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THE ANALYST

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Obituary

EDWARD HINKS

THE death of Edward Hinks, M.B.E., B.Sc., F.R.I.C., occurred on the 25th May, 1946, after an illness borne with the greatest fortitude.

His passing will be a personal loss to a large number of members, for his charm and kindly outlook endeared him to all who came in contact with him.

Hinks was born in Croydon in 1879 and was educated at the Whitgift Grammar School, where he took an interest in athletics, including running, swimming and shooting, and indeed he shot for his school at Bisley. In later years he showed little enthusiasm for any games but was fond of walking and climbing and on occasions he visited Switzerland to indulge in these pursuits.

In 1899 Hinks entered King's College, London, and studied chemistry under Professor John Millar Thomson. He successfully took the examinations for the Associateship of the Institute of Chemistry and became a Fellow in 1904.

In 1905 he became an assistant in the laboratory of Sir Thomas Stevenson of Guy's Hospital, who at that time was the Home Office Analyst and Public Analyst for Surrey, and on Stevenson's death a few years later he was appointed by the Surrey County Council as Public Analyst and later Official Agricultural Analyst. Subsequently, he obtained several appointments under local authorities in Surrey as analyst under the Sale of Food and Drugs Act. Hinks always took a deep interest in water and sewage examinations and he held for many years the position of water examiner to the Mid-Kent Water Company. His private practice was considerable and he gave the most exacting attention to analytical work. In the witness box his answers had a ring of conviction and no doubt the justices before whom he gave evidence realised that Hinks's expressions of opinion were only given after careful thought.

During the 1914–18 war he served in the Army Ordnance Corps, was drafted to Mesopotamia, was twice mentioned in despatches and for his services was awarded the M.B.E.

Generally an obituary consists mainly of a summary of the professional attainments of an individual but though these attainments were of a high order Hinks will be remembered on account of his endearing personality. He had a generous regard for his fellow men and his fellow men had an unusually high regard for him. On a problem being presented to Hinks he would think long and deeply and his views would only be expressed after mature consideration; his decisions therefore always demanded the most careful attention. So highly were his opinions esteemed that he was invariably consulted on every phase of the Society's many activities and as he gave unstintingly of his time his name may be found as a member of an unusually large number of committees, many connected with our Society. Probably most gratitude is owing to him for the many hours, over many years, that he spent as Chairman of the Standing Committee on the Uniformity of Analytical Methods. This Committee was formed in 1924 and he at once became Chairman and only resigned for reasons of health.

On several occasions Hinks appeared before Parliamentary and other bodies to give evidence on behalf of the Society on matters concerning the sale or the composition of foods and drugs.

His activities extended, too, to the Royal Institute of Chemistry, for he served altogether for six years as a member of Council and in 1930 was elected a Vice-President. He was also a past examiner for the Institute in the Final for the Associateship in the special subject—the Chemistry and Microscopy of Foods and Drugs and of Water.

Only a few years after he was elected a member of our Society he became its Treasurer and he only ceased to discharge the duties entailed when he undertook the Presidency of the Society in 1928.

He published a number of papers, of which the following appeared in THE ANALYST:—

"The Detection of Coconut Oil in Butter," 1907.
"Note on Gorgonzola Cheese," 1911.
"A Flour Improver," 1912.
"The Detection and Estimation of Benzoic Acid in Milk and Cream," 1913.
"The Persistence of Hydrogen Peroxide in Milk," 1915.
"The Determination of Total Alkaloids in Cocoa and of Cocoa-matter in Flour Confectionery" (with D. D. Moir), 1935.

His wife and two daughters survive him; he lost his only son, John, in 1940. John was a brilliant scholar and from Lancing went to Pembroke College, Cambridge, where he obtained a First in the Mechanical Science Tripos and was elected to a Fellowship. He joined the R.A.F. and ultimately became personal assistant to Air Marshal Sir Charles Burnett. During the early part of 1940 he was posted (no doubt at his own request) to Bomber Command and, in October of that year, he was shot down over Magdeburg. And so Hinks, and his country, lost a distinguished son.

Hinks will ever be remembered by his friends and colleagues for his friendliness, his courtesy and, under all conditions, his cheerfulness. The Society has indeed lost an ardent supporter and many of its members an irreplaceable friend.

F. W. F. ARNAUD

Symposium on Spectroscopic Analysis

The following four Papers on Spectroscopic Analysis were read and discussed at the Joint Meeting of the Physical Methods Group and the Scottish Section at Edinburgh, on May 23rd, 1946, The Discussion follows the last of the four papers, on p. 373.

Photoelectric Spectrophotometry Applied to the Analysis of Mixtures, and Vitamin A Oils

BY R. A. MORTON AND A. L. STUBBS

THE application of absorption spectrophotometry as a method of analysis is, in general, limited to those cases where information is available concerning the absorptive characteristics, in the relevant spectral region, of all constituents of the material under examination. In such circumstances, measurements of intensity of absorption at appropriate wavelengths may be used for the estimation of components of mixtures (for general principles, see Twyman and Allsopp[1]).

This paper describes an approach applicable to cases where constituents other than those which it is required to estimate make an unknown and variable contribution to the measured absorption.

The characteristic shape of the absorption curve of a pure solute is modified by the presence of other absorbing entities to an extent depending on the absorptive characteristics of the contaminants. The observed absorption curve is then a summation of the curves for the pure substance and the accompanying materials. Thus, the observed absorption may be used as a measure of the amount of one pure substance present, only if due allowance is made for the aggregate contribution (conveniently referred to as "irrelevant" absorption) of all other solutes.

The following method of correction for unknown irrelevant absorption is based on accurate measurements of intensities of absorption at three wavelengths, not very far apart, and the assumption is made that the irrelevant absorption is linear over this wavelength range.

In Fig. 1, let λ_1, λ_2 and λ_3 be the three chosen wavelengths, and let

$$\frac{E_{\lambda 1}}{E_{\lambda 2}} = k_1 \text{ and } \frac{E_{\lambda 1}}{E_{\lambda 3}} = k_2$$

where $E_{\lambda 1}$, $E_{\lambda 2}$ and $E_{\lambda 3}$ are the extinctions (expressed as log I_0/I) for the pure substance A at the three wavelengths.

Let E_1, E_2 and E_3 be the *observed* values for the extinctions at λ_1, λ_2 and λ_3 and x_1, x_2, x_3 be the contributions of the irrelevant absorption to these gross figures. Then E_1-x_1, E_2-x_2

and E_3-x_3 will be the contributions of A to the observed absorption at the three wavelengths, from any one of which the concentration of A may be estimated.

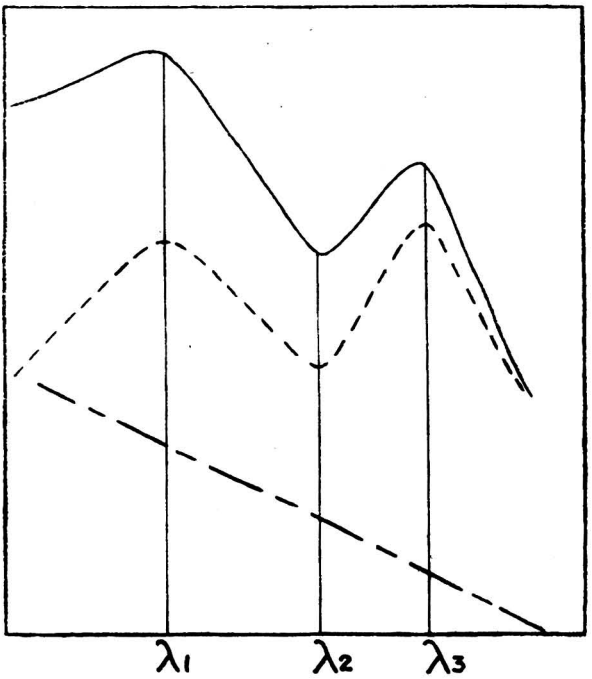

Fig. 1.

——— observed curve. – – – – – curve for pure substance. — – — irrelevant absorption.

Now since E_1-x_1, E_2-x_2 and E_3-x_3 are contributions of the substance A only,

$$\frac{E_1-x_1}{E_2-x_2} = k_1 \quad \text{and} \quad \frac{E_1-x_1}{E_3-x_3} = k_2,$$

and since the irrelevant absorption is assumed to be linear

$$x_1 = m\lambda_1 + c, \quad x_2 = m\lambda_2 + c \text{ and } x_3 = m\lambda_3 + c$$

where m and c are the constants for the straight line.

From these last three equations: $x_2 = x_1 - m(\lambda_1 - \lambda_2)$

and $x_3 = x_1 - m(\lambda_1 - \lambda_3)$.

Then substituting these values for x_2 and x_3:

$$\frac{E_1 - x_1}{E_2 - x_1 + m(\lambda_1 - \lambda_2)} = k_1$$

and $$\frac{E_1 - x_1}{E_3 - x_1 + m(\lambda_1 - \lambda_3)} = k_2.$$

Rearrangement of these equations gives:

$$k_1(\lambda_1 - \lambda_2)m = x_1(k_1 - 1) + E_1 - k_1E_2$$

and $$k_2(\lambda_1 - \lambda_3)m = x_1(k_2 - 1) + E_1 - k_2E_3.$$

Dividing the first of these equations by the second gives:

$$\frac{k_1(\lambda_1 - \lambda_2)}{k_2(\lambda_1 - \lambda_3)} = \frac{x_1(k_1 - 1) + E_1 - k_1E_2}{x_1(k_2 - 1) + E_1 - k_2E_3}$$

This may be solved for x_1, to give:

$$x_1 = \frac{E_1\{k_1(\lambda_2 - \lambda_1) + k_2((\lambda_1 - \lambda_3)\} + E_2k_1k_2(\lambda_3 - \lambda_1) + E_3k_1k_2(\lambda_1 - \lambda_2)}{k_1(\lambda_2 - \lambda_1) + k_1k_2(\lambda_3 - \lambda_2) + k_2(\lambda_1 - \lambda_3)}$$

The corrected value of E at λ_1, *i.e.*, $E_1 - x_1$, is then given by:

$$E_{\lambda 1}\text{ (corrected)} = \frac{E_1k_1k_2(\lambda_3 - \lambda_2) - E_2k_1k_2(\lambda_3 - \lambda_1) - E_3k_1k_2(\lambda_1 - \lambda_2)}{k_1(\lambda_2 - \lambda_1) + k_1k_2(\lambda_3 - \lambda_2) + k_2(\lambda_1 - \lambda_3)}$$

which may be written in the form

$$E_{\lambda 1}\text{ (corrected)} = E_1 \times A - E_2 \times B - E_3 \times C$$

where A, B and C are constants for any particular case.

The above treatment is quite general and is applicable to any three wavelengths, provided that they are sufficiently close together to justify the assumption of linearity for the irrelevant absorption. In practice, the method is not so cumbersome as might be thought from the foregoing.

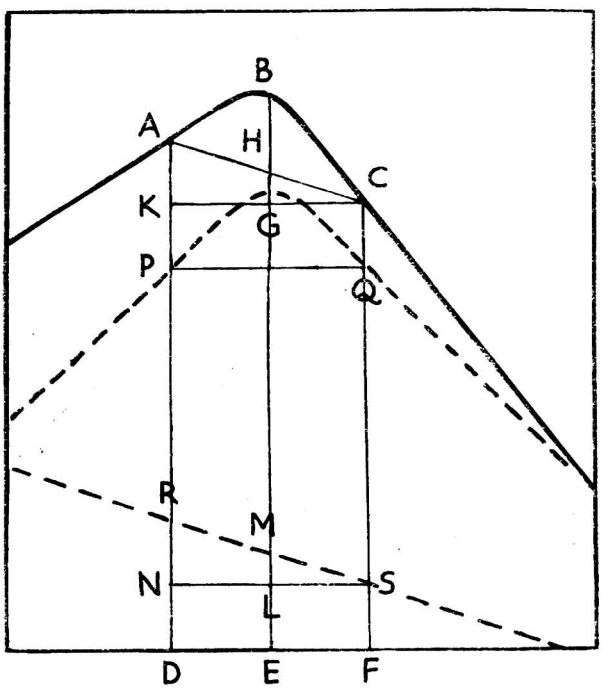

Fig. 2

A much simpler method of approach is possible if the wavelengths λ_2 and λ_3 can be chosen so that the extinctions for the pure substance at these wavelengths are equal. In Fig. 2 the dotted curve represents the absorption of the pure substance, λ_1 being the wavelength of maximum absorption and λ_2 and λ_3 wavelengths on either side of the maximum such that their extinctions are equal, *i.e.*, PQ is horizontal. The observed curve (continuous line) is a summation of that due to the pure substance and the irrelevant absorption RS.

At λ_1, the contribution of the irrelevant absorption to the gross observed absorption is ME, which must be subtracted from the gross figure to give the corrected extinction at λ_1. This irrelevant absorption may be divided into two parts, ML and LE. ML can be determined directly since it is equal to (LS/NS)RN, and RN will be the difference between the observed extinctions at λ_2 and λ_3; LS is equal to $(\lambda_3 - \lambda_1)$ and NS is equal to $(\lambda_3 - \lambda_2)$.

If LE is denoted by x, then the corrected values for the extinction at λ_1 and λ_2 are $(E_{\lambda 1}\text{ (obs.)} - \text{ML} - x)$ and $(E_{\lambda 3}\text{ (obs.)} - x)$ respectively. The ratio of these must be equal to the known ratio for the pure substance, say k.

$$i.e., \quad \frac{E_{\lambda 1}\text{(obs.)} - \text{ML} - x}{E_{\lambda 3}\text{ (obs.)} - x} = k.$$

This can be solved for x, and the value $E_{\lambda 1}$ (corrd.) obtained.

It is clear from the form of the expression for $E_{\lambda 1}$ (corrd.) that *differences* between observed extinctions are involved, which means that the precision of measurement must be

greater than that required in the final result. (It can be shown that, for the particular case of vitamin A referred to later, a deviation of ±0·5% in the measurements will give rise to a deviation of about 2·5% in the final result). Thus a photographic method of photometry is likely to be inadequate, particularly when measurements are being made on a steeply rising part of an absorption curve.

Two applications of this method of correction will be described, in both of which the measurements were made with the Beckman Photoelectric Spectrophotometer. The intensity of absorption (log I_0/I) can be rapidly determined at a given wavelength, the reproducibility being of the order of ±0·5%.

Determination of the Anthracene Content of Petroleum Oils

The problem was to determine whether a number of samples of petroleum oil contained anthracene and, if so, to determine the concentration.

Pure anthracene in hydrocarbon solvents[2] shows a number of narrow absorption bands between 300 mμ and 400 mμ, the most marked being at 356 mμ and 375 mμ, with a minimum in between at 366 mμ. The values of $E^{1\%}_{1cm.}$ at these wavelengths are 447, 447 and 89 respectively.

When the spectra of the petroleum oil samples in 0·5 mm. cells were photographed, using a rotating sector photometer and a tungsten spark under water as a continuous light source, they all showed the presence of these two bands, indicating qualitatively the presence of anthracene. The general shape of the absorption curves was, however, significantly different from that of pure anthracene, the absorption at shorter wavelengths being increased relatively to that at longer wavelengths, indicating the superposition of irrelevant absorption increasing in intensity with decreasing wavelength. The oils, after appropriate dilution with cyclohexane, were therefore examined with the Beckman Photoelectric Spectrophotometer, measurements of the extinctions being carried out at 356 mμ, 366 mμ and 375 mμ. From the measurements, and assuming that the irrelevant absorption is linear between 356 mμ and 375 mμ, the observed extinction may be corrected for irrelevant absorption as shown above.

Applying the general expression:—

$$E_{\lambda 1}\ (\text{corrd.}) = \frac{E_1k_1k_2(\lambda_3 - \lambda_2) - E_2k_1k_2(\lambda_3 - \lambda_1) - E_3k_1k_2(\lambda_1 - \lambda_2)}{k_1(\lambda_2 - \lambda_1) + k_1k_2(\lambda_3 - \lambda_2) + k_2(\lambda_1 - \lambda_3)}$$

For anthracene having $\lambda_1 = 356$ mμ, $\lambda_2 = 366$ mμ and $\lambda_3 = 375$ mμ

$$k_1 = \frac{\text{E356 m}\mu}{\text{E366 m}\mu} = \frac{447}{89} = 5{\cdot}0225$$

$$\text{and}\quad k_2 = \frac{\text{E356 m}\mu}{\text{E375 m}\mu} = \frac{447}{447} = 1{\cdot}000.$$

Then E356 mμ (corrd.)

$$= \text{E356 m}\mu(\text{obs.}) \times 0{\cdot}592 - \text{E366m}\mu(\text{obs.}) \times 1{\cdot}249 + \text{E375m}\mu(\text{obs.}) \times 0{\cdot}658$$

and

$$\text{anthracene, g./100 ml.} = \frac{\text{E356 m}\mu\ (\text{corrd.})}{447}$$

Table I shows the results obtained on 7 samples of petroleum oil of varying origin.

TABLE I

Sample	Dilution	Observed E values 356 mμ	366 mμ	375 mμ	Corrected E356 mμ	Anthracene in in original oil, % w/v
1	1:100	0·610	0·191	0·540	0·477	0·107
2	1:25	1·06	0·670	0·638	0·210	0·0117
3	1:25	1·06	0·690	0·650	0·193	0·0108
4	1:25	0·99	0·677	0·562	0·110	0·0062
5	1:25	1·02	0·690	0·573	0·119	0·0067
6	1:25	1·14	0·750	0·635	0·156	0·0087
7	1:500	1·62	1·297	1·102	0·064	0·072

Sample 1 contained 0·1% of added anthracene. The difference between this and the observed figure, 0·107%, should represent the original anthracene content of the oil. That this difference is in fact of the same order as the estimated anthracene content of similar oils, provides good justification for the correction procedure.

Estimation of Vitamin A

Three methods are available for the estimation of vitamin A: (*a*) biological tests, (*b*) the antimony trichloride colour test and (*c*) ultra-violet spectrophotometry.

The biological method is useless for routine purposes owing to the time and expense required in order to obtain any reasonable degree of precision.

The colour test is complicated by rapid fading, by a lack of complete specificity and by inhibition in certain preparations by other constituents accompanying the vitamin.

The spectrophotometric method, based on measurements of the intensity of absorption at the maximum of the vitamin A absorption band near 328 mμ, is the most satisfactory method from a practical standpoint, but depends for its success on the elimination or evaluation of absorption due to other constituents. For high potency fish liver oils it has hitherto been usual to assume that the contribution of absorbing constituents other than vitamin A

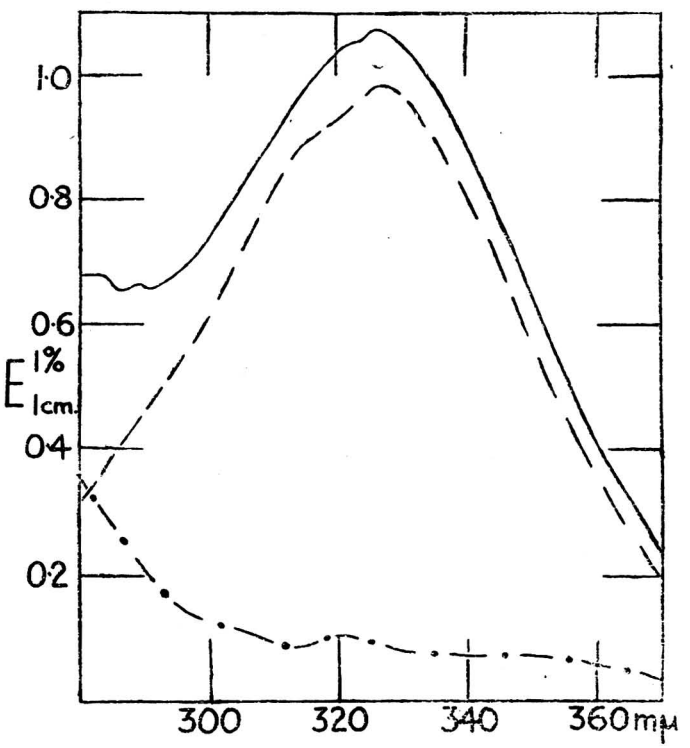

Fig. 3.

——— observed curve for cod-liver oil in cyclohexane.
– – – – observed curve for unsaponifiable matter of oil in cyclohexane.
— · — difference curve.

is negligible if the observed value of $E^{1\%}_{1cm.}$ 328 mμ is greater than 4·0, other features such as the general shape of the absorption curve and the "blue values" being "normal." For low potency oils ($E^{1\%}_{1cm.}$ 328 mμ less than 4·0), and for anomalous materials, it is usual to saponify and measure the absorption on the unsaponifiable fraction, thus eliminating unsaturated fatty acids which are, in such oils, mainly responsible for the irrelevant absorption.

Such an approach to the problem has for a number of years been broadly satisfactory, particularly in the correlation between ultra-violet absorption and the colour test. Some preparations, however, give anomalous results and the saponification procedure does not always eliminate all irrelevant absorption, *e.g.*, absorption arising from oxidative products or from "cyclised" products of vitamin A produced during the processing of the oil. In an attempt to achieve a more rigorous evaluation of vitamin A content the correction procedures outlined above have been applied to the ultra-violet absorption spectra of fish oils.

The first essential is an accurate determination of the absorption spectrum of pure vitamin A. Even at the present time, when the free vitamin and its esters have been obtained crystalline, such a determination is not easy, particularly since few crystalline specimens examined in this laboratory appear to be free from vitamin A_2 as judged by the criterion of the 693 mμ absorption band in the antimony trichloride colour test. A number of workers have published curves for various preparations of crystalline vitamin A and its esters,[3,4] but, as far as can be ascertained, none on specimens *known* to be free from vitamin A_2.

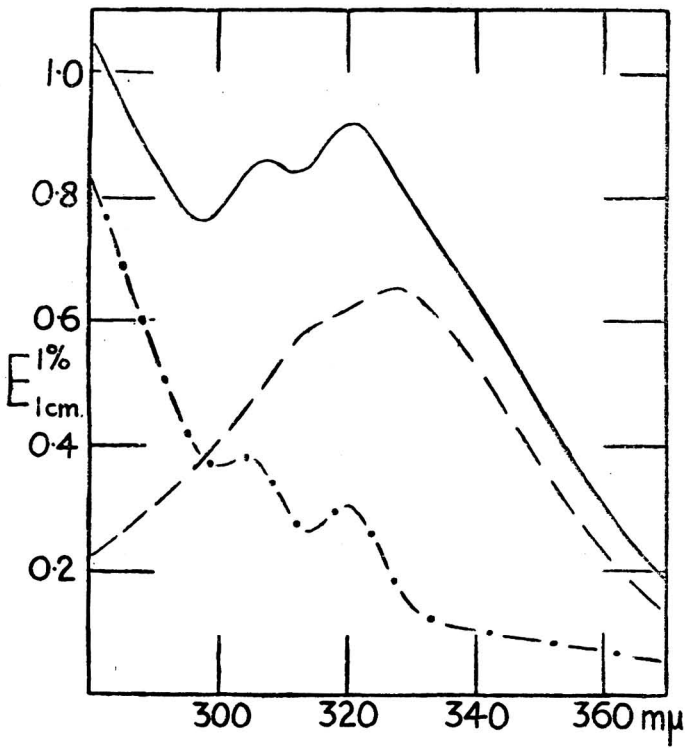

Fig. 4.

——— observed curve for cod-liver oil in cyclohexane.
— — — — observed curve for unsaponifiable matter of oil in cyclohexane.
—— · —— difference curve.

For the purpose of applying the correction procedure, all that is required is an accurate determination of the intensity of absorption at three wavelengths for a solution containing vitamin A as the only absorbing constituent. If the maximum in the absorption curve for vitamin A is taken as one of the wavelengths the correction is considerably simplified, as already shown, by choosing the other two wavelengths such that their extinctions are equal, *i.e.*, wavelengths on either side of the maximum. The only further information required is the ratio of the intensity of absorption at the maximum to that at these wavelengths. The choice of these wavelengths (or at least one of them, since choice of one fixes the other by the stipulated equality of their extinctions) is of some importance. They must be close enough to the maximum for the irrelevant absorption to approximate to linearity over the range, but sufficiently far apart for the ratio of extinctions to be large enough to give the required accuracy. Since an important application of the correction is the estimation of the vitamin A potency of cod liver oils *without* saponification, the choice of wavelengths has been made from a study of cod liver oils and their unsaponifiable fractions.

The absorption spectra of a number of cod liver oils and their unsaponifiable fractions have been measured accurately with the Beckman Photoelectric Spectrophotometer. The spectra of the "unsaponifiables" are in very good agreement, but the oils show differences which are sometimes very marked (Figs. 3 and 4). Measurements of extinctions at the maximum (328 mμ) and two other wavelengths for the oils would make it possible to correct

the observed values at 328 mμ to give corrected values for the vitamin A absorption in agreement with those obtained on the unsaponifiable fractions, provided (*a*) that the irrelevant absorption in the oil is linear over the range of the three wavelengths and (*b*) that the absorption of the unsaponifiable fraction is due to vitamin A only. Supposing, for the moment, that the second assumption is correct, (*a*) may be tested by subtracting the curves for the "unsaponifiables" from those of the original oils. For the majority of samples studied the subtraction curve, *i.e.*, the irrelevant absorption in the oil over and above that of the unsaponifiable, is in fact very close to linearity over an appreciable wavelength range (Fig. 3). Others depart markedly from linearity and show the presence of two maxima near 305 mμ and 320 mμ respectively, probably due to conjugated tetra-ene acids (Fig. 4). If the correction is to be applicable to such samples, the most satisfactory choice of wavelength on the short-wave side of the maximum will be at a point where this selective absorption makes a minimum contribution, *i.e.*, between 312 and 315 mμ. In order to include materials showing this type of irrelevant absorption, a wavelength of 313 mμ has been adopted for the point on the short-wave side of the maximum. The third point is then the wavelength at which the extinction is equal to E313 mμ for pure vitamin A.

In order to obtain sound values for this third wavelength and for the ratio E328 mμ/E313 mμ, accurate measurements have been made on two vitamin A acetate preparations; the results are shown in Table II. The results obtained on the vitamin A ester fraction from sheep liver oil, separated chromatographically by a method which has been worked out in this Department and which is to be published shortly, are also included for comparison, since normal mammalian livers contain little, if any, vitamin A_2. The extinctions have all been made equal to 1·0 at 328 mμ.

TABLE II

Wavelength mμ	Crystalline vitamin A acetate Sample (*a*) E	Crystalline vitamin A acetate Sample (*b*) E	Ester fraction from sheep liver oil E	Wavelength mμ	Crystalline vitamin A acetate Sample (*a*) E	Crystalline vitamin A acetate Sample (*b*) E	Ester fraction from sheep liver oil E
250	0·136	0·133	—	320	0·935	0·933	0·946
255	0·137	0·136	—	322·5	0·962	0·959	0·967
260	0·138	0·133	—	325	0·989	0·987	0·987
265	0·139	0·136	—	326	0·996	0·992	0·997
270	0·160	0·155	—	327	1·000	0·999	1·000
275	0·194	0·191	—	328	1·000	1·000	1·000
280	0·240	0·236	0·282	329	0·999	0·999	0·998
285	0·301	0·298	0·346	330	0·997	0·993	0·997
290	0·375	0·371	0·415	335	0·925	0·925	0·923
295	0·471	0·466	0·508	338·5	0·858	0·856	0·851
300	0·571	0·570	0·605	340	0·828	0·827	0·816
305	0·666	0·670	0·697	345	0·708	0·706	0·703
310	0·794	0·796	0·813	350	0·574	0·570	0·567
312	0·837	0·839	0·852	355	0·443	0·437	0·436
313	0·858	0·858	0·872	360	0·331	0·330	0·328
315	0·888	0·889	0·905	365	0·239	0·238	0·239
317·5	0·916	0·914	0·930	370	0·151	0·151	0·157

Both samples of vitamin A acetate were apparently free from vitamin A_2, no band being observed at 693 mμ in the colour test and the ratio E620 mμ/E693 mμ being greater than 70 even without allowance for the "spread" of the 620 mμ band in the comparatively very strong solutions used. Thus, the contribution of vitamin A_2 to the ultra-violet absorption is probably negligible for all three materials. The agreement between the results obtained on the two crystalline samples of acetate is extremely good throughout the wavelength range studied, and figures on the sheep liver oil agree very well with those for wavelengths above 325 mμ, indicating that the vitamin A_2 contribution ($\lambda_{max.}$ 345–350 mμ) is in fact negligible. At shorter wavelengths the sheep liver oil apparently possesses some irrelevant absorption increasing with decreasing wavelength.

On the basis of these results the following provisional criteria are therefore suggested for vitamin A ester in cyclohexane:

$\lambda_{max.} = 328$ mμ;

$E^{1\%}_{1cm.}$ 328 mμ = 1700 (calc. as free vitamin);

E313 mμ = E338·5 mμ;

E328 mμ/E313 mμ = 1·166.

Using these criteria, any observed value at E328 mμ may be corrected by the simpler procedure described above where

$$\lambda_1 = 328 \text{ m}\mu,\ \lambda_2 = 313 \text{ m}\mu,\ \lambda_3 = 338{\cdot}5 \text{ m}\mu \text{ and } k = 1{\cdot}166.$$

TABLE III

Sample	$E^{1\%}_{1\text{ cm.}}$ 328 mμ (obsd.)	$E^{1\%}_{1\text{ cm.}}$ 328 mμ (corrd.)	$E^{1\%}_{1\text{ cm.}}$ 328 mμ (unsaponifiable)
Cod liver oil 45	1·296	1·120	1·136
,, ,, ,, 2	0·912	0·798	0·792
,, ,, ,, 18*	0·826	0·636	0·653
,, ,, ,, 19	1·063	0·950	0·993
Vet. cod liver oil 1/(b)*	0·599	0·354	0·360
Cod liver oil 20*	0·826	0·616	0·625
,, ,, ,, 21	0·732	0·564	0·590
,, ,, ,, CV36*	0·741	0·505	0·525
,, ,, ,, 109	0·844	0·730	0·744
,, ,, ,, NM36/1A*	0·542	0·309	0·367 (corrd. 0·313)
,, ,, ,, CC23	0·850	0·731	0·728
,, ,, ,, CC26	0·842	0·664	0·692 (corrd. 0·647)
Vet. cod liver oil NM2/13*	0·552	0·306	0·342 (corrd. 0·291)
Cod liver oil CC27	0·792	0·666	0·630
,, ,, ,, CV37*	0·960	0·737	0·750
,, ,, ,, OCO90	1·196	1·096	1·062
,, ,, ,, OCO98	1·172	1·117	1·044
,, ,, 6474	0·607	0·505	0·490
,, ,, ,, CC24	0·714	0·551	0·594 (corrd. 0·560)
,, ,, ,, CC29*	0·836	0·650	0·644

Samples marked * had spectra indicating the presence of conjugated tetra-ene acid (*cf.* Fig. 4).

Table III shows results which have been obtained on a number of cod liver oils; the samples marked with an asterisk show absorption indicative of the presence of conjugated tetra-ene acids. It is clear from these results that for the great majority of samples the saponification procedure can be eliminated if the absorption of the oil is measured at three wavelengths with an accuracy of ±0·5%, and a corrected value of E328 mμ calculated. This is likely to be especially applicable to the examination of a large number of similar materials, the saponification procedure being used as an additional check in doubtful cases.

DISCUSSION

The foregoing treatment of spectrophotometric data is much more rigorous than has been possible in the past, with photographic methods of photometry. The introduction of photoelectric photometry clearly provides a basis for a considerable extension of spectrophotometric methods of analysis both in the achievement of greater precision in the analysis of simple mixtures and in the treatment of the types of system considered above.

The application to the spectrophotometric assay of vitamin A raises a number of important points. The suggested criteria for the ultra-violet absorption of vitamin A must be considered as only provisional in view of reports that isomers of vitamin A occur showing slightly different absorption characteristics,[6] and further, the absorption spectrum of the free vitamin may differ slightly from that of the esterified material. The evidence so far obtained on this latter point suggests that any such differences are likely to be small.

It is clear that the accurate determination, in an oil, of the absorption at 328 mμ which is due unequivocally to vitamin A is the first step to the precise evaluation of potency. Such a determination must involve, in most instances, some correction for irrelevant absorption and, as will be seen from Table III, saponification is not infallible in this respect and may on occasions give rise to discrepant results. Furthermore, all high potency oils so far examined need some slight correction for irrelevant absorption, including absorption due to vitamin A_2. When a fully corrected figure for the absorption at 328 mμ has been obtained, the next step, so far as spectroscopy is concerned, is a purely formal one; namely to multiply the $E^{1\%}_{1\text{cm.}}$ 328 mμ by an appropriate conversion factor so as to express the result in International Units per gram.

The selection of a factor is a matter with an involved history. If attention is confined to the three co-operative experiments organised by the Vitamin A Sub-Committee of the

Accessory Food Factors Committee (Medical Research Council), the factor lies between 1570 and 1820:[5]

	Factor	Upper limit of error P=0·95
Halibut liver oil ..	1570	140%
Cod liver oil	1820	129%
Vitamin A naphthoate..	1770	139%

It seems probable that the estimated value of $E^{1\%}_{1cm.}$ 328 mμ for the cod liver oil (determined on the unsaponifiable fraction) and for the vitamin A naphthoate (after correction for the naphthalene contribution) involved smaller irrelevant absorption contributions than the value obtained for the halibut liver oil. We suggest, therefore, that the original factor of **1600** (1934 Conference, L.N. Committee on Biological Standards) should be used, *unless the absorption at* 328 mμ *is fully corrected* for irrelevant absorption, in which circumstances a factor **1760** will be justifiable. In a wide range of commercial fish liver oils $E^{1\%}_{1cm.}$ 328 mμ *gross* $\times$ 1600 will not be very different from $E^{1\%}_{1cm.}$ 328 mμ (*corrd.*) $\times$ 1760, but whenever possible full correction is to be preferred.

The analytical problem for vitamin oils in general seems likely in the future to be made up of the following factors:

(*a*) total vitamin A content expressed in terms of mg. of vitamin (calculated as alcohol) per gram or in I.U./g.;
(*b*) vitamin A content as free alcohol and as ester;
(*c*) vitamin A_2 content;
(*d*) relative amounts of decomposition products of vitamin A and other non-glyceride contaminants.

It is proposed at a later date to discuss these problems more fully, using the experience gained on materials tested for the Ministry of Food.

We are indebted to the Medical Research Council and to British Drug Houses, Ltd., for samples of crystalline vitamin A acetate made available by Distillation Products, Inc. The Beckman apparatus was purchased from a gift made by J. Bibby & Sons, Ltd. One of us (A. L. S.) participates in the work as holder of an I.C.I. Fellowship.

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Some Modern Aids to Spectrochemical Analysis

By B. S. COOPER

Although for the past fifty years there has been steady progress in the development of spectroscopic methods and their application to chemical analysis, it is only within the last few years that the methods of emission and absorption spectroscopy have attained their full status as reliable quantitative procedures. In fact at the present time we are witnessing some notable developments in technique in both branches of spectroscopy and, judging by some of the most recent publications, quantitative routine and control work will, in future, be carried out by equipment and methods markedly different from those in general use to-day.

It is not intended to give full and detailed descriptions of the apparatus and methods now available to spectroscopists, but rather to draw attention to some of the special devices which have made possible these recent developments. The opportunity is also being taken to mention one or two other accessories which may be of interest to the general spectroscopist and to note the present position with regard to optical materials. Spectrograph design is

too specialised a subject to introduce into this review; it is best left in the hands of the expert, who to-day can provide an instrument that requires little or no adjustment by the user.

It often happens that a complete change of technique, with the possibility of new fields of application, results from the introduction of some new device or material. The application of the photographic plate to spectroscopic studies by Becquerel in 1842 is an instance in point. At the present time the introduction of the ultra-violet sensitive multiplier photocell would appear to be leading to important changes in the practice of emission spectroscopy.

The first items to be discussed may, for convenience, be grouped together.

Developments in Light Sources

On the instrumental side the main progress in emission spectroscopy lies in the development of electrical circuits which will enable spectra to be excited with the greatest reproducibility consistent with high sensitivity. The term "light source" is generally used in this branch of spectroscopy to denote the electrical circuit equipment as well as the actual spectrum-emitting material. In this country, a modern circuit has recently been described in THE ANALYST,[1] whilst a circuit which may represent an important advance in this field has been developed in the Laboratories of the British Non-Ferrous Metals Research Association.[2] Commercial equipments with which a wide variety of controlled excitation conditions can be obtained are now available in the United States.

Turning now to the electrodes themselves, it appears that for quantitative analysis of many alloys it is advantageous to cast the test samples in a mould of special design and select certain predetermined portions of the casting as sample electrodes.[3] The amount of material consumed by modern excitation techniques is often so small that it may be necessary to make some preliminary study of the conditions for obtaining a representative and homogeneous sample.

In steel analysis it now appears to be fairly common practice to use what is known as the "flat surface sparking technique."[4,5] One electrode consists of a "flat," ground or machined on the sample to be analysed, whilst the other electrode is a shaped rod of high purity carbon or graphite. It is clear that this technique is specially useful in determining the composition of samples already in fabricated form.

The only other point I wish to mention in connection with electrodes relates to high purity graphite. This is one of the most useful electrode materials both for qualitative work, particularly on trace impurities, and for quantitative methods using solutions. The grade available before the war, and still generally used, was of high purity as regards the commoner elements, but contained sufficient titanium and vanadium to render it useless where trace concentrations of these two elements were being sought. During the past few years a much higher purity material has become available that has the merit of being free from the two elements just mentioned. Each lot of the material produced is tested spectrographically, and a report on the purity is provided by the suppliers.

With regard to light sources for absorption spectroscopy, the desirable features of such a source may be summarised as follows—

(1) It should emit radiation over the entire range of wavelengths likely to be of interest in the investigation.
(2) Except for routine quantitative work on known substances the spectrum of the source should be continuous.
(3) The source "brightness" (in the sense of radiation output per unit area) should be as high as possible.

There is no one source that fulfils all these requirements throughout the ultra-violet, visible and infra-red regions. The high-current-density hydrogen discharge lamp is best for the ultra-violet region, the tungsten filament for the near ultra-violet, visible and near infra-red, and the Nernst filament (or silicon carbide rod) for the infra-red region generally.

Many different designs of hydrogen source have been produced, some considerably more complex than others. One that is likely to become generally available in this country has recently been improved by my colleagues at Wembley*; the characteristic continuous ultra-violet spectrum obtained with it is shown in Fig. 1.

Another type of electric discharge tube which is being used experimentally for spectrophotometric work contains mercury, cadmium and zinc in a small fused-silica envelope.

* This lamp was shown at the meeting.

This source gives a relatively large number of strong lines throughout the visible and ultra-violet. A suggested application is for the quantitative determination of a number of constituents in a mixture of known qualitative composition; the extinction coefficient is determined (*e.g.*, by photoelectric response), using a convenient line for the most sensitive absorption region of each constituent. Fig. 2 shows the ultra-violet spectrum of this source at different electrical loadings. It runs steadily for long periods at any selected loading, and, owing to its high brightness, provides relatively high radiant power of the required wavelengths.

The tungsten filament lamp, although of restricted application, can still be used with advantage in some spectrochemical problems. For calibration work it is valuable, since the spectral energy distribution can be derived from the filament temperature, the spectral emissivity values for tungsten and the spectral transmission curve of the lamp envelope. For work in the ultra-violet region it is not essential to use fused-silica envelopes or quartz windows. The expense and difficulty of making such lamps is considerable and it may be of interest to know that an equally satisfactory calibration source can be made using a special form of thin glass window.* A concave hemispherical window, of adequate strength to resist the pressure difference inside and outside the lamp, can be made with a thickness so small that radiation from the tungsten filament as low as 2700A in wavelength is transmitted. The same glass, in normal bulb wall thickness absorbs all radiation below 3000A. Quartz has practically no advantage over the thin glass window since the radiation from the tungsten itself becomes negligible below 2700A.

Optical Materials

The transmission ranges of optical materials used in spectroscopic dispersing systems are given in Table I.

Table I

Transmission ranges of optical materials

Ultra-violet transmission limit μ	Material	Origin	Infra-red transmission limit (approx.) μ
0·30	Special glasses	Synthetic	3
0·17	Quartz	Natural	3
0·14	Lithium fluoride	Synthetic	6
0·10	Fluorite (CaF_2)	Natural	9
—	Rocksalt (NaCl)	N or S	17
—	Sylvine (KCl)	Synthetic	20
—	Potassium bromide	,,	25
—	Thallium bromo-iodide	,,	50

The trend towards the use of synthetic materials should be noted. Of particular interest is lithium fluoride, which transmits throughout the ultra-violet and over an appreciable range of the near infra-red region; this material has the advantage of being stable to moisture.

Quartz is the principal optical material of which considerable supplies of the natural crystal are still required. Various factors have caused the price to advance very rapidly during the past few years and to-day the cost of the raw material for the manufacture of the optical parts of a quartz spectrograph represents an appreciable proportion of the total cost of the instrument. Regarding the possibility of producing synthetic quartz crystals, it may be noted that some progress is being made in this direction.[6] Perhaps it is not being too optimistic to suggest that synthetic quartz crystals of sizes suitable for spectrograph components may be produced within a few decades.

It should be borne in mind that new organic plastics may provide useful additions to the list of optical materials, although up to the present none has shown sufficient advantages to displace any of the inorganic materials listed.

Improvements in recent years in the ruling and aluminising of gratings, and in the production of accurate replica gratings, have increased their accessibility and usefulness. A ruling technique developed by R. W. Wood[7,8] results in the concentration of most of the reflected energy in one order of the spectrum and on a given side of the normal. Although grating spectrographs are now available commercially,[9] most of the instruments in use are

* This lamp was shown at the meeting.

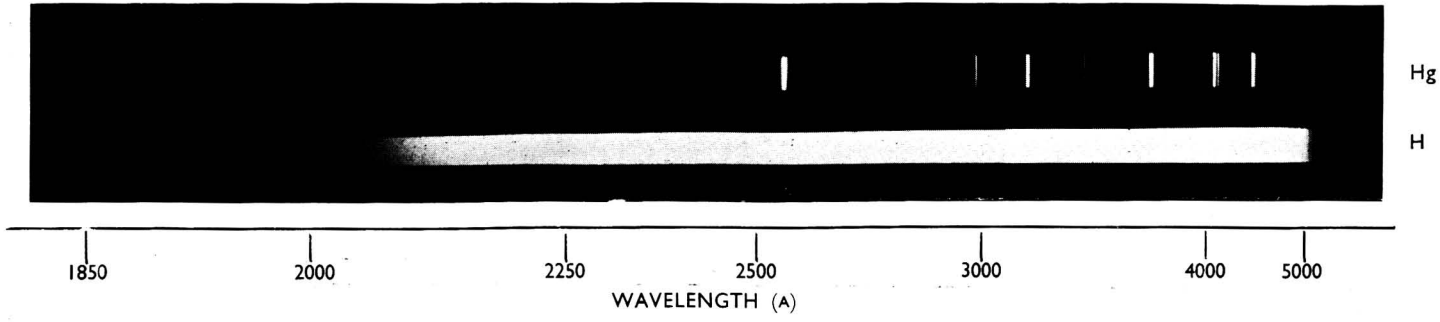

Fig. 1. Ultra-violet spectrum of high intensity hydrogen source.

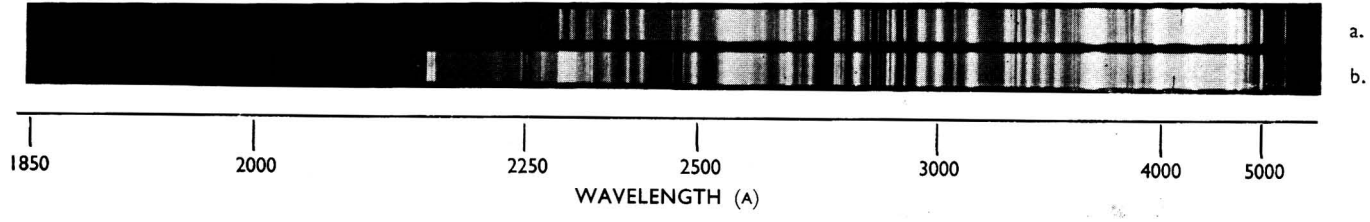

Fig. 3. Sensitisation of photographic plate to far ultra-violet, using fluorescent mineral oil.
a. Spectrum photographed on normal Kodak B.40 plate.
b. Spectrum photographed on sensitised Kodak B.40 plate.

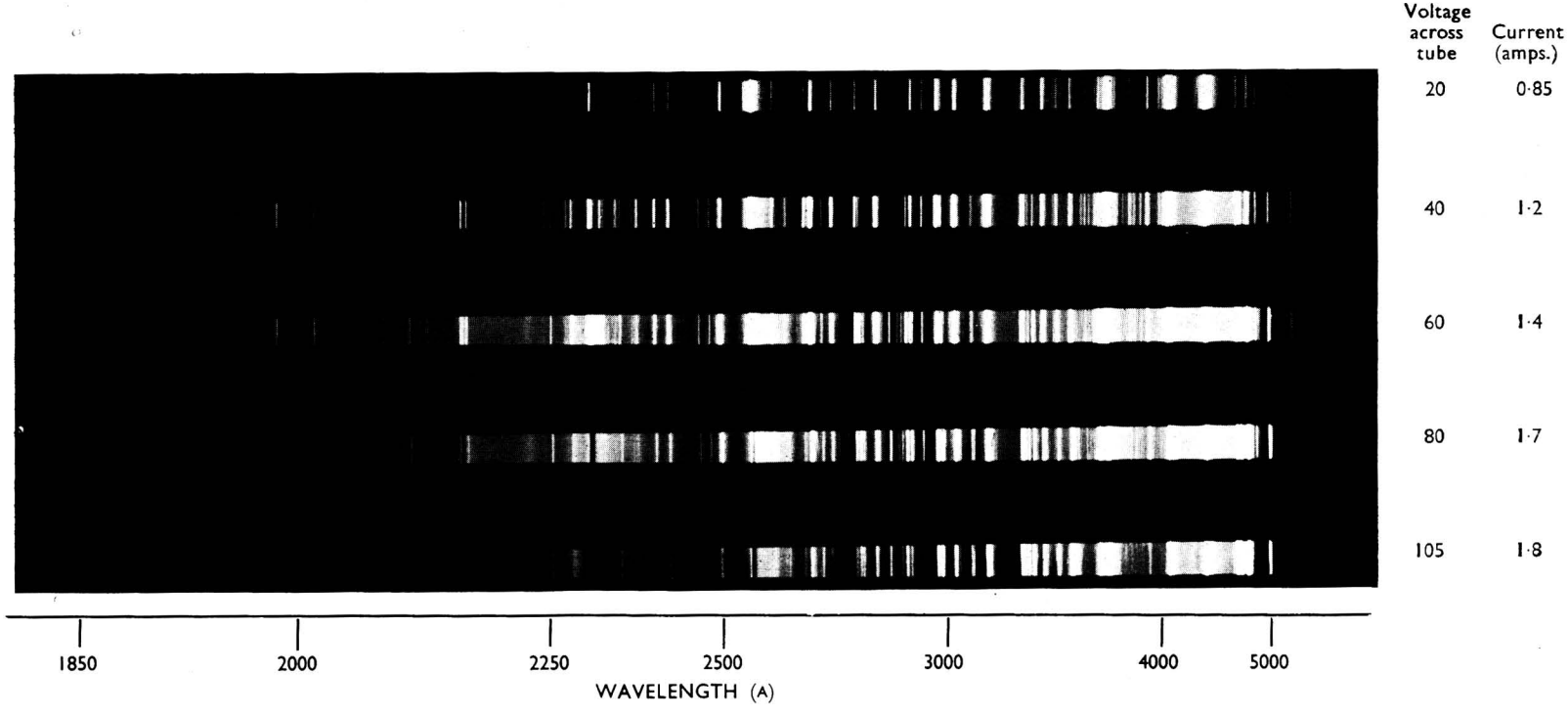

Fig. 2. Ultra-violet spectra of experimental Hg–Cd–Zn spectroscopic source at different loadings.

still of the prism type. However, should the supply of optically useful crystal quartz become very restricted, the grating instrument may become much more generally used.

Another advance in optical materials, particularly important for "abridged spectrophotometry," is in the development of colour filters having narrow transmission bands and high peak transmissions.[10] Although they are not yet available commercially, descriptions have recently been given of optical interference filters which may have transmission bands of half value width 0·005 μ to 0·15 μ and peak transmissions as high as 30% in the range 0·45 μ to 1 μ.

Developments in Energy Receivers

Photographic Plates

The first modern development that may be mentioned is by no means novel, but as it was used in studying the spectra of the electric discharge ultra-violet sources discussed earlier (see page 357) it may be appropriate to include it here. At wavelengths below about 2100A the absorption of the gelatin emulsion of the plate is sufficiently high to absorb the ultra-violet radiation almost completely before it can affect the silver halide particles. However, it is possible to obtain an adequate photographic image at these wavelengths by coating the gelatin emulsion with a fluorescent substance, which produces an emission of higher wavelength radiation to which the gelatin emulsion is much more transparent.

Fig. 3 shows two spectra of the same source, one on a sensitised and the other on a non-sensitised plate. In this instance, the emulsion was coated with a thin film of a mineral oil (found by previous trial to be the most effective of a number tested) which was removed, by washing the plate in light petroleum, prior to processing.

A recent important development is a new plate specially designed for industrial emission spectrography (the Kodak Uniform Gamma plate). This is intended for routine ultra-violet spectrography and has a practically uniform contrast factor (γ) over the wavelength range 2600A–4000A. Moreover, the actual value of γ obtained (1·0 to 1·2) appears to be the most suitable for the purpose. This range of wavelengths covers most of the requirements for metallurgical analysis, including that of steel. Those who are concerned with the calibration of photographic plates in emission spectrography will appreciate the value of having available a plate with these characteristics.[11]

Thermopiles

A range of thermopiles of new design (due to E. Schwarz) has recently become available.[12] The advantageous characteristics of this type of thermopile are:

(i) The thermo E.M.F. of the junctions is high; it may be from 5 to 40 times that of previous types of thermo-junction.

(ii) The electrical resistance of the junctions is low and can be made to suit any galvanometer.

(iii) The thermal resistance is high (*i.e.*, low conductivity).

(iv) The speed of response is extremely high; it may be appreciably less than 0·05 secs.

A large increase of sensitivity and stability can be obtained by mounting *in vacuo*. With pressures as low as 10^{-5} to 10^{-6} mm. of mercury, vacuum factors of 40 times can be obtained with the Schwarz thermopile. The evacuated envelope can be fitted with a quartz, fluorite or potassium bromide window in order to obtain the required wavelength range. A getter is provided which can be activated when required should the sensitivity fall off owing to a slight decrease in the vacuum. For spectral energy investigations or for general absorption work these thermopiles represent a notable advance.

Galvanometer Amplifiers

The older type of moving magnet galvanometer used for the highest sensitivity measurements with thermopiles or photocells has now been superseded by a more robust form of galvanometer, used with a photoelectric device which amplifies the deflection of the mirror several hundred times. Fig. 4 shows one form of galvanometer amplifier. A beam of light, reflected from the primary galvanometer (*i.e.*, the one connected directly to the thermopile or other energy-receiving device), illuminates two rectifier type photocells connected in opposition, the light being divided between the two cells so that they give equal current for zero deflection. A small deflection of the galvanometer alters the balance of illumination on the two cells and this difference is indicated by a further galvanometer, usually called the

secondary galvanometer, in circuit with the cells. This deflection may be several hundred times that of the primary galvanometer, the only limit to the degree of magnification that can be employed being imposed by the possibility of the Brownian motion effect becoming apparent.

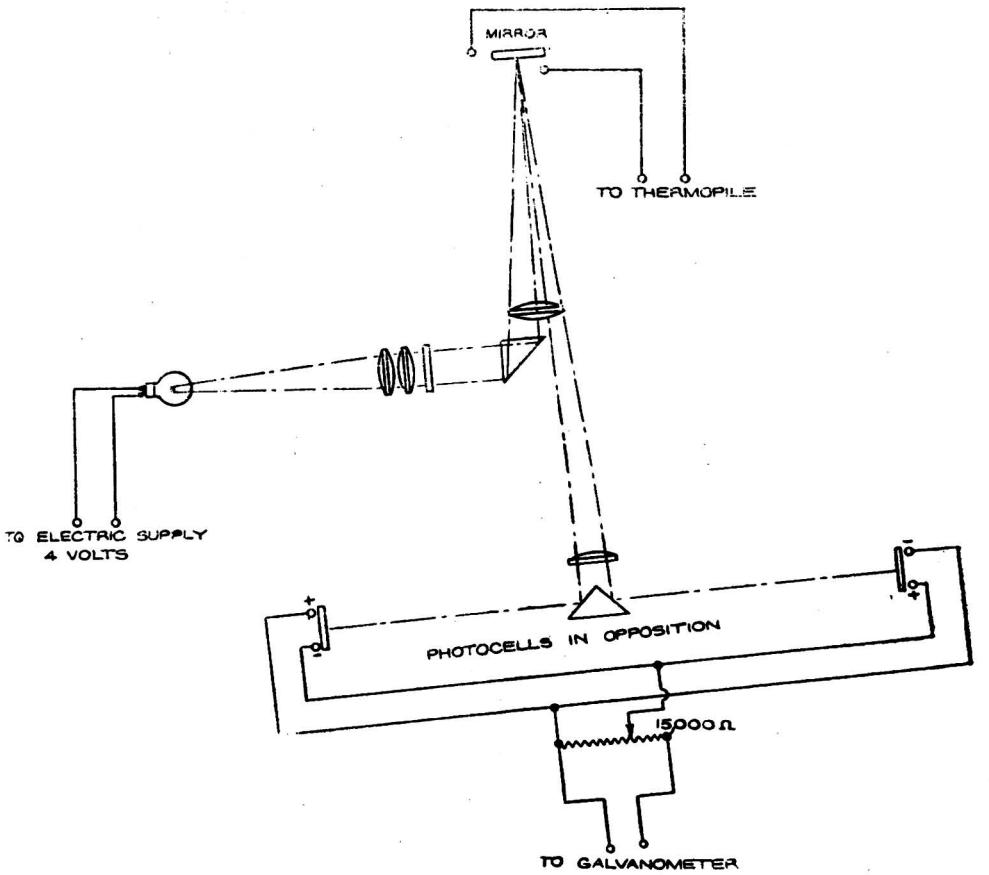

Fig. 4.

Diagrammatic layout of galvanometer relay (Hilger)

Multiplier Photocells

Some of the present novel developments in emission spectroscopy have resulted from the application of these cells in place of the photographic plate. In an electron multiplier, the photo-current produced by the incidence of radiation on a sensitised cathode is multiplied many times by secondary emission occurring at successive electrodes within the tube. By suitable focussing of the electron beam between successive stages very high overall amplification may be obtained. Either magnetic or electrostatic focussing is possible, but the latter has been developed in an ingenious manner in the compact RCA multiplier phototube with which all the new American developments have been achieved. One version (IP.28) of this multiplier is constructed in a special glass envelope transmitting in the ultra-violet down to about 2000A and is capable of amplifying feeble currents produced under weak radiation by an average of 200,000 times when it is operated at 100 volts per stage. The resulting output current is a linear function of the exciting radiation, and the response is instantaneous.

In one of the first applications of multiplier photocells to spectrum recording,[13] the exit slit and multiplier were both mounted on a little carriage that could move along the exact focal curve of the spectrograph. The photo-current was fed to an electronic recording micro-ammeter which, on scanning the spectrum, traced a curve generally similar in appearance to the microphotometer trace of a spectrogram. The noticeable absence of "ripple" on the base

line of the trace allowed peaks due to very faint lines to be identified and measured. On a corresponding microphotometer trace these small peaks were lost in the "ripple."

The next application to be published was in connection with the analysis of magnesium alloys.[14] The apparatus was arranged so that a framework in the focal plane of the spectrograph carried exit slits in positions corresponding to selected analysis lines of the alloying constituents to be determined. The multiplier cell in its mount could be moved on a carriage to receive radiation as required from each of these slits. A second photocell in a fixed position received radiation reflected from the focus of a magnesium line selected to serve as the internal standard. A ratio of the outputs of the two cells receiving analysis line and internal standard line respectively was obtained by using a valve voltmeter to indicate the difference of potential between their anodes when each was connected to a 20 megohm load. The voltmeter dial was calibrated from working curves to read directly the percentage composition of the sample under test. The constituents determined were aluminium, zinc, manganese, silicon, copper, nickel, iron and lead.

A subsequent development[15] has made the whole process more automatic, the final stage incorporating a multipen recorder which marks the percentages of ten constituents on a specially calibrated chart. The small size of the electrostatically focussed multiplier is seen to advantage in this application where no fewer than eleven tubes, with their associated optical components, are grouped at the exit end of the spectrograph.

A commercial equipment utilising the same principles is now being advertised and, although no technical data regarding performance are yet available, it is claimed that a steel can be analysed quantitatively for eleven constituents "within forty-five seconds of placing the sample in the Quantometer."

Although these last developments are clearly limited to routine control analysis, it is evident that we are now on the threshold of some very notable advances in the techniques of spectrochemical analysis as applied to this particular field.

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Applications of Spectrographic Analysis to Soil Investigations

By R. L. MITCHELL

In the work of the Macaulay Institute we are interested in the changes occurring during the processes of soil formation, and in the relationship of the soil to the yield, composition, and health of the plants and animals associated with it, as well as in the analysis of the soil itself. The recent realisation of the importance of certain trace constituents to the health both of plants and animals has led to a search for methods for their determination, and arc methods of spectrographic analysis have proved suitable. The quantities involved, often of the order of parts per hundred million, are generally smaller than those considered important in metallurgical analysis, although the demand for accuracy is possibly not quite so great, and at present we are satisfied with errors of the order of ± 10 per cent. Flame spectrographic methods, applicable to the determination of the alkalis and alkaline earths in solution,

are being employed, particularly for the routine determination of potassium in extracts of soils and plants.

A soil consists essentially of an inert framework of silicate minerals, together with an active portion of weathered, colloidally active, clay minerals and some organic matter. The colloidally active fraction carries those ions which can be termed "available" to the plant, whilst the reserves of most constituents are bound up in the crystal lattices of the silicate minerals and are only slowly released by weathering. A knowledge of the ultimate chemical composition is of little practical use as far as the major constituents are concerned, and determinations of these by spectrographic methods have not been attempted at the Macaulay Institute.

TABLE I

TRACE ELEMENT CONTENTS OF THREE TYPICAL SOILS FROM N.E. SCOTLAND DETERMINED BY THE SEMI-QUANTITATIVE CATHODE LAYER ARC SPECTROGRAPHIC METHOD, AS PARTS PER MILLION

	Wavelength A	Sensitivity	Soil derived from: Granite	Norite	Old Red Sandstone
Rb	7800·2	20	600	30	200
Li	6707·8	1	7	20	50
Ba	4934·1	5	2000	1000	700
Sr	4607·3	10	300	500	200
Cr	4254·3	1	5	50	200
Sc	4246·8	10	—	20	10
Mn	4030·8	10	700	2000	500
Co	3453·5	2	—	30	18
Ni	3414·8	2	10	15	60
Zr	3392·0	10	2000?	150	300
Zn	3345·0	300	—	—	—
La	3337·5	30	30	—	30
Y	3327·9	30	30	—	30
Ag	3280·7	1	—	—	—
Cu	3274·0		<10	10	40
V	3185·4	5	20	300	100
Mo	3170·3	1	—	—	—
Be	3131·1	10	—	—	—
Ga	2943·6	1	25	20	20
Sn	2840·0	5	5	—	—
Pb	2833·1	10	20	—	—
Tl	2767·9	50	—	—	—
Ge	2651·2	10	—	—	—

A dash indicates that the content is less than the sensitivity quoted.

A determination of the total content in a soil probably has more significance for the trace constituents than it has for the major constituents, since the amounts of the trace constituents may vary upwards of one thousand fold from soil to soil, whilst the major constituents seldom vary more than five to ten fold. Because of these large variations, and because the relationship between total content and plant availability is not exact, a semi-quantitative determination, giving an accuracy of $\pm 30\%$ under favourable conditions, is employed for such determinations. This is the original cathode layer arc technique as described by Mannkopff and Peters,[1] Preuss,[2,3] Strock,[4] Tongeren[5] and Mitchell.[6] A mixture of the finely ground material with carbon powder is filled into a deep, narrow boring (8 mm. × 0·8 mm.) in a thin (2·8 mm. diam.) carbon electrode, and burnt as the cathode in a 9 amp. direct current arc. The arc is imaged on the slit in order to take full advantage of the cathode layer effect, there being marked increase in sensitivity in the immediate neighbourhood of the cathode for elements with low ionisation potentials.

The spectrograms are compared in a spectrum comparator with those for standard mixtures similarly treated and the approximate content estimated. It is possible by this means to get an indication of any trace constituents present in deficient or excessive amount. This method has been used for the determination of the trace constituents in different layers of soil profiles,—it has shown for instance that cobalt and nickel do not follow iron during podzolization,—and it is also being used in the investigation of rocks and their individual minerals. This follows the lines of Goldschmidt's work for which the method was developed, and supports his ideas on the distribution of trace elements in rocks and their constituent

minerals. The important factors are the ionic radius and valency of the trace constituent and the possibility of its taking the place, in the crystal lattice, of one of the constituent ions. Thus $Co^{\cdot\cdot}$ (0·82A) and $Ni^{\cdot\cdot}$ (0·78A) replace $Mg^{\cdot\cdot}$ (0·78A) or $Fe^{\cdot\cdot}$ (0·83A) in ferromagnesian minerals, but are absent from the felspars in the same rock as there is no suitable lattice substitution.

As an illustration of the results obtainable by this semi-quantitative cathode layer arc method, in Table I are given the wavelengths of suitable lines, the sensitivities, and the results for three typical soils from North-east Scotland. The higher values for cobalt, nickel, chromium and vanadium in the soil derived from the more basic rock, and for rubidium, zirconium, lead and barium in that from the acidic rock, are explained by arguments similar to the above. Analyses of soils along the same general lines have been reported by Allison and Gaddum,[7] Ballard,[8] and Guelbenzu, Ruiz and Azcona.[9]

For the analysis of plant ash a similar technique, even if it were accurate enough, would be possible only for certain elements, owing to the dilution effect of the high alkali, alkaline earth and phosphate contents, and generally a chemical pre-treatment to remove these and to effect a concentration is necessary. It has been found possible to adopt the same concentration procedure for trace constituents extracted from plant ash after sodium carbonate fusion as for those removed from soils by the less energetic extractants (such as acetic acid or ammonium acetate) whose action simulates to some extent that of the plant. A concentration method has the advantage that it allows quite different types of material to be submitted to the same spectrographic treatment, as the composition of the major constituents of the resultant concentrate can be standardised. Scott[10] has pointed out the effect that the composition of a material may have on the intensity of the lines of a trace constituent, or even on the ratio of the trace constituent to internal standard intensities. Thus, chromium, vanadium and molybdenum in calcium carbonate give lines of the same strength as those given by 5 to 10 times as much in a silica base, and this intensification in presence of calcium may have marked effect on the direct determination of these elements in agricultural materials unless a standard of precisely the same composition is used for comparison. Plant ashes in particular are very variable in composition, depending on the plant species and the part analysed.

When a chemical concentration method is adopted it is possible to bring the elements recovered into a base of standard composition. Alumina has proved to be the most suitable base for the analysis of trace constituents concentrated from plant materials and from soil extracts. It is easily manipulated for filling into electrodes and its behaviour in the arc is satisfactory. We have found concentration to be carried out most conveniently by precipitation with organic reagents: 8-hydroxyquinoline precipitates cobalt, nickel, molybdenum titanium, zinc and copper together with iron and aluminium in ammonium acetate solution, at *p*H 5·1. The amounts of aluminium and iron in the solution can be adjusted, with previous experience of similar samples, to give a precipitate weighing between 30 and 50 mg. after ignition at 450° C., and containing a suitable amount of iron for use as spectrographic internal standard. Generally iron has to be added to soil extracts and aluminium to plant materials. This method, described by Scott and Mitchell,[11] has recently been developed further[12] by the use of mixed precipitants to bring down other elements, and a simultaneous precipitation by 8-hydroxyquinoline, tannic acid and thionalide recovers, in addition to the elements mentioned, chromium, vanadium, tin, lead, beryllium and germanium and probably also silver, gallium, thallium and other elements, although these have not yet been studied fully. Cadmium is recovered, and is added to the solution in known amount to serve as a second spectrographic internal standard.

For the quantitative analysis of these precipitates a modified cathode layer arc technique as described by Davidson and Mitchell[13] is employed. The previously described electrodes are used and into each about 4 mg. of precipitate, mixed with an equal weight of carbon powder, is filled. The use of such electrodes with a carbon powder mixture results in a very steady arc which strikes to the sample and the inner rim of the electrode. The gain in stability of the arc more than outweighs the slightly increased work in filling the narrow boring. Admixture with carbon helps to prevent spluttering and for certain types of material the ratio may even be increased with advantage. It should be noted that the electrodes are carbon and not graphite, as the latter, owing to its greater thermal conductivity, burns away rather slowly. The carbon electrode is burnt to the full depth of the boring in about 3 minutes at 9 amperes, and the exposure is continued for the full period. A lens at the slit of the Hilger Large Quartz Spectrograph produces an image of the arc at the collimator, where a

mask isolates light from the cathode tip and the adjoining one third of the arc column. By this means the slit would be evenly illuminated from end to end were not a rotating step sector situated in front of the slit to divide the spectrogram into strips which receive exposures increasing in steps of 2. This enables all measurements of intensity to be made at the same photographic density. In the simplest means of photometry what in fact is measured is the exposure time, relative to that for the internal standard, required to give a certain density.

In order to obtain working curves from which contents can be determined, series of standard mixtures containing several trace constituents in an alumina base with a fixed iron content are prepared by grinding the necessary oxides in an agate mortar, contents from 1 per cent. down to 1 part per million being obtained by dilution with the base in steps of $\sqrt{10}$. Separate dilution series are prepared with different contents of iron, the internal standard, a range of Fe_2O_3 from 2 to 40 per cent. being covered.

The method of photometry involves for each line the measurement of 3 steps in the spectrogram adjacent to the density chosen for measurement. This is generally a density of 0·4, which, with a clear plate setting of $i_0 = 50$ as is usual on a Hilger Microphotometer, corresponds to a galvanometer deflexion of $i = 20$. Thus three steps giving deflexions between 5 and 30 are generally measured. From each value of i is obtained the optical density, log (i_0/i). A Table giving this for values of I between 0 and 50 has been prepared and reprints are available. The same procedure is followed for the internal standard line, and, where background can be disregarded, the values of log (i_0/i) for both lines are plotted against the logarithm of the exposure given by the corresponding step of the step sector, as in Fig. 1A. Figs. 1 and 2 illustrate generally the types of curves obtained.

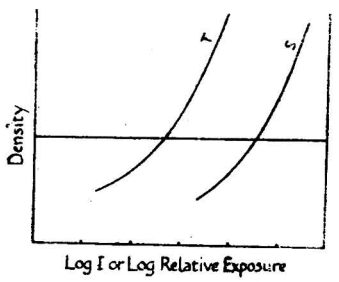

Fig. 1A. Blackening curves for lines of trace element (T) and internal standard (S), ignoring background.

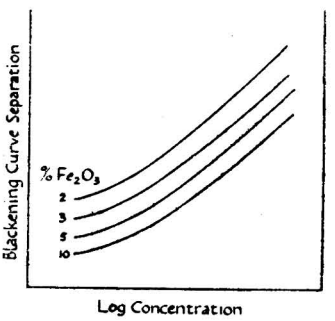

Fig. 1B. Working curves, not corrected for background, for different Fe_2O_3 contents.

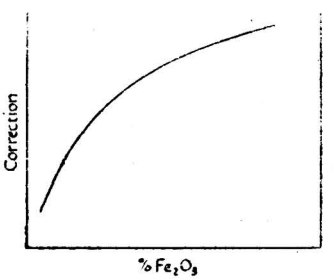

Fig. 1C. Correction curve for displacement of working curve due to change in Fe_2O_3 content.

The separation of the two curves at any density is a measure of the ratio of the exposures which the lines have to be given when producing that density. This separation, for a series of standard mixtures, plotted against the logarithm of the trace element content gives the working curve (Fig. 1B). The working curve is displaced vertically along the separation axis by changes in the content of the internal standard, and this is the basis of the variable internal standard method of Davidson and Mitchell.[13] From the graph of the displacement against change in iron content (Fig. 1C) a correction can be obtained for any iron content, enabling the standard working curve at a convenient iron content to be utilised for all determinations. The working curves are approximately straight lines except at low trace element contents, where the effect of background introduces a pronounced toe, and for accurate determinations in this region a correction for background is necessary. The accuracy is rather better over the whole range when this correction is made.

The photometric procedure is then to measure, in addition to three steps of each line, two or three steps of the background, again covering a density of 0·4 (Fig. 2A). The logarithms of the relative intensities of the background, analysis line + background, and internal standard line + background are given by the points at which the blackening curves cut the selected density. From these values the relative intensities of the two lines freed from the effect of background can be obtained. This is a somewhat tedious calculation if ordinary logarithms are used, but can be shortened by using gaussian or subtraction logarithms and is

greatly simplified[14] by means of a Table derived from subtraction logarithms, which we have prepared and of which reprints are available.[15] This method makes the assumption that in practice the Eberhard effect can be disregarded, and the results which we have obtained would appear to justify this.

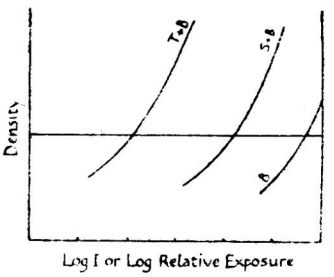

Fig. 2A. Blackening curves, for background correction method, for lines of trace element (T + B) and internal standard (S + B) and the background (B).

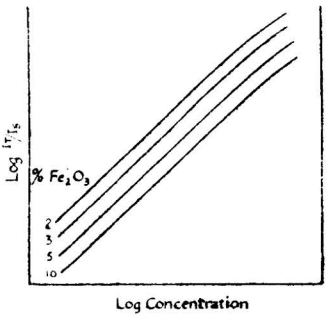

Fig. 2B. Working curves, corrected for background, for different Fe_2O_3 contents.

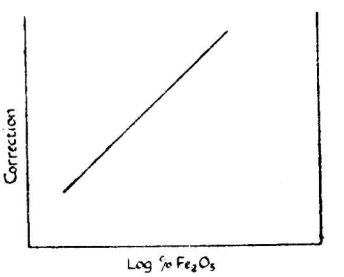

Fig. 2C. Correction curve for displacement of working curve due to change in Fe_2O_3 content, after background correction, on logarithmic scale.

When the logarithm of the ratio of the relative intensities after background correction, is plotted against the logarithm of the concentration (Fig. 2B), the working curve for most trace constituents is a straight line at the theoretical angle of 45°, and the toe disappears. At very high contents there is some falling off in slope owing to self reversal effects, as the lines which have to be employed, in order to obtain adequate sensitivity, are just those which, ending on the ground state, are liable to self reversal. The correction curve for changes in iron content (Fig. 2C) also approaches its theoretical slope when the displacement is plotted against log iron content. This allows a reasonably accurate determination of any element for which working curves are not available and where their complete experimental determination would not be justified for the purpose in view. Empirical working and correction curves can be drawn with slopes of unity through values obtained for one standard mixture close in composition to the unknown, and from these, results of sufficient accuracy are often obtainable, as Scott[16] has shown. He obtained angles of slope of 45° ± 0·5° for chromium, cobalt, nickel, vanadium, molybdenum, beryllium, gallium, tin and lead in alumina and sodium chloride bases, and similar values for the corresponding iron internal standard lines. The curves before correction for background had angles of 35–42°.

Marked changes in the composition of the base generally affect the relative intensities of the analysis and internal standard lines. Thus whilst the Co : Fe ratio is practically independent of the base material,[10] Cr : Fe or Mo : Fe ratio is very sensitive to changes, as already mentioned, but such effects can be eliminated by a concentration method. Another method for the control of this effect is the addition of a spectroscopic buffer in large amounts to each sample examined, but generally the dilution effect of this method would reduce the sensitivity too far in our type of sample. It is being employed for copper in plant material, where contamination effects make the concentration method difficult and adequate sensitivity is available.

The recovery of some elements from solution by the concentration method involving precipitation with 8-hydroxyquinoline, tannic acid and thionalide, followed by spectrographic analysis of the precipitate, is illustrated in Table II. The errors of the whole process will be seen seldom to exceed ±10% over the range of contents shown. A content of 50 p.p.m. in the precipitate analysed corresponds to 2 micrograms in the solution, or, with our normal aliquots, one part in ten million in the plant material or soil. By the concentration process a 500- to 1000-fold concentration can be effected. The amount in the electrode is about one-tenth of the precipitate, or 0·2 μg. upwards. For cobalt, amounts as low as 0·04 μg. can be determined with errors of the 10% order.

The essential requirement of any such concentration method is the simultaneous quantitative recovery of as many of the trace constituents as possible, with the removal of the major diluents, chiefly the alkalis, alkaline earths and phosphate. There would appear to be no other practical method of achieving this at present available. Extraction methods

such as those with dithizone tend to be rather selective,[17,18] whilst adsorption methods, such as those with synthetic resins, as used by Eastmond,[19] separate cations and anions but the separation of different cations does not appear practicable.

TABLE II

DETERMINATION OF VARIOUS ELEMENTS BY CONCENTRATION FROM SOLUTION WITH 8-HYDROXYQUINOLINE + TANNIC ACID + THIONALIDE FOLLOWED BY SPECTROGRAPHIC ANALYSIS

Present, parts per million ..	50	125	250	500	1250		
Found, „ „ „ :							
Cobalt	47	126	249	471	1290		
Nickel	52	135	272	520	1375		
Molybdenum	57	124	241	503	1120		
Chromium	50	126	235	432	1220		
Vanadium	53	127	244	497	1175		
Present, parts per million ..	100	250	500	1000	2500	5000	10000 (1[illegible]
Found, „ „ „ :							
Beryllium	117	255	490	1023	2660		
Germanium	162	269	525	1014	2870		
Tin		295	537	1088	2340		
Lead			544	956	2611		
Titanium	110		542	1100		5435	10700 (1·07%)
Present, Zinc per cent. ..	0·1	0·5	1·0	5·0	10·0		
Found, „ „ „ ..		0·56	1·02	5·07	10·16		

Some typical results showing the amounts of trace constituents extracted by dilute (2·5%) acetic acid from the soils for which total contents are given in Table I are to be found in Table III. It will be seen that whilst certain of the elements, for instance cobalt, show

TABLE III

TRACE CONSTITUENTS EXTRACTED BY DILUTE ACETIC ACID FROM SOILS OF DIFFERENT GEOLOGICAL ORIGINS AS PARTS PER MILLION

	Co	Ni	Mo	V	Cr	Ti	Sn	Pb	Zn
Old Red Sandstone	0·41	1·04	0·02	0·13	0·17	0·21	0·4	0·4	4
Norite	1·49	0·56	0·02	0·19	0·11	0·38	0·3	0·3	16
Granite	0·12	0·42	0·07	0·12	0·14	0·47	6·8	1·6	22

quite large variation from soil to soil, others are relatively constant despite appreciable variation in total content.

TABLE IV

TRACE CONSTITUENTS IN A PASTURE HERBAGE AS PARTS PER MILLION OF DRY MATTER

Sample	Co	Ni	Mo	V	Cr	Ti	Sn	Pb	Zn	Cu	Fe
Mixed ..	0·19	1·4	0·44	0·09	0·13	1·7	0·4	1·1	43	18·2	83
Red clover ..	0·21	1·6	0·35	0·10	0·09	2·1	0·2	1·6	42	18·6	85
Rye grass ..	0·09	0·1	0·72	0·10	0·21	2·7	—	1·1	34	6·1	41
Cocksfoot ..	0·05	0·1	0·78	0·18	0·12	2·0	—	1·5	22	11·7	26
Blank.. ..	<0·01	0·05	0·01	0·01	0·04	0·1	0·1	0·3	<1		

The results for plant material in Table IV indicate the contents of a mixed pasture herbage and of its chief constituent species. One practical point which these results bring out is the necessity for careful sampling of mixed pastures.

We have been particularly interested in the cobalt content of soil extracts and plant materials, because of a cobalt deficiency disease in sheep in various parts of Scotland. This occurs when the diet is low in cobalt, the limiting value in the herbage being of the order of 5 to 10 parts per hundred million of the oven-dry material, corresponding to a content in the soil of about 25 parts of cobalt per hundred million extractable by 2·5% acetic acid. This disease, when it occurs on normal arable soils, can be prevented by addition of 2 lbs. of cobalt chloride per acre (about 0·25 p.p.m. of Co), and in Table V are given some data for herbage contents showing the effects of adding cobalt-rich fertilizer and lime to the soil. The influence of lime, affecting principally the soil acidity, on the uptake of trace constituents by the plant is well shown. The uptake of cobalt and nickel is decreased whilst that of molybdenum is increased. Where cobalt deficiency is serious, deaths among lambs are common,

whilst cobalt manuring may produce live-weight increases compared with those of lambs which survive without cobalt manuring, of some 20 to 30 lbs. at the age of six months.[20]

Other investigations are covering animal diseases involving copper and molybdenum, and the effects of other trace constituents on plants and animals are being studied.

TABLE V

EFFECT OF COBALT ADDITION (2 lbs. of $CoCl_2.6H_2O$ per acre) AND LIMING (5 tons of ground limestone per acre) ON UPTAKE BY RED CLOVER (as parts per million of dry matter)

	No cobalt				Cobalt			
	Co	Ni	Mo	Fe	Co	Ni	Mo	Fe
Unlimed ..	0·22	2·0	0·3	65	0·89	1·6	0·3	57
Limed ..	0·18	1·4	1·5	75	0·53	1·0	1·0	68

Lundegårdh flame method.—The use of this method[21,22] represents an entirely different application of spectrographic analysis to soil investigations. It is employed as a routine method for the determination of the alkali and alkaline earth metals, magnesium and manganese in solution. The source is an air-acetylene flame, the gas pressures of which are accurately controlled. The solution to be analysed is introduced into the flame as a fine spray in the air supply. In view of the ease of preparation of standard solutions, it is usual to carry these, together with a series of solutions to be analysed, on each plate. Spectrograms of 16 unknowns and six standards in duplicate can be prepared in a little over one hour.

The low energy of the flame source gives a simple spectrogram, iron in moderately high concentration showing only a few lines, so that large dispersion is not necessary, but the ultimate lines of the alkali and alkaline earth metals and a few other elements are very sensitive.

A simplified method of photometry, taking merely the ratio of the galvanometer deflexions for the line and the flame background on which it is superimposed, is generally employed. This ratio in fact gives the transmission of the line itself (free from background), and is plotted against the concentration in the solution. In extracts of soils and plant materials, determinations of potassium, sodium, calcium, magnesium, manganese and strontium can generally be made with spectrographic errors not exceeding ±5% when duplicate spectrograms are taken, whilst greater accuracy is obtainable by increased replication, or by the use of an internal standard.

The use of the method for soil work has been principally the determination of potassium in acetic acid extracts in connection with the soil advisory service for farmers, and for this purpose it would appear that direct photometric determinations such as are now being widely employed in the United States with electron photomultiplier tubes could well be used in conjunction with the Lundegårdh flame. Suitable equipment does not however appear to be available in this country as yet.

Determinations of exchangeable cations extracted from soils by ammonium acetate, and of the alkalis in plant ash (after hydrochloric acid extraction) and in rocks and minerals (after the initial stage of the Lawrence Smith extraction) are other applications of the method. As an indication of the sensitivity, Table VI gives suitable ranges of contents, in milligrams

TABLE VI

SUITABLE RANGES OF CONTENTS (as mg. per 50 ml.) FOR DETERMINATION BY THE LUNDEGÅRDH FLAME METHOD

Element	λ, A	Range
K	4044	0·5 –10·0
Na	3302	0·5 –10·0
Li	6708	0·005– 0·1
Ca	4227	0·025– 0·5
Mg	2852	0·3 – 6·0
Sr	4607	0·025– 0·5
Mn	4031	0·03 – 0·6
Fe	3860	1·0 –20·0

per 50 ml. This volume is a convenient unit to work with; determinations can be made on much smaller volumes, but on a routine scale use of smaller volumes leads to filtration and washing difficulties.

Interference effects are found remarkably seldom; in fact the only serious effect reported is that of aluminium on calcium and strontium,[23] where marked depression occurs. This

makes precautions necessary when dealing with soil extracts made with strong acids. The cause of the depression is not clear, but it is possible to overcome it for one of the elements affected by addition of excess of the other. Some elements, notably potassium, are slightly depressed in the presence of hydrochloric acid stronger than tenth normal, but this effect is not observed if the acidity conditions in the solutions are standardised. Generally, interference troubles can be overcome by preparing standards containing the same extraneous substances.

This short description can only indicate the lines along which we are working. Generally, however, it can be said that our applications of spectrographic methods are for the accurate quantitative determination of those constituents in which we are interested. Our use of the method for purely qualitative purposes has been very limited, and it is in its quantitative applications that we have found its value.

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MACAULAY INSTITUTE FOR SOIL RESEARCH
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Spectrographic Analysis of Rare and High Purity Materials

BY D. M. SMITH

It is the purpose of this paper to indicate the methods of spectrographic control used in the preparation and testing of spectroscopically pure substances now carried out in these Laboratories.*

The work involved requires the closest co-operation between the chemical and spectrographic laboratories, since only in a few instances is spectrographic analysis alone used for the control of purity (*e.g.*, copper and, to a certain extent, lead and tin).

In general the quantities of the impurities present are so small that chemical analysis is often out of the question, especially when only small amounts of material are available. Purification processes are repeated until the spectra show a constant impurity content, indicating the stage at which such processes do not effect any further removal of impurities. It follows that the decision that a high state of purity has been attained is subject to a certain amount of discretion and it is in cases such as these where the pooling of spectrographic and chemical experience is essential.

* Since 1944, this Company has, by agreement with Adam Hilger Ltd., undertaken the supply of "H.S." and "Specpure" substances and the control of their purity. They are now known as "Matthey Spectroscopically Standardised Substances."

Specific Examples of the Type of Work Undertaken

1. Pure Copper Electrodes—Pure copper rods are used for various spectrographic purposes and special care has been taken over the preparation of what is believed to be the purest copper commercially available in relatively large quantities. The metal is refined (three times) by electrolysis, using a purified electrolyte at each stage of deposition. The pure cathode sheets are melted and cast *in vacuo* and the ingots reduced to their final form entirely by cold-working. The rods are finally pickled in hot hydrochloric acid and washed in ammonia, after which they are wrapped with cellophane.

The metal is analysed spectrographically at all stages of manufacture. First the cathode is examined and then samples from the top and bottom of each ingot and finally an analysis is made on the rods themselves. In this way it is possible to track down any impurities and determine their origin. For example, tests on the first trial cathodes showed the presence of 0·001–0·002% of lead and 0·0005–0·001% of silver. Special precautions are taken to avoid contamination by impurities (silicon in particular) from the crucible during the melting operation. Even with all the precautions taken it does not seem possible to reduce the silver, lead and nickel contents to below 0·0003–0·0004% of each element.

2. Rare Earths—While the sensitive lines for rare earth elements are given in the Massachusetts Institute of Technology Wavelength Tables, 1939 (edited by G. R. Harrison) the most persistent lines are not in all cases indicated. An attempt has been made, therefore, to ascertain these empirically, by photographing successive exposures of a continuously burning arc until the whole of the material has been volatilised. The Littrow spectrograph, set for the wavelength region 2960–6000A, was used, as this range appeared to cover most of the principal lines given by the rare earth elements. Moreover, this is in accord with the following statement by W. F. Meggers[1]: "The strongest lines of ionized rare earths are expected to lie between 3500A and 5000A. . . . Because rare earths are easily ionized it is possible that their strongest 2nd spectrum lines will surpass in intensity the strongest 1st spectrum lines in ordinary arcs. . . ." For the rare earth spectra so far examined, this statement is confirmed as regards the intermittent A.C. arc. Since the carbon and cyanogen bands are present in the wavelength range chosen, pure silver electrodes are being used in place of high purity carbon or graphite electrodes for the analysis of rare earths by this method. (Silver electrodes were used by Meggers[2] in his work on the spectra of hafnium.)

3. Pure Graphite and Carbon Electrodes—The ordinary grade of pure graphite contains about 0·03% of impurities, of which about 0·025% is calcium, and the remainder made up of traces of copper, iron, vanadium, silicon, titanium, magnesium and sometimes manganese. Highly purified carbon and graphite rods are now available. The residue after complete combustion of this material in oxygen varies from 0·001% to 0·0025%, and this gives an indication of the high degree of purity attained.

It is found that the number and intensity of the impurity lines is greatest in the first spectrogram photographed from new electrodes and these lines are listed in the report accompanying the material. In subsequent spectrograms the number and intensity of these lines diminish; in particular, the calcium lines are frequently absent from the spectra of rods arced for a second time. Thus the few impurities present are generally removed by pre-burning the electrodes, but whether such impurities are due to surface contamination, airborne or otherwise, or are actually present in the material and volatilised by the arc, it is extremely difficult to say.

Graphite electrodes are generally used in the Company's laboratory for analytical purposes, partly because the rods are more easily cleaned and shaped than rods of carbon, copper or silver and partly because a reasonable period of pre-burning brings the electrode ends to a state of spectroscopic purity.

Identification of Impurity Lines

The complete qualitative analysis of a given material requires the identification of every line in its spectrum, from the ultra-violet region to the red end of the visible region. With elements having simple spectra this does not present any great difficulties, but with many-lined spectra comparison is made with the spectrum of the purest sample available. (As a matter of interest, it may be noted that many materials may be obtained in their purest state in the form of compounds rather than as metals, since there is always the danger of introducing impurities by contamination during reduction to metal and subsequent melting and working operations). In general, lines common to both spectra (and appearing

with the same relative intensities) may be ascribed to the basis element, so that it is only necessary to identify the lines of different intensity in the two spectra, or present in one and not in the other.

In cases such as these the following procedure has given satisfactory results. The spectrum of the impurity element is obtained by placing two or three drops of a 1% "Specpure" solution of the element in a shallow cavity in the lower electrode of a graphite arc just after the arc has been run for about 30 seconds to remove any surface contamination and to warm up the electrodes. The solution rapidly dries and the arc is then exposed for about 10 seconds. The upper electrode is a pointed graphite rod. (This graphite need not be of the highest purity since, with the exposure given, the impurity lines will not show up strongly in comparison with the lines of the element or elements introduced.) With practice it is possible in this way to obtain a spectrum showing only the most persistent lines of the element. This spectrum is superposed to overlap that of the material being analysed, by suitable choice of diaphragms in front of the spectrograph slit.

Since, as a general rule, the number of possible impurities in a given material is fairly large, the photography of many superposed impurity comparison spectra is required. Several elements can be introduced together, however, when their spectra are sufficiently simple and the most persistent lines are sufficiently widely separated. For example, aluminium, manganese and chromium, in the form of drops of "Specpure" solutions, may be added together and since calcium is a common impurity in the graphite we have a single comparison spectrum giving the persistent lines of four elements at once.

This is a tedious procedure, unjustifiably so if it had to be carried out for the analysis of each subsequent sample of the same material. With the Judd Lewis Spectrum Comparator, however, plates of spectra prepared in the manner described can be used for checking the purity of further samples, the spectra of which are photographed in the normal way.

In the interpretation of the spectra difficulties have been encountered from the following causes:

1. Masking of impurity lines by lines of the basis element. (This is of more consequence when the impurity line is the most persistent line of the element sought.)
2. Presence of faint lines of doubtful origin.
3. Enhancement of impurities in the supporting electrodes (carbon or graphite) by alkali metals or compounds, thus giving false indications of impurities in the sample.
4. Spark lines having the appearance of impurity lines, in intermittent A.C. arc spectra.

In attempting to solve these problems the M.I.T. Wavelength Tables have proved invaluable.

1. MASKING OF LINES—When the most persistent impurity lines are masked by lines due to the basis element of the material, it is not possible to state with certainty that such an impurity is definitely absent. For example, the calcium line 3933·666A is not resolved (even with the Littrow spectrograph) from the iron line 3933·605A, which is a comparatively faint line. Comparison is then made with other samples of pure iron and its compounds, and if there is no enhancement of the line which could be ascribed to the superposition of an impurity line, and in the absence of other (but less sensitive) lines, calcium would be reported as "not detected."

If the masking is by a strong line of the basis element there is, of course, more uncertainty, as a superposed faint impurity line would remain undetected.

2. PRESENCE OF FAINT LINES OF DOUBTFUL ORIGIN—When a faint line appears with constant relative intensity in the spectra of different samples of the same material or different compounds, particularly when derived from different sources of supply, it is legitimate to suspect it as a line of the basis element, even if unrecorded in the literature. The following example illustrates the kind of uncertainty which may arise. A faint line at 2516A in the intermittent A.C. arc spectrum of highly purified tungsten may be the silicon line 2516·123A, an unrecorded tungsten line, or possibly the air line 2516·1A. Also either one or two lines may be superposed on the other. Except by laborious measurements using equipment not generally available in an industrial spectrographic laboratory, the question can only be answered by the type of procedure outlined in 1 above.

3. ANALYSIS OF ALKALI METALS—Impurities in carbon or graphite electrodes are enhanced by the introduction of alkali metal salts into the arc, so that these electrodes are unsuitable for the detection of traces of impurities in such compounds unless these impurities

are definitely absent from the carbon or graphite. Pure copper or pure silver electrodes may be used in such cases.

An interesting point arose in the analysis of lithium carbonate for traces of magnesium, using the intermittent A.C. arc between graphite electrodes. The lines 2795·53 and 2802·695A arise from singly ionised atoms (Mg II), whereas the line 2852·129A arises from the neutral atom (Mg I). While the Mg I line shows a marked enhancement on the introduction of lithium carbonate, the other two lines remain of practically the same intensity as in the spectrum of the graphite electrodes before the introduction of the sample. The degree of ionisation being lowered, the emission from lower excited atomic states (arc lines) is thus favoured at the expense of that from the higher states (spark lines). (The possibility of enhancement of the Mg I line by a sodium line at 2852·828A is discounted by the fact that the more persistent sodium line 5889·953A is barely visible.) Using pure silver electrodes for the analysis, all three magnesium lines were very faintly present, Mg 2852·129A being only slightly stronger than the other two, indicating no appreciable quantity of magnesium in the lithium carbonate.

A further complication in the analysis of alkali salts may arise from the fact that the exceptionally low ionisation potentials of the alkali metals tend to depress the excitation of certain other elements present.[3]

4. SPARK LINES IN INTERMITTENT A.C. ARC SPECTRA—The following spark lines, due to singly ionised cadmium, thallium and gallium, respectively, have the appearance of impurity lines and, no doubt, spark lines will be found in the spectra of other substances when triggered arc sources of excitation are used.

Cadmium Cd II	Thallium Tl II	Gallium Ga II
3071·648	2298·08	2780·15
3535·687	2530·67	
3250·17	3091·66	

The following spark lines of carbon (C II) appear in spectra obtained by means of carbon or graphite electrodes—

2509·11	2836·71
2511·71	2837·60
2512·03	

and the line C III 2296·89 is also occasionally very faintly present.

The C II lines persist in the 7 amp. intermittent A.C. globule arc of copper, a phenomenon which appears somewhat surprising considering the feeble nature of the high-frequency spark triggering.

EXCITATION SOURCES

It is generally accepted that the arc method of analysis is more sensitive in detecting traces of impurities than the spark method, but there is some divergence of opinion as to the most suitable type of arc excitation. The following types have been considered.

1. DIRECT CURRENT ARC—Although M. Milbourn[4] has successfully used the D.C. arc for the analysis of copper for impurities by the globule method, he admitted, in an earlier contribution,[5] that the high sensitivity of the method is generally obtained at the expense of reproducibility. He has shown, however, that by careful (photocell) control of the arc it is possible to achieve an adequate degree of reproducibility. Other methods of stabilising D.C. arc discharges have been proposed, but it would seem preferable to select a type of arc excitation inherently more reproducible in character than to try to compensate for the inherent instability which is associated with the internal mechanism of the arc.

2. CONSTANT CURRENT D.C. ARC—In this arc source, recently developed by Adam Hilger, Ltd., the constant current is obtained from full-wave rectification of A.C. from a circuit containing inductance and capacitance, tuned so that the current flowing is independent of the resistance of the arc. A safety switch is included in the circuit to cut off the supply in the event of the arc being broken.

Comparative tests are in progress with this type of arc discharge, but a sufficiently thorough investigation has not yet been carried out for a considered opinion to be given as to its performance. In the following instances, however, the constant current D.C. arc has proved superior to the intermittent A.C. arc, using electrodes of pure graphite.

Silica—Reproducible spectra have been more easily obtained with the constant current arc, probably on account of the refractory nature of the material, than with other types of arc source.

Gold—Traces of impurities (*e.g.*, copper and silver) are more readily detected. The constant current arc runs more steadily than the intermittent A.C. arc and there is copious volatilisation of the gold, resulting in a stronger spectrum. The sensitivity is correspondingly increased.

Scandium compounds—Although the intermittent A.C. arc gave satisfactory spectra of scandium potassium sulphate, the spectra of scandium sulphate were very faint and quite unsuitable for analysis. Improved results were obtained for the latter by using the constant current arc with the sample negative, but the best results were obtained with the sample positive, in which case the spectra were directly comparable with those of the scandium potassium sulphate obtained with the intermittent A.C. arc.

3. INTERMITTENT A.C. ARC—In view of the successful application of the intermittent A.C. arc to the quantitative determination of impurities in high purity zinc, this method has been tried out for the analysis of a wide variety of materials. Very encouraging results have been obtained with other metal rods and also with metal globules and powders, using the graphite arc. Further evidence of the wider application of this excitation source is given in a recent paper by J. A. C. McClelland.[6]

The circuit utilises the fact that an electrical condenser charged to the same potential will always liberate the same amount of energy upon discharge. The discharge is triggered by a superimposed high-frequency spark occurring at or near the voltage peak of the A.C. wave. A detailed description of the circuit and its operation is given by G. W. J. Kingsbury and J. A. C. McClelland[7] and the method is among those recommended by the British Standards Institution (B.S. 1225 : 1945).

It will be seen from the foregoing, however, that circumstances arise where this type of discharge has not proved so satisfactory and further examples of irregular running of the arc are given by pure iron and pure copper rods. Whether by modification of the discharge conditions these materials could be analysed satisfactorily is a matter for further trial and experiment. For the present, however, either the constant current arc or the graphite arc (globule) technique is an adequate alternative.

As examples of the sensitivity of the intermittent A.C. arc method may be quoted the following results obtained by comparative chemical and spectrographic analysis. All the principal sensitive lines of manganese and iron were detected in a sample of silver. The possibility of surface contamination was ruled out as, after severe pickling, the lines appeared with the same intensity in further spectra photographed. Chemical analysis of the material gave 0·00014% of manganese and 0·00016% of iron. Much less than these quantities could therefore be detected spectrographically.

Similarly, less than 0·0002% of nickel was detected in a sample of antimony.

4. GENERAL-PURPOSE SOURCE UNIT—This source unit, devised by A. Walsh,[8] gives the widest range of excitation conditions at present available in this country. (The Dietert Multi-source Unit[9] has been available in America since 1943.) It retains the principal feature of the intermittent A.C. arc, a triggered discharge of reproducible character, but has far greater flexibility, together with rectification (full- or half-wave) of the A.C. supply. To quote the author's description—"A new source unit has been constructed which will provide a simple condensed spark, a low-voltage D.C. arc and, by using a triggered low-voltage discharge, a whole series of excitation conditions intermediate between those prevailing in the conventional spark and arc. This triggered type of discharge can be arranged to give high sensitivity together with a higher accuracy than is possible with a D.C. arc, or to give a high degree of reproducibility." It is proposed to construct such a unit in the Company's laboratory.

CONCLUSION

The scope of the work undertaken is very wide, covering not only the known elements but many of their compounds. It is too much to hope that any single excitation source and set of operating conditions can be found to deal satisfactorily with such a variety of materials. Consequently much further research is needed to establish the optimum conditions for the analysis of any given substance. Unfortunately each analytical problem requires individual solution, so that the use of a variety of excitation sources or, better still, a flexible source such as the General-Purpose Unit will undoubtedly provide a means to the desired end.

It is hoped that this somewhat sketchy outline has served to indicate the steps that are being taken to ensure the provision of material of the highest purity possible.

In conclusion, I wish to acknowledge my indebtedness to my colleagues and assistants for their co-operation in this work, and to thank the Directors of Johnson, Matthey & Co., Limited, for permission to publish.

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Discussion

The papers were discussed singly as indicated by the italicised headings below.

Paper by R. A. Morton and A. L. Stubbs—

Dr. F. WOKES asked if in the old method of compensation used in absorption spectroscopy, in which the "unknown" solution was put in one cell and the solvent containing the "irrelevant substances" in the other, the same result would be obtained as if the two spectra were produced separately and the result calculated from the difference as now described.

Dr. A. L. STUBBS said the method of compensation mentioned would require that the "irrelevant substances" could be obtained by themselves, which was not always possible.

Dr. F. E. MOON said that the authors seemed to have assumed that the "irrelevant" absorption was due to a single substance. With a material such as cod liver oil was it not more likely to be due to a number of substances?

Professor R. A. MORTON replied that it did not matter whether the irrelevant absorption was due to one or a number of substances, provided it varied linearly with the wavelength over the portion of the spectrum used.

Mr. V. C. FARMER remarked that for measurements of the high precision claimed, within ± 0·5%, a very constant light source was required, and asked if simultaneous readings of light intensities were made at different wavelengths to compensate for any variation in the source.

Professor MORTON said that, thanks to very fine engineering design and construction, they did have a power unit of astonishing constancy, without which the procedure described would not be justified.

Mr. A. STEWART spoke of the advantages of combining chromatography and spectroscopy so that impurities were removed before measurement. Coloured compounds lend themselves to such treatment and suitable selection of the conditions frequently enables the components to be washed through an adsorption column without loss and in a relatively short time, say half-an-hour. Generally the components may be collected separately and measured, conveniently with a photoelectric absorptiometer; when several of the components require to be determined it is sometimes expedient to use conditions under which certain bands are not effectively resolved, the components present being determined by spectrophotometric analysis, which is facilitated by the small number of substances present. The precision is limited by that of the photometric measurements, which has a standard deviation of about 1%. The effect of this on the percentage composition is relatively small for minor components but is large for a main component, and in determining a component at high purity it is advantageous to measure the difference in optical density between the solution from the chromatograph and one of known and approximately equal concentration of the pure component placed in the compensating cell. In the spectrophotometric analysis of a two-component mixture advantage may be taken of the additive nature of the optical density of mixtures of solutions of the components to base the factors used in the simultaneous equations on measurements over the whole range of compositions. This minimises the difficulty that the wave-length of maximum absorption of one component sometimes corresponds with a steep slope and troublesome measurement for the other, as less difficulty is experienced in measuring the more uniform eyepiece field obtained with a mixture.

Professor MORTON said he entirely agreed with Mr. Stewart as to the use of chromatography. He had sometimes found it necessary to estimate free and combined vitamin A in animal liver extracts. This had been done by extracting the total fat and then filtering a solution in light petroleum through a short column of bone meal, which allowed the vitamin ester to pass readily but retained the free vitamin. The former was washed through with light petroleum and the free vitamin afterwards eluted from the column. There were many instances in which the combination of chromatography and spectroscopy was very useful. Regarding Mr. Stewart's remarks about determination of purity, Professor Morton said that supposing, for example, it was necessary to decide whether a sample of calciferol was of 97 or 99% purity, the orthodox way of obtaining the maximum accuracy was to make, say, half a dozen separate estimations and assess the results statistically. That could, in fact, be done on one plate if care was taken to get a standard plate and take a number of standard wave-lengths on the same plate and subject them to statistical analysis, because each of them should have a recognised fixed ratio to the maximum. All the computations could be done on the one plate as easily as if a dozen plates were taken and the results worked out; but this method did involve obtaining a really accurate curve on a sample of unequivocal purity.

Paper by B. S. Cooper—

Mr. D. M. SMITH said he would like to add a little to what Mr. Cooper had said about the casting of electrodes for accurate spectroscopic analysis and emphasise its importance. With aluminium alloys it was well known that the metallurgical history of a sample, *e.g.*, whether it had been cast, drawn or heat-treated, affected the intensities of the lines due to alloying components and to the basic metal.

Professor MORTON asked if the interference filters mentioned by Mr. Cooper were now being made. He had seen one that had been brought from Germany. With daylight it gave the appearance of a sodium source viewed through a spectroscope the slit of which had not been narrowed quite enough to resolve the doublet.

Mr. COOPER said he could not give much information about these filters. He saw two at the exhibition of German equipment shown in London, and he had heard that they were likely to be made in this country, but he did not know by whom.

Professor DRYERRE asked if the Schwarz thermopile was marketed in this country, and thought that perhaps it might be of use for studying the variations in temperature in the tissues of animals.

Mr. COOPER said it was made in this country by Adam Hilger, Ltd. It was a radiation-receiving thermopile and he would have thought that a low-capacity probe thermo-junction would be more likely to serve Professor Dryerre's purpose.

Paper by R. L. Mitchell—

Mr. N. STRAFFORD said he would like to have a little more detailed information about the determination of trace elements and the manner of use of the organic precipitants mentioned by Dr. Mitchell. He also asked if a low tension arc was used.

Dr. MITCHELL said that full details would shortly be published, but briefly the procedure was that, following addition of suitable amounts of 8-hydroxyquinoline, tannic acid and thionalide, the hydrochloric acid solution from soil or plant material was buffered to *p*H 5·1 with ammonium acetate after neutralisation to *p*H 2·0 with ammonia, using the yellow to green colour change of iron in the presence of 8-hydroxyquinoline. After standing overnight the precipitate was easily filterable through paper, provided the amount of tannic acid was correct. Too much tannic acid produced a gummy mass. The precipitate was washed with a little water and ignited in an electric muffle, its iron content was determined and part of it was filled into the electrode. In further reply to Mr. Strafford he said that a low tension arc was used.

Mr. B. G. MCLELLAND asked whether there was any fundamental reason for using acetic acid for simulating the plant action in extracting the trace elements from the soil, in place of the 1% citric acid commonly used for the determination of available potassium and phosphorus. He also asked how the standard mixtures were made and what precautions were taken to ensure purity of the ingredients.

Dr. MITCHELL replied that the choice of extracting agent for soil must in any event be empirical, and they had found acetic acid was as good as citric acid for the purpose and it did not give a solid residue on evaporation on the water-bath. Standard mixtures were prepared from major ingredients such as SiO_2, Al_2O_3 and Fe_2O_3 purified in the laboratory, whilst adequately pure supplies of the trace constituents were available, the requirements here not being so stringent.

Dr. STUBBS said he presumed that, in using a rotating spectrophotometer, Dr. Mitchell used a fairly long slit; had he had difficulties due to unequal densities at different parts of a line? He himself, when using a 10 mm. slit, found differences in focus between the top and bottom of a line. In using the Lundegardh apparatus, did Dr. Mitchell take any special precautions with glass-ware to avoid contamination by, *e.g.*, sodium and calcium from the glass?

Dr. MITCHELL said they had tested the intensities over the whole length of the slit and found them constant within the error of the microphotometer. They used only about one-third of the prism. They had a mask at the prism to shut off the anode and adjust the proper portion of the cathode of the arc column, and perhaps that mask improved the resolution. Regarding the Lundegardh glass-ware they worked with the less sensitive potassium and sodium lines in the ultraviolet, and no trouble had been experienced with Pyrex glass.

Dr. E. C. OWEN noted that Dr. Mitchell had said nothing about boron in the soil. It was a constituent of some value and very widely distributed. He wondered whether Dr. Mitchell had had difficulty with it on account of its presence in glass or whether he had not been concerned with it at all.

Dr. MITCHELL said the boron question was a difficult one. Boron availability in the soil seemed to depend not so much on the total boron content but rather on such factors as lime status, climatic conditions and moisture content. Boron had to be determined in the arc on copper electrodes, as boron-free carbon is not available at a reasonable cost. With the present laboratory facilities the contamination with copper arising therefrom made copper determinations uncertain, and as the latter were of interest boron determinations were not being made to any extent.

Dr. STUBBS remarked that the taking up of material from glass would apply to all standard glass-ware that one used in making up solutions for the Lundegardh apparatus.

Dr. MITCHELL replied that in his opinion it was quite safe to have glass-ware for the burner tubes and connecting tubes, because one usually found there was deposition on them rather than evaporation from them. There seemed more reason for using silica for standard flasks and the like; but he did not consider that for his work the glass-ware was an important disturbing factor.

Professor MORTON asked if cobalt had been determined in the liver of the undersized lamb that Dr. Mitchell had mentioned.

Dr. MITCHELL replied that the analyses on these lambs was not yet completed, but comparative tests on livers of other healthy and unhealthy lambs showed about fifty times as much cobalt in the former as in the latter.

Paper by D. M. Smith—

Mr. COOPER asked if the minute quantities of silver and lead found in the extremely high-purity copper had been determined by chemical methods or found by some indirect method of assessment. Mr.

Smith had mentioned determinations of the most persistent lines of different elements. He hoped that if data on this subject were accumulated, especially about the rare elements, it would be made known in print. He thought Mr. Smith went too far in saying that sometimes the only sure way to determine an element was to determine all its lines.

Mr. SMITH said that the analysis of the high-purity copper was largely based on previous experience with this type of material. Analytical tables compiled for this purpose were derived from spectra of standards, the analysis of which was in part chemical and in part spectrographic and some discretion was used in deciding what evidence to accept. Regarding the study of the rare earth elements, this work was in progress, but it was likely to take a long time to complete, for there was much still to be done. As to identifying every line in the spectrum, it was the ideal method, although impracticable in the case of many-lined spectra, *e.g.*, those of molybdenum and tungsten.

Professor MORTON mentioned the possibility that in laboratories where many different substances were analysed the air and the dust might become contaminated with a marvellous assortment of elements. Had Mr. Smith found it necessary to resort to any kind of air conditioning to cope with the risk of interference of that kind.

Mr. SMITH replied that he had come across difficulties of that kind, *e.g.*, airborne contamination by such elements as calcium and even by cadmium fume (on windy days) from a factory in the neighbourhood. He agreed as to the importance of air-conditioning and hoped that it would be possible to instal some device to obtain this. Certain American laboratories even considered humidity control to be necessary for accurate quantitative work. However, with careful working it was possible to minimise the dangers of accidental contamination and as an instance might be mentioned the fact that several of the materials tested had given spectra showing no trace of the most persistent calcium lines.

Mr. CLARK said that with regard to the wave form of the A.C. power used, he presumed that the ordinary high voltage A.C. power had a sine wave form which just broke the gap down when it reached a given voltage, when there was an intermittent A.C. arc and a synchronised device for breaking that at a given voltage. Had anyone used the square wave form for keeping this voltage practically constant? He believed that sort of wave form was used in some Radar impulse transmitters, and possibly many of these might now become available and might be adaptable to other apparatus requiring that sort of thing.

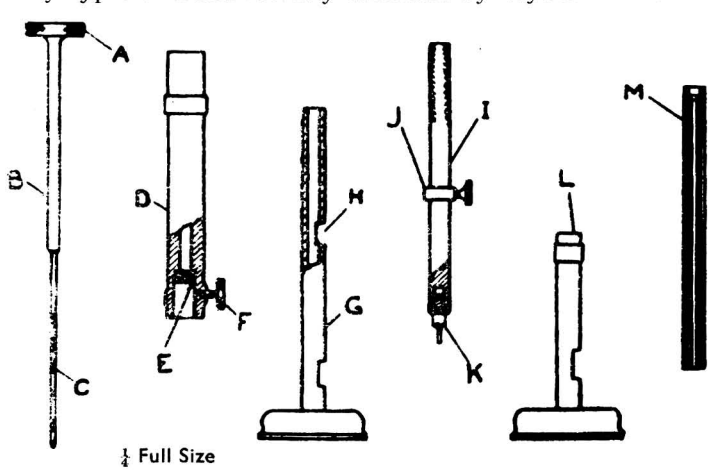
Mr. SMITH said he had no information about that. He thought most of the units used the ordinary sine wave form.

A Micro Filter Stick for Use in Spectrographic Analysis

BY G. E. WILSON

(This apparatus was described and exhibited in the course of the Discussion on the preceding symposium)

THE employment of the spectrograph for the analysis of microchemical quantities of solids generally necessitates the transfer of the material to the cathode crater from one of the many types of filters recently described by Wyatt.[1] This transfer may be effected by the



½ Full Size

A. Nurled handle.
B. Drill mount.
C. Drill 1/16″ diameter.
D. Sleeve guide.
E. Guide plate with centre hole.
F. Fixing screw.
G. Carbon holder.
H. Slots for holding carbon when drilling.
I. Recess cutter.
J. Recess depth stop.
K. Cutter head, interchangeable.
L. Holder for short lengths of carbon.
M. Carbon filter stick.

method used, for example, by Harper and Strafford,[2] in their work on the spectrographic estimation of traces of arsenic in foodstuffs, but whatever the particular procedure adopted it requires extremely careful manipulation to avoid mechanical loss.

The elimination of this danger and a speeding up of the analysis may be effected by converting the cathode carbon itself into a filter stick, similar in shape to that designed by

King.[3] This can be carried out readily by means of the hand-operated tool shown in the sketch, which is more easily controlled than a power driven lathe or drill.

PROCEDURE—Place the carbon in holder G, superimpose guide D, so that the underside of the guide plate E, rests on the top edge of G, and then tighten screw F. Insert drill and applying slight downward pressure; turn the handle in a clockwise direction. The length drilled is registered on the inscribed mount B, and for 6-inch carbons it has been found expedient to drill rather more than 3 inches into one end of the carbon, which is then reversed and the remaining length is drilled from the opposite end. The drill and sleeve are removed, and the recess cutter is positioned by inserting the guide pin in the hole of the drilled carbon. The recess guide stop is moved up the inscribed shank of the cutter a distance equal to the required depth of the recess, the fixing screw is tightened and the recess cut by revolving the tool until the stop makes contact with the top of the carbon holder. By choosing a suitable design of cutter head, any desired shape of crater may be formed, for example, that used by Mitchell.[4]

The tiny filter paper disc which fits into the recess is cut by means of a cork borer and the usual micro-filtration technique is used, filtering preferably from a centrifuge. A fresh recess may be cut when distortion takes place as a result of arcing, a new flat surface being obtained by rubbing down on emery paper.

I wish to thank the officers of the London, Midland and Scottish Railway Company for permission to publish this note.

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The Transformation of Biological Responses with Special Reference to Vitamin-D Assays

BY N. T. GRIDGEMAN

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

BIOLOGICAL assay has been defined by Bliss[1] as any "determination of potency or toxicity based upon the reaction of living matter." This definition, admirable enough in its precision and brevity, yet tends to obscure a fact that we, as analysts rather than physiologists or pharmacologists, do well to emphasise, *viz.*, that in nine cases out of ten bio-assay is simply quantitative analysis. An assay or estimation is conducted biologically whenever it happens to be more convenient or satisfactory to use, as reagents, living organisms rather than pure chemicals. Perhaps the only really important difference (apart from the fact that in chemical assays one usually adds the reagent to the sample, whereas in bio-assays it is invariably *vice versa*) is that a considerably larger number of repeat tests is necessary in biological work. As in ordinary analysis so in bio-assay the various methods of measuring the reaction between reagent and sample—in other words, of dealing quantitatively with the response of the organism to the test substance—may be grouped into a few general types. We may distinguish the following three:

The *first* concerns quantal or "all-or-none" response, in which the criterion is simply whether or not the reaction takes place. An example is mouse convulsions in one of the insulin assays. In such assays the proportion of positive reactions in a series is recorded.

The *second* utilises reaction time, *e.g.*, the time taken to cure a deficiency symptom, or to reveal a toxicity symptom, or simply for death to occur. The chick assay of vitamin K, for example, utilises time for blood-clot formation.

The *third* is the graded-response assay, so called because the degree of reaction depends on the amount of test substance administered. Among innumerable examples of this type may be instanced (i) bone-ash percentage in the rat assay of vitamin D, (ii) uterine size in the rat assay of oestrone and (iii) liver-fat increase in the mouse assay of anterior pituitary extract.

Only the graded-response assay will be considered here, and only one aspect of that, *viz.*, the way—the numerical style, as it were—in which the response or reaction is expressed. The importance of this aspect may not at first sight be obvious; in order to throw it into relief one must recall the fundamentals of design in bio-assay. These may be expressed as follows. It is necessary that graded doses of a standard preparation be given to a set of suitable animals, while, simultaneously and under strictly comparable conditions, similarly graded doses of the sample or test preparation are given to another set of animals. In these circumstances the curve relating standard doses to responses can be used to read off the potency of the sample, by way of its responses, in terms of the standard; and it will be clear that the result can be stated with the more confidence the more nearly the responses to the test preparation coincide with those to the corresponding doses of standard. This is putting the process in its simplest terms; in practice the result is worked out algebraically, which is not only more convenient and more accurate, but permits the calculation of that all-important appendage of every bio-assay—its statistical limits of error. Computational methods are described in the literature, and there is no need for recapitulation here.

In most graded-response assays the relation between response and dosage is approximately logarithmic, that is to say, the response/log-dose curve is approximately linear. (Perfect linearity, although providing a comforting element of mathematical neatness to the results, and possibly facilitating interpretation, is not, contrary to some statements, a prerequisite of assay validity.) In some assays, notably the microbiological assays of the amino acids and the water-soluble vitamins, the response/dose curve is itself linear; such assays present their own, rather special, interpretative problems,[2] and lie outside the compass of this paper.

Let us now assume that in a particular assay, or in an exploratory test designed to establish an assay technique, the response/log-dose curve and its statistical error have been estimated. The question arises, is it possible to modify the expression of response in any way that would increase its sensitivity or simplify its handling? Three approaches can be tried; they may in brief be described as: that of numerical simplification, that of mathematical transformation, and that of adjustment in terms of concomitant variables. They are elucidated *seriatim* below.

(i) By *numerical simplification* is meant taking care that the numerical expression is the shortest and neatest that the circumstances will permit. Attention to numerical form can save both time and trouble. The substance of this section may be condensed into two maxims. First: never evaluate the response to more significant figures than accuracy warrants. "Accuracy" here refers to the variance of the response and not to the sensitivity of the measuring instrument. For example, having chemical balances at hand, we find it all too easy to express bone-ash percentages to the 4 or 5 figures that the weighings will yield; yet a moment's reflection shows that nothing is sacrificed by rounding off at the third digit. This may be an extreme example, but there is much evidence that at least one digit could have been dropped in many response measurements. Similarly, when the metameter* is obtained from the response by way of 4-figure tables—log tables, for example, as in the X-ray chick assay of vitamin D_3—it is seldom necessary to use all the digits that the tables give. The second maxim is: if the response is measured in units that involve decimal fractions—and particularly if the values are less than unity—always abandon the points and use only the significant digits. Thus 0·0258 is taken as 258.

(ii) *Mathematical transformation.* Let us suppose that a response/log-dose curve based on three average points is plotted and found to be curvilinear instead of rectilinear. Such a curve can be linearised by the raising of the responses to that power n satisfying the condition

$$R_3^n - R_2^n = R_2^n - R_1^n$$

where the R's are the normal responses at the three dosage levels 1, 2 and 3 (equally spaced on the log scale). The appropriate value of n is best determined graphically, and it will be observed that

$$\text{if } R_2 \gtreqless \frac{R_1 + R_3}{2}, \text{ then } n \gtreqless 1.$$

But although this device—the use of (response)^n as metameter—has been resorted to occasionally, it is so hedged with danger as to be rarely commendable. For the transformation,

* Metameter = the response, or transformation of the response, used in the computation of an assay.

while straightening the curve and thus yielding an apparently more satisfactory equation, also introduces a variance gradation. Suppose, for example, that a set of responses, having a homogeneous standard deviation s, is transformed to linearity when n = 2, *i.e.*, when the metameter is taken as R^2. The standard deviation of this metameter can be shown to be 2Rs, which, being a function of R, is no longer homogeneous at all dosage levels. Now it so happens that heterogeneous variance is biometrically less tolerable than non-linearity of the response curve. Furthermore, the smaller the original variance in terms of the slope, the greater this distorting effect will be, and the more probably will an increase of the overall calculated error of the assay ensue. Thus this transforming device is all too likely to defeat its own purpose. Not, however, inevitably; in certain instances of initial heterogeneity of variances the transformation may have the double effect of straightening the curve and homogenising the variance.

This matter of the homogeneity of variances, implying independence of response variance and dosage level, is in fact so important that we may turn the whole problem round and lay down that the soundest use of mathematical transformation, as here understood, is to equalise variances initially found to be graded to dosage level—even if loss of linearity is thereby entailed. This means that the variances of the responses at the different dosage levels should always be tested for statistical homogeneity. If, as is quite conceivable, we find that they are not homogeneous but graded in the direction $V(R_1) < V(R_2) < V(R_3)$, it will be necessary to determine the kind of gradation and so arrive at a response-transforming factor that will yield metameters of uniform variance throughout the dosage range. An interesting example is afforded by the X-ray chick assay[3] of vitamin D_3 in which the variance of the response (tarso-metatarsal distance) is correlated with dosage level; the metameter is taken as the log of the response and has a homogeneous variance.

It so happens that, in the example cited, the transformed responses yield a linear equation. But this cannot invariably be expected, and a few words might be said here on the question of linearity. I have already parenthetically suggested that linearity is not an essential of assay validity. Wood[4] has in fact shown that a non-linear response curve can be interpreted as if it were linear, without loss of validity. But it is essential that the assay should be of symmetrical 6-point design, otherwise it may be impossible to distinguish non-linearity of the fundamental response curve from qualitative differences between the standard and test-substance curves. In the 6-point assay we have three doses each of standard and test-substance at equal log-dose intervals. When the results are subjected to an analysis of variance, these 6 points, in the form of metameter means, yield 5 degrees of freedom, giving information on the statistical significance of the following aspects of their distribution:

(1) Slope (standard and test-substance slopes combined).
(2) Samples (*i.e.*, standard versus test-material).
(3) Non-linearity (tendency to curvature).
(4) Non-parallelism (tendency for standard and test-material lines to diverge).
(5) Contrasting curvature (tendency for standard and test-material lines to curve in opposite directions).

The really important tests of validity are represented by points (4) and (5); if either or both can be shown to be true sources of variance, fundamental differences between the response curves of standard and test-material are indicated, and the assay is invalid. If, on the other hand, (4) and (5) are not sources of variance, the assay is valid whether or not (3), the non-linearity term, is a source of variance; and the assay can be adequately interpreted by means of the usual "best straight-line" equation.

(iii) *Adjustment in terms of concomitant variables.* By this is meant the withdrawal from the metameter of whatever elements of extraneous variance can be distinguished and recorded. The observed metameter and its variance are thus in effect transformed to their counterparts in derivative conditions of stricter control. The use of the observed metameter alone is based on the assumption that it is uninfluenced by other "non-treatment" variables; it is sometimes profitable to test this assumption, for, if a correlation does exist, allowance can be made for it and a more accurate assay obtained. For example, by using "line area" alone in the line-test rat-assay of vitamin D, we assume that the area is uncorrelated with initial weight. The rats are often so distributed that the mean initial weights in each dosage group are as nearly as possible equal; this is an insurance against the distorting influence of the possible emergence of a line-area correlated with initial-weight. But while this device protects the mean responses from possible falsification, it cannot protect the variance from the

inflational effect of the type of correlation envisaged. To obtain complete protection, both initial weights and responses must be tabulated in parallel, and the data analysed for variance and covariance as described in the now classic papers of Bliss and Marks.[5]

An instructive example in this field is provided by the bone-ash rat-assay of vitamin D. The usual metameter is percentage of ash (in the dried fat-free tibia). Bliss[6] drew attention to the assumption, implicit in this procedure, that "for individuals treated alike the log of the ash content plotted against the log of the organic content lost in ashing gives a straight line with slope equal to 1". Using the data of a published assay, Bliss tested this assumption, found the slope to be significantly less than unity, and therefore re-analysed the assay data with "log-weight of the ash..... adjusted by covariance for differences in the log-weight of organic matter lost by combustion" as metameter. The new assay value differed only slightly from the original, but the variance was halved, *i.e.*, "the increase in precision of the assay was equivalent to doubling the number of observations."

The present writer has employed a similar transformation in bone-ash assays,[7] but took "1000 × log-weight (in centigrams) of ash, adjusted by covariance for regression on 1000 × log-weight of prepared bone." This device also increased the precision of the assay, compared with that of the normal percentage-ash assay, by an amount equivalent to a doubling of the number of animals used.

It is always to be borne in mind that an observation of correlated variances in a particular kind of assay in laboratory A is no guarantee that it operates in laboratory B. Each assayist must examine his own data without reference to other workers' findings. Here again line-test vitamin-D assays may be instanced; some laboratories have found a significant regression of line-area on initial-weight; other laboratories have not, and are thus saved the trouble of making the extra observations and computations.

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LEVER BROTHERS & UNILEVER LTD.
PORT SUNLIGHT, CHESHIRE

An Absorptiometric Method for Magnesium in Zinc-Base Die-Casting Alloys

BY F. H. EDWARDS AND A. M. ROBINSON

THE British Standard Specification 1005 : 1942 outlines a gravimetric method for determining magnesium in zinc alloys specified by B.S. 1004. The magnesium content of these alloys varies between the limits 0·03 and 0·06%, so that the gravimetric method requires an opening sample consisting of two separate 5-gram portions which are later combined and the magnesium finally weighed as pyrophosphate. The present method comprises several modifications to the one given in B.S. 1005, such as the application of Stross's colorimetric finish[1] and the use of a smaller opening sample. This enables results of comparable accuracy to be obtained in approximately one-third of the time required by the B.S. method. As shown below, the magnesium figures are not affected by the presence of copper as an alloying element up to 3% or by small amounts of iron, lead, tin and cadmium as impurities.

METHOD

SOLUTIONS REQUIRED—*Ferric Chloride*—Dissolve 3·5 g. of ferric chloride in 20 ml. of hydrochloric acid (sp.gr. 1·16) and dilute to 100 ml. with water.

Starch—Mix 1 g. of soluble starch with a few mls. of cold water and pour into 50 ml. of boiling water. Cool and add 1·2 g. of calcium chloride. Make up to 100 ml. and filter through a No. 40 paper. Prepare fresh daily.

Titan Yellow—Dissolve 0·5 g. of Titan yellow in 1000 ml. of water, filter through a No. 40 paper, and keep in a black bottle.

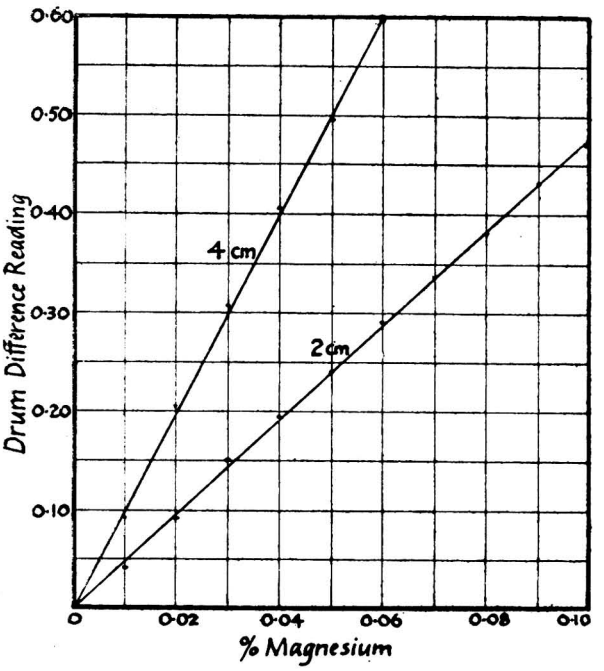
Buffer Solution—Dissolve 137·5 g. of sodium hydroxide and 36 g. of boric acid in 1000 ml. of water and filter through a No. 54 paper.

Methyl Red—Dissolve 0·2 g. of methyl red in 100 ml. of 50% alcohol.

Potassium Cyanide—Dissolve 10 g. of potassium cyanide in 100 ml. of water.

Standard Magnesium Solution for Calibration—Dissolve 5·067 g. of magnesium sulphate ($MgSO_4.7H_2O$) in water and make up to 1000 ml. in a graduated flask. One ml. of this solution is equivalent to 0·01 per cent. of magnesium on a 5-gram sample.

PROCEDURE—Dissolve 5 g. of the alloy in 20 ml. of hydrochloric acid (sp.gr. 1·16) and 5 ml. of nitric acid (sp.gr. 1·42) in a 450 ml. conical beaker. Boil down to low bulk to remove most of the excess acid. Add 150 ml. of water and 1 ml. of 3·5% ferric chloride solution. Add solid sodium hydroxide until the zinc and aluminium are re-dissolved. Add a further 150 ml. of water and digest on the hot plate until the precipitate coagulates. Filter hot



through a double paper consisting of a Whatman No. 54 inside a No. 40, and wash three times with hot 1% sodium hydroxide solution. Discard the filtrate. Dissolve the precipitate through the paper with three 10 ml. portions of boiling 20% sulphuric acid and wash five times with hot water. Add 5 g. of ammonium sulphate and 2 drops of methyl red indicator. Dilute to about 180 ml. and spot-in ammonium hydroxide (sp.gr. 0·880) till one drop turns the solution yellow. Boil for 1 minute, cool and dilute to 200 ml. in a measuring cylinder. Filter through a dry No. 40 paper into a dry beaker.

Pipette 10 ml. of the filtrate into a 250 ml. conical beaker and add successively 50 ml. of water, 10 ml. of starch solution, 10 ml. of 10% potassium cyanide solution, 5 ml. of Titan yellow solution and 10 ml. of buffer solution. Make up to 100 ml. in a graduated flask and read on the Spekker photoelectric absorptiometer, using a mercury-vapour lamp as light source, Ilford heat-absorption filters and Ilford Green 604 filters, and a water/water setting of 1·00.

PREPARATION OF STANDARD GRAPH—The standard graph was obtained upon synthetic materials treated exactly as in the method described above. This entailed the use of 4·8 g. of spectro-pure zinc and 0·2 g. of spectro-pure aluminium to which were added the requisite amounts of the standard magnesium solution. In the present case samples representing from 0·01 to 0·10 per cent. of magnesium were carried through in addition to a blank and the difference readings were plotted for 4 cm. and 2 cm. cells. It should be stated that the 4 cm. cell becomes unsuitable for contents exceeding 0·06 per cent. of magnesium owing to the high

blank, due particularly to the colour of the Titan yellow. The graph was found to remain unaltered when checked at intervals over a period of several weeks. See Fig. 1.

RESULTS

Sample	Composition					Magnesium, %	
	Al	Cu	Pb	Sn	Cd	Chem.	Spekker
1	4·12	—	—	—	—	0·013	0·010
2	3·95	0·99	—	—	—	0·013	0·014
							0·012
3	4·00	1·02	—	—	—	0·041	0·041
							0·045
4	4·02	1·03	—	—	—	0·070	0·074
							0·072
5	4·04	1·02	—	—	—	0·095	0·097
							0·097
6	High purity zinc					—	0·001
7	3·36	—	—	—	—	0·074	0·070
8	4·08	—	—	—	—	0·052	0·050
9	4·61	—	—	—	—	0·023	0·023
10	2·31	—	—	—	—	0·012	0·012
11	4·40	—	—	—	—	0·038	0·037
12	3·72	—	—	—	—	0·063	0·060
13	3·36	1·00	—	—	—	0·074	0·072
14	4·08	1·00	—	—	—	0·052	0·049
15	4·61	1·00	—	—	—	0·023	0·022
16	3·36	3·00	—	—	—	0·074	0·072
17	4·08	3·00	—	—	—	0·052	0·051
18	4·61	3·00	—	—	—	0·023	0·024
19	4·00	—	0·05	—	—	—	0·001
20	4·00	—	—	0·05	—	—	0·001
21	4·00	—	—	—	0·05	—	0·001

Fading—The colour produced by the 0·023 per cent. magnesium standard faded to the extent of 0·004 per cent. after standing for 6 hours. It is recommended that readings be taken within 30 minutes of colouring.

The authors thank the Director of Scientific Research, Admiralty, for permission to publish, and also the Research Laboratories, Imperial Smelting Corporation, Limited, for supplying several of the samples used.

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BRAGG LABORATORY, N.O.I.D.,
SHEFFIELD

February, 1946

Ministry of Food

STATUTORY RULES AND ORDERS*

1946—No. 943. Order, dated June 25, 1946, amending the Feeding Stuffs (Regulation of Manufacture) Order, 1944. Price 1d.

This Order corrects an error in a previous Amending Order, S.R. & O., No. 829, 1946: in paragraph 9 (1) (a) of Part A of the Second Schedule the figure "2¼," signifying the minimum percentage of calcium expressed as calcium carbonate, should be "2½."

— **No. 945. The Soft Drinks Order, 1946. Dated June 26, 1946.** Price 3d.

This Order revokes and re-enacts the Soft Drinks Order, 1943, and its amending Orders with certain amendments, of which the principal are as follows:

The definition of "soft drink" is amended to provide that a product used or intended to be used both as a medicine and as a drink shall be regarded as a soft drink for the purpose of the Order.

Conditions are prescribed under which Quinine Tonic Water or India Tonic Water may be manufactured and sold. Besides prescribing the ingredients and maximum price the Order requires such tonic water to bear a label with the words "Contains not less than ⅛ grain of quinine (calculated as quinine sulphate B.P.) per pint."

The sugar content of the drinks listed in Part II of the First Schedule is increased and the saccharin content reduced.

— **No. 1136. Order, dated July 16, 1946, amending the Bread (Control and Maximum Prices) (No. 2) Order, 1943.** Price 1d.

This amending Order legalises and makes compulsory Vienna loaves weighing 7 ounces instead of 8 ounces and in Northern Ireland baps weighing 7 ounces or 3½ ounces instead of 5 ounces.

It also authorises Weights and Measures authorities (or in Northern Ireland the Ministry of Commerce) to institute proceedings for offences against the Order in respect of the weight of any bread.

* Italics signify changed wording.

1946—No. 1221. Order, dated July 24, 1946, amending the Food Standards (Preserves) Order, 1944 (S.R. & O., No. 842, 1944). Price 1d.

This amending Order extends the description "special standard marmalade" to include grape fruit marmalade having a fruit content not less than 25 per cent.

— **No. 1265. Order, dated July 25, 1946, further amending the General Licence, dated September 11, 1943, under the Manufactured and Pre-Packed Foods (Control) Order, 1942.** Price 1d.

This amending Order reduces the minimum oil and fats content of manufactured and pre-packed Christmas puddings from 10% to 9%.

— **No. 1355. The Meat Products, Canned Soup and Canned Meat (Control and Maximum Prices) Order, 1946. Dated August 8, 1946.** Price 5d.

This Order, which came into force on August 18, 1946, revokes and substantially re-enacts the Meat Products, Canned Soup and Canned Meat (Control and Maximum Prices) Order, 1944, as amended.

Among the principal changes in the Order are—

(1) *An increase in the meat content of beef sausage, beef sausage meat and beef slicing sausage to 50% minimum is prescribed.*

(2) *Minimum instead of fixed percentages of meat content for sausages and other open meat products and for home-packed canned meat paste and fish paste are prescribed. The minima, given in Schedule I, are: pork sausages, pork sausage meat and pork slicing sausage 50% (of which at least 80% shall consist of pork); beef sausages, beef sausage meat and beef slicing sausage 50%; other sausages or sausage meat except liver sausage and imported Salami sausages 30%; meat roll or galantine 30%; liver sausage 45%; meat paste 55%; fish paste 70%.*

ADDITION OF CALCIUM CARBONATE TO NATIONAL FLOUR

THE Minister of Food announces that, on the advice of the Special Diets Advisory Committee of the Medical Research Council, from Sunday, August 11th, the rate of addition of calcium carbonate (*Creta Praeparata*) to National Flour will be increased from 7 ounces to 14 ounces per sack of 280 lbs.

This is to compensate for the increased phytic acid content of 90 per cent. extraction flour.

P.N. 4220
July 31, 1946

PROPOSED MAXIMUM LIMITS FOR THE FLUORINE CONTENT OF CALCIUM ACID PHOSPHATE (A.C.P.) USED IN FOOD AND OF CERTAIN SPECIFIED FOODS

As a result of recommendations made by the Inter-Departmental Committee on Food Standards,* the Ministry of Food has under consideration the issue of an Order under Regulation 2 of the Defence (Sale of Food) Regulations, 1943, prescribing limits for the fluorine content of the following foods:

(*a*) Calcium acid phosphate and sodium acid pyrophosphate when used for food purposes	300 parts per million
(*b*) Baking powder and golden raising powder	100 ,, ,, ,,
(*c*) Self-raising flour and other food products containing aerating ingredients	8 ,, ,, ,,

Any manufacturer or other party desiring to make any comments on these proposals should forward them in writing to the Ministry of Food, Miscellaneous Food Products Division, 39–40, Portman Square, London, W.1, not later than August 31st, 1946.

P.N. 4222*a*
July 31, 1946

FLUORINE IN CALCIUM ACID PHOSPHATE

REPORT OF THE INTER-DEPARTMENTAL COMMITTEE ON FOOD STANDARDS TO THE MINISTRY OF FOOD†

WE received a memorandum from the Manufactured Foods Division of the Ministry of Food requesting our advice on a proposal to make an Order prescribing maximum limits for the fluorine content of calcium acid phosphate (A.C.P.) sold for use in food, and certain articles containing it.

The memorandum stated that two processes are used for the manufacture of A.C.P. In the first process elementary phosphorus is used as the raw material and the product is of a high degree of purity. Something like 80 per cent. of the total output in the country is made in this way. In the alternative process the raw material is rock phosphate and the product is contaminated with compounds of fluorine. We were informed that manufacturers using this process claim that they have been selling A.C.P. containing as much as 3000 p.p.m. of fluorine for many years without exception being taken by Food and Drugs Authorities. During the last few years these manufacturers have had to use such rock phosphates as have been available and no doubt the resulting A.C.P. has often been more heavily contaminated than it was before the war.

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* See Report following.

† Received from the Ministry of Food.

We were also informed that the possibility of harmful contamination of foods with fluorine has been giving concern to Public Health Authorities. From 1942, Food and Drugs Authorities brought proceedings in certain cases under the Food and Drugs Act, 1938, in respect of baking powder or articles containing it contaminated with fluorine but they failed in some of them to obtain conviction.

In August, 1943, the Society of Public Analysts and Other Analytical Chemists, which had been giving consideration to this question, issued a statement recommending that no action should be taken under the Food and Drugs Act where the following proportions of fluorine, however combined, were not exceeded:

In Acid Phosphates	200 parts per million
In Baking Powder	70 ,, ,, ,,
In Golden Raising Powder	50 ,, ,, ,,
In Self-Raising Flour, Cake Mixtures and like compositions	5 ,, ,, ,,

Nevertheless, in the absence of a legal standard it is difficult to convince a Court that where any of these proportions is exceeded an offence has been committed. In at least one case subsequently a Food and Drugs Authority failed to secure a conviction and in general Authorities appear to have been reluctant to take action.

In view of this unsatisfactory situation, several Authorities have proposed that limits should be defined by Regulation. Manufacturers have also urged that steps should be taken to clarify the position, and at our invitation the manufacturers submitted a report of work carried out on their behalf by H. E. Archer, M.R.C.S., L.R.C.P., F.R.I.C., and B. Leech, M.A., F.R.I.C.

In this report it was suggested that

(i) In the combination in which it occurs in food products after aerating ingredients have interacted, fluorine is insoluble in normal gastric juice even when the acidity approaches the upper limit of normality. Further, phosphates present in a food after interaction of the aerating ingredients buffer the gastric juice at a *p*H on the alkaline side of neutrality so that any fluorine present is insoluble and unabsorbable.

(ii) Within the limits of experimental error, any fluorine ingested in the form of baking powder containing contaminated A.C.P. after interaction of the aerating ingredients is excreted completely in the faeces.

The report was discussed at a meeting between the manufacturers' representatives and the Committee We pointed out that since the experiments described related to only one adult, who was on a high milk diet, the results could not be accepted as evidence of what might take place in the case of other persons, especially children, taking a more varied diet. Dr. Archer, however, indicated that the case for the harmlessness of fluorine rested not merely on the experiments described in the report, which he recognised as being chiefly of a confirmatory nature, but also on the fact that mottling of teeth is rarely or never found except where the water supply is known to be contaminated.

He asserted that this condition was a highly sensitive indicator of fluorine absorption and since A.C.P. contaminated with fluorine was extensively used, the absence of motting of teeth showed that fluorine was not absorbed from A.C.P. It was further asserted that even if widespread mottling had occurred, the condition was to be regarded merely as a cosmetic disability and not as evidence of the toxicity of fluorine. Reference was made to work by Roholm stated to show that cryolite workers enjoyed excellent health and that comparatively large amounts had to be ingested daily for many years before any symptoms developed. It was stated further that investigations in this country had failed to reveal any radiological evidence of skeletal changes in either adults or children with mottled teeth, except in undernourished children from bad homes. The manufacturers' case therefore was that fluorine was not absorbed from A.C.P. and even if absorbed was harmless. On the other hand our attention was drawn to various statements in medical literature suggesting that mottling of teeth is more common than usually supposed. Moreover with other toxic elements, for example lead, almost complete excretion in the faeces does not necessarily justify the assumption that oral ingestion is harmless.

We felt that the arguments advanced by the manufacturers could not be dismissed without further investigation and that their validity could be assessed only by persons having the requisite physiological and toxicological knowledge.

The following questions were accordingly addressed to the Medical Research Council.

(1) Is there any authoritative evidence that injury to human health has resulted from the absorption over lengthy periods of small amounts of fluorine from food other than water?

(2) Is it true that widespread mottling of teeth in the community does not occur?

(3) If the answer to question (2) above is in the affirmative, can the absence of widespread mottling be accepted as evidence that absorption does not occur at all or not to any harmful extent?

(4) Having regard to the available evidence, including that advanced in the report prepared by Dr. Archer and Mr. Leech, is it in the opinion of the council desirable that an Order should be made limiting the amount of fluorine in calcium acid phosphate?

(5) If the answer to question (4) is in the affirmative, what limit should be imposed?

The following specific answers were given to these questions:

(1) Answer is *no*: because so far no one would have recognised the effects of such ingestion; analysts have only been interested in fluorine determinations in recent years and pathologists have not clearly established the criteria of milder forms of chronic fluorosis.

(2) Answer is *no*: in any case mottling of enamel is not the most suitable indication of fluorine ingestion except by the water supply.

(3) Animal experiments have shown that fluorine is ingested by pigs, rabbits, rats, cattle, sheep and dogs from food, pasture and rock phosphates. Widespread fluorosis occurs in cattle and humans in Algeria, one of the regions from which much rock phosphate is obtained. The ingestion of fluorine from rock phosphates, besides producing the well known bone changes, produces in different species different effects, including pathological changes in the mucous membrane of the alimentary canal, and nephrosis.

(4) Yes, an Order should be made.

(5) The limit for calcium acid phosphate should be 300 p.p.m.

In recommending that the limit be somewhat higher than was suggested by the Society of Public Analysts and Other Analytical Chemists, the Council stated that in their view a limit of 300 p.p.m. would still be safe having regard to the proportion of A.C.P., which is used in foods containing it.

In amplification of these answers the Council informed us that in their opinion it is essential that a limit be set for the amount of fluorine permissible in any material used in food production and suggested that the safest procedure would be to prohibit the use of A.C.P. made from rock phosphate for food purposes. The view was expressed that since the effects of fluorine are cumulative, absorption from the alimentary canal could only be proved or disproved by determining the urinary excretion of fluorine during administration over a period. The argument that the absence of mottling of teeth showed that absorption does not occur was criticised on various grounds. In addition it was suggested that Roholm's monograph on fluorine poisoning, the recent work of Ockerse in South Africa and Shortt in India, and reports from industries using fluorine compounds, all tended to show that fluorine was a potentially toxic contaminant.

In view of the possible effect such an Order would have on certain manufacturers we felt it desirable to acquaint ourselves more fully with the grounds underlying the Council's recommendation and Dr. Margaret Murray, who had advised the Council on the matter, kindly attended a meeting of the Committee to provide further information.

Dr. Murray suggested that it was not justifiable to base any opinion on a short-term experiment, since, for example, in the parts of India where fluorosis is endemic, the effects may not become evident for 25 to 30 years. She considered that the balance found by Dr. Archer and Mr. Leech in the intake and excretion of fluorine by their patient must have been largely fortuitous because, apart from the difficulty of accurately determining small quantities in the excreta, fluorine is cumulative and the fluorine excreted on one particular day is not necessarily that ingested the previous day. She agreed that small amounts in drinking water had a beneficial effect in tending to prevent caries but was nevertheless of the opinion that this did not justify the ingestion of fluorine in larger quantities, or from other sources.

The further evidence provided by Dr. Murray may be summed up by saying that fluorine, if absorbed in small quantities over a sufficient period of time, can produce gross lesions of bone; there is abundant evidence from animal experiments and analyses of human bones that fluorine can be cumulatively absorbed; and there is radiological evidence that a mild spondylitis occurs in some children living in areas where the drinking water contains fluorine although it is possible that the affection occurs only in those children whose nutritional state is poor.

We record our appreciation of the assistance rendered by the Medical Research Council and by Dr. Murray. In view of the unquestionable harmfulness of large amounts of fluorine, and of the cumulative effect of small doses, we feel that the risk to the public health from the repeated ingestion of small amounts of fluorine is too serious to be ignored, and that an Order should be made prescribing maximum limits for the contamination.

With the evidence at present available, it is only possible to make an approximate estimate of the maximum amount of fluorine which can be ingested daily over a period years without harmful effect, and in fixing a limit for fluorine in A.C.P. it must be remembered that products other than calcium acid phosphate may contribute small quantities of fluorine to the total intake. The Medical Research Council are, however, satisfied that a limit of 300 p.p.m. of fluorine in A.C.P. would be safe, and for the reasons indicated above, we consider that this figure should be adopted as the maximum limit.

We are of the opinion that an Order prescribing limits only for A.C.P. and sodium acid pyrophosphate would fail to achieve its object. These substances are not bought by the public as such, and samples would not often be submitted for examination under the existing arrangements for ensuring the purity of food. The articles in which these phosphates are likely to be used are those containing aerating agents, such as self-raising flour, baking powder and products sold as cake mixtures or bun mixtures. Since these contain other phosphorus compounds, it is not possible to determine the proportion of A.C.P. or sodium acid pyrophosphate with accuracy, and an analyst would have the utmost difficulty in convincing a court that a particular sample containing an abnormal amount of fluorine had been made with a phosphate not complying with the statutory requirements. It is therefore regarded as essential that maximum limits be prescribed by Order for the more widely sold foods which are likely to contain fluorine-contaminated food phosphates.

Information as to the proportions of acid phosphate in the food products now on the market has been supplied by the Manufactured Foods Division of the Ministry of Food. A wide range is shown in each type of product and if a limit for fluorine were based on the maximum proportion of acid ingredient, a phosphate containing much more than 300 p.p.m. of fluorine could be used in the manufacture of a powder of lower aerating power for which a smaller proportion of the acid phosphate would be needed. We feel that if a manufacturer wishes to supply a product having an unusually high aerating power, it is not unreasonable under present conditions to require him to use as the acid ingredient in whole or in part a substance such as tartaric acid which is not liable to contamination with fluorine.

Only a small minority of the baking powders on the market contain more than 30 per cent. of A.C.P. or sodium acid pyrophosphate. We accordingly recommend that the fluorine limit for baking powder be one-third that for A.C.P. itself, that is 100 p.p.m. "Golden raising powder" contains on the average a somewhat lower proportion of aerating ingredients than baking powder, but the difference is small and it is recommended that the same limit be prescribed for both kinds of product.

Self-raising flour, cake and bun flours, batter mixtures and similar products comprise a further class of foods containing A.C.P. In the majority of these the A.C.P. content does not exceed 2½ per cent. Since in use they generally constitute the sole farinaceous ingredient of the consumed goods, we think it would be appropriate to set a limit of 8 p.p.m. to the fluorine content of this group.

We are aware that a few products in this class may contain a higher proportion of A.C.P., but makers of these particular products should be able, by proper selection of ingredinents, to conform to the standard.

In suggesting these limits, we have borne in mind the possibilty that flour itself, especially when fortified with chalk, may contain traces of fluorine. Such traces are, however, small in comparison with the amount introduced by the phosphate and no special provision is necessary.

We accordingly recommend that an Order be made prescribing the following maximum limits for the fluorine content of the foods specified:

(*a*)	Calcium acid phosphate and sodium acid pyrophosphate, when used for food purposes	300 parts per million
(*b*)	Baking powder and golden raising powder	100 ,, ,, ,,
(*c*)	Self-raising flour and other food products containing aerating ingredients	8 ,, ,, ,,

Although not within our terms of reference, we desire to bring to the Ministry's notice the need for further research into the effects of long continued ingestion of small quantities of fluorine in food. We feel that in the areas of India and elsewhere where fluorosis is endemic, ample material is ready to hand for the investigation of this matter which may well have a considerably greater bearing on the public health than has hitherto been generally realised. We suggest that the Ministry should draw the attention of the Medical Research Council, or other organisation able to arrange for the conduct of research work of this kind, to the desirability in the public interest of a thorough investigation of the effects of fluorine ingestion being undertaken at an early date.

British Standards Institution

A FEW copies of the following draft Specifications, prepared by Technical Committee FCC/4—Solvents, and 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.

CH(FCC)5977—Draft Revision of B.S.580 for Trichlorethylene (Technical) and Trichlorethylene (Stabilised).
CH(FCC)5978—Draft Revised B.S.662 for Carbon Disulphide.
CH(FCC)5981—Draft Revised B.S.S. for Diacetone Alcohol.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Chemical Determination of Vitamin A in Dried Whole Eggs. C. R. Thompson, M. A. Ewan, S. M. Hauge, B. B. Bohren and F. W. Quackenbush (*Ind. Eng. Chem., Anal. Ed.*, **1946, 18**, 113–115)—Attempts to use published chemical methods for determination of vitamin A in dried eggs failed to give results agreeing with those obtained by bio-assay. Investigation showed that the differences were due to difficulty in extraction of the vitamin and to errors caused by the presence of large amounts of carotenols, for which arbitrary corrections could not be applied. When extraction in a Waring Blendor was tried as described by Schrenk *et al.* (*Id.*, **1944, 16**, 632) a large proportion of the vitamin remained in the residue. Of a number of solvents tried ethyl ether proved the least suitable, but solvents that were more effective (alcohol, carbon tetrachloride, 1,2-dichloroethane, trichloroethylene and mixtures of benzene and alcohol) had relatively high boiling points and prolonged heating darkened the extract. Large amounts of carotenols caused errors when attempts were made to apply Koehn and Sherman's modification of the Carr - Price colorimetric method (*J. Biol. Chem.*, **1940, 132**, 527; ANALYST, 1940, **63**, 303). Gillam and Heilbron (*Biochem. J.*, 1935, **29**, 1064; ANALYST, 1935, **60**, 564) have shown that the vitamin A of egg yolk can be separated from xanthophylls by adsorption on calcium carbonate and elution with a mixture of light petroleum and benzene. Preliminary experiments showed that calcium hydroxide is a better adsorbent than calcium carbonate for the chromatographic separation of the carotenoids of eggs and that, when the unsaponifiable extract is adsorbed from light petroleum solution and the column is developed with a mixture of 60% of benzene and 40% of light petroleum, a sharp separation is obtained. The carotenoids are eluted from the column in the order β-carotene, cryptoxanthol, vitamin A, luteol and zeaxanthol.

Method—To 5 g. of the sample in a 125-ml. Erlenmeyer flask add 20 ml. of absolute methanol and 5 ml. of sat. aq. potassium hydroxide soln., stir until suspension of the sample is complete and heat on the steam bath until all the particles are disintegrated (10 min.). Transfer the cooled mixture into a 500-ml. separator with 70 ml. of water and extract first with 35 ml. of peroxide-free ether and then four times with 25- to 30-ml. portions. The last extracts should be almost colourless. Wash the extract five times with 25-ml. portions of water and dry it over 20 g. of anhydrous sodium sulphate for 1 hr. at room temp. Evaporate the ethereal extract to about 15 ml. under reduced pressure in a water bath at 50° C., transfer it into a dry 25-ml. flask with dry light petroleum and make it up to volume with the same solvent. If the soln. is still cloudy it should be dried with a small amount of sodium sulphate. Adsorb 10 ml. of this soln. on a column (20 × 135 mm.) of 3 parts of calcium hydroxide (Braun's, Lot No. 10588) and 2 parts of Hyflo Super-Cel. Develop the chromatogram with a mixture of 60% of thiophene-free benzene and 40% of dry light petroleum. Elute the two lowest bands containing β-carotene and cryptoxanthol separately. Collect the vitamin A fraction until the luteol begins to give a yellow colour to the eluate. If the column is properly packed, the

β-carotene and cryptoxanthol bands are easily distinguished and the luteol band will be distinct as it approaches the end of the column. Fifty to 80 I.U. of vitamin A and 150 to 200 μg. of total carotenoids (as β-carotene) can be treated satisfactorily with the column described. A total vol. of 250 to 500 ml. of combined β-carotene, cryptoxanthol and vitamin eluates is the optimum range. Remove the solvent from each of the β-carotene and cryptoxanthol fractions and dissolve the residues in 10 ml. of light pertoleum. Measure the light transmission with the Evelyn photoelectric colorimeter with the standard 440 mμ alter. Recombine these solns. with the vitamin A fraction and evaporate under reduced pressure to about 15 ml. (This is necessary because small amounts of vitamin A occur in these two eluates; the effects of β-carotene and cryptoxanthol on the total blue colour development are negligible.) Transfer this to a 25-ml. flask with re-distilled chloroform. Evaporate a 10-ml. aliquot in a colorimeter tube and re-dissolve in 2 ml. of chloroform. Place the tube in the Evelyn colorimeter, add 8 ml. of antimony trichloride reagent (*infra*) and within 5 to 10 sec. measure the colour with the standard 620 mμ filter.

To establish the calibration curve of the instrument for vitamin A 1 g. of U.S.P. reference cod liver oil No. 2 was saponified and extracted by the procedure described. A sample of β-carotene purified by chromatography was used as the standard for determination of β-carotene, cryptoxanthol and total carotenoids.

Purification of the light petroleum was found necessary, as some commercial samples gave a green colour with antimony trichloride. Four to 5 gall. of commercial light petroleum (b.p. 65° to 67° C.) were percolated through a column (7 × 35 cm.) of silica gel (Davison Chemical Corporation, No. 659–528–2000). The percolate was stirred mechanically for several hr. with two successive portions of conc. sulphuric acid and after separation of the acid layer the last trace of acid was removed with dil. sodium hydroxide soln. It was then stirred for a few hr. with alkaline potassium permanganate, separated and distilled and dried over anhydrous sodium sulphate. If the humidity of the laboratory was too high the carotenoids moved too rapidly down the column, and it was then impossible to separate the β-carotene and cryptoxanthol fractions. To avoid this the adsorbent was stored in a desiccator over sulphuric acid, and removed only long enough to pack the column. The antimony chloride reagent was prepared by dissolving 22·5 g. of antimony trichloride in 100 ml. of re-distilled chloroform, filtering and storing in a brown bottle. Results obtained by this method were within 10% of those obtained by bio-assay. A. O. J.

Determination of Menthol in Peppermint Oil. Acetic Anhydride and Pyridine as Reagent. J. S. Jones and S. C. Fang (*Ind. Eng. Chem., Anal. Ed.*, **1946, 18, 130–131**)—The proposed method is a modification on the macro scale of the micro method of Petersen, Hedberg and Christensen (*Id.*, 1943, **15**, 225) for determining the hydroxyl content of pure organic compounds. Weigh accurately about 0·6 g. of peppermint oil in a tared 3-in. test tube, add about 0·5 g. of acetic anhydride (C.P.), re-weigh the tube and add 0·5 ml. of pyridine (C.P., water-free). Seal the tube with a cork previously dipped in melted paraffin wax, shake immediately once and set aside for 48 hr. Then open the tube, place it in an Erlenmeyer flask, add 50 ml. of water and titrate the mixture with 0·5 *N* sodium hydroxide (carbonate-free), using phenolphthalein as indicator. Near the end of the titration heat the flask for a few min. to ensure complete hydrolysis of the excess of acetic anhydride and continue the titration to an end point persisting for 1 min. Determine the vol. of standard alkali required to neutralise the acid derived from 1 g. of acetic anhydride. Titrate another portion of the sample with standard alcoholic sodium hydroxide to determine the amount of free acid in the peppermint oil. The amount of free menthol (%) in the sample is then given by

$$(A \times R - B_1 + B_2)\, N \times 156{\cdot}16 \times 100/W$$

where A is the wt. in g. of acetic anhydride used, R the ml. of standard alkali required to neutralise the acid from 1 g. of acetic anhydride, B_1 the ml. of standard alkali required to neutralise the residual acid after acetylation of the sample, B_2 the ml. of standard alkali required to neutralise the free acid in the sample, N the normality of the standard alkali and W the wt. of the sample in mg.

Investigation of the method showed that an acetylation period of more than 24 hr. is necessary and that one of 28 to 32 hr. is ample for maximum values. An excess of acetic anhydride of at least 100% is necessary and the ratio of acetic anhydride to pyridine must not be less than 1 : 1. Results by this method agree closely with those obtained by the method of Power and Kleber (*Pharm. Rundsch.*, 1894, **12**, 162) which is generally accepted in the industry and is official in the U.S.P. When the method was used for the recovery of known amounts of menthol up to 500 mg. added to peppermint oil containing 50·6% of free menthol the recoveries ranged from 98·5 to 100·2%. Expts. showed that the method of the U.S.P. yields very satisfactory results if the details of manipulation given in the U.S.P. are closely followed. The proposed method, however, is more convenient and the sample requires no attention during the acetylating period. Duplicate determinations by the proposed method agree more closely than those by the official method. A. O. J.

Critical Investigations on the Determination of Nicotine by the Picrate Method. H. Baggesgaard Rasmussen and H. Kofoed (*Dansk. Tidssk. Farm.*, 1944, **18**, 233–250)—Owing to the non-availability of silicotungstic acid for the determination of the nicotine content of *Nicotiniarustica*, the method proposed by Pfyl and Schmitt (*Z. Unters. Lebensm.*, 1927, **54**, 60), involving precipitation of nicotine dipicrate and subsequent determination by weighing or titration, has been used.

Method—Transfer 20 g. of the finely cut leaves of fresh tobacco, or 5–10 g. of the roughly cut dry leaves, to a 1-litre Pyrex flask by means of 100 ml. of water. Add 30 ml. of 10 *N* sodium hydroxide and steam distil into a 600-ml. beaker containing a mixture of 5 ml. of concentrated hydrochloric acid and 5 ml. of water, using an efficient condenser and no connecting bulb, and with the tip of the condenser below the surface of the acid in the receiver. Continue the distillation until 2 ml. of distillate, acidified with 2 drops of hydrochloric acid, give no precipitate or opalescence with silicotungstic acid, the total volume of distillate being about 300 ml. Adjust the reaction of the distillate to about *p*H 5, using methyl red as indicator, add 1 ml. of 0·5 *N* hydrochloric acid followed by 150 ml. of saturated (approx. 0·5 *M*) picric acid, slowly and with constant stirring. Leave for 12 hr., collect the precipitate on a tared sintered glass filter (1G2 or 1G3), and wash with the minimum quantity of a mixture of 1 vol. of saturated picric

acid solution and 3 vol. of water. Two washes each of about 20 ml. are satisfactory. Remove as much of the wash liquid as possible by suction, wipe the outside of the filter and weigh. Finally dry the filter and precipitate at 105° C. to constant weight. Correct the dry weight of the precipitate for the picric acid derived from the wash-solution and retained by the precipitate. The melting point of the isolated nicotine picrate was determined by the capillary tube method. The tube was lowered into a heated liquid paraffin bath at a temperature 5° below the expected melting point, the heating taking place at the rate of 2° C. per min. The melting points of 35 samples varied from 223·1° C. to 225·6° C., 23 of them lying between 224·0° C. and 224·5° C. Pure nicotine picrate melts at 225·0° C. The solubility of nicotine picrate in water was found to be 0·39 g. per litre and in the solution used for the precipitation (about 0·017 *M* picric acid) nephelometric determination indicated the presence of less than 0·016 g. per litre. Thus, a mixture of 300 ml. of distillate and 150 ml. of saturated picric acid solution would contain less than 7 mg. of nicotine picrate in solution. The melting points of the precipitated picrates indicate that none of the samples contain nor-nicotine and the statement of Waser (*Acta nicotiana, Berlin*, 1939, 99) that this substance is not volatile with steam from a solution made alkaline with magnesium oxide has been confirmed. J. A.

Orienting Investigations on the Morphine Content of *Papaver Somniferum* During the Growth of the Plant. H. Baggesgaard Rasmussen and K. Ilver (*Dansk. Tidssk. Farm.*, 1945, **19**, 71–105)—The various methods for the determination of morphine in poppy capsules and other parts of the poppy plant are not suitable for small amounts or series analyses, hence the following procedure has been devised. *Method*—Mix 100 ml. of water with 10 g. of the finely divided material consisting of stems, leaves or capsules and boil the mixture for 5 min. Leave overnight, separate the insoluble matter by pressing and repeat the extraction with 60 ml. of water. Again separate the insoluble material and finally extract with 40 ml. of water. Express as much of the water as possible from the residue, mix the aqueous extracts, filter through cotton wool and dilute to 200 ml. Transfer 20 ml. to a continuous extraction apparatus together with 5 ml. of 2 *N* sodium hydroxide and 1 g. of ammonium chloride and extract for 3 hr. with 60 ml. of chloroform containing 5% w/w. of methanol. Remove the solvent from the extract by evaporation, dissolve the residue in *N* hydrochloric acid and heat the solution on the water bath for 15 min. with constant shaking. Dilute the acid solution of alkaloids to 25 ml. and submit 5 ml. to the polarographic determination of morphine as described by Rasmussen, Hahn and Ilver (*Dansk. Tidssk. Farm.*, 1945, **19**, 41). The procedure was checked against the method of J. C. Jespersen (*Id.*, 1936, **10**, 16). Good agreement was obtained.

The morphine content of various portions of the poppy plant was determined at various stages of growth and it was found that the leaves contained a maximum of 0·1% of the alkaloid, falling to 0·02% during growth. The alkaloid content of the stems reached in some instances more than 0·1% but during growth the amount diminished, although not to the same extent as in the leaves. The capsules showed a strong increase in morphine content up to the semi-ripe stage, in some instances reaching 0·6%, but on ripening the amount of alkaloid fell considerably. The distribution of the component parts, the leaves, stem, capsules and seeds, of the air-dried drug has been computed. The young plants yielded a sample of which about 40% consisted of leaves, about 48% stem, 9% capsule and 2·5–5% seeds. On ripening, the proportion of leaf fell to about 10%, that of stem remained constant, while the proportion of capsule and seeds increased to 12–15% and 25–30% respectively. The oil content of the seeds remained unaltered during the ripening process and no change was found in the saponification value and the iodine value (190–193 and 136–140 respectively) during the same period. J. A.

Biochemical

Manometric Determination of Formic Acid. N. W. Pirie (*Biochem. J.*, 1946, **40**, 100–102)—Pipette a 1-ml. sample, containing 0·1–1·0 mg. of formic acid, into a distillation unit, such as that described by Markham (*Biochem. J.*, 1942, **36**, 790), and add sufficient sulphuric acid to make the solution *N*. Steam-distil until 40 ml. of distillate have collected and titrate the distillate with 0·02 *N* sodium hydroxide, using methyl red as indicator. Add 0·1 ml. excess and then evaporate to 3–4 ml. on a hot plate. Transfer the solution quantitatively to a 10-ml. test-tube having a B14 ground-glass joint and evaporate to dryness in an oven at 100° C. Add 1 ml. of oxidising solution, containing 8% of mercuric chloride, 2% of hydrated sodium acetate and 2% of acetic acid. Insert an ungreased ground-glass stopper carrying a carefully greased stopcock and evacuate with a filter-pump for 1 min. Close the tube with the stopcock, seal with a drop of mercury and immerse the tube in boiling water for 20 min. Put 2 ml. of 0·5 *N* sodium hydroxide into the chamber of a Van Slyke apparatus, replace the mercury used to seal the stopcock by 2 drops of 70% v/v. sulphuric acid and connect the tube to the Van Slyke apparatus by means of a short piece of pressure tubing. Transfer the carbon dioxide to the Van Slyke apparatus and measure the amount by the usual procedure. One mg. of formic acid should give rise to 186 mm. pressure of carbon dioxide at 20° C. and 2 ml. The error is less than 1% with 1 mg. quantities and 5–10% with 0·1 mg. quantities of formic acid. F. A. R.

Stabilisation and Estimation of Lactic Acid in Blood Samples. C. Long (*Biochem. J.*, 1946, **40**, 27–33)—A simple procedure for the estimation of lactic acid was developed from previously published methods (Friedemann, Cotonio and Shaffer, *J. Biol. Chem.*, 1927, **73**, 335; Gordon and Quastel, *Biochem. J.*, 1939, **33**, 1332). The method previously described (Long, *Biochem. J.*, 1944, **38**, 447) for stabilising the pyruvic acid concentration of blood proved to be applicable to lactic acid also.

Fit a 100-ml. Monax boiling-tube with a rubber bung carrying a thistle funnel, the end of which almost touches the bottom of the tube, and a splash-head, made by cutting short a 20-ml. pipette. Fit a similar tube with a bung carrying a piece of glass tubing and an absorption tower made by filling a glass tube, of 35 cm. length × 10 mm. internal diam., to a depth of 20 mm. with glass beads of 3–5 mm. diam.; both tubes should almost touch the bottom of the boiling-tube. Connect the piece of glass tubing to the top of the splash-head by means of rubber tubing and connect the top of the absorption tower to a vacuum-pump.

Add 5 ml. of the blood sample to a tube containing 20 mg. of 75% "Cetavlon" (cetyltrimethylammonium bromide), 21 mg. of sodium fluoride and

168 mg. of citric acid monohydrate which has been neutralised to *p*H4 with 40% sodium hydroxide solution and then evaporated to dryness, as described by Long (*loc. cit.*).

Add the stabilised blood drop by drop to a mixture of 4 ml. of 25% trichloro-acetic acid soln. and 1 ml. of water in a 15-ml. centrifuge tube, shake thoroughly and centrifuge for 30 min. at 3500 r.p.m. Decant the supernatant layer from the protein precipitate and transfer 3 ml. to the first boiling tube of the apparatus described above. Put 4 ml. of 0·9% sodium metabisulphite solution into the second boiling-tube *via* the absorption tower. Heat the tube containing the deproteinised blood in a water-bath maintained at 60° C. and apply suction to the absorption tower so that air bubbles freely through the sample, but the bisulphite solution does not rise above the level of the glass beads. Add 10 ml. of a 30% solution of ceric sulphate in *N* sulphuric acid through the thistle funnel and continue the heating and aeration for another 30 min. Disconnect the apparatus and rinse the glass tubing and absorption tower with water into the boiling-tube. Add 2 drops of 1% starch solution and remove the excess metabisulphite by addition of 0·1 *N* iodine, followed by 0·01 *N* iodine until a faint blue colour is formed. Add 3–5 g. of finely powdered $Na_2HPO_4 \cdot 12H_2O$ and titrate to the same end-point with 0·01 *N* iodine. One ml. of 0·01 *N* iodine ≡ 0·45 mg. of lactic acid.

For routine work, it is convenient to carry out 5 estimations and one blank estimation simultaneously, these occupying about 2 hr. The recovery of lithium lactate at low concentrations was 98·5 to 101% of the theoretical, even in presence of similar amounts of pyruvic acid or glucose. In presence of a 200-fold excess of citric acid alone, the amount required to stabilise lactic acid in blood, the recovery was about 90% of the theoretical. but this reduction in yield is not so great when the citric acid is buffered and recoveries of lactic acid added to blood ranged from 94·5 to 101% of the theoretical whether citric acid was added or not.

F. A. R.

Rapid Microdetermination of Glycogen in Tissue Slices. W. J. van Wagtendonk, D. H. Simonsen and P. L. Hackett (*J. Biol. Chem.*, 1946, **163**, 301–306)—Put the tissue, weighing from 50 to 75 mg., into 2 ml. of 35% potassium hydroxide solution in a 6-in. Pyrex test tube equipped with an air-condenser. Immerse the tube in a boiling water bath and reflux for 2 hr. Filter the digest through a Whatman No. 41 filter paper into a calibrated colorimeter tube containing a crystal of potassium iodide. Wash the residue on the filter paper with 1 ml. of water and make up the filtrate to 10 ml. with 95% ethanol. Add one drop of a 1% solution of phenolphthalein in 50% ethanol, and neutralise with concentrated hydrochloric acid. Cool, add one drop of acid in excess and when the glycogen has coagulated (3 min.) centrifuge for 5 min. at 3000 r.p.m. Decant the supernatant liquid and dissolve the precipitated glycogen in 1 to 2 ml. of warm water. Dilute to 5 ml. and add exactly 0·05 ml. of a solution of 1 g. of iodine and 2 g. of potassium iodide in 20 ml. of water. Mix and immediately evaluate the colour, using a No. 54 filter. Subtract from the result the value of a blank reading given by a mixture of 5 ml. of water and 0·05 ml. of the iodine - potassium iodide solution and calculate the glycogen content from the colour given under the same treatment by a mixture of 1 ml. of standard glycogen solution (25 mg. of glycogen in 25 ml. of 35% potassium hydroxide solution) and 1 ml. of 35% potassium hydroxide solution. The recoveries of glycogen added to liver slices ranged from 91 to 112% of the theoretical, with an average of 101·6%.

F. A. R.

Determination of Inorganic Phosphate in the Presence of Labile Phosphate Esters. O. H. Lowry and J. A. Lopez (*J. Biol. Chem.*, 1946, **162**, 421–428)—Phosphocreatine, acetylphosphate and ribose-1-phosphate are unstable and rapidly split by the reagents ordinarily used for the estimation of inorganic phosphate, so that with some tissue extracts high results are obtained. The present procedure is a modification of the method of Fiske and Subbarow, the *p*H being changed from 0·65 to 4·0, the molybdate concentration being reduced from 0·25 to 0·1% and ascorbic acid being substituted for the mixture of bisulphite and 1 : 4-aminonaphtholsulphonic acid. Under these conditions the phosphoric esters are more stable, being hydrolysed to the extent of not more than 5%.

Deproteinise the sample with ice-cold 5% trichloroacetic acid or 3% perchloric acid solution or, when very labile esters are present, with saturated ammonium sulphate solution which is 0·1 *N* in acetic acid and 0·025 *N* in sodium acetate (*p*H 4). With the acid precipitants, adjust to *p*H 4·0–4·2 by adding 4 vols. of 0·1 *N* sodium acetate. Dilute the extracts with acetate buffer of *p*H 4 (0·1 *N* acetic acid, 0·025 *N* sodium acetate) until the inorganic phosphorus concentration is 0·05–0·3 mg. of P per 100 ml. Dilute the ammonium sulphate extracts at least 5-fold. To the extract add 0·1 vol. of 1% ascorbic acid solution and 0·1 vol. of 1% ammonium molybdate solution in 0·05 *N* sulphuric acid. Evaluate the colour 5 and 10 min. after addition of the molybdate solution at a wave-length of 700 mμ. At the same time evaluate the colours of a blank and of a standard solution containing 0·15 mg. of phosphorus per 100 ml. If the readings at 5 and 10 min. are different, extrapolate to zero time.

With certain tissue extracts, the reaction is delayed and, in such instances, a known amount of inorganic phosphate should be added to a duplicate portion of the extract and the value of the unknown calculated from the difference between the values obtained from the unknown solution and the unknown solution with added phosphate.

F. A. R.

Determination of Nitrogen and of Protein in Pooled Samples of Human Plasma. R. P. Cook (*Biochem. J.*, 1946, **40**, 41–45)—Four samples of human plasma were obtained by pooling the blood from 67 normal persons. The acetone-precipitated, heat-coagulated proteins and "globulins" were prepared from these samples and their nitrogen contents estimated by the micro-Dumas and micro-Kjeldahl methods. After correcting for moisture and ash, the values obtained were 13·3 to 15·9, mean 15·2, for the total plasma proteins and 13·5 to 15·1, mean 14·3% for the "globulins." The distribution of nitrogen in various plasma fractions was determined and the total proteins were estimated by the gravimetric method. The results were compared with the values calculated from the nitrogen contents by the use of various conversion factors. Fairly good correlation was obtained when the total plasma-nitrogen was multiplied by 6·25 or the protein-nitrogen by 6·6, but low results were obtained when the protein-nitrogen was multiplied by 6·25. It is suggested that for accurate work results should be expressed either as protein-

nitrogen or as nitrogen precipitated by some specific reagent; this would avoid the use of a particular conversion factor. F. A. R.

Studies of the Van den Bergh Reaction. C. H. Gray and J. Whidbourne (*Biochem. J.*, 1946, **40**, 81–88)—Van den Bergh and Muller (*Biochem. Z.*, 1916, **77**, 90) showed that a red pigment was formed when diazotized sulphanilic acid was added to the sera of patients with obstructive jaundice, whereas with normal sera and sera from patients with other forms of jaundice the reaction only took place when ethanol was added. The reason for this difference in behaviour has hitherto been obscure. Subsequent workers observed that the "direct" reaction, that is, the reaction in absence of alcohol, sometimes proceeded rapidly and sometimes slowly, and methods of estimating the supposedly different types of bilirubin were described by Malloy and Evelyn (*J. Biol. Chem.*, 1937, **119**, 481) and by Rappaport and Eichhorn (*Lancet*, 1943, **1**, 62). The method described by the latter has now been slightly modified as follows. Put 1 ml. of serum into a test-tube and add 7 ml. of buffer solution (for the "indirect" reaction: 15 g. of citric acid, 5 g. of sodium citrate, 5 g. of caffeine and 24 g. of urea in 100 ml.; for the "direct" reaction: 0·9 g. of KH_2PO_4, 18 ml. of $N/15$ phosphoric acid and 24 g. of urea in 100 ml.) and 1 ml. of the diazo reagent (mix 10 ml. of a solution of sulphanilic acid (1 g.) in 15 ml. of fuming hydrochloric acid, diluted to 1 litre with water, with 2 ml. of a 0·5% solution of sodium nitrite). Prepare a blank from 1 ml. of serum, 7 ml. of buffer and 1 ml. of 0·15 N hydrochloric acid and, at intervals up to 30 min. after addition of the diazo reagent with the sera of patients suffering from obstructive jaundice or acute hepatitis or up to 6 hr. with the sera of patients with haemolytic jaundice, evaluate the colour of the test solution relative to the blank using an Evelyn photoelectric colorimeter with a Chance No. 5 green filter (maximum transmission 520–540 mμ.

This modified method gave results in good agreement with those obtained by the method of Malloy and Evelyn, whereas other published methods gave lower results owing to adsorption of the pigment on the precipitated proteins. A study of the rate of formation of the red pigment showed that this was dependent on the concentration of bilirubin; solutions containing more than 1·6 mg. of bilirubin per 100 ml. did not obey Beer's law, whilst with solutions containing 4 mg. per 100 ml. the absorption density increased relatively little with concentration. Whether a prompt or delayed Van den Bergh reaction is obtained is solely dependent on the quantity of bilirubin present and this is due to the failure of bilirubin solutions to obey Beer's law. Thus a serum with a bilirubin content of 18 mg. per 100 ml. would give a marked colour in the first 30 sec., and this would appear to increase very little thereafter because of the gross divergence from Beer's law at this concentration; a serum containing 3 mg. per 100 ml., on the other hand, would give a colour increasing steadily in intensity over a prolonged period, because at this concentration Beer's law is obeyed. The type fo Van den Bergh reaction observed in obstructive jaundice and in acute hepatitis thereford depends solely on the concentration of bilirubin in the serum, and the test has no other significance than that originally claimed by Van den Bergh, namely to distinguish between obstructive and other types of jaundice. F. A. R.

Determination of Plasma Iodine. A. Taurog and I. L. Chaikoff (*J. Biol. Chem.*, 1946, **163**, 313–322)—A modification of Chaney's method (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 179) was found to give satisfactory results for the determination of iodine in plasma (3 ml.) and small amounts of thyroid tissue. Digest 3 ml. of plasma with a mixture of 25 ml. of 70% iodine-free sulphuric acid and 2 ml. of 80% chromic acid in a two-necked 300 ml. flask with ground-glass joints on a hot plate, until the flask is filled with fumes. Cool and add 15 ml. of double-distilled water and repeat the digestion. Again cool and add 25 ml. of double-distilled water and connect the flask to a distillation apparatus. Put 3 ml. of 50% phosphorous acid into a dropping funnel inserted into the side-arm of the flask and, as soon as liquid begins to distil, put 1·2 ml. of 1% sodium hydroxide solution into the trap by way of the condenser. Now add the phosphorous acid slowly from the dropping funnel and continue the distillation. After 7 min. transfer the contents of the trap to a 15-ml. centrifuge tube and rinse the still with a minimum of water. To 4-ml. portions of the distillate add 1 ml. of water and 0·4 ml. of arsenious acid reagent (dissolve 3·71 g. of arsenious oxide in water containing 2·5 g. of sodium hydroxide, dilute to about 300 ml. and neutralise with concentrated sulphuric acid; add a further 21 ml. of acid and make up the volume to 500 ml.). To similar portions of distillates from blank samples add 1-ml. quantities of standard iodine solutions containing amounts of iodine ranging from 0 to 0·1 μg., followed by 0·4 ml. of arsenious acid reagent. Immerse all the tubes in a bath maintained at 30° ± 0·1° C., add to each tube at intervals of exactly 30 sec. 0·3 ml. of 0·1 N ceric ammonium sulphate and evaluate the colours exactly 10 and 20 min. after addition of the ceric sulphate solution; the two values should be almost identical. The accuracy of the results was checked by measuring the recovery of radioactive iodine, which was of the order of 90% of the theoretical for amounts less than 1 μg. of iodine, and 100% for amounts greater than 5 μg. F. A. R.

Determination of Thyroxine in the Thyroid Gland of the Rat. A. Taurog and I. L. Chaikoff (*J. Biol. Chem.*, 1946, **163**, 323–328)—Hydrolyse the glands with 2 N sodium hydroxide by heating on a steam bath for 15 hr. Neutralise to *p*H 3·5–4·0 by addition of 6 N sulphuric acid and add 2 ml. of butanol. Shake thoroughly and separate the two phases by centrifuging. Transfer the butanol layer to a second centrifuge tube and extract the aqueous layer with 1·5 ml. of butanol. Shake the combined butanol extracts with an equal volume of 4 N sodium hydroxide containing 5% of sodium carbonate. Centrifuge and transfer the butanol layer to another centrifuge tube, re-extract with 5 ml. of the sodium hydroxide - sodium carbonate solution and transfer the butanol layer, which now contains only thyroxine, to a two-necked digestion flask for the determination of the iodine content. The aqueous phases are combined and used to estimate the non-thyroxine iodine content. Remove the solvent completely from the butanol extract by distillation under reduced pressure in a stream of carbon dioxide. Add about 100 mg. of iodine-free wheat (to supply organic matter for the digestion) and then digest and distil as described in the preceding paper. Estimate the non-thyroxine iodine in a similar manner. The recoveries of thyroxine and di-iodotyrosine added to rat muscle hydrolysate averaged 87% and 90% respectively. F. A. R.

Polarographic Determination of Thyroxine and 3 : 5-Di-iodotyrosine. G. K. Simpson and D. Traill (*Biochem. J.*, 1946, **40**, 116–118)—3 : 5-Di-iodotyrosine gives a polarogram consisting of two steps, the half wave potentials varying with the base electrolyte used. In 0·5 *N* sodium carbonate solution containing 1% of tetramethyl ammonium iodide, the steps occurred at half-wave potentials of −1·50 and −1·70 volts *versus* the saturated calomel electrode. The height of the two steps together was approximately proportional to the concentration of di-iodotyrosine. It was preferable to remove oxygen from the solution by bubbling nitrogen through for 10 min. Thyroxine also gives a polarogram, and in a mixture (2 : 3) of ethanol and 0·5 *N* sodium carbonate solution containing 1% of tetramethyl ammonium iodide three steps were formed with half-wave potentials of −1·20, −1·42 and −1·70 volts respectively. The overall height of this composite wave was proportional to the concentration of thyroxine. With concentrations ranging from 3×10^{-5} to $1{\cdot}25 \times 10^{-4}$ *M*, removal of oxygen was necessary, but not with more concentrated solutions. It proved possible to determine thyroxine in presence of di-iodotyrosine, within certain concentration limits, by means of the step at −1·20 volts, using the ethanolic sodium carbonate solution as base solution. F. A. R.

Microbiological Method for the Estimation of Tyrosine. M. Gunness, I. M. Dwyer and J. L. Stokes (*J. Biol. Chem.*, 1946, **163**, 159–168)—Tyrosine can be estimated in proteins and foods by means of *Lactobacillus delbrückii* L D 5, using the medium and technique previously described (Stokes and Gunness, *J. Biol. Chem.*, 1945, **157**, 651; ANALYST, 1945, **70**, 265). Samples weighing 0·5 or 1·0 g. are hydrolysed with 10 ml. of 5 *N* sodium hydroxide by autoclaving at 15 lbs. pressure for 10 hr. The precipitate that forms on neutralisation of the hydrolysate is removed by centrifuging; it is washed twice with 20-ml. quantities of water to recover the tyrosine adhering to it. *l*(-)-Tyrosine is used as the standard. The method is claimed to be quite specific for tyrosine. Duplicate assays gave similar results, and the recoveries of tyrosine added to proteins prior to hydrolysis were within 10% of the theoretical. The values so obtained, however, were consistently 10 to 15% lower than those obtained by colorimetric methods, but in view of the controversy concerning the accuracy of the various colorimetric procedures it is impossible to decide whether these or the microbiological results are the more accurate. F. A. R.

Microbiological Determination of Certain Free Amino Acids in Human and Dog Plasma. S. W. Hier and O. Bergeim (*J. Biol. Chem.*, 1946, **163**, 129–135)—Arginine, histidine, *iso*leucine, leucine, lysine, phenylalanine, threonine, tyrosine and valine were estimated microbiologically in tungstic acid filtrates of plasma, using 0·1 ml. of the basal medium previously described (Hier *et al.*, *J. Biol. Chem.*, 1945, **161**, 705, 717; Abst., ANALYST, 1946, **71**, 292) in each tube. Standard and unknown solutions were added in the usual way and the volumes adjusted to 2·0 ml. The tubes were sterilised, inoculated and incubated as previously described. Tryptophan was estimated by the method of Dunn *et al.* (*J. Biol. Chem.*, 1945, **157**, 387). Recoveries were satisfactory for all the amino acids studied and duplicate assays made on the same tungstic acid filtrate showed that the results were reproducible to within 10% of the mean. F. A. R.

Some Culture Studies on *Lactobacillus arabinosus* and *L. helveticus*. M. C. Nymon and W. A. Gortner (*J. Biol. Chem.*, 1946, **163**, 277–282).—Several methods of culturing micro-organisms are used in microbiological assays, and the differences between them may well account for the discrepancies sometimes observed between the results from individual laboratories. It was found that cultures transferred every 3 or 4 weeks in yeast-extract - glucose - agar with incubation at 37° C. showed a gradual decline in the linearity of the response to added nicotinic acid. For example, a fresh culture of *L. arabinosus* gave a linear response up to 0·3 μg. per tube and this fell in 4 months to about 0·2 μg. and in 10 months to 0·15 μg. The culture was rejuvenated by changing the incubation temperature to 30° C. and transferring weekly from liver-tryptone broth to liver-tryptone agar and thence through broth again to the basal medium; after 5 months the response had increased to 0·5 μg. In the same way cultures of *L. helveticus* were rejuvenated by transfer through broth to agar but, in this instance, incubation was continued at 37° C. F. A. R.

Organic

A Sensitive and Selective Test for Gallotannin (Tannic Acid) and other Tannins. F. Feigl and H. E. Feigl (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 62–63)—A violet flocculent ppt. is slowly produced when an ammoniacal soln. of tannic acid is warmed with a soln. of ferrous α,α′-dipyridyl sulphate. The ppt. forms quickly and completely if the ammoniacal soln. or suspension is treated with acetic acid and warmed. A soln. of ferrous α,α′-phenanthroline sulphate gives a similar reaction with tannic acid. The reaction is probably due to formation of an adsorption complex by combination of the oxidation products of tannin with ferrous α,α′-dipyridyl hydroxide, since the ppt. is formed slowly at the surface of the liquid and does not appear if air is excluded or if much alkali sulphite is present. The probability that auto-oxidation of tannic acid is responsible for the reaction with $Fe(\alpha,\alpha'\text{-dip})_3^{\cdot\cdot}$ ions suggested that other auto-oxidisable phenolic compounds would behave similarly. Pyrogallol reacted very strongly, gallic acid (cold sat. soln., 1%) less strongly, hydroquinone weakly and phloroglucinol, resorcinol and pyrocatechol not at all. Vegetable tannins, being phenolic in structure and oxidisable in alkaline solns., were also found to react. Synthetic tanning agents, however, most of which are condensation products of formaldehyde and sulphonated phenols, do not respond to the test.

Dissolve 0·25 g. of α,α′-dipyridyl and 0·146 g. of ferrous sulphate heptahydrate in 50 ml. of water. This soln. is stable in closed containers. Before applying the test boil a portion of the reagent made ammoniacal and remove the resulting slight ppt. of hydrous ferric oxide; this purification is essential when testing for small amounts of tannins. To 1 ml. of the test soln. in a test-tube add 1 ml. of the ammoniacal reagent and heat to boiling point. Add acetic acid until the odour of ammonia disappears and again heat to boiling point. A flocculent violet ppt. forms in presence of tannins. With minute amounts the ppt. has a brownish tinge. Turbidities produced by very small amounts of ppt. can be easily discerned in the red soln. if the tube is held toward an intense source of light with a sheet of frosted glass interposed. Identification limit, 8 μg. of tannic acid; limiting concn., 1 in 125,000. The test is three times as sensitive as

the non-specific phenolic reaction of tannic acid with ferric chloride and sodium acetate.

The behaviour of all samples towards ammonia and acetic acid should be determined before adding the reagent. Remove any ppt. that appears when acetic acid is added to the warm ammoniacal soln. and apply the test-tube procedure to the filtrate after again making it ammoniacal. The following natural tannins answered to the test: gallotannin, tara powder, gambier, myrobalan, quebracho and extracts of wattle, mangle, sumach and fustic.

To apply the test to leather boil about 0·5 g. of leather, cut into very small pieces, in ammonia water for 2 min., filter and use the filtrate for the test. Preliminary tests with ammonia and acetic acid are essential with leather coloured with coal tar dyes, any ppt. produced being removed and the test applied to the filtrate. With 19 specimens of leather out of 20 the test revealed the nature (vegetable or synthetic) of the tanning agent. The exception was a leather tanned with a sulphited quebracho extract. For detection of tannin in beverages 3 ml. of the samples were used. Positive reactions were obtained with varieties of red and white wines and with aq. extracts of tea, maté and guarana. Cinzano of Italian origin, light and dark beer and aq. extracts of raw and roasted coffee did not respond to the test. A. O. J.

Direct Volumetric Determination of the Organic Sulphonate Content of Synthetic Detergents. T. U. Marron and J. Schifferli (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 49–50)—Under standardised conditions *p*-toluidine hydrochloride reacts with alkyl or alkyl-aryl sulphonates, giving amine-sulphonate salts which can be subjected to rapid and relatively precise quantitative analysis. A neutral detergent mixture containing the sodium salts of alkylbenzene sulphonic acids as the active organic ingredients may be made to react in an aqueous medium with an amine salt of a strong inorganic acid to produce the sulphonic acid salt of the amine and the sodium salt of the inorganic acid. Removal of the sulphonate-amine complex into a phase immiscible with water will displace the equilibrium to promote completion of the reaction. The organic sulphonate can then be determined by suitable treatment of the solvent extract of the reaction mixture. Direct titration with standard alkali in presence of a suitable indicator was found rapid and convenient, the weakly basic amine causing no interference. After numerous trials, *p*-toluidine hydrochloride was selected as the amine salt and carbon tetrachloride as the solvent.

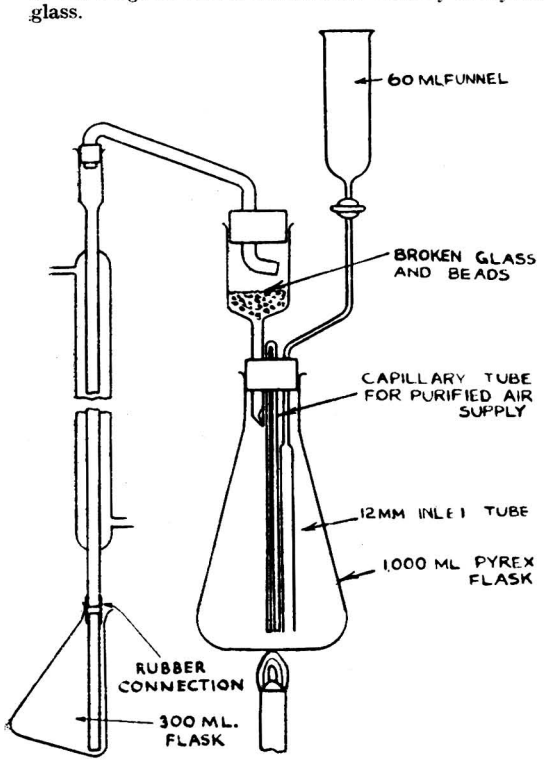
The procedure for solid detergents containing 30 to 60% of sodium alkylbenzene sulphonate, the remainder being neutral inorganic salts, is as follows. Place the weighed sample containing 3 to 4 g. of the organic material in a 250-ml. separating funnel (Corning No. 6340) with a stem cut to 1·25 cm., add 50 ml. of carbon tetrachloride and 100 ml. of an aq. soln. containing 3·40 g. of *p*-toluidine hydrochloride and shake the stoppered funnel mechanically at room temp. for 5 to 10 min. or until all the solid phase has disappeared. When separation is complete, remove the carbon tetrachloride layer and re-extract the aqueous phase with 25 ml. of carbon tetrachloride, a brief shaking being now sufficient. Combine the carbon tetrachloride extracts in 100 ml. of 25% ethanol previously neutralised with 0·1 *N* sodium hydroxide to the faint purple end-point of *m*-cresol purple. Titrate the mixture in a glass-stoppered 500-ml. Erlenmeyer flask with 0·1 *N* sodium hydroxide until the emulsion obtained by vigorous intermittent shaking during titration tends to remain of a lavender colour. Calculate the organic content of the sample from the organic equivalent of the standard sodium hydroxide soln. found by applying the method to a suitable reference sample of known organic content. The organic content of the reference sample may be found by the "difference" method (Berkowitz and Bernstein, *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 239; Hart, *Id.*, 1939, **11**, 33), *i.e.*, by determining moisture, sodium sulphate, sodium chloride and ether-soluble matter and deducting their sum from 100%. To avoid limiting the application of the method, calculations on a stoichiometric basis are avoided in the general procedure. The weight of the average of the alkyl groups (hydrocarbon species obtained by fractional distillation of petroleum) is not always known or is subject to variation with the manufacturing process so that some products would not be accurately analysed if calculated factors were employed. Expts. showed that with constant initial concn. of the amine and varying amounts of sample the alkali equivalent per g. remained constant when the molecular ratio of amine to sulphonate exceeded 2·5. With constant sample wt. and varying initial concn. of amine it appeared that the alkali equivalent per g. is regularly affected by the initial concn. of the amine salt. Physical limitations fix the range of reagent concn. Concn. of amine salt above 8% causes phase separation difficulties; concn. much below 2·5 times the molar concn. of the sulphonate and below about 3·4 g. per 100 ml. of reagent causes emulsification by unattacked detergent. To avoid occult errors, reagent concns. at which there is an apparent stoichiometric relationship have been chosen. These concns. were determined by application of the method to mixtures of commercial detergent with pure sodium dodecylbenzene sulphonate. When the stoichiometric point is found by use of a suitable pure compound of known molecular wt., the average molecular wt. of the mixture of homologues in a commercial detergent can be found. For routine purposes it is not necessary to determine the optimum amine salt concn. If a reference sample is used with the unknown sample under identical conditions the concn. may be between 3 and 8%. Perchloroethylene may be used instead of carbon tetrachloride, but other solvents should be used only after adequate study of the effects involved. With slight variations in technique the method has been applied successfully to liquid detergent and wetting preparations containing non-reactive diluents. Replacement of benzene by toluene, phenol or naphthalene as the source of aryl group in the detergent introduced no complexity of manipulation. Sodium benzenesulphonate alone or mixed with detergent samples remained undetected under the conditions of the method. A. O. J.

Inorganic

Determination of Nitrogen in Refractory Metal Carbides and their Compositions. J. C. Redmond, L. Gerst and W. O. Touhey (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 24–26)—The carbide is finely ground and decomposed by gently fuming with sulphuric acid, potassium bisulphate and selenium oxychloride. The ammonia formed is liberated with caustic soda and absorbed in standard acid. The carbide may take eight days to decompose by this method, but there is no loss of nitrogen. Other methods of dissolving which were found unsatisfactory were:—(1) Fuming with

sulphuric acid and sodium sulphate, (2) fuming with sulphuric acid and sodium thiosulphate, (3) fusion with potassium bisulphate and solution of the melt in hydrochloric acid, (4) fuming with sulphuric acid, potassium bisulphate and hydrofluoric acid (very destructive to glassware), (5) heating in a stream of hydrogen with copper oxide and lead oxide catalysts and absorption of ammonia in sulphuric acid.

Apparatus—The distillation apparatus is shown in the diagram and is constructed entirely of Pyrex glass.



Reagents—Ammonia-free water prepared over Devarda's alloy. Selenium oxychloride soln., 1·2% in conc. sulphuric acid, sp. gr. 1·84. Tartaric acid soln., 50% in ammonia-free water. Sodium hydroxide soln., 60%: dissolve 600 g. in 1000 ml. of distilled water and digest overnight on the water bath with zinc-copper couple. Sodium hydroxide, 0·01 *N*. Sulphuric acid, 0·01 *N*. Methyl red soln., 0·1% in 95% ethyl alcohol.

Method—The material must be ground to less than 200 mesh, preferably in a motor-driven pestle and mortar with a tungsten carbide insert and tip. Weigh 1 g. of sample, transfer it to a dry 250-ml. Erlenmeyer flask and add 15 g. of potassium bisulphate, 30 ml. of sulphuric acid and 1 ml. of selenium oxychloride soln. Place on a hot plate and keep the solution just fuming, with a small beaker inverted over the neck of the flask, until solution is complete. Cool and add carefully 50 ml. of tartaric acid soln. and then 75 ml. of ammonia-free water. Boil gently for 5 min. until all soluble salts are dissolved.

Rinse the entire distillation apparatus with distilled water, place 150 ml. of 60% sodium hydroxide soln. and 400 ml. of water in the flask and distil rapidly while passing a gentle stream of carbon-dioxide-free air to prevent bumping, until 150–200 ml. of distillate have come over. Reject the distillate, rinse the outlet tube with ammonia-free water and check that the system is free from ammonia by distilling over 50 ml. more into a flask rinsed with ammonia-free water and containing methyl red soln. (this should remain pink). Remove the heat and put in position a rinsed collecting flask containing 5 ml. of 0·01 *N* sulphuric acid and 4 drops of methyl red soln. Increase the air flow slightly and transfer the sample soln. to the tap funnel. Run the soln. carefully into the distillation flask, rinse the sample flask into the funnel 3 times with ammonia-free water and run these rinsings and a total of 500 ml. of ammonia-free water into the distillation flask. Reduce the air stream and proceed with the distillation until 200 ml. have been collected. Remove the receiving flask from the outlet tube, rinsing this down with ammonia-free water. Titrate with 0·01 *N* sodium hydroxide until the pink colour just disappears, then add 2 more drops of methyl red soln. and titrate to a full yellow colour. Run a blank with the same quantities of reagents, omitting only the fuming. The method gives very consistent results and nitrogen added as ammonium sulphate was recovered quantitatively, but as carbide samples of standard nitrogen content are not available this type of check was not possible. C. F. P.

Agricultural

Determination of Inorganic Phosphorus in Plant Materials. W. A. Pons jr. and J. D. Guthrie (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 184–186)—The sample is treated with trichloroacetic acid and an absorptiometric determination of the extracted phosphorus is made. The method may be used with samples rich in protein. *Reagents*—Trichloroacetic acid, 0·75 *N*—Make 123 g. of the pure acid up to one litre with distilled water; prepare when required, or store in a refrigerator. Molybdate reagent—Dissolve 50 g. of ammonium molybdate in 400 ml. of 10 *N* sulphuric acid and 500 ml. of water, dilute to 1 litre and store in a paraffin-lined bottle. Stannous chloride solns.—Dissolve 10 g. of stannous chloride hexahydrate in 25 ml. of conc. hydrochloric acid, dilute 1 ml. of this stock solution to 200 ml. with approx. *N* sulphuric acid shortly before use. Standard phosphate soln.—Dissolve 4·3929 g. of purified dry monobasic potassium phosphate in 300 ml. of water and 200 ml. of approx. *N* sulphuric acid. Add a few drops of 0·1 *N* potassium permanganate as preservative and dilute to 1 litre. One ml. ≡ 1·0 mg. of phosphorus. The soln. is a stable one, from which more dilute solns. are made as required.

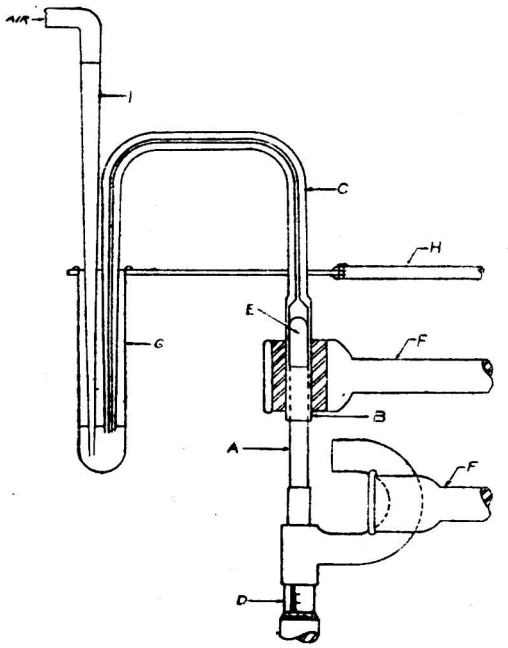
Method—Weigh 1 g. of the ground sample (60 mesh or finer) into a 125 ml. glass stoppered conical flask. Add 50·0 ml. of 0·75 *N* trichloroacetic acid and shake mechanically for 1 hr. Filter through an ashless filter paper, discarding the first part of the filtrate. Transfer aliquot portions containing from 0·005 to 0·045 mg. of inorganic phosphorus (usually 2 ml.) to 125 ml. separating funnels with a mark at 20 ml. Add 5 ml. of molybdate reagent, dilute to 20 ml. with distilled water, add 10 ml. of *iso*butyl alcohol (boiling range 106° to 110° C.) and shake for 2 min. Discard the aqueous layer and wash the alcohol soln. once with 10 ml. of approx. *N* sulphuric acid. Add 15 ml. of the diluted stannous chloride soln., shake for 1 min. and discard the aqueous layer. Transfer the blue *iso*butyl alcohol layer to a 50-ml. graduated flask, rinsing the funnel and diluting to the mark with 95% ethyl alcohol.

Determine the transmission at about 730 mμ in an absorptiometer or spectrophotometer at any time between 40 min. and 19 hr. after colour development. Prepare a calibration curve from standard phosphate solutions (0–0·045 mg. of P) by similar procedure.

Spectrophotometric curves of the colour system are given. The results of analysis of such materials as cottonseed meal, peanut meal, wheat, straw, crude phytins and dialysed proteins are listed. Some samples yielded colouring matter to the *iso*-butyl alcohol, but the extracted colour had no absorption at 730 mμ and did not interfere. The precision is indicated by 16 determinations on a sample of solvent-extracted peanut meal over a period of 6 months, which gave an average of 0·0717% of inorg. phosphorus with a standard deviation of ± 0·0017% and 25 determinations on another sample which gave an average of 0·0696% of inorg. phosphorus with a S.D. of ± 0·0012%. Glucose-1-phosphate is rapidly hydrolysed by 0·75 *N* trichloroacetic acid, fructose diphosphate is hydrolysed very slowly and sodium β-glycerophosphate, adenylic acid and phytin show no hydrolysis after 24 hr. in contact with the acid. To establish the absence of easily hydrolysed organic phosphorus compounds from the sample the result of trichloroacetic extraction for 1 hr. was compared with that of extraction for 24 hr. The second figure was considerably larger than the first when glucose-1-phosphate was present, and appreciably larger when fructose diphosphate was present. Hydrolysis of glucose-1-phosphate in the solvent-extraction part of the process is very slight when the total time taken for this step is about 5 min. It is suggested that this part of the method may consequently be of value in following enzymatic synthesis by measuring changes in inorg. phosphorus. L. A. D.

Microchemical

Microdetermination of the Saponification Number of Fats and Oils. Decigram, Centigram and Milligram Procedures. K. Marcali and W. Rieman III (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 144–145)—The literature contains no satisfactory method for the micro-determination of the saponification number of fats and oils. The double indicator method of Rieman (*Id.*, 1943, **15**, 325) is more easily adaptable to micro procedures than the standard method (*Methods of Analysis of the A.O.A.C.*, 1940, 443). *Apparatus*—For the decigram procedure a 10-ml. burette with 0·05-ml. graduations and with a tip constricted to deliver 0·01-ml. drops is suitable. The burette for the centigram procedure is shown in the figure. A ground-glass rod *A* of diam. 5·2 mm., serving as piston, fits snugly inside a ground-glass tube *B*, the upper end of which is sealed to a capillary tube *C* bent twice at right angles and constricted at the tip. A 25-mm. micrometer *D*, the head of which has been removed, serves to measure accurately the position of the piston *A*. Above the piston is a layer of mercury, *E*, forming an effective seal and above the mercury is 0·5 *N* hydrochloric acid. Metal clamps, *F*, hold the micrometer and glass tube rigidly in position. The titration vessel *G*, is a 13 × 100 mm. Pyrex test-tube. A movable arm, *H*, taken from a Rehberg burette, supports the titration vessel. A finely drawn capillary tube, *I*, admits a stream of air free from carbon dioxide into the titration vessel. The burette for the milligram procedure is similar, but a smaller test-tube (10 × 75 mm.) and ground-glass rod and tube (3·1 mm.) are used. To fill these burettes wipe the tip with Kleenex tissue and immerse it in the 0·5 *N* hydrochloric acid. Turn the micrometer spindle down and slowly push down the piston until it touches the spindle. A fluorescent illuminator is used for all titrations.



Reagents—Prepare alcoholic potassium hydroxide as previously described (Rieman, *loc. cit.*). Prepare alcoholic 0·001 *M* bromophenol blue by dissolving 65 mg. of the indicator in 1 ml. of 0·1 *N* sodium hydroxide and adding a mixture of 60 ml. of purified 95% alcohol and 40 ml. of benzene. The alcohol and benzene rinsing mixture contains 60 ml. of benzene and 40 ml. of 95% alcohol. Standardise the hydrochloric acid (approx. 0·5 *N*) with pure sodium tetraborate decahydrate, using for the centigram procedure 400 to 600 mg. of a carefully prepared 0·5 wt.-normal soln. of borax, adding 1 drop of 0·001 *M* methyl red and titrating to the first pink colour, and for the milligram procedure about 10 mg. of borax accurately weighed on a microbalance, adding 4 drops of water and 1 drop of 0·00008 *M* methyl red and titrating. Express the results as mg.-equivalents of acid per mm. of the micrometer scale.

Decigram procedure—To about 500 mg. of the sample in a 50-ml. Pyrex Erlenmeyer flask add about 5 ml. of the alcoholic potassium hydroxide soln. and immediately connect a reflux condenser having its upper end protected by an Ascarite tube. Boil gently for 30 min., add 2 drops of 1% alcoholic phenolphthalein soln. and titrate with 0·5 *N* hydrochloric acid to the disappearance of the pink colour. Add 3 drops (0·18 ml.) of aq. 0·001 *M* bromophenol blue and 1 ml. of benzene and continue the titration to a green colour that does not become blue on further agitation.

Centigram procedure—Weigh to within 0·2 mg. about 50 mg. of sample into a tared Pyrex test-tube, add about 21 drops (0·5 ml.) of alcoholic potassium hydroxide soln. and immediately attach an Ascarite tube. Support the tube vertically so that the

bottom rests on the flat surface of a micro-drying block kept at 145° ± 5° C. The test-tube serves both as reaction vessel and reflux condenser, and saponification is complete in 30 min. Then add 1 drop (0·03 ml.) of 0·2% alcoholic phenolphthalein soln., insert the tip of the micro burette (previously wiped with Kleenex tissue) and the tip of the air delivery tube and support the tube in the apparatus (Fig.). Adjust the air stream to about 80 bubbles per min., add the 0·5 *N* hydrochloric acid until the colour just disappears and read the micrometer. Without removing either capillary tube add 1 drop (0·06 ml.) of aq. 0·001 *M* bromophenol blue and 7 drops (0·12 ml.) of benzene, reduce the air stream to 50 bubbles per min. and continue the titration until the soln. has a milky appearance; then add 7 drops of benzene, increase the air stream to 100 bubbles per min. and continue the titration to a green end point. The amount of acid used between the two end points is equivalent to the potassium hydroxide required for saponification.

Milligram procedure—Weigh a platinum boat (4 × 3 × 2 mm. made from foil 0·06 mm. thick) on a microchemical balance and place it on a clean surface. Dip the end of a finely drawn-out glass rod into the sample, let most of the oil drain off the rod and touch the centre of the boat with the tip of the rod and re-weigh the boat. The sample should weigh from 3 to 8 mg. With platinum tipped forceps place the boat just inside a horizontally-held 10 × 75 mm. Pyrex test tube and tilt the tube so that the boat slides to the bottom. If any oil sticks to the side of the tube the expt. must be started anew. Let 2 drops of the alcoholic potassium hydroxide fall from the siphon of the storage bottle to the bottom of the test tube, immediately connect an Ascarite tube and support the test tube vertically so that its bottom rests on the flat surface of a micro drying block heated to 130° ± 5°. After 30 min. remove the Ascarite tube, add 2 drops (0·06 ml.) of 0·02% alcoholic phenolphthalein, insert the wiped tip of the micro burette and the air delivery tube and support the test tube in the titration apparatus taking care that the boat does not hinder delivery of acid from the burette. Adjust the air stream to 60 bubbles per min., note the micrometer reading *a* and titrate to the disappearance of the pink colour. Rinse down the inside of the test tube with 0·1 to 0·2 ml. of alcohol-benzene mixture by means of a measuring pipette, continue the titration, if necessary, and note the reading *b* of the micrometer. Add 2 drops (0·06 ml.) of the alcoholic bromophenol blue soln., continue the titration, rinsing the sides of the tube again as the end point is approached and note the micrometer reading *c* when the green end point is reached. Contamination of the alcoholic potassium hydroxide with atmospheric carbon dioxide cannot be avoided in this procedure although it is not encountered in the other procedures and therefore a blank determination is necessary. Titrate 2 drops of the alcoholic potassium hydroxide in the test tube as described noting the initial micrometer reading *x*, the reading at the phenolphthalein end point, *y*, and the reading at the bromophenol blue end point *z*. Calculate the saponification value by means of the formula

$$[(b - c) - B\,(a - c)]\ 56{\cdot}11\ F/W$$

where B is $(y-z)/(x-y)$, F is the concentration of the hydrochloric acid in mg.-equivalents per mm. and W is the wt. of the sample in grams.

In determinations of the saponification numbers of 11 different oils and fats the mean differences, irrespective of sign, from results by the standard method were ± 0·2, ± 0·3 and ± 0·6 for the decigram, centigram and milligram methods respectively. None of the procedures is applicable to acetylated oils. A. O. J.

Physical Methods, Apparatus, etc.

Spectrophotometric Determination of Traces of Nickel. O. R. Alexander, E. M. Godar and N. J. Linde (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 206–208)—Quantities of nickel ranging from 5 to 80 μg. may be determined, *e.g.*, in food products, biological materials and steels with an accuracy of ± 5%. The nickel is separated by treating a sulphate soln. with ammonium citrate and ammonia, adding dimethylglyoxime soln., extracting with chloroform and washing the extract with ammonia soln. The nickel is removed from the chloroform soln. by extraction with dilute hydrochloric acid and this soln. is then treated with ammonium citrate and ammonia. Sodium diethyldithiocarbamate soln. is added and the coloured nickel complex is extracted with *iso*-amyl alcohol. The evaluation of the nickel content is by spectrophotometric measurement of the transmission at a wavelength of 385 mμ. The coloured soln. has its absorption maximum very near the edge of the visible spectrum and is not considered suitable for comparisons by eye. For full details of the method and the preparation and purification of the reagents the original paper should be consulted. L.A.D.

Compression Resistance of Paperboard (Ring Crush Test). T.A.P.P.I. Tentative Standard T472 m-46. Anon. (*Paper Trade J.*, 1946, **122**, March 28, T.A.P.P.I., Sect. 137)—The conditioned specimen is mounted, in the form of a ring or tube, in a horizontal plate having an annular groove (depth 0·25, outside diam. 0·625 or 2·00 in., according to the size of the specimen). The plate is placed centrally between 2 parallel platens, which are brought together at 0·5 ± 0·125 in. per min., and the load at crushing is noted. Tests are made with samples cut in each direction of the sheet. J. G.

Instrumentation Studies. LIV. Penetration of Papers by Water Vapour. VIII. Controlled Relative Humidity Cabinet in which Specimens may be Weighed without Removal from the Conditioned Atmosphere. Institute of Paper Chemistry (*Paper Trade J.*, 1946, **122**, February 21, *T.A.P.P.I. Sect.*, 81–86)—The cups to be weighed are carried on 3 annular, horizontal ring-shaped shelves in the humidity cabinet. These shelves may be rotated in a horizontal plane, by means of a shaft passing through the wall of the cabinet, so as to bring each cup at will on to one of 3 pairs of triangularly-shaped hooks on the two arms of a fork, which is suspended by a rod from the left-hand arm of a balance placed on top of the cabinet. Normally, each pair of hooks is slightly below the level of the shelf it serves, so that when the shelf is lowered (by depressing the rotating shaft) the cup is deposited on the pair of hooks, and is then ready to be weighed. Detailed technique for sealing and operating the cabinet, for controlling the relative humidity inside it by means of sat. salt solns., and for the measurement of the relative humidity inside it by the dew point, wet-and-dry-bulb thermometer and electrical hygrometer methods, is described. J. G.

Ink-Line Test for the Determination of Degree of Sizing in Writing Paper. M. O. Schur and R. M. Levy (*Paper Trade J.*, 1946

122, March 21, *T.A.P.P.I. Sect.*, 121–125)—A method of making an ink line under standard conditions is described. The "pen" is a brass frame which can be weighted as desired, carried on three wheels (diam. 0·5 in.), one of which has a circumferential slot of uniform width and depth with bevelled edges, which carries the ink. The sample is placed flat on a glass plate, and the "pen" is drawn across it on a cord, the other end of which is attached to the face of a drum which is rotated mechanically at any desired speed (normally to give a pen travel of 1 in./sec.). Three 8-in. parallel lines are usually drawn, using a standard ink (1·0% medicinal grade methylene blue in 0·5% phosphoric acid) to which is added up to 2% of diethylene glycol if it is desired to test the effect of a quick drying fountain pen ink. The lines are examined under the microscope (× 100), the max. widths of the lines at five 1·0-cm. intervals being recorded and averaged (x). The wheel slot width is also measured microscopically (y). Then % spread (S) = 100 $(x-y)/y$. S increases markedly with a rise in relative humidity, *e.g.*, from 29 to 56 between 20 and 47% (at 70° F.). Typical data are presented. The method enables the wire and felt sides of paper to be differentiated, and demonstrates that S for a line drawn in the cross direction is greater than that in the machine direction. The effects of pH on sizing and of the use of various sizing agents are also illustrated. J. G.

Reviews

METHODS OF ANALYSIS OF THE ASSOCIATION OF THE OFFICIAL AGRICULTURAL CHEMISTS. Sixth Edition, 1945. Pp. xii + 932. Association of Official Agricultural Chemists, P.O. Box 540, Benjamin Franklin Station, Washington 4, D.C. Price $6.25.

Methods of Analysis, A.O.A.C., is an old friend to chemists in this country and its quinquennial revision is looked forward to with great interest. The general form of the new edition is as before, but the total number of pages represents an increase of 23 per cent. over the last and the number of illustrations has been proportionately increased. Two chapters previously blank and reserved for Sewage and Agricultural Dust have now been allocated to Cosmetics and Enzymes. Forty per cent. of the increased matter is due to a new chapter on Extraneous Materials in Foods and Drugs and to expansion of the chapter on Vitamins. The remaining increase is more or less evenly distributed over the 42 other chapters. A decimal system of numbering the sections facilitates reference.

The immense amount of collaborative effort expended in testing methods before inclusion ensures that each is a real standard method of approved value. No praise is too high for the part played in the production of this work by the Editorial Board, the Committee on Editing and the unnamed collaborators.

J. R. NICHOLLS

THORPE'S DICTIONARY OF APPLIED CHEMISTRY. Fourth Edition. Vol. VII, IO—METE. Pp. 629. London: Longmans Green & Co., Ltd. Price 80s.

This volume marks in some degree a change of policy in the production of *Thorpe*. It is the first to appear under the guidance of an Editorial Board, of which the Chairman is Sir Ian Heilbron and which includes Drs. Emeléus, Melville and Todd, with Dr. Whiteley as Editor and Dr. A. J. Welch as Assistant Editor. Some innovations appear; they are designed to balance the space allocated to specific branches of chemistry as it is to-day. They provide more space for physico-chemical subjects, by elimination of many short articles of a historic nature, and by omitting many individual members of large groups such as alkaloids, dyestuffs or synthetic drugs, which are to be treated in comprehensive general articles.

In the achievement of these ends it is natural that many new names appear in the list of contributors, and these speak well for the editor's efforts to secure contributions from the leaders in particular new subjects of industrial importance.

The volume has its own index, occupying 15 pages—a very useful feature in view of the time which must elapse before the whole edition is complete.

Having regard to the extraordinarily wide scope of the Dictionary, it is impossible for any reviewer to do more than test the monographs on those few subjects of which he happens to have knowledge, and to note the scope and references in others. This volume, like the others of the series, fully comes up to expectations. The information is right up-to-date, thorough and well documented. *Thorpe* is the first place to turn to for information on a new industrial problem, and it seldom fails to afford something useful and references to more. This volume has a wealth of new material, whether on iron and steel, vitamin K, lard substitutes, lichens, luminescence, margarine, mesomerism or a hundred other subjects. The analytical aspect has been kept in mind; most of the articles dealing with metals or

organic compounds have some information on appropriate methods of analysis, *e.g.*, those on magnesium, manganese and mercury are by Dr. Schoeller and Mr. Lambie.

We venture to congratulate the Editorial Board on their arrangement and choice of materials and on the way they have tackled the difficult problem of producing so great a work under present conditions. The binding and production are in the now familiar form, but we regret the lack of uniformity in colour and grade of paper—no doubt it was unavoidable, but it does mar such valuable volumes, the cost of which is necessarily considerable.

Those who wish to keep their reference books up-to-date, and to have the latest information at hand, will acquire this volume as a matter of course, and they will be amply repaid.

H. E. Cox

PHYSICAL METHODS GROUP

A JOINT MEETING of the Physical Methods Group with the Cardiff and District Section of the Royal Institute of Chemistry will be held at 6.30 p.m. on Friday, October 11th, 1946, at University College, Cardiff. The subject of the meeting will be Electrometric Analysis. Further particulars will be announced later.

The Annual General Meeting of the Group will be held on Tuesday, November 26th, 1946, at Burlington House, London W.1.

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