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Volume 74 Price 3s. 6d.
No. 883, Pages 529—576 October, 1949

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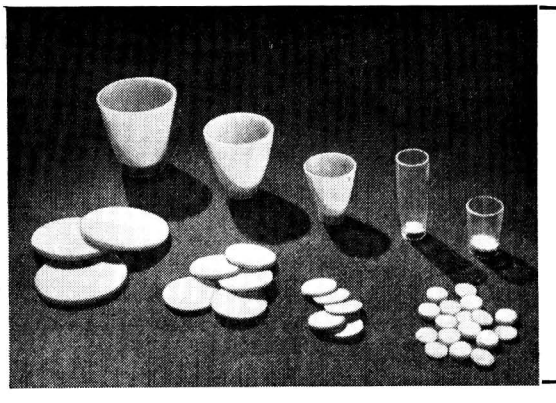

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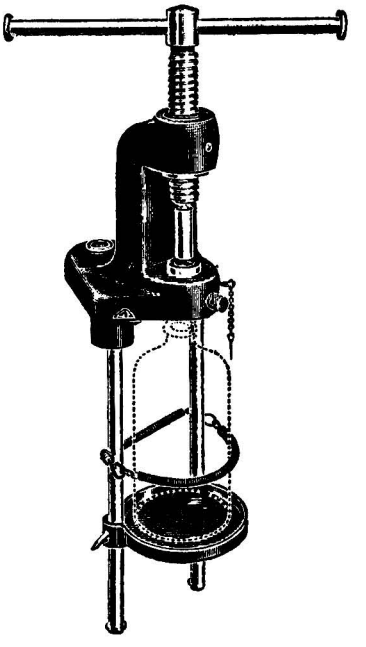

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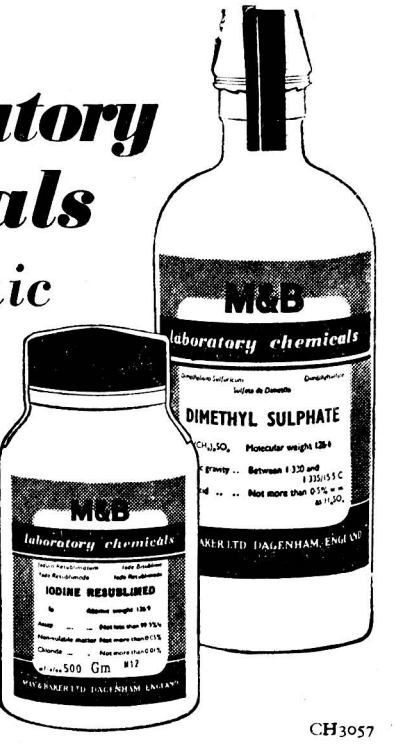

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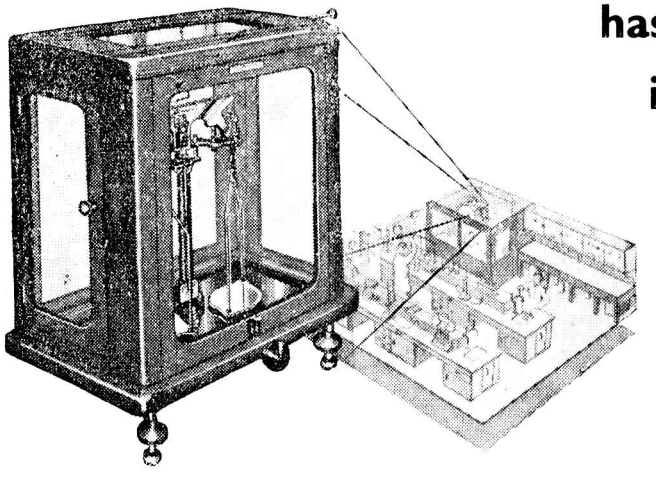

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

Applications of the Intermittent A.C. Arc. Part II. Determination of Rare Earths

By J. A. C. McCLELLAND

(*Paper presented at the Joint Meeting of the North of England Section and the Physical Methods Group at Liverpool, on October 2nd*, 1948)

The chemical separation and quantitative determination of rare earth elements is a laborious procedure, and when the need arose for analysis of complex minerals for these elements, a spectrographic finish was obviously desirable provided that adequate sensitivity and precision could be obtained.

One factor affecting sensitivity must be the interference by substances other than those to be determined, and this effect may be minimised by separating the desired elements from the major constituents of the sample.[1]

Chemical extraction of the rare earths, as distinct from their individual separation, is a comparatively simple process and by this means very small amounts may be determined, depending only on the quantity of sample available. Such a process has been used.

Accuracy as well as ultimate sensitivity will depend largely on the means of excitation used. The intermittent A.C. arc[2,3] has been found to give a good sensitivity and reproducibility in this determination. A precision of the order of ±10 per cent. of the proportion of the element being determined was obtained, using prepared mixtures of pure rare earth oxides with iron oxide.

Some difficulty was at first experienced in obtaining by fusion a consistently smooth and even coating on the electrode. Investigation showed this failure to be associated with the particle size of the iron oxide; a remedy was found in the control of conditions of precipitation and ignition.

Experimental

(*a*) Preliminary chemical concentration—

For spectrographic purposes the essential point in any chemical separation of the rare earths is to obtain by the simplest means a concentration of rare earths substantially free from all other elements that might cause interference in the spectrographic technique. The amount of sample used for the determination will be governed by the amount of rare earth it is suspected of containing, and it will be necessary at the outset to take the sample to complete solution. When the material is a silicate rock, prolonged treatment with hydrofluoric acid will be necessary before any separation of the rare earths can be accomplished. Materials of this nature usually contain large amounts of other elements, such as iron and aluminium, from which the rare earths must be separated.

If appreciable quantities of uranium salts are present the method described by Short and Dutton[4] or by Hirt and Nachtrieb[6] may be used. The former describe a process which

separates rare earths from large amounts of uranium salts, involving precipitation of the rare earths as fluorides and hydroxides, thereby avoiding the less satisfactory separation of the rare earths as oxalates.

The separation of rare earths from uranium salts has not, however, arisen in the present work, and a simpler method of precipitation has been used, an iron salt being added to serve as an internal standard.

Taking 1 g. of the original sample from which the rare earths are to be concentrated and adding 0·25 g. of iron oxide to the rare earths obtained, the following percentages of rare earth oxides may be determined. The figures given are for determination of rare earths by means of the lines given in Table II, which have been selected, where possible, with emphasis on precision rather than sensitivity; therefore these ranges do not necessarily record the lowest possible limit of detectability—

Y	La	Ce	Pr	Nd
0·0005–0·025%	0·001–0·025%	0·005–0·05%	0·002–0·05%	0·002–0·05%
Sm	Eu	Gd	Tb	Dy
0·002–0·05%	0·0002–0·01%	0·002–0·05%	0·002–0·05%	0·0005–0·02%
Ho	Er	Tm	Yb	Lu
0·0002–0·01%	0·0005–0·01%	0·001–0·025%	0·0002–0·01%	0·0002–0·01%
Sc	Th			
0·0002–0·01%	0·025–0·25%			

The particle size of the iron oxide was found to be important in obtaining consistent results by the fusion technique, and conditions of precipitation and of ignition were adjusted to give the desired results in this respect. Electron micrographs, for which I am indebted to Mr. B. S. Cooper of the Research Laboratories of the General Electric Company Ltd., were taken in an attempt to solve this problem.

In preparing the samples where the amount of iron oxide is relatively small, it is necessary to avoid too fine a particle size. To achieve this the precipitate is washed to the bottom of the filter paper and ignited in as compact a mass as possible.

(*b*) STANDARDS—

For the preparation of standards the iron oxide is prepared from Specpure iron and the final precipitation carried out in presence of filter paper pulp. Subsequent ignition at 800° C. produces an iron oxide that is not too coarse.

The following standards were prepared. Percentages given are based on the oxide mixtures: subsequent dilution with potassium hydrogen sulphate reduces the percentage of rare earth in the actual mixture used on the electrode to one-fifth of these figures.

Ho—1·6, 0·8, 0·4, 0·16, 0·08 and 0·04 per cent.
Ce—3·0, 1·5, 0·75, 0·4 and 0·2 per cent.
Th—10·0, 5·0, 3·3 and 1·0 per cent.
Others—2·0, 1·0, 0·5, 0·2, 0·1, 0·05 and 0·02 per cent.

Note—All the rare earth oxides used were pure except that of holmium which contained 80 per cent. of holmia together with dysprosia and yttria (Johnson, Matthey & Co., spectrographically standardised).

From these single standards, composite standards were prepared: these standard mixtures, as will be seen from Table I, usually included about four different rare earth elements. Owing to the cost of some of the rare earth oxides it was not possible to prepare one standard to embrace all those investigated, although such a standard would be exceedingly useful in routine analysis of this nature.

The sensitivity of thorium in the arc discharge is much lower than that of the other elements investigated in this work, and is not included in the standard mixtures tabulated below. It is, however, of not inconsiderable importance in analysis involving the determination of rare earths.

(*c*) SPECTROGRAPHIC TECHNIQUE—

The concentrate of rare earth oxides in iron oxide, obtained from the preliminary chemical treatment of the sample, such that the ferric oxide content is not substantially less than ten times the total rare earth content, is finely ground with a weight of potassium hydrogen sulphate four times that of the ferric oxide.

Standards are prepared from pure rare earth oxides and pure iron oxide well ground and mixed with potassium hydrogen sulphate so that $KHSO_4 : Fe_2O_3 = 4 : 1$ as before.

TABLE I

STANDARD MIXTURES

Percentage calculated on total oxide before addition of $KHSO_4$

	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Sc
1	0·07	—	1·0	—	—	—	0·07	—	—	—	—	—	—	—	—	—
2	0·12	0·25	0·75	—	—	—	0·12	—	—	—	—	—	—	—	—	—
3	0·25	0·12	0·4	—	—	—	0·25	—	—	—	—	—	—	—	—	—
4	0·33	0·33	—	—	—	—	0·03	—	—	—	—	—	—	—	—	—
5	—	0·5	1·5	—	—	—	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	0·66	0·33	—	—	—	—	—	—	—	0·07	—	—
7	—	—	—	1·0	—	1·0	—	—	—	—	—	—	—	—	—	—
8	—	—	—	0·5	0·12	0·5	—	—	—	—	—	—	—	0·50	—	—
9	—	—	—	0·25	0·25	0·12	—	—	—	—	—	—	—	0·25	—	—
10	—	—	—	0·16	0·33	—	—	—	—	—	—	—	—	0·16	—	—
11	—	—	—	0·5	0·5	0·5	—	—	—	—	—	—	—	0·25	—	—
12	—	0·25	1·5	—	—	—	—	—	—	—	—	—	—	—	—	—
13	—	0·165	1·0	—	—	—	—	—	—	—	—	—	—	0·165	—	—
14	—	0·66	0·5	—	—	—	—	—	—	—	—	—	—	0·065	—	—
15	—	0·33	0·25	—	—	—	—	—	—	—	—	—	—	0·33	—	—
16	—	—	—	—	—	—	—	1·0	—	—	—	—	—	—	—	0·25
17	—	—	—	—	—	—	—	0·66	—	0·33	—	—	—	—	—	0·165
18	—	—	—	—	—	—	—	0·33	—	0·165	—	—	—	—	—	0·07
19	—	—	—	—	—	—	—	0·165	—	0·07	—	—	—	—	—	0·03
20	—	0·66	—	0·66	—	—	—	0·165	—	—	—	—	—	—	—	—
21	—	0·5	—	1·0	—	—	—	—	—	—	—	—	—	—	—	—
22	—	0·33	—	0·33	—	—	—	0·66	—	—	—	—	—	—	—	—
23	—	0·17	—	0·17	—	—	—	0·33	—	—	—	—	—	—	—	—
24	—	0·25	—	—	—	—	—	1·0	—	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—	—	0·4	—	0·15	0·04	0·04	—	0·2	—
26	—	—	—	—	—	—	—	—	0·2	—	0·08	0·1	0·1	—	0·1	—
27	—	—	—	—	—	—	—	—	1·0	—	—	—	—	—	0·05	—
28	—	—	—	—	—	—	—	—	0·66	—	—	0·33	0·33	—	—	—
29	—	—	—	—	—	—	—	—	—	—	0·4	—	0·5	—	—	—
30	—	—	—	—	—	—	—	—	—	—	0·28	0·66	—	—	0·33	—
31	—	—	—	—	—	—	—	—	0·4	—	0·16	0·2	0·2	—	0·2	—
32	—	—	—	—	—	—	—	—	0·66	—	0·28	—	0·33	—	—	—

The resulting powder is placed in a cupped graphite electrode having a crater 1 to 1·5 mm. deep, and levelled off. The electrode is heated on a hot-plate until fusion just occurs, whereupon the melt is spread evenly and rapidly over the surface with a steel blade to give a smooth coating to the electrode.

Pairs of such electrodes are used in the intermittent A.C. arc discharge under the following conditions—

Spectrograph	Large Littrow instrument setting 2900–6000 A.
Source to slit	38 cm. with lens F.1025.
Slit width	1½ divisions, 0·015 mm.
Electrodes	Graphite ¼-inch diameter J.M.4.
Electrode separation	4 mm.
Capacitance	48 μF.
Current	4 amp.
Plate	Ilford Isozenith developed 5½ min. in I.D.2 at 66° F.
Exposure	0/90 sec.

Note—Correct exposures give Fe 4337·0 A. a density of 0·6 to 0·7. The spectrograph on which this work was done was fitted with an old type of slit for which 1 division ≡ 0·01 mm. and it required some preliminary experiments to adjust the slit width with sufficient accuracy to obtain the correct density.

(*d*) LINE PAIRS—

A preliminary survey of the rare earth spectra was made as a guide to the choice of suitable line pairs and to the sensitivity and reproducibility to be expected under the conditions

of excitation used. In this, the survey recently made by Smith and Wiggins[5] on the rare earth spectra with special reference to the wavelength and intensity of the most sensitive lines proved very valuable.

Difficulties were encountered in the selection of suitable line pairs, as many of the more sensitive lines are masked by the bands that are produced in the graphite arc. Furthermore, lines due to other rare earth elements are sometimes superimposed upon those of the element being determined; as for instance the line Ce 4012·4 A. which is coincident with Nd 4012·25 A. and which becomes visible at a concentration of about 0·25 per cent. of cerium. In this case the only satisfactory alternative lines for neodymium are 4061·1 A., which is weaker and somewhat masked by background, and 4303·6 A., which is available only at concentrations of neodymium above 0·5 per cent.

Possibly the most serious hindrance has been the variable quality of the graphite. Two different effects have been encountered that have in different ways influenced the results obtained. In the first place impurities present in the graphite have seriously interfered with the rare earth and iron comparison lines which would otherwise have been selected. The graphite originally used was of American origin and contained, among other less important impurities, titanium and vanadium in varying proportions; it was frequently possible, however, to select rods that were free from these impurities and when this was done the results obtained were superior to any obtained with subsequent supplies of graphite. Latterly the supply of graphite was obtained from sources in this country and it was immediately apparent that, although free from vanadium, it was never free from titanium; in fact it contained the latter element in almost constant amount throughout all batches of rods. This has interfered with the determination of erbium by means of the line Er 3372·75 A. and more seriously with the determination of other elements by use of Fe 3370·8 A. for comparison. Although the line Fe 3369·5 A. has proved useful it is generally less satisfactory than Fe 3370·8 A. owing to its relatively weaker density.

A small amount of high-purity graphite has been obtained but, owing to its high cost, the amount of work that could be performed with it has been limited. By its use, however, it has been shown that the absence of titanium makes it possible to use with advantage the lines mentioned above.

A further disadvantage of the later supplies of graphite is that the character of the bands in the graphite arc has shown a change which gives an increased background effect. The bands are less pronounced in spectra produced in the intermittent arc with graphite electrodes than in similar spectra produced by D.C. arc excitation—markedly so when the earlier supplies of graphite were used. It appears that the newer type of graphite contributes more to the exposure relative to the sample placed upon it than did the original graphite used. The high-purity material did, however, show background effects comparable with those of the graphite of American origin. The rather smaller diameter of the high-purity graphite was disadvantageous in other respects compared with the material used in the earlier part of the work.

The well known differences in spectral response of the rare earth oxides to arc excitation will of necessity give rise to considerable differences in the lowest limit of detectability of the various elements; *e.g.*, the limit of detection of cerium in the mixed rare earth and iron oxide concentrate is about 0·1 per cent. For this reason it has been necessary in some cases to utilise the most sensitive line, whether this is the best line for reproducibility or not, but for other elements a better reproducibility may be obtained by choosing a line that may not be the most sensitive line available; preference has been given where possible to higher precision at the expense of some slight loss in sensitivity.

Calibration curves have in general been plotted as ratio of deflection rare earth to deflection iron against percentage concentration of rare earth oxide in the oxide concentrate. In order to cover the widest possible range of composition the densities of the weakest lines are such that they may sometimes fall outside the straight line portion of the characteristic curve of the plate (density 0·1 to 1·1).

Tabulated below are the line pairs used for each element determined, together with recommended concentration limits and explanatory notes.

RESULTS AND PRECISION—

The following results were obtained in the determination of rare earths in minerals. Figures obtained in control samples, where known amounts of rare earths were added, will

TABLE II

LINE PAIRS

All wavelengths are in Ångstrom units

Element	Range, %	Rare earth lines	Iron lines	Remarks
Y	0·02–1·0	3600·7	3603·2	
		3710·3	3701·1	Stronger line than 3600·7 but somewhat masked by Fe 3709·3.
		4374·9	4352·7	Note. Fe 4376·0, Gd 4373·8.
La	0·05–1·0	4333·7	4337·0	Tb 4336·5 interferes with Fe.
		4333·7	4352·7	Note. Ce 4337·8, Sm 4334·1, 4336·1.
		3988·5	4009·7	Note. Yb 3988·0, V 4332·8.
		3995·75	4009·7	
		3337·5	3370·8	Useful above 2%; weak Fe 3337·7 may interfere.
Ce	0·25–2·0	4562·4	4603·0	
		4572·3	4603·0	Ce masked by V 4571·8.
		4012·4	4009·7	Useful in absence of Nd 4012·25.
Pr	0·1–2·0	4008·7	4009·7	Note. Er 4008·0.
		3908·4	3898·0	Somewhat masked by Fe 3907·9. Note. Sc 3907·5.
Nd	0·1–2·0	4012·25	4009·7	Masked by Ce 4012·4.
		4061·1	4009·7	Somewhat masked by background.
		4303·6	4337·0	Visible at 0·5%.
Sm	0·05–2·0	3592·6	3594·6	Note. Y 3592·9.
		4577·7	4603·0	Stronger line, though some background effect.
Eu	0·01–0·5	3972·0	3977·7	Note. Fe 3971·3, Pr 3972·2.
		4661·9	4603·0	Strong line useful for quantities less than 0·1%.
		4522·6	4476·0	Useful for quantities greater than 0·2%.
Gd	0·1–2·0	4347·3	4337·0	Use Fe 4352·7 in presence of Tb. Gd 4347·3 slightly masked by background.
		3362·2	3369·5 or 3370·8	
		3350·5	3369·5 "	
		3358·6	3369·5 "	
Tb	0·1–2·0	3509·2	3445·2	Slightly masked by background.
		3324·4	3369·5 or 3370·8	Weaker line but the most reliable above 0·5%.
		4338·45	4352·7	
Dy	0·02–1·0	4000·45	4009·7	Note. Pr 4000·2.
		4211·7	4233·6	More sensitive line but background rather heavy.
		3531·7	3603·2	Slightly masked by background.
Ho	0·01–0·5	3456·0	3369·5 or 3370·8	
		4053·9	4009·7	More sensitive line but background rather heavy.
Er	0·02–1·0	3499·1	3445·2	For 0·5% upwards.
		4008·0	4009·7	Recommended in absence of Pr 4008·7.
		3372·75	3369·5 or 3370·8	Note. Ti 3372·8.
Tm	0·05–1·0	3462·2	3369·5 or 3370·8	
		3362·6	3369·5 "	
		3131·3	3369·5 "	
Yb	0·01–0·5	3464·4	3445·2	
		3289·4	3286·8	Yb 3289·4 stronger line than 3464·4.
Lu	0·01–0·5	3359·6	3369·5 or 3370·8	
		3312·1	3369·5 "	Note. Er 3312·4.
Sc	0·01–0·5	4023·7	4009·7	
		4020·4	4009·7	Note. Er 4020·5.
		3613·8	3603·2	
		3642·8	3603·2	
Th	1·0–10·0	4019·1	4009·7	

Note—All percentages mentioned above are calculated as rare earth oxide in the total oxide concentrate. The iron line 3370·8 is superimposed upon a titanium line and is only usable if the graphite is titanium-free.

indicate the accuracy obtainable by this method. Preliminary chemical separation in this case was carried out in far greater detail than is usually required; the preparation involved about eight precipitations, so that some loss of rare earth is to be expected.

MILLIGRAMS OF RARE EARTH OXIDE PER GRAM OF ORIGINAL MATERIAL

	Found in sample	Added to sample	Found in sample after addition
Y	nil	0·00010	0·00009
La	nil	0·00020	0·00017
Ce	nil	0·0015	0·0011
Eu	0·00009	0·00022	0·00030
Yb	traces	nil	traces

Pr, Nd, Sm, Gd, Tb, Dy, Ho, Er, Tm, Lu, Sc and Th were not detected in the sample.

Determination of rare earths in fluorites has also been carried out; it is considered that the fluorescence of fluorite may be due to the presence of certain elements of this group.

As a measure of reproducibility, replicate exposures were made of a number of standard mixtures. Although the number of replicates is not large the results given will afford a fair indication of the reproducibility obtained by the method.

Tests were also carried out on both types of graphite that were used in the latter part of the work, namely, the variety that contained titanium and the high-purity graphite. The test involved about ten duplicate exposures of a given standard sample on each type of graphite. Where comparison was possible, that is, for lines not affected by titanium, the two types of graphite showed very little difference in the precision obtained, but such differences as occurred seemed to be slightly in favour of the ordinary variety. Similarly, comparisons between the two iron lines Fe 3369·5 A. and Fe 3370·8 A. for the internal standard line, which could only be made on the high-purity graphite, showed again a slight advantage in favour of the stronger iron line.

The table below shows the standard deviation of ratio deflection rare earth/deflection iron, σR, which has been translated to standard deviation on the amount of rare earth present, by comparison with a standard calibration curve.

TABLE III

Element	No. of exposures	% of element	σR	Standard deviation %
Y	12	0·12	0·09	± 10
La	12	0·25	0·08	± 8
Ce	12	0·7	0·035	± 8
Pr	16	0·5	0·085	± 15
Nd	16	0·5	0·06	± 10
Sm	16	0·5	0·055	± 10
Eu	12	0·12	0·05	± 10
Gd	14	0·66	0·055	± 10
Th	18	0·5	0·04	± 6
Dy	14	0·33	0·095	± 10
Ho	18	0·30	0·065	± 7
Er	20	0·2	0·025	± 6
Tm	20	0·3	0·05	± 9
Yb	16	0·25	0·045	± 8
Lu	20	0·2	0·035	± 7
Sc	13	0·165	0·058	± 8

CONCLUSIONS—

The technique described, by which the sample is fused *in situ* on a graphite electrode, has provided a simple and efficient method of ensuring that the material is firmly held on both the lower and the upper electrode, thereby enabling a discontinuous source to be used. In this way the characteristic properties of the intermittent A.C. arc, namely, the high sensitivity of arc excitation together with a reproducibility comparable with that of a spark source,[2] may be utilised. Furthermore, a fusion technique permits the analysis of insoluble samples which are excluded when a solution of the material is evaporated on the electrode.[3] A method for general analytical problems is proceeding on these lines.

It may also be possible by the use of a technique such as that described by Wiggins[5] to eliminate the band spectrum of a graphite arc and thereby broaden the choice of lines available for analysis.

TABLE IV

COMPARISON OF "STANDARD" AND HIGH-PURITY GRAPHITE

Number of exposures, 10

Line pair	Standard deviation %	
	Standard graphite	High purity graphite
Lu 3359·6 A. / Fe 3369·5 A.	± 5	± 7½
Lu 3359·6 A. / Fe 3370·8 A.	—	± 7½
Er 3372·75 A. / Fe 3369·5 A.	—	± 7½
Er 3372·75 A. / Fe 3370·8 A.	—	± 7
Ho 3456·0 A. / Fe 3369·5 A.	± 10	± 8
Ho 3456·0 A. / Fe 3370·8 A.	—	± 7
Tm 3462·2 A. / Fe 3369·5 A.	± 8	± 9
Tm 3462·2 A. / Fe 3370·8 A.	—	± 7½

SUMMARY

A method is described for the spectrographic determination of small amounts of rare earth elements present in minerals, to include all known members of the rare earth group proper, that is, the lanthanides, and other elements usually associated with the group, namely, yttrium, scandium and thorium.

The rare earth elements are first separated from the major mineral constituents by chemical means, and ignited to oxide after addition of an iron salt to serve as an internal standard. Standards are prepared from pure rare earth oxides mixed with iron oxide.

The concentrates and standards are ground with potassium hydrogen sulphate, and fused on to cupped graphite rods, pairs of which are used as electrodes in the intermittent A.C. arc discharge.

I wish to express my thanks to Dr. P. J. Hardwick of this Department for the chemical work referred to above and to Mr. C. Park for carrying out some of the spectrographic operations and finally to the Government Chemist for permission to publish this report.

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DEPARTMENT OF THE GOVERNMENT CHEMIST
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DISCUSSION

Mr. J. HASLAM asked if Dr. McClelland had considered the possibility that differences in the behaviour of the iron oxide might be associated with different contents of Fe_3O_4.

Mr. B. S. COOPER thought that the differences might be related to size and shape of particles and suggested a closer examination of X-ray patterns and if that gave no information a comparison of samples by means of the electron microscope.

Dr. D. M. SMITH said that, although in recent papers he and G. M. Wiggins had stressed the advantages of the constant-current D.C. arc for work on the rare earths, they did not wish to imply that the intermittent A.C. arc was unsuitable, and he welcomed this paper as showing that under the conditions standardised by Dr. McClelland reliable quantitative data could be obtained with it.

Mr. F. J. WOODMAN asked if, as the rare earth oxides were presumably somewhat hygroscopic and some of them, according to the literature, were unstable on ignition, any particular precautions were taken in weighing and general handling, in the preparation of the quantitative standards used in the work described.

Dr. MCCLELLAND said, in reply to Mr. Woodman, that the rare earth oxides used in the preparation of the standard mixtures were weighed directly from the sealed tubes in which they were supplied. In the fusion technique, the temperature was raised only just sufficiently to fuse the potassium hydrogen sulphate mixture and it was considered unlikely that any loss of rare earth oxide would occur in the short time that the electrodes were maintained at that temperature.

In reply to questions on the character of the iron oxide, X-ray examination showed no differences in composition, but X-ray films would be re-examined to see if any differences in particle size had been revealed. Difference in ultimate particle size seemed to be the most likely cause of variation in character of the iron oxides and investigations on these lines would be undertaken.

Isolation and Determination of Morphine, Codeine and Heroin from Viscera and Body Fluids by Adsorption

BY A. STOLMAN AND C. P. STEWART

A SURVEY of the literature shows clearly that the existing methods for the determination of morphine and its derivatives in body fluids are unsatisfactory when the amount of alkaloid is small. Even when several centigrams are present in approximately 100 g. of the material, good results can only be achieved by the most meticulous attention to the details of a long and cumbersome process.

Adsorption offers a possible alternative to the usual methods involving extraction with immiscible solvents. Mutch[1] showed that various alkaloids were adsorbed by magnesium trisilicate, though rather inefficiently under his conditions. Stewart, Chatterji and Smith[2] used adsorption on kaolin for the isolation of several alkaloids in toxicological analysis and obtained promising preliminary results. On the other hand, Daubney and Nickolls[3] discarded adsorption methods as inefficient. All three of these groups used the rather crude procedure of agitating a suspension of the adsorbent in the alkaloid-containing solution. In the toxicological analyses, moreover, it was insufficiently realised that particular adsorbents must be expected to vary in effectiveness when the extracts used as analytical material contain high concentrations of electrolytes. It therefore seemed desirable to investigate further the possibility of removing small amounts of alkaloid from tissue extracts by adsorption methods.

EXPERIMENTAL

ADSORBENT—

We were fortunate in finding a commercially available synthetic silicate, Florisil,* which was more effective than a number of other adsorbents tested in removing morphine from pure solution. It is a hard, white granular substance, obtainable in various standard particle sizes (of which 60 to 100-mesh, standard U.S. equivalent sieves, proved most satisfactory) and, in distilled water, gives a suspension having pH 9·8. Its physical properties make Florisil particularly convenient in use, as it allows fairly rapid percolation of fluids and does not readily clog. For use it was cleaned by heating under reflux with a mixture of formic acid, ethyl alcohol and ethyl acetate in the apparatus described below.

REAGENTS—

The purity of the reagents was found to be an important factor.

Ethyl alcohol and methyl alcohol—Each litre of the 95 per cent. alcohol, laboratory grade, was mixed with 4 g. of silver nitrate dissolved in a minimum quantity of water and about 1 ml. of 40 per cent. sodium hydroxide solution. After the mixture had been allowed to stand for 24 hours, more sodium hydroxide was added until no further precipitation of silver occurred. The precipitated silver salt was removed by filtration and the alcohol heated under reflux for half an hour and then distilled.

Ethyl acetate—Each litre of this reagent was heated under reflux for half an hour with approximately 25 g. of phosphorus pentoxide and then distilled.

* Obtainable from the Floridin Co. Inc., Penna, U.S.A. Manufactured in accordance with U.S. Patent No. 2,393,025.

Oxalic acid—This reagent was rendered anhydrous by heating to between 60° and 70° C. and then purified by sublimation at temperatures between 140° and 157° C. No special apparatus was required. The sublimation was carried out in a large glass-stoppered conical flask partially immersed in an oil-bath at the stated temperature. The sublimed product condensed on the cooler surfaces of the flask.

Formic acid—Analytical reagent grade of 90 per cent. formic acid was used.

Sodium carbonate solution—A saturated solution of the pure salt (AnalaR) was used.

Formaldehyde - sulphuric acid reagent—1 ml. of 40 per cent. formaldehyde solution was mixed with 99 ml. of pure sulphuric acid (sp.gr. 1·84).

Formaldehyde - ferric sulphate - sulphuric acid reagent—0·2 ml. of the formaldehyde - sulphuric acid reagent was mixed with 60 ml. of sulphuric acid (sp.gr. 1·84) and then 8 ml. of 10 per cent. (w/v) aqueous ferric sulphate solution were added with cooling.

Phenol reagent—Commercially prepared Folin - Ciocalteu reagent was used.

APPARATUS—

Adsorption columns—The columns were prepared in Pyrex glass tubes of 14-mm. diameter and 90-mm. or 120-mm. length. The Florisil was packed in the usual way (Zechmeister and Cholnoky[4]) on a base of cotton wool with the aid of a wood plunger, and was covered by a small wad of glass wool to prevent disturbance of the upper surface during manipulation.

Elution apparatus—In order to avoid the distribution of 1-mg. quantities of alkaloid Cholnoky[4]) in a large volume of solvent, elution of the adsorbed alkaloids was carried out in the apparatus shown in Fig. 1, which was made in the laboratory. A 100-ml. flask is used to contain the solvent. The lower ground glass joint can be either a B19 or B24 and the upper one a B24 or B32. Any type of condenser can be used, provided that its lower end has an extension centred over the funnel, to facilitate the flow of the condensed solvent into the adsorption tube. The stem of the inserted funnel is of sufficient length to enter the neck of the adsorptiontube. Four projections on the inner side-walls of the body of the apparatus act as supports for the adsorption tube, without hindering the return flow of the solvent into the flask. The opening at the bottom of the body for the return flow is 1 to 1·5 mm. in diameter. This eluting apparatus worked very efficiently. Very little vapour came up through the bottom and at no time was there interference with the return flow of the liquid.

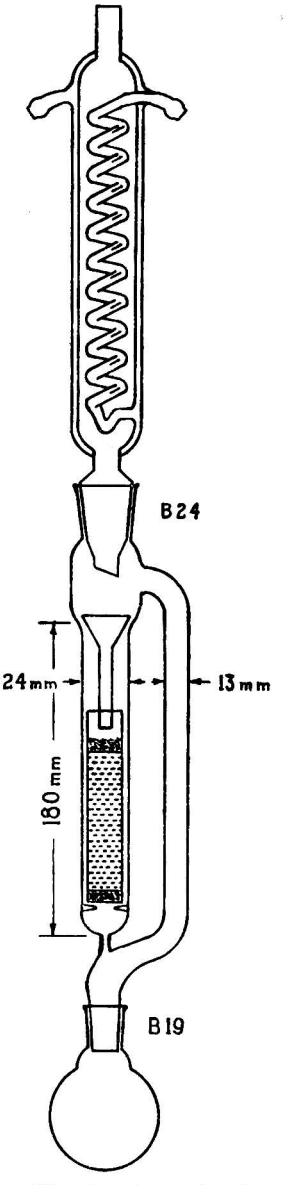

Fig. 1. Apparatus for elution with small volume of solvents

The preliminary cleaning of the adsorption columns and the elution of the alkaloid were both accomplished in the eluting apparatus. The procedure was simple. The cleaning or eluting solvent was boiled at a rate so adjusted as to ensure a constant small layer of fluid above the Florisil. At times the rate of percolation through the column was slow at the start, but it invariably increased to a satisfactory rate within a short time. In order to detect the occasional sudden formation of an air lock in the top part of the column only a small layer of liquid, which served as an indicator for the rate of percolation of the solvent through the column, was permitted to collect above the Florisil. Moreover, if the adsorption tube above the adsorbent was filled to overflowing with the liquid, there was no certainty that the liquid was percolating through the column.

In the procedures where the Florisil was overlaid with a salt necessary for the required elution, the salt was placed on the glass wool mat and then covered with a wad of cotton wool. The use of the latter was to prevent the drops of solvent from falling on the salt

and splashing it over the sides of the adsorption tube. It was not uncommon for an air-lock to form between the salt layer and the adsorbent. This, however, was easily remedied by discontinuing the heating for a minute or two, and then disconnecting the condenser and inserting a wire through the funnel into the salt layer to break the air-lock.

QUANTITATIVE METHODS USED—

No attempt was made to develop new methods of determination for the three alkaloids. With the small amounts used in this investigation it was found expedient to adopt existing colorimetric methods.

The reagents containing sulphuric acid are very liable to give "off colours" with slightly impure samples of the alkaloids, but it should be emphasised that the alkaloids adsorbed on Florisil and eluted as described invariably gave colours as true as those given by the standard solutions prepared from pure alkaloids. This is an important advantage of the adsorption procedure in the actual identification of the alkaloids.

(*a*) *Determination of morphine*—The Oberst method[5] for the colorimetric estimation of morphine was used, with substitution of the Folin - Ciocalteu phenol reagent[6] for the Folin - Denis reagent. The morphine solution, prepared from the residue after evaporation of the eluate, was transferred to a 100-ml. volumetric flask and made alkaline with 20 ml. of saturated sodium carbonate solution; 2 ml. of the phenol reagent were then added and the solution made up to volume. An intense blue colour developed rapidly, reaching maximal intensity within 2 hours and then remaining stable for many hours. All comparisons of the colour were made after 2 hours, in a photo-electric colorimeter, using a red filter (Ilford 204).

A freshly prepared heroin hydrochloride solution having the same concentration as the morphine solution gives only a faint trace of a blue colour with this reagent. A solution that has stood for several days gives a considerably deeper colour, indicating a slow hydrolysis of the heroin with liberation of the phenolic group. A pure codeine solution gives no colour with the phenol reagent.

(*b*) *Determination of heroin*—With the formaldehyde - sulphuric acid reagent, heroin gives first a red colour changing gradually to reddish-blue.

The dried residue obtained after the evaporation of the eluate was mixed with the reagent and stirred until completely dissolved. The volume was then made up in a volumetric flask to 50 ml. with more of the reagent. The colour became stable within ½ to 1 hour and readings were made after 1 hour, in the photo-electric colorimeter, using the red filter (Ilford 204). The standard follows Beer's law for low concentrations.

Morphine and codeine give colours similar to that given by heroin with this reagent, which is non-specific; although the colour shades differ, the method can only be used for determination of a single pure alkaloid.

(*c*) *Determination of codeine*—The formaldehyde - ferric sulphate - sulphuric acid reagent of Fulton[7] serves as a convenient colorimetric reagent for codeine, giving a reddish-blue colour. In the original paper 0·6 ml. of formaldehyde - sulphuric acid reagent was suggested, but we found 0·2 ml. of this reagent preferable.

The method can be standardised with any quantity of formaldehyde reagent, but the blue colour predominates with the smaller amounts of formaldehyde. The dried residue left by evaporation of the eluate was completely dissolved in some of the reagent, and the volume made up to 50 ml. with more of the reagent. The colour was read, after 1 hour, in the photo-electric colorimeter, using the red filter (Ilford 204). There was a linear relation between colorimeter readings and alkaloid concentration within the range used. Like the heroin colour reagent, this reagent is non-specific, giving colours with morphine and heroin similar to that obtained with codeine.

DEVELOPMENT OF THE METHOD

BASIC CONDITIONS OF ADSORPTION AND ELUTION—

One milligram of the alkaloid (morphine, heroin or codeine) was dissolved in 100 ml. of distilled water and the solution was run through a column of untreated Florisil. The adsorbed alkaloid was eluted by refluxing with methyl alcohol for 1 hour. Quantitative determination of the eluted alkaloid showed an apparent recovery of 105 to 120 per cent. with morphine or codeine and 94 per cent. with heroin.

Cleaning of the Florisil columns by refluxing for about 2 hours with a mixture of formic acid (1 vol.), ethyl alcohol (2 vols.) and ethyl acetate (2 vols.) in the apparatus described,

and then washing with 250 ml. of distilled water, the last 50 ml. of which were used for pH determination, removed the interfering impurities and improved the adsorption of heroin so that recovery was nearly complete for all three alkaloids. The slight loss of alkaloid observed might be due to incomplete adsorption or incomplete elution or both. Obviously the elution must first be made optimal. Increasing the time of elution within reasonable limits did not solve the problem. The treatment of the columns with formic acid had decreased its "alkalinity" to the range pH 7·0 to 7·5 (*i.e.*, the washing water from the Florisil so treated had a pH lower than that from untreated Florisil and even, with long refluxing, a pH below 7·0). Any columns from which the washing water had a pH below 7·0 were discarded. It was considered that elution might be helped by restoring the alkalinity of the column, and therefore solid sodium carbonate was placed on top of the column before elution With this procedure the solvent returning to the flask from the column is strongly alkaline. Since morphine is somewhat unstable in hot alkaline solution it was considered necessary to maintain an acid reaction in the flask by addition of a suitable non-volatile acid. This was achieved by using as the eluting fluid in the flask 25 ml. of methyl alcohol containing 0·5 g. of pure oxalic acid. With this procedure the recovery of the alkaloids became complete and exact. After the elution period of 45 to 60 minutes, the alcohol solution was transferred to an evaporating dish or beaker. Distilled water in sufficient amounts was used to complete the transfer. The solution was placed on a water-bath for evaporation to a small volume. For morphine the volume was reduced only to 10 to 15 ml., as its colorimetric determination was made in aqueous solution. For codeine and heroin, the solution was evaporated on the steam-bath to the first signs of salt crystallisation, never to complete dryness. For the final stage of evaporation, *i.e.*, complete drying, the beakers were placed in a warm air current (40° to 45° C.). Overheating on the steam-bath is a source of error generally overlooked, yet it is essential to avoid it, as heroin and codeine in minute quantities can be destroyed rapidly by excessive heat.

APPLICATION TO SOLUTIONS RESEMBLING TISSUE EXTRACTS—

Our problem was to determine the alkaloids present in tissue extracts, whether prepared by the Stas - Otto process (dilute alcohol), the Dragendorff method (acidified water) or with trichloroacetic acid as suggested by Stewart *et al.*,[2] although we were not concerned, at this stage, with the efficiency of these processes in extracting the alkaloids from tissue. We had therefore to consider the effects, upon adsorption, of high concentration of salts and organic acids and of alcohol. Further, such extracts are not absolutely free from proteins and lipoids.

To simulate these conditions we used solutions containing 1 mg. of alkaloid with 1·0 to 4·0 g. of sodium chloride per 100 ml., and to these we added 25 to 150 ml. of ethyl alcohol, or 5 to 20 g. of trichloroacetic acid, or both of these reagents within the ranges mentioned.

After one of these solutions had percolated through the column, the column was washed with about 50 ml. of aqueous alcohol (4 vols. of water : 1 vol. of alcohol) and excess fluid was forced out before elution began.

When ethyl alcohol formed half or more of the total volume of the solution, adsorption was incomplete, but when it formed only one-third or less it did not appreciably interfere (Table I *a*).

With the solutions containing 5 per cent. of trichloroacetic acid, the adsorption was incomplete even when the alcohol was present in amounts which, alone, did not decrease adsorption efficiency (Table I *b*).

These solutions, of course, were strongly acid (pH about 2·0), whereas all solutions previously tried had been neutral. We therefore repeated the series, adding sodium hydroxide solution to neutralise the trichloroacetic acid (final pH about 6·5 to 7·0). Under these conditions adsorption was again complete, provided the alcohol concentration was not excessive (Table I *c*).

The object of testing solutions containing both alcohol and trichloroacetic acid was to prepare the way for a composite method of obtaining tissue extracts by adding trichloroacetic acid to a Stas - Otto or Dragendorff extract to precipitate protein, or by adding alcohol to a trichloroacetic acid extract to prevent adsorption of residual pigments.

Consistently complete adsorption of various amounts of alkaloid was achieved with trichloroacetic acid (neutralised) concentration of about 5 g. in 125 ml. of solution containing 25 ml. of ethyl alcohol. With these proportions the sodium chloride concentration could safely be raised even to 4 g. per 100 ml. It was even possible to raise the concentration of

trichloroacetic acid to 16 g. per 100 ml. (Table I *d*). This shows that the conditions are remarkably elastic. In fact, the lower concentration of 1 g. of sodium chloride, 5 g. of trichloroacetic acid and 25 ml. of ethyl alcohol per 125 ml. represents the usual tissue extract, and in any case such an extract can in practice be diluted to any desired extent, since increase in volume has no other effect than to increase the percolation time.

TABLE I

ADSORPTION OF MORPHINE FROM AQUEOUS-ALCOHOL SALT SOLUTIONS

	Morphine added mg.	NaCl g.	Water ml.	C_2H_5OH ml.	CCl_3COOH g.	$CH_3COOC_2H_5$ g.	Morphine recovered %
a	1·0	—	100	100	—	—	85·0
	1·0	—	100	50	—	—	99·7
b	1·0	1·0	100	75	5·0*	—	85·0
	1·0	1·0	100	50	5·0*	—	92·0
	1·0	1·0	100	25	5·0*	—	95·0
c	1·0	—	100	—	5·0	—	100·0
	1·0	—	100	—	20·0	—	98·0
	1·0	1·0	100	150	5·0	—	80·0
	1·0	1·0	100	100	5·0	—	81·0
	1·0	1·0	100	50	5·0	—	99·5
	1·0	1·0	100	25	5·0	—	100·0
d	0·1	1·0	100	25	5·0	—	99·5
	0·2	1·0	100	25	5·0	—	100·0
	0·5	1·0	100	25	5·0	—	100·0
	2·0	1·0	100	25	5·0	—	100·0
	5·0	1·0	100	25	5·0	—	99·8
	1·0	4·0	100	25	20·0	—	99·0
e	1·0	1·0	100	25	5·0	5·0	100·0
	1·0	1·0	100	25	5·0	10·0	100·0
	1·0	1·0	100	50	5·0	20·0	85·0
	1·0	1·0	100	50	5·0	10·0	95·0

* Not neutralised.

In a few experiments it seemed that the presence of a little ethyl acetate or *iso*butyl alcohol improved adsorption. Quantitative experiments, however, showed that this was not so, but that ethyl acetate did not prevent adsorption when added in amounts up to 10 ml. per 125 ml. of the mixed alcohol - saline - trichloroacetate solution (Table I *e*).

EFFECT OF THE pH OF THE SOLUTION ON ADSORPTION—

Adsorption of morphine was complete at pH 6·5 to 7·0 but was incomplete in 5 per cent. (w/v) solution of trichloroacetic acid (pH about 2·0), and at this low pH codeine and heroin were also incompletely adsorbed. The importance of pH had also been shown by the necessity of treating the adsorbent with acid to ensure complete adsorption of heroin. Evidently more detailed examination of the effect of pH was necessary. Using the standard treatment of the column already described, we measured the recovery of each of the three alkaloids (in separate solution) from solutions of which 125 ml. contained 1·0 mg. of alkaloid, 100 ml. of 1 per cent. (w/v) sodium chloride solution, 25 ml. of ethyl alcohol and 5 g. of trichloroacetic acid, the pH being adjusted to the desired level (measured electrometrically) by titration with sodium hydroxide solution. As Table II shows, recovery of all three alkaloids was

TABLE II

ADSORPTION OF 1 MG. OF ALKALOID FROM SOLUTIONS AT DIFFERENT pH VALUES

pH of solution	Morphine %	Codeine %	Heroin %
9·0	98·0	—	—
8·0	100·0	100·0	99·3
7·0	100·0	100·0	100·0
6·5	100·0	100·0	100·0
6·0	100·0	100·0	100·0
4·0	95·9	100·0	83·8
2·0	84·0	77·0	—

substantially complete within the pH range 6·0 to 8·0. Above pH 8·0 there were indications that morphine was not quite completely adsorbed; below pH 6·0 adsorption of morphine and heroin was incomplete, but codeine was still adsorbed at pH 4·0, though not at pH 2·0.

METHOD APPLIED TO TISSUE EXTRACTS, BLOOD FILTRATES AND URINE FILTRATES

PREPARATION OF TISSUE EXTRACTS—

(*a*) For the alcoholic extracts, 100 g. of liver were macerated in a Waring Blendor with 200 ml. of 95 per cent. alcohol and enough tartaric acid to give an acid reaction. This mass was mixed with another 300 ml. of alcohol and then filtered. The filtrate was clear and deep yellow in colour. Each volume of filtrate was mixed with 4 volumes of 5 per cent. (w/v) aqueous trichloroacetic acid solution and filtered, in order to remove some of the proteins which were dissolved by the alcohol and which, if allowed to remain, were partially adsorbed by the Florisil. This filtrate was used for adsorption experiments after the adjustment of the pH and the addition of specified amounts of alkaloid.

(*b*) For the preparation of the aqueous trichloroacetic acid extract the method of Stewart, Chatterji and Smith[2] was used. Small pieces of liver (totalling 100 g.) were macerated in a Waring Blendor with 200 ml. of 10 per cent. (w/v) trichloroacetic acid solution. The filtrate obtained was clear and light yellow in colour. Each volume of filtrate was mixed with an equal volume of water in order to reduce the concentration of trichloroacetic acid to 5 per cent. For adsorption work, each 100 ml. of the 5 per cent. trichloroacetic acid solution was mixed with 25 ml. of 95 per cent. alcohol, which had been found to prevent the adsorption of the impurities by the Florisil but to cause no interference with the adsorption of the alkaloids (Table I).

PREPARATION OF BLOOD FILTRATES—

Trichloroacetic acid precipitation of the blood proteins produced a water-clear filtrate. One volume of blood was mixed with 1 volume of a 10 per cent. (w/v) aqueous solution of trichloroacetic acid and one-half volume of 95 per cent. alcohol. The coagulated proteins were removed by filtration and the filtrate was used for adsorption experiments after the adjustment of the pH and the addition of a known amount of alkaloid.

PREPARATION OF URINE FILTRATES—

Deep yellow-coloured normal human urine was used. As the urine contained a negligible amount of protein the use of trichloroacetic acid was unnecessary. The urine was mixed with an equal volume of water and half of its volume of 95 per cent. alcohol. The solution was filtered if necessary and, the pH having been adjusted, the alkaloid was added for adsorption measurements.

RECOVERY OF ADDED ALKALOIDS—

Tissue extracts, blood filtrates and urine filtrates, treated in the same way as the solutions in the "model" experiments, behaved peculiarly in that the recovery of added alkaloid was very small. These fluids had a final pH of about 6·5. Since the model solutions gave good adsorption over the range pH 6·0 to 8·0, it was obviously necessary to test the tissue extracts over the whole of this range. It was then found that the tissue, blood and urine extracts allowed complete adsorption only on the alkaline side of neutrality and indeed at a pH higher than is satisfactory for the model solutions, the optimal range being pH 8·0 to 9·0. There are several possible explanations of this discrepancy, but as yet we are unable to say which is correct.

Urine filtrates, and to a less degree tissue extracts, still contain pigment which is partially adsorbed under our conditions, and since the pigments are eluted by methyl alcohol, they interfere with the colorimetric determination of the alkaloids. They can, however, be removed by washing the column, prior to elution, with 200 ml. of a mixture containing water, ethyl alcohol and ethyl acetate in the proportions 10 : 3 : 2 by volume. This treatment completely removed the pigment from the column without disturbing the alkaloid.

One difficulty remained in the case of extracts that still contained traces of protein. Part of this protein was adsorbed and eluted with the alkaloid, appearing as a flocculum during the evaporation of the eluate. When this occurred the fluid was filtered through a small wad of lightly packed cotton wool and the filtrate, together with the washings (of 1 : 4 alcohol - water mixture) was evaporated to the required state for colorimetry.

With the method modified in these ways each of the three alkaloids gave complete

recovery (Table III). Although the Folin - Ciocalteu reagent, used for the final determination of the morphine, reacts with other phenolic substances, blank controls on the extracts showed either no colour at all or only a negligible trace. We have reason to believe that the method can be applied successfully to much smaller amounts of alkaloid than 0·5 mg., the limiting factor being rather the sensitivity of the quantitative methods of determining the alkaloid in the final eluate than the manipulations involved in the adsorption and elution.

TABLE III

RECOVERY OF ALKALOIDS ADDED TO EXTRACTS OR FILTRATES

Alkaloid	Amount added mg.	Extract or filtrate of—	Volume of extract ml.	Alkaloid recovered mg.
Morphine	**1·00**	**Tissue**	**250**	**1·00**
	0·50		200	0·50
	1·00	Urine	200	0·99
	0·50		250	0·50
	0·50	Blood	225	0·50
Codeine	1·00	Tissue	200	1·00
	0·50		250	0·49
	1·00	Urine	250	1·00
	0·50		250	0·49
	0·50	Blood	225	0·49
Heroin	1·00	Tissue	200	0·98
	0·50		250	0·48
	1·00	Urine	100	0·99
	0·50		250	0·48
	0·50	Blood	200	0·50

SUMMARY

Morphine, codeine, or heroin, in amounts ranging from 0·5 to 5·0 mg., contained in 100 to 250 ml. of alcohol or trichloroacetic acid extract of tissue, blood or urine, can be quantitatively adsorbed on a synthetic magnesium silicate (Florisil) and quantitatively eluted by methyl alcohol. The conditions are discussed and a method of determination is developed.

The eluted alkaloids are shown, by the purity of their colour reactions, to be readily identifiable. The method is therefore convenient for this purpose as well as for quantitative analysis.

The authors are grateful to T. & H. Smith, Ltd., Edinburgh, for generous samples of the pure alkaloids. They have also to thank Professor Sir Sydney Smith for his interest in the work.

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CLINICAL LABORATORY
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February, 1949

The Separation and Determination of Mixtures of Morphine, Heroin, Codeine and Barbiturates by Adsorption

By A. STOLMAN and C. P. STEWART

Since it had proved possible, by a process of adsorption and elution, to determine accurately as little as 0·5 mg. of morphine, codeine or heroin in extracts of body fluids,[1] it seemed desirable to investigate possible extensions of the method—to the differentiation of alkaloids present together, to the separation and identification and determination of alkaloids other than the morphine group, and to the separation of non-alkaloidal toxic substances. Adsorption techniques may eventually replace the classical but less convenient procedure of fractional extraction with various solvents as a general procedure in toxicological analysis.

In this paper we describe preliminary experiments in this direction—showing the possibility of separating completely such closely related alkaloids as morphine and heroin, as well as the possibility of determining barbituric acids in presence of morphine.

Experimental

The adsorbent, reagents and (except as described below) the conditions were as previously described by us.[1]

Separation of morphine, heroin and codeine—

The separation of the individual alkaloids from a mixture can be accomplished only if conditions can be found for their selective adsorption or selective elution. The conditions found to be optimal for the adsorption of morphine from tissue extracts or pure solutions were satisfactory also for heroin and codeine or for a mixture of all three. The obvious line of approach therefore was to test the possibility of selective elution—either by varying the pH of the eluting solvent, or by using a variety of solvents. Some control of the pH of the solvent can be accomplished by covering the column with a layer of an acid or alkaline salt, which is slowly dissolved by the hot solvent. The control is very uncertain, however, and although there were indications that, *e.g.*, heroin was more readily eluted by ethyl alcohol at a low pH than were morphine and codeine, the results were too irregular to be useful. Selective solvents were therefore sought, and it was found that acetone would elute codeine and heroin but not morphine, whereas ethyl acetate would elute codeine but not morphine or heroin when these alkaloids were present alone. Unfortunately, when the three alkaloids were present on the column together or in pairs, these solvents gave no differential elution whatsoever.

Fractional elution having failed, we directed our attention to the possibility of selective adsorption, but here it was desirable to confine alteration of conditions to the column of adsorbent, leaving the composition of the extract unchanged and uniform for all alkaloids.

Early in our work we had attempted to re-use columns on which alkaloids had been adsorbed and then eluted with methyl alcohol; we had found that such columns would not adsorb morphine from "model" tissue extracts, but that they would still adsorb codeine and heroin. On retesting methanol-treated columns with mixtures of the alkaloids, we found that selective activity remained—no more than mere traces of morphine were adsorbed, whereas adsorption of codeine and heroin was complete. Table I shows how completely morphine could, by this means, be separated from codeine or heroin. In the experiments summarised in this table the codeine or heroin was adsorbed on a methanol-treated column (refluxed for 3 hours with methyl alcohol), eluted with methyl alcohol by the method described by us[1] and determined colorimetrically. The liquid and washings from this column were passed through a second column which had been treated in the usual way by refluxing with the formic acid - ethyl alcohol - ethyl acetate mixture; the morphine was adsorbed on this second column, from which it was eluted with methyl alcohol for colorimetric determination.

Evidently, in the small quantities tested, morphine can be successfully separated from codeine or heroin, the two alkaloids being separately determined with a high degree of accuracy. Recovery of morphine was not quite complete, which is not surprising in view of the small amount present and the extra manipulations involved in the two-column technique. There was still, however, no separation of codeine from heroin under the conditions we had imposed.

Indications have been obtained that by altering the conditions—in particular by changing the composition of the model extracts—some separation may be possible. Apart from the undesirability of such changes we have to confess that complete separation has not yet been achieved. Procedures that appeared to differentiate the two alkaloids when either was

TABLE IA

SEPARATION OF MORPHINE FROM HEROIN

	Mixture	Amounts	Recovery	
			Heroin	Morphine
		mg.	mg.	mg.
(1)	Morphine	1·0		0·96
	Heroin	1·0	1·00	
(2)	Morphine	1·0		0·94
	Heroin	0·5	0·49	
(3)	Morphine	0·5		0·49
	Heroin	1·0	0·99	

TABLE IB

SEPARATION OF MORPHINE FROM CODEINE

	Mixture	Amounts	Recovery	
			Codeine	Morphine
		mg.	mg.	mg.
(1)	Morphine	1·0		0·94
	Codeine	1·0	1·00	
(2)	Morphine	1·0		0·98
	Codeine	0·5	0·50	
(3)	Morphine	0·5		0·50
	Codeine	1·0	1·00	

present alone, invariably failed when applied to a mixture. The heroin and codeine appeared to interfere with each other, a phenomenon similar to that previously encountered in our attempts at selective elution.

SEPARATION AND DETERMINATION OF BARBITURATES—

A number of experiments with Barbital (diethylbarbituric acid) and Phenobarbital (phenylethylbarbituric acid), using Florisil columns and acid or alkaline solutions similar to the model tissue extracts from which the three alkaloids had been adsorbed, showed that there was no adsorption of these barbiturates. It therefore appeared that it would be possible to separate a mixture of these alkaloids from barbiturates.

Removal of the barbiturates from the eluate of the Florisil column by adsorption on another solid was attempted. Many otherwise possible solids were unsuitable on account of their fine particle-size, which made them very inconvenient for use in an adsorption column, owing to slow percolation rate. Activated carbon with its well known adsorptive capacity for many substances, among them the barbiturates, was therefore tried for the removal of the barbiturates from the water - alcohol - trichloroacetic acid solution. Cocoa-nut shell charcoal ground to "60 to 100-mesh" particle-size was found to be the most suitable of those tried for this specific purpose.

The charcoal column was cleaned by refluxing for 2 hours with ethyl acetate in the apparatus previously described.[1] The ethyl acetate was then removed by further refluxing with ethyl alcohol and finally washing with 200 ml. of water.

The colorimetric method as described by Levvy[2] was used in this work with minor modifications. Quantitative estimations for 5 mg. of barbiturate were made by dissolving it in 2 to 3 ml. of chloroform, adding 0·2 ml. of cobaltous acetate solution and 0·6 ml. of *iso*propylamine solution. The final volume was made up to 5 ml. with chloroform. The bluish-pink colour developed immediately and was stable for more than half an hour. Comparison of the colour with that of a standard solution was made within half an hour of the development of the colour in a photo-electric colorimeter, using a blue-green filter (Ilford 302).

For the estimation of 1 mg. of barbiturate the same quantities of *iso*propylamine and

cobaltous acetate reagents were used but the final volume was made up to 2 ml. with chloroform. It was not satisfactory to employ one barbiturate as a standard for the quantitative estimation of the other barbiturates, as the various barbiturates did not produce the same amount of colour per unit concentration.

Experiments with either Barbital or Phenobarbital added to model solutions containing water, salt, alcohol and trichloroacetic acid showed that the substances were adsorbed over a wide range of pH (5·5 to 8·5), but that ethyl acetate, in concentrations of only 5 ml. per 100 ml. completely inhibited the adsorption. For these experiments the adsorbed barbiturate was eluted by refluxing for 1 hour with ethyl acetate, and the eluate carefully evaporated to dryness. The crystalline residue was taken up in 10 ml. of water acidified with hydrochloric acid and the barbituric acid was extracted from this by ether, which was then evaporated to dryness, the residue being used for colorimetric determination.

In the first attempts to isolate the barbiturates from tissue extracts and urine and blood filtrates, the barbiturates were added to the filtrates from the Florisil columns on which alkaloid had been adsorbed. Precisely the same results were obtained when barbiturate, together with morphine, codeine or heroin was added to the tissue or blood extract and the alkaloid was first removed by adsorption on a Florisil column by our standard procedure. Since it had already been found that ethyl acetate interfered with the adsorption of barbiturates and since this substance was ordinarily employed in washing the Florisil columns after adsorption of alkaloids, all filtrates from the Florisil column that were to be used for the adsorption of barbiturates had to be removed before the final washing with the water - ethyl alcohol - ethyl acetate solution. The pH of the filtrate was then adjusted for, unlike the pure solution, the tissue extracts gave poor recovery of the barbiturate from the alkaline range (Table II). At pH 5·5, however, almost complete recoveries were obtained from all the solutions. Very little loss of the barbiturate was found to take place on passage through the Florisil column. Crystalline barbital was obtained from the ether residue of all the solutions tried, including the urine filtrates. No quantitative estimation of the barbiturates in the urine was attempted at this time because the urine pigments were completely adsorbed on the charcoal column and on elution a slight amount was removed with the barbiturates. This slight amount was insufficient to interfere with the crystallisation of the barbiturate but did interfere with its colorimetric estimation.

TABLE II

RECOVERY OF BARBITURATES ADDED TO TISSUE EXTRACTS, BLOOD FILTRATES AND URINE FILTRATES

Barbiturates	Amount added mg.	Solution used	pH of solution	Barbiturate found mg.
Barbital	5·0	Tissue	8·5	3·50
	5·0	Tissue	8·5	2·30
	5·0	Tissue	5·5	4·98
	1·0	Tissue	5·5	1·00
	1·0	Blood	5·5	0·99
	5·0	Blood	5·5	4·99
	5·0	Blood	8·5	4·95
Phenobarbital	1·0	Tissue	5·5	0·97
	5·0	Tissue	5·5	5·00
	5·0	Tissue*	5·5	4·98
Barbital	1·0	Tissue*	5·5	0·96
	5·0	Tissue*	5·5	4·95

* Solutions containing morphine and barbiturate percolated through a Florisil column. In the unmarked tests the barbiturate was added to the filtrate from the Florisil column.

It must be emphasised that the experiments described in this paper are of a purely preliminary character, serving only to establish the possibility of extending to mixtures and to other substances the adsorption method previously worked out for the related alkaloids, morphine, codeine and heroin. Much more detailed work is needed before the method can be accepted as of general value in toxicological analysis. The exploratory work, however,

does indicate that such further work may be undertaken with reasonable hope of success, and it is for that reason that it has been reported at this stage.

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February, 1949

Micro-Diffusion Method for the Determination of Calcium in Blood Serum

BY M. F. MURNAGHAN

MOST of the methods for the determination of serum calcium have been based on its precipitation as oxalate, which is then determined titrimetrically (Clark and Collip, 1925[1]), colorimetrically (Sendroy, 1942[2]) or manometrically (Van Slyke and Sendroy, 1929[3]; Sendroy, 1944[4]). Titration with potassium permanganate, although the simplest and most widely used procedure, has certain disadvantages: 2 ml. of serum are generally required, the titration should be carried out at 70° to 80° C. and difficulty is experienced in obtaining a sharp end-point except in bright daylight. The manometric method possesses the advantage over the permanganate procedure that smaller amounts of serum may be used without loss of accuracy; but each estimation takes about 20 minutes, so that, if a large number are required, this method becomes time-consuming. The micro-diffusion method to be described is particularly useful where a large number of estimations have to be carried out and is as accurate as the manometric method.

In this the calcium is precipitated and isolated as oxalate, which is then dissolved in *N* sulphuric acid and oxidised by a suitable oxidant in the outer chamber of a No. 2A Conway unit[5] to carbon dioxide, which diffuses into the central chamber where it is absorbed by a barium hydroxide solution. After diffusion is complete the excess of barium hydroxide is titrated with 0·02 *N* hydrochloric acid, with phenolphthalein as indicator. A blank unit is set up at the same time, the difference between the blank and sample figures giving the amount of carbon dioxide evolved, from which the amount of oxalate present can be deduced, and from this the amount of calcium.

METHOD

REAGENTS—

Oxidant solution—Saturated ceric sulphate in *N* sulphuric acid, containing a trace of manganous sulphate.

0·05 *N Barium hydroxide solution*—25 ml. of 0·2 *N* barium hydroxide, 20 ml. of absolute alcohol, 3 ml. of 1 per cent. phenolphthalein solution in alcohol and distilled water to 100 ml.

Saturated ammonium oxalate solution—Filtered before use.

Dilute ammonia solution—2 per cent. (v/v) solution of concentrated aqueous ammonia in water.

0·02 *N Hydrochloric acid*—Prepared exactly from the *N* acid.

APPARATUS—Besides the micro-diffusion units and Conway burette, centrifuge tubes of 3-ml. capacity with pointed ends are required, and the syphoning apparatus shown in Fig. 1.

PROCEDURE—

Precipitation and isolation as oxalate—Into a 3-ml. centrifuge tube, pipette 1 ml. of distilled water and 0·5 ml. of the serum (or 1 ml. when a very low value is anticipated). Add 0·5 ml. of saturated ammonium oxalate solution, which should not touch the sides of the tube. Mix with a glass rod, and rinse the rod with distilled water until the total volume is about 3 ml. Set aside for 30 minutes and then centrifuge.

Remove the supernatant fluid with the syphoning arrangement shown in Fig. 1, until the surface of the fluid is 1 to 2 mm. above the opening in the capillary tube. The rate of

syphoning should not exceed 1 ml. per minute. Rinse the sides of the tube with 2 ml. of dilute ammonia solution without disturbing the precipitate, centrifuge and syphon. Repeat the washing, centrifuging and syphoning once more and put the tube in an oven to dry at 105° C. When the tube is dry, pipette into it exactly 0·5 ml. of *N* sulphuric acid so that the acid washes the sides of the tube. Place in a hot water-bath, stir with a fine glass rod to dissolve the precipitate and allow to cool.

Determination of oxalate—Pipette exactly 0·4 ml. of the solution into the outer chamber of the unit (No. 2A). Then pipette 0·2 ml. of the 0·05 *N* barium hydroxide into the central chamber and seal the unit as quickly as possible. (Seal each unit before pipetting barium solution into the next.) Displace laterally the cork that closes the hole in the lid, rapidly pipette 0·3 ml. of the oxidant solution through the hole into the outer chamber and rapidly replace the cork to form a firm seal. Rotate the units several times, to facilitate intimate mixing, and set them aside at room temperature for 90 minutes. Then remove the lids and titrate the excess of barium hydroxide with 0·02 *N* hydrochloric acid from the Conway burette until the pink colour of the phenolphthalein disappears. Carry out blank determinations with *N* sulphuric acid.

Calculation—If 0·5 ml. of serum was taken, the volume of 0·02 *N* hydrochloric acid used, in large divisions of the burette, divided by 2 ≡ mg. of calcium per 100 ml. of the serum. (Each large division = 0·01 ml.)

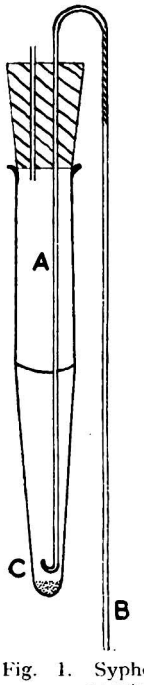

Fig. 1. Syphoning apparatus
A. Capillary with upturned end
B. Constructed outlet
C. Calcium oxalate ppt.

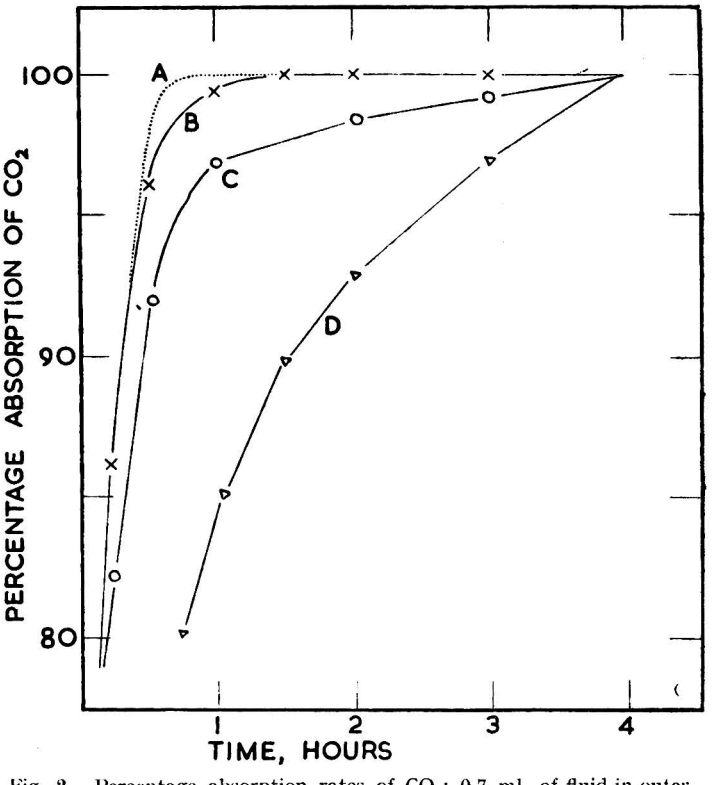

Fig. 2. Percentage absorption rates of CO_2; 0.7 ml. of fluid in outer chamber of Number 2A unit
Curve A. Acidification of bicarbonate
Curves B, C and D, 134 μg. of $(COONa)_2$ with:
B—$Ce(SO_4)_2$ (saturated) and $MnSO_4$ (trace) in *N* H_2SO_4
C—$Ce(SO_4)_2$ (saturated) in *N* or 0.2 *N* H_2SO_4
D—$Ce(SO_4)_2$ (saturated) in 5 NH_2SO_4

FACTORS INFLUENCING THE RATE OF CARBON DIOXIDE EVOLUTION—

The oxidant—Ceric sulphate has long been recognised as a suitable oxidant for oxalate. As shown in Fig. 2, Curve C, 4 hours are required for the complete evolution and absorption

of the carbon dioxide from 0·4 ml. of 0·005 *N* sodium oxalate in *N* sulphuric acid with 0·3 ml. of a saturated solution of ceric sulphate in *N* sulphuric acid as oxidant. It has been found that the presence of manganese ions enhances the rate of evolution of carbon dioxide. This is shown in Fig. 2, Curve B, only 1½ hours being required for the complete evolution and absorption when a trace of manganous sulphate is added to the oxidant solution. (For comparison, Curve A shows the absorption rate of carbon dioxide liberated by acidification of bicarbonate.) A large number of trace elements, including manganese, were tried as catalysts, but they did not increase the rate of carbon dioxide production beyond that obtained with manganous sulphate. When potassium permanganate was used as oxidant, the absorption curve was similar to that with ceric sulphate alone (Curve C, Fig. 2), but permanganate was abandoned at an early stage because it involves the use of a special fixative (a mixture of paraffin wax and liquid paraffin), as permanganate reacts with petroleum jelly, the usual fixative used in micro-diffusion procedures.

Effect of acidity with ceric sulphate alone as oxidant—The absorption curve obtained when 0·2 *N* acid was used with the cerium oxidant did not differ from that with *N* acid (Curve C, Fig. 2). On the other hand, increasing the acidity to 5 *N* caused a marked decrease in the rate of carbon dioxide evolution (Curve D, Fig. 2).

FACTORS AFFECTING THE ACCURACY OF THE DETERMINATION—

In the early stages of the investigation it was noted that the coefficient of variation for the results on a given standard solution was unduly high. This was found to arise from marked variations in the time elapsing between the pipetting of the barium hydroxide solution into the central chamber and the sealing of the units. The results shown in Table I, relating to two sets of blank units (No. 2A), illustrate this point.

TABLE I

INFLUENCE OF TIME ELAPSING BETWEEN THE PIPETTING OF THE BARIUM HYDROXIDE SOLUTION INTO THE CENTRAL CHAMBER AND THE SEALING OF THE UNITS

	Rapid sealing	Delayed sealing
Number of analyses	16	15
Mean, ml. 0·02 *N* hydrochloric acid ..	0·464	0·455
Coefficient of variation	0·9%	3·0%

It can be seen that, with rapid sealing of the units after pipetting the barium hydroxide solution, (1) the mean is greater and (2) the spread is less. This is evidently due to lessened absorption of carbon dioxide from the atmosphere. Therefore sealing should be carried out immediately after pipetting the barium hydroxide solution. Assistance at this stage, to place lids and corks in position, is advisable. This principle is applicable to all estimations carried out with the Conway unit when relatively strong alkali is used as the absorbing reagent. The glass stoppers, supplied by the makers with the Conway No. 2A unit, have a diameter of only 10 mm. and perfect sealing is occasionally unobtainable. Rubber corks of about 16 to 18 mm. in diameter at the large end and smeared with vaseline always give a perfect seal.

RESULTS

(1) *Results with standard sodium oxalate solutions*—Table II illustrates the results obtained on standard oxalate solutions (0·01 *N*, 0·005 *N*, 0·0025 *N* and 0·00125 *N* disodium oxalate in *N* sulphuric acid), with 90 minutes absorption period. When only 60 minutes were allowed for absorption, the mean recoveries were 1 to 3 per cent. low.

TABLE II

RESULTS OBTAINED WITH STANDARD SODIUM OXALATE SOLUTIONS

Absorption time: 90 minutes

Disodium oxalate present, μg.	Equivalent to calcium, μg.	Disodium oxalate found, μg.	Number of analyses	Coefficient of variation, %
268·0	80	269·0	75	1·6
134·0	40	134·0	141	2·2
67·0	20	66·7	29	3·2
33·5	10	33·5	43	6·6

(2) *Results with standard calcium solutions*—Table III shows results obtained with solutions containing 10, 5, 2·5 and 1·25 mg. of calcium per 100 ml., prepared from a stock solution containing 1 mg. per ml. (5·478 g. of $CaCl_2.6H_2O$ per litre).

TABLE III

RESULTS OBTAINED WITH STANDARD CALCIUM SOLUTIONS

Absorption time: 90 minutes

Calcium present, μg.	Calcium found, μg.	Number of analyses	Coefficient of variation, %
80	81·0	30	1·4
40	40·0	41	2·2
20	20·0	22	3·8
10	9·3	18	8·6

(3) *Results on serum by micro-diffusion compared with the Clark and Collip method*—Table IV shows the results obtained on four different human sera by the two methods.

TABLE IV

SERUM CALCIUM VALUES. COMPARISON OF THE MICRO-DIFFUSION WITH THE CLARK AND COLLIP METHOD

2 ml. of serum used in the Clark and Collip method; 0·5 ml. in the micro-diffusion method

Serum	Method	Mean calcium found, mg. per 100 ml.	Number of analyses	Coefficient of variation, %
A	C. & C.	10·50	5	3·0
A	M-d.	10·49	15	2·0
B	C. & C.	12·00	3	3·5
B	M-d.	12·15	18	2·1
C	C. & C.	10·20	5	2·5
C	M-d.	10·30	8	1·8
D	C. & C.	11·00	3	3·0
D	M-d.	11·08	12	2·2

SUMMARY

1. A micro-diffusion method, using the Conway unit No. 2A, is described for estimating quantities of oxalate ranging from 30 to 270 μg., expressed as sodium oxalate (equivalent to 10 to 80 μg. of calcium). With values between 130 to 270 μg. an accuracy to within 2 per cent. is obtainable.

2. The adaptation of this procedure to the determination of 40 to 80 μg. of calcium in blood serum (about 0·5 ml.) with an accuracy to within 2 per cent. is described.

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DEPARTMENT OF BIOCHEMISTRY AND PHARMACOLOGY
UNIVERSITY COLLEGE, DUBLIN

September, 1948

Report of the Analysts' Sub-Committee of the Ministry of Health Conference on the Differential Assay of Penicillin

Part II

The Determination of Total Penicillins by the Iodimetric Method

THE Sub-Committee appointed was as follows—

C. R. Bond, M.Sc.Tech., F.R.I.C., Imperial Chemical Industries Ltd. (*Chairman*).
L. J. Bellamy, B.Sc., Ph.D., Ministry of Supply.
A. R. Graham, Ph.D., A.R.I.C., Distillers Company Ltd. (from October, 1947).
T. Henshall, M.A., D.Phil., Distillers Company Ltd. (until October, 1947).
T. E. V. Horsley, B.Sc., F.R.I.C., Glaxo Laboratories Ltd.
G. F. Hall, M.B.E., B.Sc., F.R.I.C., Boots Pure Drug Co., Ltd. (*Hon. Secretary*).

INTRODUCTION

In Part I of this Report,[1] we have described the determination of benzyl penicillin on a weight/weight basis by precipitation with N-ethylpiperidine. At a meeting of the Conference on March 5th, 1948, it was agreed that crystalline penicillin G offered for sale ought to have a benzyl penicillin content of not less than 90 per cent. of the total penicillins present. In order to express the benzyl penicillin content on this basis it is necessary to know the percentage of total penicillins. The Sub-Committee has therefore investigated the iodimetric method originated by Alicino[2] and subsequently modified by Mundell, Fischbach and Eble.[3] Various modifications have been employed by several members of the Sub-Committee, and comprised essentially the use of—

(1) Buffered reactants to reduce the effect of non-penicillin impurities to a minimum;
(2) Different strengths of alkali for inactivation of the penicillin;
(3) Longer or shorter periods for inactivation and iodination;
(4) Temperatures of inactivation and iodination ranging from room temperature to 30° C.;
(5) Higher concentrations of potassium iodide in the standard iodinating reagent.

These modifications are detailed for the three methods described in Table I.

TABLE I

MODIFICATIONS IN USE BY MEMBERS OF THE SUB-COMMITTEE

	Method I	Method II	Method III[4]
Concentration of solution, mg./ml.	0·6	1·2	0·6
Volume of aliquot, ml.	5·0	10·0	5·0
Solvent	water	water	buffer
Concentration of sodium hydroxide solution	0·1 *N*	0·1 *N*	1 *N*
Volume of sodium hydroxide solution, ml.	5·0	5·0	1·0
Time of inactivation, minutes	30	60	30
Temperature of inactivation, °C.	30	30	room temp.
Concentration of hydrochloric acid	0·11 *N*	0·11 *N*	1 *N*
Volume of hydrochloric acid, ml.	5·0	5·0	1·0
Concentration of iodine solution	0·01 *N*	0·02 *N*	0·01 *N* (in buffer)
Volume of iodine solution, ml.	15·0	25·0	10·0
Concentration of KI in iodine solution, %	0·2	2·52	0·2
Time of iodination, minutes	15	60	30
Temperature of iodination, °C.	room temp.	30	20
Concentration of sodium thiosulphate solution	0·01 *N*	0·01 *N*	0·01 *N*
Standing time of blank, minutes	nil	nil	5
Factor (1 mg. of sodium benzyl penicillin ≡ ml. of 0·01 *N* iodine)	2·52	2·796	2·31

It will be observed that Method I is very similar to the method of Mundell, Fischbach and Eble, the two main differences being the quantity of alkali added, an amount equivalent to that used in the original method of Alicino being retained, and the introduction of a longer period and a higher temperature for inactivation.

Experimental

Preliminary work to determine the effects of modifying time and temperature in Method I gave the results shown in Table II.

Table II

Effects of time and temperature in Method I

Inactivation		Iodination				
Time min.	Temp. ° C.	Time min.	Temp. ° C.	Iodine ml.	Penicillin %	Remarks
30	20	15	20	10	94·7; 95·1	
30	20	15	20	15	95·2; 95·3	
30	30	15	20	15	97·4; 97·7	*Cf.* Method I
30	30	15	30	15	97·3	
30	30	30	30	15	98·0; 98·1	
30	30	45	30	15	99·1	
30	30	60	30	15	99·4; 99·7	
30	30	75	30	15	99·5; 99·7	
30	30	90	30	15	99·0; 99·2	
60	30	30	30	15	99·5; 99·8	
60	30	60	30	15	100·9; 101·3	*Cf.* Method II

It was thus confirmed that the conditions must be carefully standardised, if consistent results are to be obtained.

The Sub-Committee decided to make a series of tests on a standard sample of crystalline sodium benzyl penicillin (the A.S.C. Standard—*vide* Report, Part I[1]) and on a crude sodium salt (B.1005), using the methods described in Table I, with a view to selecting the procedure that gave the most reproducible and uniform results.

The composition of B.1005 was as follows—

Potency u./mg. (*S. aureus*)	% Penicillin (bio-assay)	Penicillins (*B. subtilis* units) % of total penicillins (micro-chromatogram)					
		X	U_1	G	F	D	K
1120 (3)	67	trace	trace	95	2	2	1

The figures in parenthesis here and in the following tables denote the number of determinations.

The results obtained are given in Tables III and IV.

Table III

Results obtained by the three methods on the A.S.C. standard

Member		Method I %	Method II %	Method III %
A	Range	98·0–98·1 (2)	98·1–98·2 (2)	100·7–100·9 (3)
	Mean	98·1	98·2	100·8
B	Range	97·4–98·4 (3)	96·9–99·9 (3)	98·7–101·0 (3)
	Mean	98·0	98·4	100·1
C	Range	99·1–100·0 (6)	95·8–99·6 (8)	99·6–99·7 (4)
	Mean	99·3	96·9	99·6
D	Range	99·1–99·5 (2)	97·3–97·7 (4)	101·6–101·7 (2)
	Mean	99·3	97·5	101·7
E	Range	96·7–99·2 (15)	95·1–97·2 (12)	100·7–103·0 (10)
	Mean	97·6	96·1	101·9
Difference between extreme means	..	1·7	2·3	2·3

TABLE IV

RESULTS OBTAINED BY THE THREE METHODS ON SAMPLE B.1005

Member		Method I %	Method II %	Method III %
A	Range	64·5–65·8 (4)	65·8–66·9 (4)	66·7–69·2 (6)
	Mean	65·3	66·4	67·6
B	Range	64·8–65·8 (5)	65·4–69·5 (5)	68·7–69·2 (4)
	Mean	65·3	66·7	68·9
D	Range	67·7 (2)	69·0–69·4 (2)	72·0–72·4 (2)
	Mean	67·7	69·2	72·2
E	Range	66·1–67·1 (6)	62·9–64·5 (4)	66·3–68·4 (6)
	Mean	66·8	63·7	67·5
Difference between extreme means	..	2·4	5·5	4·7

As Method I gave rather more consistent results than the others, the use of the buffer in Method III and the addition of more potassium iodide in Method II indicating no advantages in this respect, it was adopted as the basis for further work. In Method I, however, the quantities of sample and reagents used were rather small, and it was considered that these could be increased with advantage. Preliminary experiments had shown that the degree of inactivation and iodination were affected by temperature changes; as "room temperature" was insufficiently precise, it was agreed to use 30° C., this being generally the lowest temperature that could be easily controlled.

Incorporation of these modifications provided the "Tentative Method" of the Sub-Committee, which differed from Method I in using 10-ml. aliquots of a solution of the sample containing 1·2 mg./ml. and 30 ml. of 0·02 *N* iodine containing exactly 0·4 per cent. of potassium iodide and in employing 30° C. for both inactivation and iodination.

The Sub-Committee examined by this method four samples of penicillin, ranging in purity from crystalline benzyl penicillin (the A.S.C. Standard) to a very crude sample containing about 26 per cent. of penicillin, with a view to obtaining information on the reproducibility and general level of the results with penicillins of various degrees of purity.

The composition of the two samples not already described was found to be as follows—

Sample	Potency u./mg. (*S. aureus*)	% Penicillin (bio-assay)	Penicillins (*B. subtilis* units) % of total penicillins (micro-chromatogram) X	U_1	G	F	D	U_4	K
48/0058	1444 (5)	86	trace	trace	72	10	8	trace	9
M.O.S.1	438 (3)	26	—	trace	96	4	trace	—	trace

The results obtained by the different members are given in Table V.

TABLE V

RESULTS OBTAINED BY DIFFERENT MEMBERS USING THE "TENTATIVE METHOD"

Member		A.S.C. %	48/0058 %	B.1005 %	M.O.S.1 %
A	Range	99·5–100·6 (5)	82·0–82·4 (2)	65·3–66·8 (6)	27·3–27·4 (2)
	Mean	99·9	82·2	66·1	27·4
B	Range	102·8–103·7 (4)	84·8–85·3 (2)	—	26·4 (1)
	Mean	103·1	85·0	—	26·4
C	Range	98·0–102·0 (6)	77·5–85·0 (4)	60·7–65·9 (12)	26·2–27·1 (3)
	Mean	100·5	81·9	64·7	26·6
D	Range	101·3–102·3 (4)	85·6–87·2 (4)	65·9–68·7 (4)	28·1–28·8 (4)
	Mean	101·8	86·4	67·3	28·5
E	Range	97·3–98·8 (4)	81·0–82·0 (4)	63·9–66·2 (4)	25·2–26·8 (4)
	Mean	98·1	81·5	65·3	26·2
Grand Mean		100·7	83·4	65·8	27·0
Difference between extreme means	..	5·0	4·9	2·6	2·3

The conclusions reached from consideration of the above results were—

(1) Member D obtained consistently higher results than the others with all samples and member B sometimes obtained higher results. Thorough examination of all reagents and working conditions, followed by exchange of reagents by members A and D, failed to account for this.

(2) Non-penicillin impurities did not have as great an effect as might have been expected.

In an effort to reduce the variation in results, further experiments were made in which the concentration of alkali present and the temperature of inactivation and iodination were varied.

The results obtained on the A.S.C. Standard, using Method I except for the modifications stated, are given in Table VI.

TABLE VI

EFFECT OF ALKALI CONCENTRATION AND TEMPERATURES OF INACTIVATION AND IODINATION ON IODINE ABSORBED

Member	Temp. of inactivation ° C.	Temp. of iodination ° C.	Alkali added: 0·1 *N* 5 ml. %	0·1 *N* 10 ml. %	*N* 0·5 ml. %	*N* 1 ml. %	*N* 2 ml. %	*N* 5 ml. %
D	30	room temp.	98·0					100·3
	24	room temp.	98·0					100·3
	5	5	93·6					98·4
E	30	30	96·4	96·4		95·3	98·0	
	30	0	93·3	94·1		95·3		
	0	30	70·4	85·2		94·5		
	0	0		77·0	85·2	95·5	96·8	

These results show that variation in the temperature of inactivation has a considerable effect on the amount of iodine absorbed when inactivation is carried out with 0·1 *N* sodium hydroxide. This effect is, however, considerably reduced when *N* sodium hydroxide is used for inactivation.

Further experiments were made with the tentative method to find out whether the use of *N* sodium hydroxide in place of 0·1 *N* sodium hydroxide would so reduce the effects of small variations in temperature as to permit of the test being carried out at room temperature, with its normal fluctuations, without thereby introducing any appreciable error in results: at the same time the adequacy of the proposed periods of inactivation and iodination, using *N* sodium hydroxide, were examined. The results obtained are shown in Table VII. In each separate experiment the only variable was the one under investigation.

TABLE VII

EFFECT OF VARIATIONS IN TEMPERATURE AND TIME OF INACTIVATION AND IODINATION

Time of inactivation, minutes		20	30	40
Per cent. found		102·8	102·8*	102·6
Time of iodination, minutes		10	20	30
Per cent. found		102·9	102·6	102·8
Temperature of inactivation, ° C.		14	23	30
Per cent. found		102·0	101·6	101·9*
Temperature of iodination, ° C.	0	14	23	30
Per cent. found	98·2	98·6	103·8	104·1*
Temperature of inactivation and iodination, ° C.		14	23	30
Per cent. found		101·2	103·9	104·4*

* Indicates conditions of final method.

It should be noted that in Table VII the results marked with an asterisk were obtainep under the conditions of the final method. These show appreciable variation from one to another and are indicative of the variation in results to be expected with this method.

These experiments confirmed that the effects produced by variation in temperature

were too large to be ignored, particularly at the iodination stage Extension of the periods of inactivation and iodination beyond those proposed had little effect on the amount of iodine absorbed. It was concluded therefore that the tentative method should be modified in the light of these data by the use of 5 ml. of *N* sodium hydroxide in place of 5 ml. of 0·1 *N* sodium hydroxide. It was obvious, however, that with the modified conditions of assay a different conversion factor was necessary. In order to determine this factor, each member made a number of determinations on the A.S.C. Standard, which contained (*vide* Report, Part I) 98·0 per cent. of penicillins, including approximately 97·5 per cent. of benzyl penicillin.

Using a conversion factor of 0·3822 mg. of sodium benzyl penicillin per 1 ml. of 0·01 *N* iodine, which preliminary work had shown to approximate to the correct value, the results given in Table VIII were obtained.

TABLE VIII

RESULTS OF DETERMINATIONS ON THE A.S.C. STANDARD BY THE FINAL METHOD

(Replicate weighings with duplicate assays)

	Sodium benzyl penicillin found, %				
Member	Weighing 1	Weighing 2	Weighing 3	Weighing 4	Mean
A	97·7 97·9	96·9 97·2	96·5 97·5	97·1 98·1	97·36
B	98·7 99·7	98·9 99·5			99·20
C	98·0 99·0	99·0 99·0	97·5 97·0		98·25
D	99·7 99·7	98·2 98·5	98·4 98·4		98·82
E	96·4 97·1	97·3 98·8	96·8 95·5	97·5 97·5	97·11

Statistical analysis of the data in Table VIII shows that there is a significant difference between laboratories and also between duplicates. Based on the mean of duplicates and on the assumption that the variation within each laboratory is the same, the variance due to error within laboratories is 0·49; allowing for this, the variance between laboratories is 0·62. The variance of each laboratory mean and the weight to be attached to each mean are given in Table IX.

TABLE IX

WEIGHT ATTACHED TO EACH LABORATORY MEAN

Laboratory	Mean, %	Variance V	Weight 1/V
A	97·36	0·74	1·35
B	99·20	0·86	1·16
C	98·25	0·78	1·28
D	98·82	0·78	1·28
E	97·11	0·74	1·35

The weighted mean is 98·11, with a standard deviation of 0·39. Approximately four degrees of freedom may be associated with this estimate of error, which gives 95 per cent. fiducial limits of 97·03 to 99·19. This is due to differences between laboratories which in practice it has not been found possible to eliminate.

Taking these data into consideration and regarding the A.S.C. Standard as 98·0 per cent. sodium benzyl penicillin, the best estimate of the conversion factor is

1 ml. of 0·01 *N* iodine ≡ 0·382 mg. of sodium benzyl penicillin.

The error due to assuming the iodine absorption of pent-2-enyl penicillin to be the same as that of benzyl penicillin and to disregarding the difference in molecular weights between these two penicillins is negligible compared with the error of the method, in view of the very small amount (0·5 per cent.) of pent-2-enyl penicillin present in the A.S.C. Standard penicillin.

The method recommended is therefore as follows.

METHOD

REAGENTS—

0·1 *N* Sodium thiosulphate solution.

0·01 *N* Sodium thiosulphate solution prepared by dilution of the above daily.

0·1 *N* Iodine in 2 per cent. potassium iodide solution.

0·02 *N* Iodine solution prepared by dilution of the above daily.

1 per cent. Starch solution prepared by pouring a paste of 2 g. of soluble starch and 5 ml. of distilled water into a boiling solution of 40 g. of sodium chloride in 200 ml. of distilled water.

N Sodium hydroxide.

1·1 *N* Hydrochloric acid.

PROCEDURE—

Weigh accurately about 60 mg. of the sample, dissolve in distilled water and dilute to a volume of 50 ml. Transfer a 10-ml. aliquot to a stoppered flask, add 5 ml. of *N* sodium hydroxide and allow to stand for 30 minutes in a water-bath at 30° C. Acidify with 5 ml. of 1·1 *N* hydrochloric acid, add 30 ml. of 0·02 *N* iodine, close the flask with a wet stopper and place it in a water-bath at 30° C. for 15 minutes. Titrate the excess of iodine with 0·01 *N* sodium thiosulphate, adding 1 ml. of starch solution near the end-point.

Complete a blank determination by transferring a 10-ml. aliquot of the penicillin solution to a stoppered flask. Add 30 ml. of 0·02 *N* iodine and titrate immediately with 0·01 *N* sodium thiosulphate.

The difference between the two titrations represents the amount of iodine that has reacted with the penicillin. Each titration should be made in duplicate.

Each millilitre of 0·01 *N* iodine is equivalent to 0·382 mg. of sodium benzyl penicillin.

The Sub-Committee is indebted to all those who have assisted in the experimental work, and to Dr. O. L. Davies for his statistical analysis of the data in Table VIII.

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The Estimation of Trichloroethylene in Air

BY F. H. BRAIN

INTRODUCTION

IN the course of a recent investigation of the use of trichloroethylene in midwifery, the results of which are shortly to be published by Helliwell and Hutton, the need arose for a simple and rapid, but reasonably accurate, method for determining small concentrations of this drug in air. The methods recorded in the literature for the estimation of halogen-containing hydrocarbons in air fall into four classes.

1. Lamson and Robbins[1] and Robbins[2] utilised the effect of carbon tetrachloride vapour on the rate of loss of heat from a hot wire and were able to detect one part in 10,000 to 20,000 parts of air. Though the method is elegant it calls for somewhat specialised and expensive apparatus.

2. Dudley[3] decomposed carbon tetrachloride and trichloroethylene in a known volume of air by passing through a tube at 800° C. and Schayer and Ackermann[4] these and other chloro-hydrocarbons by combusting in a gas flame, the resulting chlorine and hydrogen chloride being absorbed in an alkaline solution of a reducing agent. The chloride ion was estimated gravimetrically, turbidimetrically or by Volhard's method. Dudley determined in this way carbon tetrachloride and trichloroethylene in concentrations down to 0·15 mg. per litre with high accuracy but required air samples of from 12 to 65 litres, the passage of which through the furnace took up to 30 minutes, whilst Schayer and Ackermann claim recoveries of 91 to 95 per cent. at concentrations of 100 to 400 p.p.m., using only 275-ml. air samples and 95 to 98 per cent. using 500-ml. samples. Tebbens[5] and Olsen, Smyth, Ferguson and Scheflau[6] have described apparatus similar to that of Dudley.

3. Wells[7] adsorbed the chlorohydrocarbon from a known volume of air on 40 to 50 g. of charcoal, the chloride content of a sample of which was determined by silver nitrate after hydrolysis with alcoholic potash or in a Parr bomb. The method was time-consuming and considerable difficulty was experienced

in obtaining uniform samples of charcoal for analysis. The quantities of carbon tetrachloride recovered ranged from 4 to 700 mg.

4. The remaining methods relied on the removal of the halogen-containing vapour from a measured quantity of air by passing it through a known volume of a solvent, the concentration in which was then determined in some convenient manner. Barrett[8] estimated trichloroethylene absorbed in alcohol from 1 to 3 litres of air, making use of the colour reaction discovered by Fujiwara.[9] The colour intensity was compared visually with those produced simultaneously from standard solutions of trichloroethylene in alcohol. The concentrations so found were always high, and to correct for this they had to be multiplied by the empirical factor 0·68 to bring them within the limits of error of ±11 per cent. It was claimed that as little as 20 p.p.m. could be detected though the error at this low concentration was extremely high. Webb, Kay and Nichols,[10] however, absorbing carbon tetrachloride by passing through a sintered glass plate in acetone, record a recovery of 144 ± 20 per cent. Rogers and Kay[11] improved on this, using fritted glass bubblers, designed by Rogers[12] for absorption and obtained recoveries of 103 ± 9, 100 ± 10 and 96 ± 7 per cent. of carbon tetrachloride at concentrations of 50, 112 and 209 p.p.m. respectively, with acetone as solvent. Recovery varied somewhat with the rate of air flow. These authors stated that, whilst 1-litre samples sufficed, they used, in fact, as much as 5 litres for concentrations of the order of 100 p.p.m. Wells[7] was unable to obtain satisfactory results in this way, absorbing carbon tetrachloride in alcohol, hydrolysing with alkali and estimating as chloride. That there should be some discrepancy in the results obtained by these authors is not surprising considering the volatility of the solvents employed and, in some cases, the volume of air sample passed through them. This difficulty was partially overcome by Powell[14] who employed toluene as solvent and designed an apparatus that had a sufficiently low air flow resistance to permit of its use for estimating trichloroethylene in expired air. This employed three absorption bottles of special design in parallel, for which the efficiency was estimated to be 92 per cent. for air streams containing up to 3·5 mg. per litre at a total rate of 1 litre per minute. The volume of toluene employed, however, amounted to 90 ml. in all and it was necessary to pass, according to the concentration, between 15 and 40 litres of air, which was subsequently collected in a Haldane bag for volume measurement.

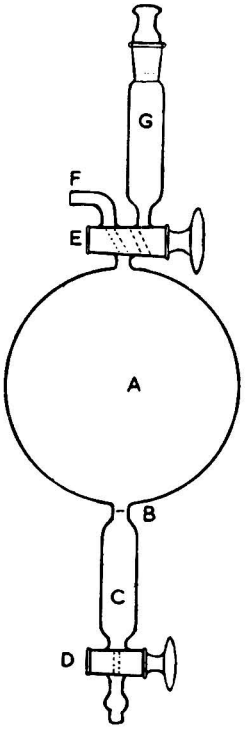

Fig. 1

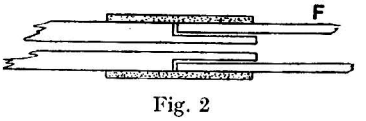

Fig. 2

For the present purpose a different method has been devised and is now described. The trichloroethylene is absorbed in anisole from an air sample of limited volume and estimated colorimetrically by means of a modified form of the Fujiwara reaction. The method presents no difficulties due to air flow and is very suitable for the estimation of trichloroethylene in expired air.

Method

Apparatus—

A glass sampling and absorption apparatus, represented in Fig. 1, was constructed. In this a calibrated bulb, A, of 200 to 250-ml. capacity, is connected by a constricted section, B having a graduation mark, to a smaller chamber, C, of about 10-ml. capacity. C terminates in a tap, D, and A in a two-way tap, E, connecting it either to the air - trichloroethylene mixture via F, or to the third stoppered chamber, G, of capacity about 10 ml. The bore of E should be at least 3 mm.

Operation—After cleaning and washing the apparatus, but before drying, lubricate the taps with a paste of fine graphite and glycerine and then wash out the apparatus with water to remove surplus lubricant. Rotate each tap a number of times during the washing until the water stream, as judged by the colour imparted to it, removes only an insignificant further amount of lubricant. Then wash out the apparatus liberally with acetone, allow to drain and thoroughly dry by sucking air through it, rotating the taps until all trace of acetone has been completely removed. The taps now have dry channels from which the lubricant has been washed away, thus minimising absorption of trichloroethylene by the lubricant. After mounting the apparatus on a stand, attach a mercury reservoir to the lower

extremity by rubber tubing and connect F to the source of the trichloroethylene - air mixture by a short capillary tube as in Fig. 2, to reduce contact with the rubber tubing to a minimum. A little fine graphite on the rubber tube facilitates detachment later. Fill A with mercury, expelling the air via G, and sweep out F and the connecting capillary by partially filling A via F–E and discharging via E–G. Then stopper G, fill A with air sample to the graduation mark of B and close D and E. Detach F and, after G has been blown out with clean air, introduce a known volume of anisole and stopper G. The volume of anisole used can be varied according to requirements, but 6 ml. has usually been found suitable. Then run out the remaining mercury in C, the slight reduction in pressure preventing seepage of solvent round the taps, detach the mercury reservoir and connect A and G. Distribute the anisole between A, C and G and shake the apparatus for 15 minutes. Ensure uniformity of the anisole solution by passing it to and fro once or twice between G, A and C. Finally invert the apparatus and transfer the solution and allow it to drain as completely as possible into G. Close E and sample the anisole as below.

ESTIMATION OF TRICHLOROETHYLENE IN THE ANISOLE SOLUTION—

The method employed for estimating the trichloroethylene in the anisole extract, depending on the measurement of a cherry-red colour formed on heating an aliquot portion with pyridine and 40 per cent. sodium hydroxide solution, has been fully described by Braih and Helliwell.[14] The method is summarised below.

REAGENTS—

Pyridine—"Pure anhydrous" or AnalaR pyridine, dried over potassium hydroxide sticks and redistilled. Lower grade pyridine should not be used.

Anisole—washed twice with dilute sodium hydroxide solution and three times with distilled water, dried over anhydrous calcium chloride and redistilled.

Sodium hydroxide solution—10 *M* in water, standardised.

Tetraethylammonium hydroxide reagent—prepared by adding to 10 *M* sodium hydroxide sufficient of the quaternary hydroxide to make it 0·025 *M* with respect to that base.

PROCEDURE—

Measure with a pipette a suitable aliquot of the anisole solution and sufficient pure anisole to make the combined volumes up to exactly 5 ml., and add both to 5 ml. of dry colourless redistilled pyridine and 3 ml. of 10 *M* sodium hydroxide in a 6-inch by $\frac{3}{4}$-inch Pyrex test tube; immediately place the test tube in a boiling water-bath and stir the mixture mechanically for 10 minutes at such a rate as to ensure thorough mixing of the two layers. This and the subsequent operations must be carried out in subdued daylight or artificial light, as bright sunlight causes rapid fading of the colour. Then remove the water-bath, add exactly 1 ml. of the tetraethylammonium hydroxide reagent to the mixture, continue stirring for exactly 1 minute, remove the tube and cool thoroughly. The two layers separate rapidly. Remove the upper (pyridine) layer by means of a pipette that has been swept out with carbon dioxide-free air (acid gases discharge the colour) and deliver it into a prepared 1-cm. absorptiometer cell by admitting carbon dioxide-free air to the pipette. The cell should have been prepared 10 minutes before use by placing in it 0·5 ml. of 10 *M* sodium hydroxide and covering with a vaselined slide, which is displaced to admit the coloured solution and then replaced. Measure the colour intensity immediately with a Spekker photo-electric absorptiometer, using a water cell in comparison and Ilford Spectrum Blue-green filters No. 603. The colour is stable in subdued light for about 20 minutes, allowing ample time for a series of readings to be taken. The first reading of a series is sometimes a trifle low.

Calibration of the absorptiometer—Standard solutions of trichloroethylene were prepared as follows. The mean weight, *W*, of anisole delivered by the 5-ml. pipette reserved for this solvent was determined. A sealed thin-glass ampoule containing approximately 80 mg. of trichloroethylene, accurately weighed, was broken under a known weight of the anisole in a stoppered bottle and the solution thoroughly mixed. This primary standard was then broken down in steps, by weight, until a solution was obtained containing exactly 10 mg. of trichloroethylene per *W'* g. of solution. From this, sub-standards were prepared by dilution by volume, using always the same pipette for both trichloroethylene solution and pure anisole. Five-ml. portions of each sub-standard were submitted in duplicate to the colour development process described above and a calibration equation derived from the mean Spekker readings so obtained for each concentration.

In the results recorded in Table I the relationship between the Spekker drum readings, S, and the weight of trichloroethylene, T mg., in the volume of anisole solution taken were related by the expression $T = 0{\cdot}1434\ S$. It was convenient if the volume of anisole used for extraction and the size of the aliquot taken were such as to give a Spekker reading of the order of 0·5 to 0·8. Each estimation (excluding the time taken in preparing the trichloroethylene - air mixtures for the experiments recorded in Table I) required approximately 1 hour and the apparatus, after cleaning the taps, washing with acetone and flushing well with warm water, was ready for use again.

RESULTS—

In testing the apparatus, standard trichloroethylene concentrations were put up as follows. Standard solutions of trichloroethylene in dry redistilled benzene were prepared by weight and the mean weight of each solution delivered by a 1-ml. pipette was determined. One ml. of each solution in turn was delivered into a dry, calibrated, approximately 10-litre

TABLE I

SUMMARY OF RESULTS

Volume of bottle, 11·25 litres. Correction for volume of benzene vapour, 0·24 litre. Volume of anisole used for extraction, 6 ml. Volume of air sample taken, 223 ml.

Concentration of trichloroethylene taken		Volume of anisole extract used	Number of estimations	Concentration found		Mean recovery for each concn. range
mg./litre	p.p.m.	ml.		mg./litre	%	
5·31	904	0·5	1	5·12	96·5	
5·76	981	"	5	5·54–5·79	96·1–100·3	98·1%
1·83		2·0	1	1·74	95·0	
1·76	300	"	1	1·74	98·6	
1·70		"	1	1·68	98·5	
1·59		"	1	1·56	98·0	
1·15	196	"	1	1·14	98·9	97·8%
0·525		5·0	3	0·512–0·532	97·5–101·1	
0·500	85	"	7	0·502–0·527	100·3–105·1	101·3%
0·351	60	5·0	2	0·366–0·368	104·2–104·8	104·6%
0·175	30	5·0	4	0·175–0·181	100·0–103·5	
0·168		"	2	0·170–0·173	100·9–102·8	101·4%

Over-all mean recovery 100·3%
Average deviation from the mean 2·3%

stoppered bottle, which was immediately stoppered and inverted into a bowl of mercury and left for 2 hours. This time was found to be sufficient for uniform distribution of the vapour. In calculating the actual trichloroethylene concentration it was assumed that the small quantity of benzene present augmented the volume of mixture as if it were behaving as a perfect gas at N.T.P. The sampling apparatus was prepared, and F (see Fig. 1) was attached to a bent capillary tube long enough to reach to the bottom of the 10-litre bottle and provided with a cardboard collar to cover the mouth of the bottle when, after unstoppering, the capillary tube was thrust into it. The sample was taken, extracted and estimated as described above.

TABLE II

Trichloroethylene, vols. % P	Concentration, C, in mg./100 ml. of blood: Sample A, Found, C_A	Sample A, Calculated*	Sample B, Found, C_B	Sample B, Calculated†
0·53	1·84	1·85	2·15	2·21
0·70	2·30	2·45	2·77	2·91
1·49	5·29	5·21	(6·76)§	6·20

* $C_A = 3{\cdot}50\ P$ †$C_B = 4{\cdot}16\ P$

§ This figure is too high, as the average value of the ratio C_B/C_A for the figures given, together with others, not quoted, for which P was not determined, was 1·19 (*i.e.*, 4·16/3·5), and C_B for a value of P = 1·49 should therefore be of the order 6·2 mg./100 ml.

An account of the use of the method by Helliwell and Hutton in connection with work on trichloroethylene as an anaesthetic for midwifery purposes will appear elsewhere in due course. I am indebted to them for permission to include the following figures amongst the results of this preliminary investigation. The table below records the concentration, C, of trichloroethylene in mg. per 100 ml., determined by the method of Brain and Helliwell,[14] in two samples of blood exposed to three different partial pressures of the drug, P, expressed in volumes per cent., which were estimated by the method described above. C should, of course, be proportional to P and this is verified for sample A and to a lesser extent for sample B, bearing in mind the normal variation of estimations on physiological material. Values calculated on the assumption of such a proportionality are included for comparison.

SUMMARY

A method is described for estimating the concentration of trichloroethylene in air samples. It has been shown to give recoveries of 95 to 105 per cent. with concentrations from 5·76 down to 0·17 mg. per litre (981 to 29 p.p.m.) using only 223 ml. of the air sample. Of the twenty-nine estimations recorded, nineteen gave results within the limits 100 ± 2 per cent. and the mean of all was 100·3 per cent.

The method would appear to be applicable to the estimation of other substances.

The cost of materials used in this work was covered by the Association of Anaesthetists of Great Britain and Ireland.

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CHEMISTRY DEPARTMENT
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Addendum to paper by Klatzkin, Norris and Wokes in the August issue, 1949, p. 454. Please add the following acknowledgement in the final paragraph—

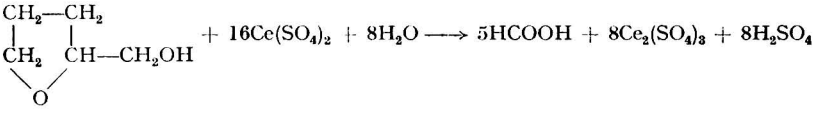
"Our thanks are due to our colleague Miss Nora Baxter for providing spectroscopic data."

Notes

NOTE ON THE DETERMINATION OF SMALL AMOUNTS OF TETRAHYDROFURFURYL ALCOHOL

RECENTLY we were called upon to determine small amounts of tetrahydrofurfuryl alcohol in aqueous solution. It was shown that this alcohol could be determined by oxidation with ceric sulphate in sulphuric acid solution at boiling temperature.

The oxidation appears to proceed as follows:—



The presence of the formic acid in the oxidation products can readily be demonstrated by steam distillation and reduction of mercuric chloride by the steam distillate; previous workers[1,2,3] have observed

that oxidation of organic compounds by the ceric sulphate procedure often results in the formation of formic acid.

The method is as follows.

SOLUTIONS REQUIRED—(1) Ceric sulphate solution, 0·1 *N*. (2) Ferrous ammonium sulphate solution, 0·1 *N*. (3) Sulphuric acid solution, 6 *N*. (4) N-Phenyl anthranilic acid indicator solution: dissolve 0·0533 g. of N-phenyl anthranilic acid in a solution of 0·05 g. of Na_2CO_3 in 5 ml. of water, and dilute the solution to 50 ml. with distilled water.

PROCEDURE—To 10 ml. of the solution under examination add 50 ml. of 0·1 *N* ceric sulphate solution, 20 ml. of 6 *N* sulphuric acid solution and 50 ml. of water and boil the mixture under reflux in an all-glass apparatus for 1 hour and then cool. Titrate the cold solution with 0·1 *N* ferrous ammonium sulphate solution until a slight excess is present, and determine this excess by titration with 0·1 *N* ceric sulphate solution, using two drops of N-phenylanthranilic acid indicator solution. The end-point change is from yellow to rose madder.

Make a blank determination on 50 ml. of the 0·1 *N* ceric sulphate solution under corresponding oxidation conditions. It is desirable that in the determination itself not more than one half of the ceric sulphate should be used up in the oxidation.

1 ml. of 0·1 *N* ceric sulphate solution = 0·000638 g. of tetrahydrofurfuryl alcohol.

The following results have been obtained on oxidation of known amounts of tetrahydrofurfuryl alcohol.

Tetrahydrofurfuryl alcohol taken, g.	0·147	0·0065	0·0017
" " found, g.	0·148	0·0063	0·0017

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

J. HASLAM
L. H. RUDDLE

February, 1949

THE DETERMINATION OF ETHYL ALCOHOL IN ETHYL ETHER

SOME technical supplies of ether used during the war for the extraction of propellant mixtures were found to contain as much as 0·5 to 1·0 per cent. of ethyl alcohol as impurity and it was found that this was not removed by the usual treatment with potassium permanganate and potassium hydroxide followed by drying and distillation. The impurities present in this ether were isolated by careful re-fractionation and shown to consist of ethyl alcohol together with a trace of acetone and of mineral jelly.

Since small quantities of ethyl alcohol in supposedly pure ether may cause variable solubility losses when nitrocellulose is determined as the residue after prolonged extraction with ether, it was important to detect and determine the amount of alcohol present and to ensure its complete removal.

In the determination of acetone in ether by Messinger's method[1] it has been shown that, whilst the method is satisfactory for acetone even at high dilution, no reaction occurs with ethyl alcohol at similar concentrations and the presence of this impurity is not disclosed. An additional determination of the alcohol content of ether was therefore necessary and one that has been found to be particularly suitable depends on the formation of a red co-ordination complex between alcohols and ceric ammonium nitrate solution.[2] A method based on this reaction is described by F. Duke,[3] who recommends the use of a photo-electric spectrophotometer to measure the intensity of the colour produced, and corrects for the fading caused by oxidation of the alcohol by extrapolating to zero time. However, the determination may be made more simply and rapidly by the method described below, requiring only the simplest apparatus. A single determination can be made in a very few minutes with an accuracy quite adequate for the purpose, the effect of fading being minimised by the short period required for the matching of the colour produced.

It should be noted that the colour is given by all the lower alcohols and that aldehyde, amines, etc. interfere.[3]

METHOD—

To prepare the ceric ammonium nitrate solution, dissolve 667 g. of ceric ammonium nitrate, $(NH_4)_2Ce(NO_3)_6$, in the minimum quantity of water and make up the total volume to 1 litre. Filter the solution if cloudy. This reagent will keep for an indefinite period.

Extract 50 ml. of the ether to be tested in a separating funnel with three 15-ml. portions of water. Place the combined extract in a 50-ml. Nessler cylinder. Into a second cylinder measure 40 ml. of distilled water and 5 ml. of the reagent. Finally, add 5 ml. of the reagent to the cylinder containing the aqueous extract and rapidly titrate the contents of the second cylinder with a 3 per cent. (w/v) solution of alcohol

in water. When the colour of the blank almost matches that in the original tube adjust the volume of liquid in the two tubes to the same level before completing the titration. When the colour matches

the alcohol content of the ether, g./100 ml. = ml. of 3% alcohol required × 0·06
and " " " " " g./100 g. = ml. of 3% alcohol required × 0·06/0·720.

This method was used to determine the alcohol content of known ether - alcohol mixtures with the result shown below.

TABLE I

Alcohol present in ether, g./100 ml.	Titre of 3% alcohol ml.	Alcohol in ether, determined g./100 ml.
0·10	1·65	0·099
"	1·70	0·102
"	1·65	0·099
"	1·65	0·099
"	1·60	0·096
0·20	3·20	0·192
"	3·30	0·198
"	3·30	0·198
"	3·25	0·195

As will be seen from Table I the extraction of alcohol is complete under the conditions of the method and subsequent estimation of quantities between 0·10 and 0·20 per cent. is satisfactory. The end-point may be judged to within 0·1 ml., corresponding to an error of 3 per cent. on 0·10 per cent. of alcohol.

The method will detect the presence of 0·01 per cent. of alcohol.

PREPARATION OF ALCOHOL-FREE ETHER—

Ether may be rendered completely free from alcohol by washing with a 10 per cent. solution of sodium chloride. One litre of ether can be conveniently treated in a 2-litre separating funnel with three successive 300-ml. portions of salt solution. The solubility of ether in 10 per cent. salt solution is small and the loss of solvent on this account is negligible.

The alcohol-free ether is dried and distilled in the usual way.

Acknowledgment is made to the Chief Scientist, Ministry of Supply, for permission to publish this paper.

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CHEMICAL INSPECTORATE
MINISTRY OF SUPPLY

J. LAMOND
April, 1949

A NEW METHOD FOR THE DETECTION OF SEMI-MICRO QUANTITIES OF ZINC IN COBALT SALTS

FEW methods are available for the detection of small amounts of zinc in cobalt salts. Removal of cobalt by the addition of alkali always leads to excessive co-precipitation of zinc. Most of the methods available are only of use when the zinc/cobalt ratio is of the order 1/1000, and two polarographic methods have been described[1,2] that give a means of separation of the two elements provided that cobalt is not in great excess. An accurate but lengthy dithizone separation may be used[3] but the procedure described below serves as the basis for a rapid limit test which will detect 50 p.p.m. of zinc in cobalt salts. The procedure is similar to that described by Miller[4] but the separation of the zinc depends on the greater solubility of zinc thiocyanate in ether. Part of the cobalt is also extracted as the thiocyanate but the bulk is retained in the aqueous phase. The small amount of cobalt that is extracted with the zinc can be removed from active interference by conversion to potassium cobalticyanide, which then serves as a precipitant for zinc in acid solution.

METHOD—

Dissolve 5 g. of cobalt chloride, nitrate or sulphate in 20 ml. of water, add 1 ml. of 5 *N* hydrochloric acid and 5 ml. of 7·5 *M* ammonium thiocyanate and extract with 15 ml. of ether, shaking vigorously. Evaporate the ether from the ethereal extract and to the residue add cautiously 0·5 ml. of nitric acid (sp.gr. 1·42). When the vigorous reaction has ceased, evaporate to dryness, dissolve the residue in 1 drop of 5 *N* hydrochloric acid and 4 ml. of water, boil and filter. To the filtrate add 1·5 *M* potassium cyanide solution drop by drop until the precipitate formed just redissolves. Pass a current of air through the solution

for 5 minutes, add 2 drops of hydrochloric acid (sp.gr. 1·18) and allow to stand for 1 hour. In presence of 0·25 mg. of zinc a turbidity will be formed.

Thanks are due to the Directors of Hopkin & Williams Limited for permission to publish this work.

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E. A. JOHNSON
W. C. JOHNSON

April, 1949

Ministry of Food

CIRCULAR MF15/49

(England and Wales)

This circular, dated August 31st, 1949, of which the following is a summary, draws attention to

The Milk (Special Designation) (Raw Milk) Regulations, 1949,

The Milk (Special Designation) (Pasteurised and Sterilised Milk) Regulations, 1949,

(see below), which re-enact with amendments the provisions of the Milk (Special Designations) Regulations, 1936 to 1948. The major changes introduced in these regulations are consequent upon the relevant provisions of the Food and Drugs (Milk and Dairies) Act, 1944 (which comes into operation on October 1st, 1949) as amended by the Food and Drugs (Transfer of Functions) Order, 1948, and the Milk (Special Designations) Act, 1949. Other changes of detail which experience has shown to be desirable and, in some particulars, necessary have been made.

4. For the time being the use of special designations in relation to milk will remain a voluntary act on the part of the person or council concerned; in due course, however, when areas have been specified under the Milk (Special Designations) Act, the position will be radically different in as much as the use of special designations in specified areas will be obligatory upon all who wish to sell milk by retail in such areas. Local authorities and Food and Drugs Authorities concerned with the execution and enforcement of the regulations in specified areas will have added responsibilities as the agents of the central government for carrying into effect the policy which has been given legislative form in the Milk (Special Designations) Act, 1949.

Paragraphs 5 to 9 of the Memorandum are concerned with provisions relating to dealers' licences.

Paragraphs 10 to 12 relate to the Milk (Special Designation) (Raw Milk) Regulations. The designations authorised by these regulations are, as at present, "Accredited" and "Tuberculin Tested." The use of the former designation is, however, now limited to a period of five years from the commencement of these regulations, *i.e.*, until October 1st, 1954; in addition, it should be noted that section (2) of the Milk (Special Designations) Act restricts the use of the designation "Accredited" in specified areas to milk derived from a single herd.

Paragraphs 13 to 16 relate to the Milk (Special Designation) (Pasteurised and Sterilised Milk) Regulations. The designations authorised under these regulations are "Pasteurised" and "Sterilised"; the latter is a new designation and has been incorporated into the regulations in anticipation of the time when areas are specified and it will be necessary to legalise in them the sale of sterilised milk.

14. *Licensing authorities*—Responsibility for the granting of dealers' (pasteurisers' and sterilisers') licences has been transferred from local authorities to Food and Drugs Authorities who are responsible for the execution and enforcement of many allied provisions of the Milk (Special Designations) Act.

15. *General and special conditions subject to which licences are granted*—The general and special conditions laid down in the schedules to these regulations are additional to any relevant provisions of the Milk and Dairies Regulations and of the Milk (Special Designation) (Raw Milk) Regulations. The regulations include details of the pasteurising and sterilising processes, they provide for both the Holder and the Higher Temperature Short Time processes and in addition give the Authority, with the Minister's approval, discretionary power to approve any other time and temperature, a provision which will enable the Authority, with the Minister's approval, to keep in step with new methods of heat treatment which tests have shown to be satisfactory. The Minister, on the advice of the best authority available, has reduced the minimum temperature under the H.T.S.T. process by 1° F. Under properly operated conditions there is, on present evidence, no risk to health, but the change will result in an improved "cream line" on the milk and will doubtless help to popularise pasteurised milk among consumers. The Authority will note that as from October 1st, 1950, until October 1st, 1954, milk which is pasteurised in bottles (in-bottle pasteurisation is provided for in the regulations) shall be delivered to the consumer in those bottles, and milk which is pasteurised by the usual method in other containers shall be put into the bottles or other containers in which it is to be delivered to consumers only by the person holding a licence to use the designation "Pasteurised"

in relation to such milk and on registered premises named in that person's licence. As from October 1st, 1954, all pasteurised milk shall be put into bottles or other containers in which it is to be delivered by retail on the premises on which it is pasteurised.

16. Both the Raw Milk Regulations and the Pasteurised and Sterilised Milk Regulations make provision for keeping designated milk apart from all other milk. In each case the dealers' arrangements must be adequate to ensure that the milk to which the licence applies is not contaminated by any other milk. The Minister is aware that the corresponding provision in the Milk (Special Designations) Regulations, 1936 to 1948, has given rise to difficulty and variation of interpretation, with the result that, in some districts, Tuberculin Tested milk is being handled in small and congested premises and the main object of the provision is in danger of being defeated. The present regulations have a proviso contained in paragraph 2 of the First Schedule, which has been inserted with the object of assisting licensing authorities in the execution and enforcement of the condition. The Minister sees no objection to undesignated milk and designated milk or to two or more kinds of designated milk being handled, etc., on the same premises provided that adequate arrangements are made to prevent the milk to which the designation applies being contaminated by any other milk, and he is of the opinion that the cleansing of vessels and plant can be dispensed with when the circumstances are such as to exclude any possibility of contamination of the designated milk by any other milk. The use of vessels and plant for handling Accredited milk *immediately* after such vessels and plant had been used for handling Tuberculin Tested milk would, in his opinion fall into this category; on the other hand, the handling of pasteurised milk immediately after raw milk (whether designated or not) should not be permitted, as in such circumstances there may be some risk of contamination of the pasteurised milk by the unpasteurised milk.

Ministries of Food, Health, and Agriculture and Fisheries

STATUTORY INSTRUMENTS*

1949.—No. 1588. The Milk and Dairy Regulations, 1949. Pp. 17. Price 6d.

These Regulations, issued jointly by the Ministry of Health, the Ministry of Agriculture and Fisheries and the Ministry of Food, came into operation on October 1st, 1949. They re-enact with amendments the Milk and Dairies Regulations, 1926 to 1943.

The principal changes are consequent upon the provisions of the Food and Drugs (Milk and Dairies Act), 1944, and the Agriculture (Miscellaneous Provisions) Act, 1949.

The Minister of Agriculture and Fisheries thus becomes responsible for the registration of dairy farms and dairy farmers and for the enforcement of the regulations on dairy farms (except in so far as they relate to diseases communicable to man).

The local authorities retain responsibility for those provisions which apply outside dairy farms, for the provisions relating to diseases communicable to man and for the registration of dairies that are not dairy farms and of dairymen who are not dairy farmers.

A Central Milk and Dairies Advisory Committee and County Milk and Dairies Advisory Committee are set up to review and make recommendation as to the operation of these Regulations and the Milk (Special Designation) Regulations.

New provisions are made for the inspection of cattle on dairy farms by veterinary inspectors of the Ministry of Agriculture and Fisheries.

There are a number of minor changes relating to buildings, the cleansing of vessels and utensils and the distribution of milk and its protection against contamination and infection and, in particular, provision is made for modern practices in dairying, including the use of mechanical refrigeration for cooling and of approved chemical agents for cleansing apparatus and appliances.

— **No. 1589. The Milk (Special Designation) (Pasteurised and Sterilised Milk) Regulations.** Pp. 18. Price 6d.

These Regulations made jointly by the Ministry of Health and the Ministry of Food came into operation on October 1st, 1949. They revoke and re-enact with amendments the Milk (Special Designations) Regulations, 1936 to 1948 (S.R. & O., 1936, No. 356; 1938, No. 218; P.R. & O., July 9th, 1941; S.R. & O., 1942, No. 771; 1943, No. 1645; 1946, No. 10; and S.I., 1948, No. 1117), so far as they relate to pasteurised milk and provide for a new special designation, "sterilised milk."

The principal changes are consequent upon the provisions of the Food and Drugs (Milk and Dairies Act, 1944, and the Milk (Special Designations) Act, 1949. In accordance with the first-mentioned Act, as modified by the Transfer of Functions (Food and Drugs) Order, 1948, regulations dealing with the special designations of raw milk are made jointly by the Minister of Health, the Minister of Agriculture and Fisheries and the Minister of Food, and are contained in separate regulations, the Milk (Special Designation) (Raw Milk) Regulations, 1949. These new regulations are made jointly by the Minister of Health and the Minister of Food; they are, therefore, concerned only with the special designations of heat-treated milk.

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* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

The special designations which may be used in accordance with these regulations in relation to milk are "Pasteurised" and "Sterilised."

The regulations provide also for the use of the special designations "Tuberculin Tested Milk (Pasteurised)" and "Tuberculin Tested Milk (Sterilised)."

The conditions under which milk shall be pasteurised or sterilised are closely defined and the provisions for sampling laid down.

The procedures for the phosphatase and methylene blue tests for pasteurised milk, and the turbidity test for sterilised milk are described in full detail (pp. 4).

— **No. 1590. The Milk (Special Designation) (Raw Milk) Regulations.** Pp. 21. Price 6d.

These Regulations made jointly by the Ministry of Health, the Ministry of Agriculture and Fisheries and the Ministry of Food came into operation on 1st October, 1949. They revoke and re-enact with amendments the Milk (Special Designations) Regulations, 1936 to 1948, so far as they relate to raw milk (the revoked orders are the same as those noted under Regulation No. 1589, above).

The special designations authorised by these regulations which may be used in relation to milk are "Tuberculin Tested" and "Accredited."

After October 1st, 1957, the special designation "Tuberculin Tested" may only be used in respect of milk from a herd which is on the Register of Attested Herds kept by the Minister of Agriculture and Fisheries.

After October 1st, 1954, the special designation "Accredited" will no longer be permitted.

These regulations provide that milk sold under a special designation shall satisfy a prescribed methylene blue reduction test.

Provisions as to sampling, the transport and keeping of samples, and identification of samples are laid down.

The procedure to be followed in the methylene reduction test and its interpretation are described in full detail.

British Standards Institution

DRAFT SPECIFICATIONS

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

Draft Specification prepared by Technical Committee ISE/18—Sampling and Analysis of Iron and Steel.

CK(ISE) 5633—Draft on the Determination of Chromium in Ferro-Chromium (Superseding CK(ISE) 4375).

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

CK(OSC) 6427—Draft for Crude Sperm Oil (Revision of B.S.997—1941).

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Biochemical

Colorimetric Determination of Thyroxine. D. Winikoff and V. M. Trikojus (*Biochem. J.*, 1948, **42**, 475–480)—The method is based on the colour formed by treating thyroxine with N^1-diethylsulphanilamide in alkaline solution.

REAGENTS—(1) *N^1-diethylsulphanilamide*—Dissolve 40 g. of 4-acetamido-benzenesulphonyl chloride in 200 ml. of acetone and add this solution to a cold aqueous solution of diethylamine prepared from 44 g. of diethylamine hydrochloride and 200 ml. of 2 *N* sodium hydroxide. Cool and add 16 g. of powdered sodium hydrogen carbonate. Keep at room temperature for some hours, shaking frequently. Remove the acetone by heating under reduced pressure on a water-bath, chill the residue, and add 200 ml. of water. Boil the precipitate for a few minutes in 40 ml. of 6 *N* hydrochloric acid. Add water and heat for a few minutes. Cool the hot filtered solution and make it alkaline with 2·5 *N* sodium hydroxide. Wash the resulting precipitate with water and re-crystallise first from 60 per cent. ethanol with the aid of charcoal and then from a mixture of ethanol and light petroleum (b.p. 60° to 80° C.). The product should be colourless prisms, m.p. 105° to 106° C. *Diazotisation*—Dissolve 1·14 g. of the amide in 9 ml. of 10 *N* hydrochloric acid. Dilute to 100 ml. with water. Take 5 ml. of this solution and, keeping it at 0° C., add 5 ml. of 4·5 per cent. (w/v) sodium nitrite solution. Add 20 ml. of ice-cold water, and use the reagent after 10 min. standing.

(2) n-*Butanol*—Purify by distillation over solid sodium hydroxide and use the fraction boiling at 116° C.

PROCEDURE—Use 2 ml. of a solution containing 1 to 5 mg. of thyroxine per 100 ml. in 0·1 *N* sodium

hydroxide. Place in a glass-stoppered, 15-ml. centrifuge tube, add 2 ml. of *n*-butanol and then 1 ml. of 2 *N* sodium hydroxide. Mix by shaking, but do not let the liquid reach the neck of the tube. Add 3 ml. of a solution containing 1 ml. of 2 *N* sodium hydroxide and 2 ml. of the diazonium solution. Stopper the tube and shake well for 15 sec. Set aside for 5 min., remove the stopper, and then keep the tube for 24 hr. on a gently-shaken rack in a thermostat at 25° C. Also place on the rack a control tube containing 2 ml. of 0·1 *N* sodium hydroxide plus reagents.

Stopper the tubes and centrifuge for 30 min. at 2500 r.p.m. Transfer the butanol phase with a Pasteur pipette to a small dry tube. To 0·75 ml. of this solution add 0·25 ml. of *N* alcoholic sodium hydroxide to prevent the formation of a turbidity. The solution can be stored for 24 hr. if evaporation of solvent and access of carbon dioxide are prevented. Determine the absorption with a Hilger Spekker absorptiometer, using the Ilford green filter (maximum absorption is at 540 mμ.). Prepare a calibration curve based on pure thyroxine.

A preliminary treatment of some biological material may be required. Treat with ether before extracting the acid-insoluble thyroxine precipitate with butanol or else extract the thyroxine into aqueous alkali from the butanol extract by adding light petroleum. Re-extract the aqueous phase with butanol and continue as above.

W. S. Wise

Determination of 4-Methyl-2-Thiouracil in Animal Tissue and Blood. H. van Genderen, K. L. Van Lier, and J. De Beus (*Biochim. et Biophys. Acta*, 1948, **2**, 482–486)—Difficulties encountered in the determination of low concentrations (*e.g.*, 1 mg. per 100 g.) of 4-methyl-2-thiouracil by previous methods for the determination of thiouracil (Williams *et al.*, *J. Clin. Invest.*, 1944, **23**, 613; *J. Lab. Clin. Med.*, 1944, **29**, 329) were due not to the lack of sensitivity of the colour reaction with Grote's reagent, but to the method of precipitation. The recovery of added methylthiouracil was poor, and red-coloured reaction products of Grote's reagent with impurities were formed.

From a study of the effects of pH and the distribution of methylthiouracil between different buffer - organic solvent systems along the lines indicated by Morch (*Acta Pharmacol. Toxicol.*, 1945, **1**, 106), a routine experimental procedure has been evolved.

The purification of a chloroform - ethanol (20 per cent.) extract of the acidified and dried blood or tissue pulp, by partition between an acid aqueous phase and chloroform (without ethanol), makes possible constant, high recoveries of added methylthiouracil. With a buffer of pH 8 almost all the methylthiouracil remains in the aqueous phase.

In presence of ethanol, the development of the colour with Grote's reagent is slower, but the colour is very stable.

Method—*Preparation of Grote's reagent*—Dissolve 1 g. of sodium nitroprusside and 1 g. of hydroxylamine hydrochloride in 20 ml. of water, add 2 g. of sodium bicarbonate, shake until the effervescence stops, add 0·1 ml. of bromine, and shake again. Filter the solution into a volumetric flask and dilute with water to 50 ml. The reagent can be kept in the ice-box for about 10 days. Variations between different batches of the reagent are difficult to avoid, and it is therefore necessary to conduct an experiment with a standard solution of methylthiouracil once a day.

Colorimetric reaction—Adjust the extract of serum, blood or tissue to a pH of from 7·9 to 8·1 with a boric acid - borax buffer solution of pH 8·0. Add 10 per cent. of 96 per cent. ethanol to the extract, followed by 0·4 ml. of Grote's reagent per 10 ml. of extract, and allow the mixture to stand at room temperature for 30 min. Measure the light extinction in a spectrophotometer at 6650 A. in a 3-cm. cell. The Beer - Lambert law holds for the extinction - concentration curve over a wide range.

Precipitation method for serum—Add 5 ml. of a freshly prepared mixture of equal parts of 10 per cent. sodium tungstate solution and 0·66 *N* sulphuric acid to 5 ml. of blood serum in a centrifuge tube. Shake the tube vigorously and centrifuge. Take 5 ml. of the clear supernatant fluid and add 2 ml. of borate buffer (pH 8·0) and 1 ml. of ethanol. Adjust the pH of the sample to 8·0 and make the final volume 10 ml. Of 1 mg. of methylthiouracil added to the serum per 100 ml., 82 to 88 per cent. can be recovered.

Extraction procedure for blood and tissue—Grind 10 g. of tissue in a mortar with sand and add 7 to 10 ml. of 0·5 *N* alcoholic hydrochloric acid, to bring the pH of the material to below 4. Transfer the material to a distilling flask with a few millilitres of ethanol and evaporate the extract to dryness under reduced pressure on a water-bath at 70° C. Repeat the evaporation after addition of 10 ml. of methanol. For blood, pipette 10 ml. of blood directly into the distilling flask, add 10 ml. of 0·5 *N* alcoholic hydrochloric acid and, after mixing, follow the evaporation procedure described for tissue. Disperse the dried tissue or blood cake with 10 ml. of ethanol and add 50 ml. of chloroform or of methylene chloride. Heat under refluxing conditions for 15 min. on a water-bath and decant the liquid through a filter into a distilling flask. Repeat twice the extraction with the chloroform - ethanol mixture, combine the three extracts and filter. Evaporate the filtrate to dryness on the water-bath. With successively 5 ml., 3 ml., and 2 ml. of chloroform (or methylene chloride) and 3 ml. of distilled water transfer the residue to a centrifuge tube. Transfer most of the clear water layer to a tube and extract the chloroform with two more 3-ml. portions of water. Combine the three aqueous extracts, adjust the pH to 8 by means of borate buffer and sodium hydroxide solution. Add 1·5 ml. of ethanol before filtering through a hard filter if necessary. Dilute to a volume of 15 ml. with the buffer solution and add 0·6 ml. of reagent. Continue the determination as previously described.

Results—Blank determinations on blood or tissue without methylthiouracil usually give extinction readings slightly higher than those of aqueous "blanks." The colour is yellow, but light red or

brown discolorations, which do not necessarily interfere with the measurement of the green reaction product of methylthiouracil, may appear.

For a single determination in the range of 100 μg. of methylthiouracil per 10 g. of tissue the result is expected to have an error not greater than ±6 μg. G. A. STEWART

Use of Sodium Hypochlorite in the Determination of Alanine, Valine, and Leucine. E. Aubel and J. Asselineau (*Biochim. et Biophys. Acta*, 1948, **2**, 198–206)—The general reaction and the method for alanine are dealt with in a previous paper (*Idem., Bull. Soc. Chim.*, 1947, 114 M; *Analyst*, 1948, **73**, 693).

Determination of valine and leucine in a mixture of the two—The process involves the separation of *iso*butyraldehyde (b.p. 63° C.) and *iso*valeraldehyde (b.p. 92° C.). The aldehydes are therefore entrained from the reaction flask into carbon tetrachloride, which has an intermediate boiling-point (76° C.), and the *iso*butyraldehyde is then separated by the use of a 1·5-metre fractionating column packed with glass rings and jacketed with a water-cooled condenser on the upper portion. The boiling-flask has an air inlet tube that passes into the liquid, and the head of the column consists of a swan-neck leading into a trap containing potassium bisulphite solution. The *iso*butyraldehyde concentrates at the top of the column and is entrained during 1·5 hr. into the bisulphite by a suitably regulated air current. The excess of bisulphite is titrated with iodine in the usual manner. Results are up to 5 per cent. high and results for *iso*valeraldehyde, which are obtained by difference, are correspondingly low.

Analysis of mixtures of alanine, valine, and leucine—When alanine is also present the acetaldehyde produced is entrained with the *iso*butyraldehyde during the fractionation. The acetaldehyde is then determined by the colorimetric method (*loc. cit.*). Valine + alanine are determined iodimetrically, and valine by difference. Leucine is also calculated by difference after straight entrainment of all three aldehydes followed by iodine titration. (In the example quoted, alanine is determined in the mixture of the *three* aldehydes.) In a table of results for various mixtures of the three amino acids the maximum errors range from +6 to −4·5 per cent. for alanine, from +4 to −7·6 per cent. for valine, and from +6·7 to −15 per cent. for leucine.

Determination of valine in protein hydrolysates—When applied to a complex mixture of amino acids the usual process of air entrainment leads to low results for valine and leucine; steam distillation is therefore adopted. Under these conditions the aldehydes derived from glycine, phenylalanine, and methionine are also entrained; consequently leucine cannot be determined by difference. *Procedure*—Decompose the chloroamino compounds by adding the reaction mixture during 15 to 20 min. to 60 ml. of boiling 0·1 *M* phosphate buffer, receiving the distilled aldehydes in 10 ml. of water cooled in ice. Transfer the receiver to a Fuchs apparatus (*Z. physiol. Chem.*, 1933, **221**, 271), heat the solution to boiling, maintain an air current of 500 ml. per min., and receive the aldehydes in carbon tetrachloride cooled in an ice - salt mixture. This operation requires 15 min. Fractionate the carbon tetrachloride solution of aldehydes, determine the total aldehydes in the distillate by iodine titration, the acetaldehyde colorimetrically, and the *iso*butyraldehyde, and thence the valine, by difference.

Determination of leucine in a complex mixture of amino acids—The mixture considered contains 3 to 6 mg. each of leucine, alanine, valine, glycine, aspartic acid, phenylalanine, and methionine. The solution is passed through a column of 5 g. of "acid" alumina (Asselineau, *Bull. Soc. Chim.*, 1947, 1065 M), concentrated under reduced pressure, adjusted to pH 6·5 to 6·8, and passed through a column of activated silver sulphide (Hamoir, *Biochem. J.*, 1945, **39**, 485). The usual hypochlorite treatment is applied. The formaldehyde derived from the glycine is retained by the addition of asparagine to the phosphate buffer (Peynaud, *Bull. Soc. Chim.*, 1946, **40**, 685). The distillate contains only the aldehydes derived from leucine, valine, and alanine. The analysis is completed according to the principles already described.

W. C. JOHNSON

Determination of Copper and Lead in Biological Material. P. R. v. d. R. Copeman (*Union of South Africa, Dept. of Agric., Science Bulletin No.* 251)—Satisfactory procedures have been evolved from well known methods for the determination of these elements in biological material.

Preparation of the sample—Treat a known weight of material (not more than 50 mg.) with 100 ml. of 10 per cent. nitric acid in a Kjeldahl flask, gradually bringing the solution to the boiling-point, until the material is completely disintegrated. If any undissolved material remains, filter it off and treat it with a small amount of nitric acid in another Kjeldahl flask. Add 10 to 15 ml. of concentrated sulphuric acid to the combined solutions and evaporate, adding a small amount of concentrated nitric acid to the hot solution when it begins to darken. When a clear solution remains and white fumes appear, cool, and add 20 ml. of water or of a 10 per cent. solution of ammonium oxalate. Evaporate again until white fumes appear, cool and dilute the solution to a known volume in a volumetric flask.

Procedure for the determination of copper—To 5 ml. of a solution representing 50 mg. of tissue in 50 ml. add 5 ml. of a 20 per cent. solution of citric acid and 2 ml. of a 4 per cent. solution of sodium pyrophosphate. Make the solution alkaline to litmus with concentrated aqueous ammonia solution adding 0·5 ml. in excess, cool, and add 2·5 ml. of carbon tetrachloride followed by 1 to 2 ml. of a 2 per cent. aqueous solution of sodium diethyldithiocarbamate. Shake the mixture well, and after allowing the liquids to separate, run off the golden-yellow carbon tetrachloride solution into a dry measuring cylinder. Repeat the extraction with further 2·5-ml. portions of carbon tetrachloride until no further colour is extracted. Dilute to a known volume with carbon tetrachloride and com-

pare the colour with that of a standard prepared in the same way. It is necessary to carry out a blank determination and apply the appropriate correction.

The carbon tetrachloride extract must be free from water and, if necessary, it can be dried by adding a small amount of anhydrous sodium sulphate and shaking. It should not be filtered through a filter paper, since such paper usually contains traces of copper. Using the above procedure, no substance normally interferes. Excellent recoveries are obtained for determinations on various animal tissues as well as on materials such as paper, cereals, and salt.

Procedure for the determination of lead—Transfer a portion of the solution (usually 25 ml.) to a separating funnel, add 5 ml. of a 20 per cent. solution of citric acid, and make the solution alkaline to litmus with concentrated aqueous ammonia solution, adding a slight excess. Cool, add 2 ml. of a freshly prepared 10 per cent. solution of potassium cyanide and extract with 5 ml. of a 0·05 per cent. solution of dithizone in chloroform. (If the volume of the original solution is greater than 25 ml., a correspondingly larger volume of potassium cyanide solution must be added.) Allow the liquids to separate and run the chloroform layer into a clean tube. Continue to extract the aqueous solution with the dithizone solution until the chloroform layer is no longer red, but green, finally washing with pure chloroform. Transfer the combined extracts to a test tube, and distil off the chloroform. Add 1 ml. of concentrated sulphuric acid and 1 ml. of concentrated nitric acid and heat the contents of the tube over a small flame, adding two glass beads to minimise bumping. Add a few drops of nitric acid at intervals to obtain a colourless solution, and continue to heat until white fumes appear. Cool, add 10 ml. of 50 per cent. ethyl alcohol, and allow the solution to stand overnight while the lead sulphate precipitates. Filter the solution through a tightly packed pad of filter paper with the aid of suction, and wash the test tube and pad with 10 to 15 ml. of 50 per cent. ethyl alcohol acidified with a few drops of concentrated sulphuric acid. Dissolve the lead sulphate in a 20 per cent. solution of ammonium acetate, and pour two 5-ml. portions of warmed ammonium acetate through the pad. Wash the pad with 5 ml. of warm water and dilute the combined solution to 25 ml. Transfer 5 ml. of the final solution to a 50-ml. separating funnel, add 2 drops of phenol red indicator solution, and sufficient aqueous ammonia solution to give a red colour. Add 1 ml. of a 10 per cent. solution of potassium cyanide and then add a 0·005 per cent. solution of dithizone in chloroform in small amounts from a burette. After each addition, shake the mixture, allow it to settle and run off the red chloroform layer. When the chloroform layer shows a purple tint, reduce the volume of the additions of dithizone solution and wash the aqueous solution with pure chloroform, leaving a few drops in the funnel each time. The titration is complete when the chloroform solution remains green after the addition of dithizone. A blank determination must be carried out and the necessary correction applied.

Satisfactory results have been obtained for the determination of lead in various tissues, as well as in urine and in paper, cereals, and salt.

J. G. WALLER

Biochemistry of Scandium and its Separation as Phytate. G. Beck (*Mikrochem.*, 1948, **34**, 62–66)—Although scandium is more abundant than many more familiar elements (*e.g.*, 10 times more than iodine) it has been little studied. Its wide distribution and availability to plants has prompted this investigation of methods of detection in the animal organism.

The known affinity of scandium for the phosphate ion led to the use of phytic acid (inositol hexaphosphoric acid) as precipitant. Scandium phytate, $Sc_6C_6H_6P_6O_{27},36H_2O$, is obtained as a white flocculent precipitate of very stable constitution and is insoluble in concentrated hydrochloric acid. It is unaffected by nitric acid, *aqua regia*, and hydrogen peroxide even at the boiling-point. A solution of scandium containing 30 μg. of the element per ml. and acid with hydrochloric acid yields a turbidity on addition of sodium phytate. Titanium, zirconium, hafnium and thorium also yield acid-insoluble precipitates, but zirconium and hafnium phytates dissolve in oxalic acid. Zirconium, hafnium, and titanium phytates are also soluble in alkali fluoride, whilst thorium phytate dissolves in cyanacetic acid. Scandium phytate is stable towards alkali and towards ammonium carbonate; it dissolves in oxalic acid solution buffered with sodium acetate, but only over a narrow pH range. The phytates of aluminium and of the rare-earth metals are soluble in dilute mineral acids but not in acetic acid or in ammonium carbonate. Aluminium phytate is also soluble in alkali hydroxide. The gallium compound is the only phytate mentioned that is soluble in aqueous ammonia. Beryllium and indium phytates are somewhat soluble in ammonium carbonate.

Detection of scandium in urine—Evaporate 6 litres of urine in a porcelain basin, decompose the organic matter with nitric acid, and dissolve the residue in dilute nitric acid. Filter off the carbon particles, ignite them with potassium nitrate in a platinum dish, dissolve the mass in nitric acid and add to the main solution. Add 1 ml. of a 30 per cent. solution of sodium phytate and a drop of ferric chloride solution. If necessary reduce the acidity by adding aqueous ammonia solution. The precipitated ferric phytate carries down scandium and zirconium. Centrifuge, wash the sediment, fuse it with a small amount of sodium hydroxide with addition of some potassium nitrate, and leach out with water (Solution 1). Dissolve the residual ferric hydroxide in hydrochloric acid, precipitate the iron by ammonia, add a few drops of ammonium sulphide, and boil with potassium cyanide until all the iron goes into solution. Add ammonium chloride to destroy alkali carbonate and centrifuge the trace of white residue. Boil Solution 1 with ammonium chloride for a considerable time and then centrifuge it with the residue obtained from the dissolution of the ferric hydroxide. Boil [the combined residues, presumably] with hydrochloric acid and

centrifuge from traces of silicic acid. Neutralise 3 ml. of the clear solution with ammonia, add ammonium carbonate, and filter from precipitated aluminium hydroxide. Place one or two drops of the filtrate on a microscope slide and add a drop of saturated sodium fluoride solution and a few granules of hexamminocobaltic chloride or nitrate. After 5 min. observe under the microscope with high magnification. Scandium forms yellowish cubes of $[Co(NH_3)_6]ScF_6$ (*Idem., Mikrochim. Acta*, 1937, **2**, 9). Mix another portion with sodium phytate; scandium yields a turbidity unaffected by hydrochloric acid or oxalic acid. The estimated quantity of scandium is usually 10 to 20 μg. per litre (limit of detection with phytate). No reaction for titanium is obtained with hydrogen peroxide. Treat another portion of the solution with a very little alizarin sulphonic acid solution. The resulting red colour persists on addition of hydrochloric acid but disappears with sodium fluoride. Sodium phosphate causes a slight precipitate insoluble in hydrochloric acid. These reactions indicate zirconium in μg.-quantities.

Scandium in milk—Two litres of milk treated similarly showed a scandium content similar to that of urine.

Scandium is also found in ox blood to the extent of 50 to 100 μg. per litre, in pig's liver in similar concentration, and about 10 μg. per kg. in the human brain.

Scandium can also be used as a reagent for phytic acid. A solution of the latter, containing 300 μg. per ml., gives with scandium sulphate a turbidity that does not clear on adding a drop of dilute sulphuric acid. W. C. JOHNSON

Micro-polarographic Determination of Chloride Ions in Biological Fluids. F. Santavy (*Coll. Czech. Chem. Comm.*, 1948, **13**, 557–560)—The anodic wave given by chloride ions can be used for the polarographic determination of chloride in biological material. The method has the advantage that albumens need not be removed and also that it can be carried out rapidly on small volumes.

Procedure—Make the solution to be examined 0·1 *N* with respect to sulphuric acid, remove dissolved oxygen by passing a stream of inert gas through the solution, and record a polarogram from +0·4 to 0 v. *versus* the mercury pool. The chloride ion concentration in the final solution should not exceed 0·004 *N*.

Determinations on a number of biological fluids, using as little as 1 cu. mm. of liquid, gave results in good agreement with those obtained by other methods. J. G. WALLER

Organic

Electric Furnace for Semi-micro-analysis of Carbon and Hydrogen. H. E. Ungnade and K. Zilch (*Anal. Chem.*, 1948, **20**, 996)—The copper oxide filling of commercial micro-furnaces is inadequate with larger samples, 15 to 25 mg., but a longer copper oxide section and correspondingly longer furnace give excellent results. A 33-cm. furnace (Hevi Duty Electric Co.) has been modified by using a Pyrex 172 micro-combustion tube about 11 mm. in outside diameter resting in a stainless steel or iron pipe about 12 mm. in inside diameter, held in place by steel collars. A chromel - alumel thermocouple is introduced into the space between the furnace and the iron pipe, readings being taken on a millivoltmeter calibrated against a pyrometer. The furnace is operated at 80 v. with a V.5 Variac transformer.

No special precautions are necessary for compounds not containing nitrogen, but with the longer filling incorporating a platinum gauze, combustion must be carefully done. A semi-micro sample can be analysed as quickly as a micro-sample by Niederl's method, and the deviations from the theoretical values for a large number of samples has been ±0·1 per cent. M. E. DALZIEL

Determination of Benzoyl Peroxide in Organic Media. S. Siggia (*Anal. Chem.*, 1947, **19**, 872–873)—In iodimetric methods for peroxides the use of any common solvent other than ethyl and *iso*propyl alcohols is not recommended. Methods in which iodine is liberated are also inapplicable in presence of unsaturated compounds, as in polymerisation work. The method described uses arsenious oxide to reduce the peroxide and determines 0·01 g. of active oxygen with an accuracy to within ±0·5 per cent.

Procedure—Prepare a 0·1 *N* arsenious oxide solution containing 25 g. of sodium hydrogen carbonate per litre, and transfer 25 ml. to a 125-ml. Erlenmeyer flask. Add a quantity of the peroxide solution containing 0·005 to 0·010 g. of active oxygen. Add ethyl alcohol, if necessary, to make the solution homogeneous. If the substance for examination is a polymer, dissolve it in benzene. When benzene is used with a polymer add alcohol as usual. A precipitate of polymer may be ignored when the benzoyl peroxide content is less than 5 per cent. When the peroxide content exceeds 5 per cent. pour off the liquid from the precipitate, redissolve, reprecipitate with alcohol, and combine the extracts. Add boiling-chips to the solution and boil down to about 25 ml. in an air current. Dilute with water to 40 ml. Repeat the evaporation and dilution until all volatile organic substances are eliminated. Make just acid with *N* sulphuric acid and add 0·5 g. of sodium hydrogen carbonate. Chill the solution and titrate the excess of arsenious oxide with 0·05 *N* or 0·1 *N* iodine.

W. C. JOHNSON

Spectrophotometric Determination of Vanillin and Related Compounds. H. W. Lemon (*Anal. Chem.*, 1947, **19**, 846–849)—When solutions of *p*-hydroxyaldehydes and *p*-hydroxyketones in ethyl alcohol are made alkaline the long-wave bands of their ultra-violet absorption spectra are displaced into the high ultra-violet and their absorption intensities are considerably increased. The concentration of any one of these compounds can be determined by measuring the optical density of an alkaline solution in comparison with that of a non-alkaline solution at the wavelength of maximum absorption. The alkali concentration should be

equivalent to more than 1 ml. of 0·2 per cent. potassium hydroxide solution in 100 ml., since below this figure the absorption is dependent on the alkali concentration. For *p*-hydroxybenzaldehyde, vanillin, syringaldehyde, and acetovanillone no change in absorption characteristics occurs within 22 hr., or as a result of altering the width of the slit. A procedure is also given for the analysis of mixtures of two components.

Determination of vanillin—The quantity of vanillin in the aliquot should be such that the optical density reading is between 0·2 and 1·2. Pipette two such aliquots into 100-ml. flasks, to one add 7 ml. of 0·2 per cent. alcoholic potassium hydroxide solution, and dilute each to 100 ml. with 95 per cent. ethyl alcohol that has been boiled under reflux with zinc dust and potassium hydroxide and distilled in glass apparatus. Determine the absorption of the alkaline solution in comparison with the non-alkaline solution using the Beckman spectrophotometer, after confirming the exact position of the maximum (about 353 mμ.). Calculate the concentration of vanillin from the equation $C = D/E^{1\%}_{1\,cm.} L$, where C = concentration in g. per 100 ml., D = density reading, $E^{1\%}_{1\,cm.}$ = 1950 = the extinction value at 353 mμ. of vanillin in alkaline solution as compared with a non-alkaline solution, L = thickness of solution in cm.

Analysis of solutions containing two components—Any two-component mixture of *p*-hydroxyaldehydes or *p*-hydroxyketones can be analysed by the method if sufficient spectroscopic differences exist. The total concentration is obtained by determining the absorption at a coincident point on their $E^{1\%}_{1\,cm.}$ curves, and using the equation $C = D/EL$. Then the concentration of one component can be found, after determining the absorption at a non-coincident point, by the use of the equation

$$C_2 = \frac{(D/L) - E_1 C}{E_2 - E_1}$$

In these equations, C = total concentration in g. per 100 ml., C_2 = concentration of component 2 in g. per 100 ml., D = optical density, L = thickness of solution in cm., E = extinction coefficient at coincident wavelength, E_1 and E_2 = extinction coefficients of components 1 and 2 at non-coincident wavelength selected. The procedure is applied, with satisfactory results, to solutions containing known quantities of vanillin (2) and syringaldehyde (1). For these two substances the coincident point is at 364 mμ. and E = 1480. A suitable non-coincident point is at 353 mμ., where E_2 = 1950 and E_1 = 1080.

Determination of vanillin in vanilla extracts—Dilute 10 ml. of the extract with absolute alcohol to 100 ml. After a few minutes filter off the flocculent precipitate that forms. Dilute a portion of the filtrate 10-fold with absolute alcohol and take two suitable aliquots of the diluted liquid in two 100-ml. flasks, add alcoholic potassium hydroxide solution to one, and dilute both to 100 ml. with absolute alcohol. Determine the absorption and calculate the vanillin content as described for the determination of vanillin (*supra*).

Determination of vanillin and coumarin in vanilla extracts—The $E^{1\%}_{1\,cm.}$ curves for these two substances coincide at 315 mμ. The absorption of coumarin is negligible at 353 mμ., where the absorption by vanillin is at its maximum. When both are to be determined lead acetate treatment and ether extraction (Englis and Hanahan, *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 501) is necessary. Dilute the ether extract with absolute alcohol, prepare neutral and alkaline solutions, and determine the vanillin in the usual manner. Determine the total concentration of vanillin plus coumarin by measuring the absorption at 315 mμ. of the alkaline solution in comparison with absolute alcohol.

The absorption of an alkaline solution of coumarin alters rapidly if water is present, the maximum changing from 275 mμ. ($E^{1\%}_{1\,cm.}$ = 750) to 330 mμ. ($E^{1\%}_{1\,cm.}$ = 380). It is possible to distinguish qualitatively between genuine and artificial extracts by observing the change in absorption at these wavelengths during 1 hr. W. C. JOHNSON

Inorganic

Rapid Micro-Method for the Photometric Determination of Potassium. P. Jordan (*Helv. Chim. Acta*, 1948, **31**, 1483–1487)—Various conditions of time and temperature are studied for the precipitation of potassium as cobaltinitrite (*cf.* Wrangell and Beutelspacher, *Z. anal. Chem.*, 1932, **90**, 401). Complete precipitation is not attained, but the adopted conditions involve only a small and reasonably constant error. The use of a concentrated solution of sodium cobaltinitrite contributes to the reduction of the error and results in the formation of a precipitate of the composition $[Co(NO_2)_6]K_2Na,2[Co(NO_2)_6]KNa_2$. The precipitate obtained by Wrangell and Beutelspacher (*loc. cit.*) was of the composition $[Co(NO_2)_6]K_2Na$. The precipitate is dissolved in alkali and the solution treated with indole, and acidified. A red-violet colour results from the formation of nitroso-indole and the corresponding light absorption is measured.

REAGENTS—*Sodium cobaltinitrite solution*—Dissolve 14 g. of the pure salt in 100 ml. of water and 4·5 ml. of glacial acetic acid. Keep the solution for a day before use, store it in a brown bottle in a refrigerator, and filter the required quantity immediately before use through a compact cotton-wool plug. *Indole solution*—Dissolve 0·15 g. of indole in 10 ml. of alcohol and dilute to 100 ml. with water. Store in a brown bottle. *Comparison solution*—Dissolve 0·40 g. of sodium nitrite in 1000 ml. of water and dilute 10-fold immediately before use. Each millilitre of the diluted solution corresponds to 0·005 mg. of potassium. This solution is used for preliminary operations, such as the choice of the colour filter.

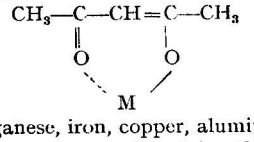
Procedure—The sample solution may be neutral, but preferably should contain 3 per cent. of acetic acid. For the method for removing ammonium salts, see Wrangell and Beutelspacher (*loc. cit.*). Measure 1 ml. of the solution into a 10- to 15-ml. centrifuge tube with a conical bottom. Add 1 ml. of sodium cobaltinitrite solution dropwise with

thorough agitation. Set aside in a vertical position for 15 to 18 hr. at 0° to 5° C., then centrifuge for 20 min. at 3000 r.p.m. Decant the clear liquid by means of a syphon made of 2-mm. tubing drawn off finely and bent at a right-angle at the inner end and equipped with a Mohr clip at the outer end; the surface of the tube is coated with paraffin wax to prevent adhesion of particles of precipitate. To the residue add 2 ml. of water without disturbing the precipitate, centrifuge for 5 min., and decant again. Repeat this operation twice. Add 5 ml. of 0·1 *N* sodium hydroxide and heat for 15 min. on a water-bath, shaking to dissolve the precipitate completely. Dilute with water and add indole solution and diluted sulphuric acid (1 + 1) in the quantities shown in the following table, the approximate quantity of potassium being found by preliminary experiment. After 5 min. determine the light absorption by the method appropriate to the instrument employed. Derive the quantities of potassium from the readings by the use of three graphs, one for each range in the table, previously determined with known quantities of potassium.

Quantity of potassium in mg.	Dilute with water to—	Add	
		indole solution	sulphuric acid
0·02–0·07	48 ml.	1 ml.	1 ml.
0·07–0·14	96 ml.	2 ml.	2 ml.
0·14–0·30	192 ml.	4 ml.	4 ml.

The scheme of dilution avoids excessive concentration at the final stage. A brownish colour is obtained beyond the concentrations employed in the method and the concentration - extinction relationship is no longer linear. The average loss of potassium is about 0·007 mg. and the dilution limit for the detection of the coloured compound is 1 part in 2,800,000. W. C. JOHNSON

Separation of Interfering Metals by Extraction with Acetylacetone in the Colorimetric Determination of Magnesium with Titan Yellow. E. Abrahamczik (*Mikrochem.*, 1947, **33**, 209–216)—In the determination of magnesium with Titan Yellow the following metals interfere: manganese, iron, copper, cadmium, zinc, bismuth, tin, aluminium, cobalt, and nickel. Separation of these metals by precipitation leads to co-precipitation of magnesium. Acetylacetone yields compounds of the type



with manganese, iron, copper, aluminium, titanium, vanadium, uranium, and certain other less common elements, and these compounds are insoluble in water but soluble in an excess of acetylacetone and in organic solvents. Thus is provided a means of removing the principal interfering elements in magnesium determination.

Procedure—To the magnesium solution containing interfering metals add sodium hydrogen carbonate to produce an alkaline reaction. Shake the solution with a solution of 1 part of acetylacetone in 4 parts of carbon tetrachloride in a cylindrical separating funnel. One or two extractions are usually sufficient to remove the bulk of the interfering metals. A film of precipitate forms on the liquid/liquid interface and remains in the aqueous phase on separation. Extract with carbon tetrachloride to remove the residual metallic compounds of acetylacetone. Remove the acetylacetone dissolved in the enolic form by acidifying slightly and extracting again. Destroy the last trace of acetylacetone by adding bromine water and reduce the excess of bromine with sulphite. Then follow the usual procedure for the determination of magnesium with Titan Yellow (*e.g.*, Kreibich and Bäumler, *Aluminium*, 1938, **20**, 528). W. C. JOHNSON

Electrolytic and Polarographic Determination of Zinc in Thorium. J. H. Patterson and C. V. Banks (*Anal. Chem.*, 1948, **20**, 897–900)—The optimum conditions for determining zinc in thorium - zinc alloys by electrolytic and polarographic methods are described. The electrolytic method can be used for samples containing from 1 to 100 per cent. of zinc; alloys with smaller zinc contents can be examined polarographically.

Procedure for the electrolytic method—Dissolve a sample of alloy containing 15 to 200 mg. of zinc and not more than 3 g. of thorium by cautiously adding 15 ml. of concentrated hydrochloric acid. After the vigorous reaction has subsided, add 5 ml. of 70 per cent. perchloric acid solution and evaporate until the perchloric acid fumes. Wash the sides of the beaker and again evaporate to fuming. Add 50 ml. of water, cool, and add 25 ml. of a 60 per cent. solution of sodium citrate. After making the solution just alkaline to methyl red indicator with sodium hydroxide solution, warm to dissolve any precipitate, dilute to 100 ml., and add 15 ml. of acetone. Electrolyse the solution with a copper-plated platinum cathode, at a current of 1 amp. for 1 hr. Wash the electrode with water with the current on and then wash with alcohol and ether, dry for 1 min. at 110° C., and weigh.

Under these conditions a suitable deposit of zinc free from any thorium, is formed.

Procedure for the polarographic method—Add carefully 50 ml. of concentrated hydrochloric acid to about 10 g. of alloy covered with water in a 600-ml. beaker. Add about 1 mg. of sodium silicofluoride and boil until the black residue is dissolved. Evaporate the solution to 20 ml., add 25 ml. of 2·0 *M* sulphosalicylic acid, and 4 ml. of 0·5 per cent. gelatin solution, followed by 20 ml. of 15 *N* aqueous ammonia. Stir until the precipitate has redissolved, adjust to pH 8·5 ± 0·2, and dilute to 100 ml. in a volumetric flask. Transfer a sample of this solution to a polarographic cell and, after

removing dissolved oxygen, record a polarogram.

Add a known amount of pure zinc to a similar sample of the alloy, and treat it in the same way. The zinc content of the alloy is determined from the heights of the steps formed in the two cases.

The zinc contents of alloys containing 20 to 150 p.p.m. of zinc have been determined. Under these conditions no interference from thorium is encountered. J. G. WALLER

Polarographic Determination of Iron. L. Meites (*Anal. Chem.*, 1948, **20**, 895–897)—The polarographic oxidation of ferrous ions in an acidic buffer solution containing oxalate ions is suitable for analytical purposes. Two methods are described for preparing a solution of the sample, free from interfering ions.

Procedure 1—Dissolve a portion of the sample, containing not less than 10 mg. of iron, in 10 to 20 ml. of concentrated hydrochloric acid in a Kjeldahl flask, adding sufficient diluted nitric acid (1 + 1) to give a clear solution. Add 40 ml. of water and 20 g. of silver metal and boil the mixture vigorously for 5 min. (The silver should be prepared by adding copper to silver nitrate solution or by an electrolytic method.) Continue to boil for a few minutes after 5 g. more of silver have been added and then filter the solution into a 100-ml. volumetric flask. Add 1 g. of anhydrous sodium sulphite and dilute the filtrate and washings to 100 ml.

Procedure 2—Dissolve the sample in hydrochloric and nitric acids in the same way as Procedure 1. Evaporate the solution almost to dryness and add 20 ml. of concentrated hydrochloric acid. Again evaporate almost to dryness and then dissolve the residue in 25 ml. of 7·75 *M* hydrochloric acid, transfer the solution to a 100-ml. separating funnel, and extract it with three 30 to 40-ml. portions of peroxide-free *iso*propyl ether. Extract the combined ether solutions with 10 ml. of water, adding a saturated sodium carbonate solution until evolution of carbon dioxide ceases and the hydrated ferric oxide in the aqueous layer coagulates. If the ether layer is not colourless, re-extract it with further quantities of dilute sodium carbonate. Transfer the combined aqueous layers to a 100-ml. volumetric flask, add just sufficient concentrated hydrochloric acid to dissolve the precipitate and, after adding 1 g. of sodium sulphite, dilute to 100 ml.

An aliquot portion of the solution obtained by Procedure 1 or 2 is added to a polarographic cell containing a known volume of air-free, 1·0 *M* potassium oxalate adjusted to pH 4·8 by adding dilute sulphuric acid. The height of the anodic wave at −0·05 v. *versus* the saturated calomel electrode is proportional to the ferrous ion concentration.

The iron contents of a series of standard alloys have been determined by these procedures with an accuracy to within ±1 per cent. of the correct result. J. G. WALLER

Presence and Determination of Molybdenum and Rare Earths in Phosphate Rock. W. O. Robinson (*Soil Science*, 1948, **66**, 317–322)—The determination of 1 to 200 p.p.m. of molybdenum in various phosphate rocks by the colorimetric thiocyanate method is described. The small quantity of silica present must first be removed to avoid emulsification on extraction with *iso*propyl ether. Rhenium, if present, would give the same colour. The rare-earth content is determined on the water-insoluble residue from the sodium carbonate fusion, Reynosos' method being used to separate the calcium and phosphorus.

REAGENTS—*Standard ammonium molybdate solution*—Dissolve 1·149 g. of ammonium molybdate in 1 litre of water and stabilise the solution with 2 to 3 ml. of concentrated aqueous ammonia; determine the molybdenum as lead molybdate and dilute to give a solution containing 0·05 mg. of molybdenum trioxide per millilitre. *Stannous chloride solution*—Ten per cent. in diluted hydrochloric acid (1 + 9).

PROCEDURE—*Molybdenum determination*—Mix 5 g. of finely ground rock with 15 g. of sodium carbonate in a large platinum crucible. Heat over a burner of the Meker type, at first uncovered to oxidise organic matter and then covered, to about 1050° C. and swirl every 5 min. until the mass is quiescent (about 20 min.). Cool rapidly on the sides of the crucible and detach the cake into a 250-ml. beaker half filled with water. Heat on a steam-bath for 2 hr.; break up the cake as it softens and grind to a fine slurry. Heat for 15 min. more and then filter into a large platinum dish. Acidify the filtrate with a moderate excess of hydrochloric acid, evaporate to dryness, and treat the residue with 7·5 ml. of concentrated hydrochloric acid and water. Filter off the silica and wash until the volume of the filtrate is 120 ml. Transfer to a 200-ml. separating funnel, add 5 ml. of 5 per cent. potassium thiocyanate solution, shake, add 5 ml. of stannous chloride solution, and shake again. After 30 sec. add 10 ml. of *iso*propyl ether and shake for 30 sec. Allow to stand until the ether layer is clear and transfer it to a 25-ml. measuring flask. Repeat the extraction with a further 10 ml. of ether. Dilute the contents of the flask to volume and compare with a standard prepared as follows. Measure 125 ml. of 5 per cent. sodium carbonate solution into a 200-ml. separating funnel and add 0·1 to 10 ml. of the standard molybdate solution. Add 25 ml. of concentrated hydrochloric acid and cool with swirling. Develop the colour as above and compare immediately in a colorimeter. Satisfactory comparisons can also be made in the separating funnels in the presence of the aqueous layers. *Determination of rare-earth content*—Combine the leached residues from two molybdenum determinations, dissolve in nitric acid, and evaporate. Filter off the silica, wash, and decompose it with hydrofluoric and nitric acids; add the residual solution to the main filtrate. Add 10 g. of mossy tin and heat on the steam-bath for 2 hr. Filter and determine the rare-earth metals in the filtrate by two precipitations with ammonia, two with oxalic acid in very small volumes, and an intervening precipitation with ammonia.

C. F. HERBERT

Volumetric Micro-determination of Water in Minerals. C. L. Rulfs (*Mikrochem.*, 1948, **33**, 338–343)—The method is shorter and simpler than the modification of the Brush and Penfield technique developed by Hybbinette and Benedetti-Pichler (*Ibid.*, 1942, **30**, 23) and uses the Karl Fischer reagent, the end-point being determined electrometrically. Ignition of the sample is retained so that the "total" water content is still determined.

Method—Apparatus—The sample is ignited in a Pregl micro-muffle, air being circulated over the sample, placed as near as possible to the exit of the ignition tube, *T*, by directing a burner against the short leg, *L*. The collecting tube, *A*, is made of 12- to 14-mm. internal diameter resistance glass

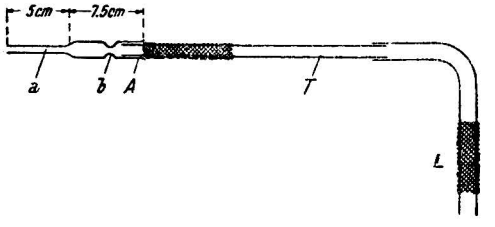


Fig. 1. *Pregl* muffle with water collecting tube

tubing, which slides loosely over the end of the ignition tube. The constriction, *b*, is about 6 mm. wide. The open end of *A* is flared and fire-polished, and the opposite end is sealed to 5 mm. of 6- to 8-mm. outer diameter tubing, *a*. During operation a blast of air is directed at *b* to ensure quantitative condensation at this point. Koch micro-burettes are used for dispensing the ethanol - water solution and the Karl Fischer reagent, and their tips should

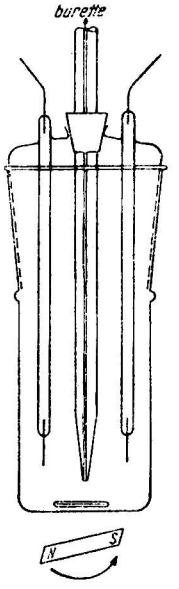


Fig. 2. Titration cell

reach the bottom of the titration cell. The titration cell, as shown, is made of 30- to 36-mm. outer diameter resistance glass and is about 90 to 100 mm. high, being topped by a ground-glass cap with four outlets. Two of these carry 26-gauge platinum wire electrodes extending to 6 to 10 mm. from the bottom of the cell, the third outlet carries the burette tip, held in a rubber stopper, and the fourth is for the escape of air as the titrant is added and consists of a drawn-out capillary. Magnetic stirring avoids the need for a stirrer shaft.

Reagents—The solutions required are Karl Fischer reagent, a 0·2 to 0·3 per cent. solution of water in anhydrous synthetic methanol for back-titration, and a standard water-ethanol mixture. Standardise the Karl Fischer reagent by adding a weighed amount of the standard water - ethanol mixture to a measured excess of the reagent, and titrate back with the methanol solution; this gives a sharper end-point than direct taxation.

Procedure—When not in use store the dry apparatus over a good desiccant. Weigh a sample containing between 2 and 15 mg. of water into a micro-combustion boat and place it in the muffle tube, through which a gentle air stream is already passing. Slip *A* over the exit, and direct a blast of cold air against the constriction before bringing the second burner into place under the sample. When the sample reaches red heat, remove the collecting tube, and insert its narrow end, *a*, through the central opening of the titration vessel. Add 5 ml. of Karl Fischer reagent to the cell through the collecting tube and, while withdrawing it, rinse the tube with a few drops of a strictly anhydrous solvent (pyridine, methanol, ethanol, or ether), and place the burette tip immediately in position. Begin stirring, and titrate as in the standardisation; the end-point is somewhat sluggish.

Results—With samples containing not more than 13·5 mg. of water the precision of duplicate determinations is about 0·5 per cent., and the accuracy is to within ±1 per cent. M. E. DALZIEL

Titanium. Polarographic Determination in Clays and Clay Products. D. F. Adams (*Anal. Chem.*, 1948, **20**, 891–895)—A polarographic method is described for determining titanium in clay, calcined alumina, leach residues, and leach liquors. The best results are obtained when the supporting electrolyte consists of 1·0 *N* sulphuric acid saturated with sodium oxalate and containing 0·8 per cent. of urea. The titanium concentration should be between $0{\cdot}06 \times 10^{-3}$ and $0{\cdot}10 \times 10^{-3}$ *M* and the ratio of iron to titanium less than 10 : 1. An empirical correction for the concentration of iron must be applied to the results.

Procedure for leach residues, original clay, and calcined alumina—Weigh a sample of dry, ground material, containing approximately 7 mg. of titanium, into a 200-ml. beaker and fuse the sample with 3·0 g. of potassium hydrogen sulphate. Cool the melt, leach it with dilute sulphuric acid, and dilute to 100 ml. in a volumetric flask. Transfer 10 ml. of the solution to a 25-ml. volumetric flask, add 8 ml. of a 25 per cent. solution of urea and sufficient 10·0 *N* sulphuric acid to give a final concentration that is 1·0 *N* with respect to sulphuric acid. Dilute to 25 ml., transfer a portion of the solution to a polarographic cell, saturate the solution with sodium oxalate and, after removing

dissolved oxygen, record a polarogram from 0 to −0·8 v. *versus* the saturated calomel electrode.

Procedure for leach liquors—Add 10 ml. of liquor, containing approximately 0·3 mg. of titanium, to 25 ml. of 1·0 *N* sulphuric acid and evaporate the solution to remove sulphur dioxide. Cool, dilute to 50 ml. in a volumetric flask, and then transfer a 10-ml. portion to a 25-ml. flask containing 8 ml. of a 25 per cent. solution of urea. The procedure is then the same as that described above.

J. G. WALLER

Determination of Chloride in Mixtures Containing Hypochlorite, Chlorite, and Chlorate. C. Loeb, J. Chabert, A. Banderet, and J. Meybeck (*Analyt. Chim. Acta*, 1948, **2**, 316–320)—In the determination of chloride with silver nitrate the various ions considered interfere in the following ways. Hypochlorite yields part of its chlorine as silver chloride while the rest passes to a higher state of oxidation. Chlorite causes low results for chloride in potentiometric determinations, but only in acid solutions. Chlorate interferes only in strongly acid solutions.

Hypochlorite is reduced quantitatively by arsenite solution and none of the oxyacid ions is reduced if the solution is alkaline. The hypochlorite is first determined, therefore, by addition of arsenite in excess, followed by bromometric titration of the excess of arsenite. Another portion of the solution is treated with an excess of arsenite, and the chloride produced by reduction plus the chloride originally present is titrated with silver nitrate in the solution buffered at pH 7·4. At this pH neither chlorite nor chlorate interferes and arsenite and arsenate are precipitated as their silver salts only after all the chloride has been precipitated.

Procedure—Run 10 ml. of the solution into an excess of 0·01 *N* sodium arsenite. Add 20 ml. of 0·1 *N* sodium borate and 4 drops of a 0·1 per cent. solution of a mixture of equal parts of phenol red and bromothymol blue. Allow to stand for a few minutes. Add 0·1 *N* boric acid until the indicator shows a pale violet colour (pH 7·4) and titrate potentiometrically with 0·1 *N* silver nitrate from a micro-burette.

From the figure found for total chloride subtract the figure already found for chlorine as hypochlorite.

W. C. JOHNSON

Rarer Elements in Qualitative Analysis. II: Uranium. T. P. Chao and S. C. Chen (*J. Chem. Educ.*, 1948, **25**, 686–687)—In qualitative analysis, uranium is detected in group III.

Procedure—After expelling hydrogen sulphide from the filtrate from group II, add solid ammonium chloride and aqueous ammonia solution until the solution is alkaline, and without filtering, saturate with hydrogen sulphide. Cold, *N* hydrochloric acid extracts all but cobalt and nickel sulphides from the precipitate. Treat the extract with aqueous ammonia solution and hydrogen sulphide, and filter the suspension. Reject the filtrate, and extract the precipitate with cold, *N* hydrochloric acid. Treat this extract with sodium hydroxide, sodium peroxide, and sodium carbonate to obtain a precipitate containing the hydrated oxides of manganese and iron, traces of the hydrated oxides of zinc, cobalt, and nickel, and if phosphate or oxalate is present, the carbonates of barium, strontium, calcium, and magnesium, and a solution containing aluminate, chromate, zincate, and peruranate. Acidify the solution with hydrochloric acid, and add a slight excess of aqueous ammonia to precipitate aluminium hydroxide and ammonium diuranate, which are filtered from the zincate and chromate solution. Dissolve the precipitate in dilute nitric acid, and add solid sodium hydrogen carbonate and a slight excess of aqueous ammonia, thus precipitating aluminium hydroxide and forming a soluble complex uranyl carbonate. Acidify the filtered solution with acetic acid and add a slight excess of sodium phosphate to precipitate white ammonium uranyl phosphate. Confirm uranium by dissolving the precipitate in dilute hydrochloric acid, concentrating the solution to a small volume, and adding sodium chloride and potassium ferrocyanide, whereupon a brown-red precipitate of uranyl ferrocyanide, which turns yellow on addition of potassium hydroxide to the suspension, should be formed.

M. E. DALZIEL

Phenoxthin as a Reagent for the Detection of Small Amounts of Palladium. O. Konig and W. R. Crowell (*Mikrochem.*, 1948, **33**, 298–299)—Phenoxthin (phenoxathin) is a sensitive reagent for palladium, and is easily prepared (*Organic Syntheses*, Vol. 2, p. 485). On the drop-reaction plate, a 10 per cent. solution of the reagent in acetone with a drop of the palladium solution in 0·1 *N* hydrochloric acid gives a yellow colour; on paper impregnated with the reagent, a yellow to red-brown spot is obtained. The reaction is sensitive to 0·01 μg. of palladium and the limiting concentration is 1 part in 100,000.

Gold salts react to give a red colour on the drop-reaction plate and a diffuse violet spot on paper; ammonia, ammonium salts, tin,^{II} cyanide, thiocyanate, fluoride, oxalate, and tetraborate also interfere. Osmate, osmium,^{III} ruthenium,^{III} and ruthenichloride ions interfere because of their colour. Mercury^{I} reduces the sensitivity of the test. Dimethylglyoxime, lead, silver, ferrous and ferric iron, tin,^{IV} cobalt, nickel, copper,^{II} nitrite, sulphate, chloride, bromide, mercuric chloride, or iridic chloride do not interfere. One part of palladium can be detected in the presence of 200 parts of platinum or 100 parts of rhodium.

The palladium and rhodium compounds can be isolated, but not those of platinum, iridium, or gold. The 2-sulphonic acid and 2 : 8-disulphonic acid derivatives of the reagent each give yellow colours with palladium, but they are less sensitive, the limit of identification being 0·1 μg., and their use has the sole advantage that gold does not interfere.

M. E. DALZIEL

Thio-Acids as Drop Reagents for Palladium. O. Konig and W. R. Crowell (*Mikrochem.*, 1948, **33**, 300–302)—Aqueous solutions of thioglycollic and thiomalic acids give yellow colorations with bivalent and quadrivalent palladium salt solutions at pH values between 0·5 and 9·0; the limit of

identification is 0·05 μg. of palladium and the limiting concentration 1 part in 100,000. At concentrations greater than 5 parts per 1000, a yellow precipitate forms; this dissolves readily on dilution.

At concentrations exceeding 10 g. per litre, gold, platinum, and iridium salts give white, amorphous precipitates, which dissolve on dilution to give colourless solutions; rhodium salts give a pink precipitate, which dissolves on dilution to give a pink solution and turns brown on standing, or yellow if the solution is very dilute. Brown iridic chloride is reduced to a colourless tervalent form.

One part of palladium can be detected in presence of 250 parts of gold, platinum, or iridium, or any mixture of these and in presence of 25 parts of rhodium with or without 75 parts of a mixture of the other three. Ruthenium gives a grey spot, osmium a greenish-grey spot, mercuric mercury a yellow precipitate, silver an orange precipitate, and cupric copper a yellow precipitate. Ferric iron reacts only in the presence of ammonia, a violet coloration being formed.

The following do not interfere: nitrate, sulphate, thiocyanate, chloride, bromide, iodide, fluoride, chlorate, perchlorate, oxalate, tetraborate, ammonia, ammonium, and dimethylglyoxime. Cyanide inhibits the reaction and nitrite gives a deep violet spot, but 0·1 μg. of palladium can be detected in the presence of 10 μg. of nitrite.

The compounds formed cannot be isolated, but the composition of the palladium thioglycollic acid compound has been deduced by indirect methods.

M. E. DALZIEL

Electro-reduction of Periodate at the Dropping Mercury Electrode. Part I. Behaviour in Acidic Solutions of pH less than 3. R. H. Coe and L. B. Rogers (*J. Amer. Chem. Soc.*, 1948, **70**, 3276–3281)—In the pH range from 0·8 to 3·0, periodate ions give two reduction steps, which represent reduction to iodate and to iodide. These steps have a limited application for analytical purposes. Acidic solutions of potassium metaperiodate can be determined with an accuracy to within ±1 per cent. by using the second step, but solutions of orthoperiodic acid cannot be determined with an accuracy better than to within ±2·5 per cent. J. G. WALLER

Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 986)—The crystallography of ascorbic acid is described under the headings given in the first paper of the series (*Ibid.*, 1948, **20**, 275; *Analyst*, 1948, **73**, 579). M. E. DALZIEL

Polarography. Some Factors Affecting Drop-Time. F. L. English (*Anal. Chem.*, 1948, **20**, 889–891)—The effect of a number of factors on the drop-time has been examined. These factors include vibration, the applied voltage, the purity of the mercury, the cleanliness of the capillary tip, the current flowing, and the nature of the solution.

Slight vibration of the capillary has a measurable effect on the drop-time. The purity of the mercury has little effect, ordinary laboratory-grade material giving the same drop-time as carefully purified material. Liquid amalgams of various metals, however, have a considerable effect on the drops. Traces of oil on the capillary tip produce irregular drops, but this can be avoided by immersing the tip in ethyl alcohol when not in use.

The presence of capillary-active substances in the solution has a considerable effect as does the solvent used, but the effect of the concentration of the supporting electrolyte is small.

The results show that for accurate calculations involving the Ilkovic equation, it is important to determine the drop-time under the exact conditions of the analyses. J. G. WALLER

Reviews

PHOTO-ELECTRIC METHODS IN CLINICAL BIOCHEMISTRY. By G. E. DELORY, M.Sc., Ph.D. Pp x + 90. London: Hilger & Watts, Ltd. 1949. Price 15s.

There is no doubt that photo-electric methods of analysis are popular. From their introduction somewhere about twenty years ago they have made rapid strides in popularity, and these strides became almost those of seven-leagued boots with the appearance on the market of suitable photo-electric instruments. It is a truism that any method of analysis that lends itself to the quantitative application of visual colorimetry can be better applied photo-electrically; for the uncertainties and vagaries of the human eye are nowhere more apparent than in the laboratory.

With so wide a range of application, photo-electric analysis could clearly not wait long before its own literature came into existence. Much of the pioneer work in the adaptation of suitable methods was carried out on Messrs. Hilger's instruments, and as their instruments become more and more widely distributed the makers assist the users by the publication of books such as the one under review.

It describes modifications of a number of well-known biochemical colorimetric determinations suitable for use with Hilger instruments, the range of tests described covering all those more commonly wanted. Needless to say, only slight further modification would be required to adapt the tests as here recorded for use in any other photo-electric instrument. In addition, three introductory chapters describe simply and adequately the general principles of colorimetry, of colorimetric measurements and of the special technique of photo-electric colorimetry. These chapters are followed by descriptions of the Hilger instruments.

The subject is set out in very simple language, and the author has adopted very straightforward means for describing the various methods of test. If some of these, at a casual glance, seem to differ in not unimportant details from earlier descriptions of the same methods appearing elsewhere, no doubt the author is sure of his ground and can recommend the latest version. K. A. WILLIAMS

RECENT ADVANCES IN ANALYTICAL CHEMISTRY. FRONTIERS IN CHEMISTRY. Volume VII. Edited by R. E. BURK and OLIVER GRUMMITT. Pp. 209. New York and London. Interscience Publishers. 1949. Price 27s.

This volume is the seventh of the Frontiers in Chemistry series published under the auspices of the Western Reserve University, Ohio. As with the previous volumes, this book gives permanent form to a number of lectures on related subjects given at the University by a group of specialists.

Those unfamiliar with the series should be warned that these volumes do not give as exhaustive a treatment of the subject as their titles may appear to suggest. For example, if the present title were prefaced with the word "Some," this would in no way detract from the merits of the book and would be generally fairer to the authors and the reader.

The contents include: Voltammetry (Polarography) and Amperometric Titrations, by I. M. Kolthoff; Inorganic Analysis with Organic Reagents; Some Recent Colorimetric and Gravimetric Organic Reagents, both by J. H. Yoe; Applications of Infra-red Spectroscopy in Analysis, by O. Beeck; Electron Microscopy and Micro-analysis, by J. Hillier; Fractionation, Analysis and Purification of Hydrocarbons, by F. D. Rossini; Applications of the Mass Spectrometer, by J. A. Hipple.

Electron Microscopy and Infra-red Spectroscopy have already been dealt with in Volume IV of this series, "Major Instruments of Science and their Application to Chemistry" (reviewed in *The Analyst*, **1946, 71**, 345), but the treatment in the present volume is not only by a new team of specialists, but also takes account of three years' progress in these rapidly advancing subjects; duplication is thus more apparent than real. Both these and several of the other subjects included have received attention at meetings of the Physical Methods Group of our Society; these more detailed accounts of recent progress will be welcomed by those whose interest has already been stimulated in such branches as Polarography and Mass Spectrometry.

Reading these essays emphasises once again how diverse have become the tools and methods of modern analytical chemistry or rather chemical analysis. The only feature remaining in common appears to be the ultimate purpose of the operation. This very diversity precludes authoritative comment on all the sections of the book. Those within the reviewer's own field of interest are authoritative, lucidly written and stimulating to read; those dealing with less familiar matters appear to be of an equally high standard.

No analyst can fail to profit by attending this course of lectures through the medium of the printed word; teachers of chemistry should find this account of modern advances particularly valuable. Potential research workers in the field are specially recommended to read Professor Yoe's "Outline for Development of a New Colorimetric Method."

B. S. COOPER

ALCOHOL: A FUEL FOR INTERNAL COMBUSTION ENGINES. By S. J. W. PLEETH, B.Sc., F.Inst.Pet. Pp. xv + 259. London: Chapman and Hall Ltd. 1949. Price 28s. net.

Modern civilisation runs on fuel, and a constantly recurring exercise of economists is to estimate how long it will be before the natural resources of fuel are exhausted. The estimates are very variable and agree only that an end will come. However remote that may be, the deduction is general that natural assets must be conserved and other sources of energy must supplement if not supersede them. For the internal combustion engine, as we know it, liquid fuels will probably always be essential and any that can be produced in unlimited quantity must be of value. Ethyl alcohol is not an ideal fuel but it is an important one having many desirable characteristics, including that of production from vegetable matter available throughout the world and renewable annually.

The author first surveys solid and liquid fuels and then describes the production of ethyl and other alcohols from natural raw materials and by synthesis. Modern methods of dehydration are indicated by flow-sheet diagrams. The principles of the internal combustion engine are dealt with and the essential requirements and characteristics of liquid fuels are discussed, including fuel volatility, knock rating, water tolerance, gum formation and subsidiary effects such as corrosion and cylinder wear. About 16 pages are devoted to the testing of alcohol and alcohol blends.

The methods of test described are adequate for the purpose, but no mention is made of the Karl Fischer reagent when the water content is required with accuracy.

The composition of Power Methylated Spirit given is not quite in accordance with the regulations, and the fact that it must be coloured with a specified amount of dye is not referred to.

The Appendix gives 12 tables relating the specific gravity or density of alcoholic solutions with their strength. The first six tables, dealing with aqueous ethanol, are labelled "by permission of the U.S. Bureau of Standards" and the seventh is an abstract from the British Official Tables. It is pointed out that there is a discrepancy between Table VII and the previous ones and inspection shows that the differences are in the higher strengths and are of the order of 0·1 per cent. alcohol. It may, however, be mentioned that the first six tables do not agree with those given in the United States Pharmacopoeia or in the Methods of Analysis of the Association of Official Agricultural Chemists, all of which are in agreement with the British Official Tables to within 0·01 per cent. alcohol. It would appear that the American Tables cited are not the current ones and caution should be observed in their use.

Although at the moment power methylated spirit is little used in this country as a fuel, the position may change in the near future. Coal of poor quality must be processed at the collieries, and some of the products of low temperature carbonisation and of hydrogenation will need to be blended before they are suitable as motor fuels. Alcohol is particularly promising for such blending and the information given in this book will be valuable to those concerned in marketing such products capable of competing with petrol.

J. R. NICHOLLS

REPORTS OF THE ANALYTICAL METHODS COMMITTEE OBTAINABLE THROUGH THE EDITOR

The Reports of the Analytical Methods Committee listed below may be obtained direct from the Editor of THE ANALYST, 7-8, Idol Lane, London, E.C.3 (not through Trade Agents), at the price of 1s. 6d. to Members of the Society, and 2s. 0d. to non-Members. Remittances must accompany orders and be made payable to "Society of Public Analysts."

The Reichert-Polenske-Kirschner Process. (Test for Butter Fat.) *To be reprinted.*

Milk Products Sub-Committee:

Reports Nos. 1 and 2. Analysis of Condensed Milks.
Report No. 3. Analysis of Sweetened Condensed Milk in which the Sucrose has altered during Storage. *To be reprinted.*
Report No. 4. Determination of Water, of Total Solids and of Fat in Dried Milk.

Sub-Committee on Dirt in Milk. Report. Determination of Dirt in Milk.

Report on the Determination of Total Solids in Fresh Liquid Milk.

Essential Oil Sub-Committee:

Report No. 1. Estimation of Cineole in Essential Oils. (1) Cajaput and Eucalyptus Oils.
Report No. 2. Physical Constants (1).
Report No. 3. Physical Constants (2).
Report No. 4. Interim Report on the Determination of Acetylisable Constituents in Essential Oils.
Report No. 5. Determination of Phenols in Essential Oils.
Report No. 6. Determination of Citral in Lemon Oil.
Report No. 7. Determination of Solubilities.
Report No. 8. Determination of Cineole in Essential Oils. (2) Camphor Oil. (3) Other Oils.
Report No. 9. Determination of Carvone and Menthone.
Report No. 10. Determination of Citronellal.
Report No. 11. Determination of Aldehydes other than Citronellal.
Report No. 12. Determination of Ascaridole.
Report No. 13. Determination of Esters.
Report No. 14. Solubility Test for Ceylon Citronella Oil. (Gratis.)

Metallic Impurities in Foodstuffs Sub-Committee (formerly **Sub-Committee on the Determination of Arsenic, Lead, etc. in Food Colouring Materials**):

Report No. 1. Determination of Arsenic. *Out of print.*
Report No. 2. Determination of Lead.
Report No. 3. Determination of Copper.
Report No. 4. Determination of Zinc.

Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps:

Report No. 1. Determination of Unsaponifiable matter in Oils and Fats.
Report No. 2. Determination of Unsaponified Fat in Soap.
Report No. 3. Determination of Free Alkali in Soaps.
Report No. 4. Determination of Free Alkali and Silica in Silicated Soaps.
Report No. 5. Determination of Rosin in Soaps.
Report No. 6. Determination of Phenols in Soaps.

Poisons Sub-Committee appointed to investigate Methods of Assay for Various Substances appearing in the Poisons Schedules of the Poisons Regulations, 1935:

Report No. 1. Assay of Lobelia (*Lobelia Inflata*).
Report No. 2. Assay of Gelsemium
Report No. 3. Assay of Aconite.
Report No. 4. Assay of Yohimba.
Report No. 5. Assay of Jaborandi.
Report No. 6. Assay of Ephedra and of Ephidrine in Nasal Sprays.

Fluorine in Foods Sub-Committee:

Report on the Determination of Fluorine in Foods.
Addendum to above Report. (Gratis.)

Sub-Committee on Vitamin Estimations. Microbiological Panel:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.

Tragacanth Sub-Committee:

Report No. 1. Evaluation of Powdered Tragacanth.
Report No. 2. Evaluation of Flake Tragacanth.

COUNTY BOROUGH OF BARNSLEY
WATERWORKS DEPARTMENT
APPOINTMENT OF CHEMIST

APPLICATIONS are invited for the above appointment from persons with experience in the examination of public water supplies.

Preference will be given to applicants possessing a University Degree or other professional and technical qualifications.

The successful candidate will be required to take charge of the Water Examination Laboratory and to exercise supervision of the operation of filtration and treatment plants of the Undertaking.

The salary will be in accordance with A.P.T. Grade V (£520–£570 per annum) of the National Scales, with car allowance. A house will be provided. The post is subject to the provisions of the Local Government Superannuation Acts and the successful candidate will be required to pass a medical examination. The appointment will also be subject to the general conditions of service for officers of the Corporation as varied from time to time.

Forms for the guidance of candidates may be obtained from the Waterworks Engineer and Manager, Town Hall, Barnsley, Yorkshire, and completed applications should reach him by the 19th December, 1949.

Canvassing, either directly or indirectly, will be deemed a disqualification.

A. E. GILFILLAN,
Town Clerk.

Town Hall, Barnsley.
14th October, 1949.

B.Sc. or A.R.I.C., with pharmaceutical experience, required for analytical laboratory in East Midlands. Commencing salary £600–£750 per annum, depending on qualifications. Write Box 3724, THE ANALYST, 47, Gresham Street, London, E.C.2.

ANALYST required for laboratory in the Midlands engaged in the examination of fine chemicals and medicinals. Applicants should possess an Honours Degree in Chemistry or A.R.I.C. and preferably should have had some industrial experience. Familiarity with modern physical methods of analysis would be an advantage. Salary to be commensurate with qualifications and experience. Write Box 3723, THE ANALYST, 47, Gresham Street, London, E.C.2.

CHEMIST required for work involving the analysis of raw materials and investigation of problems relating to the use in the Cable and Telecommunications Industry. London area. Salary up to £420 per annum according to age and qualifications. Degree in Chemistry preferred. Write, stating age and qualifications, to Box 3722, THE ANALYST, 47, Gresham Street, London, E.C.2.

COUNTY BOROUGH OF BOURNEMOUTH
PUBLIC ANALYST

APPLICATIONS are invited for the appointment of Public Analyst and Official Agricultural Analyst under the Fertilisers and Feeding Stuffs Act, 1926, for the County Borough of Bournemouth, from persons possessing the qualifications prescribed by the Public Analysts Regulations, 1939, or such other qualifications as may be approved under the Regulations.

Remuneration will be in accordance with the following scale of fees:—

Two guineas for each of the first 100 samples of any kind per annum.

One guinea for each sample above 100 in any year.

The sum paid in respect of analytical work on this basis during the past six months was approximately £600, but no guarantee can be given as to the number of samples which will be sent for analysis in any year.

The Analyst appointed will be required to provide laboratory accommodation and materials, etc.

Applications endorsed "Public Analyst," stating age, qualifications, expereince and past and present appointments and the names of two persons to whom reference can be made as to character and ability, must reach the undersigned not later than the 19th November, 1949. Forms are not provided.

Canvassing, either directly or indirectly, will disqualify.

A. LINDSAY CLEGG,
Town Clerk, Bournemouth.

SOMERSET COUNTY COUNCIL
DEPUTY COUNTY ANALYST

APPLICATIONS are invited for the appointment of Deputy County Analyst and Deputy Official Agricultural Analyst. Candidates must be Fellows of the Royal Institute of Chemistry and hold the Branch E certificate and should be experienced in the analysis of Food and Drugs, Fertilisers and Feeding Stuffs and other work generally carried out in Public Analysts' Laboratories.

The post is allocated to Grade VIII of the A.P.T. Division, and the commencing salary will be within the scale of £685 × 25 × £760 according to qualifications and experience. It is subject to superannuation, and the successful candidate will be required to pass a medical examination.

Applications with the names of two referees should be forwarded to the County Medical Officer of Health, County Hall, Taunton, and marked "Deputy County Analyst," and should be received not later than one week after the date of publication of this advertisement.

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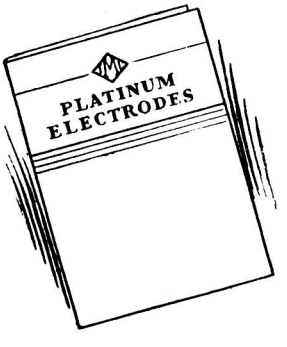

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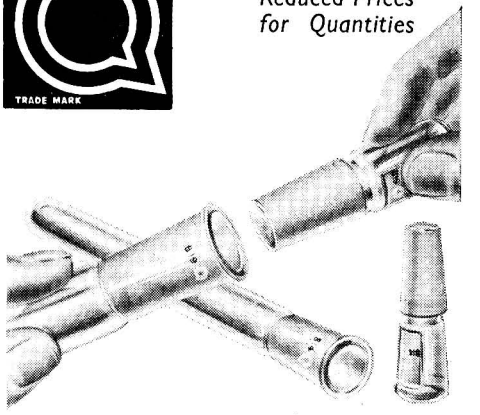

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Printed and Published for the Society of Public Analysts and other Analytical Chemists by W. Heffer & Sons Ltd., Cambridge, England. **Communications to be addressed to the Editor, J. H. Lane, 7–8, Idol Lane, London, E.C.3.** Enquiries about advertisements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

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