_2$, where k is an arbitrary constant. (We could have made the more formal assumption of two constants, k_1 and k_2, corresponding to x_1 and x_2, respectively, but there is no loss of generality in putting that of x_1 equal to unity, since any constants proportional to these factors can be used without affecting the coefficient of variation of the combination, and the alternative chosen gives simpler expressions.) Then, since x_c is a linear function of x_1 and x_2, the combined quantity will have a mean $\bar{x}_c = \bar{x}_1 + k\bar{x}_2$ and standard deviation $\sigma_c = \sqrt{\sigma_1^2 + 2kr\sigma_1\sigma_2 + k^2\sigma_2^2}$. This latter expression is exact if σ_1 and σ_2 are the true values for the constituents, but in practice only a small number of observations are usually available for estimating these quantities. If s_1 and s_2 are the standard deviations estimated from a total of n samples of ingredient analysed, then s_c, the estimate of σ_c, should be regarded as based on (n — 2) degrees of freedom. The reduction in the number of degrees of freedom is reasonable if it is considered that *two* constants have really been fitted to the data in arriving at the variance of the optimum combination, one being the mean value and the other the factor k. If only two samples of the ingredient had been analysed a value of k could be found such that the resulting combination was a constant (for the two samples), but this would have no practical significance. By analogy with multiple regression theory, one degree of freedom is lost every time a fresh constant is fitted to the data. Since s_1 and s_2 are themselves calculated by dividing the sum of squares by (n — 1), the estimated standard deviation of the combination will be

$$s_c = \sqrt{(s_1^2 + 2krs_1s_2 + k^2s_2^2) \times (n-1)/(n-2)}.$$

The necessary value of k for the optimum combination is that which minimises the coefficient of variation given by $V_c = 100s_c/\bar{x}_c$. This may be found by differentiating V_c with respect to k, and equating to zero, leading to the value:

$$k = s_1(V_1 - rV_2)/s_2(V_2 - rV_1) \quad \ldots \quad (1)$$

where V_1 and V_2 are the observed coefficients of variation of the two constituents.

If this value of k is inserted in the above expressions for s_c and $\bar{x}_c$, we find for the coefficient of variation of the optimum combination:

$$V_c = V_1V_2\sqrt{\frac{1 - r^2}{V_1^2 - 2rV_1V_2 + V_2^2} \times \frac{n-1}{n-2}} \quad \ldots \quad (2)$$

Equations (1) and (2) enable the value of the optimum combination $x_1 + kx_2$ and its coefficient of variation to be calculated for any pair of constituents available for estimating an ingredient.

From a consideration of equation (2) it is possible to draw general conclusions on the benefit to be gained from combining constituents in this way. If the constituents are uncorrelated (r = 0) the greatest gain will occur when the two coefficients of variation are equal; in this event a reduction of about 30 per cent. in the probable range is effected. If the correlation is negative, a still greater reduction results, increasing as r approaches minus one. If the correlation is positive, the gain is greatest when the ratio of smaller to larger coefficient of variation differs most from the correlation coefficient numerically. No improvement is possible when these two quantities are equal, so that even a high positive correlation (as considered on page 430) is useless when the coefficients of variation are similar. This is obvious if the correlation coefficient is unity and the coefficients of variation equal, since one set of values is then a multiple of the other and provides no fresh information. These conclusions are borne out in Table I.

TABLE I

OPTIMUM COMBINATION OF SOLUBLE SOLIDS WITH FREE ACID (AS HYDRATED CITRIC ACID) IN SOME COMMON FRUITS

Fruit	Soluble solids: Mean value, % $\bar{x}_1$	Soluble solids: Coefficient of variation V_1	Free acid: Mean value, % $\bar{x}_2$	Free acid: Coefficient of variation V_2	Correlation coefficient r	Number of samples n	Optimum combination: Free acid factor k	Optimum combination: Mean value $\bar{x}_c$	Optimum combination: Coefficient of variation V_c
Apple ..	11·99	10·5	1·09	42·2	− 0·59	37	1·99	14·16	7·4
Blackcurrant	14·52	13·3	3·53	11·0	− 0·12	20	5·91	35·38	8·2
Gooseberry ..	8·28	9·7	2·21	14·5	+ 0·39	48	0·95	10·38	9·4
Plum	12·59	17·9	1·74	27·9	+ 0·34	50	1·77	15·67	17·2
Raspberry ..	8·70	17·4	1·70	18·0	+ 0·17	29	4·67	16·64	13·7
Strawberry ..	9·01	10·3	0·96	24·2	+ 0·23	43	0·86	9·84	10·2

It is evident that in order to find the optimum combination of two analytical constituents it is necessary to calculate, not only their means and standard deviations, but also their correlation coefficient. This can only be done where determinations of the constituent values have been made on the *same* samples of the ingredient and imposes restrictions on the usefulness of published data in this respect. For fruits, however, many data of the required type are available and some of them have been published.[2]

Table I shows the values of k and V_c for the optimum combination of soluble solids with free acid in some common fruits. It is immaterial to which constituent it is decided to apply the factor k so long as it is remembered that s_2 and V_2 in equation (1) relate to measurements of this constituent (*i.e.*, x_2). In Table I, k is applied to the free acid constituent, the soluble solids factor being taken as unity. To obtain the most accurate estimate of fruit content in a fruit pulp from these two measurements, the free acid percentage in the pulp is multiplied by the appropriate value of k and added to the soluble solids percentage. The quantity so obtained, multiplied by 100, is then divided by the corresponding quantity for the fruit itself, (*viz.*, the mean percentage of free acid, multiplied by the same factor) and then added to the soluble solids in the fruit. Thus, for the strawberry pulp considered previously, this leads to an estimated fruit content of $\frac{(0{\cdot}8 \times 0{\cdot}86) + 7{\cdot}0}{(0{\cdot}96 \times 0{\cdot}86) + 9{\cdot}01} \times 100 = 78{\cdot}1$ per cent. Table I shows the mean values of the optimum combination for the various fruits, to use in estimating fruit contents of pulps when these two constituents are employed in analysis. They are calculated from the mean values shown for the separate constituents.

Reference to Table I shows that with some fruits, notably apple, blackcurrant and raspberry, a substantial reduction in the coefficient of variation is effected by the optimum combination. The manner in which the coefficient of variation affects the accuracy of the estimation, as measured by the range within which the fruit content is likely to lie, has been previously shown.[1] For the sample of strawberry pulp considered above (p. 430), the limits within which the fruit content is likely to lie (probability = 0·95) are 64·7 and 98·5 per cent. Although the range is large the apparent fruit content of 78·1 per cent. is a more reliable estimate than the one obtained by averaging the two estimates obtained from the soluble solids and free acid separately. As remarked above, this estimate is equivalent to the use of a combination in which each constituent is divided by its mean value in the fruit. The coefficient of variation of such a combination may be found by inserting the value $\bar{x}_1/\bar{x}_2$ for k, giving $\frac{1}{2}\sqrt{V_1^2 + V_2^2 + 2rV_1V_2}$. Limits to the apparent fruit content of 80·5 per cent. are then 62·4 and 113·3 per cent. The estimate from the average is, in fact, less reliable than that from the use of the soluble solids figure alone, owing to too great emphasis being placed on the free acid content, which in strawberries has an extremely high coefficient of variation.

Although two constituents can, therefore, always be combined to form an optimum combination as shown, it does not follow that their combined use will necessarily lead to a more accurate estimation than by the use of one of them alone. In fact, whilst the coefficient of variation of the optimum combination can never be greater than that of either of the constituents, it may sometimes be equal to the lower one, and then there is obviously no advantage in carrying out both analytical determinations. With strawberries, for example,

equal accuracy in estimating fruit contents is obtained by the use of soluble solids determination alone. Whether or not the optimum combination results in improved accuracy can always be seen by comparing the coefficients of variation. As mentioned above, the only case where no improvement occurs is where the ratio of the smaller to the larger coefficient of variation of the two constituents equals the correlation coefficient. In all other cases some improvement must occur, but this will not be appreciable (as with strawberries) unless these two values differ considerably. A negative correlation between the constituents always enables an improvement to be made. However, working out the optimum combination is worth while, even if no improved accuracy results, since it then establishes that one of the analytical determinations may be omitted without increasing the error of the estimation.

Extension to more than two constituents

Where other constituents are also available for determining the proportion of an ingredient, any pair can be treated as above, and the two constituents showing least variation in their optimum combination could then be selected for use. A more general line of approach is to find the optimum combination of all the available constituents taken together. This involves a more laborious calculation but can lead to a lower coefficient of variation than is obtainable from any pair of constituents.

As before, determinations of each constituent are required on the *same* samples of ingredient in order that their correlations may be calculated. The number of correlation coefficients necessary increases rapidly as more constituents are added. Thus four constituents involve six correlations, whilst five would require ten correlation coefficients. But it is rare for more than four constituents to be useful for estimating an ingredient. With fruit, data for the four constituents insoluble solids, soluble solids, sugars and free acid have been collected in these laboratories. The method of combining four measurements will be given; the treatment is general and can be extended to any number of constituents.

In generalising the procedure to more than two constituents the device of putting the first multiplying factor equal to unity has not been followed, as the advantage of symmetry in the resulting equations would be lost by so doing. The method employed is similar to the technique of multiple regression.[3] Calculation of the actual correlation coefficients may be avoided, though the corresponding cross-products are required.

Let x_1, x_2, x_3, x_4 denote the values of four different constituents (determined on the same sample of the ingredient). Also let $S_{11} = \Sigma(x_1 - \bar{x}_1)^2$, $S_{12} = \Sigma(x_1 - \bar{x}_1)(x_2 - \bar{x}_2)$, etc., where the summations extend over the n samples of the ingredient analysed, and $\bar{x}_1$, $\bar{x}_2$, etc., denote the means for the respective constituents. Then if the combination $x_c = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4$, where a_1, a_2, a_3 and a_4 are constants, the coefficient of variation of x_c is given by:

$$V_c = \frac{100s_c}{\bar{x}} = \frac{100\sqrt{(a_1^2S_{11} + \ldots + a_4^2S_{44} + 2a_1a_2S_{12} + \ldots + 2a_3a_4S_{34})/(n-4)}}{a_1\bar{x}_1 + a_2\bar{x}_2 + a_3\bar{x}_3 + a_4\bar{x}_4} \qquad (3)$$

The divisor (n — 4) is used in the numerator because four constants are now being fitted to the data, giving (n — 4) degrees of freedom for estimating the variance of the combination.

Values of a_1, a_2, etc., that make V_c a minimum are found by partial differentiation of the right-hand side of (3) with respect to each factor in turn and equating to zero, leading to the set of equations (apart from common factors):

$$\left.\begin{aligned} a_1S_{11} + a_2S_{12} + a_3S_{13} + a_4S_{14} &= \Sigma x_1 \\ a_1S_{12} + a_2S_{22} + a_3S_{23} + a_4S_{24} &= \Sigma x_2 \\ a_1S_{13} + a_2S_{23} + a_3S_{33} + a_4S_{34} &= \Sigma x_3 \\ a_1S_{14} + a_2S_{24} + a_3S_{34} + a_4S_{44} &= \Sigma x_4 \end{aligned}\right\} \qquad (4)$$

These equations may be solved in any of the standard ways of dealing with multiple regression equations. For reasons given below, it is best to use the c-matrix method of Fisher. Full working details of this method of solution are given in statistical works[3,4] and will be illustrated below with data from analyses of blackcurrants. Solutions to (4) can be written:

$$\left.\begin{aligned} a_1 &= c_{11}\Sigma x_1 + c_{12}\Sigma x_2 + c_{13}\Sigma x_3 + c_{14}\Sigma x_4 \\ a_2 &= c_{21}\Sigma x_1 + c_{22}\Sigma x_2 + c_{23}\Sigma x_3 + c_{24}\Sigma x_4 \\ a_3 &= c_{31}\Sigma x_1 + c_{32}\Sigma x_2 + c_{33}\Sigma x_3 + c_{34}\Sigma x_4 \\ a_4 &= c_{41}\Sigma x_1 + c_{42}\Sigma x_2 + c_{43}\Sigma x_3 + c_{44}\Sigma x_4 \end{aligned}\right\} \qquad (5)$$

Here the factors c_{11}, c_{12}, c_{13}, c_{14} are the values of a_1, a_2, a_3, a_4 that satisfy the four simultaneous equations obtained from (4) when Σx_1 is replaced by 1 and Σx_2, Σx_3 and Σx_4 by 0. The factors c_{21} to c_{24} are obtained by replacing Σx_2 by 1 and the others by 0 and solving the equations. Similar procedures give the values of c_{31} to c_{34} and c_{41} to c_{44}. Since it may be shown that $c_{12} = c_{21}$, $c_{13} = c_{31}$, etc., only ten c-factors need be calculated in theory. In practice, however, these equalities provide useful checks on the arithmetic.

If we multiply the first of equations (4) by a_1, the second by a_2 and so on, then adding up the left-hand side and dividing by the appropriate number of degrees of freedom gives the variance of the optimum combination. As four constants are fitted, (n − 4) degrees of freedom are available, hence:

$$(n - 4) \text{ var } x_c = a_1\Sigma x_1 + a_2\Sigma x_2 + a_3\Sigma x_3 + a_4\Sigma x_4$$

or

$$s_o = \sqrt{n\bar{x}_c/(n - 4)}$$

This gives a simple formula for calculating the coefficient of variation of the optimum combination as

$$V_c = 100s_c/\bar{x}_c = 100/\sqrt{\frac{n-4}{n}(a_1\bar{x}_1 + a_2\bar{x}_2 + a_3\bar{x}_3 + a_4\bar{x}_4)} \quad \ldots \quad \ldots \quad (6)$$

There is a resemblance between the combination of measurements to give minimum coefficient of variation and the discriminant function introduced by Fisher. The latter quantity represents the combination of various measurements that best discriminates between two different varieties, or species. For example, we might be interested in the combination of insoluble solids and soluble solids that would best distinguish blackcurrants from strawberries (if no other means of identification were available). The present combination may be regarded as the discriminant function that best distinguishes the original fruit from the population of all pulps containing a fixed proportion, P, of fruit. The coefficients calculated for this discriminant are found to be proportional to the coefficients as calculated above (for any given P). Since any set of constants *proportional* to a_1, a_2, etc., leaves the coefficient of variation of the combination unchanged, the discriminant function interpreted in this way leads to the same solution for the best linear combination as above.

The values of the coefficients calculated from equations (5) may be in error from the true values which would be obtained if all possible samples of the ingredient could be analysed. In practice, only a small amount of data may be available, particularly as only data from samples in which all constituents have been determined can be utilised. In many cases the correlations are not statistically significant although their numerical values are incorporated in the calculation. From analogy with the discriminant function, the standard error of the coefficients a_1, a_2, etc., may be easily calculated by multiplying the standard error s_c of the combination by $\sqrt{c_{11}}$, $\sqrt{c_{22}}$, etc.

Equations (5) and (6) above enable the multiplying factors and the coefficient of variation of the optimum combination to be worked out for the four constituents. When the optimum combination is evaluated it is necessary to decide whether all the measurements involved are useful in lessening the variation. This is not obvious, as it is with two constituents only, because even if the coefficient of variation of the combination is less than that of any one constituent this may be due entirely to the combined effect of only three or perhaps two constituents. Any constituents that are not useful can be omitted in the chemical analysis without reducing the accuracy of the final estimate of the percentage of ingredient. A criterion for rejecting or retaining any constituents in the combination is provided, however, by the standard errors of their coefficients a_1, a_2, etc. Any constituent whose coefficient is numerically less than its standard error can be omitted without increasing by more than 1 per cent. the coefficient of variation of the optimum combination of the remaining constituents. These standard errors can be easily calculated, as indicated above, if Fisher's c-matrix method of solving equations (4) is employed.

If more than 50 observations are involved, the coefficient needs to be approximately $\sqrt{n}/7$ times its standard error for the constituent to be usefully retained in the combination. The exact value for the estimated coefficient of variation of the optimum combination after rejecting any constituent can be calculated from the value before rejection by dividing by the factor

$$\sqrt{\frac{n-p+1}{n-p}\left\{1 - \frac{a^2}{(n-p)\text{ var } a}\right\}}$$

where a = value of the coefficient of the rejected constituent, n = number of observations and p = number of constituents combined (before rejection).

When a constituent is discarded it becomes necessary to recalculate the coefficients for the optimum combination of the remaining constituents. There is no need, however, to solve the original equations afresh, leaving out the unwanted measurement. If any constituent, say x_p, is to be rejected, then the new c-matrix may be calculated[3] from the old by relations of the type $c'_{ij} = c_{ij} - \frac{c_{ip}\, c_{jp}}{c_{pp}}$, where c'_{ij} is the new value. The appropriate values of the new coefficients, with their standard errors, then follow as above, except that one more degree of freedom is now available.

It is the advantage of the c-matrix method of solution that it enables the standard errors of the coefficients to be readily calculated and also permits easy adjustment of their values when one or more constituent is discarded for any reason. In practice it is possible to round off the calculated values of the coefficients or to reduce them, in approximate proportion, to small whole numbers without appreciably altering the coefficient of variation of the combination. It is desirable for the coefficients to be small integers, if possible, in order to minimise the arithmetical labour of calculating the proportion of ingredient. The final coefficient of variation of the combination adjusted in this way must, however, be checked from the expression (3) and not from equation (6), inserting the modified coefficients. This should not differ from the optimum value by more than, say, 1 per cent.

APPLICATION TO BLACKCURRANTS AND OTHER FRUITS—

The following data from 20 samples of blackcurrants illustrate the method of arriving at the optimum combination of the constituents insoluble solids, soluble solids, sugar and free acid. Table II shows the sum of squares and cross-products (of deviations from the means) for the four constituents, which are the values S_{11}, S_{12}, etc., above. Thus the entry of 66·116 in column 3, line 2 (or column 2, line 3) denotes the cross-product $S_{23} = \Sigma(x_2 - \bar{x}_2)(x_3 - \bar{x}_3)$ of soluble solids and sugars. The table is symmetrical about the diagonal, since $S_{ij} = S_{ji}$, where S_{ij} is the value in column i, row j. The last row in Table II shows the sums Σx of each constituent for the 20 samples.

TABLE II

SUMS OF SQUARES AND CROSS-PRODUCTS OF FOUR CONSTITUENTS (MEASURED FROM MEANS) IN 20 SAMPLES OF BLACKCURRANTS

	Insoluble solids x_1	Soluble solids x_2	Sugars x_3	Free acid x_4
x_1	+ 11·119	− 7·035	− 7·207	+ 1·360
x_2	− 7·035	+ 71·214	+ 66·116	− 1·721
x_3	− 7·207	+ 66·116	+ 81·099	− 8·573
x_4	+ 1·360	− 1·721	− 8·573	+ 2·890
Σx	117·4	290·4	132·0	70·6

Values for the c-matrix are then calculated by solving the four sets of four equations, equating the left-hand side to each column of the right-hand side in turn.

$$\left.\begin{aligned} 11{\cdot}119\, a_1 - 7{\cdot}035\, a_2 - 7{\cdot}207\, a_3 + 1{\cdot}360\, a_4 &= 1,\ 0,\ 0,\ 0 \\ -7{\cdot}035\, a_1 + 71{\cdot}214\, a_2 + 66{\cdot}116\, a_3 - 1{\cdot}721\, a_4 &= 0,\ 1,\ 0,\ 0 \\ -7{\cdot}207\, a_1 + 66{\cdot}116\, a_2 + 81{\cdot}099\, a_3 - 8{\cdot}573\, a_4 &= 0,\ 0,\ 1,\ 0 \\ 1{\cdot}360\, a_1 - 1{\cdot}721\, a_2 - 8{\cdot}573\, a_3 + 2{\cdot}890\, a_4 &= 0,\ 0,\ 0,\ 1 \end{aligned}\right\} \quad .. \quad .. \quad (7)$$

These values are shown in Table III (mathematically the inverse matrix of Table II). Again the table is diagonally symmetrical, as $c_{ij} = c_{ji}$.

TABLE III

c-FACTORS CALCULATED FROM EQUATIONS (7)

	x_1	x_2	x_3	x_4
x_1	+ 0·12447	+ 0·10260	− 0·10535	− 0·30988
x_2	+ 0·10260	+ 0·38463	− 0·41567	− 1·05192
x_3	− 0·10535	− 0·41567	+ 0·46749	+ 1·18844
x_4	− 0·30988	− 1·05192	+ 1·18844	+ 3·38983

Equations (5), in which c_{ij} is the figure in row i, column j, of Table III, may now be solved, giving for the coefficients $a_1 = 8{\cdot}624$, $a_2 = -5{\cdot}392$, $a_3 = 12{\cdot}534$ and $a_4 = 54{\cdot}339$. From these we calculate $n\bar{x}_c = 1012{\cdot}458 - 1565{\cdot}837 + 1654{\cdot}488 + 3836{\cdot}333 = 4937{\cdot}442$, whence $\bar{x}_c = 246{\cdot}872$, $s_c = \sqrt{4937{\cdot}442/16} = 17{\cdot}57$ and $V_c = 7{\cdot}12$.

The standard errors of the coefficients are 6·20 ($= 17{\cdot}57 \times \sqrt{0{\cdot}12447}$), 10·89, 12·01 and 32·34 respectively. The standard error of a_2 is about twice its numerical value, so that the soluble solids constituent may be omitted from the combination. A recalculation of the c-matrix, omitting x_2, is therefore made (see p. 435) by means of equations of the type $c'_{ij} = c_{ij} - (c_{i2}c_{j2}/0{\cdot}38463)$.

These values are shown in Table IV.

TABLE IV

c′-FACTORS CALCULATED FROM TABLE III WHEN x_2 IS OMITTED

	x_1	x_3	x_4
x_1	+ 0·09710	+ 0·00553	− 0·02928
x_3	+ 0·00553	+ 0·01827	+ 0·05163
x_4	− 0·02928	+ 0·05163	+ 0·51294

Values of the coefficients with their standard errors are now $a_1 = 10{\cdot}062 \pm 5{\cdot}27$, $a_3 = 6{\cdot}706 \pm 2{\cdot}28$, $a_4 = 39{\cdot}591 \pm 12{\cdot}11$, and the coefficient of variation of the combination is 6·96. This is actually less than the optimum value with all four constituents, owing to the fact that the variance is now estimated from 17 instead of 16 degrees of freedom. All coefficients are now greater than their standard errors and the three constituents must all be regarded as useful in reducing the variation of the combination. The coefficient a_1 of the insoluble solids has the greatest proportional standard error, but it cannot be omitted from the combination without increasing the estimated variation (coefficient of variation of the optimum combination of sugars and free acid alone = 7·63). Thus, on the evidence available from the analysis of 20 samples of blackcurrants, the maximum precision in analysis arises from the use of the combination [10·1 (insoluble solids) + 6·7 (sugars) + 39·6 (free acid)] and no further improvement is achieved by including a determination of soluble solids.

If all four analytical determinations are made on a blackcurrant pulp and an average is taken of the estimates of fruit content given by each constituent separately, it may be shown that this is equivalent to using a single combined factor with a coefficient of variation of 9·81. Thus the above statistical treatment enables a more accurate estimation of the fruit content to be made with one less analytical determination, and makes more efficient use of the available data.

It may be that when a constituent is rejected a larger number of samples becomes available for calculating the optimum combination of the remaining constituents. In this event a recalculation should be made, and the new values employed, even though the additional data happen to lead to an increase in the estimated coefficient of variation of the combination. Such an increase (or decrease) is quite likely to occur through sampling variations. In calculating the mean value of the optimum combination, the mean value inserted for each constituent should be estimated from as large a number of samples as possible. For this purpose it is not necessary to restrict the data to samples on which all constituents have been determined. Such a mean value, of course, cannot be used in calculating the coefficient of variation of the optimum combination, the best estimate of which is already obtained as above. But *solely* for the purpose of calculating the percentage of ingredient this will be the most accurate mean value to use.

Table V has been drawn up in this way, and gives for various fruits the optimum combination out of the four determinations insoluble solids, soluble solids (by specific gravity), sugars (as invert sugar) and free acid (as hydrated citric acid) together with the mean value of the combination based on data collected over a number of years. The reducing sugars were determined by Fehling's titration on a suitably diluted aqueous extract of the fruit. The other analytical determinations were carried out as described by Hinton and Macara.[2] The exact values of the factors have been rounded off, as explained above, to give integral values as small as possible without significantly increasing the coefficient of variation of the combination. It should be noted that some of the combinations involve the difference between constituents rather than their sum. This is the case where a multiplying factor turns out

to be negative owing to a relatively large positive correlation between the constituents. The numerical value of the whole combination is, of course, always positive.

The table also shows the coefficient of variation, V_o, of the optimum combination, together with the number of samples, n, on which it is based. For comparison are shown the coefficient of variation, V_s, of the best single constituent and the coefficient of variation, V_a, of the combination equivalent to averaging estimates from all four single constituents.

TABLE V

OPTIMUM COMBINATION OF THE CONSTITUENTS INSOLUBLE SOLIDS, SOLUBLE SOLIDS, SUGARS AND FREE ACID (AS HYDRATED CITRIC ACID)

Fruit	Optimum combination: Factors	Mean value	V_o	n	Best single factor: Constituent	V_s	V_a
Apple	Sol. solids + 2 (free acid)	14·2	7·4	37	Sol. solids	8·4	11·2
Blackcurrant ..	Insol. solids + sugar + 6 (free acid)	32·7	7·0	20	Free acid	11·0	9·8
Damsons ..	3 (Sol. solids) − 4 (sugar) + 9 (free acid)	38·8	9·6	10	Free acid	15·0	14·7
Gooseberry ..	Insol. solids + 6 (soluble solids) − 3 (sugar)	42·4	7·2	48	Sol. solids	9·7	12·2
Plum	9 (Insol. solids) + 2 (sol. solids) + 3 (free acid)	41·7	15·2	41	”	17·9	15·2
” (sieved) ..	Sol. solids + 2 (free acid)	16·1	17·2	50	”	17·9	—
Raspberry ..	5 (Sol. solids) − 4 (sugar) + 4 (free acid)	33·5	11·8	29	”	17·4	16·3
Strawberry ..	Insol. solids + sol. solids	11·2	·9·8	65	”	10·5	11·1

The optimum combination for sieved plums has been included in view of the common practice of sieving this fruit. This has been calculated by omitting the insoluble solids factor and recalculating the combination for the remaining three constituents in the manner indicated above. Strictly speaking, therefore, this does not represent the optimum combination of the four constituents, but is the best available in the absence of data for insoluble solids in sieved plums.

CONCLUSIONS

From a consideration of Table V it is evident that for the common fruits not more than three (in some cases only two) constituents need be determined in a pulp to obtain the percentage of fruit with the maximum precision. The gain in precision (as measured by the coefficient of variation) over the use of the best single constituent varies from about 36 per cent. for blackcurrants and damsons to only 4 per cent. for sieved plums. It is interesting to note that out of the four constituents, the soluble solids appears as the most reliable by itself in all cases except two. It should be noted that if separate estimates from all four constituents are averaged, the percentage of fruit may be obtained with *less* precision (indicated by V_a) than if one constituent only is used (V_s). This is notably so with apples and gooseberries, owing to the relatively large variation in some constituents.

In order to obtain the most reliable estimate, therefore, of the fruit content of a pulp the constituents shown in the combinations of Table V are determined in the pulp, multiplied by the factors given, and divided by the mean value shown. Limits to the estimate may be calculated from the coefficient of variation V_o, bearing in mind that this is based on (n − 4) degrees of freedom.

Consideration of the optimum combination has been confined to the examples of fruit, but it is of perfectly general application. The combination represents that linear function of the constituents which is most constant in samples of the ingredient. This provides the most reliable factor for estimating the percentage of ingredient in a food product if each constituent, taken by itself, leads to an estimate of the percentage in direct proportion to the amount present. It is assumed also that the amount of constituent in the food product is known exactly, that is, that no analytical errors or other uncertainties affect its determination. If this does not hold, the combination worked out to show minimum variation in samples of the ingredient may not also show minimum variation in samples of the food product containing a certain fixed proportion of ingredient, and consequently may not lead to the most reliable estimate. Considerations of this type arise with jams where uncertainty

enters into the analytical determinations largely owing to use of added pectin. Sampling errors may also be appreciable, particularly in the insoluble solids determination, owing to non-uniform filling into jars from a batch of jam. For these reasons the case of jam is more complex and requires separate treatment, which it has not yet been possible to give owing to lack of suitable data.

Apart from these limitations, the optimum combination can always be used where two or more constituents, each of which show variation in the ingredient, are available for the determination of the ingredient. For more than two constituents the calculation becomes somewhat tedious unless a machine is available, but the gain in precision may make the time spent well worth while, particularly if some analytical determinations may subsequently be omitted as a result.

SUMMARY

A linear combination of two or more chemical constituents of an ingredient may be calculated that shows less variation in samples of the ingredient than any other linear combination. If the value of this optimum combination is determined for a sample of a food product and divided by the mean value for the ingredient the most reliable estimate of the true proportion of ingredient is obtained. Where several constituents are available for the purposes of the estimation it is possible to reject any that do not contribute to the reliability of the estimate. The use of the optimum combination may enable greater accuracy to be achieved, with fewer analytical determinations, than if estimates from all available constituents are averaged. The method is applicable to all food products where the whole of the values of the chemical constituents determined are contributed by the ingredient estimated. The calculation of the optimum combination is illustrated for blackcurrants and the combination given for various fruits, applicable to the analysis of pulps.

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A Study of the Methods Prescribed by the F. & F. S. Regulations (1932) for the Estimation of Oil, Albuminoids and Fibre in Feeding Stuffs

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DAILY use, over many years, of the methods for estimating oil, albuminoids and fibre in feeding stuffs, laid down in the Fertilisers and Feeding Stuffs Regulations, 1932 (S.R. & O., 1932, No. 658), has shown the urgent need for their reconsideration. These methods are dealt with in turn below.

ESTIMATION OF OIL

The Regulations state—

(*a*) "A weighed quantity of the sample shall be placed in an extraction thimble, which shall then be placed in an extraction apparatus and extracted with petroleum spirit, b.p. 40° to 60° C. At the end of 3 to 4 hours the thimble shall be removed from the apparatus, dried and its contents finely ground, preferably with sand,

in a small mortar previously rinsed with petroleum spirit. The substance shall then be returned to the thimble, the mortar being washed out with petroleum spirit, and the extraction containued for another hour. The extract should be free from suspended matter. After evaporation of the solvent, the oil shall be dried at 100° C. and weighed.

(*b*) In the case of samples containing saccharine matter, the weighed portion in the thimble shall be washed with water and then dried, previous to extraction."

It is assumed that procedure (*b*) must be applied to all compound feeding stuffs containing molasses and procedure (*a*) to all other feeding stuffs. It might, however, be contended that practically all feeding stuffs should be water-washed, for most of them contain natural sugars, as shown in Table I.

TABLE I

NATURAL SUGARS IN FEEDING STUFFS

Material	Sugars as cane sugar %	Material	Sugars as cane sugar %
Barley	1 to 2	Milk whey dried	40 to 45
Bran (wheat)	5 to 6·5	Oats	1 to 3
Beet residue	3 to 9	Palm kernel cake	2 to 3
Coconut cake (copra)	10 to 15	Potato flakes	3 to 4
Carrot pomace	24	Rape meal	4 to 6
Cocoa cake	4	Rice	0 to 1
Cotton cake (undecorticated)	3 to 5	Rice bran	2 to 3
Fish meal	nil	Rice bran (parboiled)	0 to 1
Groundnut cake (decorticated)	6 to 9	Soya bean meal	9 to 11
Linseed cake	5 to 6	Sunflower cake	5 to 6
Locust bean meal	38 to 42	Tapioca	0 to 3
Maize meal	1 to 3	Wheat	2 to 3
Maize gluten	0 to 1	Wheat flour (seconds)	3 to 5
Milk powder (skimmed)	30 to 35		

These figures for sugar content show the ranges found in the course of our experience and they are in broad agreement with those in the scientific literature.

Owing to the presence of sugars in most feeding stuffs it appeared desirable to ascertain (1) how different types of feeding stuffs behave when subjected to the water-washing process and (2) what is the percentage of molasses above which it is necessary to water-wash in order to obtain the correct figures for oil. With that object, estimations of oil were made on the following feeding stuffs, with and without previous water-washing.

	Natural sugars %	Added molasses %	Total sugars %	Added calcium carbonate %
Palm kernel meal	3·2	nil	3·2	1
Mixed bran and palm kernel meal (1 + 1)	4·7	nil	4·7	0·5
Poultry meal	6·0	nil	6·0	2·8
Poultry pellets	5·3	3·0	6·8	2·8
National Cattle Food*	4·3	5·0	6·8	2·0

* Contained 17·5 per cent. of linseed cake.

In the oil estimations the thimble specified in the Regulations was not used, but the meal was wrapped in filter paper and placed in a Tate tube for extraction.

Water-washing of the samples was carried out as follows: 5 g. of the meal were stirred in a beaker with 5 ml. of cold water, filtered and washed twice, each time with 25 ml. of cold water. The damp meal was transferred to a basin and dried on a steam-bath, the filter paper being dried in a steam-oven. The drying time was in this way reduced to 1 hour and the difficulty due to the meal adhering strongly to the paper was avoided. After drying, the meal was transferred to the dry filter paper, wrapped in another filter paper and extracted. The residue was re-ground and re-extracted.

The results are shown in Table II.

The observed standard errors were calculated: for direct extraction, A, the average standard error was ±0·044 per cent. and that for extraction after water-washing, B, ±0·059

TABLE II

YIELDS OF OIL, PER CENT.

A. From sample direct. B. From water-washed sample

	Palm kernel meal		Bran and P.K. meal		Poultry meal		Poultry pellets		N.C.F. meal No. 1	
	A	B	A	B	A	B	A	B	A	B
Replicate determinations	9·74	9·35	7·58	6·92	6·34	5·46	5·52	4·80	6·36	5·66
	9·74	9·45	7·66	7·00	6·32	5·48	5·48	4·73	6·40	5·64
	9·79	9·52	7·56	6·85	6·26	5·52	5·54	4·82	6·29	5·64
	9·80	9·46	7·58	6·81	6·32	5·44	5·54	4·83	6·34	5·66
			7·56	6·80					6·36	5·64
			7·66						6·41	5·62
Means	9·77	9·45	7·60	6·88	6·31	5·48	5·52	4·80	6·37	5·64
Difference A − B ..	0·32		0·72		0·83		0·72		0·73	

per cent. Direct extraction gave more consistent results than extraction after water-washing, but both methods were satisfactory in respect of standard error. Analysis of variance showed that variations between the methods far outweighed the variations caused by the experimental errors of each method.

These results have been confirmed in a number of laboratories in our organisation. Table III shows the extent to which the water-wash method resulted in low oil figures for 78 samples of feeding stuffs of various types containing low percentages of added molasses.

TABLE III

Oil per cent. found by direct extraction minus oil per cent. found after water-washing	Number of samples
0·25 to 0·50	17
0·51 to 0·75	23
0·76 to 1·00	20
1·01 to 1·50	13
above 1·50	5

It has thus been established that feeding stuffs with or without added molasses can yield appreciably lower oil figures after the water-wash treatment, prescribed by the Feeding Stuffs Regulations.

Experiments were made to ascertain the cause of the difference, attention being directed to possible changes in free fatty acid content, loss of oil by emulsification, formation of lime soap and the effect of moisture.

FREE FATTY ACID CONTENT OF THE EXTRACTED OILS—

The free fatty acid contents of the oils extracted directly or after the water-wash were determined, the true molecular weights being calculated from the saponification equivalents. The figures, which refer to the same feeding stuffs as were used in the preceding experiments, are set out in Table IV and related to the loss of oil in the water-washing process.

TABLE IV

FREE FATTY ACIDS OF EXTRACTED OILS

A. From sample direct. B. From water-washed sample

	P.K. meal		Bran and P.K. meal		Poultry meal		Poultry pellets		N.C.F. meal	
	A	B	A	B	A	B	A	B	A	B
F.F.A. in extracted oil, %	6·1	4·6	12·5	7·9	15·4	10·0	16·8	13·8	18·1	13·7
F.F.A., calculated on meal, %	0·60	0·43	0·88	0·54	0·97	0·55	0·98	0·66	1·15	0·77
Deficiency of oil due to water-washing, % on meal	0·32		0·72		0·83		0·72		0·72	
Deficiency of F.F.A. due to water-washing, % on meal	0·17		0·34		0·42		0·32		0·38	

The oil extracted after washing contained appreciably less free fatty acid than the oil obtained by direct extraction and the extent of the reduction of the free fatty acid content corresponded to about half of the oil lost by the water-washing process. No appreciable amount of fatty matter could be extracted from the filtrate from the water-wash.

POSSIBLE FORMATION OF CALCIUM SOAP DURING WATER-WASHING—

Addition of calcium carbonate to certain stock foods had been customary in the industry for a number of years, and in 1940 its addition to certain feeding stuffs became compulsory. In July, 1942, this regulation became general, apart from special foods such as calf meals and foods for which a special licence has been obtained. Hence there arises the possibility of a reaction between the calcium carbonate in the cattle food and free fatty acid in the oil when the meal is in the wetted condition in the water-wash process. The residues after direct extraction and after water-washing and extraction were moistened with water followed by a small quantity of concentrated hydrochloric acid, and then dried on the water-bath and re-extracted. Under these conditions the figures shown in Table V were obtained.

TABLE V

EFFECT OF ACIDIFICATION AND RE-EXTRACTION

A. Sample direct. B. Water-washed sample

Treatment	P.K. meal		Bran and P.K. meal		Poultry meal		Poultry pellets		N.C.F. meal	
	A	B	A	B	A	B	A	B	A	B
Straight extraction ..	9·73	9·39	7·60	6·88	6·19	5·21	5·30	4·68	6·22	5·45
Further grind plus 1-hour extraction	0·04	0·06	0·05	0·04	0·12	0·27	0·22	0·12	0·15	0·19
Acidification and re-extraction	0·08	0·19	0·41	0·67	0·34	0·57	0·11	0·42	0·11	0·26

After acidification the residue from the first extraction of the water-washed meal yielded more fatty matter than that from the directly extracted meal. This additional fatty matter was found to contain appreciable quantities of fatty acid. These effects may be connected with the formation of lime soaps from the calcium carbonate and the free fatty acid in the wetted mass.

EFFECT OF DRYING ON OIL EXTRACTION—

Oil extractions were carried out on various meals, with and without prior drying, Series I for 3 hours at 102° C. and Series II for 2 hours at 105° C. The results are shown in Table VI.

TABLE VI

EFFECT OF DRYING ON OIL EXTRACTION

	Moisture %	Oil: on meal as received %	Oil: on dried meal calculated to original meal %
Series I			
Palm kernel meal	10·3	9·77	9·74
Bran plus P.K. meal	11·0	7·6	7·38
Poultry meal	10·9	6·31	6·14
Poultry pellets	11·8	5·52	5·46
N.C.F. meal	12·3	6·36	6·13
Series II			
Pig Meal No. 1	10·8	3·95	3·6
Calf Nutlettes	11·75	6·4	6·45
Poultry Mash No. 1	10·9	3·0	2·8
National Cattle Nuts No. 1A ..	12·5	3·9	3·5
Linseed cake..	10·8	9·55	9·3
Extracted palm kernel meal ..	8·4	1·5	1·55
Extracted rape meal	9·8	2·2	2·2
Fish meal	7·75	6·85	5·65
Bran	10·7	4·4	4·25
Decorticated groundnut cake ..	8·75	9·5	9·5

The figures show that considerable reduction in oil content can result from drying the meal, which is a necessary part of the water-wash process.

A serious objection to the water-wash process is that some of the feeding stuffs, *e.g.*, those containing linseed cake, are apt to form gelatinous masses that are difficult to handle and to filter.

POSSIBLE LOSS THROUGH CHANGES IN THE DAMP MEAL—

In some cases during the water-wash process the meal may remain in a wet condition for a comparatively long time. This may result in an increase in free fatty acid and other changes, including the formation of lime soap. In order to get some idea of the effects of leaving the meal in a wet condition, washed poultry meal was spread on a dish and allowed to dry in an evacuated desiccator; with fresh sulphuric acid the drying took 48 hours and with once-used sulphuric acid 4 days. The effect is shown in Table VII.

TABLE VII

Treatment of sample	Period during which sample was damp	Oil, %
Extracted directly	—	6·3
Washed, dried at 102° C.	3 hours	5·47
Washed, dried in desiccator	48 hours	5·12
Washed, dried in desiccator	4 days	3·58
Washed, and later dried at 102° C.	4 days	2·54

In keeping the washed meal damp for 4 days, the storage conditions were purposely exaggerated, but the figures show clearly that in the wet condition changes do occur that lead to serious reduction in the figures for oil content.

The preceding sections have shown that the low oil figures obtained after water-washing can be partly due to (1) the formation of calcium soaps in the wet mass, (2) the effect of drying the wet mass before extraction with solvent and (3) the effect of changes in the wet mass if it is allowed to remain unduly long in the wet condition.

EFFECT OF VARIOUS PROPORTIONS OF MOLASSES UPON OIL EXTRACTION—

To determine the effect of different proportions of molasses upon the apparent oil content, palm kernel cake meal (without added calcium carbonate) and mixtures of the meal with molasses were extracted with light petroleum (b.p. 40° to 60° C.) directly and after water-washing. The oil content found by direct extraction of the meal without molasses was taken as the "theoretical" value and used as the basis for calculating the theoretical oil contents of the molassed samples, given in the second column of Table VIII (upper part).

TABLE VIII

Extraction with light petroleum (b.p. 40° to 60° C.) only
Oil content of original meal by direct extraction 7·05%

Molasses in sample	Oil in sample, theoretical	Oil by direct extraction	Oil by extraction after water-wash	Theoretical figure minus	
				Direct figure	Water-wash figure
%	%	%	%	%	%
0	—	7·05	6·55	—	0·50
15	5·99	5·83	5·58	0·16	0·41
20	5·64	5·43	5·44	0·21	0·20
25	5·29	4·96	5·07	0·33	0·22
30	4·93	4·47	4·82	0·46	0·12

Extraction with methylated ether followed by extraction of resulting oil with light petroleum
Result on original meal 7·28% oil

Molasses in sample	Oil in sample, theoretical	Oil found	Theoretical figure minus figure found
%	%	%	%
0	—	7·28	—
15	6·18	6·03	0·15
20	5·82	5·90	− 0·08
25	5·46	5·60	− 0·14
30	5·10	5·18	− 0·08

A similar set of estimations was carried out on the mixtures after they had been cooked and shaken in their glass-stoppered bottles in a steam-oven for several hours, but these results are not quoted, as they were similar to those obtained on the uncooked mixtures.

The 1906 Act prescribed the use of methylated ether as a solvent and it was decided to carry out estimations by extraction with methylated ether in the first instance and after removal of the ether from the extract, extracting the latter with light petroleum (b.p. 40° to 60° C.); the idea was that the methylated ether would probably extract all the oil, plus a little sugar, and then the light petroleum would take up only the oil. It was realised that methylated ether by itself tends to give high figures for oil, sometimes 1 per cent. high.[1] The results are given in the lower part of Table VIII.

From the first set of figures, with light petroleum alone, it will be seen that direct extraction gives a slightly low oil figure at 20 per cent. of molasses, but decidedly low figures at 25 and 30 per cent. of molasses, whilst the water-wash method gives very low figures at 0 and 15 per cent. of molasses and a reasonably accurate figure at 30 per cent. Hence highly molassed feeds should be water-washed before extraction. The dividing line, above which water-washing should be practised, appears to be about 25 per cent. of molasses. With the second method reasonably accurate results are obtained at all the levels of molasses tested. Similar results were obtained on mixtures of palm kernel cake and molasses, to which calcium carbonate had been added.

SUMMARY—

The water-washing method is cumbersome and lengthy and its effect, whether or not the feeding stuffs contain added molasses, is to reduce the apparent oil content to a varying and unpredictable degree, which may be as much as 1·5 per cent. This reduction is associated with formation of soap from the calcium carbonate and the free fatty acid in the oil, lowered extraction efficiency on dried meal and possible changes in the wet mass on standing.

When the proportion of molasses does not exceed 25 per cent., direct extraction is satisfactory; with higher percentages it is desirable to wash out the molasses before extracting the oil, or alternatively, the oil can be first extracted with methylated ether, the solvent evaporated from the extract and the latter extracted with light petroleum. The use of two solvents would be an added complication in carrying out numerous daily routine estimations.

ESTIMATION OF ALBUMINOIDS

The Fertilisers and Feeding Stuffs Regulations, 1932, prescribe the use of 25 ml. of concentrated sulphuric acid, 10 g. of potassium or sodium sulphate and a small crystal of copper sulphate or a globule of mercury in the Kjeldahl digestion flask. Mercury is well known as an effective catalyst for the digestion, but it requires addition of sodium sulphide to the liquor before distillation. In recent years, selenium has been coming into regular use in the Kjeldahl process and has been the subject of numerous publications.[2] Alcock gives figures to show the increasing rapidity of the process with increasing quantities of sodium sulphate in presence of 0·2 g. of copper sulphate.[3]

It was considered desirable to obtain further information on the effect of increasing quantities of selenium in presence of various quantities of sodium sulphate.

Kjeldahl determinations were carried out in the normal manner on 1 g. of high-protein cattle meal or 1 g. of dried pure sulphanilic acid, with either 8 or 18 g. of anhydrous sodium sulphate, 25 ml. of concentrated sulphuric acid and different quantities of selenium metal. For details of the test conditions see Table IX.

The figures for the protein meal in Table IX show the marked accelerating effect of selenium on the destruction of organic matter. Increasing the amount of sodium sulphate from 8 to 18 g. reduced the time of clearing in absence of catalyst and in presence of very small quantities of it (*e.g.*, 0·01 g. of selenium). Nothing appears to be gained by using more than 0·1 g. of selenium and there is some evidence that 0·2 g. gives slightly lower results.* Analysis of variance by Fisher's method indicated that in both sets of experiments significant increase in albuminoids found occurred when 0·1 g. of selenium was used (compared with 0·01 g.), and in the experiments with the protein meal when 3 hours boiling was given after

* Patel and Sreenivasan (*Anal. Chem.*, 1948, **20**, 63) state that prolonged boiling with selenium leads to loss of nitrogen and that this can be corrected by the addition of mercuric oxide; their basal salt mixture contained copper sulphate, and thus they never worked with selenium as sole catalyst.

clearing, instead of the 1 hour prescribed. Increase in the amount of sodium sulphate from 8 to 18 g. had no significant effect.

TABLE IX

DETERMINATIONS ON HIGH-PROTEIN MEAL AND ON SULPHANILIC ACID

1 g. of sample with 25 ml. of concentrated H_2SO_4 in Kjeldahl flask, with additions as below

Catalyst	Na_2SO_4 g.	Clearing time, min.	Additional heating time, min.	Total heating time, min.	Albuminoids %
Determinations on a high-protein meal					
None	8	120	60	180	35·29
None	18	95	60	155	35·36
Selenium metal, 0·01 g.	8	43	60	103	35·40
,,	8	40	180	220	36·09
,,	18	33	60	93	35·57
,,	18	36	180	216	36·10
0·05 g.	8	30	60	90	36·09
0·10 g.	8	25	60	85	36·09
,,	8	25	180	205	36·22
,,	18	25	60	85	36·02
,,	18	25	180	205	36·21
0·15 g.	8	25	60	85	35·84
0·20 g.	8	25	60	85	35·96
Copper sulphate, 0·1 g.	8	60	60	120	35·86
,,	8	60	180	240	36·00
,,	18	50	60	110	35·92
,,	18	50	180	230	36·14
Determinations on sulphanilic acid (N = 8·093%)					Nitrogen %
None	8	150	60	210	7·74
Selenium metal, 0·01 g.	8	30	60	90	7·88
,,	8	30	180	210	8·03
,,	18	30	60	90	8·04
,,	18	30	180	210	8·07
0·05 g.	8	30	60	90	8·00
0·10 g.	8	30	60	90	8·07
,,	8	30	180	210	8·07
,,	18	25	60	85	8·08
,,	18	25	180	205	8·06
0·15 g.	8	25	60	85	8·02
0·20 g.	8	25	60	85	8·01

Some further tests were carried out on blood meal and fish meal, which have high albuminoid contents and might conceivably present difficulties in obtaining accurate figures. Mercury (one globule) was included in these tests and copper sulphate was used at the augmented amount of 0·5 g. The results are shown in Table X.

TABLE X

ESTIMATION OF ALBUMINOIDS IN BLOOD MEAL AND FISH MEAL

Total time of heating	Found, per cent. With 0·5 g. of mercury	With 0·5 g. of copper sulphate	With 0·1 g. of selenium
Blood meal:			
1½ hours	82·1	80·7	81·5
2½ ,, ...	82·2	81·5	81·5
3½ ,, ...	82·1	81·5	81·9
6 ,, ...	82·0	81·9	82·0
Fish meal:			
1½ hours	74·1	72·6	73·3
2½ ,, ...	73·8	73·3	74·0
3½ ,, ...	74·1	73·6	73·8
6 ,, ...	74·5	74·2	74·0

For blood meal the clearing times were much the same with each catalyst, about 25 minutes; but for the fish meal the mercury and the selenium caused clearing in about 25 minutes and the copper sulphate in 35 minutes.

It would appear that 0·5 g. of mercury is the best catalyst in the sense of yielding the maximum albuminoid figure in the shortest time and that, as in the previous tests, 0·1 g. of selenium is a distinctly better catalyst than copper sulphate. The Regulations specify that heating should be continued for 1 hour after clearing. Our results have shown that 0·1 g. of selenium requires some 2½ to 3 hours heating after clearing in order to obtain the maximum figure and that copper sulphate requires more time than selenium. Alcock[3] considers that it is important to specify a total digestion time rather than 1 hour after clearing; this opinion is supported by our figures for selenium and copper sulphate.

Estimation of fibre

For the estimation of fibre the Regulations provide that if the sample is sufficiently fine to pass through a sieve having apertures about 1 mm. square it should be used as it is; if it is not it should be powdered until it passes through the sieve.

In the first case the analyst must work with the material as received, but in the other the sample upon which the test is to be made may in the main be ground very finely or only just sufficiently so to pass through the sieve. Fineness of grinding is an important factor in the estimation of fibre and in the following tests the samples were sieved after each grinding, only the portion that failed to pass the sieve being reground. Except for the sample that was very finely ground (Table XIII), the sieve used was B.S.S. No. 16.

The method is empirical and therefore the results obtained are particularly dependent upon individual interpretation, in the absence of precise directions on details.

A few points call for special mention: (i) 2·5 or 3 g. of sample are taken; (ii) after removal of the oil the residue is dried (method not stated); (iii) during the treatment with acid and caustic soda, the contents of the flask are boiled gently and continuously for exactly 30 minutes; (iv) during these boilings the flask is to be rotated every few minutes in order to mix the contents and remove particles from the sides.

A series of co-operative tests was undertaken in nine separate laboratories where feeding stuffs were daily analysed. The results are summarised in Table XI.

TABLE XI

Feeding stuff	Number of labs.	Number of operators	Number of tests	Fibre, % Highest	Lowest	Average	Highest minus Lowest result
Bran A	8	—	39	10·7	9·0	10·0	1·7
Bran B	9	21	42	12·12	10·03	10·92	2·09
Extracted palm kernel meal:							
Sample A	9	17	30	14·8	10·88	13·06	3·92
Sample B	9	20	35	14·9	10·50	12·69	4·4
Dairy meal	9	32	64	6·48	5·50	5·98	0·98

The Regulations allow for a variation of one-eighth above and below the guarantee fibre content; this allowance covers the variation from the average with the bran samples and dairy meal, but not with the extracted palm kernel meal, where 10 per cent. of the results were outside the permissible limits for sample A and 15 per cent. for sample B. The spread between the highest and lowest figures is far too great for the method of estimation to be considered satisfactory, and further tests were designed to throw light on the possible causes of the variations.

A study of factors affecting the accuracy of fibre estimations—

In this estimation the sample has to be boiled with aqueous acid and alkali of specified concentration for a definite time, the rate of boiling being defined as "gentle and continuous." This definition may be interpreted in widely different ways. The results of varying the conditions and overstepping the boiling are given in Table XII.

In number (1) test, by the official procedure, an electric heater was used, according to our normal custom. The thoroughness of the agitation was determined by the vigour of

TABLE XII

	Variation in boiling procedure	Fibre content of palm kernel cake %
(1)	Official procedure	10·4
(2)	Vigorous boiling	10·8
(3)	Simmering over a bunsen flame	12·8
(4)	Simmering in a boiling water-bath	19·2
(5)	Acid boil of 1 hour	8·8
(6)	Alkali boil of 1 hour	9·1
(7)	Acid and alkali boils of 1 hour each	7·4

the boiling and it will be seen that as ebullition became milder, the fibre figure increased. In the official method, the acid and alkaline boiling periods are specifically limited to exactly 30 minutes, but if this time were extended by oversight, it would lead to lower fibre figures, as shown in the above table.

During vigorous boiling, the foam tends to deposit solid particles in the flask outside the area of the reaction, and experiment showed that the use of an air tube, supplying a gentle and steady jet of air from a mechanical blower on to the surface of the boiling liquid, prevented bad frothing and washing of the meal above the general liquor level; this device gave more consistent figures for palm kernel meal than the normal procedure, and it is mentioned in an American publication.[4] Ebullition, as a means of controlling temperature and agitation in a heterogeneous reaction, cannot be considered satisfactory and it is suggested that mechanical agitation with a suitable stirrer would make for much more consistent results.

To determine the effect of fineness of grinding, a sample of palm kernel cake was ground in the normal way to pass a B.S.S. No. 16 mesh sieve (apperture 1/25 inch or 1 mm.). Part of this ground sample was further ground to pass a sieve having an apperture of 1/64 inch, or 0·4 mm. Fibre was estimated on each of these portions, using both 2 and 3 g. (Table XIII).

TABLE XIII

EFFECT OF FINENESS OF MEAL ON FIBRE ESTIMATION

Feeding stuff	Number of labs.	Number of operators	Number of tests	Fibre, % Highest	Lowest	Average
Palm kernel cake:						
Ordinary powder: 2 g.	9	24	44	14·6	10·0	11·7
3 g.	9	24	44	14·1	10·1	12·2
Fine powder: 2 g.	9	24	44	13·0	9·6	10·8
3 g.	9	24	44	13·5	9·4	11·5

On applying the regulation allowance of one-eighth above and below the average figure, it was found that 23 per cent. of the figures obtained with 2 g. of ordinary powder were outside the permissible limits, and 14 per cent. of the figures on 3 g. of ordinary powder. Similarly for the fine powder, 11 per cent. of the figures fell outside the permissible limits with 2 g. and 16 per cent. with 3 g.

The finer powder gave significantly lower fibre figures. A study of variance by statistical methods suggests that this is due not to mechanical loss of meal during filtration but rather to a more ordered reaction between the powder and the liquids and to easier manipulation in the flask. The variance factor for the different laboratories suggested that they differed somewhat in their technique and also there was a very significant difference between operators, some obtaining consistently high and others consistently low figures.

The results of these tests suggest that the official method requires tightening up, or modification, in the following respects—

The fineness of the sample and the weight to be taken should be stated without an alternative. The method of agitation should be improved. In addition, the method of drying after oil extraction should be specified and it is recommended that a reflux condenser should be attached to the conical flask during the boiling.

Nine oil-mill laboratories of Lever Brothers and Unilever Limited have taken part in this work.

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THE BRITISH OIL AND CAKE MILLS LIMITED
STONEFERRY, HULL

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The Chemical Estimation of Nicotinic Acid, using *p*-Aminopropiophenone

BY CHLOE KLATZKIN, F. W. NORRIS AND F. WOKES

PREVIOUS workers[1,2] have shown that with various foods satisfactory agreement can be obtained between microbiological and chemical assays of nicotinic acid, the latter by the cyanogen bromide method. The aromatic amines used have included metol, aniline, procaine and *p*-aminoacetophenone (PAAP). Of these it was found[2] that *p*-aminoacetophenone was the most satisfactory for examining certain cereal products of low nicotinic acid content, since it produced under the specified experimental conditions the greatest intensity of colour with a given amount of nicotinic acid. However, with some foods, especially low-extraction wheat flours, maize and oats, marked discrepancies occurred, the chemical method giving considerably higher results than the microbiological method. After we had begun to investigate this problem, supplies of *p*-aminoacetophenone failed, and an alternative aromatic amine had to be found. Procaine did not prove satisfactory, and metol and aniline gave too feeble colours. We then tried *p*-aminopropiophenone (PAPP), and found this to give results equal to those given by *p*-aminoacetophenone. This paper describes our findings with the new amine, and also shows how discrepancies between chemical and microbiological results can be largely eliminated by using a more complete set of blanks.

CHEMICAL METHOD

REAGENTS—

p-Aminopropiophenone (*PAPP*)—Commercial samples were found to require purification, being strongly coloured, almost insoluble in water and only partly soluble in alcohol. They should be extracted with several lots of ether, and the pooled ether extracts decolorised with "charcoal for adsorption." After filtration, the ether should be removed and the purified amine recrystallised several times from alcohol. Solutions should be freshly prepared each day, by dissolving 1·25 g. in a mixture of 1·76 ml. of concentrated hydrochloric acid, 17·5 ml. of alcohol and water to make up the volume to 25 ml. These solutions should be kept in brown bottles in the dark. (The quantity of acid was chosen to give the most suitable degree of acidity in the final reaction mixture.)

The other reagents required have been described previously.

PREPARATION OF EXTRACT—

This is carried out as in the previous method,[2] except that in the preparation of the "final extract" the volume should be made up to 25 ml. *not* with water but with alcohol. If any precipitation occurs, centrifuge and use the supernatant liquid.

DEVELOPMENT OF COLOUR—

Prepare the following mixtures (in ml.)—

	U	U + NA	SB	TRB	AB	ARB	CB	CRB
Final extract	3	3	3	—	3	—	3	—
Standard nicotinic acid solution ..	—	1	—	—	—	—	—	—
Alcohol	3	2	7	6	5	8	4	7
Timing now becomes critical. At known times add—								
CNBr	2	2	—	2	—	—	2	2

Put in water-bath at 56° to 58° C. for 4 minutes and then in water-bath at 20° C. for 5 minutes in the dark. Then add—

Alcohol	—	—	—	—	—	—	1	1
Mixed amine reagent	2	2	—	2	2	2	—	—
Total volume	10	10	10	10	10	10	10	10

Keep the mixtures in the dark, and at 4½, 5 and 5½ minutes after adding the mixed amine reagent measure their optical densities against water, according to a timetable as suggested below (in minutes).

	U	U + NA	CB	CRB	U	U + NA
Add CNBr and place in water-bath at 56° to 58° C.	0	3	7	10	13	16
Transfer to water-bath at 20° C. in dark	4	7	11	14	17	20
Take out of bath	8½	11½	15½	18½	21½	24½
Add amine reagent, mix well and put in cuvettes.						
Take measurements of optical density as follows—						
1st reading	13½	16½	20½	23½	26½	29½
2nd reading	14	17	21	24	27	30
3rd reading	14½	17½	21½	24½	27½	30½

This allows for duplicates of U and U + NA. Duplicates of CB and CRB can be added if desired, also duplicates of other blanks, for which timing is not critical.

OPTICAL MEASUREMENTS—

As described previously.[2]

CALCULATION—

Let the mean readings of the respective tubes be—

- U for final extract of sample.
- U + NA for final extract of sample to 3 ml. of which 10 µg. of nicotinic acid have been added.
- TRB for total reagent blank.
- SB for solution or test blank.
- CB for cyanide or cyanogen bromide blank.
- CRB for cyanide reagent blank.
- AB for amine blank.
- ARB for amine reagent blank.

Then the nicotinic acid content of the sample is—

$$\frac{U - TRB - [(AB - ARB) + (CB - CRB) - SB]}{(U + NA) - U} \times \frac{25}{5^*} \times \frac{20}{3} \times \frac{\text{vol. of eluate}}{\text{vol. of sample used}} \ \mu g./g.$$

* Assuming the measured volume of the supernatant liquid used in preparation of the final extract was 5 ml., as given in the directions. If it was not, the appropriate correction must be made.

which simplifies to—

$$\frac{U - TRB - AB + ARB - CB + CRB + SB}{(U + NA) - U} \times \frac{100}{3^*} \times \frac{\text{vol. of eluate}}{\text{vol. of sample used}} \ \mu g./g.$$

MICROBIOLOGICAL METHOD

The microbiological assay of nicotinic acid using *L. arabinosus* 17/5 is now well known, and does not call for detailed description. The method adopted in these studies is almost exactly as described and recommended by the Microbiological Panel of the Vitamin Estimations Sub-Committee of the Society of Public Analysts and Other Analytical Chemists.[3]

The possibility that the organism is sensitive to fats and fatty acids has been recently confirmed (Lynes and Norris[4]). In order to avoid complications of this kind, some of the

samples were pre-extracted with light petroleum to remove fat. In all cases, however, the final extract was shaken with light petroleum prior to filling the assay tubes.

The results quoted are the mean of a number of assays, each at three or four levels of dosage. The calculation is made by reference to the equation to the standard curve of best fit. Confirmatory calculations based on the ratio of the slopes of calculated standard and sample curves were also made. Only results of assays of undoubted validity have been included in compiling Table I. The accuracy of a single determination by microbiological methods has somewhat arbitrarily been regarded as ±10 per cent.; but with careful working greater accuracy may be expected, and in the present series the figure has been reduced, on average, to about ±5 per cent. This limitation is of some importance when considering the closeness of agreement of results by microbiological and by chemical methods.

TABLE I

ASSAY OF NICOTINIC ACID BY MICROBIOLOGICAL METHOD

Results in μg. per g. of original material

Sample assayed	Content	Standard error of determination ±	Coefficient of variation ± %
Barley	73·5	2·6	3·5
Malted barley	82·9	2·9	3·5
Malt and oil	84·8*		
Malt extract	94·8	4·9	5·2
Malt and yeast food	91·2	7·1	7·8
Dried yeast	329·0	15·6	4·7
Wheat, whole	50·5	3·4	6·7
Wheat flour, 80% extraction	17·5	0·4	2·3
Wheat flour, fortified	51·0	3·0	5·9
Wheat, germinated	57·5	2·0	3·4
Yeast extract A	540·0	21·9	4·1
Yeast extract B	488·0	17·4	3·6

* Average of results previously published.

The above was written before publication of a paper by Kodicek and Pepper.[5] Detailed comment on their findings in respect of the microbiological assay of nicotinic acid is inappropriate in this communication. It may be mentioned, however, that many variations in the composition of the medium were tested by the Microbiological Panel, and the most suitable was that finally published. Subsequent use of this medium in a large number of assays would seem to support the conclusions of the Panel. It is agreed that the somewhat optimistic estimates of coefficients of variation anticipated by some workers have not been justified; but we have, in general, found lower coefficients than those indicated by Kodicek and Pepper. Where the variation has been high it has always been found that this is due to the inclusion of assays which, for various reasons, were invalid.

SPECTROSCOPIC STUDY OF COLOUR GIVEN BY DIFFERENT AMINES

The selection of the secondary filter used in the photo-electric estimation of the colour given by nicotinic acid with the amine and cyanogen bromide should be based on the absorption curve of the colour. We determined this, using the Beckman Model DU Spectrophotometer, on a series of freshly prepared colours, the average reading at each wavelength being measured at the same number of minutes after mixing, so that allowance was made for any errors that might have arisen from the instability of the colour. The curves obtained by using p-aminopropiophenone and p-aminoacetophenone were determined separately. The results, plotted in Fig. 1, showed, with p-aminopropiophenone a rather broad band with a peak at about 445 mμ., very similar to that given by p-aminoacetophenone, which has a peak at about 455 mμ. No significant shifts occurred in these maxima during the time taken to carry out an assay, although the densities were of course changing. The peak given by p-aminoacetophenone at 455 mμ. is not shown in the extinction curve of the p-aminoacetophenone colour published by Kodicek[6] in 1940 and obtained with the Zeiss step-photometer using filters which provided only a rough indication of the curve. It was, however, found by Dann and Handler[7] (at about 440 mμ.) using the Coleman DM Spectrophotometer. These spectroscopic data are summarised in Table II; and show that the No. 7 Chance's violet filter, with maximum transmission at about 440 mμ., is equally suitable when using

p-aminoacetophenone, *p*-aminopropiophenone or aniline, but less satisfactory when using metol as the aromatic amine.

TABLE II

SPECTROSCOPIC DATA ON COLOURS GIVEN BY DIFFERENT AMINES IN CNBr TEST FOR NICOTINIC ACID

	Absorption maxima (in mμ.) obtained with			
	Metol	Aniline	PAAP	PAPP
Dann and Handler	400	435	440	—
Klatzkin, Norris and Wokes ..	—	—	455	445

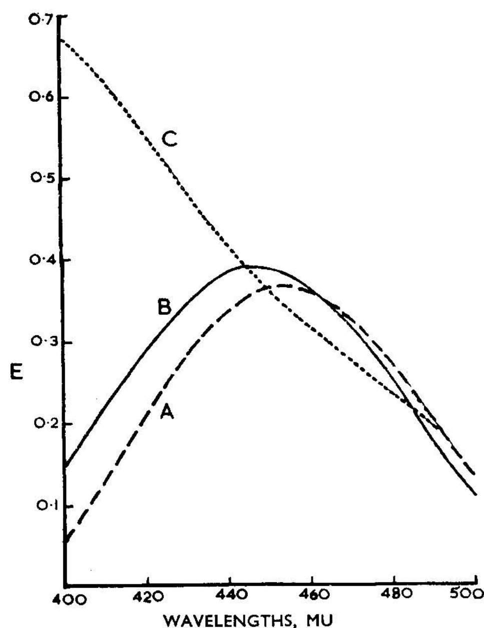

Fig. 1. Absorption curves of yellow colour given by nicotinic acid with CNBr and PAAP or PAPP

Curve A. PAAP (at 2–4 min. after mixing) against TRB

Curve B. PAPP (at 1¼–4¼ min. after mixing) against TRB

Curve C. PAPP (at 1¼–4¼ min. after mixing) against alcohol

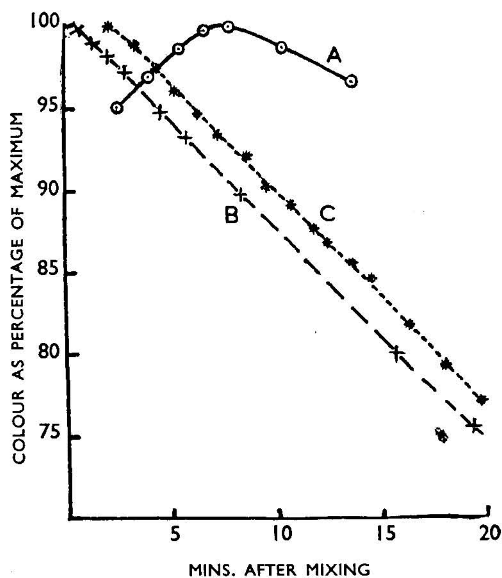

Fig. 2. Rates of development and fading of colour given by nicotinic acid with CNBr and PAAP or PAPP

Curve A. PAAP in alcoholic solution

" B. PAAP in aqueous solution

" C. PAPP in alcoholic solution

RATE OF FADING OF COLOUR—Readings of the colour obtained when using *p*-aminopropiophenone as aromatic amine, under the given experimental conditions, were taken with the Spekker absorptiometer at different times after mixing. The results of a number of experiments are plotted in Fig. 2. They show that the rate of fading of the colour resembled that previously observed with *p*-aminoacetophenone in aqueous solution, but was more rapid than was given by that amine in alcoholic solution. The need for careful timing therefore applies equally to both amines if satisfactory results are to be obtained. It is of interest to note that the presence of alcohol in the reaction mixture retarded the development of the colour, so that the maximum was not reached until about 3 minutes after mixing, whereas in aqueous solution it was reached in less than a minute. A similar effect of alcohol was previously observed when using *p*-aminoacetophenone.

CALIBRATION OF ABSORPTIOMETER—Table III summarises our results when calibrating the absorptiometer with pure nicotinic acid, using *p*-aminopropiophenone under the given experimental conditions. They show that Beer's law is obeyed over the range 5 to 40 μg. per 10 ml., which amply covers the working range. The accuracy of the results compares favourably with that previously found with *p*-aminoacetophenone.

RECOVERY EXPERIMENTS WITH ADDED NICOTINIC ACID—

These were carried out with *p*-aminopropiophenone in the same manner as they had been applied to *p*-aminoacetophenone, and gave satisfactory percentage recoveries, ranging from 97 to 105 and averaging 101 in 130 assays on 34 different samples. This compares favourably with the percentage recoveries of 94 to 102 previously obtained with *p*-aminoacetophenone in 157 assays. We also obtained satisfactory recoveries when adding a known amount of nicotinic acid to the food before extraction in order to test the behaviour of the Lloyd's reagent.

TABLE III

CALIBRATION OF ABSORPTIOMETER WITH PURE NICOTINIC ACID USING *p*-AMINOPROPIOPHENONE

Concentration of NA µg. in 10 ml.	Mean optimum density (D) at 5 min. after mixing	$\frac{D \times 1000}{\text{concentration}}$
5	0·105	21·0
10	0·221	22·1
20	0·434	21·7
30	0·629	21·0
40	0·846	21·2
	Mean	21·4
	Standard error of mean	0·22

EXPERIMENTS ON VARYING PROPORTION OF ALCOHOL AND OF AMINE IN REACTION MIXTURE—

The maximum solubility of *p*-aminopropiophenone in alcohol at room temperature was about 1 in 60 or 1·5 per cent. By using 5 ml. of such a solution in 15 ml. of reaction mixture we obtained a final concentration of 0·5 per cent. of *p*-aminopropiophenone, giving with 20 µg. of nicotinic acid an absorptiometer reading of about 0·35 under the given experimental conditions. The amine could be rendered more soluble by adding to the alcohol the 6 per cent. hydrochloric acid used to obtain the necessary degree of acidity in the reaction mixture. We were thus able to prepare a 5 per cent. solution of *p*-aminopropiophenone, and by using 2 ml. of this in 10 ml. of reaction mixture obtained in the latter a final concentration of 1·0 per cent. of the amine. This gave, with 20 µg. of nicotinic acid, an absorptiometer reading of about 0·42. Thus, an increase of 100 per cent. in the concentration of amine gave an increase of only about 20 per cent. in the intensity of colour. We decided that even if the concentration of amine in the reaction mixture could have been further increased by using some other solvent, any resulting further increase in colour intensity would not have been of practical significance. When we came to apply this technique to foods we found that on mixing the alcoholic reagent with an aqueous extract of the food there might be gradual precipitation not detected until colour measurements had begun, when it was too late to make any correction. This difficulty was overcome by using alcoholic extracts of the food. Any opalescence appearing in these was removed by centrifuging before developing the characteristic colour. Fifty per cent. alcohol in the reaction mixture proved less satisfactory than about 80 per cent., the concentration finally adopted.

EFFECT OF pH—

The pH for adsorption and elution is of course critical, as shown by numerous workers. The effect of the pH of the reaction mixture, although equally critical, seems to have received very little attention. Previous work in these laboratories had shown that for *p*-aminoacetophenone and for procaine the optimal pH for the reaction is below 1, the colour intensity falling rapidly as the pH rises above 2, and reaching a minimum between 4 and 5. Our experiments with *p*-aminopropiophenone gave similar results (see Fig. 3). We therefore adopted for this amine the same reaction pH as for *p*-aminoacetophenone.

PREPARATION AND PURIFICATION OF EXTRACT—

The food material (in No. 30 or 40 powder) should be extracted with 4 *N* hydrochloric acid for 40 minutes in a boiling water-bath, as previously described.[2] In general, the findings of Kodicek and Pepper[5] confirm our belief that acid extraction is preferable to alkaline extraction, but we have obtained better results with slightly stronger acid than theirs. Alkaline extraction of cereals involves the production of greater amounts of interfering substances, and this is more marked where germinated cereals are under test. Our results

for wheat, using acid extraction and amine blank, agree with those of Kodicek and Pepper, close concordancy between microbiological and chemical assays being obtained. We differ from them in that we do not obtain similar concordancy with ungerminated maize and oats.

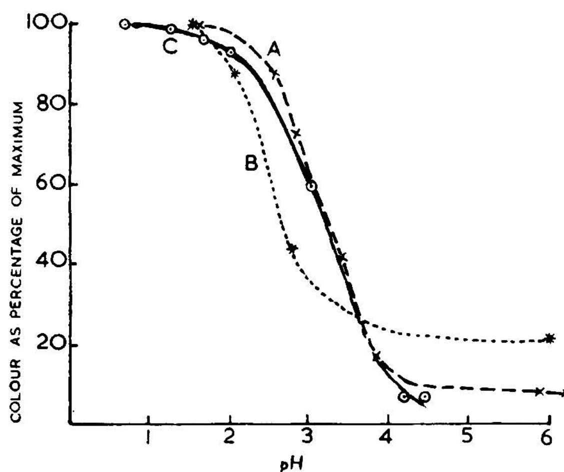

Fig. 3. Effect of pH on intensity of colour given with different amines in the cyanogen bromide test for nicotinic acid
Curve A. Results with PAAP
" B. Results with PAPP
" C. Results with procaine

It has been shown[2] that the use of potassium permanganate to remove interfering colours may lead to varying results, perhaps because of the development of slight haziness scarcely detectable visually but exerting a marked effect on the photo-electric cell. We have also found decolorisation with peroxide to be unsatisfactory and have obtained the most satisfactory results by adsorbing the nicotinic acid on Lloyd's reagent at an acid pH, eluting it with alkali and removing further interfering colour by adsorption on freshly prepared lead hydroxide as previously described.

ALLOWANCE FOR INTERFERING SUBSTANCES

These substances, which are incompletely removed by the purification processes, include—

(*a*) natural pigments;
(*b*) "amine chromogens"—developing colour with the aromatic amine;
(*c*) "cyanide chromogens"—developing colour with the cyanogen bromide.

In addition, allowance must be made for—

(*d*) the colour of the reagent; and
(*e*) the bleaching effect of the cyanide on certain colours.

Various blanks have been introduced to correct for these disturbing factors, *e.g.*, "total reagent blank" (TRB) for (*d*),[8] cyanide blank (CB) for (*a*) + (*c*),[9] amine blank (AB) for (*a*) + (*b*).[10] The bleaching effect of the cyanide may be diminished by adding acid immediately before adding the cyanide[11] in a special "acid blank" applied to urine, but this does not necessarily effect complete correction when applied to foods. More accurate allowance for the bleaching effect can be made by adding to the total colour, U, the solution blank plus cyanide reagent blank minus cyanide blank (SB + CRB − CB). This quantity is usually positive, hence correction for "bleaching" involves an *increase* in the colour measured, whereas correction for amine chromogens involves a *decrease*. With certain food materials the magnitude of the bleaching effect is similar to that of the amine chromogens, so that these two factors more or less counterbalance each other, and may both be ignored without seriously affecting the result. With some other food materials, these two factors cannot safely be neglected, and the complete set of blanks should be employed, as given above under "Chemical Method."

The magnitude of these different blanks is indicated by data obtained in 39 assays on various foods, using *p*-aminopropiophenone, and summarised in Table IV. The amine blank, AB, is seen to be the chief offender, providing 30 to 65 per cent. of the total colour measured. About half of AB is due to the amine, as can be seen by deducting from AB the blank ARB. The difference between AB and ARB was always greater than the solution blank, SB, the extra colour due to amine chromogens ranging from 6 to 19 per cent. of the total colour. Since the total reagent blank did not vary significantly from the sum of ARB and CRB there was little change in colour when mixing the two halves of the reagent. On the other hand, the bleaching effect of the cyanogen bromide was sometimes quite marked, SB + CRB − CB ranging from 3 to 13 per cent. of the total colour.

TABLE IV

MAGNITUDE OF BLANKS IN CHEMICAL ASSAYS OF NICOTINIC ACID USING PAPP

All results given as percentage which the given blank represents of the total colour given by final extract with reagent

Material	No. of assays	AB	ARB	AB − ARB	SB	AB − ARB − SB (amine chromogens)	CB	CRB	TRB	SB* + CRB − CB
Wheat, 80% extraction	6	65	35	30	11	19	5	7	42	13
Wheat, whole	3	56	36	20	12	8	7	6	38	11
Barley	4	44	26	18	7	11	5	5	27	7
Malted barley	4	46	27	19	8	11	6	4	32	6
Malt extract preparations	16	49	26	23	6	17	4	4	29	6
Yeast preparations ..	6	30	21	9	3	6	3	3	25	3

* Bleaching effect of cyanide.

COMPARISON OF METHODS USING DIFFERENT BLANKS—

Table V shows the wide deviation in results that may be obtained on a given sample by using different blanks. For facilitating comparison, the results are all expressed as percentage of the mean microbiological result. They show that the more completely allowance is made for interfering substances by means of blanks, the nearer the chemical results come to the microbiological results.

TABLE V

CHEMICAL ESTIMATION OF NICOTINIC ACID USING BLANKS INCLUDED IN DIFFERENT METHODS

All results quoted as percentage of mean microbiological result

Material	Results using blanks employed by: Bandier and Hald	Bandier	Harris and Raymond	Dann and Handler	Friedemann and Frazier
Barley 41	127	114	97	110	109
Malted barley 42	138	123	102	123	111
Malt and oil	133	133	104	122	104
Malt extract	128	128	100	122	96
Malt and yeast	119	119	116	111	111
Dried yeast	129	129	110	121	101
Wheat, whole	129	112	96	107	111
Wheat flour, 80% extraction ..	150	126	84	108	114
Wheat flour, fortified	113	97	76	87	90
Wheat, germinated	118	105	79	91	102
Yeast extract A	122	122	105	116	102
Yeast extract B	109	102	112	103	110
Means	129	118	98	110	105
Standard error of mean	± 3·2	3·3	3·7	3·4	2·1

Taking into consideration the combined errors of the chemical and microbiological methods, it may be assumed that on an individual sample a deviation of 20 per cent. between the mean results given by the two methods is probably significant, and on the average for the 12 samples included in the table a deviation of 10 per cent. between the results given by the two methods is almost certainly significant. When using Bandier and Hald's blanks,

the chemical results on 8 out of 12 of the samples significantly exceeded the microbiological results, and a highly significant difference was also observed for the mean of all samples. With Bandier's blanks, the chemical results on 6 out of 12 of the samples significantly exceeded the microbiological results, and a significant difference was also observed for the mean of all samples. With Harris and Raymond's blanks, the chemical results on 2 out of 12 of the samples were significantly lower than the microbiological results, but the average for all samples did not differ significantly from the microbiological result. The latter was also true when using the blanks of Dann and Handler[7] or of Friedemann and Frazier.[12] Hence, the blanks used by Bandier and Hald, and by Bandier, give less satisfactory results than those used by the other workers mentioned in the table. For many types of foodstuffs the blanks of Harris and Raymond and of Dann and Handler will probably give as reliable and satisfactory results as the more complete set of blanks used by Friedemann and Frazier. However, with certain foodstuffs (*e.g.*, low-extraction wheat flour, oats and maize) discrepancies between chemical and microbiological results may arise, which can be markedly reduced by the use of the full set of blanks. There is, of course, the possibility that the discrepancy between chemical and microbiological results may, in certain instances, be due to the occurrence of an anti-vitamin diminishing the microbiological activity without affecting the chemical results, or alternatively the presence of a nicotinic acid precursor giving a positive response to the chemical but not the microbiological assay. This raises important questions which must be left over for future consideration. These questions do not seem likely to arise with the materials examined in the present investigation.

COMPARISON OF PAAP AND PAPP RESULTS—

In assays on 24 samples of cereals and cereal products the mean results with *p*-aminopropiophenone were 99 per cent. and ranged from 82 to 110 per cent. of the results with *p*-aminoacetophenone when examining the same sample. Two of these samples were a barley and a malted barley, which were the first we examined using the full series of blanks with *p*-aminopropiophenone, and gave results 82 and 86 per cent. of those obtained with *p*-aminoacetophenone. Omitting these 2 samples, the results on the remaining 22 gave mean results with *p*-aminopropiophenone ranging from 94 to 110 per cent. and averaging 100 per cent. of the results with *p*-aminoacetophenone.

COMPARISON OF PAPP WITH MICROBIOLOGICAL RESULTS—

The data in Table V showed that when using *p*-aminopropiophenone with a full set of blanks the average chemical result on 12 samples was 105 per cent. of the average microbiological result. Equally good agreement was obtained with other samples examined by both methods (for example, the nicotinic acid content of a barley sample was 98 μg. per g. chemically and 96 μg. per g. microbiologically). We therefore think that when estimating nicotinic acid in the types of material included in Table V the chemical method using *p*-aminopropiophenone with, if necessary, the full set of blanks, can be relied upon to give satisfactory results.

We are indebted to Miss Janet Horsford and Mr. R. Evans for assistance. The chemical work in this paper will form part of a thesis to be submitted for a Ph.D. in the University of London.

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January, 1949

Colorimetric Determination of Streptomycin B (Mannosido-streptomycin)

By W. B. EMERY and A. D. WALKER

(*Read at the meeting of the Society on Wednesday, May 4th,* 1949)

No method has so far been published for the direct determination of streptomycin B (a mannoside of streptomycin, isolated by Titus and Fried[1]) in presence of streptomycin itself (which for convenience we shall call streptomycin A). By assuming a certain ratio (about 1 : 5) between the biological and chemical "potencies" of pure streptomycin B, it is possible to calculate the proportion of this antibiotic present in a sample of streptomycin. Since the ratio is not accurately known and will, of course, be constant only for a given strain of bacterium, no great trust can be put in results obtained in this way. The development of an accurate method of determining streptomycin B is described in this paper.

Morris[2] has reported the use of a 0·2 per cent. w/v solution of anthrone in 95 per cent. v/v sulphuric acid for the quantitative determination of carbohydrates. It seemed possible that this reagent might be applied to the assay of streptomycin B by determining the mannose residue in the molecule, always provided that the glucosamine and streptose moieties in the molecules of both streptomycins do not react with the reagent. Results obtained by the method described below have shown that they are not.

Experimental

Preparation of the reagent—Cautiously add 950 ml. of AnalaR concentrated sulphuric acid to 50 ml. of cold distilled water. It is essential that AnalaR sulphuric acid be used, since solutions of anthrone in commercial acid deteriorate rapidly. Cool the 95 per cent. sulphuric acid to room temperature and dissolve in it 2 g. of recrystallised anthrone, m.p. 153° to 154° C. (prepared as described by Morris[2]). The reagent is stored at 0° C. and is stable for at least a fortnight.

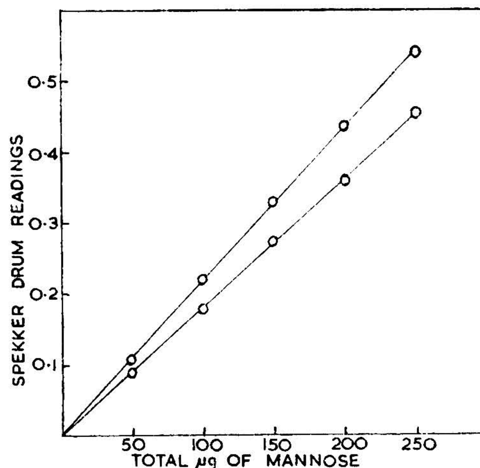

Fig. 1. Typical standard curves

Construction of a standard curve—Prepare solutions of pure mannose containing 10, 20, 30, 40, 50, 60, 80 and 100 µg. per ml. Add from a burette 10 ml. of the reagent to 5 ml. of each of these solutions, contained in 6 × 1 inch boiling tubes, which should be of uniform wall-thickness and diameter. Prepare a blank by adding 10 ml. of the reagent to 5 ml. of water. Gently shake the tubes to insure thorough mixing of the reagent with the solution, and after at least 20 minutes measure the absorption of the green solutions against the blank by means of a Spekker photo-electric absorptiometer, using Chance glass OR2 red filters

(peak transmission 640 mμ.) and cells 2 cm. long. Plot the readings obtained against the number of micrograms of mannose in the 5 ml. of solution. The curve so constructed is a straight line passing through zero for absorptiometer readings up to at least 0·6, but occasionally flattens out at higher levels. The slope of the line varies slightly from day to day. Two typical curves are shown in Fig. 1.

ASSAY PROCEDURE AND CALCULATION OF RESULTS—

Dilute a solution of the streptomycin sample to be assayed until the concentration of streptomycin B is between 60 and 200 units per ml., corresponding to about 20 to 60 μg. of mannose per ml. (see calculation below). Add 10 ml. of reagent to 5 ml. of the solution and read the resultant yellowish-green solution against the blank of water plus reagent in the manner described above. Find the concentration of mannose (in μg. per ml.) in the streptomycin solution by reference to the standard curve. It is usual to assay the streptomycin solution at two different levels and average the results. Thus, one might take 2 and 4 ml. respectively of the solution, making each up to 5 ml. with water before assaying. The total streptomycin (in units per ml.) present in the test solution is determined by chemical assay based on the maltol - ferric ion reaction.[3,4] The molecular proportion of streptomycin B (*i.e.*, the molecular percentage of the total streptomycin present) in the sample may be calculated as follows.

Let a solution of streptomycin at X chemical units per ml. contain Z μg. of mannose per ml.

Then, since the molecular weight of mannose = 180
and the molecular weight of streptomycin B base = 743,

the concentration of streptomycin B base $= \frac{743Z}{180}$ μg. per ml. $= \frac{743Z}{180{,}000}$ mg. per ml. .. (1)

The expression (1) can be used to calculate the weight percentage of streptomycin B in a solid sample of streptomycin.

By definition, 1 mg. of streptomycin A base ≡ 1000 chemical units, and as the molecular weight of streptomycin A base is 581,

$\frac{743}{581}$ mg. of streptomycin B base ≡ 1000 chemical units .. .. .. (2)

From (1) and (2),

$$\text{Concentration of streptomycin B} = \frac{743Z}{180{,}000} \times \frac{581}{743} \times 1000 \text{ chemical units per ml.}$$
$$= \frac{581Z}{180} \text{ units per ml.}$$

$$\text{Hence molecular proportion of streptomycin B} = \frac{581Z \times 100}{180\,X} = \frac{323Z}{X}\ \%$$

RESULTS—

The method described above has been applied to a number of samples of streptomycin hydrochloride and streptomycin calcium chloride complex. Samples chosen have contained from 3 to 95 per cent. of streptomycin B. The hydrochlorides containing over 50 per cent. of streptomycin B were prepared from production residues by modifications of the method of Titus and Fried.[1] The results obtained on a few samples are recorded in Table I, together with the biological/chemical assay ratios, and the proportions of streptomycin B calculated from these ratios. Ratios of 0·2 and 1·0 for pure streptomycin B and streptomycin A respectively have been assumed.

It seemed desirable to demonstrate the reliability of our method in some way other than by comparison with results obtained as indirectly as those tabulated. Accordingly, we assayed a sample of streptomycin calcium chloride complex, recrystallised the sample and assayed the recrystallised material and the mother liquor. Of 330 mg. of streptomycin B present in the starting material (5 g.), 168 mg. were recovered in the mother liquors and 148 mg. in the recrystallised complex (96 per cent. recovery). This, in conjunction with the results above, appears to indicate that streptomycin B will form a calcium chloride complex, but that this salt is more soluble than that of streptomycin A.

TABLE I

Sample No.	Type of compound	Assay ratio	Molecular proportion of streptomycin B: Calculated from ratio	Molecular proportion of streptomycin B: By anthrone assay
1	Hydrochloride	0·240	95	90
2	"	0·240	95	97
3	"	0·245	94	94
4	"	0·560	55	54
5	"	0·592	51	54·5
6	"	0·760	30	40·5
7	"	0·620	47·5	43·0
8	"	0·720	35·0	39·0
9	"	0·760	30·0	28·5
10	$CaCl_2$ complex	1·000	0·0	3·6
11	"	0·930	8·8	7·1
12	"	0·870	16·3	9·5
13	"	0·880	14·8	9·1

SUMMARY—

The use of Morris's reagent (0·2 per cent. of anthrone in 95 per cent. sulphuric acid) for the quantitative estimation of streptomycin B is described. Results obtained by the method given are in accord with those calculated from chemical and biological assays, assuming the relative biological activities of the two streptomycins are as stated.

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August, 1948

DISCUSSION

The PRESIDENT congratulated the authors on their interesting contribution on what was to most present a new problem. He asked what were the relative antibiotic values of streptomycins A and B and what was the nature of anthrone.

Dr. J. H. HAMENCE enquired if the method could be applied to soils or soil extracts; he was interested to know what means were available for investigating the antibiotic substances in soils.

Mr. EMERY, replying to the President, said that in terms of activity towards *B. subtilis*, streptomycin A was about five times as potent as streptomycin B. Anthrone was partially reduced anthraquinone. Streptomycin A did not respond to the test described to any appreciable extent.

Mr. WALKER said that in soils there might be many substances other than antibiotics that would react with anthrone, but few that would react to both the anthrone and the maltol - ferric ion tests.

The Reduction of Antimonial Tin Solutions with Metallic Nickel and Cobalt

BY H. HOLNESS

(*Read at the meeting of the Society on Wednesday, May 4th*, 1949)

THE reduction of tin solutions containing antimony by means of metallic iron was studied by Järvinen[1] who showed that the precipitated antimony co-precipitated appreciable amounts of tin. More recently, Evans and Higgs[2] studied the use of metallic lead, iron, nickel, aluminium and zinc for the same purpose and reported that "only nickel causes no co-precipitation of tin with either copper or antimony." As a result, Schoeller and Holness[3] used nickel powder to remove antimony in their gravimetric determination of tin in alloys by the tannin method and in furtherance of this work I have tried to apply it to the determination of minor amounts of tin in antimony regulus.

It was in this connection that the protective action on nickel of strong hydrochloric acid solutions of antimony trichloride was encountered, an effect previously studied by Clarke[4] and more recently by Piontelli and Fagnani.[5] In both cases the conditions determined were those affording protection, not those causing a maximum attack on the nickel. In a recent paper[6] these latter conditions were established as requiring: (1) an acid concentration of about 2·5 *N*, (2) heating to be carried out on a water-bath, and (3) the solution on no account to be boiled or mechanically agitated.

It should be pointed out that Evans and Higgs reached the general conclusion quoted above, using only one concentration of antimony and tin, *viz.*, 0·2 g. of each in 100 ml. of acid. Their technique was to boil the solutions for 30 minutes with sheet nickel, to filter hot and to wash the precipitate thoroughly with hot, diluted hydrochloric acid (1 in 10) and "subsequently to test the metallic precipitate for tin and, if it is present, to determine its proportion in the bulk of the precipitate." No details are given, however, of the method used to determine this tin.

In the work recorded below, antimonial tin solutions were treated with nickel powder using a technique similar to that quoted above. The tin was determined in the filtrate and any negative error in recovery attributed to co-precipitation. The ratios of tin to antimony were of an order calculated to test critically the validity of the statement regarding co-precipitation by nickel.

There seems to be no record of the use of metallic cobalt as a reductor in tin determinations and since hydrochloric acid solutions of antimony behave towards cobalt in much the same way as towards nickel,[4] a few experiments were included to establish its behaviour so far as co-precipitation was concerned.

Experimental

Small amounts of tin were added to large concentrations of antimony to give ratios ranging from 1 to 0·1 per cent. of tin in antimony. After reduction with nickel powder the precipitated antimony was filtered and washed and the filtrate oxidised with bromine water. Iron in the form of ferric chloride was then added to act, first as a collector of the tin, and then as an indicator in the subsequent recovery of the tin by precipitation with tannin—a technique which had proved its worth in previous work.[3]

Procedure—

Carefully weighed pieces of A.R. granulated tin were added to 1·0 g. lots of antimony metal powder ("ex tartar emetic") and each was placed in a 750-ml. conical flask and treated with 100 ml. of diluted hydrochloric acid (1 + 1) containing sufficient bromine to dissolve it. After solution the excess of bromine was removed by boiling and 100 ml. of water were added to reduce the acidity to about 2·5 *N*. Five g. of fine nickel powder were then added and the whole heated on a water-bath until reduction was complete—about 15 to 20 minutes—this stage was denoted by the evolution of *small* gas bubbles from the unused nickel, which continually forced to the surface of the liquid the black mass of nickel plus antimony. This mass was filtered through a pad of paper pulp sprinkled with a little nickel powder, and washed well with hot *N* hydrochloric acid. The filter pad with its precipitate was then transferred to a beaker and boiled with 100 ml. of *N* hydrochloric acid, filtered and again well washed. This technique of filtering and washing was observed in all the experiments using metal powders. The combined filtrates and washings were treated with a little bromine water to oxidise the tin to the stannic state, 5 to 10 drops of a 10 per cent. solution of ferric chloride, 5 g. of ammonium chloride and sufficient diluted aqueous ammonia (1 + 1) to make the solution just neutral to litmus. The solution was boiled, a little paper pulp added and the precipitated ferric hydroxide containing the stannic hydroxide filtered through a No. 41 Whatman filter. The filtrate was discarded and the precipitate washed with a hot 2 per cent. solution of ammonium chloride before being dissolved on the paper, in a hot solution of 5 g. of ammonium oxalate in 20 ml. of *N* hydrochloric acid. After the filter had been well washed with hot water, the filtrate and washings were treated with tannin and the tin determined by the method described in a previous paper.[3]

The first few experiments (Nos. 1 to 5) clearly showed a small co-precipitation of tin with the antimony and, in so far as conditions allowed, minor changes in technique were made with a view to the recovery of this small amount. Evans and Higgs carried out their reductions in boiling solutions, but this was only possible here by substantially increasing

the bulk of liquid, owing to the protective action of the high antimony concentration. Compromises were therefore effected; in Experiment 6 the solution was boiled for 5 minutes after the reduction was complete and before filtration, and in Experiment 7 the precipitated metal, after filtration, was boiled with 1 g. of nickel powder in 100 ml. of 2·5 *N* hydrochloric acid for 5 minutes and filtered, and the filtrate and washings were added to the previous filtrate. These modifications in procedure however, brought about no change in the amount of tin co-precipitated and it was decided to conduct at least one experiment in boiling solution—Experiment 8. The antimony concentration was reduced by dilution with 2·5 *N* hydrochloric acid to a value equal to that used by Evans and Higgs, the nickel was added and the solution boiled for 30 minutes, after which the experiment was continued as before. It was noted that reduction under these conditions proceeded more slowly, but the result appeared to be the same.

TABLE I

Expt. No.	Antimony taken	Tin taken	Nickel used	Tin found	Error (tin co-pptd.)
	g.	g.	g.	g.	g.
1	1·0	0·0012	5	0·0007	− 0·0005
2	1·0	0·0052	5	0·0039	− 0·0013
3	1·0	0·0036	5	0·0027	− 0·0009
4	1·0	0·0065	5	0·0051	− 0·0014
5	1·0	0·0093	5	0·0077	− 0·0016
6	1·0	0·0019	5	0·0012	− 0·0007
7	1·0	0·0080	5 + 1	0·0065	− 0·0015
8	1·0	0·0042	5	0·0031	− 0·0011
9	1·0	0·0010	5	(*a*) 0·0005	
			5	(*b*) 0·0003	− 0·0002
10	1·0	0·0022	5	(*a*) 0·0013	
			5	(*b*) 0·0004	− 0·0005
11	1·0	0·0081	5	(*a*) 0·0061	
			5	(*b*) 0·0011	− 0·0009

Re-treatment of the precipitated antimony was next tried—Experiments 9 to 11. The precipitate was dissolved in hydrochloric acid and bromine, diluted and treated as before with nickel powder: a small recovery of tin resulted. This recovery furnished further proof that tin was co-precipitated to a small degree with nickel-reduced antimony.

It seemed reasonable to suppose that the co-precipitation of the tin would vary with the amount of antimony present, in which case the amount co-precipitated by the nickel-reduced antimony in the work of Evans and Higgs would have been too small to be apparent. Experiments 12 to 16 were designed to test this supposition. Equal aliquots of a standard solution of ammonium stannichloride were used and different weights of antimony trioxide added. The acidity was then adjusted to 2·5 *N* in hydrochloric acid and reduction effected with 5 g. of nickel powder as before. The results were surprising, for there was little or no alteration in the amount of co-precipitation, in spite of the fact that the amount of antimony used ranged from 1 to 0·1 g.

This unexpected result focussed attention on the method used to recover the tin after reduction, and alternative procedures were explored.

TABLE II

Expt. No.	Tin taken	Sb_2O_3 taken	Sb equiv.	SnO_2 found	Sn equiv.	Error (Sn co-pptd.)
	g.	g.	g.	g.	g.	g.
12	0·0085	1·2	1·0	0·0089	0·0070	− 0·0015
13	0·0085	0·9	0·75	0·0088	0·0069	− 0·0016
14	0·0085	0·6	0·5	0·0090	0·0071	− 0·0014
15	0·0085	0·3	0·25	0·0090	0·0071	− 0·0014
16	0·0085	0·12	0·1	0·0092	0·0072	− 0·0013

Several methods of precipitating the tin together with the iron from a large concentration of nickel or cobalt offered themselves and four methods were examined. Using a stock solution of tin, 25-ml. portions were reduced for 5 minutes in hot 2·5 *N* hydrochloric acid solution with 5 g. of nickel powder, after which the unused metal was removed by filtration and well washed with hot *N* hydrochloric acid in the manner described above. The filtrate and washings were oxidised with bromine water and 5 to 10 drops of a 10 per cent. aqueous

solution of ferric chloride added. The tin was then determined by each of the following methods:—

(*a*) Solid ammonium chloride was added followed by an excess of ammonia—sufficient to form the blue nickel hexammine. The solution was boiled and filtered, and the precipitate dissolved in 50 ml. of *N* hydrochloric acid containing 5 g. of ammonium oxalate, the tin being determined by tannin precipitation.

(*b*) Solid ammonium chloride was added and sufficient ammonia to render the solution just neutral to litmus. The solution was boiled and filtered and the precipitate treated as in (*a*).

(*c*) Solid ammonium chloride and 10 g. of ammonium acetate were added and the solution was neutralised to litmus with ammonia. It was then boiled and filtered and treated as in (*a*).

(*d*) Solid ammonium chloride and a solution of 1 g. of tannin in 25 ml. of water were added and the solution boiled. Ammonia was then added to the gently boiling solution until the purple colour of the iron tannin complex was just apparent; the precipitate was then filtered and treated as in (*a*).

The stock solution of tin, prepared by dissolving recrystallised ammonium stannichloride in *N* hydrochloric acid, was standardised by the tannin method and also by sulphide precipitation; 25 ml. gave 0·0517 g. and 0·0516 g. of SnO_2 respectively.

Two determinations with 25 ml. of the stock solution treated by method (*a*) gave only 0·0442 g. and 0·0466 g. of SnO_2. With methods (*b*), (*c*) and (*d*), however, 0·0503 g., 0·0505 g. and 0·0504 g. of SnO_2 respectively, were obtained. From these results two facts emerge: (i) the use of excess of ammonia must be avoided, as some ammine formation probably takes place, and (ii) the nickel itself would appear to remove a small amount of tin. This latter possibility was examined.

Portions of the stock solution of tin were added to 200 ml. of 2·5 *N* hydrochloric acid, boiled and treated with 5·0 g. of the nickel powder for 5 minutes. The powder was then removed by filtration and well washed as usual with hot *N* hydrochloric acid. The tin was determined in the filtrate by the method (*b*) described above. The unused metal together with the filter were boiled with 200 ml. of 2·5 *N* hydrochloric acid until all the metal had dissolved. The solution was filtered and the filtrate treated as above with a view to the recovery of any tin that may have become attached to the nickel. This was repeated with different amounts of nickel powder.

TABLE III

No.	Ni used	Tin taken SnO_2	Tin found: In filtrate SnO_2	Tin found: Recovered from Ni SnO_2	Tin found: Total SnO_2	Error SnO_2
		g.	g.	g.	g.	g.
17	5 g.	0·0233	0·0224	0·0008	0·0232	− 0·0001
18	5 g.	0·0103	0·0088	0·0013	0·0101	− 0·0002
19	5 g.	0·0103	0·0091	0·0011	0·0102	− 0·0001
20	3 g.	0·0103	0·0095	0·0007	0·0102	− 0·0001
21	1 g.	0·0103	0·0100	0·0003	0·0103	0·0000
22	5 g.	nil	—	nil	—	—
23	12 sq. in.	0·0103	0·0103	nil	0·0103	0·0000
24	36 sq. in.	0·0233	0·0230	trace	0·0230	− 0·0003
25	36 sq. in.	nil	—	nil	—	—

The results of Experiments 17 to 21 show that there is a small retention of tin by the metallic nickel but the amount is less than a milligram. This small quantity does not wholly account for the errors noted in the previous experiments, and it must be concluded that these are composite errors, the loss of tin being caused partly by the unused nickel powder and partly by the precipitated antimony.

In view of these results and the widespread use of nickel as reductant in the volumetric determination of tin, Experiments 23 and 24 were carried out with thin sheet nickel in place of nickel powder.

It was realised that the fine nickel powder used in this work offered a very much larger surface area than could be conveniently obtained by the use of sheet nickel and it was to be expected that no really conclusive results would be obtained, but it is worth recording,

that the *tendency* for the sheet nickel to retain tin is present, for with 36 square inches of surface, a trace of tin was detected. Any errors resulting from this tendency are usually compensated for in practice by the use of nickel-reduced tin solutions for the standardisation of the iodine. It is, however, of interest to note that in a recent paper, Miller and Currie tried to determine 10-mg. quantities of tin by iodine titration after nickel reduction and observed "a persistent negative error which occasionally reached 2·5 per cent."[7] Here the iodine solution was standardised by arsenious oxide, so that a part of their error could well be attributed to the retention of some tin by the nickel.

In general, this investigation has shown that some small loss of tin results from the use of nickel as a reductant, that the loss is increased if antimony is present, but that even then the amount is only very small. It does not invalidate the use of nickel in the volumetric determination of tin on the macro scale, but it would seem advisable to use nickel-reduced tin solutions to standardise the iodine solution. The use of nickel powder to remove antimony in the determination of tin in alloys by the tannin method[3] would appear to be permissible, only provided the amount of nickel powder used does not exceed 1 g. Where larger amounts are necessary, as in the determination of tin in antimony regulus, the precipitated metal must be dissolved and re-treated.

EXPERIMENTS WITH METALLIC COBALT—

It was assumed that much the same conditions would be required for the reduction of antimony solutions by metallic cobalt as were required by the nickel. The procedure followed, therefore, was that described above.

It was found that the cobalt was more vigorous than nickel in its action, and the reductions were complete in a much shorter time (from 3 to 5 minutes). The precipitated antimony, however, differed somewhat in character from that reduced by nickel, for it floated and made filtration tedious; with nickel the antimony had remained at the bottom of the flask except that it was carried to the surface from time to time by the accumulated gas; it was also possible to decant most of the liquid through the filter when nickel was used.

TABLE IV

Expt.	Antimony taken	Tin taken	Cobalt used	Tin found	Error (tin co-pptd.)
	g.	g.	g.	g.	g.
26	1·0	0·0043	5·0	0·0017	− 0·0026
27	1·0	0·0111	5·0	0·0051	− 0·0060
28	1·0	0·0163	5·0	0·0120	− 0·0043
29	1·0	0·0407	5·0	0·0321	− 0·0086

SUMMARY

The statement that only nickel causes no co-precipitation of tin with antimony, has been critically examined. Antimonial solutions containing from 1 to 0·1 per cent. of tin were reduced by nickel powder and small co-precipitations of tin were observed. These amounts were much smaller than those observed by Evans and Higgs using lead, iron, aluminium and zinc—they amounted at most to 1 part of tin in 500 parts of precipitated antimony.

The small losses of tin were caused partly by co-precipitation with the antimony and partly by retention on the surface of the remaining nickel powder. When sheet nickel was substituted for the powder, only a trace of retained tin was noted.

Experiments using metallic cobalt in place of nickel showed it to be less satisfactory.

Of all the metals used for the reduction of antimonial tin solutions it would appear that nickel causes the least co-precipitation of tin, with cobalt next, followed by iron, lead, aluminium and zinc in that order.

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January, 1949

Discussion

Mr. F. L. Okell said that the investigation would be of interest to chemists who have to deal with ores and alloys of tin containing antimony; for it substantiated the opinions of those who hold that the reduction by nickel foil of stannic chloride solutions containing antimony does not lead to low tin figures. In addition, the author has shown that when the precipitated antimony and the excess nickel powder is filtered on a pad sprinkled with nickel powder and washed with *N* hydrochloric acid, the filtrate is free from antimony. This adds a useful separation to the analytical chemistry of antimony.

Had the author, during his experiments with nickel as a reductant for tin, had any experience of inactive nickel that fails to effect complete reduction? This phenomenon, possibly caused by the formation of a protective film, had been reported many times, recently by Miller and Currie (1948). He had met with but one example, a low grade metal containing carbon and copper. Pure nickel, etched by boiling with hydrochloric acid and an oxidiser, chlorate, ferric chloride or bromine, gave complete reduction.

Dr. B. S. Evans congratulated Mr. Holness on what seemed to be a valuable piece of work. It was no particular surprise to him to learn that there was, after all, evidence of a small co-precipitation of tin with the antimony thrown down by nickel. He had expected such co-precipitation and had been very perplexed when it did not seem to occur. A very notable occurrence, however, with the nickel they had used, was the smallness of the total precipitation of antimony, sometimes not much more than a trace; this he did not remember having heard mentioned by other workers.

There was one point that he thought should be mentioned, though it might well be a misunderstanding. In Table I the amount of tin co-precipitated would appear to be based on a difference figure and this after the main bulk had been boiled to dispel bromine. Tin in the presence of hydrobromic acid is very much more volatile than if hydrochloric acid alone is present and it might well be that at least some of the loss of about 1·5 milligrams might be put down to this cause.

With regard to Higgs's and his own work, he did not clearly remember the method they used to determine the co-precipitated tin in the antimony, but it was a *positive* method and was something like this— The washed precipitate was dissolved in bromine - hydrochloric acid, a large excess of oxalic acid added and the antimony removed by Clark's method by precipitation with hydrogen sulphide. The tin was recovered as stannous sulphide from the filtrate by a method he had published in 1932 (*Analyst*, 1932, **57**, 362), which at the time seemed to work very well.

Mr. Holness, in reply to Mr. Okell, said that he had only met with inactive nickel when trying to reduce tin solutions containing antimony. In investigating this condition, he had come to the conclusion that, when antimony was present, it was wiser to activate the nickel by boiling with a hydrochloric acid solution of sodium chloride to which a very small amount of antimony trioxide had been added, rather than to use the method suggested by Mr. Okell. He could confirm that this method gave complete reduction with antimony-free tin solutions, but he had experienced trouble when antimony was present. He was of the opinion that nickel etched by hydrochloric acid and an oxidiser would become inactive in a more dilute antimony solution, than nickel activated by hydrochloric acid and sodium chloride containing a trace of antimony.

Referring to the possible loss of tin during the expulsion of bromine mentioned by Dr. Evans, he said that this had been envisaged, and in Experiments Nos. 12 to 16 (Table II) a standard tin solution was used together with antimony trioxide, in order to avoid the use of bromine. The results, however, showed a similar loss of tin to that obtained when bromine was used and the matter had not been pursued further.

Erratum: June (1949) issue, p. 351.

At the end of the first item in the list of References, *for* "540" *read* "54".

Notes

RAPID ESTIMATION OF FAT IN SAUSAGES AND SAUSAGE MEATS

The following method was found to be very useful and gave results that agreed closely with those by the more standard Soxhlet method of fat extraction.

Place 10 ml. of sulphuric acid of sp.gr. 1·820 to 1·825 in a butyrometer. Thoroughly mince the sample of sausage (freed from casing) or sausage meat and place a representative portion on a watch glass, together with a small spatula. Weigh these, transfer about 2 g. of the sample carefully into the butyrometer and reweigh to ascertain the weight transferred. Introduce into the butyrometer also 1 ml. of amyl alcohol (of the standard required for the Gerber estimation of fat in milk) and sufficient water at approximately 80° C. to fill the butyrometer to the shoulder below the neck. Insert the stopper and carefully mix the contents of the tube by shaking until no more particles of meat are visible. Subsequently immerse in water at 68° C. and then centrifuge for 5 min. at about 1000 revs. per minute. At the end of this period return the tubes to the water-bath, where the readings are taken on the scale.

Multiply the percentage reading on the butyrometer by 11·2 and divide by the weight of sample taken, in grams, to obtain the percentage of fat in the sample.

Interference by the cereal content is overcome by immersion in boiling water, when the fat column rises above the cereal matter. The following results are typical of a number of experiments carried out on samples differing widely in fat content; the fat was determined both by Soxhlet extraction and by the above method.

By Soxhlet extraction	29·2	42·6	20·7	4·0	24·1	22·1 per cent.
By above method	29·7	42·8	20·85	3·2	24·3	22·4 per cent.

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A. TALBOT
September, 1948

THE DETERMINATION OF CAFFEINE IN COFFEE AND COFFEE PRODUCTS

THE estimation of caffeine in coffee and coffee products by the method of Kidd, Nanji, and Edwards,[1] in which zinc ferrocyanide is used as a defecating agent, has been found in the authors' experience to give low or erratic results. This is particularly noticeable when the precipitate is allowed to stand for any length of time and especially overnight. An investigation of the method showed that the precipitate adsorbed caffeine on standing. The use of normal lead acetate in place of zinc ferrocyanide obviated this difficulty. The method finally adopted for this determination was as follows.

The infusion from ground coffee (concentrated if necessary) or the solution of coffee extract, or coffee and chicory essence, is transferred to a 100-ml. graduated flask and diluted to approximately 70 ml. and 5 ml. of 20 per cent. normal lead acetate solution are added slowly, dropwise, with shaking. The mixture is made up to the mark, mixed, and filtered. The filtrate is de-leaded by addition of a little solid powdered sodium oxalate and refiltered. Fifty ml. are then transferred to a separating funnel, 5 ml. of 0·880 aqueous ammonia are added and the caffeine is extracted with successive portions of chloroform, 40, 30, 20 and 10 ml. The combined chloroform extracts are washed with 10 ml. of *N* sodium hydroxide, the chloroform layer is separated and the caustic soda layer extracted with a further 15 ml. of chloroform. The chloroform is removed from the combined extracts by distillation and the residue of impure caffeine is dissolved in a small quantity of hot water and transferred to a 100-ml. Kjeldahl flask. The nitrogen determination is carried out in the normal manner, using 10 ml. of sulphuric acid, 5 to 10 g. of potassium sulphate and a crystal of copper sulphate.

With quantities of caffeine ranging from 10 to 70 mg. in coffee and coffee products and with aqueous solutions of pure caffeine, consistent and satisfactory results were given by the lead acetate method and it has been adopted for routine estimations.

The authors' thanks for permission to publish are due to Saml. Hanson & Son, Ltd., Eastcheap, London, in whose laboratories most of this work was carried out.

REFERENCE

1. Kidd, J. D., Nanji, H. R., and Edwards, F. W., *Analyst*, 1941, **66**, 240.

37 KELMSCOTT ROAD
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A. TAYLOR
D. J. TAYLOR (Mrs.)
December, 1948

THE INACTIVATION OF BIOTIN BY CHLORINE

DURING a series of experiments in which the relative activities of D-biotin and DL-desthiobiotin to *Saccharomyces cerevisiae* were compared by a modification of the technique of Hertz,[1] it was observed that biotin gave variable low responses. The cause was traced to the occasional presence of small amounts of chlorine in the glass-distilled water used in one laboratory. Chlorine was found to inactivate aqueous solutions of crystalline D-biotin to different degrees, according to the chlorine concentration and time of contact. At the concentrations of biotin tested (10^{-6} to $10^{-9}\,M$) and at constant time of contact t, $\log [B/(B-x)]$ was approximately proportional to chlorine concentration, where B = original biotin concentration and $(B-x)$ = biotin activity at time t. After 2 hr. contact at 0·01 parts per million, chlorine had no measurable effect on biotin activity, whereas at 3 parts per million, no detectable biotin activity remained. A concentration of 3 parts per million of chlorine did not affect the activity of aqueous solutions of DL-desthiobiotin. It may therefore be postulated that the sulphur atom of the biotin molecule is involved in a chemical reaction with chlorine and that the biotin is thereby inactivated.

The same concentration of chlorine did not affect biotin in the presence of other constituents of the assay medium, indicating that the chlorine was preferentially reduced by other oxidisable substances in the medium.

Since tap water may contain chlorine, which in certain types of stills is redissolved in the distilled water, it is important that water used for the dilution of biotin for microbiological assays should be tested for freedom from chlorine. If no visible colour is developed by the standard *o*-tolidine test,[2] the water is sufficiently free from chlorine to have no perceptible effect on the activity of biotin towards *S. cerevisiae.*

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RESEARCH AND DEVELOPMENT DEPARTMENT
DISTILLERS COMPANY LIMITED
EPSOM, SURREY

J. S. HARRISON
E. J. MILLER
January, 1949

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* (1949, **74**, 262).

Public Analyst	*Appointments*
HATFULL, Ronald Stanley (Deputy)	Metropolitan Borough of Battersea.
JAMES, George Vaughton (Temporary Deputy)	County Borough of Bournemouth.
LEATHER, Alfred Norman	County Borough of Manchester.
LYNE, Francis Arthur (Deputy) ..	Royal Borough of New Windsor.
,, ,, ,, (Joint) ..	City of Oxford.
McLACHLAN, Thomas	City of Oxford. Royal Borough of New Windsor. Urban District of Thurrock.
PAULLEY, William Minterne.. ..	Metropolitan Borough of Stepney.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENT

NOTIFICATION of the following appointment has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1949, **74**, 262).

Official Agricultural Analyst	*Appointment*
LEATHER, Alfred Norman	County Borough of Manchester.

Ministry of Food

STATUTORY INSTRUMENTS

1949—No. 1303. The Meat Products and Canned Meat (Amendment No. 2) Order, 1949. Price 1d.

This Order, which came into force on July 12th, 1949,

(a) *provides that the use of meat fat in the manufacture of whale meat, vegetable, or fish products shall not make them meat products;*
(b) *prohibits the use of whale meat in meat products; and*
(c) *provides maximum prices for Mexican Meat and Gravy.*

—No. 1536. The Labelling of Food (Amendment No. 2) Order, 1949. Price 1d.

This Order, as from August 20th, 1949,

(a) *imposes special requirements as to the labelling of frozen peas; and*
(b) *transfers Christmas puddings from Table C in the First Schedule of the principal Order* (*S.R. & O.*, 1943, *No.* 1553) *to item* 8 *of Table A in that Schedule, thereby providing that notwithstanding the revocation of the Manufactured and Pre-packed Foods* (*Control*) *Order,* 1942 (see *Analyst*, 1949, **74**, 413), *the ingredients of Christmas puddings need not be specified in accordance with Article* 2 (3) *of the principal Order.*

—No. 1584. The Feeding Stuffs (Rationing) Order, 1949. Price 6d.

This Order, as from September 1st, 1949, *replaces the Feeding Stuffs* (*Rationing*) *Order,* 1943, *as amended.*

British Standards Institution

DRAFT SPECIFICATIONS

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, J. H. Lane, 7–8, Idol Lane, London, E.C.3.

Draft Specification prepared by Technical Committee M/22—Cleanliness of Hessian for Upholstery.
CK(M)5607—Draft for Cleanliness of Used Hessian.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Biochemical

Estimation of Serum Vitamin A with Activated Glycerol Dichlorohydrin. A. E. Sobel and S. D. Snow (*J. Biol. Chem.*, 1947, **171**, 617–632)—The vitamin A contained in 1 ml. of serum can be estimated by measuring the colour formed with activated glycerol dichlorohydrin (G.D.H.). This reagent, unlike the Carr - Price reagent, is not sensitive to moisture, and so a simplified procedure can be used.

Reagents—N *Potassium hydroxide* in 90 per cent. ethanol, prepared from a stock solution of potassium hydroxide and absolute alcohol. *Activated G.D.H.*—Distil glycerol dichlorohydrin in presence of 1 per cent. by weight of antimony trichloride at a pressure of 10 to 40 mm. of mercury. Discard the first and last fractions. The distillate should be free from antimony trichloride, as shown by its remaining clear when water is added.

Procedure (with Coleman Spectrophotometer)—To 1 ml. of serum in a $\frac{3}{8} \times$ 4-in. test tube, add 1 ml. of 95 per cent. ethanol, and then mix the contents of the tube by tapping (or for saponification add 1 ml. of *N* potassium hydroxide in 90 per cent. ethanol to the serum, mix the contents, and then place the tube in an oven at 60° C. for 20 min.). Add 2 ml. of A.R. light petroleum (b.p. 30° to 60° C.), shake for 10 min. and then centrifuge for about 30 sec. Remove the solvent layer with a fine-tipped dropper and place in a $\frac{1}{2} \times$ 4-in. test tube. Extract with a further 2 ml. of light petroleum, shaking for only 5 min. (If the carotene is to be determined at 440 mμ., place the light petroleum in a cuvette graduated at 4 ml., make up to the mark, and read the absorption with filter PC–4. Transfer the light petroleum extract quantitatively to a $\frac{1}{2} \times$ 4-in. test tube.)

Evaporate the extract to dryness by placing the tube in a water-bath at 40° to 50° C. and passing a stream of nitrogen over the contents. Add 1 ml. of A.R. chloroform and then 4 ml. of G.D.H. Mix the solution with a flat-tipped stirring rod for 2 min., and then place it in a 50-mm. cuvette of capacity 2·8 ml. Measure the absorption with a 555-mμ. filter, against a blank of 4 ml. of G.D.H. and 1 ml. of chloroform. Turn the wavelength dial to 800 mμ. and, at 4 min. after the mixing of the reagents, measure the absorption, using filter PC–5.

Prepare a calibration curve for vitamin A, by means of standards in chloroform solution containing from 0·2 to 5·0 μg. per ml. Bring the vitamin A standard and the G.D.H. solution to 25° C. before use. Mix 1 ml. of standard and 4 ml. of G.D.H. solution in a glass-stoppered cylinder, and then place the latter in a water-bath at 25° C. for 2 min. Pour the solution into a 50-mm. cuvette and read the absorption with the 555-mμ. filter exactly as above. Plot a graph of optical density against the vitamin A concentration of the standard.

The calibration curve of carotene can be determined at 440 mμ. against a light petroleum blank, using standards containing 1 to 10 μg. of carotene per 4 ml. of light petroleum. The carotene calibration curve can also be determined with G.D.H. at 800 mμ., with standards in chloroform containing 1 to 10 μg. of carotene per ml. Proceed as for the vitamin A calibration, reading the absorption 4 min. after mixing the reagents.

Prepare a calibration curve as though for vitamin A with carotene at concentrations of from 1 to 10 μg. per ml. This graph is used to correct the sample readings for interference by carotene, the amount of carotene being determined at 555 mμ. or at 800 mμ.

The procedure is slightly modified when other photometers are used. With a Beckman spectrophotometer, determine the vitamin A at 555 mμ. and the carotene at 830 mμ. Dissolve the residue from the light petroleum extract in 0·2 ml. of chloroform and add 0·8 ml. of G.D.H. Measure the absorption in a 10-mm. cell of capacity 1 ml. The amount of serum required can be reduced by using smaller cells.

With visual colorimeters, dissolve the dried extract from 1 ml. of serum in 0·2 ml. of chloroform and then add only 0·8 ml. of G.D.H. Compare the colour of the sample solution and a standard in 1-ml. micro-cups of length 40 mm. Prepare a standard for each unknown with 1 ml. of a chloroform solution containing 2·5 μg. of vitamin A to which are added 4 ml. of G.D.H.

The G.D.H. method has been compared with the Carr - Price method, and good agreement found. Higher values were obtained with the Carr - Price reagent when saponified serum was used. Using the G.D.H. reagent, however, the same value was obtained on saponified and unsaponified serum, unless the vitamin A content of the serum was large.

Working in a humid atmosphere, the use of A.R. light petroleum without further purification, and the use of redistilled waste G.D.H. did not interfere with the determination. The carotene content could be estimated at 440 mμ. or, with sufficient accuracy, at 800 mμ. using the same solution as for the vitamin A determination.

W. S. Wise

Determination of Amounts of Purine Nitrogen of the Order of 10 to 40 micrograms. Application to Nucleic Acids, Nucleoproteins, Tissues, and Micro-organisms. R. Vendrely (*Biochimica et Biophys. Acta*, 1947, **1**, 95–100)—A simple and rapid method is described for estimating small amounts of purine nitrogen of the order of 10 to 50 μg. The material to be analysed is hydrolysed in *N* hydrochloric acid. The purines liberated are separated in a pure state by two successive precipitations as a copper complex. The nitrogen in the purine bases is determined by the micro-Kjeldahl method.

Hydrolysis—Measure the liquid sample with a Linderstrøm-Lang pipette (or weigh tissues) into a small flask of total capacity 3 to 4 ml. and fitted with a ground-glass stopper. Add sufficient hydrochloric acid of a convenient concentration to give a

final volume of 1·2 to 2·0 ml. of normal acid and connect the flask to a condenser consisting either of a long narrow glass tube, or, for hydrolyses of longer duration, a water-cooled condenser. Heating is best carried out in a paraffin-bath fitted with a thermostat. Immerse the micro-flasks to the rim in the liquid and heat for 2 hr., with stirring, for a nucleic acid, and 8 hr. for a complex substance. After hydrolysis, cool by immersion in cold water. Remove the hydrolysate with a teat-pipette and filter through a micro-filter (Jena 12 G3) directly into 5-ml. conical centrifuge tubes, where the precipitation of the purines is carried out. Filtration may be aided by slight suction, the centrifuge tube being placed inside a vacuum receiver. Rinse the small flasks two or three times with 0·5 ml. of water, which is then passed through the filter. The same filter can be used several times. The filtrate, 2 to 3 ml., contains all the purines. Make it alkaline to methyl red with 40 per cent. sodium hydroxide solution, stirring with a fine glass stirrer, then make acid again with several drops of 10 per cent. acetic acid solution.

PRECIPITATION OF THE PURINE—Place the tubes containing the hydrolysed sample in a small water-bath containing boiling water. If the heating at this stage causes the formation of a precipitate, remove it by centrifuging, transfer the liquid to fresh tubes by means of a teat-pipette, wash the precipitate with 0·5 ml. of water acidified with acetic acid, and add this wash water to the main fraction.

First precipitation—Add successively to the hydrolysate, with stirring, 5 drops of 30 per cent. pyrosulphite (metabisulphite, $Na_2S_2O_5$ or $K_2S_2O_5$) solution, and 4 drops of 10 per cent. copper sulphate solution. A fine precipitate forms, and gradually becomes coloured by liberation of copper oxide. Remove the stirrer and wash it with a few drops of water. Centrifuge for 5 min. at 3000 r.p.m. and collect the brownish precipitate, removing the supernatant liquid with a teat-pipette; 2 or 3 mm. of liquid should be left above the precipitate.

Decomposition of the copper complex—Take up the precipitate in 1·5 ml. of warm water and replace in the water-bath. Add 4 drops of neutral sodium sulphide solution made by saturating a 1 per cent. sodium hydroxide solution with hydrogen sulphide and adding an equal volume of 1 per cent. sodium hydroxide solution. Add 6 drops of 10 per cent. acetic acid solution, followed by 2 drops more if the liquid is slow in clearing, and stir. A more or less colloidal black precipitate of copper sulphide and sulphur is gradually formed by warming on the water-bath for 20 to 25 min. The liquid should be almost colourless and quite clear. Centrifuge and transfer the liquid, without loss, by means of a teat-pipette to a fresh series of conical tubes. Wash the pipette with about 0·5 ml. of water, which is then run on to the precipitate, repeat twice, again centrifuge, and add the supernatant liquid to the first one obtained.

Second precipitation—Again precipitate the combined liquids with pyrosulphite and copper sulphate as before, centrifuge for 5 min., remove the supernatant liquid and take up the precipitate in 3 or 4 successive small amounts of water (0·4 ml.) and transfer by pipette to special digestion tubes, 9 cm. long, 1 cm. in internal diameter, blown out to a bulb, 2 cm. across, on one side of the closed end.

DETERMINATION OF NITROGEN IN THE PRECIPITATED PURINE—*Digestion*—To prepare the digestion reagent, boil 50 ml. of pure sulphuric acid for 15 min. with an excess (5 g.) of a catalyst made by mixing 1 part of selenium with 5 parts of mercuric sulphate, and 30 parts of potassium sulphate. Decant the completely dehydrated acid, while boiling, into a pre-heated ground-glass stoppered tube, stopper, and allow to cool. Use the clear supernatant liquid as the reagent; it will keep in good condition for over a month if precautions are taken to prevent re-hydration.

Evaporate an aqueous suspension of purine complex to dryness in the digestion tube in an oven at 105° C. Drop into the tube several strands of glass wool that has previously been washed with chromic acid and water. Cut a small wick from very fine strands of the glass wool to a length of 3 to 5 mm. above the axis of the tube. Add 80 μl. of the digestion reagent, and place the tubes fan-wise on a 4-mm. thick asbestos plate perforated with small holes 2 mm. in diameter corresponding to the base of each tube, with the upper ends of the tubes resting on a metal frame. Heat the asbestos plate by a 600-watt electric element. The heating, for a total time of 30 min., is discontinuous, the current being so adjusted that the boiling liquid rises and falls up the side of the tube for a distance of about a third of the total length. When the digestion is complete, cool the tubes and add 1 ml. of water.

Distillation—Markhams' apparatus is used (*Biochem. J.*, 1942, **36**, 790). Transfer the liquid to the apparatus by means of a teat-pipette and wash out the digestion tubes twice with 1 ml. of water. Introduce 0·6 ml. of sodium hydroxide by slowly raising the stopper of the charging funnel, and rinse with an equal volume of water. Collect the distillate in 3·5-ml. receptacles, made by cutting off the neck of 5-ml. ampoules, and containing 90 μl. of $N/30$ sulphuric acid, 1 drop of 0·08 per cent. methyl red, and 4 drops of water. A narrow tube connected to the end of the condenser dips into the acid and at the conclusion of the distillation this tube is rinsed on the outside with 2 drops of water. Distillation of 2 ml. takes 5 to 7 min.

Titration—Use a 100-μg. Linderstrøm-Lang burette (*Compt. rend. Lab. Carlsberg*, 1931, **19**, No. 4). When determining the normality of the $N/30$ acid, choose a given colour for the end-point and use this colour in the following tests. At least 3 blanks and 3 tests should be run.

Calculation of results—A correction factor is applied according to the time of hydrolysis. For hydrolysis times of 0, 1, 2, 4, 8, and 16 hr. the values obtained for adenine are multiplied by 1·000, 1·010, 1·030, 1·045, 1·104, and 1·256, respectively. The corresponding correction factors for guanine are 1·000, 1·000, 1·010, 1·012, 1·036, and 1·075, and for an equimolecular mixture of adenine and

guanine, 1·000, 1·012, 1·022, 1·038, 1·071, and 1·165. Reproducible results were obtained by this method on natural materials with an accuracy of about 5 per cent. J. S. HARRISON

Determination of Phytic Acid by Oxidation of the Inositol with Periodic Acid. M. H. M. Heggen and J. F. Reith (*Pharm. Weekblad*, 1948, **83**, 801–805)—Phytic acid can be hydrolysed by boiling under refluxing conditions for 8 hr. with 35 per cent. sulphuric acid. The inositol can then be determined by oxidation with periodic acid under definite conditions, under which 6·73 atoms of oxygen are used for 1 molecule of inositol. The oxidation is carried out at 37° C. for 19 hr., after which sodium hydrogen carbonate is added, followed by excess of arsenite and potassium iodide. After 10 min., the excess of arsenite is determined by back titration. The presence of iron, as in precipitated iron phytate, does not interfere.

G. MIDDLETON

Gas Analysis

Rapid Determination of Low Concentrations of Carbon Monoxide in Air. M. Katz and J. Katzman (*Canadian J. Res.*, 1948, **26**, F, 318–330)—A granular form of 69 mol. per cent. of silver permanganate deposited on 31 mol. per cent. of zinc oxide oxidises carbon monoxide in air completely. The presence of some water vapour is necessary to initiate oxidation, but changes in humidity from 30 to 100 per cent. have no further effect on the reaction.

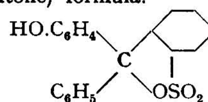
The granular reagent has an average particle size of 0·90 mm. and is prepared by uniform deposition of the silver salt on the zinc oxide carrier, pressing of the moist filter cake in a mould at 3 to 5 tons per sq. in., and air drying of the graded material at 60° C. for 72 hr. Dried samples stored in brown bottles retain their activity towards carbon monoxide during storage for 2 years at room temperature. Loss of oxygen from the silver salt during use eventually causes loss in activity. 20 to 25 Ml. of the reagent are sufficient for an 8-hr. continuous test on concentrations of carbon monoxide below 0·1 per cent. before the reagent loses its efficiency. The reagent was used in two vessels One was a glass tube of 1-in. diameter with ten copper-constantan thermocouples embedded in the reagent at half-inch intervals. Concentrations of carbon monoxide of 183 p.p.m. were completely oxidised at rates of flow up to 12 litres per min. The other vessel contained 20 ml. of granules and fifty-five differential thermocouples. A straight line relationship between concentration of carbon monoxide (0 to 600 p.p.m.) and microvolts (0 to 11,000) was obtained at a rate of flow of 6·5 litres per min. Hydrogen is also oxidised but less effectively: no appreciable errors are introduced provided the concentration of hydrogen is not appreciably greater than that of the carbon monoxide. The method is readily applicable to the determination of carbon monoxide in the air in aircraft, motorised vehicles, mines, and buildings, for concentrations of 5 to 200 p.p.m.

W. J. GOODERHAM

Organic

Acid-Base Reactions in Organic Solvents. Behaviour of Some Halogenated Derivatives of Phènolsulphonephthalein with Various Classes of Organic Bases in Benzene. M. M. Davis, P. J. Schuhmann, and M. E. Lovelace (*J. Res. Nat. Bur. Stand.*, 1948, **41**, 27–40)—The previous investigation of bromophthalein magenta (Davis and Schuhmann, *Ibid.*, 1947, **39**, 221; *Analyst*, 1948, **73**, 467) is extended to the sulphonephthaleins, and similar experimental methods are employed.

A number of the halogenated sulphonephthaleins have been isolated as colourless or nearly colourless solids and the colour of commercial specimens is believed to be due to impurities or to hydration. This colourless form is believed to possess the lactone (sultone) formula.



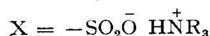
Solutions of these indicators in benzene are also colourless or nearly colourless, their structure being unaffected by such inert solvents. The pale yellow colour observed on standing is attributed to traces of moisture or to alkali in the glass surface. The yellow colour indicates conversion to one of the quinoid forms that are assumed in aqueous media. It disappears on addition of acetic acid. Transmittancy curves are plotted for benzene solutions of chlorophenol blue, bromophenol blue, iodophenol blue, tetrabromophenolphthalein, and bromophthalein magenta E down to 280 mμ.

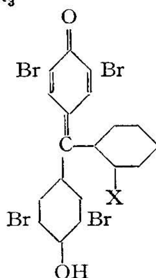
The reactions of bromocresol green, bromophenol blue, and tetrabromophenol blue with various bases in benzene solution are illustrated by transmittancy curves. Qualitative observations are recorded for iodophenol blue, chlorophenol blue, bromochlorophenol blue, chlorophenol red, bromophenol red, bromocresol purple, and bromothymol blue. The following summary applies to all the above indicators with the exception of tetrabromophenol blue and bromothymol blue; chlorophenol red also shows slight variations.

Small quantities of either strong or weak bases cause the appearance or intensification of the yellow colour and this stage of the reaction is usually complete when approximately one equivalent of the base has been added. Further changes occur on addition of amines in excess of one equivalent and the final colour varies with the type of amine. Primary aliphatic amines cause precipitation, but a momentary red-purple colour is observed with some of the indicators. Secondary aliphatic amines produce a purple-blue colour. Tertiary aliphatic amines or *sym.*-di- or tri-arylguanidines produce a magenta colour. Quaternary ammonium salts give blue solutions. This second stage may require up to several hundred equivalents of amine for its completion. The unhalogenated sulphonephthaleins are not sufficiently soluble for similar investigation.

The transmittancy curve for the yellow form of bromophenol blue, produced by addition of one

equivalent of 1 : 2-diphenylguanidine, is almost identical with the curve for bromophthalein magenta E with no added base. This is to be anticipated from the reasoned assumption that bromophenol blue, when treated with one equivalent of base, forms an acid salt of the structure represented by the following formula, where





The same structure, with X = $-COOC_2H_5$, is the only structure that can be assigned to bromophthalein magenta E. The colour changes that accompany the further addition of various types of amines to solutions of the halogenated sulphonephthaleins in benzene are similar to those that occur with bromophthalein magenta E, and are attributed to the changes in configuration already postulated for bromophthalein magenta (Davis and Schuhmann, *loc. cit.*).

The usefulness of the sulphonephthaleins as indicators in hydrocarbon solvents is severely limited by their low solubility in such liquids and by the overlap in the steps in their neutralisation. The first change from the colourless to the yellow form is, however, brought about by aromatic, as well as by aliphatic amines, whilst bromophthalein magenta is not sufficiently acid to react with aromatic amines. W. C. JOHNSON

Piperazinium Salts for Utilisation in Identification of Organic Acids. M. Prigot and C. B. Pollard (*J. Amer. Chem. Soc.*, 1948, **70**, 2758–2759)—Thirty-six new piperazinium salts suitable for use in identifying organic acids have been prepared by an improved technique: they have been characterised and their properties are listed in the accompanying table.

DATA CONCERNING PIPERAZINIUM SALTS DERIVED FROM VARIOUS ORGANIC ACIDS

	Piperazinium salts			
			Neutral equivalents	
Acid	Yield per cent.	Melting-point ° C. corrected	Calcd.	Found
o-Benzoylbenzoic	78	186·2–186·6	287	289
o-Bromobenzoic	90	227–230 decomp.	244	241
m-Bromobenzoic	74	169–171	244	250
p-Bromobenzoic	49	224–226	244	240
α-Bromopropionic	73	195 decomp.	196	192
Dichloroacetic	62	181 decomp.	344	340
n-Capric	49	92·5–93·5	215	219
n-Caprylic	32	97·5–98·0	187	184
o-Chlorobenzoic	81	217–218 decomp.	200	194
p-Chlorobenzoic	67	219–220 decomp.	200	199
trans-Cinnamic	92	206 decomp.	191	190
Citric	100	141–142	139	137
Ethoxyacetic	73	120–121	147	145
p-Ethoxybenzoic	76	176·2–177·0 decomp.	209	211
Fumaric	98	240 decomp.	101	105
α-Furoic	94	234–236 decomp.	155	160
Gallic	84	209·0–209·7 decomp.	213	*a*
Hippuric	82	182–184 decomp.	222	222
Lauric	77	92·0–92·5	243	243
Maleic	92	148	101	102
Methoxyacetic	80	155·7–156·4	133	132
o-Methoxybenzoic	92	190·4–191·4	195	196
m-Methoxybenzoic	89	136·9–138·5 decomp.	195	202
α-Naphthoic	48	131·5–139·0 decomp.	215	216
β-Naphthoic	89	194·0–195·0 decomp.	215	217
p-Nitrocinnamic	90	247·9–248·7 decomp.	232	232
p-Nitrophenylacetic	96	205·5–205·9 decomp.	224	225
4-Nitrophthalic	96	201·5–204·5 decomp.	149	149
Pelargonic	60	95·1–96·2	201	198
Phenoxyacetic	84	183·7–184·2 decomp.	195	195
o-Phthalic	91	187–188	139	139
*iso*Phthalic	77	251·7–252·2 decomp.	126	126
Terephthalic	52	Decomp. above 350	126	127
D-Tartaric	86	248–254	118	117
meso-Tartaric	87	140–141	118	119
p-Toluic	79	203·0–203·3	179	181

a Solution too dark to titrate.

Preparation of derivatives—Dissolve some of the organic acid in anhydrous ether or *iso*propyl alcohol and gradually incorporate the calculated amount of piperazine, added as a 1 *M* solution in *iso*propyl alcohol. Separate the precipitate by filtration, add it to fresh ether or *iso*propyl alcohol, stir thoroughly, filter again, and dry in a desiccator over phosphorus pentoxide. If the sample is quite insoluble in ether or *iso*propyl alcohol, dissolve in water before adding piperazine, and evaporate over boiling water to recover the salts.

Determine the melting-point by using a bronze block pre-heated to within 5° of the m.p.

To determine the neutral equivalent, dissolve some of the salt in a 50 per cent. aqueous solution of *iso*propyl alcohol and titrate with 0·1 *N* sodium hydroxide, using either thymolphthalein or Orange II as indicator.

By the method employed the following acids failed to give salts of practical value for qualitative organic analysis: α-bromo-*n*-butyric, anthraquinone-β-sulphonic, barbituric, 2-chloropropionic, 2 : 5-dichlorobenzenesulphonic, 2 : 4-dichlorophenoxyacetic, diethylacetic, diphenylacetic, erucic, glycine, *p*-hydroxybenzoic, iodoacetic, itaconic, levulinic, DL-methylethylacetic, mucic, 1-naphthol-4-sulphonic, sulphosalicylic, thioglycolic, and trimesic.

A. H. A. Abbott

Determination of Aromatics and Olefines in Hydrocarbon Mixtures. C. Berg and F. D. Parker (*Anal. Chem.*, 1948, **20**, 456–457)—The determination is carried out by a modification of the acid-solubility method. The reagent used is 15 per cent. fuming sulphuric acid, which is added to a solution of the sample in glacial acetic acid. Experiments with fuming sulphuric and glacial acetic acids showed that addition of a mixture of these acids to the hydrocarbon resulted in only partial dissolution of aromatic compounds, whereas addition of fuming sulphuric acid to a solution of the hydrocarbon in glacial acetic acid was successful. Fuming sulphuric acid containing 15 per cent. of sulphur trioxide was more effective in dissolving olefines than acid of lower concentrations.

Solubility of hydrocarbons—*Iso*-octane (2 : 2 : 4-trimethylpentane) was used as one of the most sensitive of saturated hydrocarbons for solubility tests in four different mixtures of glacial acetic and fuming sulphuric acids. The solubility was only 0·5 per cent. compared with 3·0 per cent. for 98 per cent. sulphuric acid. Benzene, which is completely soluble in 98 per cent. sulphuric acid, was found to be soluble in a mixture of equal quantities of the two acids. The solubility of olefines was tested with di-*iso*butylene, diamylene (a mixture of 3 : 5 : 5-trimethylheptene-2 and 3 : 4 : 5 : 5-tetramethylhexene-2) and *iso*-octene (a mixture of isomeric octenes). The solubilities in the recommended mixed acids and in 98 per cent. sulphuric acid are given in Table I.

Procedure—Place 15 ml. of glacial acetic acid in a 60-ml. graduated bottle (Stoddard solvent test bottle, A.S.T.M. designation, D484–40) and add, by means of a pipette, 10 ml. of the sample followed by 25 ml. of fuming sulphuric acid (15 per cent. sulphur trioxide content) in 5-ml. portions. Shake the bottle in a bath of iced water after each addition and until no further heat is generated. Rotate the bottle for 15 min. at 30 r.p.m. Fill the bottle to the level with fuming sulphuric acid and allow to stand 2 hr. before reading the volume solubility. Add 0·5 per cent. to the upper meniscus reading to correct for the difference in curvature between the upper and lower menisci.

Table I

Composition of sample, per cent. by volume							Solubility in	
n-Heptane	*iso*-Octane (*a*)	Di-*iso*-butylene	Di-amylene (*b*)	*iso*-Octene (*c*)	Toluene	Total aromatics and olefines %	Mixed acid	98% Sulphuric acid
100·0	—	—	—	—	—	0·0	0·0	0·0
—	—	100·0	—	—	—	100·0	80·0	42·0
—	—	—	100·0	—	—	100·0	93·0	45·0
—	—	—	—	100·0	—	100·0	99·0	49·5
—	—	—	—	—	100·0	100·0	100·0	100·0
50·0	—	30·0	—	—	20·0	50·0	50·0	32·0
50·0	—	—	30·0	—	20·0	50·0	50·0	31·0
50·0	—	—	—	30·0	20·0	50·0	50·0	33·5
50·0	—	10·0	—	—	40·0	50·0	50·0	45·0
50·0	—	—	10·0	—	40·0	50·0	50·0	45·0
50·0	—	—	—	10·0	40·0	50·0	50·0	50·0
20·0	20·0	10·0	10·0	10·0	30·0	60·0	60·0	48·5

Data for the solubility of certain pure hydrocarbons and the results of analyses of fuels are given.

The solubility method using 98 per cent. sulphuric acid is subject to error owing to the polymerisation of olefines and the formation of saturated hydrocarbons by complex reactions, *e.g.*, di-*iso*butylene and diamylene are particularly difficult to dissolve. Active saturated hydrocarbons are likely to dissolve giving rise to an error of the opposite kind that increases with increasing concentration of acid.

Results—The increased absorption given with this method compared with the 98 per cent. sulphuric acid method is particularly advantageous in the analysis of cracked gasolines. The latter method suggests a lower olefine content than is given by bromide - bromate determinations. Typical results are recorded in Table II.

TABLE II

Gasoline	Olefine, % (bromide - bromate)	Solubility: In 98% sulphuric acid	Solubility: In mixed acids
Straight-run gasoline from Los Angeles Basin crude oil	1·0	14·5	14·5
200° to 240° F. cut from thermally reformed Los Angeles Basin raffinate	21·4	22·0	29·0
Thermally reformed gasoline from Santa Maria Valley crude oil (0·44% sulphur)	32·0	28·0	37·0
Catalytic polymer gasoline (from C_3 – C_4 olefines)	76·0	38·5	74·5

W. C. WAKE

Determination of Asphaltenes, Oils, and Resins in Asphalt. R. L. Hubbard and K. E. Stanfield (*Anal. Chem.*, 1948, **20**, 460–465)—A method is described for determining three fractions of petroleum and native asphalts. These fractions are separated as asphaltenes, oils, and resins, and are recovered quantitatively. Since the separation of asphalts into these three components is necessarily arbitrary, it is essential, in order to obtain consistent and reproducible results, that definite chemical compounds rather than mixtures of specified boiling range should be used for solvent extractions, and that the extractions should be carried out at definite, reproducible temperatures. The authors use *n*-pentane and methanol-benzene for extractions at controlled temperatures in a special apparatus.

Apparatus—The two special pieces of apparatus used are shown in Figs. 1 and 2. Fig. 1 shows a glass adaptor for removing solvents and drying extracts at atmospheric or reduced pressures. It

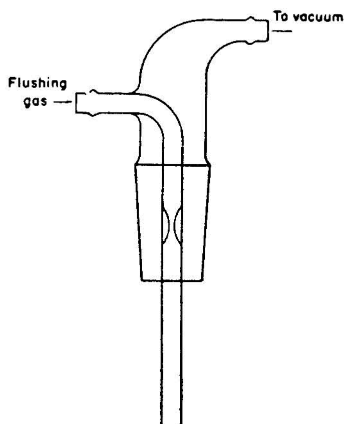


Fig. 1. Adaptor for removing solvent

is used with 300-ml. flasks having flat bottoms and standard ground-joints, and flushing gas is passed through one side-tube on to the surface of the extract and out through the opposite tube. The constant temperature extraction apparatus shown in Fig. 2 allows the continuous extraction by liquid of solid or semi-solid materials in an inert atmosphere at specified temperatures. The water-bath D surrounding the solvent cooling-coil is maintained at the desired temperature by water circulating from a thermostatically controlled bath of larger capacity. The mercury-sealed paddle stirrer H is operated by an oscillating motor, G,

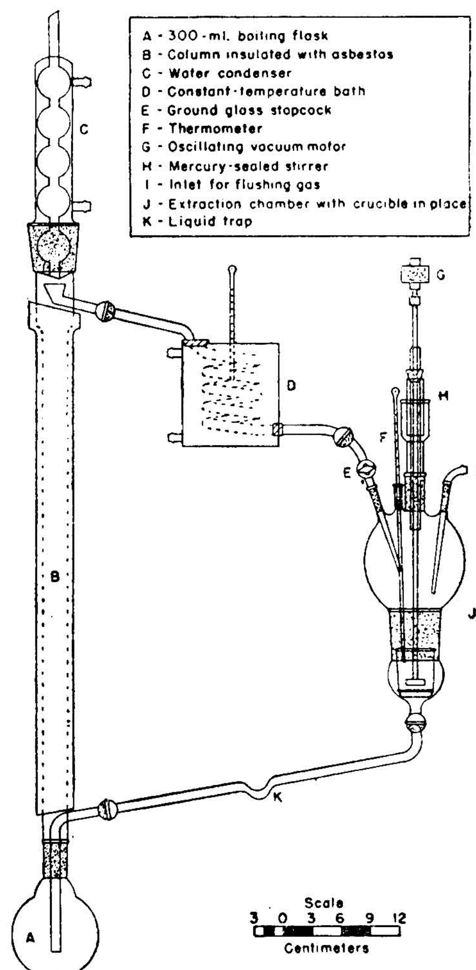


Fig. 2. Constant-temperature extraction apparatus

of the type used for car windscreen wipers. The liquid trap K in the solvent return line aids observation of the colour of the extract. The sintered-disc crucible, shown in place in the extraction chamber, J, is of 50-ml. capacity with a disc diameter of

40 mm. and should be of such a porosity that 15 to 20 ml. of acetone will pass in 30 sec.

Preparation of asphalt sample—Weigh accurately into a 100-ml. centrifuge tube a sample containing 1·25 to 1·5 g. of asphalt, the lower weight being taken when the asphalt is known to contain a high proportion of resins or asphaltenes. Warm the tube to soften the asphalt and distribute it evenly over the lower part of the tube.

Determination of asphaltenes—Add to the sample in the centrifuge tube 40 ml. of *n*-pentane per g. of asphalt. Disperse by hand with a screw-type stirring rod made by flattening and then twisting a section 5 cm. long and situate 1 cm. from the lower end of a glass rod 6 mm. in diameter and 25 cm. long. Transfer the tube to a water-bath at 15·6° C. and, with the same rod, stir at 2500 r.p.m. for 10 min. Remove the stirring rod, place a stopper in the tube, and allow to stand for 12 hr. or overnight in darkness or subdued light. Return the tube to the constant temperature bath for 20 min., stir at 2500 r.p.m. for 10 min., and then remove it from the bath and centrifuge for 5 min. with a relative force of 975 **g**. Decant the clear pentane solution into a 300-ml. boiling flask and note the approximate volume of the pentane-insoluble fraction. Add to the residue 25 ml. of *n*-pentane per ml. of residue, stir for 10 min. at 15·6° C., and centrifuge for 5 min. as before. Repeat this washing process three times more, using the same volume of *n*-pentane each time. Add the washing liquid to the original pentane extract in the 300-ml. flask.

Dissolve the pentane-insoluble asphaltene fraction in benzene and filter the solution through filter paper into a tared boiling-flask. Use a few drops of methanol in eluting the final traces of asphaltenes from the filter paper. Connect the adaptor shown in Fig. 1 to the flask and distil to dryness on a steam-bath, a current of carbon dioxide, nitrogen, or helium being passed through the adaptor during the operation at a reduced pressure of 5 to 10 in. of water. After removal of the solvent, complete the drying of the extract for 25 min. at 105° C., all the time passing a stream of the flushing gas at reduced pressure. When dry, cool in a vacuum desiccator, flush with air, remove the adaptor, and weigh to determine the yield of asphaltene fraction.

Determination of oils—Distil the combined pentane extract and washings from the initial extraction to remove most of the solvent. If this extract is allowed to stand before further treatment, the air in the flask should be replaced by an inert gas. Evaporate this concentrated extract to approximately 5 ml. and pour it evenly over 25 g. of alumina in a Gooch crucible. (The alumina, of technical grade, should pass a 100-mesh per inch sieve and be retained on 200-mesh sieve. Heat the alumina for 2 hr. at 700° C. and cool in a desiccator before use.) Rinse the material on to the alumina with 3- to 5-ml. portions of *n*-pentane, continuing the rinsing until the base of the crucible becomes moistened with solvent. When correctly done the upper portion of the alumina is dark and the lower portion colourless. Place the crucible in the extraction chamber of the apparatus shown in Fig. 2 and dry the material to a powder by stirring gently in a stream of inert gas. Place *n*-pentane that has been dried by passing through alumina, in the boiling flask A, bubble gas slowly through the liquid, and heat the latter with a water-bath. Adjust the stopcock E so that *n*-pentane, cooled to 15·6° C. by the cooling coil, is added slowly to the sample in the crucible, the flow being such as to maintain it one-half to three-quarters full. The initial percolate from the crucible should be colourless or slightly yellow. If it is distinctly coloured, repeat the analysis with a smaller sample. After percolation has commenced, start the stirrer, and continue the extraction for 1 hr. Then wash the extraction chamber and the return line with *n*-pentane, remove the boiling flask, and concentrate the extract and washings to 25 ml. by distillation. Filter through filter paper, dry, and weigh as described for the asphaltene fraction.

Determination of resins—Remove the crucible containing the mixture of resins and alumina from the extraction chamber and mount on a filter funnel. Elute resins by alternately stirring with 10- to 20-ml. portions of a methanol - benzene mixture (10 + 90) and withdrawing liquid by suction. Distil the resin extract to a small volume, centrifuge to remove fine particles of alumina, filter into a tared boiling flask, dry, and weigh as before.

The determination of all three fractions can be completed in one 8-hr. day after the sample has been dispersed in *n*-pentane and allowed to stand overnight.

Results—Table I, based on 41 analyses of 16 asphalts, indicates the accuracy of the method. The deviations refer to those from the mean for each asphalt and are given in grams per 100 g. of sample.

Comparison of the new method with that of Strieter (*J. Res. Nat. Bur. Standards*, 1941, **26**, 415–418) is given for 6 coating asphalts. The average yields of asphaltene, oil, and resin fractions by the present method (referred to as the Bureau of Mines method) were 0·6 per cent. higher, 1·9 per cent. lower, and 1·2 per cent. higher, respectively, than those of the corresponding fractions determined by the Strieter method.

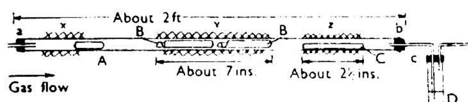
TABLE I

	Asphaltenes	Oils	Resins
Mean deviation	0·19	0·40	0·43
Maximum deviation	0·6	1·3	1·2
Percentage of samples with deviations greater than 0·50%	2	20	32

W. C. WAKE

Estimation of Chlorine in Polymeric Materials. W. M. Phillips (*Plastics*, 1948, **12**, 587–589)—The method described is a simple combustion not requiring the use of special apparatus. Results are quoted for polychloroprene, polyvinylchloride, and a copolymer of the latter with vinylidene chloride.

Apparatus—The apparatus is shown in the diagram and consists of a hard glass tube of internal diameter $\frac{3}{8}$ in. covered with rolls of wire gauze at X, Y, and Z. Oxygen is passed into the tube from the end a and the sample is contained in the platinum boat A placed at a short distance from Z-sectioned platinum contacts B, which are heated with a long burner over the length of the wire gauze. The long, porcelain boat, C, contains halogen-free barium carbonate and is heated by a burner. Rubber bungs are used for the connections at a, b, and c. A guard bubbler containing acidified silver nitrate solution is provided at D. Should the solution in this become turbid during combustion the combustion must be abandoned since it shows that the hydrochloric acid formed has not all been absorbed by the barium carbonate.



Procedure—Support the combustion tube at both ends by retort stands, insert the platinum contacts after re-etching in warm dilute *aqua regia*, fill the long boat, C, level with halogen-free barium carbonate and insert through the end b. Pass oxygen at 11 to 12 ml. per min., and heat the tube (surrounded by wire gauze) to about 600° C. at positions Y and Z. While the tube is heating, weigh out about 20 mg. of sample and then disconnect momentarily at a and insert the sample. Burn slowly so that complete combustion takes about 1 hr. After combustion is complete cool the tube in a stream of oxygen. When cool, withdraw the boat of barium carbonate and suspend its contents in water in a porcelain dish. Titrate the barium chloride formed with 0·025 *N* silver nitrate in the presence of a few millilitres of starch solution and dichlorofluorescein indicator.

Results obtained—Re-crystallised *o*-chlorobenzoic acid was used as a standard reference compound. The results obtained with polymeric materials are compared in the table with those expected and with some obtained by the Carius method. A micro-balance was used in earlier work, but later results were obtained with an aperiodic balance of the usual sensitivity.

Polymer	Weight of sample	Chlorine, per cent.			
		Individual	Mean	Expected	Carius method
Polychloroprene	20 mg.	40·34; 40·33	40·34	40·05	—
Polyvinylchloride	20 mg.	55·98; 55·65; 55·61; 55·63	55·64	56·73	55·9
Vinylchloride-vinylidenechloride copolymer	15 mg.	65·42; 65·30; 65·71; 66·12	65·64	64·93	65·3

W. C. Wake

Carborundum Boiling Stones in Micro-Kjeldahl and Other Digestion Procedures. D. Fraser and R. S. Baker (*Anal. Chem.*, 1948, **20**, 1124)—Carborundum has the advantages that it is non-porous, indestructible, and heavy enough to settle readily even when finely-divided, and it is quantitatively removed in decanting the supernatant liquid. No nitrogen-error is incurred by its use and digestion is more efficient than with glass beads. Twenty- to 50-mesh particles are most satisfactory; they should be washed several times by boiling with distilled water, and oven-dried before storing ready for use. M. E. Dalziel

Selenium as Catalyst in Kjeldahl Digestions. S. M. Patel and A. Sreenivasan (*Anal. Chem.*, 1948, **20**, 63–65)—A summary of the published observations on the use of selenium as a catalyst in Kjeldahl digestions is given, and the results of a study of the effect of time of digestion on recovery of nitrogen are presented. The standard apparatus and procedure were used. Samples containing 10 mg. of nitrogen were treated with 20 ml. of concentrated sulphuric acid, 6 g. of potassium sulphate, and 0·2 g. of crystalline copper sulphate. As catalyst, 50 mg. of selenium were added and, in some experiments, 0·5 g. of mercuric oxide was added in addition to the selenium. Mercury-ammonium complexes were destroyed by adding 0·5 g. of sodium thiosulphate before distillation.

With ammonium sulphate and using selenium, the recovery of nitrogen was 99·7 per cent. after 10 min. digestion and was reduced to less than 95 per cent. by 3 hr. digestion. With mercuric oxide and selenium present, recovery was complete after 20 min. digestion, and 98·6 per cent. after 3 hr., the slower rate of loss of nitrogen being due to the formation of mercuric-ammonia complexes. No loss of nitrogen occurred when neither of the catalysts was added. In the digestion of casein in absence of selenium, the liquid became clear after 40 min.; recovery of nitrogen was complete only after another hour's digestion and was slightly low if the digestion was further prolonged. In presence of selenium, the liquid was clear after 10 min., recovery was complete after another 15 min., and fell to 95·6 per cent. when the time of digestion after the liquid cleared was 6 hr. When mercuric oxide was present the effect of over-long digestion was not so great. Loss of nitrogen caused by the selenium takes place only when conversion of nitrogen to ammonium sulphate is complete.

Recovery of nitrogen from nicotinic acid and from quinoline was not complete when the time of

digestion after the liquid became clear was 6 hr. Recovery of nitrogen increased with digestion time, but after 6 hr. the liquids began to bump to an undesirable extent and the digestion could not be continued. Only when both selenium and mercuric oxide were present was the recovery of nitrogen over 98 per cent. after 6 hr. digestion.

The time of after-boiling required for obtaining theoretical yields of nitrogen must be standardised for each type of nitrogenous organic matter, and selenium cannot be recommended as a general reagent for Kjeldahl determinations.

B. ATKINSON

Detection and Estimation of Micro-quantities of Cyanide. A. O. Gettler and L. Goldbaum (*Anal. Chem.*, 1947, **19**, 270–271)—The sensitivity of the Prussian blue test, which is specific for cyanide, is enhanced by conducting hydrogen cyanide through a piece of filter paper impregnated with ferrous sulphate and sodium hydroxide.

METHOD—The exit tube from the reaction vessel is in two parts, the adjacent ends of which are flanged, ground, and lugged, to enable a disc of test paper to be held across the tube firmly when the lugs are held together by rubber bands. The paper is prepared by dipping a sheet of Whatman No. 50 smooth glazed, acid- and alkali-treated paper for 5 min. in a filtered, 10 per cent. solution of hydrated ferrous sulphate, drying it in air, dipping it in 20 per cent. sodium hydroxide solution, and again drying in air.

Procedure—Place 2 ml. of test solution or 2 g. of finely-macerated tissue in a 50-ml. aeration tube with 3 ml. of water. Acidify with dilute sulphuric acid, or with 20 per cent. trichloroacetic acid solution for blood samples. Connect one end of the assembled exit tube to the aeration tube and the other end to an aspirator. Place the aeration tube in water at 90° C., submerged so that the liquid levels are the same, and apply suction at the maximum rate for 5 min. Remove the test paper and place it in diluted hydrochloric acid (1 + 4) to remove iron hydroxides, rinse with water, and dry. The intensity of the blue stain is proportional to the cyanide concentration; the limit of sensitivity is 0·1 μg. of hydrogen cyanide.

The quantity of cyanide is estimated by comparison with standard stains. For best results, the diameter of the flange orifice should be adjusted according to the amount of cyanide, *i.e.*, 4-mm. diameter for 0·2 to 1 μg., 10-mm. diameter for 1 to 5 μg., and 15-mm. diameter for 5 to 20 μg. Results are correct to within 0·1 μg. on quantities up to 1 μg., and to within 1 μg. on quantities between 1 and 5 μg.

To detect nitrogen in organic compounds, embed a small crystal of the compound in a small piece of metallic sodium and fuse in a piece of Pyrex tubing sealed at one end. Plunge, while red hot, into 2 ml. of water and treat the solution as above.

M. E. DALZIEL

Inorganic

Determination of Hydrogen Chloride in Presence of Chlorine. H. N. Barnam and T. R. Thomson (*Anal. Chem.*, 1948, **20**, 60–61)—From an aqueous solution containing hydrochloric acid and chlorine the chlorine is removed by boiling the solution under refluxing conditions and the hydrochloric acid is then titrated with standard alkali. With the ordinary type of reflux condenser removal of chlorine is slow, but with the apparatus

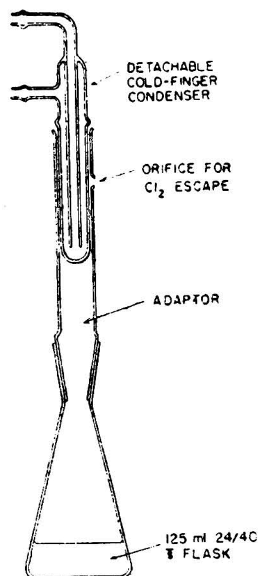


Fig. 1. Recommended boiling apparatus

illustrated removal of chlorine is complete after boiling for 15 min. Analyses of 0 to 1 *N* solutions of hydrochloric acid containing dissolved chlorine showed that all the results for normalities above 0·008 were high by about 0·0007 and the error was higher for solutions containing less acid. Standard hydrochloric acid should therefore be added to raise the normality above 0·1 before the chlorine is boiled out.

B. ATKINSON

Electrometric Titration of Nitric Acid in Oleum. C. D. McKinney (jun.), W. H. Rogers, and W. H. McNabb (*Anal. Chem.*, 1947, **19**, 1041)—An electrometric method is described for the determination of the amount of nitric acid added to oleum as an anti-freeze. Bowman and Scott (*Ind. Eng. Chem.*, 1915, **7**, 766) recommend titration of the nitric acid with a standard solution of ferrous sulphate, in which the first appearance of the red-brown colour of the ferrous nitrosyl sulphate is taken as the end-point of the titration. The end-point is easily obscured if the oleum is dark in colour, as in the reclaimed sulphuric acid used as drip acid for absorption of sulphur trioxide in the manufacture of oleum.

A previous report by Treadwell and Vontobel (*Helv. Chim. Acta*, 1937, **20**, 573) on the electrometric titration of nitric acid required the use of a potentiometer and a specially prepared electrode

and titration cell. In the present work the Serfass electron-ray titration apparatus (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 536) fitted with a platinum-tungsten electrode combination was used. This has the advantage of convenience, elimination of interference of the dark colour with the end-point, and elimination of the empirical blank correction of about 0·2 ml. Any similar assembly should serve equally well.

Procedure—To prepare the ferrous sulphate solution (approximately 0·6 *N*) dissolve 176·5 g. of ferrous sulphate heptahydrate in about 400 ml. of water, add 500 ml. of 60 per cent. sulphuric acid, dilute to 1 litre, and standardise the solution against 0·5 *N* potassium dichromate in presence of 10 ml. of 18 *N* sulphuric acid in the manner described (*infra*) for determination of nitric acid.

Weigh a sample of oleum containing about 0·4 g. of nitric acid from a Lunge pipette into a 250-ml. beaker containing 100 ml. of concentrated (95 per cent.) nitric-free sulphuric acid. Turn the sensitivity control of the titration unit to position eight and set the polarisation control in the off position. Place the electrodes and the mechanical stirrer in the beaker and mix the solution thoroughly. Adjust the cathode-ray tube to the closed position by means of the "eye control." Titrate with the ferrous sulphate solution; addition of the first few millilitres will cause the "eye" to open momentarily but further slow addition will bring it back to the closed position. Continue the titration until the eye remains open for 30 sec.

Determine the nitrosyl sulphuric acid by titration with standard potassium permanganate. Weigh a 10-g. sample of oleum into a 100-ml. volumetric flask containing about 20 ml. of 95 per cent. sulphuric acid, dilute to the mark with sulphuric acid, and then transfer an aliquot (25 ml.) to a 250-ml. beaker containing water and a large piece of ice. Titrate the solution with standard potassium permanganate to an end-point persisting for 1 min.

When the procedure was applied to two samples of oleum that had been analysed by the familiar nitrometer method, it was noted that the electrometric end-point preceded the visual end-point by 0·12 ml., thereby indicating that this excess of ferrous sulphate solution is needed to produce a detectable colour change. No correction is necessary in calculating the results of the electrometric method.

The ferrous sulphate titration of nitric acid in oleum measures only the actual nitric acid content of the sample, whereas the nitrometer method measures the nitrosyl sulphuric acid also. The nitrosyl sulphuric acid occurs owing to reaction between oxides of nitrogen and oleum. To place the two methods on the same basis therefore, it was necessary to determine the nitrosyl sulphuric acid content of the oleum samples and to correct the results of the ferrous sulphate titration.

Concordant results were obtained by this method over a wide range of sample size and the values obtained agreed well with those of the nitrometer method. Although the method has been applied only to oleum in this investigation, it can be applied equally well to inorganic and organic nitrates.

A. O. JONES

Rapid Oxidimetric Procedure for Determining Nitrate. W. Leithe (*Mikrochem.*, 1947, **33**, 48-50)—Ferrous sulphate is oxidised to the ferric state by nitric acid on boiling in a solution containing about 65 per cent. of sulphuric acid by weight. The excess of ferrous iron is determined with potassium dichromate or permanganate.

For large amounts of nitrate—Dissolve 1 g. of the nitrate-containing material in water and dilute accurately in a 500-ml. volumetric flask. Pipette 25 ml. into a 250-ml. Erlenmeyer flask. Pipette 25 ml. of ferrous sulphate solution (55 g. of the heptahydrate dissolved in 100 ml. of water containing a few drops of sulphuric acid, and diluted to 1 litre with 50 weight-per cent. sulphuric acid of sp.gr. 1·40) into the flask, followed by 25 ml. of concentrated sulphuric acid, and 3 small boiling beads, and heat to boiling for 3 min. The dark brown solution becomes light yellow. Cool the solution in water, add 3 to 5 ml. of 70 to 80 per cent. phosphoric acid solution and 50 ml. of water, and cool again. Add 2 drops of 0·025 *M* ferroin solution and titrate with 0·1 *N* potassium dichromate to the appearance of a blue-green colour, or titrate with 0·1 *N* potassium permanganate without an indicator. Carry out a blank determination.

For small quantities, 0·03 to 3 mg., of nitrate ion—Into a 100-ml. Erlenmeyer flask pipette 5 ml. of 0·02 *N* ferrous sulphate (5·5 g. of heptahydrate dissolved in 20 ml. of water and diluted to 1 litre with 50 per cent. sulphuric acid) and 5 ml. of concentrated sulphuric acid. Remove the air by adding about 0·2 g. of sodium or potassium hydrogen carbonate and add the sample dissolved in 5 ml. of water. Mix the solution and boil it gently for 3 min. in presence of beads to prevent bumping; then add 1 ml. of phosphoric acid and 5 ml. of water and cool the solution. Titrate with 0·01 *N* potassium dichromate, using 0·0025 *M* ferroin as indicator.

Ammonium salts, chloride, and ferric iron do not interfere with the method, but nitrite interferes with the back-titration.

M. E. DALZIEL

Accumulation of Traces of Arsenate by Co-precipitation with Magnesium Ammonium Phosphate. [Determination of Small Amounts of Arsenic in Steel.] I. M. Kolthoff and C. W. Carr (*Anal. Chem.*, 1948, **20**, 728-730)—A systematic study to determine the best conditions for the quantitative co-precipitation of arsenate has been made.

Procedure—Add to the arsenic solution sufficient bromine water to oxidise all the arsenic present and produce a yellow colour. Add sufficient mono-potassium phosphate to provide 1 mg. of P_2O_5 per ml. of solution, add 1 ml. of hydrochloric acid, and 10 ml. of magnesia mixture (50 g. of magnesium chloride hexahydrate and 100 g. of water). Neutralise with aqueous ammonia solution and, when most of the precipitate has formed, add 5 ml. of concentrated aqueous ammonia solution in excess. Filter the solution after 2 to 4 hr., wash the precipitate with diluted aqueous ammonia solution (1 + 19) and determine the arsenate in the precipitate by a suitable volumetric procedure

(*Volumetric Analysis*, I. M. Kolthoff and N. H. Furman, Vol. 2, p. 410, 1929).

A blank determination should be made as a check on the reagents.

In 500 ml. of solution, 0·075 mg. of arsenic can be determined to within 2 per cent.

Determination of arsenic in steel—Treat a sample containing not less than 0·1 mg. of arsenic in a 250-ml. Erlenmeyer flask with 25 ml. of 6 *N* nitric acid and a small excess of bromine water, boil for a few minutes, and cool the solution. Add 10 g. of tartaric acid for each gram of steel taken, 0·2 g. of potassium dihydrogen phosphate, and 50 ml. (a ten-fold excess) of magnesia mixture. Transfer to a 250-ml. bottle and add, at least, a 10-ml. excess of concentrated aqueous ammonia solution. Shake for 4 hr., filter off the precipitate, and dissolve it in 3 *N* hydrochloric acid. Add 1 g. of tartaric acid, 10 ml. of magnesia mixture, and an excess of aqueous ammonia solution. Shake the vessel for 2 hr., filter, and wash the precipitate with diluted aqueous ammonia solution (1 + 19). Re-dissolve the precipitate in 3 *N* hydrochloric acid and determine the arsenate as usual.

For amounts of the order of 0·1 mg. of arsenic, use a micro-burette and 0·005 *N* iodine.

On a sample containing 0·012 per cent. of arsenic, the eight values obtained ranged from 0·011 to 0·014 per cent. On samples containing less than 0·01 per cent. of arsenic, the blank corrections are too large for the method to be satisfactory.

A similar method can be used for determining small amounts of arsenic in the presence of antimony, tin, aluminium, or zinc, the precipitation of these metals by ammonia being prevented by adding tartaric acid; with antimony, a re-precipitation may be necessary. M. E. Dalziel

Preparation of Phosphomolybdic Acid from Phosphoric Acid and Molybdic Trioxide. T. J. Hastings, jun. and H. A. Frediani (*Anal. Chem.*, 1948, **20**, 382–383)—The method of preparation suggested by Linz (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 459) uses only water as solvent and is convenient for preparing commercial quantities of the acid. Reaction between 1 g.-mol. of molybdic trioxide and 0·083, 0·1, or 0·166 g.-mol. of phosphoric acid, filtration, and crystallisation gave initial products containing phosphorus : molybdenum ratios 1 : 10·11, 1 : 8·74, and 1 : 6·15 respectively, but on extraction of their aqueous solutions with ether, addition of water and a small amount of nitric acid, and crystallisation all gave good yields of an acid containing one phosphorus to 11·93 ± 0·08 molybdenum atoms.

When amounts of tungstic trioxide and phosphoric acid calculated to yield the 1 : 12 acid were treated by the Linz method no 1 : 12 acid was obtained; a ten-fold excess of phosphoric acid also failed to give this acid. Freshly-precipitated tungstic acid was not tried. M. E. Dalziel

Iodine Monochloride End-point in Titration of Tripositive Antimony. Titration with Iodate, Permanganate, and Ceric Solutions. E. W. Hammock, R. A. Brown, and E. H. Swift (*Anal. Chem.*, 1948, **20**, 1048–1050)—Accurate results are obtained only in a narrow range of hydrochloric acid concentrations.

Iodate solutions—At acid concentrations below 2 *M*, iodate and iodine monochloride are fairly rapidly reduced by tervalent antimony to iodine, which is only slowly oxidised to iodine monochloride by iodate. Above 4 *M*, reduction is slow, and at 5 *M*, it is incomplete. A concentration between 2·5 and 3·5 *M* is recommended; increase of either hydrogen ion or chloride ion concentration results in a decreased rate of oxidation of the antimony.

Permanganate solutions—When the iodine monochloride end-point is used, the concentration of hydrochloric acid must be not greater than 3 *M* indicating that permanganate has a greater tendency than iodate to oxidise iodine selectively at high acid concentrations. Moderate variations in iodine monochloride and antimony concentrations do not cause marked changes in titration characteristics.

Ceric sulphate solutions—Precipitates are liable to be formed in solutions less acid than 2 *M*. Below 3 *M*, the tendency is to over-run the end-point because of the slow oxidation of the iodine. Above 3 *M*, the slow oxidation of the antimony tends to cause premature end-points. Thus the iodine monochloride end-point is not recommended with ceric solutions. In absence of iodine monochloride, antimony is more quickly oxidised by ceric ions in 6 *M* than in 2 *M* hydrochloric acid, so it appears that iodine is preferentially oxidised by ceric ions and the slow oxidation of antimony by iodine monochloride is the limiting factor.

Titrations were carried out (1) with permanganate, essentially according to McNabb and Wagner (*Ind. Eng. Chem., Anal. Ed.*, 1930, **2**, 251), but in 2 *M* acid and below 5° C.; (2) with ceric sulphate in 3 to 3·5 *M* acid, using methyl orange as indicator; (3) with iodate in 2·5 to 3 *M* acid; and (4) with permanganate, using the iodine monochloride end-point in 2·5 to 3·5 *M* acid. All values agreed to within 1 part in 1000. M. E. Dalziel

Determination of the Gases in Meteoritic and Terrestial Irons and Steels. L. K. Nash and G. P. Baxter (*J. Amer. Chem. Soc.*, 1947, **69**, 2534–2544)—The methods available for the determination of gases in steels and meteorites are reviewed. Thermal methods of extraction, such as fusion *in vacuo*, are considered to be inadmissable owing to reactions at high temperatures between constituents of the evolved gases and between the gases and the sample. Dissolution of the sample in aqueous solutions of salts overcomes this difficulty and tests have been carried out to investigate the relative merits of solutions of potassium cupric chloride, iodine, and mercuric chloride for this purpose.

Gas-free steels prepared by fusion *in vacuo* were used to determine the blanks caused by the reagents, and the recovery of synthetic gas mixtures maintained in contact with the reagent solutions was also studied. The use of hot solutions was found always to be undesirable, the evolution of gases from side-reactions being considerable. Subsequent

tests were carried out with cold solutions, using mechanical shaking to increase the rate of dissolution of the sample.

Potassium cupric chloride—Considerable amounts of carbon dioxide were obtained in blank tests. The recovery of carbon monoxide was incomplete owing to retention by cuprous salts; the recovery of nitrogen and hydrogen was satisfactory.

Iodine—The amounts of carbon monoxide and carbon dioxide evolved in blank tests were very much greater than for the other two reagents.

Mercuric chloride—The recovery of synthetic gas mixtures was satisfactory. The carbon monoxide and carbon dioxide blanks were small except with steels of high carbide contents. The evolution of blank gases continued after dissolution of the sample was complete, and this secondary evolution was related to the primary evolution of blank gases during dissolution of the sample. Hence by analysis of the gases evolved during the secondary evolution, a correction could be deduced for the gases evolved from side-reactions during a determination.

Mercuric chloride is the most satisfactory reagent, but potassium cupric chloride may be used if results for nitrogen and hydrogen only are required. The following general procedure is recommended.

Procedure—The reaction is carried out in a flask fitted with a stop-cock and a short condenser, and having a sealed side-arm to contain the sample. Load the sample into the side-arm, and transfer the reagent solution to the flask, using a volume of about 250 ml. containing sufficient reagent to give a 20 per cent. excess. Boil the solution gently under slight suction to remove air, close the stop-cock, and freeze the reagent solution to minimise the vigour of the initial reaction. Tip in the sample, remove the flask from the freezing bath, and shake it mechanically until dissolution is complete. Draw off and analyse the evolved gas. If mercuric chloride solution has been used, continue the shaking, and apply a correction based on the composition of the secondary evolution.

H. J. CLULEY

Identification of Free Silica in Dusts and Fumes. W. H. Gitzen (*Anal. Chem.*, 1948, **20**, 265–267)—Free silica can be detected in dusts or fumes by observing in the electron microscope the effect of exposing samples to hydrofluoric acid vapour. The method has been used for detecting silica in fumes occurring in the production of fused alumina from siliceous materials.

The dust sample is prepared for examination by dispersion in a resin solution (*e.g.*, Formvar), which is spread into a film by pouring it on to glass or water. Alternatively, the sample is prepared during collection by lining a small electrostatic precipitator with a nitrocellulose film supported on a screen.

After preliminary examination under the electron microscope, the sample film is exposed for 1 to 5 min. to the vapour from 48 per cent. hydrofluoric acid solution at room temperature. The fine particles of silica are completely vaporised as silicon tetrafluoride and leave in the film voids that are readily observed on re-examination under the electron microscope. Silicates are indicated by voids containing some unvaporised material. Alumina is unaffected by the treatment.

H. J. CLULEY

Inorganic Chromatography. L. Sacconi (*Gazz. Chim. Ital.*, 1948, **78**, 583–591)—The hypothesis has been advanced that the adsorption of metallic ions on a column of alumina is determined by the alkalinity of the alumina.

To test this, trials have been made with two types of alumina, the first being technical alumina activated by heating to 600° C. for 10 hr., and the second being prepared by oxidation of amalgamated aluminium cuttings in moist air and subsequent heating of the oxide thus formed to 650° C. for 10 hr. Thus prepared, the alumina contained no trace of sodium and its suspension in water had pH 5·4. An aqueous suspension of technical alumina has pH about 9·4.

Columns prepared with these two types of alumina were tested with solutions of mercuric nitrate and of thallic chloride. The coloured zones obtained, yellow for mercury and brown for thallium, were similar with each alumina specimen.

Suspensions of alumina were made by shaking with water in test tubes. These were allowed to settle and a solution of mercuric chloride was added cautiously. In the tube containing technical alumina, a pale yellow precipitate was formed in the clear liquid and, on shaking and allowing to settle again, the alumina under the stratum of yellow assumed a reddish colour. With the alumina from amalgamated metal no precipitate was formed in the clear liquid but, on shaking, the alumina assumed a colour varying from ochre to reddish. Similar results to these latter ones were obtained with technical alumina that had been subjected to prolonged washing with water.

It is concluded therefore that whilst the adsorption of cations is due to the formation of basic compounds this is caused not by the alkalinity of the liquid but by a reaction at the surface of the alumina itself.

Experimental—The columns employed were 3 mm. in diameter and 10 cm. long, and were filled with technical alumina activated as described and sifted through sieves of 625 to 1600 apertures per square centimetre. The cations separated in the following order:—

$$
\begin{array}{c}
Ti^{\cdot\cdot\cdot\cdot} \\
U^{\cdot\cdot\cdot\cdot} \\
As^{\cdot\cdot\cdot},\ Sb^{\cdot\cdot\cdot},\ Bi^{\cdot\cdot\cdot},\ Cr^{\cdot\cdot\cdot},\ Hg^{\cdot\cdot},\ UO_2^{\cdot\cdot},\ Pb^{\cdot\cdot}, \\
Fe^{\cdot\cdot\cdot} \\
Tl^{\cdot\cdot\cdot} \\
Ce^{\cdot\cdot\cdot}
\end{array}
$$

$$
\begin{array}{c}
Ni^{\cdot\cdot} \\
Co^{\cdot\cdot} \\
Cu^{\cdot\cdot},\ Ag^{\cdot},\ Zn^{\cdot\cdot},\ Fe^{\cdot\cdot},\ Tl^{\cdot},\ Mn^{\cdot\cdot\cdot} \\
Cd^{\cdot\cdot}
\end{array}
$$

The separation and identification of $Ti^{\cdot\cdot\cdot\cdot}$, $U^{\cdot\cdot\cdot\cdot}$, $Ce^{\cdot\cdot\cdot}$, and $Tl^{\cdot\cdot\cdot}$ was studied employing various developers. Titanium was distinguished from bismuth, silver, thallium, mercury, and copper by development with an alcoholic solution of benzoyl acetone slightly acidified with hydrochloric acid;

this gave a yellow-orange colour to the titanium band. To distinguish from iron, a neutral solution of the ketone was employed followed by diluted hydrochloric acid (1 in 20). The red-brown band at first produced descended the column leaving a yellow band in its place, the titanium complex being stable to acids while the iron reacts. The separation from chromium, thallium, and lead was imperfect.

For uranium, potassium ferricyanide was employed and gave a red-brown colour. Good separation was not obtained from arsenic or chromium. For thallium, the separation was generally imperfect. Ammoniacal potassium chromate gave a brown colour and dithizone in 4 per cent. sodium hydroxide solution, a cherry red.

With cerium, a solution of benzidine in acetic acid gave an intense blue colour.

A. H. BENNETT

Physical Methods, Apparatus, etc.

Micro-effusiometry. L. K. Nash (*Anal. Chem.*, 1948, **20**, 258–262)—The apparatus described is for determining the molecular weight of 0·5 ml. of a permanent gas or 0·5 mg. of a volatile liquid.

Apparatus for gases—The manometer M (Fig. 1), containing distilled mercury, is 6 to 7 cm. high and is made from 24-mm. tubing. The bore of the tap S is at least 2 mm. in diameter. The right branch of

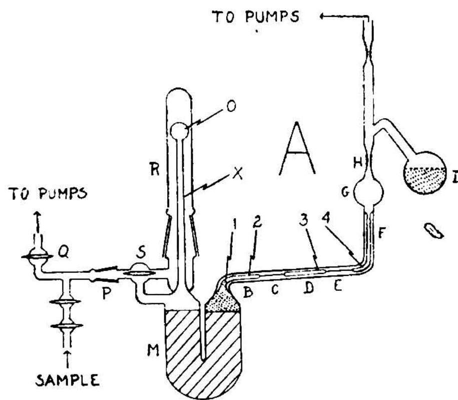


Fig. 1.

the manometer is sealed to a length of capillary tubing. Sections B and D are 3-cm. lengths of 2-mm. tubing, sections C and E are 2-cm. lengths of 0·5-mm. capillary, and section F is a 6-cm. length of 2-mm. capillary. The bulb I contains the auxiliary manometer liquid, tetralin. The bulb O is prepared by blowing a thin-walled bulb, 0·01 to 0·02 mm. in thickness and 12 mm. in diameter, on the end of a length of 6-mm. tubing. A small orifice in the bulb is made by placing an earthed copper wire in the tube and a Tesla coil leak tester on the outside. The voltage of the discharge is increased until the discharge strikes through the bulb. The bulb is sealed to the apparatus at point X.

Standardisation—Evacuate both sides of the manometer. Distil tetralin from I into the right branch of the manometer until the tetralin level is close to point 1. Close the tap Q and turn S to connect P to M. Introduce about 0·5 ml. of dry nitrogen. The tetralin level should reach the region of point 4. Turn S to connect the outer space R to P and open Q. Measure the time taken for the tetralin meniscus to move from point 3 to point 2. Alter the amount of tetralin in the manometer until the time taken is 6 to 8 min. and then seal the right arm of the manometer at H. Repeat the introduction of nitrogen and measurement of the time for effusion until concordant results are obtained.

Procedure—Introduce about 0·5 ml. of the gas into the space over the left branch of the manometer, turn S to connect P to outer space R, open Q, and measure the time for effusion as above. Repeat several times. From the time of effusion for nitrogen, and using Graham's law, calculate the molecular weight of the gas.

The average error of a series of molecular weight determinations was 1 per cent.

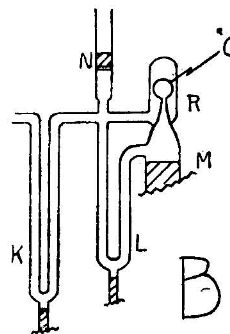


Fig. 2.

Apparatus for volatile organic liquids—This is illustrated in Fig. 2. The taps are replaced by mercury cut-offs and the joint in the outer jacket is eliminated. Introduce the liquid to the evacuated apparatus by touching the tip of a capillary pipette containing the sample against the surface of the fritted disc N, which is sealed against the atmosphere by a small quantity of mercury. Raise the mercury in the U-tube L, lower the mercury in K, and proceed as above. Benzene is a suitable liquid for use as a standard. The error of the estimation is usually not greater than 3 per cent.

B. ATKINSON

Improved Horizontal Micro-burette. A. Lacourt (*Metallurgia*, 1948, **38**, 355–356)—To ensure a regular, slow, reproducible rate of flow with micro-burettes made from tubing up to 3·5-mm. bore, a very fine tip is necessary and this introduces difficulty in filling and cleaning and causes a variation in flow due to change in position.

Introduction of a 7- to 8-cm. vertical ascending capillary tube between the horizontal tube and the descending one gives a reproducible flow without the necessity for a very narrow tip, and the flow ceases when contact of the tip with the liquid or glass surface is broken. Mount the micro-burette on a board bearing a paper millimeter scale, and

calibrate it by using either bi-iodate and thiosulphate or the solutions to be used subsequently. Clean the apparatus with water, ethanol, or dilute nitric acid, but not sulphuric acid or sulphuric-chromic acid, and allow it to dry before use; the apparatus must be cleaned and dried immediately after use.

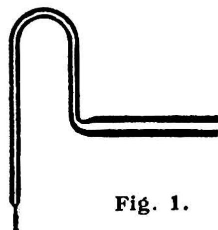


Fig. 1.

Fig. 2.

Fig. 1.—Construction of modified burette.
Fig. 2.—Meniscus in wide-bore tubing.

With instruments of wider bore, the meniscus takes a somewhat different form from that usually obtained, but good reproducibility is attained when the level is taken as shown in Fig. 2.

M. E. DALZIEL

Miscellaneous Microchemical Devices. XV. Device for Testing Gases. J. T. Stock and M. A. Fill (*Metallurgia*, 1948, **38**, 356)—The device is constructed from 2-mm. bore glass tubing, the guide-arm, A, being of glass rod and able to slide easily into the cap C. A strip of filter paper, D, impregnated with a suitable reagent is gripped between the gas-tube B and C, being held firmly in place by a rubber band, E. Gas penetrating the test paper escapes round A. Proper contact between the gripping tube-ends is ensured by rotating a small copper disc bearing carborundum

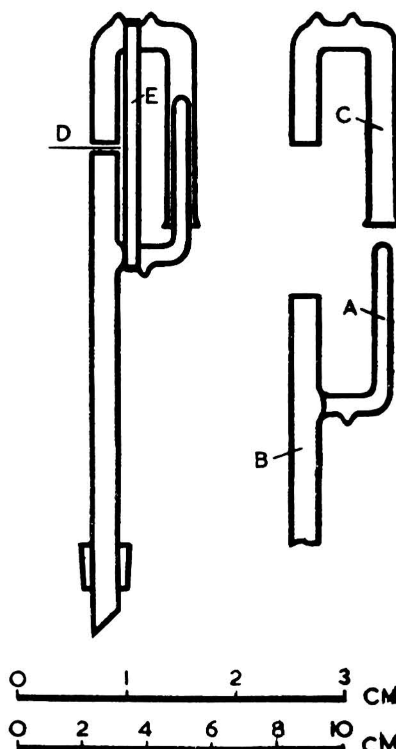


Fig. 1. Gas-testing apparatus (upper scale, micro version; lower scale, larger version)

on both sides between the ends, after they have been cut as cleanly and squarely as possible.

M. E. DALZIEL

Reviews

PRACTICAL PUBLIC HEALTH PROBLEMS. By SIR WILLIAM SAVAGE, B.Sc., M.D. Second Edition. Pp. vii + 197. London: J. A. Churchill Ltd. 1949. Price 14s. net.

This well known book, the first edition of which was reviewed in *The Analyst* in 1940, deals with many of the problems of disease prevention which confront the Medical Officer of Health and the Sanitary Inspector. The first five chapters are devoted to water, sewage and effluents, including the disposal of effluents containing milk or whey, a special problem in Somerset. Then follow four valuable chapters on the bacteriological control of milk supplies and on the supervision and control of milk pasteurisation. The author's analysis of the available statistics on milk-borne tuberculosis amply justifies his description of the present position as "deplorable."

The chapter on the Food and Drugs Act, 1938, which appeared in the first edition, has been replaced by one on hygienic food control, which deals, *inter alia*, with slaughter houses, unsound food, ice-cream and communal eating places. The author lists the hygienic requirements that he regards as essential for restaurants, etc., and expresses the hope that the time will come when only those who have attended a suitable course will be eligible for employment in catering establishments.

A chapter on field investigation of food poisoning summarises the latest knowledge on this subject, and is full of valuable hints based on personal experience of the difficulties associated with these inquiries.

It is surprising that the inspection of canned foods, a subject to which the author has made such notable contributions in the past, is allotted only eight pages, but in his view the industry is now so scientifically organised that the amount of spoilage is small and the danger to health trifling.

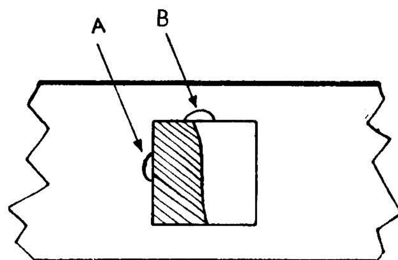
Other questions dealt with are the investigation of enteric and infectious disease outbreaks, housing inspection and disinfection. One activity that closely concerns the Medical Officer of Health—the control of household vermin—is not touched upon: some account of modern practice in this field would be welcome in future editions.

Sir William Savage expresses decided opinions on many matters, including such controversial points as the replacement of chemical by bacteriological analysis in water examination. His views may not always be accepted without question, but they represent the conclusions of long practical experience as County Medical Officer of Health. The book is of great value as a concise, interesting and informative survey by one of our foremost authorities on public health administration. G. W. MONIER-WILLIAMS

MIKRO-METHODEN ZUR KENNZEICHNUNG ORGANISCHE STOFFE UND STOFFGEMISCHE. By LUDWIG KOFLER and ADELHEID KOFLER. Pp. viii + 337. Innsbruck: Universitätsverlag Wagner Ges.M.B.H. 1948. Price about 36s.

Instruction on how to identify organic "spots" formerly insisted that the student should first ascertain the characteristic elements present, then study the reactions of the substance to find groups of elements; if the substance was solid, the melting-point could then be determined and suitable derivatives prepared to clinch the identification. It must be admitted that many students were tempted and succumbed: they determined the melting-point FIRST and short-circuited the official procedure by reference to Clarke's *Handbook of Organic Analysis*. The reviewer has been told by some modern students that it is no longer thought necessary to defer the melting-point determination, but Drs. Kofler and Kofler have carried the tendency further than the most wishful student can have imagined. From a melting-point study, the identification of an unknown solid is almost completed.

The authors determine the melting-points on a microscope hot-stage, two types of which are described. In one the temperature is measured by means of a copper-constantin thermocouple, in the other a thermometer is used and is calibrated by observing compounds having known melting-points. The use of the melting-point as a test of purity is well known and the authors claim that, in general, their method will detect about 0·25 per cent. of impurity, whereas the capillary tube method will not show less than about 2 per cent. The purification of an impure substance, or the isolation of one component from a mixture, can be effected by heating until melting just begins and absorbing the eutectic melt (*e.g.*, on filter paper), repeating the procedure as many times as may be found to be necessary.



After the melting-point of an unknown substance has been determined a special "contact slide" is prepared, on which the unknown is in contact with a selected reference compound. To prepare this slide the higher melting of the two substances is fused and a drop placed at one edge *a* (see Fig.) of a square cover glass so that it runs below the latter. After this has solidified (as shown in Fig.) the melt of the lower melting substance is introduced at *b*. The junction of the two solids is observed through the microscope, using crossed Nicols, while the hot-stage is warmed gradually. If a eutectic is formed, a dark band of liquid is seen between the two slides and the melting-point of the eutectic may be determined. The reference compounds selected for use on the contact slide depend upon the melting-point of the unknown substance; two are used, separately, with each unknown. The complete list of reference compounds is as follows:—

M.p. of unknown	Reference compounds
20° to 100° C.	Azobenzene, benzil
100° to 120°	Benzil, acetanilide
120° to 140°	Acetanilide, phenacetin
140° to 170°	Phenacetin, benzanilide
170° to 190°	Benzanilide, salophene
190° to 340°	Salophene, dicyandiamide

It is obvious that all binary systems do not form simple eutectics, but this point is adequately covered by the authors, who illustrate and describe the usual phase-rule diagrams and reproduce photomicrographs showing the contact boundaries for a number of systems. The contact slide method is developed and it is shown how the micro-thermal analysis of a system is possible: three-component systems are also considered briefly.

Further information is obtainable by determining the molecular weight of the unknown substance, using as solvent bornyl chloride between slide and cover glass, or camphor in a capillary tube on a modified hot-stage. Greater use is made, however, of the estimation of the refractive index of the unknown substance in the liquid state. Glass powders of known refractive indices are immersed in the melt and the temperature is raised until examination under the microscope shows that the refractive indices of the glass and melt are equal. The refractive index range from 1·3400 to 1·6718 is covered by 23 glasses.

The authors have examined over a thousand organic compounds by these methods and have tabulated the results, the compounds being arranged in order of melting-point. The table includes the eutectic temperatures with the reference compounds, the refractive index of the melt and "special characteristics" (*viz.*, mode of crystallisation, occurrence of sublimates, polymorphs, hydrates, liquid crystals, etc.). It

seems to the reviewer that the novel procedures described in this book could form a simple and very rapid means of identifying organic compounds: such a method would be welcome in industrial laboratories. However, it is realised that, in order for the application to be widespread, many more substances must be examined and the results tabulated for reference: L. and A. Kofler have done much work in compiling their table, but this must be extended.

Readers in this country may be disappointed to find that a book of this price should be bound in paper covered boards, only the spine being cloth, but the binding appears to be strong. The text and illustrations are clearly printed on good quality paper. The book is adequately indexed and a separate index is provided for the substances in the table. There are 204 references to the literature. G. H. Wyatt

BIOLOGICAL METHODS GROUP

A Joint Meeting of the Group with the Society for Endocrinology will be held on Thursday, October 20th, 1949, at the Medical Society of London, Chandos Street, Cavendish Square, W.1, from 2.15 p.m. until 7 p.m. The meeting will take the form of a symposium on Hormone Assay, and the Chair will be taken by Professor J. H. Gaddum, F.R.S.

The following aspects of the subject will be presented and discussed:—

(1)	General Approach to Biological Assay	Dr. H. O. Schild.
(2)	Assay of Thyroxine and Thyroidal Activity	Dr. G. F. Somers.
(3)	Assay of Insulin	Mr. K. L. Smith.
(4)	Assay of Gonadotropins	Dr. J. A. Loraine.
(5)	Assay of Posterior Pituitary Extracts	Mr. G. A. Stewart.
(6)	Clinical Methods of Assaying Oestrogens	Dr. P. M. F. Bishop.

Light refreshments will be available at 4 p.m.
Visitors will be welcome.

METHODS OF WATER ANALYSIS

In 1947 a Joint Committee representing the Institution of Water Engineers, the Royal Institute of Chemistry and the Society of Public Analysts and Other Analytical Chemists was set up by the Institution of Water Engineers for the purpose of standardising methods of water analysis.

The Report of this Committee laying down standard methods for the physical and chemical examination of potable waters is shortly to be published in the Journal of the Institution of Water Engineers. Reprints of this Report will be available to members of the Society, price 3s. 6d., post free. Application should be made, with remittance, and not through Trade Agents, to the Secretary, Society of Public Analysts and Other Analytical Chemists, 7–8, Idol Lane, London, E.C.3.

MINISTRY OF SUPPLY (CHEMICAL INSPECTORATE) invites applications from CHEMISTS for appointment in following grades for analytical work offering scope and opportunity for advancement and extended scientific experience:—

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Posts are unestablished and are mainly in N.W. England, with a few in S.E. England. Starting pay will be assessed within the above ranges.

Write, quoting F.542/49A, to Technical and Scientific Register (K), York House, Kingsway, London, W.C.2, for application forms which must be returned by 8th October, 1949.

HIS MAJESTY'S COLONIAL SERVICE, MALAYA

VACANCIES exist for CHEMISTS in the Joint Department of Chemistry, Federation of Malaya and Singapore. The work carried out by the Department includes all chemical work connected with liquors, toddy, opium, petroleum, textiles, etc.; the examination of exhibits related to poisons, counterfeiting, arson, firearms, etc.; toxicological investigations, blood-testing, and clinical analysis; the testing of petroleum-carrying vessels, drinking water and sewage control (chemical and bacteriological); analysis of food and drugs, etc. The posts are on contract for three years in the first instance at a salary of $750 per month (£1,050 per annum) plus expatriation allowance of $150 per month (£210 per annum) plus variable cost of living allowance, which is at present: $150 per month (£210 per annum) for single men, $300 per month (£420 per annum) for married officers without dependent children, $375 per month (£525 per annum) for married officers with dependent children, *i.e.*, total emoluments for a single man amount to £1,470 per annum, for a married man without family £1,680 per annum, for a married man with children £1,785 per annum.

There is a further allowance of $50 per month for officers stationed in Singapore and the contract carries a gratuity of £37 10s. 0d. for each completed period of three months' service.

Government quarters, if available, are at a nominal rent, and free first-class passages to and from Malaya are provided for an officer, his wife and children under 10 years up to four persons in all. A tour of service earns approximately 5 months paid leave in the United Kingdom. Income tax is at local rates which are very much lower than those in the United Kingdom. Candidates should preferably be under 45, and must possess an Honours degree in Chemistry from a British university, together with the Associateship or Fellowship of the Royal Institute of Chemistry. In addition, they should have had at least two years' specialised experience in some line relevant to the work of the Department, or research experience indicated by, *e.g.*, a Ph.D. qualification.

Write, giving brief details of age, qualifications and experience, to the Director of Recruitment (Colonial Service), Sanctuary Buildings, Great Smith Street, London, S.W.1.

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LABORATORY ASSISTANT required by London firm of Analytical Chemists. Must have some experience in assaying base metals, alloys, residues, etc. Young applicant preferred, if male ex H.M. Forces. Write full particulars of age, experience and salary required to Box 3718, THE ANALYST, 47, Gresham Street, London, E.C.2.

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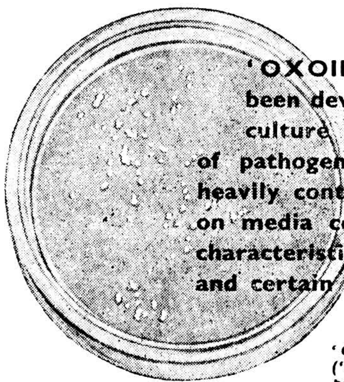

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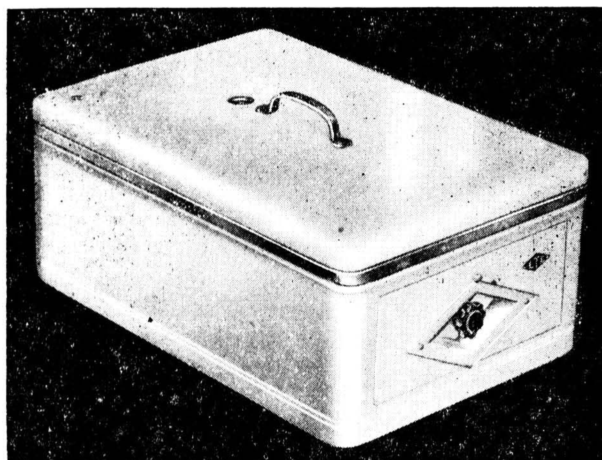

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