FEBRUARY, 1947 Vol. 72, No. 851

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS
AND OTHER ANALYTICAL CHEMISTS

PHYSICAL METHODS GROUP

THE Second Annual General Meeting of the Group was held at 6 p.m. on Tuesday, November 26th, 1946, in the rooms of the Chemical Society, Burlington House, London, W.1. The Group Chairman, Mr. R. C. Chirnside, presided, and 32 members were present. The Committee's Report and the Financial Statement were presented and adopted.

The Officers and Members of Committee for the forthcoming year are as follows. *Chairman*, Mr. R. C. Chirnside. *Vice-Chairman*, Dr. J. G. A. Griffiths. *Honorary Secretary*, Dr. J. E. Page.* *Committee*, Mr. B. S. Cooper, Dr. J. R. Edisbury, Mr. J. Haslam, Dr. S. Judd Lewis, Mr. D. M. Smith, Dr. F. Wokes and, *ex-officio*, the President, Honorary Treasurer and Honorary Secretary of the Society and the Editor of THE ANALYST. Mr. C. A. Bassett and Dr. D. C. Garrett were reappointed Honorary Auditors.

POLAROGRAPHIC DISCUSSION PANEL

The Chairman said that the Group Committee had decided to form a Polarographic Discussion Panel, the objects of which would be to hold and sponsor informal discussions on polarographic analysis. Draft rules of the panel had been approved by the Committee of the Group and the Council of the Society. The following members were elected to serve on the Committee of the Panel: Dr. W. Cule Davies, Mr. J. Haslam and Mr. J. T. Stock† (Honorary Secretary), with Dr. J. E. Page as the representative of the Group Committee.

ORDINARY MEETING

Immediately following the Annual General Meeting, an Ordinary Meeting of the Group was held, at which about eighty members and visitors were present. The following papers on the subject of Polarographic Analysis were presented and discussed: "Amperometric Titrations," by J. T. Stock, M.Sc., F.R.I.C.; "The Rotating Platinum Electrode," by C. J. O. R. Morris, B.Sc., Ph.D.; "The Application of the Cathode Ray Oscillograph to Polarography," by J. E. B. Randles, M.A., B.Sc., and L. Airey, B.Sc., who demonstrated their apparatus.

SCOTTISH SECTION

A MEETING of the Scottish Section was held at the N.B. Station Hotel, Glasgow, on Friday, November 29th, 1946. Mr. James A. Hunter presented a paper on "A Semimicro-Method for the Determination of Magnesium," which he hopes later to submit for publication in THE ANALYST. Dr. C. W. Herd, B.Sc., F.R.I.C., presented "Some Observations on the new Ice Cream Order" as a basis for discussion on the subject of the Order.

DEATHS

WE regret to record the death of

Joseph John Blackie
Robert Selby Morrell

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Obituary

PROFESSOR W. H. ROBERTS

WILLIAM HENRY ROBERTS was born in Liverpool on January 11th, 1877. He was educated at Liverpool College and University College, Liverpool, where he studied under Professor Campbell Brown. Having taken his B.Sc. degree at Victoria University he proceeded to the M.Sc. at Victoria and Liverpool University, and in 1902 he passed the examination for the Associateship of the Institute of Chemistry.

On leaving the University, Roberts became assistant to Campbell Brown, who was not only Professor of Chemistry but also Public Analyst to the County of Lancaster.

On the death of Campbell Brown the County appointment was given to Mr. Collingwood Williams—still with us—who had held the Liverpool City Analyst's post and, as the City was contemplating the building of new City Laboratories, considerable discussion took place as to who was able to undertake satisfactorily the duties of City Analyst to Liverpool. Eventually, despite expressed doubts, Roberts was chosen and he was given as his Deputy the late Mr. E. Gabriel Jones.

Thus on January 1st, 1912, Roberts entered upon his real life's work. One of his first tasks was to plan and equip the new laboratories, which were, and are, the admiration of everyone privileged to inspect them. Those who had doubted the wisdom of the appointment were speedily converted when they saw the amazing administrative ability that the new Analyst possessed.

Roberts soon gathered round him a loyal band of able young chemists, by whom he was affectionately known as "The Chief," an appellation that later generations sometimes expanded to "The Great White Chief." The laboratories were in a new building which housed also the School of Hygiene and the City Bacteriological Laboratories, and qualified medical men reading for the D.P.H. diploma attended lectures there. It was almost inevitable that Roberts should be asked to lecture on Public Health Chemistry, and the University regularised the appointment by making him an Associate Professor.

He was allowed to use the laboratories for Public Analyst work for other towns and boroughs and held the appointments of Public Analyst for the County Boroughs of Barrow-in-Furness, Birkenhead, Blackburn, Bootle, Preston and Southport and the Boroughs of Crosby, Kendal and Widnes. He was also permitted to undertake analytical and consultative work for private firms and individuals, and the assistance that he gave to commercial enterprises still further enhanced his influence and gained a desirable publicity for the laboratories.

In 1917 he served on the Council of the Society and again in 1927 and 1936. In 1934 he was made a Vice-President and in 1938 President. This was the proudest moment in Roberts's life. The writer saw him just after he received the news and can record that this recognition by fellow workers of work well done was, to him, the highest honour which could be conferred on any chemist.

From the time he passed the examination of the Institute of Chemistry, Roberts was one of the Institute's stoutest supporters and fought for the highest standards of professional conduct. Even at the time of his retirement he was planning an attack on what he considered a reflection on the integrity of the profession. From the year 1915, with one break, his name appeared in the list of the Institute's Council, and from 1933 to 1936 and again from 1944 to 1946 he was a Vice-President; from 1925 to 1929 he acted as the Institute's examiner in Branch E (Food and Drugs). He was for many years Treasurer to the Liverpool and North-Western Section, at whose meetings he was almost invariably present and which he served zealously, being Chairman in 1921.

His interest in the Society of Chemical Industry dated back to the time he served under Campbell Brown, but naturally his influence was chiefly expended on the Liverpool Section. Even when his name did not appear on the official lists as member of Committee or Chairman of the Section, his private rooms were always at its disposal and he afforded every facility to the officers of the Section. His advice on matters of policy was greatly valued, and he was looked upon as the Father of the Section.

He encouraged any young chemist who wished to start in practice and gave every possible assistance, even allowing him to work in the Laboratory to familiarise himself with methods and apparatus, but always with the proviso that the newcomer should never attempt to cut fees or otherwise degrade or disgrace the Profession.

It would be impossible to enumerate his many benefactions, for he was the soul of generosity, and, apart from his former assistants who now hold responsible positions as Public Analysts, and managers and directors of business undertakings, hundreds of chemists have been helped and inspired by him.

He was at his best in the witness-box, and it is not too much to say that he revelled in this part of his duties.

He was a lover of humanity who tried, vainly, to hide his sympathies behind a mask of austerity. Hence he was popular with all sorts and conditions of men, from judges to criminals and from leading medical specialists to laboratory boys. Criminals have even been known to tell him a joke after he had given damning evidence against them. Socially he was what is known as a "success," but this was, to him, merely much-needed relaxation and no more. His one interest in life was his profession.

He was due to retire, under an age limit clause, in January, 1942, but the Corporation felt that his services were too valuable to lose and requested him not only to retain his post but to add to his duties those of supervision of all the preparations for war-gas identification, gas damage, other damage by enemy action, water and food preservation, and so forth, "for the duration."

The death of Mrs. Roberts, a gracious and gifted lady, whose appearances at the summer meetings of the North of England Section were hailed with delight because of the atmosphere of sociability that she unconsciously diffused, and who "mothered" every member of the laboratory staff, affected Roberts profoundly. She had been his helpmeet and counsellor, restraining his impetuosity and encouraging him in his more ambitious schemes, and, with her passing, in 1942, something in Roberts died. Though he bravely tried to carry on his work as usual, those nearest to him realised how difficult he found it and how heavy was the strain under which he laboured.

He felt himself failing physically, and, from a chance remark, the writer is under the impression that he wished to "die in harness." This he nearly achieved, for during his brief period of retirement he was involved in several cases, some of which are still pending, but which he handed over to chosen confreres.

Among the large concourse that attended his funeral were representatives of the University, the City Constabulary, the Health Department and other departments of the Corporation, the legal profession, his Masonic Lodge, the Royal Institute of Chemistry, the Chemical Society, the Society of Chemical Industry, and other local Societies. The staff of the City Laboratories and a number of Public Analysts from the North also attended.

Mr. S. E. Melling (Past President) officially represented the Society.

F. ROBERTSON DODD

Some Remarks on the Statistical Background in Bio-Assay

BY E. C. FIELLER

(*Read at the Annual General Meeting of the Biological Methods Group, February 25th, 1946*)

1. In properly conducted biological assays (and this is now well recognised) the experimenter has a dual aim—firstly, to estimate the activity of a test preparation in terms of that of a standard, and secondly, to derive an objective measure of the reliability of his estimate. The task of evolving an assay procedure is a joint one for the biochemist and the statistician, but it is unwise, as well as increasingly difficult, to attempt any precise definition of their respective fields; they can collaborate effectively only through mutual adaptation. Broadly speaking, however, it is impossible to conceive of a rational assay-procedure for which there is an experimental technique without an accompanying arithmetical technique, and it is with the latter that I shall be concerned.

2. Like Mr. Gridgeman,[12] I shall restrict myself mainly to the last of the three assay-types that he distinguishes, that in which the response to any particular dose is regarded as a continuous variate whose mean value is linearly related, throughout the effective dosage-range, to the logarithm of the dose. This assumption of linearity is not in itself sufficient to provide a basis for deriving, by a rigid mathematical argument, the formulae by which such assays are usually interpreted. We need two further assumptions, that the distributions of responses about their mean values follow the normal law of errors, and that the variances of these distributions are, over the effective dosage-range, equal. If these three conditions

of linearity, normality and stability of variance are assumed to be satisfied, the design and interpretation of an assay become relatively simple. The safest procedure is to have experimental groups on at least three different dose-levels both of the standard and of the test preparation. The assay then becomes self-contained: in order to interpret it we need not appeal to previous experience for estimates of the log (dose) - response line* or of the variance of individual responses, and we can, moreover, check what for the time being I shall take for granted—the adequacy of the assumptions that we have made.

3. Let us suppose now that in an assay in which the experimental groups have been chosen at random from the laboratory stock, the doses of standard (measured on a logarithmic scale), the numbers of individuals responding to them, and their average responses are respectively

$$\begin{array}{cccc} x_{11} & x_{12} & x_{13} & \cdots\cdots \\ n_{11} & n_{12} & n_{13} & \cdots\cdots \\ y_{11} & y_{12} & y_{13} & \cdots\cdots \end{array}$$

while the corresponding quantities for the test preparation are

$$\begin{array}{cccc} x_{21} & x_{22} & x_{23} & \cdots\cdots \\ n_{21} & n_{22} & n_{23} & \cdots\cdots \\ y_{21} & y_{22} & y_{23} & \cdots\cdots \end{array}$$

If we write

$$n_1 = n_{11} + n_{12} + n_{13} + \ldots\ldots = \Sigma n_{1i},$$

$$x_1 = \Sigma n_{1i} x_{1i}/n_1, \qquad y_1 = \Sigma n_{1i} y_{1i}/n_1,$$

$$p_1 = \Sigma n_{1i}(x_{1i} - x_1)^2, \qquad q_1 = \Sigma n_{1i}(x_{1i} - x_1)(y_{1i} - y_1),$$

with similar meanings for n_2, x_2, y_2, p_2, q_2, and finally

$$p = p_1 + p_2, \qquad q = q_1 + q_2,$$

then we estimate β, the slope of the log (dose) - response line, as $b = q/p$, and the activity of the test preparation from the familiar formula[11,13]

$$\log \frac{\text{activity of test}}{\text{activity of standard}} \simeq \mathrm{L} = (x_1 - x_2) - \frac{y_1 - y_2}{b} = \mathrm{X} - \frac{\mathrm{Y}}{b}, \text{ say.} \quad \ldots \quad (1)$$

The estimate L is subject to sampling variations, since both the numerator and the denominator of the fraction Y/b are; if the protocols of the assay supply an estimate s^2 of the common variance σ^2 of individual responses, then in assays of the unrestricted design under consideration Y and b are distributed normally and independently with variances that we can estimate as

$$\mathrm{V(Y)} = s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right), \qquad \mathrm{V}(b) = \frac{s^2}{p}.$$

Suppose now that t is, say, the 5 per cent. point of the Student-distribution[10] with the same number of degrees of freedom as s^2. Then as long as $[b^2 - t^2\mathrm{V}(b)]$ is positive, the 95 per cent. fiducial limits for $\lambda - \mathrm{X}$, where λ is the true logarithm of the activity ratio, are given by the quadratic equation [6,7,9]

$$\lambda^2[b^2 - t^2\mathrm{V}(b)] + 2\lambda b\mathrm{Y} + [\mathrm{Y}^2 - t^2\mathrm{V(Y)}] = 0 \quad \ldots \quad \ldots \quad \ldots \quad (2)$$

It follows easily, on solving this equation, that if

$$\mathrm{C} = b^2/[b^2 - t^2\mathrm{V}(b)],$$

$$\mathrm{L}' = \mathrm{X} - \mathrm{CY}/b$$

and

$$s_{\mathrm{L}}'^2 = \mathrm{C}\frac{s^2}{b^2}\left[\frac{1}{n_1} + \frac{1}{n_2} + \frac{\mathrm{C}}{p}\left(\frac{\mathrm{Y}}{b}\right)^2\right], \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

then the fiducial limits for λ are $\mathrm{L}' \pm ts_{\mathrm{L}}'$. A numerical illustration is given in B.S.I. Specification No. 911[5]: the calculation of limits set out there might possibly be simplified by replacing (3) by the equivalent formula

$$t^2 s_{\mathrm{L}}'^2 = (\mathrm{C} - 1)\left[p\left(\frac{1}{n_1} + \frac{1}{n_2}\right) + \mathrm{C}\left(\frac{\mathrm{Y}}{b}\right)^2\right] \quad \ldots \quad \ldots \quad \ldots \quad (4)$$

4. It may conveniently be remarked at this stage that although the assumption of normality is necessary for a rigid derivation of the above formulae, its failure in practice entails

* *I.e.*, the line showing the relation between x and η, where η is the mean response in a large group of animals each receiving a dose the logarithm of which is x.

little loss. This is in keeping with the general fact, established by many practical and theoretical investigations (*e.g.*, Welch[22]), that statistical tests based on the analysis of variance remain stable even when the parent population departs quite widely from the normal form. In particular, the part played by the assumption of normality in the mathematical argument of paragraph 3 is only a minor one; it consists principally in ensuring the normality of the sampling distributions of the estimates Y and b, but since these estimates are weighted means of all the responses y observed in the assay, their distributions will in any case be effectively normal if each y has the same error-variance σ^2. Accordingly, we can regard the argument of paragraph 3 as dependent only on the assumptions of linearity and stability of variance.

5. The fiducial-limit formulae in the form given above hold whenever, as in completely balanced litter-mate assays, Y and b are uncorrelated. I have indicated elsewhere[6] two other methods by which they may be established; one of them, the geometric approach suggested by Fisher's account[9] of the work of Working and Hotelling and by Bliss,[3] is very clearly elaborated by Irwin in his well-known 1943 paper.[14] The present method is to be preferred, since it can be adapted immediately to the calculation of limits for assays in which Y and b are not estimated independently. This is, in general, the position in the twin cross-over test,[21] for example, and in litter-mate assays in which some of the "responses" have to be estimated. All that is necessary in such instances is to replace the second term in (2) by $+ 2\lambda[b\mathrm{Y} - t^2\mathrm{Cov}(b\mathrm{Y})]$.

6. In a self-contained assay carried out on an assigned number of animals (or other test organisms), in which the assumptions of linearity and stability of variance are sufficiently realistic working hypotheses, we shall achieve maximum precision by minimising $s_{\mathrm{L}}'^2$. This entails four requirements, which can conveniently be considered in turn,—minimising $\left(\frac{1}{n_1} + \frac{1}{n_2}\right)$ and $\left(\frac{\mathrm{Y}}{b}\right)^2$, and maximising p and b^2/s^2 (which will incidentally minimise C).

(i) It is easily seen that, if $(n_1 + n_2)$ is fixed, $\left(\frac{1}{n_1} + \frac{1}{n_2}\right)$ is least when n_1 and n_2, the numbers of responses to test and standard, are equalised. In practice, if there is any great doubt about the activity of the test preparation, it may pay to have more dosage groups on test than on standard, to allow for the possibility that the extreme ones may eventually be rejected as falling beyond the range of linearity. More generally, in a multiple assay in which k test preparations are being compared simultaneously with standard, we need to minimise $\left(\frac{1}{n_1} + \frac{1}{n_2}\right)$ subject to $(n_1 + kn_2)$ being fixed; the solution is $n_1 = n_2\sqrt{k}$.

(ii) To minimise $(\mathrm{Y}/b)^2$, it is necessary—assuming for the moment that the slope β is fixed—to minimise Y^2, *i.e.*, to aim at equalising the average responses to test and standard.

(iii) To maximise p, we need to maximise both p_1 and p_2. If with each preparation we are willing to sacrifice the test for linearity, and can determine the extreme doses between which the log (dose) - response line is straight, it is most economical to put equal groups on these doses.

(iv) We may regard b^2/s^2 as an approximation to β^2/σ^2 ($1/\lambda^2$ in the notation of Bliss and Cattell[4]), where β and σ^2 are, as above, the true slope of the log(dose) - response line, and the true residual variance about it. Maximising β^2/σ^2 is primarily a matter for the biochemist rather than the statistician, since β^2 increases with the sensitivity of the test-organisms to changes of dose, and $1/\sigma^2$ with the uniformity of their responses; the fact that the precision of the assay depends on the ratio of β^2 to σ^2 is worth stressing—it implies that it is pointless to steepen the slope β if so doing entails a proportionate, or more than proportionate, increase in σ.

7. There is, however, one contribution that the statistician may make to the reduction of β^2/σ^2. In many assays the response of the individual animal can be shown to depend not only on the dose it receives, but also on the value of some characteristic, such as its weight or blood-sugar level, at the time it is dosed. This may well happen, in particular, when the metameter is obtained by comparing the final value of the characteristic with its initial value, and is provisionally calculated by the arbitrary device of taking a difference or a percentage. In such instances, the disturbing effect of differences in the initial values can, as Gridgeman has mentioned,[12] be estimated and removed by Fisher's technique of covariance analysis, which produces a modified metameter, uncorrelated with the initial readings, for which σ^2 is reduced but β^2 unaltered. Many examples will be found in the work of Bliss and

his associates. In this connection one computational point may be mentioned, since it is frequently overlooked. If we estimate the covariance correction from the internal data of an assay, the logarithm of the activity ratio is estimated not as in the second member of (1), but as

$$X - (Y'/b') = X - (Y - hZ)/(b_y - hb_z), \quad \ldots \quad \ldots \quad \ldots \quad (5)$$

where h is the estimated residual regression of the responses y on the initial readings z, and Z and b_z are calculated from the recorded values of z by the same steps that produce Y and b_y from the recorded values of y. This method of allowing for initial differences thus introduces a covariance $Zb_zV(h)$ between Y' and b', and to calculate fiducial limits correctly we must modify the quadratic equation (2) in the way described in paragraph 4. The necessity for this modification vanishes, of course, if we can estimate h from previous experience, as proves possible in the assay of insulin by the rabbit method.[8,21]

8. The ratio β^2/σ^2 gives the best indication of the reliability of an assay method of the type we are considering, because it is inversely proportional to the number of animals needed, on the average, to give any assigned degree of accuracy. If a laboratory is repeatedly carrying out routine assays by the same technique, the estimate b^2/s^2 is the most appropriate single quantity to watch in a control chart, although it may be profitable to run subsidiary charts of b^2 and s^2 as well.[17] If rival assay-methods are available for the same purpose, as are the line-test and the bone-ash test for vitamin D_2, and if the reliability of each method is substantially constant, then the respective values of β^2/σ^2, divided by the cost per animal, provide an appropriate criterion for judging between the methods. If their reliability may change from time to time (which is what we assume may happen, when we insist that each assay should be self-contained) then we must judge between the rival methods by judging between the respective distributions of β^2/σ^2, divided by the cost per animal. In this latter case it is open to debate whether we should decide solely by the mean values of the criteria, and if not, how far we should be influenced by their stability about their mean values.

9. The considerations of paragraph 8 can profitably be extended to any assay in which the response is a continuous variate. Let us suppose that doses are still measured on a logarithmic scale, but that the mean response to any particular dose x is now a non-linear function $\bar{y}(x)$ of x, and the variance of individual responses another function $V\{y(x)\}$ of x. In place of the slope β of the straight log (dose) - response line, it is natural to consider the gradient $\frac{d}{dx}\bar{y}(x)$ of the log (dose) - response curve, and in place of the single criterion of reliability β^2/σ^2 ($= \alpha$, say), we can consider the *local* criterion of reliability*

$$\alpha(x) = \left\{\frac{d}{dx}\bar{y}(x)\right\}^2 \div V\{y(x)\} \quad \ldots \quad \ldots \quad \ldots \quad (6)$$

which will in general vary with x. If we knew $\alpha(x)$ precisely, we should choose, as the most economical one in which to carry out assays, the region of x in which $\alpha(x)$ is greatest; if the conditions of linearity and stability of variance are exactly satisfied over some range of x, then $\alpha(x)$ must be constant over that range. To the first order of approximation at any rate—and this is in keeping with the last two remarks—$\alpha(x)$ is independent of the scale on which we choose to measure the response. To demonstrate this, consider the effect of the transformation

$$z = f(y) \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (7)$$

on the group of responses to some constant dose. If $\bar{y}$ is their mean, we can represent the typical response as $\bar{y} + (y - \bar{y})$, and its transform by

$$z = f(\bar{y}) + f'(\bar{y})(y - \bar{y}) \text{ approximately.} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (8)$$

As we move from one response to another within the group, $f(\bar{y})$ and its first derivative $f'(\bar{y})$ remain constant, and on the average $(y - \bar{y})$ is zero, so that

$$\bar{z} = f(\bar{y}), \text{ and } z - \bar{z} = f'(\bar{y})(y - \bar{y}),$$

whence we derive immediately the familiar result

$$\sigma_z = f'(\bar{y})\sigma_y \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (9)$$

* The sense in which we can regard $\alpha(x)$ as a local criterion of reliability becomes apparent if we consider the idealised case in which an assay is carried out on very large dosage groups, the successive doses of test and standard lying so close together that over their range the variations in the numerator and denominator of $\alpha(x)$ are negligible. If we interpreted the protocols of this imaginary assay by the formulae of paragraph 3, we should expect to find b^2/s^2 approximating to $\alpha(x)$.

or, in the notation of equation (6),

$$V\{z(x)\} = \left\{\frac{d\bar{z}(x)}{d\bar{y}(x)}\right\}^2 \{Vy(x)\}.$$

Since $\frac{d\bar{z}(x)}{d\bar{y}(x)} \frac{d\bar{y}(x)}{dx} = \frac{d\bar{z}(x)}{dx}$, it follows that approximately

$$\left\{\frac{d}{dx}\bar{z}(x)\right\}^2 \div V\{z(x)\} = \left\{\frac{d}{dx}\bar{y}(x)\right\}^2 \div V\{y(x)\} \qquad (10)$$

i.e., $\alpha(x)$ is unaffected by the transformation (7).

10. The scale of measurement on which responses can conveniently be recorded in the laboratory is not necessarily the one on which the arithmetic of the assay should be performed. By transforming the response, it is frequently possible to bring within the scope of the familiar formulae of paragraph 3 an assay-procedure that at first sight seems to lie outside it. The considerations of paragraph 9 imply that if a metameter can be found that satisfies the two conditions of linearity and stability of variance, and if the recorded responses are transformed so that they fulfil one of these conditions, then they will automatically fulfil the other. Nevertheless, it seems preferable to aim primarily at stabilising the variance. I believe that usually this procedure will in fact reveal a sufficiently wide range of doses over which the curve relating responses to logarithms of doses may be taken as linear, and in which, for maximum precision, assays should be planned; even if it does not, it is not difficult to see how to allow for curvature in estimating the logarithm of the activity ratio and calculating its fiducial limits.[6] It seems likely, on the other hand, that to allow for real changes in the variance, as we move along the dosage range, would inevitably entail more complicated arithmetic, the results of which, moreover, could only be regarded as approximate.

11. The transformation required to stabilise the variance follows immediately from equation (9). Let us suppose that by plotting the standard deviation against the mean, for different dosage groups, we find them to be connected by the relation

$$\sigma_y = \phi(\bar{y}). \qquad (11)$$

The transformation (7) will produce a metameter z for which

$$\sigma_z = f'(\bar{y})\,\phi(\bar{y}),$$

and σ_z will therefore be constant if $f'(\bar{y})\,\phi(\bar{y})$ is, so that we must take $f(y)$ proportional to

$$z = \int \frac{dy}{\phi(y)}. \qquad (12)$$

It may be useful to draw attention, in particular, to the logarithmic and square-root transformations. The former should be applied if σ_y is proportional to $\bar{y}$, the latter if it is proportional to $\sqrt{\bar{y}}$, since

$$\int \frac{dy}{y} = \log y, \qquad \int \frac{dy}{\sqrt{y}} = 2\sqrt{y}.$$

12. As examples of the use of these two transformations, we may refer to the assay of vitamin D_3 by the radiographic technique, [19, 2] and to the assay of vitamin B_1 by the rat-bradycardia method. Olsson[20] gives details of a typical D_3 assay in which, with the tarso-metatarsal distance as metameter, the observed mean responses and estimated standard deviations within dosage-groups of 28 to 30 chicks were as follows (Table I):

TABLE I

OLSSON'S VITAMIN D_3 ASSAY DATA

(Metameter y = TMT distance)

Cod liver oil I				Cod liver oil II			
Group No.	Dose g.*	Mean $\bar{y}$	S.D. s_y	Group No.	Dose g.*	Mean $\bar{y}$	S.D. s_y
1	0·20	2·06	0·68	1	0·25	2·14	0·74
2	0·40	1·63	0·41	2	0·50	1·70	0·62
3	0·80	1·22	0·39	3	1·00	1·26	0·53
4	1·60	0·96	0·19	4	2·00	1·00	0·16
5	3·20	0·96	0·22	5	4·00	0·98	0·18

* Per kilogram of food.

In the early assays carried out in this country in 1939 and 1940 by the same method, it was observed that σ_y decreased with $\bar{y}$ in a similarly rapid manner, but that the use of the logarithmic transformation usually reduced the differences between the group variances to insignificance.[2] In B.S.I. Specification No. 911 it was accordingly recommended that

$$100 \log_{10} (10 \times \text{TMT distance in mm.})$$

should be used as metameter.[5] Jones and Elliot[16] later found, for their own assays, that in many instances the calculations of activity and of fiducial limits produced virtually identical results, whether carried out on the observed distance or on its logarithm. This does not imply that there is no need to transform the distance, since in other instances the results are not identical, and since in the laboratory in question the variance of the untransformed distance is by no means stable,[15] but it does suggest that the topic might bear re-examination.

13. The square-root transformation is of course particularly easy to handle, since the recorded responses are themselves the squares of the metameter used in the arithmetic, and it appears to apply with remarkable fidelity in the rat-bradycardia assay of vitamin B_1. Table II, based on Table II of Baker and Wright's survey[1] of assays carried out during 1937–9, shows that with the duration of cure as metameter the mean response was almost exactly proportional to the group variance. (Pooling the data is legitimate because, as has been

TABLE II

MEAN RESPONSES AND GROUP VARIANCES IN RAT-BRADYCARDIA ASSAYS FOR VITAMIN B_1

1937–9 Data: Metameter y = duration of cure in days

Dose mg.*	No. of responses	Mean $\bar{y}$	Variance s_y^2	Ratio s_y^2/y
15	501	3·10	1·59	0·50
25	483	4·49	2·50	0·55
40	475	5·97	3·24	0·54

* Old International Standard (acid-clay adsorbate).

reported elsewhere,[18] the log (dose) - response line for the laboratory in question undergoes little variation in slope or position.) The use of the square-root transformation is clearly indicated; Table III shows the extent to which it succeeded in stabilising the variance when applied to tests carried out, six years later, in the summer of 1945.

TABLE III

MEAN RESPONSES AND GROUP VARIANCES IN RAT-BRADYCARDIA ASSAYS FOR VITAMIN B_1

1945 Data: Metameter y = duration of cure in days

Dose μg.*	No of responses	Mean $\bar{y}$	Variance s_y^2
3	41	1·61	0·057
6	41	1·89	0·063
9	41	2·24	0·064

* New International Standard (crystalline vitamin B_1).

14. The well known result expressed by equation (12) could easily be applied (although as far as I know it never has been) without the intervention of formal mathematics. From the experimental records we could plot on a graph one point for each separate dosage group, the abscissa of the point being the mean response in the group, and the ordinate the *reciprocal* of the estimated standard deviation. A smooth curve drawn through the swarm of points thus obtained would approximate to the graph of $1/\phi(y)$, and the transformed value of any particular response y could be obtained mechanically as the area enclosed by this curve, the axes of $\bar{y}$ and s_y, and the ordinate at $\bar{y} = y$. It would thus be possible to build up empirically what we finally require for application—a table showing the values of z corresponding to selected values of y.

ACKNOWLEDGMENT—

I am indebted to the directors of Vitamins Limited for permission to quote paragraph 13 above from an unpublished survey of the rat-bradycardia method of assaying vitamin B_1, and, more generally, for affording me from 1942 onwards the opportunity to remain in touch with a subject that my war-time duties would otherwise have compelled me to abandon.

References

1. Baker, A. Z. and Wright, M. D., *Biochem. J.*, 1939, **33**, 1370.
2. —— ——, ANALYST, 1940, **65**, 326.
3. Bliss, C. I., *Ann. Appl. Biol.*, 1935, **22**, 134, 307.
4. —— and Cattell, McK., *Ann. Rev. Physiol.*, 1943, **5**, 479.
5. *"British Standard Method for the Biological Assay of Vitamin D_3 by the Chick Method,"* B.S.I. Specification No. 911, 1940.
6. Fieller, E. C., *Supp. J. Roy. Stat. Soc.*, 1940, **7**, 50.
7. —— *Quart. J. Pharm.*, 1944, **17**, 117.
8. —— Irwin, J. O., Marks, H. P. and Shrimpton, E. A. G., *Ibid.*, 1939, **12**, 724.
9. Fisher, R. A., *"Statistical Methods for Research Workers,"* Oliver and Boyd, 6th Edn., 1936, 10th Edn., 1946.
10. —— and Yates, F., *"Statistical Tables for Biological, Agricultural and Medical Research,"* Oliver & Boyd, 3rd Edn., 1946.
11. Gaddum, J. H., *"Methods of Biological Assay depending on a Quantal Response,"* M.R.C. Special Report Series No. 183, H.M. Stationery Office, 1933.
12. Gridgeman, N. T., ANALYST, 1946, **71**, 376.
13. Irwin, J. O., *Supp. J. Roy. Stat. Soc.*, 1937, **4**, 1.
14. —— *J. Hygiene*, 1943, **43**, 121.
15. Jones, J. I. M., *Biochem. J.*, 1945, **39**, 324.
16. —— and Elliot, J. F., *Ibid.*, 1943, **37**, 209.
17. Knudsen, L. S., and Randall, W. A., *J. Bacteriol.*, 1945, **50**, 187.
18. Nixon, W. C. W., Wright, M. D., and Fieller, E. C., *Brit. Med. J.*, 1942, i, 605.
19. Olsson, N., *Archiv. für Geflügelkunde*, 1936, **10**, 11.
20. —— *Kungl. Fysiografiska Sällskapets i Lund Förhandlingar*, 1939, **9**. 1.
21. Smith, K. L., Marks, H. P., Fieller, E. C., and Broom, W. A., *Quart. J. Pharm.*, 1944, **17**, 108.
22. Welch, B. L., *Biometrika*, 1937, **29**, 21.

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A New Titrimetric Method for the Estimation of Fluorine

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INTRODUCTION—

Despite the importance attached to the distribution of fluorides in micro quantities in foods, water, etc., and the consequent effect upon teeth, it is only comparatively recently that it has been possible to estimate such traces with any degree of accuracy. The colorimetric method of De Boer,[1] based upon the fading of a zirconium alizarin lake, was used for many years as the standard micro procedure, and modifications of this are still favoured in waterworks practice. The work of Willard and Winter[2] showed the limitations of this procedure, particularly with regard to rate of fading of the lake when fluorides were added. They suggested instead a method based on titration of the fluoride solution with thorium nitrate and use of zirconium alizarin sulphonate to indicate the point when an excess of thorium over and above that required to combine with fluorine was present in the solution. This technique was improved by Armstrong,[3] who suggested a dilute alizarin sulphonate solution as the indicator, by Hoskins and Ferris,[4] by Rowley and Churchill[5] and finally by Eberz, Lamb and Lachele.[6] The technique advocated by the last-named authors is capable of giving reproducible results after some experience, but the end-point is rather vague and there is some lag in the completion of the reaction. This factor has been reduced to some extent by the back-titration technique of Dahle *et al.*,[7] and Elsworth,[8] and has been further clarified in the Society's official publication on the subject.[9]

The possibility of using other dyestuffs instead of alizarin in the formation of the thorium lake has been investigated, and we have shown that Solochrome Brilliant Blue B.S. (Colour Index 723), which is the sodium salt of sulpho-dichlorohydroxy dimethylfuchsin dicarboxylic acid, is suitable for this purpose. It possesses the following advantages over alizarin:

(1) The colour change is sharp from pink to blue, and is more sensitive than that usually given by alizarin methods.

(2) The reaction is immediate, no lag being observed.

OUTLINE OF METHOD—

Samples containing fluorides, after suitable treatment, are buffered to *p*H 3·0 and titrated with thorium nitrate in presence of Solochrome Brilliant Blue B.S. until the colour obtained matches a blank consisting of a trace of thorium nitrate added to a buffered solution of the dyestuff. The method is standardised against known amounts of fluoride and a calibration curve is prepared relating titre of thorium solution to fluorine content. The method is suitable for amounts of fluorine from 2 μg. to 100 μg. With larger quantities, thorium fluoride comes out of solution and affects the colour matching. Suitable aliquots are therefore titrated to keep within these limits. Alternatively a protective colloid may be used whereby thorium fluoride is maintained in solution and the titratable range increased accordingly.

INVESTIGATION INTO OPTIMUM CONDITIONS—

(*a*) *Volume of titrating solutions*—

Varying the amount of the final titrating solutions has been investigated to ascertain the effect of dilution upon the end-point. It is established that up to 100 ml. can be titrated as readily as 25 ml. without increasing the concentration of the indicator. Thus although the depth of colour is weaker, it is still quite readily matched. The method should, however, be standardised on a volume equal to that to be titrated in the particular determination.

TABLE I

Volume of sample solution titrated, ml...	10	25	100	10	25	100
Fluorine present, μg.	20	20	20	40	40	40
0·004 *N* Thorium nitrate required, ml. ..	0·71	0·72	0·71	1·40	1·39	1·44

(*b*) *Use of buffer*—

The use of a buffer prepared from half-neutralised chloroacetic acid (0·24 *M*) assures that the titration is carried out at *p*H 3·0 or thereabout. At this point very reproducible results are obtained. Titration in neutral solution is indefinite, and the sharpness of end-point is increased with increasing acidity until *p*H 3·0 is reached. Solutions more acid than this do not give good reproducibility on titration. The use of the acid buffer also allows of greater sensitivity—thus twice as much thorium solution is required at *p*H 3·0 (3·12 ml. per 100 μg. of F) as at *p*H 7·0 (1·25 ml. per 100 μg. of F).

(*c*) *Concentration of dye*—

The amount of dye used does not seem to affect the titration figure of a solution, but ease of matching is greatly influenced. Thus 0·5 ml. of 0·01 per cent. solution in 50 ml. of liquid gives a very pale colour the change of which is not easy to judge, and 3·0 ml. of 0·01 per cent. solution has so much basic residual red colour that the blue colour of the thorium lake is masked. In practice the aim is to obtain the sharpest colour change with the largest amount of dye, and this occurs with 2·0 ml. of a 0·01 per cent. solution, in 50 ml. volume.

(*d*) *Excess of thorium solution added*—

The end-point of the titration is assessed by comparing the tube with a similar tube to which all the reagents and a small quantity of thorium solution are added. The amount of the excess of thorium added greatly affects the titration and it is essential that the calibration curves relate to the conditions of experiment. It is not clear why the titration figure should vary with the excess of thorium added, and it would appear that the thorium fluoride itself enters into the formation of the lake. Precautions must be taken that the amount of thorium nitrate added to the comparison tube is always the same as that used when the calibration curve is prepared. Table II shows the volumes of 0·004 *N* thorium solution required for 50 μg. of fluorine corresponding to different volumes added to the blank.

TABLE II

Thorium solution added to blank ml.	Thorium solution required by 50 μg. F. ml.	Thorium solution required by 50 μg. F. corrected for blank ml.
0·05	1·55	1·50
0·08	1·62	1·54
0·10	1·84	1·74
0·15	2·09	1·94
0·20	2·36	2·16
0·30	2·78	2·48

Although higher titration figures are obtained with increased additions to the blank, if the quantity added is above 0·10 ml. the end-point tends to become difficult to recognise.

EFFECT OF SUBSTANCES OTHER THAN FLUORINE ON THE TITRATION—

The method is not specific for fluorine, as many substances that form complex ions with thorium will behave in a similar manner.

The effects of some interfering substances are recorded in Table III.

TABLE III

EFFECT OF INTERFERING SUBSTANCES ON TITRATION OF 40 μg. OF FLUORINE

Alcohol: ml. present	0	1	5	
Titration figure, ml.	1·41	1·41	0·91*	
Sodium chloride: mg. present	0	10	100	500
Titration figure, ml.	1·41	1·53	1·54	1·58*
Sodium carbonate: mg. present	0	1	3	
Titration figure, ml.	1·41	1·38	—†	
Sodium sulphate: mg. present	0	1	10	
Titration figure, ml.	1·41	1·46	1·70*	
Sodium perchlorate: mg. present	0	10	100	
Titration figure, ml.	1·41	1·43	1·43	
Sodium borate: mg. present	0	1	10	
Titration figure, ml.	1·41	1·40	—†	
Sodium acetate: mg. present	0	1	10	
Titration figure, ml.	1·41	1·39	1·08	
Sodium citrate: mg. present	0	1	2	
Titration figure, ml.	1·41	(Basic colour only)		
Potassium chloride: mg. present	0	10	100	
Titration figure, ml.	1·41	1·43	1·47	
Sodium silicate: mg. present	0	10	100	
Titration figure, ml.	1·41	1·41	1·04	
Sodium phosphate: mg. present	0	5	100	
Titration figure, ml.	1·41	—†	—†	
Sodium tartrate: mg. present	0	1	5	
Titration figure, ml.	1·41	—†	—†	

* Poor end-point. † Decolorised.

It is concluded from these experiments that the method is applicable only to relatively pure solutions of fluorides and that with few exceptions most other ions modify the titration value. This is also the experience of workers on the de Boer technique and the alizarin-thorium titration method of Willard and Winter. It is usually necessary, therefore, to separate the fluorine from such interfering substances and this is most conveniently done by distillation as silicon tetrafluoride by heating with silica and an acid of high boiling-point.

DETAILS OF DISTILLATION OF FLUORINE—

The experimental work on the effect of interfering substances indicates that distillation of the fluorine should best be carried out with perchloric acid, since traces of any acid used are certain to distil over and the salts of this acid have least effect on the subsequent titration. Other workers[6] have shown that distillation is effective with this acid, and with glass wool as a source of silica. Ellsworth[8] investigated the temperature of distillation and showed that satisfactory results are obtained if the range is kept between 135° and 145° C. An all-glass distillation apparatus is preferred. The sample, together with about 10 ml. of perchloric acid, is placed in the distillation flask. A thermometer in an elongated ground jointed mercury pocket which is of such length that it dips below the surface of the liquid. Water is added from a funnel at such a rate and the bunsen so adjusted that distillation proceeds at between 135° and 145° C. To ensure complete distillation of all the fluorine, about 200 ml. of distillate must be collected, although about 80 per cent. of the fluorine comes over with the first 50 ml. If the temperature of distillation falls below 135° C., the removal of fluoride becomes incomplete even with 200 ml. of distillate. A distillation temperature above 145° C. results in the distillation of excessive amounts of perchloric acid, which may modify the subsequent titration.

Blank distillations show that a titration figure is always obtained, equivalent to about 4 μg. of fluorine. This is said to be derived from the glass distillation apparatus. Higher blanks than this are usually due to impurity in the perchloric acid, which should be heated to 140° C. and then redistilled before use.

The type of silica used in the distillation is important. Some authors suggest powdered glass, but glass wool seems to be most satisfactory. Precipitated silica gel is definitely unsatisfactory. Some experiments in which silica gel, obtained during heating of the sample in glass vessels with caustic soda, was present, gave results corresponding to only about 40 per cent. recovery. When silica gel was added to standard fluoride solution before distillation not more than 80 per cent. recovery was obtained. Complete recovery could be obtained by using glass wool in the distillation flask.

CALIBRATION CURVE—

Although the method as outlined is capable of giving reproducible results without difficulty, it is suggested that a calibration curve correlating titration figure and fluorine content be made each time a change in the conditions is encountered. The lake formed with thorium salts and the dye is of variable composition and in consequence slight modifications in the conditions may influence the end-point.

Taking the concentrations of reagents found to be optimum and titrating with 0·004 *N* thorium nitrate solutions, a typical calibration series is as follows.

TABLE III

Fluorine present, µg.	5	10	20	40	60	80	100
0·004 *N* Thorium nitrate solution required, ml.	0·25	0·40	0·72	1·39	2·00	2·69	3·28

METHOD FINALLY ADOPTED

REAGENTS—*Perchloric acid*: 60 per cent. (pure); redistilled. *Glass wool*: fluorine-free. *Standard thorium nitrate solution*: 0·004 *N*; dissolve 552 mg. of $Th(NO_3)_4.4H_2O$ in 1 litre of water. *Solochrome Brilliant Blue B.S.*: 0·02 per cent. solution in water. *Chloroacetic buffer*: dissolve 22·7 g. of chloroacetic acid to 100 ml. in water; titrate 50 ml. of this solution with 6 *N* sodium hydroxide to neutralise; combine the two portions and dilute with water to 1 litre.

PROCEDURE—Ash the sample containing the fluoride after heating to dryness with calcium oxide according to the procedure recommended in the Society's publication.[9] Transfer the ash with the aid of a minimum quantity of water to a distilling apparatus (described above), add a small quantity of silver perchlorate to precipitate any chloride present, and allow the distillation to proceed. Place about 0·1 g. of glass wool and 15 ml. of perchloric acid in the distillation flask with the sample and add water *via* a dropping funnel continuously at such a rate that, with the heating suitably adjusted, the distillation temperature is maintained between 135° and 145° C. Distil about 200 ml. of liquid.

Dilute the distillate to a known volume, and measure an aliquot containing less than 100 µg. of fluorine for titration. Transfer the aliquot to a Nessler glass standing on a white tile, and neutralise it to phenolphthalein end-point with dilute alkali. Then just discharge the pink colour by adding a dilute solution of perchloric acid. Add 1 ml. of dye solution and then dilute perchloric acid solution until the yellow colour of the dye just changes to pink. Then add 0·5 ml. of chloroacetic buffer solution. Into a similar Nessler tube introduce a volume of distilled water equal to that of the sample, one ml. of dye solution and 0·5 ml. of chloroacetic buffer. To this tube add also 0·1 ml. of 0·004 *N* thorium nitrate solution accurately from a micro-burette. The colour changes from pink to bluish purple. Then titrate the unknown sample titrated with 0·004 *N* thorium nitrate until it exactly matches the blank in colour. From the titration figure subtract 0·1 ml. and refer the result to the calibration curve to obtain the concentration of fluorine in the sample.

Table IV shows results of some determinations carried out in triplicate on known amounts of fluorides, submitted to the distillation technique and titrated by the method described.

TABLE IV

Fluorine in luoride taken µg.	Fluorine found µg.
1·9	1·5; 1·7; 2·1
3·8	3·7; 3·5; 4·0
7·6	7·6; 7·4; 7·4
38	37·6; 37·6; 38·2
76	75·2; 76·1; 75·8
95	95·0; 94·6; 94·4

ADAPTATION OF THE METHOD TO COLORIMETRIC TECHNIQUE—

The principle of the method described above may be applied to a colorimetric technique. In this case there is formed a blue lake with thorium nitrate and the dye. The fluorine solution is then added and the colour of the solution is diminished proportionately to the formation of the thorium fluoride complex. This reduction of colour intensity may be measured—most conveniently on the Spekker absorptiometer. The method is standardised by means of a number of standard fluoride solutions treated in the same manner. As in the titrimetric technique, rigid adherence to the conditions of calibration is necessary since the colour intensity is dependent upon the concentration of all substances in solution.

The use of the colorimetric technique is not recommended. It requires more careful control and is not capable of the same degree of accuracy.

SUMMARY—

1. A new method is given for the estimation of small amounts of fluorine. It is based upon titration of the fluoride solution with thorium nitrate solution until excess is shown by the formation of a lake with Solochrome Brilliant Blue B.S. Optimum conditions for the estimation are described.

2. The method has the following advantages over the existing alizarin technique.

(*a*) The end-point is more definite and easily judged and therefore allows of greater sensitivity;
(*b*) The end-point is immediate;
(*c*) The colour does not fade.

3. The method allows the estimation of from 2 μg. to 100 μg. of fluorine within the accuracy of titration from a micro-burette, *i.e.*, 0·02 ml., or equivalent to about 0·5 μg. of fluorine.

We are indebted to the Director-General of Scientific Research (Defence) Ministry of Supply, for permission to publish this work.

REFERENCES

1. De Boer, J. H., *Chem. Weekblad*, 1924, **21**, 404.
2. Willard, H. H., and Winter, O. B., *Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 7.
3. Armstrong, W. D., *J. Amer. Chem. Soc.*, 1933, **55**, 1741; *Ind. Eng. Chem., Anal. Ed.*, 1936, **8**, 384.
4. Hoskins, W. M., and Ferris, C. A., *Ibid.*, 1936, **8**, 6.
5. Rowley, R. J., and Churchill, H. V., *Ibid.*, 1937, **9**, 551.
6. Eberz, W. E., Lamb, F. C., and Lachele, C. E., *Ibid.*, 1938, **5**, 259.
7. Dahle, D., Bonnar, R. V., and Wichmann, H. J., *J. Assoc. Off. Agric. Chem.*, 1938, **21**, 459.
8. Elsworth, F. F., and Barritt, J., ANALYST, 1943, **68**, 298.
9. Society of Public Analysts and Other Analytical Chemists, Sub-Committee Report on Determination of Fluorine in Foods, ANALYST, 1944, **69**, 243; 1945, **70**, 442.

23–24, WELBECK WAY
LONDON, W.1

October, 1946

The Estimation of the Volatile Matter Content of Propellant Explosives

Part 2.* The Estimation of Ethyl Alcohol and Ether

BY T. G. BONNER

IN the manufacture of nitrocellulose powders a mixed solvent of ethyl alcohol and ether is invariably used to facilitate the mixing of constituents and to ensure homogeneity of the product. The removal of these solvents in the final stage of manufacture is never complete, and a small residuum is always tenaciously retained in the propellant. A knowledge of the exact amount of this volatile matter is of considerable importance in the chemistry of propellants, particularly in relation to the effect on ballistic stability of changes in the volatile matter content arising from variations in the temperatures and humidities under which the propellant is stored and used.

Several attempts have been made to estimate this residual volatile matter in simple nitrocellulose powders containing only cellulose nitrate and diphenylamine, but most of the

* For Part 1, see ANALYST, 1946, **71**, 483–490.

methods described are liable to considerable error. Desmaroux[1] obtained an aqueous solution of the organic solvents by heating under reflux and distilling with aqueous sodium hydroxide solution, and then estimated them by physical methods. The same author[2] later estimated the ethyl alcohol and ether in the aqueous distillate by oxidation with potassium dichromate in acid solution; the ethyl alcohol was separately estimated by the method of Fischer and Schmidt,[3] involving conversion into ethyl nitrite, which was removed by a stream of carbon dioxide and passed into acid potassium iodide solution, the iodine liberated being titrated with thiosulphate. Dalbert[4] also estimated the ethyl alcohol and ether together by dichromate oxidation but replaced the method of Fischer and Schmidt for ethyl alcohol by one depending on surface tension effects. Lalande[5] estimated ether alone by drawing a stream of air through the aqueous distillate, removing the ethyl alcohol in a strongly alkaline solution of potassium permanganate and then absorbing and oxidising the ether to acetic acid with dichromate in diluted sulphuric acid (1+1), the unchanged dichromate being estimated iodimetrically.

A preliminary investigation of these methods indicated that the estimation of mixtures of ethyl alcohol and ether at concentrations of about 0·1 per cent. in aqueous solution was possible with an accuracy to within 1 or 2 per cent. The ethyl alcohol is readily oxidised quantitatively to acetic acid by the method of Szeberenyi[6] by boiling with potassium dichromate in a 1:10 by volume mixture of concentrated sulphuric acid and the aqueous alcohol-ether solution. The ether is not attacked under these conditions and can be estimated by difference after oxidation of another portion of the aqueous solution in a 1:1 by volume mixture of sulphuric acid and solution, which converts both the ethyl alcohol and ether quantitatively to acetic acid; this oxidation is carried out by allowing the mixture to stand at room temperature for 1½ to 2 hours.

In applying this method to the estimation of an aqueous distillate obtained by heating under reflux and distilling a sample of propellant with aqueous sodium hydroxide two major difficulties were encountered. First, a small but appreciable amount of volatile oxidisable matter, from the disintegration of the propellant, distilled with the ethyl alcohol and ether and secondly, in modern nitro-cellulose powders dibutyl phthalate is often present and from it butyl alcohol is formed by the hydrolysing action of the sodium hydroxide; the butyl alcohol distils and interferes in the subsequent dichromate oxidation of the ethyl alcohol and ether.

The first difficulty was met by distilling with sodium hydroxide solution a synthetic mixture of cellulose nitrate, diphenylamine and any other constituents in the amounts present in a 25-g. sample of the propellant and determining the amouut of oxidisable matter in the distillate obtained; a correction was then applied for this amount. The presence of butyl alcohol in the distillate, however, required the development of a method of estimating ethyl alcohol, ether and butyl alcohol together in dilute aqueous solution.

THE ESTIMATION OF ETHYL ALCOHOL, BUTYL ALCOHOL AND ETHER IN DILUTE AQUEOUS SOLUTION

Attempts to effect complete hydrolysis of the dibutyl phthalate and subsequent distillation of the whole of the butyl alcohol were unsuccessful; prolonged hydrolysis and distillation did not achieve more than about 80 per cent. recovery of butyl alcohol from known quantities of dibutyl phthalate. Methods of estimating similar simple aliphatic compounds in dilute aqueous solution have been described by Christiansen and Fulmer[7] for mixtures of ethyl alcohol, butyl alcohol and acetone, by Bayly,[8] who investigated the oxidation of aliphatic alcohols in about 0·5 per cent. aqueous solution with potassium dichromate in 45 per cent. sulphuric acid solution; by Skrabal,[9] who claims that with minor modifications the Fischer and Schmidt method (*loc. cit.*)[3] could be applied to an aqueous solution of any simple aliphatic alcohol with an accuracy to within about 1 per cent.; and by Fresenius,[10] who estimated aqueous butyl alcohol solutions by oxidation with dichromate followed by distillation and titration of the acid products in the distillate with sodium hydroxide solution.

Application of Lalande's method—As a first step, the direct estimation of ether in dilute aqueous solutions containing ethyl and butyl alcohols by Lalande's method (*loc. cit.*)[5] was investigated. It was found that an alkaline potassium permanganate solution absorbed both alcohols, whilst the ether, unaffected by passage through this solution, could be absorbed and oxidised quantitatively to acetic acid by potassium dichromate in a 1:1 by volume mixture of sulphuric acid and water. By drawing a slow stream of air through the aqueous solution of the three constituents at 30° to 40° C. for 5 to 6 hours, then through

alkaline potassium permanganate solution and finally through the acid potassium dichromate solution, it was established that a quantity of ether of the order of 50 mg. could be estimated with an error of about 1 to 2 per cent.

Oxidation of butyl alcohol—The oxidation of butyl alcohol was next studied and it was found that, under the conditions that convert ethyl alcohol quantitatively into acetic acid in Szeberenyi's method (*loc. cit.*),[6] 1 molecule of butyl alcohol consumes 3 atoms of oxygen; this oxidation procedure is subsequently referred to as the "mild" oxidation method. Attempts to discover other oxidation conditions giving a simple stoichiometric relation between butyl alcohol and oxygen but not affecting ether were unsuccessful, and it was evident that the only immediate possibilities were the complete combustion of the three constituents to carbon dioxide and water, or the quantitative conversion of all three into acetic acid. The former possibility was rejected when the wet combustion method of Williams,[11] employing potassium dichromate or potassium iodate in concentrated sulphuric acid solution, gave erratic results with aqueous solutions of these constituents. Since the oxidation of ether to acetic acid in diluted sulphuric acid (1+1) appeared to be sensitive to any change in the ratio of acid to water, the investigation of the oxidation of butyl alcohol to acetic acid had to be confined to this acid concentration. Employing a dilute aqueous solution of butyl alcohol of known concentration and this concentration of acid, the oxidation was carried out at different temperatures for varying periods of time, and it was finally established that if the temperature was maintained at 0° C. for 24 hours and then raised to and maintained at room temperature for a further 1½ hours, the oxidation proceeded quantitatively to acetic acid; no further change then took place in the potassium dichromate content of the oxidising solution and the amount of potassium dichromate consumed corresponded to 8 atoms of oxygen per molecule of butyl alcohol. Under these conditions of oxidation ethyl alcohol and ether were both quantitatively oxidised to acetic acid. This oxidation procedure was designated the "total" oxidation method. Results of the direct estimation of synthetic aqueous butyl alcohol solutions by the mild and total oxidation procedures are given in Table I. An accuracy of to within 1 or 2 per cent. is evident.

TABLE I

ESTIMATION OF BUTYL ALCOHOL IN AQUEOUS SOLUTION

Butyl alcohol per 100 ml. of aqueous solution taken g.	Type of oxidation	Potassium dichromate consumed g.	Butyl alcohol found g.	Error per cent.
0·0810	Mild	0·319	0·0802	−1·0
0·1084	,,	0·426	0·1074	−1·0
0·1200	,,	0·482	0·1212	+1·0
0·1170	Total	1·224	0·1154	−1·3
0·1170	,,	1·232	0·1162	+0·6
0·1200	,,	1·260	0·1188	−1·0
0·1200	,,	1·258	0·1187	−1·0

Application of methods above—These oxidation methods were applied to synthetic aqueous solutions of ethyl alcohol, butyl alcohol and ether. Three aliquot portions of the solution were separately treated (1) by the "mild" oxidation method, (2) by the "total" oxidation method and (3) by Lalande's method for ether; the quantity of dichromate consumed in each was determined iodimetrically and calculated to 100 ml. of the original solution. The ether was thus obtained directly while the alcohols were obtained indirectly by the following method of calculation.

For 100 ml. of the aqueous solution, let the amounts of potassium dichromate in grams required in the various oxidations be

M. for the preferential oxidation of the two alcohols by the mild oxidation method.
T. for the total oxidation of the three constituents to acetic acid by the total oxidation method.
A. for the oxidation of ethyl alcohol to acetic acid.
B. for the oxidation of butyl alcohol to acetic acid.
C. for the oxidation of ether to acetic acid.

The amounts of dichromate represented by M, T and C are experimentally determined values; the amount of dichromate consumed by the butyl alcohol in 100 ml. of the aqueous

solution in the mild oxidation is 3B/8, since one molecule of butyl alcohol requires 3 atoms of oxygen for the mild oxidation and 8 atoms for the total oxidation.

Then for the mild oxidation of 100 ml. of the solution

$$M = A + (3B/8)$$

and for the total oxidation of 100 ml. of the solution

$$T = A + B + C.$$

From these two equations,

$$5A = 3C + 8M - 3T \quad \text{and} \quad 5B = 8(T - M - C)$$

the amount of dichromate equivalent to both alcohols is therefore obtained. The relationship between potassium dichromate and each of the three constituents is given by

1 g. of potassium dichromate ≡ 0·1886 g. of ether
≡ 0·02344 g. of ethyl alcohol
≡ 0·0944 g of butyl alcohol,

from which the amount of each constituent present can be calculated. Results of the estimation of synthetic aqueous solutions of ethyl alcohol, butyl alcohol and ether are given in Table II, and indicate an accuracy to within 1 per cent. for the ether estimation and to within about 2 to 3 per cent. for the alcohols.

TABLE II

ESTIMATION OF MIXTURES OF ETHYL ALCOHOL, BUTYL ALCOHOL AND ETHER IN AQUEOUS SOLUTION

Aqueous solution taken contained: g./100 ml.		Type of oxidation	Potassium dichromate		Found, per 100 ml.		
			Calculated equivalent g.	Amount consumed g.	Ether (direct) g.	Ethyl alcohol (indirect) g.	Butyl alcohol (indirect) g.
Ethyl alcohol	0·0499	Mild	0·624	0·626			
Butyl ,,	0·1037				0·0504	0·0490	0·1051
Ether	0·0500	Total	1·576	1·588			
Ethyl alcohol	0·0502	Mild	0·650	0·646			
Butyl ,,	0·1095				0·0517	0·0491	0·1100
Ether	0·0510	Total	1·644	1·648			
Ethyl alcohol	0·0453	Mild	0·597	0·595			
Butyl ,,	0·1015				0·0625	0·0435	0·1033
Ether	0·0620	Total	1·597	1·610			

In applying this method to estimations on aqueous distillates from propellants containing dibutyl phthalate it was necessary to apply corrections for oxidisable impurities in the distillate, in both the mild and the total oxidation, and these increased the error of the method to the order of about 5 per cent. In the absence of any other suitable method, however, this procedure was regarded as satisfactory for providing a preliminary estimate of the true ethyl alcohol and ether contents of nitrocellulose powders. In the wide variety of powders investigated the ether content was invariably higher than the alcohol content, the former usually ranging from about 0·2 to 2 per cent. and the latter from about 0·1 to 1 per cent.; some old types of powders contained over 5 per cent. of residual solvent.

When the necessity arose for an accurate routine method for the estimation of these volatile constituents certain unsatisfactory features of the method described became apparent. These included the large amount of sample required (25 to 50 g.), which is not always available, and the length of time required for an estimation (2 to 3 days); further, certain special investigations required greater accuracy than was possible with this procedure. As an alternative, the possibility was considered of vaporising the ethyl alcohol and ether in a current of air without decomposition of the propellant and subsequently absorbing and differentially oxidising them in acid dichromate solutions of different concentrations.

THE SEMIMICRO-ESTIMATION OF ETHYL ALCOHOL AND ETHER VAPOURS ENTRAINED IN AIR

Somogyi[12] describes a method for estimating mixtures of ethyl alcohol and ether vapours in air by passing the air first through 9 N sulphuric acid, which preferentially absorbs the alcohol, and then through a solution of potassium dichromate in diluted sulphuric acid (1+1), in which the ether is absorbed and oxidised to acetic acid; the ethyl alcohol is estimated by

subsequently oxidising its solution in sulphuric acid with potassium dichromate. Quantities of the order of 0·25 to 0·025 g. of each constituent were estimated by this method with an accuracy to within about 3 per cent. Komar, Sergunin and Fainberg[13] criticise certain features of this method and apply a slightly modified form of it to the estimation of much smaller quantities of ethyl alcohol and ether in air; their results show that when the amount of ether present is less than 5 mg. the recovery of it is less than 90 per cent., but with larger quantities the method is accurate to within about 3 to 4 per cent.

It was evident that this method might be further modified to include the simultaneous absorption and oxidation of the ethyl alcohol; to this end the conditions necessary for the quantitative oxidation of small amounts of ethyl alcohol (5 to 30 mg.) to acetic acid at room temperatures were investigated. It was found that this could be achieved quite readily with potassium dichromate in diluted sulphuric acid (1+10) in about 1¼ hrs. at ordinary room temperatures. To test the modified method, weighed quantities of ethyl alcohol and ether contained in thin sealed glass tubes were introduced into a 500 ml. flask fitted with an inlet and an outlet tube each carrying a stop cock, the inlet tube reaching to the bottom of the flask. After the stop cocks were closed the tubes were fractured by shaking the flask. The flask was placed in an absorption train similar to that shown in the diagram, in the position occupied in the diagram by the three-necked flask. The first absorption spiral tube contained the acid dichromate solution for the oxidation of the ethyl alcohol, the second absorption spiral tube alkaline potassium permanganate solution (prepared as described later) and the third the usual acid dichromate solution for oxidation of the ether. The alkaline potassium permanganate solution was used because in determinations on propellants its presence was effective in absorbing any oxidisable volatile constituents that would not be affected by the weak oxidising solution in the first absorption tube but that might be oxidised by the stronger solution in the third tube. At the head of the absorption train were two conical flasks, the first containing a concentrated chromic acid solution to remove any oxidisable impurities in the air drawn through the train and the second serving as a trap for any acid spray from the first. The last absorption tube in the train was connected to a bottle in which a partial vacuum was maintained by means of a water pump. After all the stop-cocks had been opened a slow stream of air was drawn through the apparatus, carrying the ethyl alcohol and ether vapours into the absorption tubes. After 3 to 4 hours the air flow was stopped, the acid dichromate solutions were washed out and the unchanged potassium dichromate was estimated iodimetrically. Results for several such determinations are given in Table III, and these show that the method is accurate to within about 2 per cent. for quantities of ethyl alcohol and ether not less than about 5 mg.

TABLE III

ESTIMATION OF MIXTURES OF ETHYL ALCOHOL AND ETHER VAPOURS ENTRAINED IN AIR

Taken in mixture: Ethyl alcohol g.	Taken in mixture: Ether g.	Ethyl alcohol found g.	Error per cent.	Ether found g.	Error per cent.
0·0365	0·0795	0·0361	−1·1	0·0803	+1·0
0·0343	0·0475	0·0340	−0·9	0·0491	+3·4
0·0227	0·0152	0·0216	−4·8	0·0155	+2·0
0·0187	0·0155	0·0185	−1·1	0·0160	+3·2
0·0097	0·0110	0·0096	−1·0	0·0109	−0·9

APPLICATION TO THE ESTIMATION OF ETHYL ALCOHOL AND ETHER IN PROPELLANTS—

Various methods were considered for removing the ethyl alcohol and ether from nitrocellulose powders free from volatile impurities oxidisable by the acid dichromate solution used in the first absorption spiral tube. It became evident that their complete removal could only be effected by dissolution of the sample of powder in a suitable solvent. Kraus[14] gives a comparison of the solubilities of cellulose nitrates, of nitrogen contents ranging from 10·20 to 12·29 per cent., in 70 different solvents; from this list a selection of the most likely solvents was made and of these, mononitrobenzene proved by far the most suitable.

Dissolution of a 2 to 3-g. sample of a nitrocellulose powder was achieved in about 2 hours at 100° C. Blank determinations on the solvent alone, and also on solutions of the usual constituents of nitrocellulose powders in it, indicated complete absence of interference by

volatile oxidisable products. The method adopted therefore was to agitate the sample of powder (2–3 g.) with 25 ml. of nitrobenzene by means of a gas-tight stirrer arrangement in the three-necked flask shown in the diagram. The flask was immersed in a boiling water bath and a stream of air was bubbled through the solution to remove the ethyl alcohol and ether. Other constituents of nitrocellulose powders volatile to some extent at 100° C., *e.g.*, diphenylamine, were almost completely retained by passing the vapours through a condenser before drawing them into the first absorption tube. Acetone and camphor, which are present in some types of powders, were removed by the alkaline potassium permanganate solution (both of these volatile constituents are unaffected by the oxidising solution in the first absorption tube but would be oxidised in the stronger medium in the third absorption tube). To test the method, weighed amounts of ethyl alcohol and ether were dissolved in nitrobenzene and an aliquot portion of the solution was transferred to the three-necked flask; the other constituents of a typical nitro-cellulose powder were added in the amounts present in a 2-g. quantity of sample and the estimation was carried out as indicated above. The time necessary to effect complete removal, absorption and oxidation was about 4 hours. Results given in Table IV indicate errors of 2 to 3 per cent. for both ethyl alcohol and ether. Duplicate results obtained with different types of nitrocellulose powders are given in Table V; excellent

TABLE IV

ESTIMATION OF MIXTURES OF ETHYL ALCOHOL AND ETHER IN NITROBENZENE SOLUTION

Taken in mixture					
Ethyl alcohol g.	Ether g.	Ethyl alcohol found g.	Error per cent.	Ether found g.	Error per cent.
0·0188	0·0455	0·0186	−1·1	0·0465	+2·2
0·0105	0·0142	0·0105	0	0·0144	+1·4
0·0105	0·0142	0·0104	−1·0	0·0143	+0·7
0·0097	0·0262	0·0097	0	0·0266	+1·6
0·0094	0·0164	0·0093	−1·1	0·0170	+3·7
0·00323	0·0074	0·0031	−3·7	0·0072	−2·7

TABLE V

THE ESTIMATION OF ETHYL ALCOHOL AND ETHER IN PROPELLANT EXPLOSIVES

Sample of propellant	Ethyl alcohol per cent.	Ether per cent.
Modern nitrocellulose powders:		
(1) MA.668	0·08; 0·09	0·57; 0·58
(2) MA.621	0·13; 0·12	0·99; 0·97
(3) Dupont 4825	0·40; 0·40	0·99; 1·01
Neonite (small flake)	0·75; 0·77	0·10; 0·11
German igniter (acetone present)* ..	0·38; 0·39	0·00; 0·00
Celluloid (camphor present)	0·21; 0·20	0·00; 0·00

* Acetone found 1·08 and 1·12 per cent. Estimation to be described later.

reproducibility is evident. The table also includes the result of analysis of one sample containing camphor and one containing acetone in place of ether; the acetone content was 1·10 per cent., but this was completely removed by the alkaline potassium permanganate solution, for no oxidation was found to have occurred in the third absorption tube. The method used for estimating this acetone will be given in Part 3 of this paper. The fact that nitroglycerine does not interfere in the method was demonstrated by carrying out an estimation on a sample of solventless cordite that contained a high percentage of nitroglycerine but no solvent; no oxidation took place in either of the acid dichromate solutions. The method has been found completely satisfactory for all types of propellants. Full details of the solutions and procedure employed are given below.

DETAILS OF METHOD—

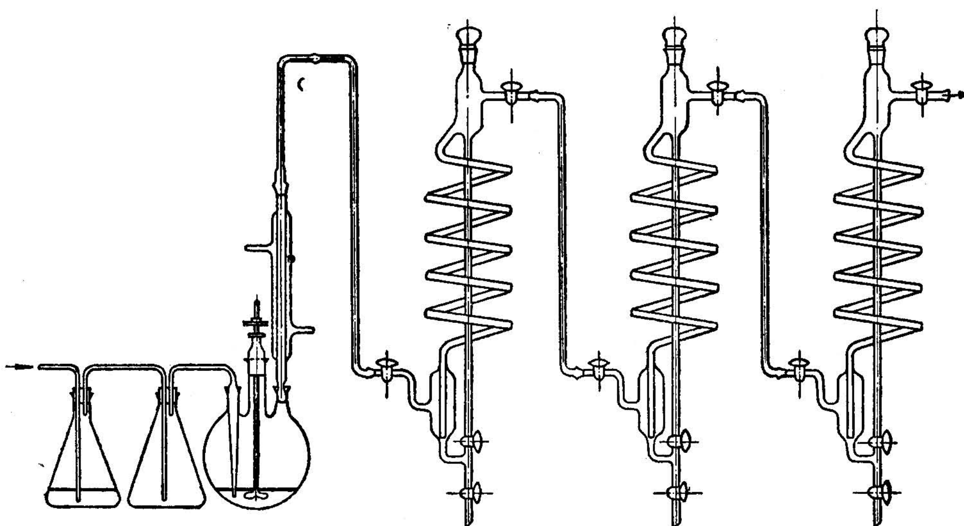
Apparatus—This is shown in the diagram. The absorption spiral tubes have an over-all length of about 18 inches and the diameter of the spiral is about 2 inches; the total internal volume is about 60 ml.

Solutions—To prepare the acid dichromate solution for the oxidation of the ethyl alcohol, transfer 25 ml. of an aqueous solution containing exactly 8 g. of potassium dichromate per litre to a clean dry 100 ml. graduated flask and add 2·5 ml. of concentrated sulphuric acid.

Introduce the bulk of this solution into the first absorption spiral tube shown in the diagram. Retain the flask, as the solution is eventually returned to it.

To prepare the acid dichromate solution for the oxidation of the ether, transfer 25 ml. of an aqueous solution containing exactly 16 g. of potassium dichromate per litre to a clean dry 200 ml. flask and add, while cooling, 25 ml. of concentrated sulphuric acid. Introduce this solution into the third absorption spiral tube shown in the diagram and retain the flask.

To prepare the alkaline potassium permanganate solution boil gently under reflux for 10 hours 104 g. of potassium permanganate and 261 g. of potassium hydroxide with 1 litre of water. After cooling, pour off the supernatant solution and introduce about 30 ml. of it into the second absorption spiral tube.



Procedure.—Crush the propellant sample into small pieces (about 3 mm. across) with a steel pestle and mortar, weigh accurately 2 to 3 g. and add it to 25 ml. of nitrobenzene (AnalaR quality) in the 250 ml. three-necked flask shown in the diagram. (The relative effects of crushing and grinding samples will be described in Part 4 of this paper.) Stir the nitrobenzene solution vigorously and, with the flask immersed in a boiling water bath, draw a stream of air through the solution at the rate of 1 to 2 bubbles per second for 5 hours.

Then stop the flow of air, wash the dichromate solutions back into their respective flasks and make up the volumes to the marks with water. Of the 100 ml. of oxidised ethyl alcohol solution add 25 ml. to a solution of 2 to 3 g. of potassium iodide in about 30 ml. of water and, after 2 minutes, dilute the solution to about 100 ml. with water and titrate the liberated iodine with 0·05 *N* sodium thiosulphate solution, using starch solution as indicator. Treat the oxidised ether solution similarly except that, as the acid concentration is much higher, add the 25-ml. aliquot portion to a solution of the potassium iodide in 100 ml. of water and, after 2 minutes, dilute to about 200 ml. with water and titrate. Calculate the amounts of ethyl alcohol and ether present from the relation to the potassium dichromate given previously (p. 50).

Summary

An account is given of the methods of determining the residual contents of ethyl alcohol and ether in nitrocellulose propellants. A new method is described which employs dissolution of a small sample of the propellant in nitrobenzene at 100° C.; the ethyl alcohol and ether vapours are removed in a current of air and are differentially absorbed and oxidised in acid potassium dichromate solutions of different concentrations. The method is suitable for all normal types of nitrocellulose and nitroglycerine powders and is applicable in presence of the usual constituents of propellants including acetone, camphor, dibutyl phthalate, diethyl diphenylurea and diphenylamine. Arising out of the preliminary investigation of the problem a method is described for the estimation of mixtures of ethyl alcohol, butyl alcohol and ether at concentrations of 0·05 to 0·10 per cent. in aqueous solution.

Acknowledgment is made to Imperial Chemical Industries (Explosives Division) for making available a valuable report in which the earlier attempts at estimating ethyl alcohol and ether in simple nitrocellulose powders were reviewed and critically assessed.

In conclusion, the author wishes to thank Mr. G. L. Hutchinson of the Armaments Research Department for his helpful discussion of the problem and the Director-General of Scientific Research (Defence), Ministry of Supply, for permission to publish this material.

REFERENCES

1. Desmaroux, J., *Mem. des Poudres*, 1924, **21**, 211.
2. —— *Ibid.*, 1928, **23**, 285.
3. Fischer, W. M., and Schmidt, A., *Ber.*, 1926, **59**, 679.
4. Dalbert, R., *Mem. des Poudres*, 1934–5, **26**, 312.
5. Lalande, A., *Ibid.*, 1932–3, **25**, 104.
6. Szeberenyi, P., *Z. anal. Chem.*, 1915, **54**, 409.
7. Christiansen, L. M., and Fulmer, E. I., *Ind. Eng. Chem., Anal. Ed.*, 1935, **7**, 180.
8. Bayly, W. A., *J. Soc. Chem. Ind. Vict.*, 1940, **40**, 267.
9. Skrabal, R., *Z. anal. Chem.*, 1940, **119**, 222.
10. Fresenius, R., *Zeit. Anal. Chem.*, 1941, **122**, 459.
11. Williams, R. J., *J. Amer. Chem. Soc.*, 1937, **59**, 288.
12. Somogyi, E. V., *Z. angew. Chem.*, 1926, **39**, 280.
13. Komar, N. P., Sergunin, N. A., and Fainberg, P. B., *J. Appl. Chem.*, U.S.S.R., 1934, **7**, 424.
14. Kraus, A., *Nitrocell.*, 1941, **12**, No. 4, 63–4.

November, 1946

The Determination of Small Amounts of Aluminium by the Aurintricarboxylate Method

BY N. STRAFFORD AND P. F. WYATT

IN a previous paper[1] we describe a procedure for the separation of very small amounts of aluminium and iron in water, with subsequent determination of the aluminium by means of ammonium aurintricarboxylate. This method gives sufficiently accurate results for waters, but we had occasion to attempt to apply it to the precise determination of small amounts of aluminium in organic compounds, after wet decomposition of 2 g. of the organic material by means of sulphuric, nitric and perchloric acids.[2] The resulting acid solution contained up to 10 mg. of iron and 0 to 300 μg. of aluminium, and two difficulties were met with in the analysis of this solution, *viz.* (1) the amount of iron present was too great, and the conditions were otherwise unsuitable, for complete separation by extraction as ferric thiocyanate, (2) measurements of optical density by means of the Spekker photoelectric absorptiometer showed that the fading of the aluminium aurintricarboxylate colour, rapid at first, then gradually becoming slower, which occurs in slightly basic medium, cannot be controlled sufficiently well to give accurately reproducible results, even when the conditions are carefully standardised.

The first of these difficulties was overcome by converting the iron into its "cupferron" complex, and separating it by extraction with chloroform from a solution containing sulphuric acid at $4N$ to $5N$ concentration. The excess of cupferron reagent can then be extracted sufficiently well to avoid interference with the subsequent aurintricarboxylate reaction. With regard to the second difficulty it was found that after development of the aluminium complex colour as usual in acetate buffered solution, removal of the colour due to excess of reagent could be effected by buffering with ammonium borate to $pH = 6{\cdot}0 \pm 0{\cdot}5$, and that it was unnecessary to raise the pH to 7·5. With buffering just on the acid side of neutrality in this way, no fading of the aluminium lake occurs, so that results are highly reproducible, and the sensitivity of the method is increased. Under the modified conditions, no interference has been found with up to 10 mg. of calcium, magnesium or phosphate (P_2O_5), or up to 1 mg. of common heavy metals, and it is possible that even larger amounts may be tolerated. Beryllium interferes, giving a colour of intensity similar to that produced by the same weight of aluminium. Iron and copper are removed in the cupferron extraction; it is necessary to ensure thoroughly complete removal of iron, as this element gives a colour about half as intense as that given by aluminium, but small amounts of copper can be tolerated. The procedure recommended for organic compounds is as follows.

REAGENTS—The reagents used are as described in the previous papers.[1,2] The cupferron reagent is a freshly prepared and filtered 6 per cent. solution in water. It must be prepared from material of analytical quality; B.D.H. reagent is suitable. An aqueous solution should be only slightly straw coloured, and a chloroform solution should show no brown discoloration due to decomposition products.

PROCEDURE—Decompose 2 g. of the organic material in a 100-ml. Kjeldahl flask with sulphuric, nitric and perchloric acids,[2] and remove nitric acid by repeated evaporation with water, in the usual manner. Dilute the acid solution until it is 5 *N* to 6 *N* in sulphuric acid, and transfer it to a graduated 50-ml. separating funnel, using the minimum amount of wash water to effect the transference. To this solution, which usually has a volume of 20 to 25 ml., and which should contain not more than 10 mg. of iron, add 2·5 ml. of 6 per cent. cupferron solution, mix well and add 10 ml. of chloroform, B.P. Shake vigorously for 40 seconds, allow to separate, and run off and reject the chloroform layer. Wash with a few ml. of chloroform, without mixing, to displace the drops of strong iron "cupferrate" solution remaining on the surface of the aqueous layer and in the stem of the funnel. Then shake for 30 seconds with 5 ml. of chloroform, allow to separate, and reject the chloroform layer. Add 0·5 ml. of cupferron solution; the turbidity produced should be white and show no colour due to iron. Extract successively with 10 ml. followed by two 5-ml. portions of chloroform, shaking for 30 seconds each time, to remove all but a trace of the free cupferron from the aqueous layer. After each extraction the stopper of the funnel should be rinsed with a little water and the washings added to the contents of the funnel, but the final acidity of the solution should not be allowed to fall below 4 *N*, or traces of aluminium may tend to be extracted.

Transfer the aqueous layer to a 100-ml. conical flask and wash the funnel with small amounts of water. Heat the solution to boiling, and boil for a few seconds to expel any chloroform present; then cool to room temperature. Add one drop of methyl red indicator, and ammonium hydroxide solution, 10 *N*, until the solution is just alkaline. Add dilute hydrochloric acid, 5 *N*, drop by drop, until the solution is just acid, and then 5·0 ml. in excess, followed by 2 drops of saturated bromine water, which should immediately bleach the indicator. Failure of the indicator colour to disappear indicates that the excess of cupferron has not been extracted properly. Finally, add 0·5 ml. of 10 per cent. hydroxylamine hydrochloride solution, to reduce the excess of bromine, and dilute the solution to 100 ml. in a measuring flask (Solution A).

Pipette 20·0 ml. of Solution A into a 100-ml. conical flask and dilute to 30 ml. with distilled water. Add 1·0 ml. of gum arabic solution (5 per cent. in water), 5·0 ml. of ammonium acetate buffer solution, and 2·0 ml. of 0·2 per cent. ammonium aurintricarboxylate solution. Mix well, heat to boiling, and boil gently on a hot plate for 5 minutes. Cool to room temperature, add 4·0 ml. of 0·8 *N* ammonium borate solution, mix, dilute to 50·0 ml. and allow to stand for not less than 5 minutes. Determine the optical density of the test solution and of a reagent blank solution (similarly obtained) on the Spekker photoelectric absorptiometer, using a 4 cm. cell for the range 0 to 10 μg. of aluminium and a 1 cm. cell for the range 10 to 70 μg. of aluminium, with either Chance blue-green glass filters No. 6 (OB2) or Ilford green No. 604 filters. If the amount of aluminium present exceeds 70 μg, take a smaller aliquot portion of Solution A and adjust the acidity so that 1·0 ml. of 5 *N* hydrochloric acid is present, before adding the acetate buffer solution.

Preparation of standard curve—Establish the standard curve required by measuring known amounts of standard aluminium solution (1 ml. ≡ 10 μg. of Al) into 100-ml. conical flasks, adding 1·0 ml. of 5 *N* hydrochloric acid and diluting to 30 ml. Then develop the colour in these solutions as described for the test solution, and determine the optical density of each solution in turn against the first solution (containing no added aluminium) as reference solution. A typical series of results, using a 1 cm. cell, is as follows:

Aluminium present, μg.	10	20	30	40	50	60	70
Optical density:							
1. With Ilford green No. 604 filters ..	0·14	0·285	0·43	0·57	0·72	0·86	1·00
2. With Chance blue-green No. 6 filters	0·10	0·20	0·305	0·405	0·51	0·62	0·71

It is advisable to test Solution A for absence of iron. Take a 50-ml. aliquot portion, add 2 ml. of 1 *M* sodium citrate solution, 1 ml. of 10 per cent. thioglycollic acid solution, and finally 5 ml. of 10 *N* ammonium hydroxide, and mix. The solution should show no trace of pink colour due to iron.

Application to water samples—For waters, we recommend that the procedure previously described[1] be modified as follows. After extraction of the iron as thiocyanate, boil the solution to remove ether, cool and dilute to 50 ml. in a measuring flask. Take a 20-ml. aliquot portion and determine its acidity by titrating with N sodium hydroxide to methyl red indicator. Reject this test, take a second 20-ml. aliquot portion and add the same amount of N sodium hydroxide as was required by the first test. Treat the reagent blank similarly. Add to blank and test 1·0 ml. of 5 N hydrochloric acid and then develop the colour of the aluminium complex exactly as described above. The optical density of the solution may be measured by the Spekker absorptiometer, or the colour may be matched with the iron thioglycollate colour disc in the B.D.H. Lovibond Nessleriser. With the above modified procedure, the disc reading (μg. of Fe) divided by 20 represents μg. of aluminium, and if a 20/50 ml. aliquot portion is taken, 8·0 μg. of iron (disc reading) $\equiv$ 1 μg. of aluminium in the total solution which corresponds to 0·10 parts of aluminium per million on a 10-ml. sample or 0·04 parts per million on a 25-ml. sample, as before.[1]

REFERENCES

1. Strafford, N., and Wyatt, P. F., ANALYST, 1943, **68**, 319.
2. Strafford, N., Wyatt, P. F., and Kershaw, F. G., *Ibid.*, 1945, **70**, 232.

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October, 1946

A Colour Reaction of Certain Classes of Azo Dyes with Copper

BY W. A. ALEXANDER

A CHANCE observation of the behaviour of the dyestuff Bordeaux B with dilute copper solutions led to an investigation from which it appears that a certain class of dyes can be distinguished by means of the characteristic reaction described below. The reaction also serves, under certain conditions, as a sensitive test for copper.

TEST—Add a few drops of a dilute (about 0·05 per cent.) aqueous solution of the dye to be tested to 10 ml. of a weakly acid solution of a copper salt (0·0001 M to 0·001 M), and then add sufficient sodium acetate to bring the pH to about 8·5. Carry out a control test—without copper. If the dye belongs to the reacting class, a distinct colour change will be noticed. Most of the reacting dyes are pink or red—and with these the colour becomes yellow-brown; in two instances the colour change in presence of copper is from blue-violet to blue-grey. Some of the reacting dyes, *e.g.*, Bordeaux B, are more sensitive than others; these sensitive dyes may be used as reagents for copper. When so used, the reaction provides a delicate test for that metal, and is capable of detecting as little as 1 μg. of copper in 10 ml., *i.e.*, a concentration of 1 in 10,000,000. In testing for copper, the solution must be dilute; if sufficiently concentrated for cupric hydroxide to be precipitated the colour change is not observed. In testing for copper, too, the amount of dye solution taken should be the minimum necessary to give a distinct colour to the control; if too much be taken, the excess tends to mask the colour change.

The test is particularly suitable for detecting copper in water for domestic use, where there is little interfering material.

INTERFERENCE—The remarks under this heading, of course, apply either when the copper ion is used to distinguish certain dyes or when a sensitive dye is used for a qualitative test for copper.

High concentrations of all salts (over 1·0 M) lower the sensitivity of the reaction somewhat. This applies to substances such as sodium chloride and potassium sulphate. Borates, fluorides, chromates, nitrites and nitrates, at concentrations below 0·1 M, do not interfere. Phosphates and silicates mask the test somewhat, but not at concentrations below 0·001 M. Cyanide, even in traces, masks the test completely.

Of the metals tested, only nickel reacts similarly to copper—but only when the pH is greater than 8·5 and the concentration of the nickel greater than 1 in 200,000. Manganese, cobalt, lead, silver, barium, strontium, calcium, magnesium, cadmium and arsenic do not interfere at low concentrations. Antimony, tin and bismuth do not interfere when the

reagent is added after the solution has been filtered at *p*H 8·5 (test for copper). Chromium, aluminium and ferric iron adsorb the dye when their hydroxides are precipitated in its presence —there is no colour change, however, and traces do not interfere. Ammonium ion lowers the sensitivity and causes the formation of a dark brown in place of a yellow-brown tint with sensitive dyes of a red colour.

REACTIVE DYES—Of the dyes examined—representative of several classes—only certain azo dyes are reactive, and the conclusions drawn may be summarised as follows.

(1) Copper-sensitive dyes contain an azo group linked in the 2 or 1 position to sulphonated α- or β-naphthol respectively.

(2) Reactive dyes derived from α-naphthol are substituted in the 4 and/or 5 positions with SO_3' groups, while those derived from β-naphthol are substituted thus in the 3 and 6 positions.

(3) In both classes, the 8 position may be occupied by SO_3' or OH.

(4) Dyes that are insensitive include those closely related azo dyes derived from α- or β-naphthol, in which the above order of substitution is reversed or substituents are not present.

(5) The remaining part of the dye structure appears to have little influence on the reaction with copper.

Tables I to IV illustrate these conclusions, together with the structure of the dyes. Of the insensitive dyes, only those closely related to the sensitive class are shown. Table I shows the reactive dyes derived from α-naphthol and Table II the inactive dyes from the same source. Table III gives the reactive dyes derived from β-naphthol—these are rather more sensitive as a class than those in Table I and include the very sensitive Bordeaux B, No. 88. Table IV shows the non-reacting β-naphthol dyes. The numbers in all four tables refer to the Colour Index. Only one exception to the substitution order mentioned above was noted, No. 54 (Table I), which has sulphonic groups in the 3 and 6 positions, whereas one would expect it to be a 4:5 or 4:8 disulphonate for a reacting α-naphthol dye. This may, however, be due to the presence of an excessive proportion of these reactive isomers.

With all the reactive dyes except two the colour change is similar—from red to yellow-brown; the two exceptions are Azo Acid Violet AL (Table I) and Azo Acid Blue (Table I, No. 59), with both of which the change is from violet to blue-grey.

MECHANISM OF REACTION—Several workers have studied the lakes formed by the interaction of certain metals with azo and other dyes. Many of the former have been shown by Drew and Landquist,[1] Beech and Drew,[2] and Boyle, Cumming and Steven,[3] to be inner co-ordination complexes. When copper is the metal, these are frequently soluble in water and amorphous in structure. Such compounds are usually formed under alkaline conditions; for example, the authors named have shown that if sulphonic groups be present a sulphonate may be first formed, and no inner co-ordination results until the sulphonic groups are neutralised. Again, a great many of the compounds which have been recorded are brown, or reddish-brown in the solid phase. Lake formation, too, generally requires the presence of a hydroxyl group in the *ortho* position with respect to the azo group.

These facts indicate that the delicate colour change noted at extreme dilution may be due to the formation of a copper lake.

There are, however, one or two facts that are not so readily reconciled with this view. Lake formation, it appears, can also occur with compounds having an *ortho* carboxyl or amino substituent in place of the hydroxyl group, and such compounds do not react with copper at extreme dilution in the manner described. Again, the authors cited make no deductions with respect to substitution rules and lake formation similar to those given above; copper lakes are obtained from a number of *ortho* hydroxy azo dyes whether derivatives of naphthalene or not, but only certain sulphonated naphthol dyes give the characteristic reaction with copper. For example, Boyle, Cumming and Steven show that dye No. 151 (Table IV) forms a reddish-brown copper lake, but it does not react characteristically at extreme dilution.

A comparison, at great dilution and equivalent concentrations, between the colours obtained on dissolving the solid Bordeaux B - copper lake and that resulting from addition of the dye to the copper ion at great dilution showed that the former was considerably darker (browner) in shade.

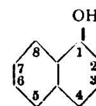


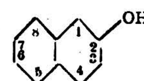
TABLE I: REACTIVE DYES DERIVED FROM α-NAPHTHOL

Name of dye or Colour Index No.	Positions of substituents							
	1	2	3	4	5	6	7	
Fast Red VR	-OH	$-N_2-$	—	—	$-SO_3'$	—	—	—
Azo Acid Violet AL	-OH	$-N_2-$ $-N_2-$	—	$-SO_3'$	—	—	—	-OH
No. 54	-OH	$-N_2-$ $-NH.CO.CH_3$	$-SO_3'$	—	—	$-SO_3'$	—	—
No. 59	-OH	$-N_2-$ $-N(CH_3)_2$	—	$-SO_3'$	—	—	—	-OH
No. 74	-OH	$-N_2-$ $-CH_3$	—	$-SO_3'$	—	—	—	—
No. 179	-OH	$-N_2-$ $-SO_3'$	—	$-SO_3'$	—	—	—	—
No. 194	-OH	$-N_2-$ $-SO_3'$	—	$-SO_3'$	—	—	—	—

TABLE II: NON-REACTIVE DYES DERIVED FROM α-NAPHTHOL

Name of dye or Colour Index No.	1	2	3	4	5	6	7	
No. 32	-OH	$-N_2-$	$-SO_3'$	—	—	$-SO_3'$	—	$-NH.SO_2-$ $-CH_3$
No. 57	-OH	$-N_2-$ $-NH.CO.CH_3$	$-SO_3'$	—	—	$-SO_3'$	—	$-NH.CO.CH_3$
No. 77	-OH	$-N_2-$	$-SO_3'$	—	—	$-SO_3'$	—	—
No. 85	-OH	$-N_2-$	$-SO_3'$	—	—	$-SO_3'$	—	—
No. 91	-OH	$-N_2-$	$-SO_3'$	—	—	$-SO_3'$	—	—
No. 201	-OH	$-N_2-$ $-SO_3'$ OH	—	—	—	—	—	—
No. 246	-OH	$-N_2-$	$-SO_3'$	—	—	$-SO_3'$	$-N_2-$ $-NO_2$	$-NH_2$
No. 387	-OH	$-N_2-$ $-N_2-$ OH	$-SO_3'$	—	—	$-SO_3'$	—	$-SO_3'$
No. 520	-OH	OCH_3 OCH_3 $-N_2-$ $-N_2$ SO_3' -OH $-NH_2$ SO_3'	$-SO_3'$	—	—	$-SO_3'$	—	$-NH_2$

TABLE III: REACTIVE DYES DERIVED FROM β-NAPHTHOL



Colour Index No. of dye	Positions of substituents 1	2	3	4	5	6	7	8
No. 79	CH_3 $-N_2-$ $-CH_3$	-OH	$-SO_3'$	—	—	$-SO_3'$	—	—
No. 88	$-N_2-$	-OH	$-SO_3'$	—	—	$-SO_3'$	—	—
No. 89	$-N_2-$	-OH	—	—	—	$-SO_3'$	—	$-SO_3'$
No. 252	$-N_2-$ $-N_2-$	-OH	—	—	—	$-SO_3'$	—	$-SO_3'$

TABLE IV: NON-REACTIVE DYES DERIVED FROM β-NAPHTHOL

Colour Index No. of dye	1	2	3	4	5	6	7	8
No. 151	$-N_2-$ $-SO_3'$	-OH	—	—	—	—	—	—
No. 196	$-N_2-$ SO_3'	-OH	—	—	—	—	—	—
No. 196	$-N_2-$ SO_3'	-OH	—	—	—	—	—	—
No. 201	$-N_2-$ -OH	-OH	—	$-SO_3'$	—	—	—	—
No. 201	OH $-N_2-$	-OH	—	$-SO_3'$	—	—	—	—
No. 202	OH $-N_2-$ $-SO_3'$	-OH	—	—	—	—	—	—
No. 28[illegible]	SO_3' $-N_2-$ $-N_2-$ $-SO_3'$	-OH	—	—	—	—	—	—
No. 286	CH_3 CH_3 $-N_2-$ $-N_2-$ $-SO_3'$	-OH	—	—	—	—	—	$-SO_3'$

The formation of the co-ordination compounds studied by the authors cited requires heating in concentrated solutions, and it is difficult to see how the rapid reaction at extreme dilution described above can be due entirely to lake formation of this type. It seems more reasonable to suppose that certain dyes are adsorbed by colloidal cupric hydroxide.

REFERENCES

1. Drew, H. D. K., and Landquist, J. K., *J. Chem. Soc.*, 1938, 292.
2. Beech, W. F., and Drew, H. D. K., *Ibid.*, 1940, 608.
3. Boyle, J. L., Cumming, W. M., and Steven, A. B., *J. Roy. Tech. Coll., Glasgow*, 1940, 617.

THE ROYAL TECHNICAL COLLEGE
GLASGOW *July*, 1946

Notes

THE DETERMINATION OF COPPER IN FOOD

IN a study of methods for the determination of copper in food, it was found that certain difficulties associated with the preparation of samples by dry-ashing could be overcome and that the procedure for photometric absorption measurement, when using sodium diethyldithiocarbamate as the colouring agent, could be simplified.

ASHING—Whilst a dry-ashing procedure is more convenient and less liable to contamination from reagents than a wet-ashing one, it is generally accepted[1,2,3] that it gives low recoveries of copper if the ashing temperature exceeds 500° C. This comparatively low temperature not only needs control but may cause a difficulty in obtaining reasonably complete ashing of some products.

The generally accepted explanation of the loss of copper is that it is due to formation of an acid-insoluble compound and not to volatilisation.[1] Piper[4] has suggested that the compound is a complex silicate of copper, and support for this is given by Bailey and McHargue,[5] who obtained lower results when using silica dishes for dry ashing than when they ashed by the wet method; by Comrie,[6] who obtained greater losses when using old silica dishes than when new ones were used, and by Van Niekerk,[7] who observed slagging of copper salts during dry ashing when they came into contact with the sides of the silica dishes he used.

It is the general practice to extract the ash with hydrochloric acid and to determine the amount of copper present in the extract, and an alternative explanation of the loss of copper is that it is due to the reduction of the copper compounds present to metallic copper by the carbon which is necessarily formed during dry ashing. This reduction occurs at relatively low temperatures[8] and, as copper is only feebly attacked by hydrochloric acid,[8] losses are to be expected when this acid alone is used for extraction. If this view is correct it is to be expected that the use of a more suitable extracting acid will result in a full recovery of copper.

The results of some recovery experiments with a farinaceous product ashed at 650° to 800° C. are given in Table I. These clearly show that the presence of nitric acid in the extracting acid results in a much more complete recovery of added copper.

TABLE I

RECOVERY OF COPPER FROM ASH WITH DIFFERENT EXTRACTING ACIDS

Hydrochloric acid			Mixture of hydrochloric and nitric acid		
Copper added µg.	Copper found µg.	Recovery %	Copper added µg.	Copper found µg.	Recovery %
—	8·0	—	—	21·6	—
50	13·2	10·4	50	70·2	97·2
50	12·6	9·2	50	70·6	98·0
50	9·2	2·4	50	67·0	90·8
50	9·2	2·4	50	71·2	99·2
50	11·2	6·4	50	67·0	90·8
50	11·2	6·4	50	68·8	94·4

It is possible to obtain consistently satisfactory recoveries of added copper when hydrochloric acid alone is used to extract the ash, provided the ashing temperature is kept at 450° to 490° C. This does not, however, show that the copper already present in the product is completely recovered, nor are the satisfactory recoveries obtained when nitric acid is used a proof of the accuracy of the nitric acid method. A series of analyses by the two dry-ashing methods were, accordingly, run in parallel with a standard wet-ashing method.[1] The results,

given in Table II, whilst showing excellent agreement between the nitric acid and the wet ashing methods, show also that the hydrochloric acid method is not reliable.

TABLE II

DETERMINATION OF COPPER IN DIFFERENT PRODUCTS

Product	Dry-ashing: HCl extraction	Dry-ashing: $HCl + HNO_3$ extraction	Wet-ashing
	p.p.m.	p.p.m.	p.p.m.
Peas	6·3	7·4	7·4
	6·3	7·3	7·4
	6·4	7·3	7·3
	6·3	7·4	7·4
Treacle pudding ..	2·1	6·0	5·9
	2·0	6·0	6·0
	2·1	6·0	6·1
		6·1	5·9
Mixed pickles ..	5·4	8·9	8·8
	5·2	9·1	9·0
	5·7	9·1	8·8
	5·6	9·0	9·0

When nitric acid has been used in the treatment of ash,[9,10,11] it has either been used as a part of what is virtually a wet-ashing procedure carried out in open dishes rather than in a flask[9] or because a subsequent electrolytic separation of copper makes the presence of hydrochloric acid undesirable.[10,11] In none of these instances is the nitric acid used to ensure a more complete extraction of the ash than would be obtained if hydrochloric acid alone is used.

Measurement of photometric absorption—The use of nitric acid often results in a yellow-coloured extract. The colour varies from sample to sample and it is, therefore, necessary to measure or allow for the blank of each extract. With food products containing low amounts of copper, a solution of the extract as concentrated in respect of copper as is practicable should be used and the dilution necessary if separate aliquots of the extract are taken for blank and colour measurement should be avoided if possible.

TABLE III

PHOTOMETRIC ABSORPTION OF A COPPER SOLUTION* AFTER ADDITION OF VARIOUS AMOUNTS OF SODIUM DIETHYLDITHIOCARBAMATE

Weight of sodium diethyldithiocarbamate added, mg.	Photometer reading†
2	50·8
5	57·0
10	69·0
20	82·8
50	82·8
75	83·2
100	82·6
250	83·0
500	85·4‡

* Containing 94 μg. of copper in 50 ml.
† Water setting 47·8.
‡ Slight cloudiness in the solution.

As can be seen from Table III, the absorption of a copper solution is independent of the amount of sodium diethyldithiocarbamate present once the maximum absorption has been reached. It is, therefore, practicable, by using the solid reagent for colour development, to measure the blank and the copper absorptions on the undiluted extract.

There is apt to be waste when the pure solid reagent is used, as, at most, only 20–30 mg. are required for full colour development. Further, if too much is used, the test solution tends to be cloudy owing to incomplete solution of the diethyldithiocarbamate. It is therefore suggested that the reagent be diluted with a salt which is readily soluble and is without influence on the copper colour. A 1 : 25 mixture with sodium chloride will be found to be a convenient one to use.

METHOD—Dry-ash a suitable sample of the product in a silica basin in the normal manner, igniting at 600° to 850° C. Extract the ash by warming it with 5 to 10 ml. of a

mixture of 2 volumes of diluted hydrochloric acid (1+1) to 1 volume of diluted nitric acid (1+1), both of analytical reagent purity.

Transfer the extract to a 100-ml. beaker, add about 2 g. of A.R. citric acid or ammonium citrate and 2 ml. of 1 per cent. gum arabic solution, neutralise with A.R. ammonium hydroxide (sp.gr. 0·880), and add 1 to 2 ml. in excess. Allow the solution to cool and transfer, with filtration if necessary, to a 50- or 100-ml. volumetric flask and make it up to the mark with water.

Set the photometer to a predetermined setting with a part of the contents of the flask, using Ilford 601 spectrum violet filters. Transfer another part to a beaker or, using the solution already in the photometer cell, add about 0·5 g. of a 1:25 mixture of sodium diethyldithiocarbamate and A.R. sodium chloride. Stir to dissolve the mixture and measure the absorption of the solution. The increase is that due to the copper colour.

Calibrate the photometer by means of a standard solution prepared from A.R. cupric sulphate or pure copper foil, with the same size of measuring cell as that used in the test, and measuring the absorption as directed above.

Thanks are due to the Metal Box Co., Ltd., for permission to publish, and to Dr. H. Liebmann for invaluable advice and constructive criticism.

REFERENCES

1. Sandell, E. B., "*Colorimetric Determination of Traces of Metals.*" Interscience Publishers Inc., New York, 1944, Chap. XVII.
2. Snell, F. D., and Snell, C. T., "*Colorimetric Methods of Analysis.*" Chapman & Hall, London, 1936. Chap. XVI.
3. Greenleaf, C. A., *J. Assoc. Off. Agric. Chem.*, 1942, **25**, 385.
4. Piper, C. S., "*Soil and Plant Analysis,*" University of Adelaide, 1942.
5. Bailey, L. F., and McHargue, J. S., *Plant Physiol.*, 1945, **20**, 74.
6. Comrie, A. A. D., ANALYST, 1935, **60**, 532.
7. Van Niekerk, P. le Roux, *Onderstepoort J. Vet. Sci. Anim. Indust.*, 1937, **9**, 623.
8. Partington, J. R., "*A Textbook of Inorganic Chemistry,*" Macmillan & Co., London, 1939, pp. 791 and 796.
9. Hanak, A., *Z. Untersuch. Lebensm.*, 1930, **59**, 511.
10. Grandel, *Pharm. Weekblad.*, 1930, **69**, 913 and 1345.
11. Haddock, L. A., and Evers, N., ANALYST, 1932, **57**, 495.

RESEARCH DEPARTMENT
THE METAL BOX CO., LTD.
ACTON, LONDON, W.3

JOHN H. HIGH
October, 1946

NOTE ON THE ASSAY OF SOLUTIONS OF *d*-TUBOCURARINE CHLORIDE FOR INJECTION

THE renewed interest in *d*-tubocurarine chloride, resulting from its extensive use for muscular relaxation during surgical operations,[1] has made it desirable that analysts should be familiar with chemical and physical methods which, apart from the fundamental biological assay, may be used for the standardisation of injections. In our laboratories it has been found that the colorimetric and polarimetric methods, described below, may be used for the routine examination of solutions, which usually contain 1 per cent. w/v of crystalline *d*-tubocurarine chloride.

POLARIMETRIC ASSAY—Crystalline *d*-tubocurarine chloride, $C_{38}H_{44}O_6N_2Cl_2.5H_2O$, possesses a specific rotation of +190° (*C* 1·0 in water) equivalent to $[\alpha]_D^{20}+215°$ for the anhydrous salt. The specific rotation in water, however, is practically independent of the concentration and consequently the optical rotation of a solution is a linear function of its *d*-tubocurarine content. This property forms a useful method for the assay of solutions, the optical rotation of an aqueous 1 per cent. w/v solution of crystalline *d*-tubocurarine chloride being +1·90°.

COLORIMETRIC ASSAY—*d*-Tubocurarine chloride yields with the Folin-Ciocalteu phenol reagent[2] a brilliant blue colour, which will detect as little as 0·01 mg. of the alkaloid. The reaction may be made quantitative when carried out under the following conditions.

Standard solution—Dissolve 10 mg. of crystalline *d*-tubocurarine chloride, $C_{38}H_{44}O_6N_2Cl_2.5H_2O$, in water and make up to 100 ml.

Test solution—Dilute the solution under examination to approximately the same concentration as the standard solution.

Reagent—Folin-Ciocalteu phenol reagent.[2] For use, dilute 1 volume of strong stock solution with 2 volumes of water.

Colour reaction—Measure 2 ml. of the standard or test solution into a 25-ml. glass-stoppered measuring cylinder, add 3 ml. of reagent and adjust the volume to 25 ml. with water. Add 2 ml. of 20 per cent. w/v sodium carbonate solution, mix, and heat a suitable volume (5 ml.) in a test tube placed in a boiling water bath for 3 minutes Cool the reaction mixture.

Prepare under identical conditions reaction mixtures from the standard and test solutions and compare them in a suitable colorimeter. From the readings obtained calculate the *d*-tubocurarine chloride content of the test solution.

A phenol, which may be present as an antiseptic in a solution for injection, will interfere with the colorimetric assay, and either a correction must be applied to the colorimeter reading or the phenol must be removed before addition of the reagent. When dealing with preparations of uncertain composition the analyst must proceed on the assumption that a phenolic antiseptic is present. For example, if a single 1-ml. ampoule, containing 10 mg. of *d*-tubocurarine chloride, is available, the following procedure should be adopted. The ampoule is opened, a portion of the solution (about 0·5 ml. required) is transferred to a 50-mm. micro-polarimeter tube and the optical rotation is determined. This polarimetric assay consumes no material and the solution is returned to the ampoule, from which 0·5 ml., accurately measured with a pipette or a micrometer syringe, is transferred to a separating funnel containing 25 ml. of water, and the resulting diluted solution, rendered faintly acid if necessary, is extracted with three portions (2·5 ml.) of chloroform. The chloroform extracts are discarded and the aqueous layer run into a 50-ml. standard volumetric flask to which the aqueous washings from the separating funnel are added to adjust the volume to 50 ml. The solution is well shaken and used for the colorimetric assay. After the polarimetric and colorimetric work has been completed sufficient of the original solution will remain in the ampoule for a biological examination, if this is required.

The methods described in this communication have yielded results correct to within ±5 per cent. It might be feared that the polarimetric assay would be unreliable on account of possible racemisation of the *d*-tubocurarine but, in our experience, solutions of the chloride undergo little change when subjected to the usual process of heat sterilisation.

I am indebted to the Directors of The Wellcome Foundation for permission to publish this information.

REFERENCES

1. Gray, T. C., and Halton, J., *Proc. Roy. Soc. Med.*, 1946, **39**, 400; Prescott, F., Organe, G., and Rowbotham, S., *Lancet*, 1946, II, 80.
2. "*Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*," 5th Edn., 281.

THE CONTROL LABORATORIES
WELLCOME CHEMICAL WORKS
DARTFORD, KENT

G. E. FOSTER
December, 1946

OXALIC ACID EXTRACTANT IN VITAMIN C ASSAY

SOLUTIONS of oxalic acid have advantages over metaphosphoric or trichloroacetic acid as extractants for vitamin C in being cheaper and more stable, and avoiding precipitation of protein, whilst effectively inhibiting the action of ascorbic acid oxidase. In a collaborative investigation on vitamin C in tomatoes, involving the preparation of large volumes of extracts, the opportunity was taken to compare the results obtained on a given fruit with oxalic and metaphosphoric acids respectively, using sufficiently large numbers of fruit to obtain averages in which the sampling error was reduced to a low level. With 1 per cent. and 2 per cent. solutions of oxalic acid the average results were respectively 98 and 99 per cent. of those obtained when 5 per cent. metaphosphoric acid solution was used. The *p*H of 10 to 40 per cent. extracts in 1 per cent. oxalic acid solution was 1·8 to 2·0 and that in 2 per cent. solutions 1·1 to 1·2. The *p*H of the tomato flesh was 4·5 to 5·5 and that of the skin 7 to 8. Control experiments showed the presence of much ascorbic acid oxidase. The extracts in metaphosphoric acid showed no appreciable loss after a week's storage in the refrigerator in brown bottles filled with nitrogen—the routine procedure applied to metaphosphoric acid extracts. With these precautions, oxalic acid can safely be used as an extractant in vitamin C assays on tomatoes and similar large fruit. It is, however, less suitable for extracting unripe walnuts containing much "apparent vitamin C" of which the dye titration value depends on the *p*H (*cf. Nature*, 1946, **158**, 133).

OVALTINE RESEARCH LABORATORIES
KING'S LANGLEY, HERTS

FRANK WOKES
November, 1946

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments of Public Analysts has been received from the Ministry of Health since the last record in THE ANALYST (1946, **71**, 586).

Public Analysts	*Appointments*
BRANSON, Victor Cecil (Deputy)	County Borough of Hastings.
COX, Henry Edward	Isles of Scilly.
HAWKINS, Ernest Stephen..	Boroughs of Folkestone, Gravesend, Maidstone and Margate.
JENKINS, Daniel Ceiriog Evans	Borough of Colne.
LOVETT, Thomas Whittaker (Deputy) ..	County Borough of Rochdale.
MOIR, Daniel Donald	Urban District of Coulsdon and Purley.

For the following particulars of Public Analyst Appointments in Scotland, held at January 1st, 1947, we are indebted to the Department of Health for Scotland.

Public Analyst	*Appointments*
DARGIE, Andrew	*Counties:* Angus, Fife, Perth and Kinross. *Burghs:* Arbroath, Dundee, Dunfermline and Kirkcaldy.
HAWLEY, John William, and WILSON, William (additional)	*Counties:* Dumfries, Kirkcudbright and Wigtown. *Burgh:* Dumfries.
JAMIESON, Archibald R., and HERD, Magnus (additional)	*Burgh:* Glasgow.
McKEAN, John Brown	*Counties:* Argyll and Bute. *Burghs:* Greenock, Port Glasgow, Paisley, Dumbarton and Motherwell and Wishaw.
MUNDY, Mrs. Lilian M.	*Counties:* Ayr and Renfrew. *Burghs:* Ayr and Kilmarnock.
ROBB, Marshall J., and .. .. .. RITCHIE, John E.	*Counties:* Aberdeen, Banff, Caithness, Kincardine, Ross & Cromarty and Sutherland.
SCOTT-DODD, Alexander	*Counties:* Berwick, Inverness, Moray and Nairn, Orkney, Roxburgh, Selkirk and Zetland. *Burghs:* Edinburgh and Inverness. *Small Burghs:* Dunbar, North Berwick and Peebles.
THIN, Russell Gibson	*Counties:* Clackmannan, East Lothian (excluding Burghs of Dunbar and North Berwick), Midlothian, Peebles (excluding the Burgh of Peebles) and West Lothian.
THOMSON, Robert Tatlock, and McKEAN, John Brown	*Counties:* Dunbarton and Stirling. *Burghs:* Airdrie, Clydebank, Coatbridge, Falkirk, Hamilton, Perth, Rutherglen and Stirling.
WEIR, Alexander B., and MACDONALD, Joseph (assistant)	*Burgh:* Aberdeen.
WILSON, Andrew, and .. McKEAN, John Brown	*County:* Lanark.

Ministry of Food

STATUTORY RULES AND ORDERS*

1946—No. 2046. The Meat Products, Canned Soup and Canned Meat (Control and Maximum Prices) (Amendment No. 3) Order, 1946. Dated December 5, 1946. Pp. 6. Price 2d.

This Order amends further the Meat Products, Canned Soup, and Canned Meat (Control and Maximum Price) Order, 1946, *as previously amended by S.R. & O.,* 1946, *Nos.* 1355, 1542, *and* 1727. *The principal amendments are concerned with maximum prices. Soups, both meat and vegetable, have been entirely removed from the scope of the Order.*

This Order came into force on the 9*th of December,* 1946.

— **No. 2124. The Feeding Stuffs (Regulation of Manufacture) Amendment No. 10) Order, 1946. Dated December 13, 1946.** Pp. 6. Price 2d.

The purpose of this amending Order is to prescribe a formula for the manufacture of a new cattle food to be known as National Cattle Food No. 2*; to prescribe a minimum wheat by-products content and to decrease the minimum cereals content of all National Compounds except National Baby Chick Food. The Order also increases the minimum percentage of fish meal to be included in*

* Italics signify changed wording.

certain pig and poultry compounds and makes a minor alteration in the grade of veterinary cod liver oil to be included in those compounds of which cod liver oil is a required ingredient.

The analytical requirements of the various compounded rations are specified.

This Order came into force on the 21st of December, 1946.

1946—No. 2169. The Labelling of Food Order, 1946. Dated December 19, 1946. Pp. 20. Price 5d.

This Order revokes and substantially re-enacts in a consolidated form the Labelling of Food (No. 2) Order, 1944, and its amending Orders. New provisions have been introduced, particularly as to the labelling of intoxicating liquors. Cocktails, cordials, processed peas and vitamins are now included.

The general purport of the Order is:—

(*a*) *to provide that all intoxicating liquors sold in bottle be labelled with an indication of their true nature, their country of origin and, with certain exceptions, their strength (Article 2 (5));*

(*b*) *to provide that intoxicating liquors for which tonic, restorative or medicinal properties are claimed, be labelled with a statement indicating the quantity of the ingredients on which the claim is based (Article 2 (5));*

(*c*) *to provide that liquids packed in advance by a retailer on the premises where they are sold be labelled with an indication of their true nature (Article 2 (7));*

(*d*) *to include provisions as to liquors sold in bottle which may be labelled under descriptions which suggest that they are or resemble spirits, cocktails, sweetened liqueurs or alcoholic cordials (Article 4);*

(*e*) *to make special provision as to the labelling of canned processed peas (Article 6);*

(*f*) *to provide that a statement of ingredients be included on the labels of pickles, canned soups and canned meats which are now no longer exempted from this requirement by inclusion in the First Schedule to the Order.*

The Order contains four Schedules, as under:

(1) Foods exempt or partly exempt from Article 2 of the Order.

(2) Part I Vitamins. Part II Minerals. Units to be used on labels.

(3) Dates of revocation of provisions of the Labelling of Food (No. 2) Order, 1944, as *now* amended.

(4) Dates of coming into force *of the various articles and paragraphs, between the 1st of February,* 1947, *and the 1st of January,* 1948.

Amongst new regulations of general interest, the Order requires that:

1. *All undistilled excisable, fermented liquor, not made from freshly gathered grapes, shall bear a prescribed label stating the description of fruit, fruits or fruit products used by the manufacturer in the process of fermentation and also the minimum alcoholic content, expressed either as a percentage of alcohol by volume or as a percentage of proof spirit. In addition, liquor "not made from fruit" is to be labelled with these quoted words, and brandy, gin, rum and whisky that has been diluted with water to an alcoholic strength of less than 65 per cent. of proof spirit is required to bear a label to that effect and also a declaration of its minimum alcoholic strength.*
2. *Brandy, the alcoholic strength of which has fallen below 65 per cent. of proof spirit solely on account of maturing in the cask is not required to be labelled "diluted," but must bear a statement of its minimum alcoholic strength, expressed either as a percentage of proof spirit or in degrees proof.*
3. *On all labels,* the declaration shall be printed in dark block type not less than ⅛ inch in height upon a light coloured ground and shall be enclosed by a surrounding line and no matter other than that hereinbefore described shall be printed within such surrounding line, provided that in the case of brandy, gin, rum and whisky, the alcohol content of which is not less than 65 per cent. proof spirit and of any other intoxicating liquor the alcohol content of which is not less than 40 per cent. proof spirit, it shall be sufficient for the declaration to be printed in dark black type not less than ⅛ inch in height upon a light coloured ground or in light block type not less than ⅛ inch in height upon a dark coloured ground in the following form:

   (Z) PROOF

   inserting at (Z) the figure which represents the percentage of proof spirit.
4. *A cocktail is required to contain not less than 40 per cent. of proof spirit,* but so long as no name or description usually associated with a cocktail containing spirits or with a sweetened liqueur is used, nothing shall prevent the use of:

   (i) the description "Wine Cocktail" for a product which is derived from wine obtained by the fermentation in the district of its origin of the juice of freshly gathered grapes and which contains not less than 35 per cent. proof spirit, or

   (ii) the description "British Wine Cocktail" or the words "wine cocktail" immediately preceded by the name of the fruit or other saccharine material from which the product is derived, for a product which is made from wine other than that referred to in the preceding sub-paragraph and which contains not less than 24 per cent. proof spirit, or

   (iii) the description "Alcoholic Cordial" for a suitably flavoured compounded spirit which has been rendered sweet and viscous only by addition of sucrose, dextrose or invert sugar and not by the use of any other ingredient and which contains not less than 10 per cent. proof spirit, or

   (iv) the description "Non-Alcoholic Fruit (or Vegetable) Juice Cocktail" for a non-alcoholic product consisting of undiluted fruit or vegetable juice and ready for consumption without dilution.

No person shall sell or have in his possession for sale any canned peas which have been dried, soaked or otherwise processed prior to canning which are described in a label attached to or printed on the wrapper or container

(a) as "peas," unless the word "peas" wherever it appears on the label is immediately preceded by the word "processed" printed in such a manner as to be substantially as conspicuous as the word "peas."

(b) as being "fresh," or "garden" or "green," or by the use of any word which may indicate either directly or by ambiguity, omission or inference, that the peas are other than peas which have been dried, soaked or otherwise processed prior to canning.

This Order details the special requirements for foods in which the presence of vitamins or minerals is claimed on labels or in advertisements. It prohibits general claims for vitamins and prescribes the units in which the quantities of the individual vitamins claimed to be present are to be declared.

1947—No. 161. The Edible Gelatin (Control) Order, 1947. Dated January 30th, 1947. Pp. 4. Price 1d.

This Order creates by definition a product known as edible gelatin and controls its use, as from February 23rd, 1947.

The following definition is given in Article 1.

"Edible gelatin" means the clean, wholesome protein which—

(a) is obtained by extraction from collagenous material;

(b) is free from objectionable taste and offensive odour when in a warm 5 per cent. aqueous solution;

(c) contains, when air-dried, not more than 3·25 per cent by weight of mineral matter; and

(d) contains, when air-dried, in each million parts by weight, not more than 1·4 parts by weight of arsenic (expressed as arsenious oxide—As_2O_3), 10 parts by weight of lead, 30 parts by weight of copper or 100 parts by weight of zinc.

Article 2. No person shall use any gelatin other than edible gelatin in the manufacture of any food.

Article 3. No person shall use any edible gelatin in the manufacture of any food other than specified food:

Provided that nothing in this Article shall prohibit—

(a) the use of edible gelatin in the manufacture of any food otherwise than for sale;

(b) the use of edible gelatin in the manufacture of any medicine or drug, medicinal capsule, or edible container for any medicine or drug; or

(c) the use of any specified food containing edible gelatin in the manufacture of any other food.

"Specified food" means any food specified in the Schedule to this Order, *viz.*,

Bakers' prepared materials.
Biscuits, as defined in the Biscuits (Maximum Retail Prices) Order, 1943.
Canned soup.
Confectionery, as defined in the Chocolate, Sugar Confectionery and Cocoa Products (Control and Maximum Prices) Order, 1944.
Flavouring Essences, as defined in the Flavouring Essences (Current Prices) Order, 1943.
Invalid Foods.
Meat Products, as defined in the Meat Products, Canned Soup, and Canned Meat (Control and Maximum Prices) Order, 1946.
Table Jellies, as defined in the Table Jellies (Maximum Prices) Order, 1946.
Wine and spirit finings.

"Sale," in this Order, includes also the use by a caterer for the purpose of his business.

British Standards Institution

A FEW copies of the following draft Specifications, 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 Specifications prepared by Technical Committee OSC/24, British National Committee on Soaps and Fats of the International Commission for the Study of Fats.

CH(OSC)8024 — Draft for the Preparation of the Sample for Examination.
CH(OSC)8025 — Draft for Ash Content.
CH(OSC)8026 — Draft for Unsaponifiable Matter.
CH(OSC)8027 — Draft for Saponification Value.
CH(OSC)8028 — Draft for Free Acidity.
CH(OSC)8029 — Draft for Refractive Index.
CH(OSC)8030 — Draft for Iodine Value (Modified Wijs' Method).
CH(OSC)8031 — Draft for Hydroxyl Value or Acetyl Value.
CH(OSC)8032 — Draft for Volatile Acids (Reichert, Polenske and Kirschner Values).
CH(OSC)8033 — Draft for Impurities.
CH(OSC)8034 — Draft for Thiocyanogen Value.
CH(OSC)8035 — Draft for Volatile Matter and Water.

Draft Specifications prepared by Technical Committee OSC/12—Vegetable Oils—

CH(OSC)8535 — Draft for Crude Coconut Oil (Revision of B.S.628).

CH(OSC)8536 — APPENDIX I to CH(OSC)7122 — Draft for Castor Oil (First Quality) (Revision of BS.650).

Revised Draft Specification prepared by Technical Committee OSC/21 — Marine Animal and Fish Oils.

CH(OSC)8870 — Draft for Technical Compound Cod Oil.

A DRAFT SPECIFICATION, CH(RDE)7772, DRAFT BRITISH STANDARD ON METHODS OF TEST FOR SOIL CLASSIFICATION AND COMPACTION, has been issued for comment. Copies of this Draft may be obtained from The British Standards Institution, 28, Victoria Street, London, S.W.1.

The preparation of this British Standard was undertaken by a Committee appointed by the Road Engineering Industry Committee in July, 1943, to consider the preparation of standard methods of test for stabilised soils. It consists of 24 sheets of text dealing with proximate analysis, physical testing and soil compaction tests, 13 sheets of recommended report forms and 24 diagrams of apparatus, graphs and charts; and is of interest, chiefly, to the soil chemist and civil engineer.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Free Fatty Acids in Dried Egg Powders. C. M. Johnson and L. Kline (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 617–619)—In previous work (*Ibid.*, 1946, **18**, 35; ANALYST, **1946**, **71**, 289) differences were reported between the total acidity of the ethereal extract of dried egg powder as determined by the method of the A.O.A.C. and the free fatty acidity of the powder, over 70 per cent. of the acidity of the extracts of some unstored powders being due to cephalin. The amount of cephalin appearing in the extract, in contrast with the free fatty acid content, was influenced by the moisture content of the powder, so that it was necessary to dry the powders uniformly as specified in the A.O.A.C. method in order to obtain reliable total acidity values. When recovery experiments were made, it was found that oleic acid which had been added to liquid egg mixtures was apparently bound in a way that prevented its extraction from the corresponding dried egg powder unless the liquid egg was adjusted to *p*H 4 before drying. Thus reliable free fatty acid values for the dried egg powders could be obtained only by reconstituting the powder, acidifying to *p*H 4 and re-drying before extraction and, in addition, a correction for cephalin was necessary.

A more satisfactory method of determining the true free fatty acid content is now described, and additional information on the combination of free fatty acids in the liquid whole egg is presented. The proposed method makes use of the differential solvent power of acetone at room temperature. Glycerides and fatty acids are soluble in cold acetone whereas the phospholipids are only slightly soluble. Traces of dissolved phospholipid can be removed by treatment with alcoholic magnesium chloride, and the phosphorus content of the treated extract is then less than 0·01 per cent.

Procedure—To 2 g. of dried egg powder in a 40-ml. graduated conical centrifuge tube, add 15 ml. of C.P. acetone and, after 30 min., centrifuge the mixture. Repeat the extraction four times, using 10-ml. portions of acetone and only 5- to 10-min. extraction periods, and stirring the suspensions frequently during the extraction periods. Combine all the extracts in a single centrifuge tube keeping the total volume below 60 ml., add, with rapid swirling, 1 ml. of absolute ethyl alcohol saturated with magnesium chloride hexahydrate, centrifuge the liquid, and transfer the supernatant portion to a 500-ml. separating funnel with the stem cut down to 1 in. Wash the precipitate twice by suspending it in 5 ml. of acetone and centrifuging, and add the washings to the liquid in the separating funnel. To the combined extracts and washings add 30 ml. of light petroleum (Skellysolve B), mix thoroughly, add 200 to 250 ml. of water, and agitate the mixture gently. The volume of acetone in the funnel should be kept as low as possible (60 to 80 ml.) because the acetone concentration in the washing procedure must lie between 20 and 30 per cent. to prevent loss of fatty acids. After separation of the phases, discard the acetone layer and wash the petroleum layer with two 25-ml. portions of water. The washings should give no reaction for chlorides. Transfer the petroleum layer to a 125-ml. glass-stoppered Erlenmeyer flask, add 50 ml. of *iso*propanol and 10 drops of 1 per cent. alcoholic phenolphthalein solution, and bubble a stream of carbon dioxide-free air through the liquid for 10 min. Titrate the solution with 0·025 *N* sodium ethylate, maintaining the stream of carbon dioxide-free air. Express the result as oleic acid, which is said to constitute 50 per cent. of the egg fatty acids (Riemenschneider *et al.*, *J. Biol. Chem.*, 1938, **126**, 255; ANALYST, 1939, **64**, 48).

Recovery of oleic acid was determined by adding known amounts of oleic acid in acetone solution to samples of spray-dried egg powder suspended in acetone. These samples with controls were subjected to the procedure described with satisfactory recovery of the added oleic acid. Samples of the same egg powders were also exhaustively extracted with ether in a Soxhlet apparatus, the ether was removed by a stream of nitrogen, and the residue was dissolved in acetone and treated with magnesium chloride in the manner described. Thus only the effect of different types of extraction was studied. The results showed that the shorter acetone extraction procedure gave results from 2 to 7·5 per cent. lower, but an error of this magnitude is probably permissible for many purposes. The longer Soxhlet extraction with ether may be desirable with some samples. Since, in the proposed method, the cephalin content of the extract is negligible, preliminary drying of the sample is not necessary. As with ether, oleic acid, when added to liquid egg before drying, was incompletely recovered by neutral acetone. Therefore a modified procedure based upon the use of acidified acetone (0·088 *N* with respect to hydrochloric acid) was used with samples to which oleic acid had been added before drying. The acidified acetone was used in the first two extractions and neutral acetone in the remaining three, because high results due to extraction of phosphorus compounds that were not

removed by magnesium chloride treatment were obtained when acidified acetone was used in the last three extractions. Although the *p*H of the suspensions of the residues from the acid acetone extraction was near 4, only 80 to 90 per cent. recovery could be attained by the method, and it is not recommended as a means of obtaining complete extraction of fatty acids from egg samples that may contain bound fatty acids, and the procedure involving reconstitution, adjusting to *p*H 4, and lyophilising is recommended for these. The fatty acids in the dried adjusted powder may then be determined by the proposed method.

Although recovery of oleic acid added to, or present in, liquid egg mixtures before drying is not complete unless the *p*H of the liquid is adjusted to 4, extraction is complete from egg powders which have developed free fatty acidity during storage in the dry state, and the recovery is not increased by the adjustment of the acidity of either spray-dried or lyophilised powders to *p*H 4. If, on the other hand, samples of egg powders in which acidity has developed are reconstituted and allowed to stand in the liquid state at its natural *p*H for a time before being lyophilised, then part of the fatty acids is no longer extractable unless the *p*H is adjusted to 4. These results may be explained by assuming that liberation of fatty acids in the powder occurs in a substantially fatty medium, *i.e.*, the fatty acids are, in a microscopic sense, near the egg lipids, and, for this reason, or because the medium is essentially non-aqueous, the fatty acids have little opportunity to combine with bases. When fatty acids occur in the liquid egg they are free to combine with the bases to an extent determined by the *p*H of the mixture. Experiments showed that, as might be expected, egg white binds fatty acids more readily than egg yolk. The proteins of the white contain many basic groups, and lysozyme, an unusually basic protein constituent of egg white, is known to form salts with strong acids and, doubtless, would react similarly with the higher fatty acids.

The lowest moisture content at which interaction of the fatty acids and proteins occurs is not known. However, egg powders containing as much as 8 per cent. of moisture gave the same free fatty acid values before and after adjustment of the *p*H. Since few dried egg powders are prepared at higher moisture levels, no adjustment of the *p*H of dried egg powders prepared from good shell eggs seems necessary.

The production of lactic acid in certain types of egg deterioration has been reported (Brookes and Hawthorne, *J. Soc. Chem. Ind.*, 1943, **62**, 181), but, since lactic acid is soluble in water, it is not determined by the procedure outlined. The method would not be applicable to material likely to contain fatty acids that are water-soluble, since they would be incompletely partitioned between the aqueous acetone and the petroleum layers in the washing process, thus leading to low results. Since the lower fatty acids are absent from egg fat (Riemenschneider *et al.*, *loc. cit.*), such losses do not occur in the determination of egg fatty acids arising from the usual type of glyceride hydrolysis. Moderate excess of acids added to lower the *p*H of the sample do not interfere in the proposed method, since they are removed during the washing process. A. O. J.

Iodimetric Method for the Assay of Penicillin Preparations. J. F. Alicino (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 619–220)—Penicillin is inert to iodine in neutral aqueous solution, whereas penicilloic acid, formed by inactivation of penicillin by means of alkali or penicillinase, consumes from 6 to 9 equivalents of iodine, depending on the conditions used. The difference between the iodine consumed under standard conditions before and after inactivation by alkali is, with certain limitations, proportional to the penicillin content.

Procedure—Dissolve 3 to 5 mg. of crystalline sodium penicillin II (G) in 5 to 10 ml. of water and add 0·5 ml. of *N* sodium hydroxide. Leave for 15 min. and then neutralise with 0·5 ml. of *N* hydrochloric acid. Add a measured excess (*e.g.*, 10 ml.) of 0·01 *N* iodine and, after 30 min., titrate with 0·01 *N* sodium thiosulphate. Under these conditions, 1 mg. of sodium penicillin II (G) consumes 2·52 ml. of 0·01 *N* iodine, corresponding to 8·97 g. equivalents of iodine per g-mol. Sodium penicillin I (F) under the same conditions consumed 2·64 ml. per mg. or 8·8 g.-equivalents per g.-mol. Penicillin III (X) may give abnormally high values.

In the assay of an unknown, inactivate in a similar manner a solution containing 1000 to 5000 units, add iodine, and back-titrate as described above. Carry out a blank titration in which the inactivation is omitted. For relatively pure preparations (800 to 1000 μg. per mg.) the difference between the two values is proportional to the penicillin content, but crude preparations give results considerably lower than the bioassay values. The discrepancy between iodometric and bioassay values can be reduced by back-titrating the blank solution immediately after addition of the iodine solution, although the alkali-inactivated sample is allowed to stand for 30 min. before titration with the thiosulphate solution.

With this modification, the method gave results in satisfactory agreement with the bioassay values for samples containing from 10 to 97 per cent. of penicillin. F. A. R.

Biochemical

Adsorption of Aneurine on Sand. H. N. Ridyard (*J. Soc. Chem. Ind.*, 1946, **65**, 92–95)—In the course of an investigation of synthetic zeolites it was found that a control column of acid-washed sand completely removed aneurine from an acid aqueous solution, and that the vitamin could be recovered from the sand by elution with saturated potassium chloride solution. Sand was in fact found to be a very convenient adsorbent for aneurine, the best results being obtained with a china-clay sand from St. Austell and with a sand from King's Lynn (Messrs. Boam). The Norfolk sand proved to be the more homogeneous, and was selected for use in the estimation of aneurine in wheat products.

Procedure—Wash the sand about six times with 0·2 *N* hydrochloric acid to remove dirt and fine material, and transfer the acid slurry into a column, 220 mm. long and 15 mm. in diameter. Allow a few ml. of acid to run through the column and then pour 10 ml. of the extract (10 g. of flour or 5 g. of wholemeal or bran per 50 ml.) on to the column. Allow to drain and wash with two 2-ml. portions, followed by about 20 ml., of 0·2 *N* hydrochloric acid. Pour 10 ml. of a saturated potassium chloride solution in 0·2 *N* hydrochloric acid on to the column and allow to drain. Wash the column with 2 or 3 portions of acid water until 2·5 ± 0·2 ml. have collected in a graduated tube placed below the column. Replace this tube by a second tube and continue washing until 13 to 14 ml. have collected. Collect another 3 to 4 ml. in the first tube and retain this solution until it has been confirmed that the whole of the aneurine has collected in the second

tube. Estimate the aneurine in this solution by the thiochrome method, using a standard solution containing the same concentration of salt as the eluate; the presence of the potassium chloride in the eluate affects the partition of the thiochrome between the *iso*butanol and the aqueous layer. The aneurine content should not be calculated from the result obtained with a standard solution that has been passed through a 220 mm. sand column, as only 90 per cent. of the aneurine is recovered on subsequent elution, whereas 100 per cent. is recovered from flour extracts under the same conditions; the difference is due to the presence in the flour extract of adsorbable material other than aneurine. The most suitable standard is prepared by saturating 10 ml. of a standard solution with potassium chloride and diluting to 13 ml. This is used in addition to the usual four standard tubes in each run to fix the extinction-concentration curve. F. A. R.

Microbiological Estimation of Methionine in Proteins and Foodstuffs. W. H. Riesen, B. S. Schweigert, and C. A. Elvehjem (*J. Biol. Chem.*, 1946, **165**, 347–358)—The requirements of methionine by the three organisms *Lactobacillus arabinosus*, *Streptococcus faecalis*, and *Leuconostoc mesenteroides* have been investigated. The following basal media were used:

Constituents	*Lactobacillus arabinosus* (Schweigert *et al.*, *J. Biol. Chem.*, 1944, **155**, 183) mg. per tube	*Streptococcus faecalis* (Greenhut *et al.*, *J. Biol. Chem.*, 1946, **162**, 69) mg. per tube	*Leuconostoc mesenteroides* (Dunn *et al.*, *J. Biol. Chem.*, 1944, **156**, 703) mg. per tube
Amino acids			
Oxidised casein hydrolysate	20 (0·2 ml.)	50 (0·5 ml.)	50 (0·5 ml.)
l(+)-Glutamic acid	4	4	4
l-Asparagine	4	4	4
l(+)-Lysine monohydrochloride+H_2O	2	2	2
dl-Threonine	2	2	2
dl-Valine	2	2	2
dl-*Iso*leucine	2	2	2
dl-Alanine	2	1	2
l(−)-Cystine	1	2	2
dl-Leucine	2	2	2
dl-Phenylalanine	1	1	1
l(+)-Arginine hydrochloride	0·5	0·5	1
l(+)-Histidine hydrochloride+H_2O	0·5	0·5	1
l(−)-Tyrosine	0·5	1	1
dl-Tryptophan	0·5	1	1
Glycine	—	0·2	1
dl-Serine	—	0·5	2
l(−)-Proline	—	—	0·5
Glucose	200	200	200
Sodium acetate	200	—	200
,, citrate+H_2O	—	250	—
Purines and pyrimidines	(0·1 ml.)	Same as *L. arabinosus*	Same as *L. arabinosus*
Adenine sulphate+$2H_2O$	0·1	,, ,,	,, ,,
Guanine hydrochloride+$2H_2O$	0·1	,, ,,	,, ,,
Uracil	0·1	,, ,,	,, ,,
Xanthine	—	0·1 (0·1 ml.)	0·1 (0·1 ml.)
Salts A	(0·05 ml.)	—	Same as *L. arabinosus*
KH_2PO_4	5	—	,, ,,
K_2HPO_4	5	50 (0·1 ml.)	,, ,,
Salts B	(0·05 ml.)	Same as *L. arabinosus*	,, ,,
$MgSO_4.7H_2O$	2	,, ,,	,, ,,
NaCl	0·1	,, ,,	,, ,,
$FeSO_4.7H_2O$	0·1	,, ,,	,, ,,
$MnSO_4.4H_2O$	0·1	,, ,,	,, ,,
	μg. per tube	μg. per tube	μg. per tube
B-vitamins	(0·1 ml.)	,, ,,	,, ,,
Aneurine hydrochloride	5	,, ,,	,, ,,
Riboflavine	5	,, ,,	,, ,,
Nicotinic acid	10	,, ,,	,, ,,
dl-Calcium pantothenate	5	,, ,,	,, ,,
Pyridoxamine dihydrochloride	12	,, ,,	,, ,,
Pyridoxine hydrochloride	50	,, ,,	,, ,,
p-Aminobenzoic acid	5	,, ,,	,, ,,
Biotin	0·01	,, ,,	,, ,,
Folic acid	0·1	,, ,,	,, ,,
Choline chloride	25	,, ,,	,, ,,
i-Inositol	25	,, ,,	,, ,,

Stock solutions were prepared in the concentrations indicated above. Several millilitres of hydrochloric and sulphuric acid were added to Salts B to prevent precipitation. The purines and pyrimidines were dissolved in hot hydrochloric acid. Xanthine was dissolved in aqueous ammonia. The B vitamins were dissolved by heating in water. New vitamin solutions were prepared every month. Convenient volumes for all solutions were 500 ml. They were stored under toluene in a refrigerator. The amino acids were ground with a mortar and pestle, and dissolved in a few millilitres of dilute hydrochloric acid prior to each assay. Oxidised casein hydrolysate was prepared by the method of Toennies (*J. Biol. Chem.*, 1942, **145**, 667), which is as follows:

Dissolve 400 g. of casein in 2·4 litres of formic acid, and rapidly add 400 ml. of 30 per cent. hydrogen peroxide with stirring. After 2–3 mins., cease stirring and leave for 1 hour. Add 10 litres of water and add, with stirring and cooling, ammonia to *p*H 4·0 to 4·5; the temperature should not exceed 35° C. Filter and re-suspend the solid in 15 litres of water. Again filter, re-suspend in water, filter, and suspend in 12 litres of methanol; allow to settle overnight, filter, and again suspend in methanol. After standing overnight, filter, and dry the solid in a desiccator and then in a vacuum drying oven at 70° C.

The standard curves obtained with the three organisms indicated that all could be used for the assay of methionine. The maximum growth obtained with *Lactobacillus arabinosus* was greater than that obtained with the other two organisms, whilst smaller amounts of methionine resulted in a greater growth response with this organism than with the others. A suitable assay range with *L. arabinosus* was 0 to 20 μg. of *l*-methionine per tube as compared with 0 to 50 μg. for the other two organisms. Good recoveries of methionine added prior to the hydrolysis of proteins (using 2 *N* hydrochloric acid at 15 lbs. pressure for 5 to 10 hours) were obtained, and the methionine contents of the proteins analysed agreed satisfactorily with the values recorded in the literature. F. A. R.

Use of Hydrogen Peroxide-treated Peptone in Media for the Microbiological Determination of Amino Acids. C. M. Lyman, O. Moseley, S. Wood, and F. Hale (*Arch. Biochem.*, 1946, **10**, 427–431)—Media for the micro-biological estimation of amino acids can be prepared more cheaply by preferential destruction or removal of certain amino acids from protein than by the use of pure amino acids. A method of destroying methionine, cystine, tryptophan, and tyrosine in peptone by treatment with hydrogen peroxide is described (*cf.* Toennies, *J. Biol. Chem.*, 1942, **145**, 667). Smooth and regular standard curves for methionine, tryptophan, and tyrosine were obtained with media in which most of the amino acid nitrogen was supplied by hydrogen peroxide-treated peptone.

Method—Treat 50 g. of Bacto-peptone in 500 ml. of *N*-hydrochloric acid with 0·05 g.-mol. of hydrogen peroxide (5·7 g. of 30 per cent. hydrogen peroxide) and leave overnight at room temperature. Heat the solution in a steam steriliser at atmospheric pressure for 30 mins., cool, neutralise with sodium hydroxide, and steam again for 1 hr. After cooling, dilute to 1 litre.

The medium used for tryptophan is prepared by replacing the amino acids in the medium of Kuiken *et al.* (*J. Biol. Chem.*, 1943, **151**, 615; ANALYST, 1944, **69**, 156) by 50 mg. of hydrogen peroxide-treated peptone supplemented by 1 mg. each of methionine, cystine, and tyrosine per 10 ml. The medium used for tyrosine is prepared by replacing the amino acids in the medium of McMahan and Snell (*J. Biol. Chem.*, 1944, **152**, 83; ANALYST, 1944, **69**, 188) by 50 mg. of the treated peptone, supplemented by 0·5 mg. of tryptophan and 1 mg. each of methionine and cystine per 10 ml. The medium used for methionine is prepared by replacing the amino acid mixture in medium D of Dunn *et al.* (*J. Biol. Chem.*, 1944, **156**, 703; ANALYST, 1945, **70**, 182) by 75 mg. of the treated peptone, supplemented by 0·5 mg. of tryptophan and 1 mg. each of cystine and tyrosine per 10 ml. F. A. R.

Colorimetric Method for the Estimation of Vitamin D, based on Formation of Carbenium Salts. H. Schaltegger (*Helv. Chim. Acta*, 1946, **29**, 285–302)—Ergosterol and calciferol give characteristic colours with aromatic aldehydes in the presence of concentrated sulphuric acid, owing to the formation of carbenium sulphates, the sterol in the ionised state behaving as a weak base; different aldehydes produce different colours. Perchloric acid can be used in place of sulphuric acid, with benzene and glacial acetic acid as solvents. Ionising solvents, such as alcohols, should be absent. The colours formed with certain aldehydes are quite stable and can be used for the estimation not only of ergosterol and calciferol, but also of cholesterol and other sterol derivatives, the colours being evaluated at appropriate wavelengths. Three types of reaction can be distinguished, according to the number of ionisable carbon atoms present in the molecule:

Type 1—All sterols containing, besides the one ionisable ethylene group, no other double bonds or only double bonds not in conjugation with a co-ordinate linkage, give only small quantities of carbenium salt and thus give only feeble colours.

Type 2—All sterols containing conjugated double bonds with two ionisable carbon atoms, *e.g.*, ergosterol and 7-dehydrocholesterol, yield considerable amounts of carbenium salts, giving more intense colours than compounds of Type 1.

Type 3—All the D vitamins and tachysterols containing three conjugated double bonds in a chain give carbenium salts with colours 10 times as intense as the salt derived from ergosterol.

Bile acids give feeble colours with aromatic aldehydes and do not interfere with the estimation of vitamin D. β-Carotene and vitamin A, however, give colours similar to those obtained with vitamin D. Aneurine, riboflavine, ascorbic acid, tocopherol, vitamin K, and linoleic acid do not give colours with the reagent. For the estimation of vitamin D, three aldehydes proved to be particularly suitable namely, anisaldehyde, vanillin, and 4-hydroxy-1-naphthaldehyde, which give bluish-violet, blue, and green colours, respectively.

Procedure—For the estimation of vitamin D in irradiated ergosterol or irradiated 7-dehydrocholesterol, remove unchanged ergosterol by dissolving in alcohol, cooling to 0° C., and filtering off the crystalline solid. Evaporate to dryness a portion of the filtrate containing 0·2 to 0·3 g. of dry matter and leave in a desiccator for 2 hr. Dissolve the residue in benzene and make up to a volume of 20 ml. To 2 ml. of this solution add from 0·1 to 0·15 ml. of acetic anhydride and 5 ml. of benzene, and heat for half an hour at 75° C. Destroy the tachysterol in the acetylated mixture by adding 10 to 15 mg. of freshly distilled maleic anhydride and 5 ml. of benzene and then warming

for 20 to 30 min. at 75° C. Cool the solution, filter off the adduct and dilute the filtrate to 20 ml. with benzene. Dilute this solution 10-fold and mix 1 ml. of the dilute solution with 1 ml. of a 0·1 per cent. solution of 4-hydroxy-1-naphthaldehyde in benzene and 1·5 ml. of benzene (purified by being heated under reflux for 2 hr. with anhydrous aluminium chloride, distilled, washed with water, dried with sodium sulphate, and re-distilled). Heat to boiling and add 2 drops of perchloric acid reagent (mix 2 ml. of acetic anhydride, 2·5 ml. of glacial acetic acid and 0·5 ml. of 70 per cent. perchloric acid, and heat under reflux for half an hour in an oil-bath at 95 to 100° C. Use a pipette that delivers a drop weighing approximately 20 mg.) Heat for a further minute and then allow to cool to room temperature. Add 1·5 ml. of glacial acetic acid and measure the extinction value (K) of a 1-cm. layer using filter S.66. The amount of vitamin D is equivalent to $150\,K + 10$. The corresponding values when anisaldehyde and vanillin are used are $62{\cdot}1\,K + 2{\cdot}5$ and $106{\cdot}5\,K + 2{\cdot}75$, respectively. These aldehydes are also employed in the form of 0·1 per cent. solutions in benzene, but filter S.57 is used instead of S.66. This estimation gives the approximate amount of vitamin D present. To obtain a more accurate value, repeat the procedure using a volume of the final benzene solution such that 50 to 80 µg. of vitamin D are present. Mix this with 1 ml. of the anisaldehyde reagent and dilute with benzene to 3·5 ml. Heat to boiling, add 2 drops of the perchloric acid reagent, heat for a further minute, and allow to cool. Add 1·5 ml. of glacial acetic acid and evaluate the colour as before.

To estimate tachysterol repeat the estimation, omitting the maleic anhydride treatment, and from the result subtract the value previously obtained.

Vitamin D in fish oils containing at least 500,000 I.U. (12·5 mg.) per ml. can be estimated directly without saponification, but a blank estimation must be carried out on the oil. Oils containing less than 500,000 I.U. per ml. must first be saponified, and the reaction applied to the non-saponifiable matter.

The results obtained with solutions of pure vitamins D_2 or D_3 seldom differed from the theoretical by more than 5 per cent. F. A. R.

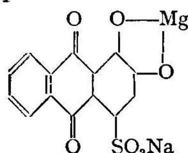
Colorimetric Method for the Estimation of Reducing Steroids. R. D. H. Heard and H. Sobel (*J. Biol. Chem.*, 1946, **165**, 687–698)—The reduction of phosphomolybdic acid to molybdenum blue has been applied to the quantitative estimation of small amounts (10 to 100 µg.) of reducing steroids related to the adrenal cortex hormone. The reaction is given by steroids containing a primary or secondary, but not tertiary, α-ketol function, or an αβ-unsaturated 3-keto group, or both.

Procedure—Put 0·1 ml. of a solution of the steroid in glacial acetic acid, containing the equivalent of about 100 µg. of desoxycorticosterone, into a small test tube and add 2·00 ml. of phosphomolybdic acid reagent prepared by mixing equal volumes of glacial acetic acid and the phosphomolybdic acid solution of Folin and Wu (*J. Biol. Chem.*, 1920, **41**, 367). Immerse the tube in a constant-volume bath of boiling water for exactly 60 min., cool immediately, and transfer the contents of the tube quantitatively with 8·00 ml. of the reagent to one of a pair of optically matched Evelyn colorimeter tubes. After allowing air bubbles to rise to the surface (about 90 sec.), read within the next 4 min. the optical density at 650 to 660 mµ. Run a blank estimation simultaneously in the same way with 0·10 ml. of glacial acetic acid and 2·00 ml. of reagent, and use the solution obtained to adjust the colorimeter to zero optical density. The results are calculated from a standard curve prepared with the aid of pure desoxycorticosterone. F. A. R.

Detection of Alkaline Earth Metals in the Cell Tissue of Plants. R. Haller (*Helv. Chim. Acta*, 1946, **29**, 8–11)—In a study of the reactions of the mucilage of the endosperm of *Ceratonia siliqua* (Carob bean) it was noticed that the sodium salt of alizarin monosulphonic acid (alizarin red SW or alizarin S), although not staining the mucilage, gave a characteristic red colour to certain cell contents. Formation of this colour is not entirely due to protein since protein yields a brown colour and not the intense red colour of this reaction. Development of the red colour indicates the presence of metallic salts forming complex compounds with alizarin. Calcium and barium compounds form vivid blue-violet lakes, and aluminium and magnesium compounds form red lakes. Aluminium is found only exceptionally in a few domestic Lycopodium species and in certain tropical plants, and, as far as the author knows, barium salts do not occur in plants. [*Abstractor's note*: Seaber, ANALYST 1933, **58**, 575, reports the occurrence of barium in Brazil nuts.] Magnesium, however, is an essential constituent of chlorophyll, and it seemed probable that magnesium salts were responsible for the formation of the red colour.

To determine if the colour can be observed in fresh cell tissue, experiments were made with sections of the leaf stalk of *Cucurbita pepo*, L. When sections are placed in a solution of alizarin monosulphonic acid and observed microscopically, a vivid scarlet colour appears after a short time especially near the vascular bundles and in the cell contents of the parenchyma. Gradually the mounting liquid is coloured red by escape of the cell contents from the tissue. The epidermis, however, with the exception of the outermost layer (the true cuticle), remains uncoloured. The cuticle is coloured intensely red, as are the tissues in the neighbourhood of the vascular bundles, and this is true also of the tissue of *Urtica dioica* L. and *Heracleum spondylum* L. Apparently, the magnesium compounds occur in the cell sap and are transported thence for the synthesis of chlorophyll. Tissues containing magnesium entirely in the form of chlorophyll do not give the reaction. If the leaf stalk of *C. pepo* is hardened in alcohol and sections are subjected to the reaction, very little colour develops, but if the hardening alcohol is treated with sodium alizarin monosulphonate a vivid red precipitate is thrown out, so that evidently the magnesium salt is extracted from the tissue. When sections of the stem of *Orobranche lucorum* L. (a parasite on *Berberis* spp.), a plant containing no chlorophyll, are placed in an aqueous solution of sodium alizarin monosulphonate and examined microscopically, no trace of colour is evident and, as might be expected, no magnesium compounds occur in the cell sap of this plant If pieces of the stem of *C. pepo* are placed in alcohol for 24 hr. and the pale green alcoholic solution is treated with alcoholic sodium alizarin monosulphonate, a red precipitate forms indicating solubility of the magnesium compound in alcohol. This red precipitate

is soluble in water forming a red solution, and it may be the compound



Its neutral aqueous solution dyes wool an intense purple red, a colour quite different from that formed with sodium alizarin sulphonate alone. When sections of the stem of *C. pepo* are mounted in silver nitrate solution and examined microscopically, a precipitate darkened by the action of daylight, and soluble in ammonia, forms in the cell sap, indicating that magnesium is present as chloride, which is appreciably soluble in alcohol. The presence of magnesium can be demonstrated by extracting the ignited red precipitate with diluted sulphuric acid and separating magnesium as magnesium ammonium phosphate; the sensitive test of Hahn with quinalizarin (tetraoxy-anthraquinone) can also be applied, and calcium does not interfere with this test. The reagent is insoluble in water, but yields a blue-violet solution when *N* alkali is added to its aqueous suspension. When a solution of a magnesium compound is added to this solution a pure pale blue colour develops and very soon a light blue precipitate forms even in very dilute solutions. The reaction is not applicable to plant sections. The cell walls are coloured a very pale blue, but the characteristic blue precipitate cannot be obtained, possibly owing to the protective colloid action of proteins.

Two East Indian plants, *viz.*, *Symplocos fasciculata* Zoll. and *S. spicata* Roxb., are used by the natives in the dyeing of cotton fabric. Fragments of the leaves of these plants, when immersed in alizarin red SW solution gave a reddish granular precipitate due to presence of aluminium compounds. Magnesium was found to be present in the ash of the leaves of *S. spicata*, but apparently plays no part in the formation of the red lake. When every trace of calcium was removed from cotton fabric and the dyeing process was carried out in a mordant solution containing aluminium and magnesium acetates, the colour produced was an unstable pale rose. In presence of calcium salts, however, an intense fast red colour was imparted to the fabric. A. O. J.

Organic

Indicator Properties of Derivatives of 4′-Nitrophenylazo-1-naphthol. K. H. Ferber (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 631–632)—Three new indicators of the 2-(4′-nitrophenylazo)-1-naphthol class, changing from a red neutral form to a blue alkaline form over various *p*H ranges, are described.

Preparation—I. *Disodium salt of* 2-(4′-*nitrophenylazo*)-1-*naphthol*-4:8-*disulphonic acid. Alpha blue*—To a stirred mixture of 1000 g. ice, 250 ml. of hydrochloric acid (20° Bé), and 2 g. of 95 per cent. sodium nitrite, add a mixture of 138 g. of *p*-nitraniline and 72 g. of sodium nitrite in 1 litre of water. After 2 hrs., filter and add the filtrate slowly to a mixture of 1 g.-mol. of 1-naphthol-4:8-disulphonic acid, 370 g. of sodium carbonate, 1000 g. of ice, and 1·5 l. of water. Agitate overnight, filter, re-slurry in 3 l. of 10 per cent. sodium chloride solution, filter, and dry at 60° C. The reported purity of the product, determined by titration with titanous sulphate, is 67·1 per cent. coal-tar dye. II. *Disodium salt of* 2-(4′-*nitrophenylazo*)-1-*naphthol*-3:8-*disulphonic acid. Epsilon blue.*—Diazotise 1 g.-mol. of *p*-nitraniline as above, and then add the product slowly to a mixture of 1 g.-mol. of 1 naphthol-3:8-disulphonic acid, 336 g. of sodium carbonate, 1000 g. of ice, and 2 l. of water. Isolate and dry the dye as above. The reported purity of the product is 93·7 per cent. III. *Disodium salt of* 2-(2′-*methoxy*-4′-*nitrophenylazo*)-1-*naphthol*-4:8-*disulphonic acid. Nitroanisole blue*—Add slowly to a mixture of 168 g. of 5-nitro-2-aminoanisole, 250 ml. of hydrochloric acid (20° Bé), and 1000 g. of ice, a solution of 74 g. of 95 per cent. sodium nitrite in 150 ml. of water. After 2 hrs.' agitation, filter and couple with 1-naphthol-4:8-disulphonic acid by the method described above for Alpha blue. The reported purity of the product is 74·1 per cent. As the products contain large amounts of inorganic salts, further purification is necessary if they are to be used in unbuffered media.

Spectral transmission curves of solutions, buffered to various *p*H values and containing 10 p.p.m. of the dye, are given for each of the indicators. The presence of boric acid in Clark and Lubs' buffers for *p*H 9 and 10 was found to interfere with the dissolved dye. The colours of the solutions are: Alpha blue, at *p*H 8, pink; *p*H 9, purplish pink; and *p*H 10 to 13, light purple. Epsilon blue, *p*H 8 to 11, pink; *p*H 12, purplish pink; and *p*H 13, light purple. Nitroanisole blue, *p*H 8 and 9, pink; *p*H 10, purplish pink; and *p*H 11 to 13, light purple. Solutions of all three dyes in water, alcohol, *N* hydrochloric acid, 5 per cent. acetic acid solution, 5 per cent. sodium bicarbonate solution, and 5 per cent. solution of $Na_2HPO_4.12H_2O$ are pink. Alpha blue and Epsilon blue are light purple with 5 per cent. sodium carbonate solution, and 0·1 *N* sodium hydroxide. Epsilon blue is pale bluish purple with a 5 per cent. solution of $Na_3PO_4.12H_2O$. Alpha blue is useful for distinguishing between carbonate and bicarbonate, and Epsilon blue is recommended for controlling the alkalinity of di- and tri-sodium phosphate mixtures. Dilute aqueous solutions are only slightly affected by storage for one month in diffused daylight. To make test papers, immerse strips of filter paper in a solution of 4 g. of the dye in one litre of water, and allow to dry. The colours obtained when various reagents are spotted on such test papers are listed in terms of the matching chips from the Munsell *Book of Colour.* B. A.

Comparative Estimations of Carbonyl and Carboxyl Groups in Chromium Trioxide and Hypochlorous Acid Oxycelluloses and Oxyxylans. B. Meesook and C. B. Purves (*Paper Trade J.*, 1946, **123**, Oct. 31, *T.A.P.P.I. Sect.*, 223-230)—(*a*) *Calcium Acetate Method* (*cf.* Yackel and Kenyon, *J. Amer. Chem. Soc.*, 1942, **121**, 64)—This depends on the titration with alkali of the acetic acid displaced from calcium acetate. Prepare a 0·5 *N* solution (*p*H 7·2 to 7·5) of the calcium acetate in boiling water, and filter it. Immerse *x* (0·2 to 0·5) g. of the sample in a 60-ml. aliquot portion of the solution, and after the requisite time (*e.g.*, 24 hr., *vide infra*), filter it, and titrate a 50-ml. aliquot portion of the filtrate electrometrically to *p*H 8·3 with 0·01 *N* sodium hydroxide. If, during the experiment, the *p*H falls to below 6·3, low

results may be expected; then, repeat the experiment using a smaller weight of sample, or in presence of a known volume of standard alkali, which is added 1 hr. after the immersion of the sample, and which is less than the amount required to bring the *p*H to above 7·0. If *y* ml. of *N* alkali is the difference between the titres obtained with the sample and with a "blank," then the carboxyl content is $6y/5000x$ g.-mols. per g. (*b*) *Silver o-Nitrophenolate Method*—Warm 10 g. of *o*-nitrophenol with 500 ml. of water to 70° C., add 15 g. of silver oxide, with stirring, and after 2 hr. filter from the excess of silver oxide. Allow the solution to cool, filter off the orange-coloured prisms, dry them, and store them in a brown bottle. Warm the filtrate with 2 to 3 g. of silver oxide, filter, dilute the filtrate to 1·5 litres, and store it in a brown bottle. This solution is the reagent (*p*H 7·3); further supplies are made by dissolving 1 g. of the crystals in 1 litre of warm water. Standardise 25-ml. portions of the solution by titration with 0·04 *N* ammonium thiocyanate in presence of 5 ml. of 6 *N* nitric acid (free from nitrous acid), and 1 ml. of saturated ferric ammonium sulphate solution as internal indicator; the silver nitrophenolate solution is 0·00495 *N*, and remains so for about 6 weeks. Immerse *x* gram (*e.g.*, 0·5 g.) of the sample in 100 ml. of the reagent at 20° C. for about 72 hr., and titrate 25-ml. portions of the resulting solution; if the reduction in the titration as compared with the standard is *y* ml. of *N* thiocyanate solution, then the carboxyl content is $4y/1000x$ g.-mol. per g. (*c*) *Methylhydroxylamine Hydrochloride Method*—Dissolve 7·0 g. of the pure methylhydroxylamine hydrochloride in a mixture of 455 ml. of water and 91 ml. of 0·49 *N* sodium hydroxide (*p*H 4·6). Immerse *x* g. of the sample (*i.e.*, approx. 2 g. of oxycellulose, or 0·2 g. of reducing sugars) in 60 ml. of the solution for about 24 hr., remove the solid matter on a dry filter, and titrate a suitable aliquot portion (*e.g.*, 50 ml.) of the filtrate to *p*H 3·5 electrometrically with 0·1 *N* hydrochloric acid; adjust the volume with distilled water to that used in a blank titration previously made, and complete the titration to *p*H 3·0. Then the carbonyl content equals $6y/5000x$ g.-mol. per g., where *y* ml. of *N* acid is the difference between the titrations obtained with the sample and with the blank. If the determination is carried out on the neutral calcium oxycellulose salt formed in Method (*a*), then the result gives carbonyl only; with acidic samples, however, carbonyl plus carboxyl are given. With all of these methods the results above can be converted to g. mol. per g. mol. of sample by multiplying by 162 for oxycellulose, or 132 for oxyxylan. Methods (*a*) and (*b*) require 1 and 3 days respectively for completion at 20° C., and under these conditions the results agree to within ±3 per cent. The sharp end-point of (*b*) makes it preferable when the number of acidic groups is small, but (*a*) is preferable for (*e.g.*, hypochlorite) oxycelluloses which contain reducing groups, since with (*b*), metallic silver is sometimes produced by reduction. The reaction of Method (*c*) is approximately 95 per cent. complete in 1 day at 20° C., and the results then agree with those obtained in 1·5 hr. with hydroxylamine hydrochloride (*cf.* Gladding and Purves, *T.A.P.P.I. Papers*, 1943, **26**, 119) to within ±3 per cent.; apart from the consideration of rapidity, (*c*) is, however, preferable to the latter method in that the reagent is stable for days instead of only for hours. Applications of the methods to the study of the oxidation of cellulose by various reagents are described. J. G.

Picric Acid Method for Determination of Aromatic Content of Aviation Gasoline. C. M. Gambrill and J. B. Martin (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 689–692)—The method of Dobryanskiĭ and Tikhonov-Dubrovskiĭ (Azerbaodzhanskoe Neftyanoe Khoz., 1935, No. 5, 84) utilised the difference in solubility of picric acid in aromatic and non-aromatic hydrocarbons to determine the aromatic content of aviation gasoline; it has been developed to a more precise and rapid form, accurate to within ±1 per cent. in the range up to 25 per cent of aromatics.

Method—60 ml. of the gasoline are measured into a 125-ml. Squibb-type separatory funnel containing 1 g. of pure picric acid, preferably dry, but in no case containing over 10 per cent. of water. The mixture is brought to between 20 and 30° C. and shaken for 5 min. mechanically or by hand; if shaken by hand, the vessel is held by the finger tips to prevent change in temperature. The temperature is recorded, and the liquid is filtered through a Whatman No. 1 or other fast paper into a 125-ml. conical flask. A 50-ml. sample of the filtrate is pipetted to a 250-ml. flask, 3 drops of *m*-cresol purple indicator are added, and the liquid is titrated with 0·05 *N* alkali until it becomes purple; a 1-ml. excess of alkali is added. The mixture is then shaken for 1 minute vigorously, the two layers are allowed to separate, and the excess of alkali is titrated with 0·01 *N* hydrochloric acid to the complete disappearance of the reddish-purple colour as viewed under a tungsten filament lamp. The number of mg. of picric acid per 100-ml. of the saturated solution = 458·2 × (ml. of *N* sodium hydroxide − ml. of *N* hydrochloric acid). This is corrected to solubility at 25° C. by multiplying it by $[1 + 0{\cdot}035\,(25-t)]$, where *t* is the temperature of determination. Percentage of aromatics is read off from a curve drawn from the following data:

Percentage of aromatics	Solubility of picric acid at 25° C. (mg. per 100 ml.)
2	18·5
5	32·8
10	67·3
15	119·8
25	297·8

The mixture of aromatics used in obtaining these figures was one of benzene : toluene : xylene, 1:3:2, which the authors claim to give a good average representation of most aviation gasoline. The presence of olefins and naphthenes necessitates corrections of −1·4 per cent. for each 10 per cent. of olefins and −0·6 per cent. for each 10 per cent. of naphthenes. Reproducibility is about ±0·2 per cent.

Determination of some Aromatic Amines and Substituted Ureas in Smokeless Powder. Improved Volumetric Bromination Procedure. T. D. Waugh, G. Harbottle, and H. M. Noyes (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 636–637)—For the quantitative determination of stabilisers in smokeless powders a volumetric bromination procedure has been widely used. This involves treatment of a solution of the powder extract with a known amount of standard bromate-bromide solution, acidification to liberate bromine, addition of iodide at the end of the bromination period, and titration of the liberated iodine with standard sodium thiosulphate solution. This method has been used in the determination of diphenylamine,

ethyl centralite (N,N′-diethyl-N,N′-diphenylurea), and acardite (N,N′-diphenylurea). Under the conditions of the procedure, 1 molecule of diphenylamine reacts with 4 molecules of bromine, and 1 molecule of ethyl centralite, or acardite reacts with 2 molecules of bromine. In the procedure developed for centralite by Levenson (*Ibid.*, 1930, **2**, 246), the bromination and titration are carried out in a one-phase system in presence of alcohol to keep the stabiliser in solution under carefully controlled conditions of temperature and time in order to obtain quantitative bromination of the stabiliser without side reactions between the bromine and the alcohol. To avoid this precise control, Ellington and Beard (*J. Soc. Chem. Ind.*, 1931, **50**, 151T) used carbon tetrachloride as solvent, and carried out the bromination and titration in the resulting two-phase system. It is now found that the advantages of both procedures may be obtained by the use of glacial acetic acid as the solvent. In most procedures for the estimation of stabilisers in smokeless powder the sample is extracted directly with ether, or is decomposed by alkali, distilled with steam, and the distillate extracted with ether. When the powder was prepared by either of these procedures for the carbon tetrachloride bromination method, results were low by as much as 10 per cent. Subsequent investigation showed this to be due to peroxides in the ether, and the replacement of ether by methylene chloride as extracting solvent led to satisfactory results.

Procedure—Place the finely divided powder containing not more than 0·075 g. of ethyl centralite, 0·02 g. of diphenylamine, or 0·06 g. of acardite into a Soxhlet extractor attached to a 250-ml., glass-stoppered iodination flask containing 100 ml. of methylene chloride. Extract the sample for 2 hr. or more, according to its state of subdivision. Detach the flask, and remove the solvent by means of a stream of dry air with the necessary precautions to prevent detonation of nitroglycerin in the extract. Dissolve the residue in 60 ml. of glacial acetic acid, and add 25 ml. of 0·1 *N* bromate-bromide (2·784 g. of potassium bromate and 15 g. of potassium bromide per litre) from a pipette. Add 5 ml. of concentrated hydrochloric acid, stopper the flask, and mix the contents. Allow the bromination to proceed for 1 ± 0·25 min. from the time of acidification if centralite or diphenylamine is being determined, or at least 5 min. for acardite. Add 10 ml. of 15 per cent. potassium iodide solution, swirl the flask, and rinse down the gutter and walls of the flask with distilled water. Titrate the solution immediately with 0·05 *N* sodium thiosulphate, adding 5 ml. of 0·5 per cent. starch solution as the end-point is approached. Make a blank determination with 60 ml. of glacial acetic acid. The amount per cent. of stabiliser in the sample is given by the expression $\frac{(1 - A/B)NVC}{W}$, where *A* (ml.) is the volume of thiosulphate solution consumed in titration of the sample, *B* (ml.) is the volume of thiosulphate solution consumed in the blank determination, *N* is the normality of the bromate-bromide solution, *V* (ml.) is the volume of the pipette, *W* is the wt. of sample (g.) and *C* is one-tenth of the equivalent weight of stabiliser (6·709 for centralite, 2·115 for diphenylamine, and 5·306 for acardite).

Nitroglycerin remaining in the solution at the end of the titration should be destroyed by boiling with an excess of ferrous chloride, or by some other appropriate method. Nitroglycerin and diethyl phthalate do not interfere with the method. The method in the form given is not applicable in presence of more than one stabiliser. Typical data are recorded. A. O. J.

Inorganic

New Principle of Fractionation of the Rare Earths with Nitrilo-triacetate. Separation of Lanthanum and Cerium. G. Beck (*Helv. Chim. Acta.*, 1946, **29**, 357–360)—The formation of stable complexes by nitrilo-triacetic acid was reported by Pfeiffer and Offerman (*Ber.*, 1942, **75**, 1), and G. Schwarzenbach *et al.* (*Helv. Chim. Acta*, 1943, **26**, 418, 452; 1945, **28**, 828, 1133). Their extreme stability is exemplified by the fact that the alkaline earth derivatives give precipitates only slowly with oxalates and carbonates, and suggests the suitability of using this reagent for separating the rare earths. Their difficultly-soluble oxalates and fluorides dissolve readily in weakly alkaline solutions of nitrilo-triacetic acid, and are reprecipitated by cautious acidification. The lanthanum salt, probably $Na_3[La(N(CH_2COO)_3)_2]$, is the least stable of the series, and gradual addition of ammonium oxalate precipitates a lanthanum-containing oxalate at a *p*H of about 6. Praseodymium and neodymium oxalates come down on further acidification, and samarium at about *p*H 5, gadolinium at approximately *p*H 4·5, and erbium at *p*H 4. These *p*H values apply in the presence of a large excess of reagent, otherwise precipitation occurs at higher *p*H.

Quadrivalent cerium in weakly alkaline sodium nitrilo-triacetate gives with hydrogen peroxide an orange, hydrated ceric oxide. The decrease in *p*H due to the liberation of acid is compensated for by adding ammonia. The cerium is free from didymium, and the reaction is selective, and sensitive to 8 γ of cerium per ml. Under similar conditions lead salts give a yellowish-brown precipitate, which is soluble in an excess of reagent; titanium gives no colouration; manganese is not oxidised by peroxide; thallium gives a precipitate under certain conditions, but both the soluble and the insoluble complexes give thallous iodide with potassium iodide. At *p*H 9 to 10, the rare earth nitrilo-triacetates give hydroxides or basic salts, lanthanum precipitating first and in a much enriched condition. Cerium, like titanium (*p*H 4·5), thorium, and zirconium ($<p$H 5·4) is soluble in weakly acid solutions. At *p*H 5·5, the zirconium complex is sufficiently stable to hinder precipitation by phosphate ions; at *p*H 5·3, precipitation of phosphate commences and increases with decreasing *p*H. Thorium dissolves in acetic acid solutions of nitrilo-acetate, and oxalate ions give no precipitate until the solution is acidified with hydrochloric acid to *p*H 3·8. Zirconium gives no precipitate under these conditions.

Precipitation of lanthanum—From a nitric acid solution of lanthanum oxide, containing about 10 per cent. of praseodymium and neodymium, precipitate the impure oxalates completely with saturated ammonium oxalate solution, dissolve the precipitate in weakly alkaline sodium nitrilo-triacetate solution, and acidify to *p*H 6·6 with 30 per cent. acetic acid to precipitate lanthanum oxalate. The oxalate thus obtained gives on ignition a pure, white oxide,—about 80 per cent. of the impure material, which after dissolution in nitric acid, gives no praseodymium spectrum and only the strongest of the neodymium lines. The remaining 20 per cent. is thrown down at *p*H 4·3 as an oxalate that yields a brownish oxide on ignition.

A solution of technical didymium oxide, containing much praseodymium, neodymium, and samarium, in 40 ml. of sodium nitrilo-triacetate solution is treated with 30 ml. of ammonium oxalate solution, and brought to *p*H 5·9 by means of acetic acid. The oxalate obtained gives about 10 per cent. of pure white oxide containing no praseodymium or neodymium . Addition of another 30 ml. of oxalate solution to the filtrate (*p*H 5·5) gives a precipitate which yields 15 per cent. of blue-white oxide containing some neodymium. A fraction at *p*H 5 yields a blue oxide containing traces of praseodymium. The next fraction contains much praseodymium. Thus, neodymium is precipitated between lanthanum and praseodymium. Absorption lines are moved slightly towards the red end of the spectrum in the nitrilo-triacetate complex.

The method can be applied also to samarium-gadolinium mixtures. Gradual acidification of a solution of the mixture in the alkaline reagent results at *p*H 5·5 to 6·9 in a precipitate giving neodymium and samarium spectra; at *p*H 5·2 to 4·4, in a precipitate giving only a weak neodymium spectrum; and at *p*H 3·4 to 3·0, in one giving only very weak spectra. On combining the precipitates obtained at *p*H 4·4 to 3 and reacidifying to *p*H 3, about 4·5 per cent. of the original material, giving a colourless solution and very faint spectra, and consisting of pure gadolinium is obtained. Earths of the erbium series precipitate at a much lower *p*H than those of the cerium series.

A solution of sodium lanthanum nitrilo-triacetate gives with cobalt hexammino nitrate an insoluble precipitate of yellow rhombic crystals of the constitution $[Co(NH_3)_6][La\{N(CH_2.COO)_3\}_2]$. Other rare earths give similar precipitates, but there is a tendency to supersaturation. Cerium gives hexagonal plates, thallium forms in a micro-crystalline condition, but thorium, zirconium, aluminium, iron, and bismuth give no precipitates. M. E. D.

Anomalous Behaviour of some Oxidising Agents. R. K. McAlpine (*J. Chem. Educ.*, 1946, **23**, 301–305)—From a consideration of the oxidation potentials and corresponding half-reactions of sodium hypochlorite, hydrogen peroxide, and potassium persulphate, it is to be expected that the efficiency of potassium persulphate as an oxidising agent will be unaffected by the acidity of the medium, whilst the reduction of the other reagents is accompanied by the production of hydroxyl ions. Thus, at *p*H 9, the persulphate is much the strongest oxidising reagent, while hydrogen peroxide is slightly the stronger of the other two. The reducing substances used were the manganous ion, the cobaltous ammonium ion, and nickelous hydroxide. The concentrations of the oxidising solutions were sodium hypochlorite 1·3 *N*, hydrogen peroxide 1·7 *N*, and saturated potassium persulphate 0·3 *N*. Theoretically, all three reagents should oxidise the manganous ion through the tervalent state to manganese dioxide, the cobaltous ammonium ion to the cobaltic ammonium ion, and the nickelous hydroxide to nickelic oxide.

For the oxidation of manganese, 5 ml. of each oxidising solution were added to 25-ml. portions of a solution containing 10 mg. of $Mn^{\cdot\cdot}$, 5 ml. of 5 *N* ammonium chloride, and 10 ml. of 5 *N* aqueous ammonia. Peroxide gives instantly a dark-brown precipitate with considerable frothing; persulphate gives slowly a brown precipitate, which turns black. The hypochlorite gives a similar effect, but the precipitate redissolves on swirling to form a faintly purple solution; on long standing, a dark brown precipitate is produced. Precipitation is complete in all but the hypochlorite-treated solution. The anomalous behaviour with hypochlorite is discussed later.

The solution of nickel contained 50 mg. of bivalent nickel in 15 ml., and 5 ml. of 3 *N* sodium hydroxide were added to form a light green precipitate. On adding 5 ml. of the oxidising agents to 20-ml. portions, persulphate instantly gives black nickel dioxide, hypochlorite gives almost as rapid an oxidation, whilst peroxide gives no change until its catalytic decomposition is complete, leaving a precipitate slightly darker in colour than the original nickelous hydroxide. Precipitation of nickel hydroxide with 15 ml. of 3 *N* sodium hydroxide in the presence of 5 ml. of 5 *N* ammonium chloride, 10 ml. of 5 *N* aqueous ammonia solution, and 5 ml. of the oxidising solution gives with persulphate a black precipitate; with hypochlorite, green nickelous hydroxide; and with peroxide, a green coloration, but no precipitate. After about 15 min. precipitation is complete, however.

The experimental cobaltous ammonium ion solution contained 10 mg. of bivalent cobalt, 5 ml. of 5 *N* ammonium chloride solution, and 10 ml. of 5 *N* aqueous ammonia in a total volume of 25 ml. Peroxide gives a deep red coloration rapidly; hypochlorite gives slowly a lighter coloured solution; persulphate forms primarily a blue-green solution, which turns dark red, showing an indirect reaction.

The anomalous behaviour of hypochlorite with manganese and nickel in ammoniacal solution is due to the intermediate formation of hydrazine, a powerful reducing reagent, which results in the redissolution of manganese in the reduced state. The slight purple coloration is attributed to the presence of colloidal $Mn(OH)_3$, oxidation to permanganate being unlikely in the strongly alkaline solution.

The reducing power of hydrazine was tested on manganese dioxide suspension, nickelous hydroxide treated in the absence of ammonia with hypochlorite, and nickelic oxide in the presence of persulphate and ammonia. The cobaltic ammonium ion was found to give no immediate colour change, although the oxidation potential is between those of nickelic oxide and $Mn(OH)_3$, which are both easily reduced. Oxidation of the cobaltous ammonia ion in the presence of hydrazine shows no variation with peroxide, but persulphate gives more slowly a red colour without the intermediate blue-green colour. Hypochlorite gives a distinct effervescence with a slight deepening of colour. This last reaction proceeds according to the theory that the oxidation of hydrazine to nitrogen takes place more readily than that of the cobaltous ammonia ion.

In the qualitative separation of manganese, nickel, cobalt, and zinc, the first precipitation of manganese in ammoniacal solution is best effected by hypochlorite, since it carries down a negligible amount of cobalt. Also precipitation of nickel in the filtrate by an excess of sodium hydroxide then shows some manganese and a trace of cobalt; if the other reagents are used, considerable cobalt is carried down although the precipitate is free from manganese. The interference of manganese in testing for nickel can be avoided by using acetic acid solution, or by removing manganese dioxide before applying the test in ammoniacal solution. The poor separation of cobalt from manganese and nickel when using persulphate is due to co-precipitation of colloidal cobaltic hydroxide with nickel dioxide but not with the hydroxide. The formation

of colloidal nickelous hydroxide when using alkaline peroxide assists in the decomposition of the excess peroxide, which occurs less readily in the absence of ammonia. M. E. D.

Microchemical

Micro-separation of Zinc and Aluminium by means of Potassium Ethyl Xanthogenate. P. Wenger, E. Abramson, and Z. Besso (*Helv. Chim. Acta*, 1946, **29**, 49–51)—The method depends on the quantitative precipitation by potassium ethyl xanthogenate, $KSCSOC_2H_5$, of zinc, aluminium being left in solution. The zinc xanthogenate is dissolved in acetic acid, and the zinc determined as the 8-hydroxyquinoline complex according to the method of Cimermann and Wenger (*Mikrochem.*, 1938, **24**, 148), or as the pyrophosphate.

Qualitative experiments showed that the conditions for obtaining the best results were as follows. A freshly prepared 2 per cent. solution of potassium ethyl xanthogenate must be used, untrustworthy results being obtained with a solution 24 hours old. Precipitation should be effected at *p*H 5 to 5·5, 1·2 ml. of reagent being used for each 2 mg. of zinc present, but not less than 0·9 ml. for smaller amounts. The reagent must be added dropwise with vigorous stirring, and the first-formed, voluminous precipitate left to stand until it is crystalline (10 to 25 min.), then filtered immediately, and washed with a 2 per cent. solution of the reagent.

Separation of zinc and aluminium. Method—To a solution containing 2·5 to 5 mg. of zinc and aluminium in an unweighed Emich beaker, add 1 drop of Merck's universal indicator, 2 drops of 10 per cent. acetic acid, and 40 per cent. aqueous sodium acetate solution until the solution is at *p*H 5 to 5·5. Add, drop by drop, 1·2 ml. of a 2 per cent. solution of the reagent for each 2 mg. of zinc present, and stir vigorously with a micro-rod; rinse the rod with 4 drops of the reagent. A flocculent precipitate forms rapidly, but on standing for 10 to 25 min. the turbid solution clears and the precipitate becomes compact and crystalline. Filter immediately into a weighed Emich beaker containing 0·4 ml. of 90 per cent. acetic acid to prevent hydrolysis of the aluminium acetate, and wash the precipitate three times with 6 drops of reagent. Concentrate the filtrate on the water-bath until the solution contains about 1 mg. per ml.; disregard a temporary turbidity during the evaporation. To the unweighed beaker, add 0·5 ml. of distilled water and 10 drops of 10 per cent. acetic acid to effect dissolution of the zinc. Filter into a tared beaker if the determination of zinc is to be made with 8-hydroxyquinoline, or into a tared crucible if the zinc is to be weighed as pyrophosphate. Wash the beaker and filter-stick with 1 ml. of distilled water.

Determination of zinc—(*a*) *8-hydroxyquinoline method*—Concentrate the zinc acetate solution to 1·5 ml. and then proceed according to Wenger and Cimermann (*loc. cit.*).

(*b*) *Pyrophosphate method*—Set the beaker containing the zinc acetate solution on the water-bath and add dropwise 1 ml. of 5 per cent. ammonium phosphate for each milligram of zinc present, 1 drop of universal indicator, and 2 to 3 drops of 1 per cent. aqueous ammonia, thus obtaining a *p*H of 6. Digest hot for 30 min., and cool for 30 min. before filtering and washing with 1 per cent. ammonium nitrate and 50 per cent. alcohol alternately. Dry in an oven, transfer to a muffle furnace, and raise the temperature slowly to 900° C., and heat for 5 min. Cool on a nickel block, and weigh after 30 min.

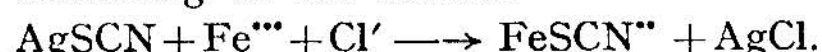
If the aluminium content is greater than 50 per cent. the pyrophosphate method is preferable, since the 8-hydroxyquinoline method tends to give slightly low results.

Determination of aluminium—The 8-hydroxyquinoline method may be conveniently employed since the aluminium is already in acetic acid solution, which should have a volume of several millilitres. Evaporate to about 1 ml. and acidify with 10 drops of 90 per cent. acetic acid, add 4 drops of 5 per cent. 8-hydroxyquinoline acetate solution for 1 to 3 mg. of aluminium, and heat on the water-bath. Buffer with 2 *N* ammonium acetate added dropwise until a permanent turbidity forms, and heat until the precipitate becomes crystalline; add a further 0·5 ml. of ammonium acetate and heat for 10 min. Filter hot, and wash 5 times with 0·3 to 0·5 ml. of boiling water before drying at 140° C. Weigh after 30 min.

Results—A hundred analyses with samples containing varying proportions of zinc and aluminium were completed, accurate results being obtained within the limits zinc, 25 to 75 per cent. and aluminium, 75 to 25 per cent. M. E. D.

Semimicro-Detection of Thiocyanate and Halide Ions. L. E. Porter (*J. Chem. Educ.*, 1946, **23**, 402)—In the detection of halides in the silver nitrate group, cyanogen anions and sulphide interfere. The ferro- and ferri-cyanide, and sulphide ions can be removed as their zinc salts (Abegg and Herz, *Z. anorg. Chem.*, 1900, **23**, 236; Weber and Winkelman, *J. Amer. Chem. Soc.*, 1916, **38**, 2000), or as their cadmium salts (Duschak and Sneed, *J. Chem. Educ.*, 1931, **8**, 1386; Pierce and Hazard, *Ibid.*, 1944, **21**, 126) before the silver nitrate is added. In the absence of cyanide, only thiocyanate and halides then remain to be precipitated. Available methods of separating thiocyanate and bromide tend to result in loss of bromide.

The following separation depends on the formation of a complex ferric thiocyanate, probably $FeSCN^{\cdot\cdot}$, when the precipitated silver thiocyanate is suspended in aqueous hydrochloric acid and treated with ferric chloride, the bromide remaining unchanged. The reaction proceeds readily, probably according to the scheme



$$AgSCN + Fe^{\cdot\cdot\cdot} + Cl' \longrightarrow FeSCN^{\cdot\cdot} + AgCl.$$

Since the separation and detection of silver thiocyanate are simultaneous, completeness of removal is easily recognised; and the subsequent test for bromide is more certain.

Procedure—Remove ferrocyanide, ferricyanide, and sulphide ions from the solution as their zinc or cadmium salts, acidify the solution with nitric acid, and add a slight excess of silver nitrate solution. Separate and wash the precipitate, and extract it with 1 ml. of 3 *M* ammonium carbonate. Test the extract for chloride by adding an excess of dilute nitric acid. The residue may contain thiocyanate, bromide, iodide, and some chloride of silver. Extract it with 7·5 *M* ammonia and detect iodide in the residue by reduction with zinc and dilute sulphuric acid, and addition of potassium nitrite and carbon disulphide to the filtered solution. Acidify the ammoniacal solution from the iodide separation with dilute nitric acid and filter. Wash the precipitate, and treat it with 1 ml. of water, 5 drops of 6 *M* hydrochloric acid and 5 drops of 2 *M* ferric chloride, and stir thoroughly. A red to reddish-brown solution indicates thiocyanate. Allow to stand for 3 to 4 min., and repeat the treatment

with acid and ferric chloride until no further coloration is produced; one repetition is usually sufficient. Warm the residue with water containing a few drops of dilute nitric acid to remove the iron, and detect bromide by reducing it with zinc and dilute sulphuric acid, and adding hypochlorite and carbon disulphide. M. E. D.

Physical Methods, Apparatus, etc.

Chromatography in the Separation and Determination of the Basic Amino Acids. M. S. Bergdoll and D. M. Doty (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 600–603)—The individual basic amino acids can be separated chromatographically from amino acid mixtures and protein hydrolysates by means of Lloyd's reagent (fuller's earth). Hydrochloric acid was the most effective eluant for spreading the lysine, histidine, and arginine bands, and it removed lysine completely before removing any histidine or arginine; it was therefore used as the first eluant. Sodium bicarbonate solution was selected as the second eluant because it separated histidine from arginine. Pyridine solution was used to remove arginine.

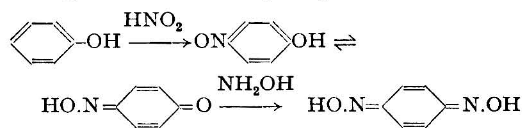
Procedure—Adjust the acidity of the amino acid solution, 10 ml. of which contain 1 to 6 mg. of lysine, 1 to 4 mg. of histidine, and 1 to 8 mg. of arginine, to about 0·3 *N* with hydrochloric acid, add 30 to 50 mg. of zinc dust for each 10 ml. of solution, heat to 80° C. and cool. Pack an adsorption tube, 400 mm. long and 19 mm. in diameter, with 20 g. of a mixture (1:2) of Lloyd's reagent and Hyflo Super-Cel and put a 0·6-cm. layer of Hyflo-Super Cel on top of the adsorbent mixture. With the aid of suction, pass the following liquids in succession through the column: 50 ml. of 1·7 *N* hydrochloric acid, 10 ml. of the amino acid solution, 180 ml. of 0·5 *N* hydrochloric acid (using the first 10 ml. to rinse the amino acid solution on to the adsorbent), 200 ml. of *N* hydrochloric acid, 150 ml. of 0·125 *M* sodium bicarbonate, 100 ml. of 10 per cent. pyridine in 0·7 *N* hydrochloric acid, and 40 ml. of 0·5 *N* hydrochloric acid. Add each new solution just as the last of the previous solution is about to disappear into the adsorbent.

Change the receiver when a total of 80, 275, 480, 625, and 730 ml. of solution has been added to the column of adsorbent, and discard the first fraction. The second fraction contains the non-basic amino acids and ammonia, the third lysine, the fourth histidine, and the fifth arginine. Run a blank column in a similar manner, with 0·3 *N* hydrochloric acid treated with zinc replacing the amino acid solution. This serves as a reagent blank for the individual amino acid estimations.

Estimate the histidine by means of Pauly's diazo reaction (*cf.* Macpherson, *Biochem. J.*, 1942, **36**, 59), and the arginine by means of Sakaguchi's reaction (*cf.* Doty, *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 169). The following method is recommended for the estimation of the lysine: make the solution just acid to phenolphthalein, and add 15 ml. of a phosphate buffer solution, *p*H 7·4 (50 ml. of 0·4 *M* potassium dihydrogen phosphate and 4·1 ml. of 1·0 *N* sodium hydroxide diluted to 100 ml.). Transfer a measured volume, containing 0·02 to 0·10 mg. of lysine, to a 25-ml. flask and make up to 3 ml. with the blank solution. Add 1 ml. of ninhydrin solution (5 mg. of triketohydrindene hydrate, 0·4 mg. of sodium hydroxide and 263 mg. of sodium chloride per ml.), and 5 ml. of glycerol. Mix, immerse for 30 min. in boiling water, cool, and dilute to a suitable volume with 95 per cent. ethanol. Evaluate the colour in a photo-electric colorimeter, and calculate the lysine concentration from a standard curve.

The recoveries of the basic amino acids when adsorbed from amino acid mixtures were 103 ± 2 per cent. for lysine, 100 ± 1 per cent. for histidine, and 99 ± 2 per cent. for arginine. The lysine and histidine values found for proteins were generally higher than the values recorded in the literature, although they were in closer agreement with the more recent results. The arginine values compared favourably with those given in the literature. F. A. R.

Chromatographic Resolution of the Quinone Oximes. D. K. Gullstrom, H. P. Burchfield, and J. N. Judy (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 613–616)—*p*-Benzoquinone dioxime is prepared by nitrosation of phenol and treatment of the resulting monoxime with hydroxylamine:



The monoxime can be separated from the dioxime and from the by-products of the reaction by chromatography on alumina.

Procedure—Dissolve a 1-g. sample of the reaction mixture in 100 ml. of acetone by heating under reflux, filter, and run the filtrate, after concentration if necessary, through a column, 15 cm. × 18 mm., of a mixture (9:1) of activated alumina and Hyflo Super-Cel. Wash with 10 ml. of 1 per cent. v/v acetic acid in acetone and then with 300 ml. of 5 per cent. v/v methanol in acetone. Remove the top brown layer by means of a spatula and discard. Transfer the green zone, which contains the monoxime, to a 250-ml. beaker and elute with successive portions of *N* sodium hydroxide, using a total volume of 100 ml. Filter, dilute the filtrate to 1 litre with water, and then dilute again to a volume suitable for measuring the optical density at 399 and 363 mμ. The green zone contains all the monoxime together with 20 to 35 per cent. of the dioxime, for which a spectrophotometric correction is applied. The percentage of monoxime is given by the expression:

$$\frac{100\ V(K'\lambda_2.D\lambda_1 - K'\lambda_1.D\lambda_2)}{G.F.(K'\lambda_2.K\lambda_1 - K'\lambda_1.K\lambda_2)}$$

where $D\lambda_1$ and $D\lambda_2$ are the measured optical densities at 399 and 363 mμ, respectively, *G* is the weight of the sample, *V* is the volume (litres) to which the solution is diluted, $K\lambda_1$ and $K\lambda_2$ are the specific extinction coefficients of the monoxime at 399 and 363 mμ, respectively (224 and 109, respectively), $K'\lambda_1$ and $K'\lambda_2$ are the specific extinction coefficients of the dioxime at 399 and 363 mμ, respectively (283 and 65·7, respectively), and *F* is the fraction of the monoxime recoverable after adsorption on alumina (generally about 0·95). When a known amount of the monoxime was added to a reaction mixture, the average deviation from the theoretical result was ± 0·23 per cent. The precision of the method, as judged by the results of duplicate analysis of 64 samples containing 2 to 55 per cent. of monoxime, was ± 3·6 per cent. of the amount present. F. A. R.

Conditions Affecting the Sequence of Organic Compounds in Tswett Adsorption Columns. H. H. Strain (*Ind. Eng. Chem., Anal. Ed.*, 1946,

18, 605–609)—It has become the practice to identify and name the components of a mixture by their relative positions on Tswett adsorption columns, and predictions concerning the structure of an organic compound have sometimes been made from its adsorptive power relative to those of known substances. It has now been demonstrated that the relative adsorption sequence on such columns varies with circumstances. Most of the work was carried out with binary mixtures, but mixtures containing 3 or more components have also been examined. Several factors affect the sequence of adsorption, and in the original paper many examples are given of the reversal of two bands. These factors are: the nature of the solvent; the presence of an impurity in the solvent; the hydrogen ion concentration; the concentration of the solutes; the nature of the adsorbent; and the temperature. This variation of the adsorption sequence with changes of conditions in the adsorption columns can often be used to advantage. Thus, the identity or non-identity of two substances may be established with greater certainty by adsorbing a mixture of the two on different adsorbents and elution with different eluants than by adsorption on one column only. Secondly, when attempting to purify a substance chromatographically, it is an advantage that the band corresponding to it should be below that corresponding to the impurity; a substance forming an upper band should therefore be readsorbed under conditions such that it forms the least readily adsorbed band.

Clearly, the relationship between adsorbability and chemical structure is more complex than was formerly supposed, and adsorbability apparently depends on competition of solvent, solution, and adsorbent for one another. Since recent work has shown that the rate of migration of a solute through an adsorption column is a function of the adsorption isotherm, variations of the adsorption sequence with different conditions in the columns must result from disproportionate variation of the adsorption isotherms. F. A. R.

Use of High-frequency Oscillators in Titrations and Analyses. F. W. Jensen and A. L. Parrack (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 595–599)—When a high-frequency oscillator is loaded by the introduction of a liquid or a solution into its tank circuit, its characteristics are altered. The magnitude of the change in the oscillator current is affected by the volume of the solution, the location of the solution within the circuit, and the conductivity of the solution due to its ionic or dipole concentration. The changes in conductivity during a reaction cause variations in loading whereby the course of the reaction can be followed, and an oscillator that is suitable for applying this principle to analytical work is now described. By the use of this instrument, the end-point in several types of titration can be determined.

A tuned-plate, tuned-grid oscillator with a sensitive metering system in the positive power supply lead is used, and details of the circuit are given. Frequencies in the range 15 to 20 megacycles give satisfactory results. The tube containing the liquid is surrounded by a shield extending well below the liquid level and is placed inside the oscillator coil. The progress of the reaction is followed by measurement of the oscillator current, and for a titration, a change in slope of the curve obtained by plotting current against the volume of titrant marks the end-point.

Results are given for a series of titrations in which 25 ml. of a 0·1 *N* solution were diluted with 100 ml. of water and titrated with another solution approximately 0·1 *N*. In titrating solutions of hydrochloric acid with sodium hydroxide or sodium carbonate; of phosphoric acid with sodium hydroxide; and of potassium chloride with silver nitrate, the current-titre curve is V-shaped, the sharp point of inflexion being the end-point of the titration. The curve for the titration of sodium carbonate solution by 0·1 *N* hydrochloric acid gave two points of inflexion, and in the titration of acidified ferrous ammonium sulphate solution with potassium permanganate solution, addition of the titrating agent beyond the end-point lowered the conductivity of the solution and so the current continued to fall beyond the point of inflexion. The V-shaped graph was obtained if the potassium permanganate used was made 0·4 *N* with respect to potassium sulphate. Potassium permanganate-sodium oxalate titrations showed similar characteristics. Titration of *o*-phthalic acid in acetone with sodium methylate in methanol gave a sharp end-point.

The end-points obtained were compared with the known end-points for the various titrations and good agreement was found; *e.g.*, silver nitrate - potassium chloride, known 24·92 ml., observed 24·90 ml.; hydrochloric acid - sodium carbonate, known 12·20, observed 12·18 and 24·40 ml. For two types of acid-alkali titrations, the end-points were compared with those obtained by the usual conductance method; agreement was again satisfactory. With solutions in organic solvents, loading due to rotation of dipoles is an appreciable part of the total effect, and an end-point can be obtained only by suitable choice of solvent.

Because silver chloride becomes more soluble as the equivalence point is approached, and dilution of ions takes place as titration proceeds, the titration of 25 ml. of 0·01 *N* potassium chloride, diluted with 300 ml. of water, by 0·01 *N* silver nitrate gave a minimum conductivity point at 23·90 ml. instead of at 25·00 ml., the true end-point. By using conductivity and solubility data, the amount of silver still in solution at the minimum point can be calculated, and the corrected end-point thus obtained was 24·99 ml.

As the curve obtained by plotting oscillator current against the concentration of hydrogen chloride in solution in dry benzene is linear, and a change in concentration from 0 to 0·275 per cent. by weight of hydrogen chloride corresponds to a change of 100 microamp., a sensitive method for determining hydrogen chloride in benzene becomes available. B. A.

Spectrophotometric Changes during Oxidation of Vitamin A Oils. G. R. Halpern (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 621–625)—Fish liver oils containing vitamin A are essentially solutions of the esters of that vitamin in a fatty carrier oil. Knowledge of the quality of the carrier oil is important for the evaluation of vitamin A oil, since carrier oils containing degradation products due to oxidation have considerable influence on the extinction coefficient at 328 mμ and on the whole vitamin A curve. The vitamin A ester itself has a sharp maximum at 328 mμ. Biologically inactive substances that may accompany it do not show this sharp peak, but have maxima at wavelengths sufficiently near to 328 mμ to produce considerable absorption at this point. Oxidised oils can also inhibit the action of vitamin A *in vivo*.

The method of Oser *et al.* (*Ibid.*, **1943**, **15**, **717**)

of plotting absorption curves of vitamin A oils in terms of absorption ratios could be useful in detecting anomalous or oxidised oils, but the method for measuring the ratio $E^{1\%}_{1cm.}$ 300/328 mμ with the limit of 0·72 for acceptable oils did not prove satisfactory, since it was found that an oil could be considerably oxidised and still give the accepted ratio. Oser *et al.* (*Ibid.*, 1945, **17**, 559) showed that oils with an acceptable $E^{1\%}_{1cm.}$ 300/328 mμ ratio did not always show the biological potency corresponding to their $E^{1\%}_{1cm.}$ 328 mμ values. It seemed desirable, therefore, to study spectrophotometric curves of the saponifiable and unsaponifiable components of the carrier oil, apart from vitamin A, during accelerated oxidation to establish their influence upon the absorption curve of vitamin A oils, and to develop better criteria for the evaluation of these oils.

Freshly prepared samples of gray fish (*Squalus suckleyi*) liver oil and ling cod (*Ophiodin elongatus*) liver oil were oxidised to different degrees by immersing them for varying periods in a water-bath at 75° C., and the absorption curves were determined in the region from 220 to 400 mμ for the whole oil and for the unsaponifiable fraction, the difference representing the absorption of the saponifiable carrier. The absorption of that part of the oxidised vitamin A which is washed out during the saponification (Oser *et al.*, *loc. cit.*, 1943) is also included in the subtracted curve. To obtain the curve of the unsaponifiable carrier, the unsaponifiable fraction was destructively irradiated by exposure in a water-cooled cell at 20 cm. for 1 hr. to a Uviarc amp (UA 32A6) with a No. 597 Corning filter. On the assumption that only vitamin A is destroyed by irradiation the curve obtained after irradiation shows the absorption of the unsaponifiable carrier, and, by subtracting the absorption values of the irradiated unsaponifiable fraction from those of the original unsaponifiable fraction, the vitamin A curve can be obtained. Values so obtained are referred to as the modified values (Little, *Ibid.*, 1944, **16**, 288). The spectrophotometric analyses were made with a Beckman quartz spectrophotometer with an ultraviolet phototube and, as light sources, a hydrogen discharge tube below 320 mμ and a tungsten lamp above 320 mμ, and all determinations were made in quartz cells with purified *iso*propanol as solvent. The saponifications were made essentially by the procedure of Oser *et al.* (*loc. cit.*, 1943), blank saponifications made on the reagents being used as spectrophotometric blanks.

With the saponifiable carrier, new maxima are formed during oxidation at 235, 275 to 280, and 335 to 345 m μ, and the shape of the curve resembles that of the curves of rancid and oxidised fats. The appearance of new maxima during oxidation is generally attributed to formation of different conjugated systems, produced by isomerisation of unconjugated fats and by destruction of a part of the four conjugated double bonds of vitamin A. Both components of the oxidised saponifiable carrier (fatty acid and oxidised vitamin A) can therefore be responsible for the formation of the 275 mμ band, the extinction coefficients of conjugated fatty acids being of a high order. The vitamin A curve is affected by formation of these three maxima. The minimum at 260 mμ steadily increases during oxidation, despite loss of potency, and this causes broadening of the curve, increase in the $E^{1\%}_{1cm.}$ 300/328 ratio, and a shifting of the 328 mμ peak to a lower wavelength in the later stages of oxidation. Examination of the curves of the saponifiable carrier shows that the point at 260 mμ (the minimum of the vitamin A curve), or at about 280 mμ (the maximum of the saponifiable carrier curve) is a better location for taking the ratio than the 300 mμ point. At these points the $E^{1\%}_{1cm.}$ values increase during oxidation and, at the same time, those at 328 mμ decrease, whereas at 300 mμ the values remain almost constant. (The point at 300 mμ was apparently chosen empirically to avoid use of a hydrogen discharge lamp.) When the ratios $E^{1\%}_{1cm.}$ 300/328, 280/328, and 260/328 mμ of the oxidised oils are plotted against loss of vitamiu A, the curves for the 260 and 280 ratios are steeper.

The extraneous absorption (per cent. of the absorption of the whole oil) of the saponifiable carrier steadily increases during oxidation, and could be used as a criterion for the quality of vitamin A oils. At 260 and 280 mμ the extraneous absorption increases significantly during the first stages of oxidation. It is most pronounced at 260 mμ because the influence of the 325 mμ maximum is greater than at 280 mμ, but both points may be used as criteria, and the 260 mμ point has the advantage that the determination can be made more accurately on the flat portion of the curve.

The determination of vitamin A on the unsaponifiable fraction of the carrier is sufficient where the extraneous absorption of the saponifiable carrier does not change considerably during the first stages of oxidation, and commercially-prepared oils, which are frequently slightly oxidised, would not give erroneously high values at 325 mμ when these are determined on the unsaponifiable fraction and calculated with a uniform factor. The vitamin A was destroyed in all samples by irradiation of the unsaponifiable fraction with the exception of a portion of ling cod liver oil which had been irradiated for 18 hr., and with both test oils a new maximum appeared at 270 mμ representing a substance formed from vitamin A during destructive irradiation (*cf.* Sobotka, *et al.*, *J. Amer. Chem. Soc.*, 1944, **66**, 1162; ANALYST, 1945, **70**, 28). The additional absorption of this substance affects considerably the curves of the modified values obtained by subtraction. During the oxidation of the test oils the absorption of the irradiated samples at 325 mμ showed a tendency to decrease, but the differences were within experimental error. It can therefore be assumed that no significant changes occur in the unsaponifiable carrier during the first stages of oxidation of either test oil, and the determination of the modified values is not justified.

The unsaponifiable carrier of the ling cod liver oil showed no pronounced maxima caused by components originally present in this fraction. The unsaponifiable carrier of grayfish oil, on the other hand, contains substances absorbing at about 238 mμ, partly masking the absorption of irradiated vitamin A at 270 mμ. The absorption at 238 mμ, however, does not affect the 325 mμ point, and remains constant in the first stages of oxidation, decreasing later. The main substance responsible for the absorption at 238 mμ was shown to belong to the glyceryl ether fraction, probably a higher unsaturated homologue of selachyl alcohol. Another substance of unknown constitution absorbing at this wavelength is present in very small amount.

Although the unsaponifiable carrier of the two test oils did not show oxidisable substances that would affect considerably the absorption curve of vitamin A, some fish liver oils can contain such substances included in the unsaponifiable fraction which produce an anomalous vitamin A curve.

In presence of such substances as kitol, vitamin A_2, anhydro-vitamin A and others, an accurate estimation of vitamin A by the means described is impossible. The commercial factor 2000 allows a certain amount of extraneous absorption in all oils because the crystalline vitamin A alcohol was shown to have a conversion factor of 2460 (Baxter and Robeson, *J. Amer. Chem. Soc.*, 1942, **64**, 2411). If the substances causing excessive extraneous absorption in the unsaponifiable fraction do not interfere with the Carr-Price reaction, colorimetric estimation of vitamin A can be used advantageously (Oser *et al.*, *loc. cit.*, 1945).

Considering the great difference in the ratios of both original test oils, it seems that the establishment of a definite limit for the magnitude of the ratio for the evaluation of the quality of fish liver oils is not satisfactory. A rigorous limit for good oils and a range for doubtful oils would be preferable. It is suggested that with doubtful samples the extraneous absorption per cent. of the saponifiable carrier, *viz.*,

$$\frac{E^{1\%}_{1\text{cm.}}\text{ (whole oil)} - E^{1\%}_{1\text{cm.}}\text{ (unsaponifiable fraction)}}{E^{1\%}_{1\text{cm.}}\text{ (whole oil)}} \times 100$$

at 280 or 260 mμ might be helpful in the estimation of the quality of these oils. From the present work, and from other experience, it appears that the value 0·72 for the $E^{1\%}_{1\text{cm.}}$ 300/328 mμ ratio indicates that oxidation has taken place especially with high potency oils. Further work, particularly biological, will be necessary to establish proper limitations of the values to be used as criteria. A. O. J.

Radioactive Studies. Utilisation of the Radioactive Isotope Dilution Procedures for Special Types of Chemical Problems. Determination of the Three Individual Components of Mixtures of Dibenzyl-Sulphide, -Sulphoxide, and -Sulphone as an Illustrative Example. F. C. Henriques, jun., and C. Margnetti (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 476–478)—As recent studies (Henriques, jun., *et al.*, *Ibid.*, 1946, **18**, 349, 415, 417, 420) have shown that some of the radioactive tracers most difficult to analyse can be determined to within 2 per cent., the accuracies of the isotope dilution method with either stable or radioactive isotopes are similar. This paper indicates the scope of the radioactive dilution method as applied to chemical problems, and gives an example of its use.

The two general procedures in applying the method are: (1) if the amount of non-radioactive compound, *P*, in the mixture is unknown, a known amount of the same compound containing a minute amount of tracer element, *P**, is added and dispersed uniformly. The amount of *P* present can be determined provided that a weighable amount of *P.P** can be isolated in a pure state; (2) if the amount of *P** in a mixture containing other radioactive compounds is unknown, then a weighed amount of *P*, sufficient to enable the isolation of a weighable quantity of pure *P.P**, is added, and the analysis becomes possible. Procedure (1) cannot be applied to the determination of traces, but (2) can be used to determine 10^{-3} μg. of some compounds.

The application of (1) to the analysis of a mixture of dibenzyl-sulphide, -sulphoxide, and -sulphone is described in detail. Samples of these compounds containing radioactive sulphur, ^{35}S, were prepared, and their radioactivities determined by the method described in an earlier paper (*Idem, ibid.*, 1946, **18**, 349). A weighed portion of each was added to the unknown mixture. Pure samples of each compound were isolated from the mixture by fractional crystallisation, and subjected to radioactive analysis. From the data obtained, the amounts present in the mixture were calculated. Analysis of a series of standard mixtures gave results to within ± 2 per cent. of the correct values. A paper to be published will describe the application of method (2) to the investigation of the mechanism of mustard gas vesication. B. A.

Radioactive Studies. Analytical Method for Determination of the Long-Life Carbon ^{14}C. F. C. Henriques, jun., and C. Margnetti (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 417–419)—Details are given of a procedure for measuring the radioactivity due to ^{14}C, half-life 1000 years, incorporated in organic compounds. The carbon-containing compounds are oxidised by a standard combustion procedure, and the carbon dioxide formed is introduced into an ionisation chamber attached to a Lauritzen electroscope. As little as 3×10^{-5} micro-curie of ^{14}C, diluted with 20 mg.-mol. of ordinary carbon, can be determined to within ± 2 per cent. The method is applicable to biological work in which ^{14}C is used as a tracer. B. A.

Radioactive Studies. Analytical Procedure for Measurement of the Long-lived Radioactive Sulphur ^{35}S, with a Lauritzen Electroscope, and Comparison of Electroscope with Special Geiger Counter. F. C. Henriques, jun., G. B. Kistiakowsky, C. Margnetti, and W. G. Schneider (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 349–353)—The paper describes a method for the determination of the radioactive tracer, ^{35}S, half-life 87 days. Samples of the sulphur-containing compounds are oxidised by the Carius method, and the sulphur is precipitated as benzidine sulphate. The radioactivity of the precipitate is measured by either a Geiger counter or a modified Lauritzen quartz-fibre electroscope. If the specific activity of the tracer is 1 milli-curie of ^{35}S per 10 mg. of compound, 10^{-3} μg. of any sulphur compound can be determined to within 2 per cent. Details of apparatus and procedure are given, and the utilities of the counter and electroscope compared. B. A.

Radioactive Studies. Analytical Procedure for Measurement of Radioactive Arsenic of 90-Day Half-life. F. C. Henriques, jun., and C. Margnetti (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 415–417)—The radioactive arsenic of 90-day half-life, believed to be ^{72}As or ^{73}As, is determined by hypophosphite reduction to the metal of the arsenic compound that is used as a carrier for this tracer element, collection of the precipitated arsenic on a filter plate, and measurement of its radioactivity by means of a Lauritzen electroscope. Details of apparatus and procedure are given. B. A.

Application of the Differential Manometer to the Measurement of Fluid Density and Specific Gravity with Special Reference to Temperature Effects. R. D. Cowherd (*Paper Trade J.*, 1946, **123**, Oct. 3, *T.A.P.P.I. Sect.*, 159–166)—In the instrument described, the density of a liquid is measured in terms of the difference in back-pressure set up when air from a common source is bubbled simultaneously through tubes immersed in a cylinder containing the liquid being tested

and in a liquid of known density (*e.g.*, mercury), under standardised conditions (*e.g.*, of head). A standard type of flow-meter unit is used for the actual measurement of the pressure difference. Automatic temperature compensation is ensured by adjusting the weight of the comparison liquid and the diameter of the container so that any decrease in the density of the contents (due to a temperature rise) will exactly offset the increase in head due to the expansion which also results; and *vice versa*. This method is preferable to the insertion of the comparison liquid cylinder in the sample liquid cylinder, so as to form a concentric heat-exchanger. The subject is treated mathematically. J. G.

Isostatic Method for Determining the Gas Permeability of Sheet Materials. D. W. Davis (*Paper Trade J.*, 1946, **123**, Aug. 29, *T.A.P.P.I.*, *Sect.*, 97–104)—In the apparatus described, the gas to be tested is passed over one side of the test sheets, which are held free from any face support in a diffusion cell, and a different "sweep" gas is passed over the other side; both gases are at approximately atmospheric pressure and at the same relative humidity. The sweep gas entrains any test gas that may permeate the test sheet, and the permeated gas is determined by chemical methods. For carbon dioxide permeability, the oxygen used as the sweep gas is passed through Ascarite, and the permeated carbon dioxide so absorbed is determined gravimetrically. For other test gases (*e.g.*, oxygen or nitrogen), the carbon dioxide used as the sweep gas is absorbed in potassium hydroxide solution, and the residual permeated test gas is determined by gas-volumetric methods. Data are given for a wide variety of sheet materials at various temperatures and relative humidities. J. G.

Reviews

CHEMICAL COMPOSITION OF FOODS. By R. A. MCCANCE and E. M. WIDDOWSON. Second Edition. Medical Research Council, Special Report Series No. 235. Pp. 156. London: H.M. Stationery Office. 1946. Price 6s.

Twice already, once on the first appearance of these tables and then when a third impression was produced, in 1940 and 1942 respectively, it has been my privilege and pleasure to welcome them in this journal on behalf of analytical chemists. Now I can do little more than repeat the substance of what was said on both those occasions.

The tables are as indispensable and authoritative as they are critically constructed by the authors on the basis of new analytical work scrupulously carried out by them. The new edition departs not at all in layout and arrangement of contents from the old, though it has additional information on a small number of foods. Thus, the data for bread, under Cereals and Cereal Foods, now include figures for "National Wheatmeal"—a defunct term already surrounded by an historical aura—as well as for white, this time specified as of 70 to 72 per cent. extraction, and for "whole meal," a term now properly used in quotation marks and defined as of 92 per cent. extraction; the reference to toasted Hovis has been omitted, presumably in part to avoid having to re-number everything subsequent. This re-numbering problem, and the preoccupation of the distinguished authors with many other lines of investigation, have doubtless prevented many additions to the original tables, though the insertion of fresh items, where space permits, could be indicated by small letters following the numbers, as already used in several places. Some space could, if it were essential, possibly be saved by removing a few of the more esoteric items; thus, to give figures for winkles purchased cooked and for winkles in shells as purchased is perhaps superfluous, the information being but rarely of great practical interest to the analyst or the dietician.

Perhaps the most interesting of the changes is the ruthless removal from this new edition of the table headed " 'Available' (Ionisable) Iron in Foods" and also of the reference to "availability" in the table giving figures for the phytic acid content of foods. Phytic acid phosphorus in the new table is expressed as a percentage of the total phosphorus: previously, figures were given for what was described with customary caution as "available" phosphorus —the quotation marks are the authors'—expressed in terms of the total phosphorus. One of the signs of increasing knowledge in physiological matters is frequently increased uncertainty —not necessarily an application of a certain much-discussed principle of modern physics.

There is one small criticism that a sympathetic reviewer would offer to the authors. When they produce further editions—and I would again express the hope that they may often be called upon to do so—they should, in their preface to those editions, call attention to any alterations to tabulated values that they have found it necessary to make, giving in the more important instances their reasons for the changes, where these are not obvious from inspection of the tables. This is, however, only a suggestion for an addition; I do not see how the would-be most carping of critics, with the utmost exercise of ingenuity, could suggest a single useful omission from this most valuable and valued compilation. A. L. BACHARACH

ADVICE TO AUTHORS

THE Council has approved the following notice by the Publication Committee, which is here given in condensed form.

The Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, including chemical, physical, biological, bacteriological and micro methods. Papers on these and allied subjects, by members of the Society or non-members, may be submitted for presentation and publication; they may:

(1) record proposals for new methods and the investigations on which the proposals are based;

(2) record the results of original investigations into known methods or improvements therein;

(3) record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;

(4) record the application of new apparatus and new devices in analytical technique and the interpretation of results.

Communications.—Papers (which should be sent to the Editor) will normally be submitted to at least one referee, on whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication. Papers or Notes accepted by the Publication Committee may not be published elsewhere except by permission of the Committee.

Abstracts.—The MS. should be accompanied by a brief abstract of about 100 to 150 words indicating the scope and results of the investigation.

Proofs.—Proofs should be carefully checked and returned within 48 hours. Two galley proofs* will normally be sent out, one of which should be retained by the Author.

Reprints.—Ten Reprints are supplied gratis to the Author. Additional reprints may be obtained at cost if the Author orders them directly from the printers, W. Heffer & Sons Ltd., 104, Hills Road, Cambridge, at the time of publication. Details are sent to Authors with the proofs.

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

The title should be descriptive and should set out clearly the scope of the paper. Degrees are now omitted after the names of Authors in the headings of papers.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Generally, the best order of presentation is as indicated below:

(*a*) General, including historical, introduction.

(*b*) Statement of object of investigation.

(*c*) Description of methods used. Working details of methods are usually most concisely and clearly given in the imperative mood, and should be given in this form, at least while economy of paper is pressing, *e.g.*, "Dissolve 1 g. in 10 ml. of water and add . . ." Well-known procedures must not be described in detail.

(*d*) Presentation of results.

(*e*) Discussion of results.

(*f*) Conclusions.

followed by a short summary (100 to 250 words) of the whole paper: items (*e*) and (*f*) can often be combined.

Illustrations, diagrams, etc.—The cost of setting up tabular matter is high and columns should therefore be as few as possible. Column headings should be brief or replaced by a number or letter to be used in combination with an explanatory footnote to the table.

Sketches or diagrams should be on white Bristol board, not larger than foolscap size, in Indian ink. Lettering should be in light pencil.

Tables or graphs may be used, but normally not both for the same set of results. Graphs should have the co-ordinate lines clearly drawn in black ink.

References.—References should be numbered serially in the text and collected in that order under "REFERENCES" at the end of the paper. They should be given in the following form:

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, **1933**, **52**, 189T.
2. Allen, A. H., "*Commercial Organic Analysis*," Churchill, London, 1882.

Notes on the Presentation of Papers before Meetings of the Society are appended to the "ADVICE," copies of which may be obtained on application to the Secretary, 7/8, Idol Lane, London, E.C.3

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