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THE ANALYST

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

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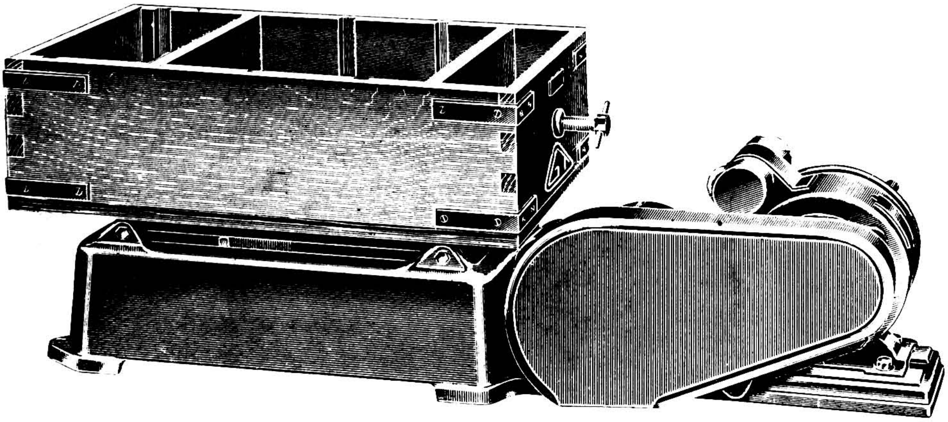

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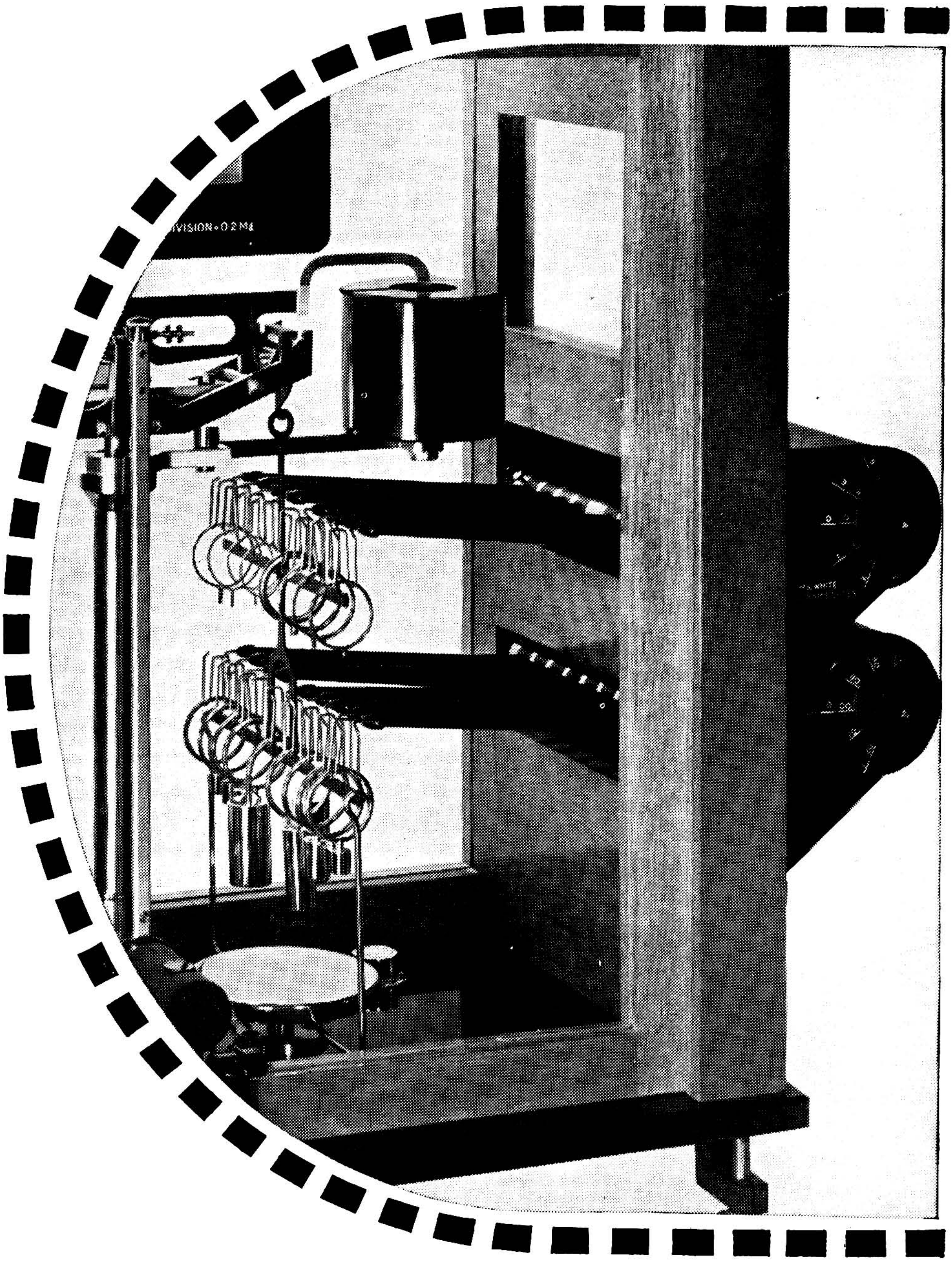

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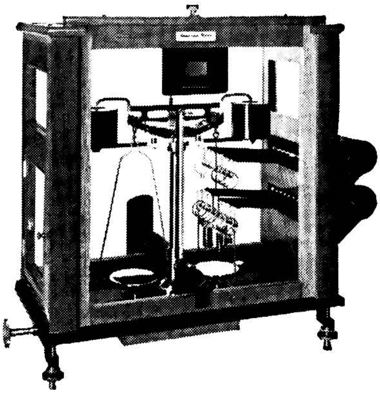

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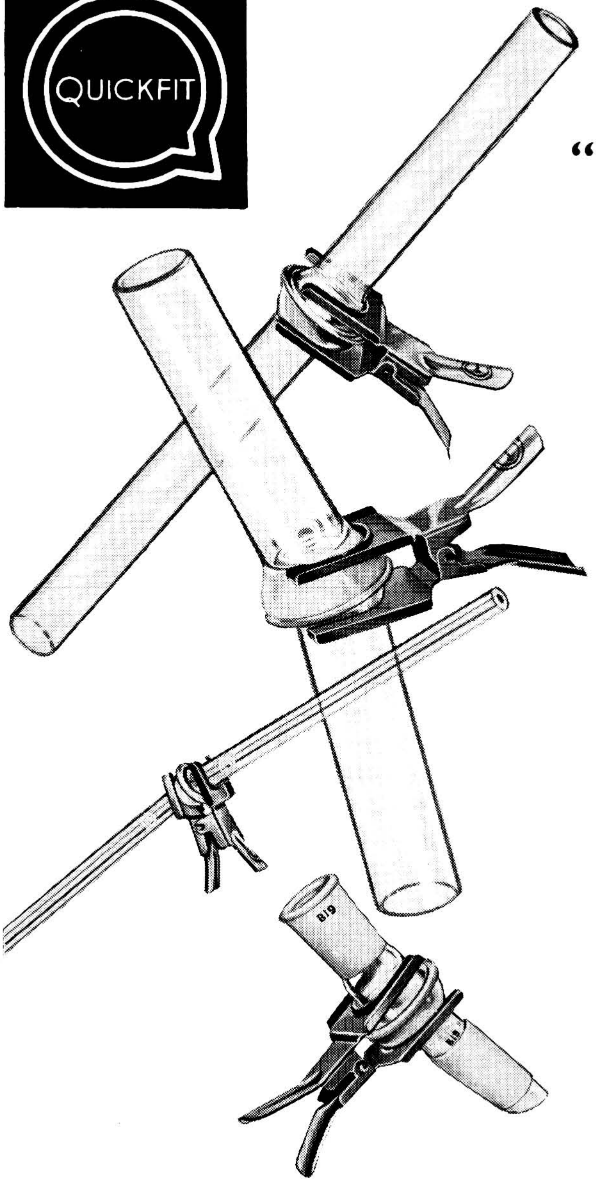

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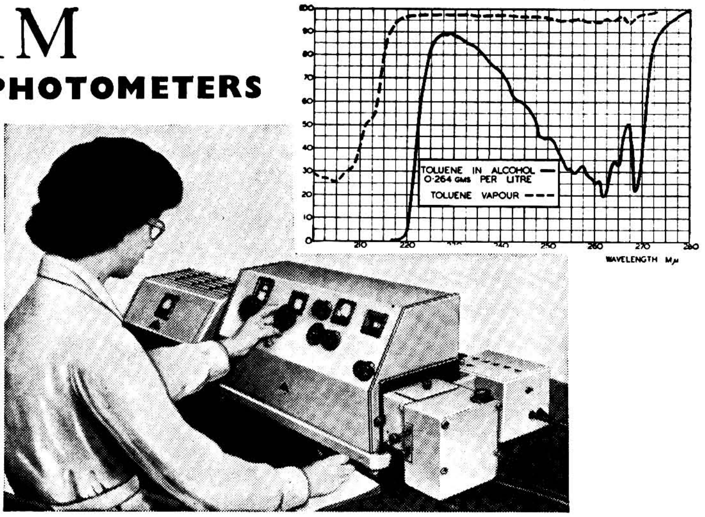

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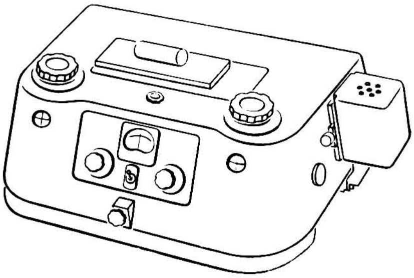

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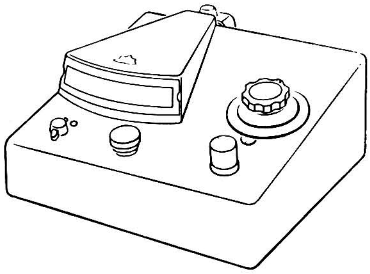

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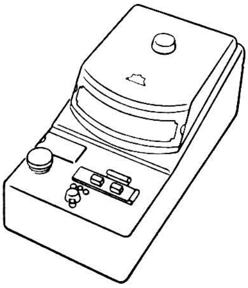

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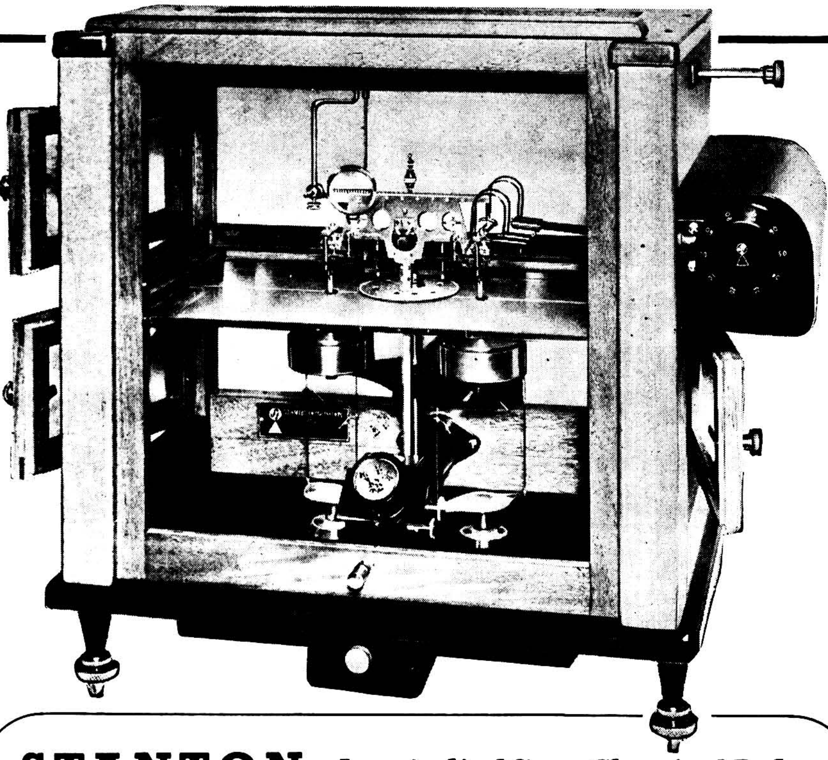

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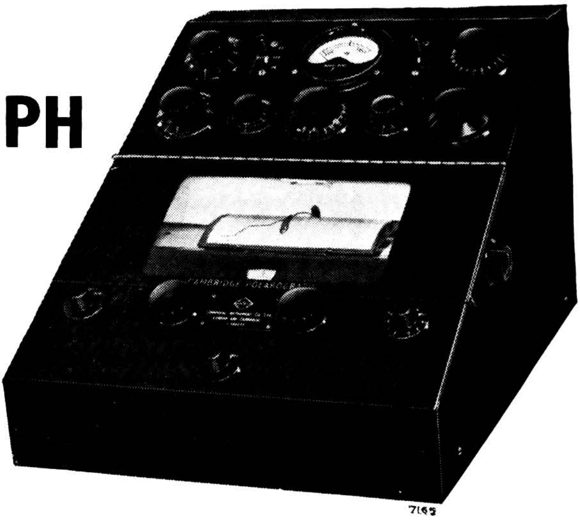

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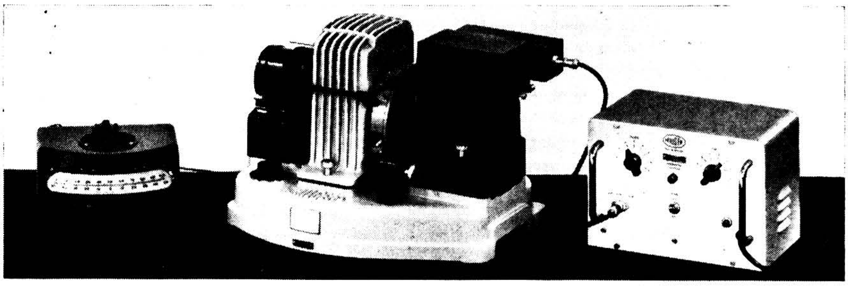

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, October 7th, 1953, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. D. W. Kent-Jones, F.R.I.C., and about 170 members and visitors were present.

At this meeting there was a discussion on "Destruction of Organic Matter." The subject was introduced by Dr. G. Roche Lynch, O.B.E., M.B., B.S., D.P.H., F.C.G.I., L.M.S.S.A., F.R.I.C., and the following papers were presented and discussed: "The Preparation of Biological Material for the Determination of Trace Metals. Part II. A Method for the Destruction of Organic Matter in Biological Material," by G. Middleton, B.Sc., F.R.I.C., and R. E. Stuckey, B.Sc., Ph.D., F.R.I.C., Ph.C.; "Determination of Lead in Foodstuffs," by H. C. Lockwood, Ph.D., F.R.I.C.

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DEATH

We regret to record the death of

William Charles Hughes

Micro-analysis of Silicate Rocks

Part IV. The Determination of Alumina

By CHRISTINA C. MILLER AND ROBERT A. CHALMERS

(*Presented at the meeting of the Society in Glasgow on Wednesday, May 6th,* 1953)

A new procedure is prescribed for the separation and direct determination of alumina in 5-mg samples of silicate rocks. Silica is volatilised by heating with hydrofluoric and sulphuric acids, and the residue is fused with potassium bisulphate and extracted with *N* hydrochloric acid. Iron, titanium, vanadium and zirconium are removed together by precipitation with cupferron and extraction with *o*-dichlorobenzene. Acetylacetone is added to the aqueous phase, and aluminium and beryllium acetylacetonates are extracted from the buffered solution at a pH of 6 to 7 by means of diethyl ether. From the ether extract aluminium and beryllium are withdrawn into 6 *N* hydrochloric acid, and the aluminium alone is precipitated and weighed as aluminium 8-hydroxyquinolinate.

The method has been applied in the presence of all the elements commonly found in silicate rocks. In the analysis of rocks, the results obtained are relatively 1 per cent. lower than those based on the classical procedure.

In the analysis of silicate rocks by the classical procedure, the component determined with the least certainty, and only after a considerable expenditure of time, is alumina. Its indirect determination depends on the weight of seldom fewer than five oxides ("mixed oxides") and the separate determination of all except alumina, which is then found by difference. On the micro-scale the inherent difficulties and uncertainties of the method are increased. Slow-filtering hydroxide precipitates can be very troublesome, and the hygroscopicity of alumina, and the many high-temperature ignitions, which significantly affect the weight of platinum crucibles, are sources of error. In microchemical work especially, one would like to abolish the hydroxides precipitation and also determine alumina more directly. Hitherto, a potent reason for retaining the precipitation of the hydroxides has been that their ability to carry down some residual silica has enabled the determination of silica to be completed. We have, however, recently proposed a method for the direct determination of total silica in a separate portion of rock.[1] Precipitation of the hydroxides is not essential for the subsequent determination of iron and titanium, which are commonly determined at this stage although they are easily determined separately, but it facilitates the determination of calcium and magnesium.

Early attempts to avoid the precipitation of the hydroxides in micro-analyses of silicate rocks were made by Schoklitsch[2] and by Hecht,[3] both of whom freed the rocks from silica, fused the residues with potassium bisulphate, precipitated together the 8-hydroxyquinolinates of iron, aluminium and titanium, and from them obtained, after destruction of organic matter, a solution of metal chlorides. Schoklitsch then precipitated and removed by filtration the cupferron complexes of iron and titanium, destroyed organic matter in the filtrate and precipitated aluminium with 8-hydroxyquinoline. Hecht, on the other hand, precipitated iron as iron sulphide in an ammoniacal tartrate solution, thus separating it from aluminium and titanium, which were then precipitated together and weighed as the 8-hydroxyquinoline complexes. After a somewhat complicated procedure, he then determined titanium as titanium dioxide and found alumina by difference.

8-Hydroxyquinoline can certainly be used to simplify the determination of alumina in silicate rocks of low complexity. McLennan[4] jointly determined iron, aluminium and titanium (TiO_2, 2 per cent.) gravimetrically as their 8-hydroxyquinolinates with a positive error of less than 0·5 per cent. One to 2 per cent. of calcium oxide and magnesium oxide, 0·5 per cent. of phosphoric oxide and 0·2 per cent. of manganous oxide were without influence at pH 5, but larger amounts of manganous oxide divided, and large (10 per cent.) amounts of calcium and magnesium oxides caused significant positive errors. As various minor constituents would accompany aluminium, one may conclude that the substitution of 8-hydroxyquinoline for ammonia is not in itself the solution of the problem when rocks of greater complexity are under consideration.

The object of our investigation was therefore to devise a method for the direct determination of alumina in 5-mg samples of more complex silicate rocks, preferably one that would not prevent the subsequent determination of calcium and magnesium, and perhaps manganese, in the same sample. The following method has been evolved.

After volatilising silica by heating with hydrofluoric and sulphuric acids, and fusing the residue with potassium bisulphate, we have quantitatively removed iron and titanium and some other elements from a *N* hydrochloric acid solution of the melt by means of cupferron and *o*-dichlorobenzene. Aluminium has then been converted into the acetylacetone complex at pH 6 to 7 and extracted quantitatively with diethyl ether, from which it has been withdrawn into 6 *N* hydrochloric acid and, after suitable adjustment of the conditions, precipitated and weighed as aluminium 8-hydroxyquinolinate. The method has been applied to some silicate rocks.

EXPERIMENTAL

QUANTITATIVE SEPARATION OF IRON, TITANIUM AND SIMILAR ELEMENTS FROM ALUMINIUM BY MEANS OF CUPFERRON—

Cupferron is frequently used in conjunction with an organic solvent for removing iron^{III} and titanium^{IV} from mineral acid solutions before determining small amounts of aluminium. When aluminium is a major component, care is required because aluminium cupferronate is extractable from solutions of low acidity. Hence the acid concentration should be reasonably high and the concentration of cupferron and the volume of organic solvent should be the minimum that permits the complete removal of iron, titanium and similar elements. The efficiency of the common solvents, diethyl ether, carbon tetrachloride, chloroform and benzene for separating iron and aluminium in *N* hydrochloric acid solution was barely adequate, and superior results were obtained with *o*-dichlorobenzene. In order to facilitate separation of the liquid phases and to minimise washings, specially designed stoppered centrifuge tubes (Fig. 1A), made water-repellent by treatment with a silicone solution, were used. The wide necks were required to prevent loss when the silicone-treated stoppers were rinsed into the tubes. As it was impossible on the micro-scale to judge when the formation of cupferronates was complete, and slight loss of aluminium occurred when more than 2 to 3 mg of cupferron in excess of theoretical requirements was used, it was necessary for maximum accuracy to know the approximate amounts of iron and titanium present, so that the quantity of cupferron added could be controlled. For maximum extraction of iron a shaking time of 2 minutes was required to overcome a slight holding effect of potassium bisulphate. Under the conditions prescribed below, about 2 parts per 1000 of iron^{III} were left in the aqueous phase.

As iron^{II} was only 85 per cent. extracted, and some is formed when hydrochloric acid solutions of bisulphate melts containing iron^{III} are held in platinum, it was necessary to limit the period of contact at 100° C to 5 minutes, or else to re-oxidise the solution before extracting. Titanium (TiO_2, 250 μg), zirconium (ZrO_2, 50 μg) and vanadium (V_2O_5, 25 μg) were completely transferred to the organic phase, whereas calcium (CaO, 500 μg), magnesium (MgO, 500 μg), manganese (MnO, 50 μg), beryllium and cerium (BeO or Ce_2O_3, 25 μg) and nickel, cobalt, chromium and platinum (NiO, CoO, Cr_2O_3 or PtO_2, 300 μg) were completely held in the aqueous layer. The presence of phosphate (P_2O_5, 150 μg) had no significant effect on the results.

QUANTITATIVE SEPARATION OF ALUMINIUM FROM CALCIUM, MAGNESIUM AND SOME OTHER ELEMENTS BY MEANS OF ACETYLACETONE—

In the analysis of potable waters, Stene[5] used acetylacetone and extraction with carbon tetrachloride or chloroform to separate aluminium, iron, beryllium, cerium and copper from calcium, magnesium, manganese, titanium, cobalt, nickel, zinc, uranium and so on, which were not extracted at a pH of 4·5 to 7·5. Abrahamczik[6] similarly separated iron, aluminium and manganese in weakly alkaline solution from magnesium and calcium. If, in silicate rock analyses, iron, titanium, vanadium and zirconium were initially removed by means of cupferron, then it seemed that aluminium might be separable from calcium and magnesium, which were the main elements to be considered at this stage, and also, by suitable control of pH, from manganese and various other subsidiary elements, excluding beryllium.

Some experiments were first made on the precipitation of aluminium with other diketones and extraction with other solvents. A reagent superior to acetylacetone was not found, but diethyl ether proved to be a more efficient solvent. Mechanical loss was again minimised by the use of appropriately designed extraction tubes, as shown in Fig. 1B. Water-repellent coatings were not permissible because of the solvent action of ether, and therefore the necks of the tubes could be narrower than before.

The effect of variations in pH on the extraction of aluminium acetylacetonate, under conditions comparable to those prescribed on p. 691, was examined for 1-mg amounts of alumina in the presence of 100 mg of potassium bisulphate. After the complex had been given time to form and the pH had been measured with a pH meter, the three extractions with ether were made and the residual aqueous layer was tested by means of aurinetricarboxylic acid. The results, which are shown in Fig. 2, indicate that extraction is essentially complete over the pH range 6 to 7.

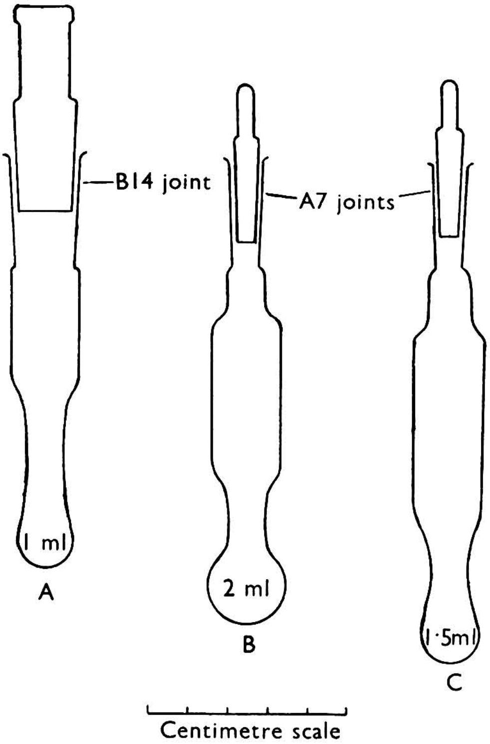

Fig. 1. Extraction tubes

The behaviour of several other elements in the procedure was investigated at a pH of 6 to 7. Calcium and magnesium (CaO or MgO, 500 μg), manganese (MnO, 50 μg), nickel and platinum (NiO or PtO_2, 250 μg) and cerium (Ce_2O_3, 25 μg) were not significantly removed by acetylacetone and ether. About 3 per cent. of cobalt and chromium (CoO or Cr_2O_3, 300 μg) were extracted by ether. These elements are minor components of rocks, and would not be expected to interfere. Beryllium accompanied aluminium. The presence of phosphate (P_2O_5, 150 μg) caused no obvious precipitation with any of the above elements except cerium and chromium. No loss of aluminium by co-precipitation with phosphate precipitates need be feared with the small amounts of these elements likely to be present in silicate rocks.

FINAL DETERMINATION OF ALUMINIUM AS ALUMINIUM 8-HYDROXYQUINOLINATE—

Attempts made to determine aluminium (in absence of beryllium) as aluminium acetylacetonate simply by evaporating the ethereal extract containing it and then heating the residue at 120° C were not a success, apparently because slow volatilisation of the metal complex occurred. The diketonate had to be broken down by treating the ethereal extracts with acid and restoring the aluminium to the aqueous phase for determination. If it were precipitated with 8-hydroxyquinoline in a solution containing acetic acid, no interference from beryllium would result. A reagent superior to 8-hydroxyquinoline for the determination of aluminium was not found.

Quantitative disruption of aluminium acetylacetonate was effected by shaking the ethereal solution vigorously for 6 minutes with 6 *N* hydrochloric acid in extraction tubes of the special type shown in Fig. 1C. Thereafter it was immaterial whether the ether was removed by evaporation in a current of air or by direct withdrawal after separation of the phases by centrifugation.

Much has been published on the determination of aluminium by means of 8-hydroxyquinoline. Its determination in faintly acid solution was required, so that, if beryllium were present, or small amounts of calcium and magnesium inadvertently accompanied aluminium, they would be prevented from interfering. Gotô[7] gave the range of complete precipitation of aluminium 8-hydroxyquinolinate as pH 4·15 to 9·80. Borrel[8] showed that precipitation began at pH 3·85 and was quantitative at pH 4·7. The Chemical Analysis Sub-Committee of the British Ceramic Research Association[9] obtained consistent results in the determination of aluminium over the pH range 4·80 to 5·14, and results distinctly lower below pH 4·48. As positive errors were prevalent in our early work, it was necessary to examine the conditions of the precipitation somewhat fully.

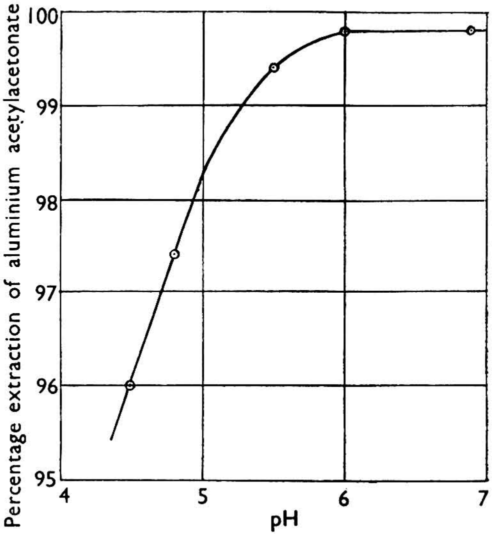

Fig. 2. Effect of pH value on the extraction of aluminium acetylacetonate with ether

It is customary to add an excess of an acetic acid solution of 8-hydroxyquinoline to the slightly acid solution containing aluminium and to effect precipitation in the heated solution by adding an excess of ammonium acetate to raise the pH appropriately.

In experiments made with 13 to 57 mg of alumina in a final volume of 120 ml, the pH after precipitation could be varied from 4·4 to 6·7 without significant effect, and it mattered not whether the solution was filtered hot or cold. Too great a rate of addition of ammonium acetate solution caused significant positive errors, the percentage errors for the same amount of added 8-hydroxyquinoline being greater for smaller amounts of alumina. When ammonium acetate solution was added very slowly, dropwise with constant stirring, the precision of the results was greatly improved, as has also been shown by Stumpf.[10] The main feature of the results was a roughly linear relationship, as shown in Fig. 3, between the weight of the excess of 8-hydroxyquinoline used and the percentage error in the weight of precipitate, from which it was deduced that a 250-mg excess was required.

On the micro-scale, one-thirtieth of all quantities was required and an excess of 8 mg of 8-hydroxyquinoline was indicated. Despite the fact that the ammonium acetate solution was added extremely slowly from a fine-tipped horizontal micro-burette[11] while the solution was vigorously stirred by a magnetic stirrer, an average error of +0·2 per cent. was incurred in determining alumina. Reduction of the excess of 8-hydroxyquinoline to 6 mg eliminated the error, under the conditions of precipitation described on p. 691. The average pH of the solution after precipitation was about 4·7. The presence of a little acetylacetone did not affect the results.

METHOD

APPARATUS—

Use Pyrex or similar glassware throughout.

REAGENTS—

Reagents should as far as possible be of recognised analytical quality.

o-Dichlorobenzene—Distil technical grade *o*-dichlorobenzene before use and collect the fraction that boils between 177° and 178° C.

Diethyl ether—Free the ether from peroxide and distil.

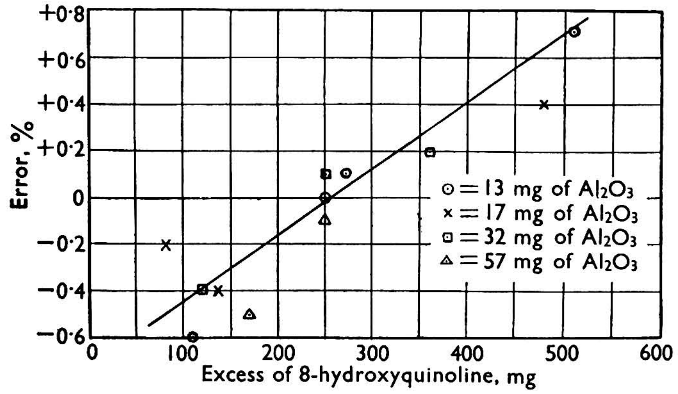

Fig. 3. Relationship between weight excess of 8-hydroxyquinoline and percentage error

Acetylacetone solution—Distil acetylacetone and collect the fraction boiling between 136° and 138° C. Mix 10 ml with 20 ml of ethanol and 70 ml of water.

Cupferron solution—Prepare freshly a cold, 5 per cent. w/v aqueous solution.

8-Hydroxyquinoline solution—Prepare a 2·5 per cent. w/v solution in 4 per cent. v/v acetic acid.

Ammonium hydroxide, 6 N—Prepare from cylinder ammonia and store in a polythene bottle.

Hydrochloric acid, 6 N—Prepare by distillation from the concentrated acid.

PROCEDURE FOR ROCKS—

With the aid of a stoppered weighing stick, weigh into a 1-ml platinum crucible about 5 mg of the dried material. Add 0·15 ml of 40 per cent. hydrofluoric acid and then, 5 minutes later, 0·15 ml of 10 *N* sulphuric acid. Evaporate to dryness on a steam-bath and cautiously expel sulphuric acid over a micro-bunsen flame. Next fuse the residue with 100 mg of potassium bisulphate, cool, place the crucible in a small porcelain capsule, and dissolve the melt in 1 ml of *N* hydrochloric acid. Heat on a steam-bath for *not more than* 5 *minutes* and transfer the solution, by means of a fine capillary attached to a suction apparatus, to a silicone-coated centrifuge tube of the type shown in Fig. 1A. Wash both capsule and crucible four times with 0·25-ml portions of *N* hydrochloric acid and finally rinse the end of the capillary with a drop of acid. Centrifuge the contents of the tube and proceed with the cupferron treatment.

Add to the solution 0·05 ml more cupferron solution than is theoretically required for removal of iron, titanium, vanadium and zirconium (0·1 ml ≡ 0·8 mg of Fe_2O_3) and 1 ml of *o*-dichlorobenzene. Shake the stoppered tube for 2 minutes at the rate of four shakes per second, then unstopper it, centrifuge and transfer the aqueous layer via a micro-filter and capillary to the appropriate centrifuge tube, Fig. 1B. The filter removes a slight opalescence that sometimes appears in the aqueous phase. Rinse the stopper into the extraction tube with 0·5 ml of *N* hydrochloric acid, insert it, and shake the tube for 15 seconds before centrifuging and removing the upper layer as before. Rinse the stopper again with 0·5 ml of acid, but do not shake the tube. Centrifuge and remove the acid. Finally wash the walls with 0·5 ml of acid, centrifuge and remove the acid.

Evaporate the solution containing the aluminium in a current of air, while heating the centrifuge tube in a bath of boiling water, until the volume is about 0·5 ml. Shake the tube to dissolve separated solids and add 0·5 ml of acetylacetone solution, followed by 0·5 ml of 5 *N* ammonium acetate solution and bromocresol purple as indicator. Mix, and then add 6 *N* ammonium hydroxide until the indicator shows blue-green (in daylight), or grey-green if some aluminium acetylacetonate precipitates. Rinse the neck of the tube with 0·1 ml of water, allow 5 minutes for complex formation and then readjust the colour of the indicator, if necessary. Add 3 ml of ether, moisten the stopper of the tube with a drop of water and insert it. Shake the tube for 3 minutes as before, unstopper it, centrifuge and transfer the upper layer by slow suction via a dry capillary to the third centrifuge tube of the type shown in Fig. 1C. Rinse the stopper into the extraction tube with 3 ml of ether and proceed as before. Repeat with 1 ml of ether and shaking for 30 seconds and then with 0·25 ml and no shaking.

To the combined ether extracts add 1 ml of 6 *N* hydrochloric acid and moisten the stopper of the tube with a drop of acid. Shake the stoppered tube as usual for 6 minutes and then remove and rinse the stopper with a little water into a 6-ml beaker. Rinse down the neck of the extraction tube with 6 drops of 6 *N* hydrochloric acid, centrifuge and discard the ether layer. Place the tube in a bath of cold water and gradually heat to boiling, while a slow current of air plays on the surface of the acid and removes residual ether. Centrifuge and transfer the contents by capillary to the 6-ml beaker. Wash the tube three times with 0·75-ml portions of water, always centrifuging briefly before transferring the liquid to the beaker.

Evaporate the solution in the beaker to about 1 ml, and adjust the pH to about 3 by means of 6 *N* ammonium hydroxide and dilute hydrochloric acid, using bromophenol blue as indicator. Add 0·25 ml more of the 8-hydroxyquinoline solution than is theoretically required for aluminium (0·5 ml ≙ 1·5 mg of Al_2O_3) and, if a precipitate forms, just redissolve it with a minimum of 0·2 *N* hydrochloric acid. Dilute the solution to about 4 ml., heat to 80° to 90° C and, while vigorously stirring it by means of a magnetic stirrer, add 1 ml of 2 *N* ammonium acetate solution *very slowly* from a horizontal burette. Next digest the precipitate on a steam-bath for 5 minutes, and then filter it hot on a sintered-glass filter stick of No. 4 porosity. Wash the beaker, filter, stirrer and precipitate with six 1-ml portions of hot water. A similar beaker brought up to weight by the addition of pieces of Pyrex-glass rod should be available as a tare. Wipe both beakers with damp flannelette and then with chamois leathers that have been kept in a desiccator containing saturated calcium nitrate solution. Heat both in an oven at 160° C for 1 hour, cool in aluminium blocks, using no desiccant, and weigh. Repeat the heating until the weight is constant; then dissolve the precipitate in chloroform, withdraw the solution, and dry and reweigh the beaker, filter stick and stirrer. The precipitate is $Al(C_9H_6ON)_3$; multiply the weight of the precipitate by 11·10 and divide by the weight of the sample to get the percentage of alumina (Al_2O_3).

RESULTS

In all the results quoted, correction has been made, where necessary, for aluminium in the reagents.

ALUMINIUM AS ALUMINIUM 8-HYDROXYQUINOLINATE—

A standard solution of aluminium chloride in *N* hydrochloric acid was prepared from Hilger and Watts's "H.H.P." aluminium (99·98 per cent.) by dissolving it in hydrochloric acid. Alumina was determined in weighed portions by the procedure given for silicate rocks, except that in half of the experiments an 8-mg excess of 8-hydroxyquinoline was added instead of 6 mg. The smaller amount gives better results, as shown—

8-mg Excess of 8-hydroxyquinoline—

Al_2O_3 taken, μg	494	534	526	1170	1216	1919	1987
Error, μg	+1	0	+1·5	+4·5	+6	+4	+4

6-mg Excess of 8-hydroxyquinoline—

Al_2O_3 taken, μg	549	533	1041	1400	1878	2059
Error, μg	+1·5	0	−1	0	+2	0

It is evident that, for accuracy to 2 or 3 parts per 1000 in the determination of alumina, the amount of 8-hydroxyquinoline added must be under control to the nearest 2 mg, that is, the alumina content of a 5-mg sample must be known to the nearest 4 per cent.

ALUMINA IN THE AQUEOUS PHASE FROM THE CUPFERRON - DICHLOROBENZENE TREATMENT—

Weighed aliquots of the standard solution of aluminium chloride, to which were added 100 mg of potassium bisulphate, were submitted to treatment with 0·05 ml of 5 per cent. aqueous cupferron solution and dichlorobenzene, as in the analysis of rocks, and the alumina content of the aqueous phase was determined by precipitating aluminium 8-hydroxyquinolinate. The results were as follows—

Al_2O_3 taken, μg ..	546	530	512	551	1916	1897	1899	2139
Error, μg	−2	−1	+1	+3	+2	+5	+4	−3

ALUMINA EXTRACTED BY ACETYLACETONE AND DIETHYL ETHER—

Weighed aliquots of the standard solution of aluminium chloride, to which were added 100 mg of potassium bisulphate, were suitably prepared and treated with acetylacetone and diethyl ether. The ether extracts were shaken with 6 *N* hydrochloric acid and the aluminium was determined with 8-hydroxyquinoline, all as described for rock analysis. The following results were obtained—

Al_2O_3 taken, μg	525	579	1169	1154	1888	1937
Error, μg	−0·5	−0·5	+1	−4·5	0	+2

ALUMINA IN PRESENCE OF OTHER ELEMENTS—

As satisfactory results were obtained in the three preceding steps, experiments were next made on the determination of various amounts of alumina in synthetic mixtures containing selected combinations of the elements that might be found in silicate rocks. All the test solutions contained 100 mg of potassium bisulphate and the procedure was that prescribed for rocks, except that the initial treatment with hydrofluoric acid and the fusion were omitted. The results given below indicate that in a 5-mg sample of rock it should be possible to determine 0 to 40 per cent. of alumina, with a reasonable degree of accuracy, in the presence of a considerable amount of all the usual components and small amounts of several others.

(*a*) *Other elements present*: CaO, 500 μg; MgO, 500 μg; P_2O_5, 150 μg; MnO, 50 μg; BeO, 25 μg; Ce_2O_3, 25 μg—

Al_2O_3 taken, μg	502	542	1969	1893
Error, μg	+2·5	+1	−0·5	−3·5

(*b*) *Other elements present*: Fe_2O_3, 1000 μg; TiO_2, 250 μg; P_2O_5, 150 μg; ZrO_2, 50 μg; V_2O_5, 25 μg—

Al_2O_3 taken, μg	546	635	1953	1910
Error, μg	+0·5	+4*	+2	−7*

* These results are corrected for 1 μg of Fe_2O_3 in the precipitate.

ALUMINA IN SILICATE ROCKS—

Finally, a number of rock samples were submitted to analysis through the whole procedure. As the method is primarily intended for use in the analysis of more complex materials, where the number and the amount of other components of the "mixed oxides" are large, and the standard samples available were lacking in this respect, it was necessary to include two synthetic samples in the series of rocks analysed. They were prepared by adding accurately weighed portions of U.S. Bureau of Standards Feldspar No. 99 (dried) to the ignited residues from the evaporation of appropriate amounts of solutions containing the elements required.

The results of the analyses are shown in Table I. The feldspars used were as issued by the U.S. Bureau of Standards. The flint clay was from the part that had passed through a 300-mesh sieve. As the percentage of silica found in this part agreed excellently with the certificate value, it was assumed that comparison with the certificate value would be valid for alumina. The olivine-basalt was part of the original sample used by Guthrie and Miller[12] and was less finely divided. All the samples, save the flint clay, which was dried at 140° C, were dried at 105° to 110° C before use.

In general, the results are about 1 per cent. lower than those shown in the last column. An attempt was made to obtain figures for comparison by other than the classical procedure. As the feldspars contained insignificant amounts of iron, titanium, manganese, calcium and magnesium, it was practicable to omit the use of cupferron and acetylacetone, precipitate together the 8-hydroxyquinolinates of aluminium, iron and titanium, and then correct for the iron and titanium content of the precipitates on the assumption that $Fe(C_9H_6ON)_3$ and $TiO(C_9H_6ON)_2$ were present (*cf.* McLennan[4]). In experiments with 70 to 90-mg samples, the results obtained were—

Feldspar No. 70—(17·60), 17·78, 17·79 and 17·81 per cent.
Feldspar No. 99—18·82 and 18·85 per cent.

which seem to suggest that the figures obtained by the classical procedure tend to be rather high. It is of interest that Guthrie and Miller,[12] working with the same sample of Feldspar No. 70, found 17·94 per cent. of alumina by a semi-micro classical procedure. The method described above was inapplicable to the flint clay on account of the complexity of the "mixed oxides."

TABLE I

DETERMINATION OF ALUMINA IN SILICATE ROCKS

Silicate	Approximate weight taken, mg	Amounts of certain components: SiO_2, %	Fe_2O_3, %	TiO_2, %	P_2O_5, %	CaO, %	MgO, %	MnO, %	Al_2O_3 by micro-method, %	Al_2O_3 by classical method or calculation, %
Feldspar No. 70* ..	4·0	67	0·03	<0·01	0·01	0·07	0·01	<0·01	17·86	18·03†
	6·3								17·71	
Feldspar No. 99* ..	4·8	69	0·07	0·02	0·14	0·36	0·05	<0·01	18·94	19·06†
	6·3								18·77	
Flint clay No. 97* ..	4·6	43	0·98	2·38	0·08	0·10	0·26	<0·01‖	38·23	38·77†
	4·5								38·43	
Synthetic rock A ..	"4·7"	40	15·0	5·0	3·0	10·0	6·0	—	11·13	11·13§
	"4·5"								10·59	10·75§
Synthetic rock B ..	"4·9"	40	10·0	3·0	1·0	6·0	6·0	—	25·43	25·69§
	"4·7"								25·31	25·55§
Olivine-basalt ..	4·2	44	13·0	2·4	0·5	10·0	13·0	0·2	12·59	12·59‡
	3·7								12·68	

* U.S. Bureau of Standards sample.

† U.S. Bureau of Standards certificate value.

‡ Guthrie and Miller's figure for semi-micro analyses.[12]

§ Calculated from the weight of Feldspar No. 99 (Al_2O_3 = 19·06 per cent.) in A, and the weight of feldspar (≏2·8 mg) plus additional Al_2O_3 in B.

‖ Also ZrO_2, 0·23; Cr_2O_3, 0·08; and V_2O_5, 0·04 per cent.

If these figures for the feldspars are accepted as being correct, and the composition of the synthetic samples is recalculated accordingly, then the errors for the four materials, calculated as weight of alumina, are as follows—

Silicate	Feldspar No. 70 (i)	Feldspar No. 70 (ii)	Feldspar No. 99 (i)	Feldspar No. 99 (ii)	Synthetic "A" (i)	Synthetic "A" (ii)	Synthetic "B" (i)	Synthetic "B" (ii)
Al_2O_3 calculated, µg..	714	1116	908	1196	476	515·5	1185	1262·5
Al_2O_3 found, µg ..	717	1111	913	1191	474	521·0	1180	1256·0
Error, µg	+3	−5	+5	−5	−2	+5·5	−5	−6·5

bringing them into line with those found for the determination of alumina in complex mixtures. Should, however, the certificate figures be correct and a negative error of 1 per cent. be genuine, it is still possible to say that the errors in terms of the rock total do not greatly exceed the limits normally allowed for duplicate analyses of gram samples by the same analyst.[13] Moreover, they are considerably less than those evident in the recent co-operative investigation of precision and accuracy in the chemical analysis of silicate rocks,[14] and rather less than those shown in a later co-operative analysis of a synthetic glass of known composition.[15] In the new micro-method, it is almost certain that the results are for alumina only and, as far as we can judge without actual experimentation, it should be possible to determine calcium and magnesium by a standard procedure in the aqueous extract left after the removal of aluminium by means of acetylacetone and ether.

We gratefully acknowledge a maintenance grant to one of us (R. A. C.) from the Department of Scientific and Industrial Research, and grants from Imperial Chemical Industries Limited and the Trustees of the Ritchie Bequest. We are indebted to Dr. M. Borrel of Lyons University for a copy of his thesis.

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NOTE—Reference 1 is to part II of this series.

CHEMISTRY DEPARTMENT
THE UNIVERSITY, EDINBURGH, 9
THE UNIVERSITY SCIENCE LABORATORIES
SOUTH ROAD, DURHAM

June 3rd, 1953

DISCUSSION

MR. R. C. CHIRNSIDE said that he had followed with interest this paper by Dr. Miller and Mr. Chalmers, as indeed he had all of Dr. Miller's papers on the subject. Those concerned with silicate and rock analysis would know that the techniques had changed very little since the time of Berzelius, and attempts to determine some of the constituents directly, as instanced in this paper, were therefore very welcome.

Some work along the same lines had been done by the Analytical Committee of the British Ceramic Research Association, and he would like to ask Dr. Miller if she was acquainted with this work, which had been published last year in the *Journal of the Ceramic Society.*

In this connection, he wished to mention that, with certain refractories where free alumina in the form of corundum was known to occur, it had been found impossible to get complete decomposition of the sample except by using a flux of sodium carbonate and borax. He wondered whether some of the low results to which Dr. Miller had referred could have arisen from incomplete decomposition of the sample.

He also wanted to mention that during the co-operative work carried out by the B.C.R.A. Committee, the cause of some apparently low results for alumina was traced to dilution of the sample with silica removed from agate mortars during the grinding of the sample.

MR. CHALMERS replied that they had read the account of the work done by the Analytical Committee of the British Ceramic Research Association, and had considered the use of sodium carbonate for the fusion of the residue left after the removal of silica by means of hydrofluoric acid, but had rejected it, partly because of the risk of loss by spattering in the subsequent dissolution in acid and partly because the bisulphate fusion was easier and seemed quite satisfactory. It was possible that the presence of free alumina might account for the apparent negative error, but it was not likely. There seemed no reason why other fluxes should not be used instead of potassium bisulphate.

MR. A. F. WILLIAMS mentioned that sodium peroxide was in universal use for breaking down ores. The technique was devised by Rafter, of New Zealand, and could be carried out in a platinum crucible at a temperature of about 400° C. The method was rapid and there was no difficulty caused by spraying. There might be difficulty in getting the sodium peroxide free from alumina, but suitable brands should be available.

MR. CHALMERS said that they were acquainted with Rafter's paper and thought that the considerations given in the previous reply would apply here also.

DR. MILLER added, in a written reply to Mr. Chirnside's comments, that the methods described by the British Ceramic Research Association did not fully lend themselves to their purpose, which was to determine alumina in association with all the elements, including some that were commonly neglected, that might be present in igneous silicate rocks, and afterwards to determine calcium and magnesium in the same sample.

With reference to the apparent negative errors recorded for the rocks, some chemical procedures tended to give biased results, and alumina determinations, as normally made, gave results that were more often too high than too low. It might be of significance that, in the co-operative analysis of the synthetic glass of known composition, the average percentage of alumina found was 16·19 instead of 15·78—a difference of 0·4. All their results for the standard samples were lower than the certificate values, the average deviation being 0·3.

The standard samples were not further ground and could not therefore be diluted with silica.

Ultra-violet Spectrophotometric Estimation of the Quality of Mineral Oils Extracted from Bread

BY M. A. COOKSON, J. B. M. COPPOCK AND R. SCHNURMANN

(*Presented at the meeting of the Society on Wednesday, May 20th,* 1953)

A method has been devised for determining in bread the degree of refining of a mineral oil that has been absorbed by the dough during bread-making. The method consists basically in sulphating, under controlled conditions, the unsaponifiable fraction of the total oils extracted from the bread, so that the natural saponifiable matter is destroyed without completely removing the unsaturated hydrocarbon constituents inherent in mineral oils refined to different degrees. The recovered mineral oil is then examined by ultra-violet absorption spectrophotometry to determine its quality. A spectrophotometric criterion, based on the absorption intensity of the sulphated oils at 2600 A, is suggested for the quality of mineral oils that can be regarded as satisfactory for the lubrication of plant used in bread production. Some properties of the naturally occurring unsaponifiable oils of bread are also described.

IN recent times bread-making has become a highly mechanised process. The nature of bread dough is such that lubrication is necessary at various stages of production to prevent adherence of the dough to metallic equipment. This is particularly so at the dough divider, where the mass of dough is separated into unit pieces, and also when the dough pieces are tinned before baking. White oils are amongst the lubricants that have been used for these purposes. Since mineral oil may also be absorbed by doughs as a result of accidental contamination from faulty machinery, it is important to maintain a reasonable standard of quality for the oil.

Mineral oils absorbed by doughs can be extracted by a solvent from the finished bread, but the naturally occurring flour-oil and other edible fats and oils added as bread ingredients are extracted at the same time.

It was recommended[1] in 1949 that mineral oils should be permitted as lubricants and greasing-aids in bread-making provided that (*a*) the quantity absorbed did not exceed 0·2 per cent. of the weight of the bread (this quantity is now the legally permitted limit[2]) and (*b*) the degree of refining of the oil was not less than that of liquid paraffin, B.P.

A method for determining the quantity of mineral oil in bread has been published,[3] and it was seen to be desirable to form some estimate of the original quality of the oil, *i.e.*, its quality before it was absorbed by the dough.

The development of a suitable method was complicated by (*i*) the small amounts of oil available for analysis, unless very large quantities of bread were laboriously extracted, (*ii*) the miscibility of mineral oils with the unsaponifiable fraction of bread-oil (*i.e.*, the natural oils in bread), which makes separation difficult, and (*iii*) the lack of suitable methods for determining mineral oil quality.

The sulphuric acid test of the British Pharmacopoeia for aromatic hydrocarbons in medicinal liquid paraffin is only suitable for fairly large amounts of white oils, and the conventional interpretation of its results has been criticised.[4] Ultra-violet absorption spectrophotometry, however, appears to be a sufficiently discriminating technique for determining the various ranges of quality of mineral oils after separating the unsaponifiable matter from bread, and it was the method chosen for this work.

EXPERIMENTAL

Five mineral oils (see Table I) of different and known degrees of refining were used in most of the experimental work.

For spectrophotometric examination, all the specimens were dissolved in purified *iso*-octane and the absorption characteristics of the solutions studied in the wavelength region 2100 to 4500 A (particularly 2400 to 3400 A). Most of the spectra were recorded by means of a medium Hilger quartz spectrograph, fitted with a Spekker photometer, at the Physics Department of the Manchester Oil Refinery Ltd.

The materials examined were prepared at the British Baking Industries Research Association, the experimental procedure being as follows. Bread doughs, to which small quantities (0·1 to 0·5 per cent.) of the mineral oils listed in Table I were added during the dough-making, were baked, care being taken that they were not otherwise contaminated with extraneous mineral oil from tins or machinery. About 18 hours after baking, the cooled bread was crumbled and digested with carbon tetrachloride for 24 hours at room temperature to extract the combined mineral and natural oils. These mixed oils were saponified and the unsaponifiable matter was extracted by standard procedures.[3]

TABLE I

THE CHARACTERISTICS OF THE MINERAL OILS USED IN THE EXPERIMENTS

		$E^{1\%}_{1\,cm}$ values at 2600 A	
Oil	Appearance	As refined	After treatment with 96 per cent. w/w H_2SO_4 at 100° C
Liquid paraffin, B.P.	Colourless (white oil)	0·030	0·051
Technical white oil	Colourless (white oil)	0·270	0·435
Transformer oil	Fluorescent, straw colour	37·0	17·4
An acid and clay treated Edeleanu extract of a lubricating-oil distillation fraction	Fluorescent, brown colour	49·0	14·2
Spindle oil*	Fluorescent, ruby colour	220·0	21·5

* This term is not connected with the spindle dough moulder; it is derived from spindling in the manufacture of textiles.

As the oil from bread to which no mineral oil has been added contains about 10 per cent. of unsaponifiable matter, further separation is required to isolate the mineral oil as free as possible from other unsaponifiable material. Two methods were tried for this purpose: (*a*) percolation of a light petroleum solution of the unsaponifiable matter through a column of activated alumina and (*b*) heating the unsaponifiable matter with sulphuric acid to remove sterols, tocopherols and hydrocarbon compounds other than those constituting the mineral oil addition, as described below.

Both methods suffer the disadvantage that some of the constituents of the less refined mineral oils are removed and part of the natural unsaponifiable oil is retained in the end product. However, the working conditions for method (*b*) have been so adjusted that a sufficiently satisfactory separation can be effected.

The separation of the oil—About 0·5 g of the unsaponifiable matter is dissolved in ether and transferred to a Babcock milk-test bottle. After removal of the solvent, 5 ml of carefully standardised 85 per cent. w/w sulphuric acid are added and the bottle is vigorously shaken for 30 minutes in a water-bath at 50° C. After cooling, sufficient 85 per cent. sulphuric acid is added to bring the liquid level into the graduated neck of the bottle, which is then centrifuged until the oil layer separates. The oil is then removed with a fine pipette for spectrophotometric examination.

RESULTS

MINERAL OILS—

Absorption values for the five oils studied are shown in the third column of Table I.

It was necessary to ensure that decomposition, particularly of liquid paraffin and technical white oil, did not occur under baking conditions, thereby making some adventitious contribution to the spectra of the total oils extracted from bread containing mineral oil. This was done by heating liquid paraffin and technical white oil at baking oven temperatures (about 250° C; apart from the crust, bread itself during baking rarely exceeds a temperature of 100° C) for baking times (about 30 minutes). It was found that even in an atmosphere of air, not of carbon dioxide and steam as in bread baking, no marked change in absorption spectra for these two oils was observed.

NATURAL UNSAPONIFIABLE OILS—

As previously stated, about 10 per cent. of the oil that can be extracted from bread, made from flour, yeast, salt and water only, is unsaponifiable. Most, if not all, of the bread-oil is derived from the flour, modified during fermentation and baking, and possibly containing traces of esters and other organic compounds produced in bread-making. It is well known that flour is a variable material, depending on the wheats from which it is made (*i.e.*, the grist), the condition of the wheats when milled, the extraction rate of the flour and the age of the flour when used. Moreover, the method of fermentation and baking produces marked differences in the nature of the resultant bread. It is not surprising, therefore, that the unsaponifiable fraction of bread-oil has been found to be of variable composition.

In Fig. 1 the two unbroken lines show the extremes of the range of absorption spectra observed over a period of 4 years from unsaponifiable oils prepared from breads produced from National flour, bleached and treated, of 81 or 85 per cent. extraction, by the same formula and baking technique. Fig. 1 also shows the spectrum of the unsaponifiable oil from a wholemeal bread; the effect of the higher extraction rate of the flour is apparent from the higher absorption intensity of the oil from this flour.

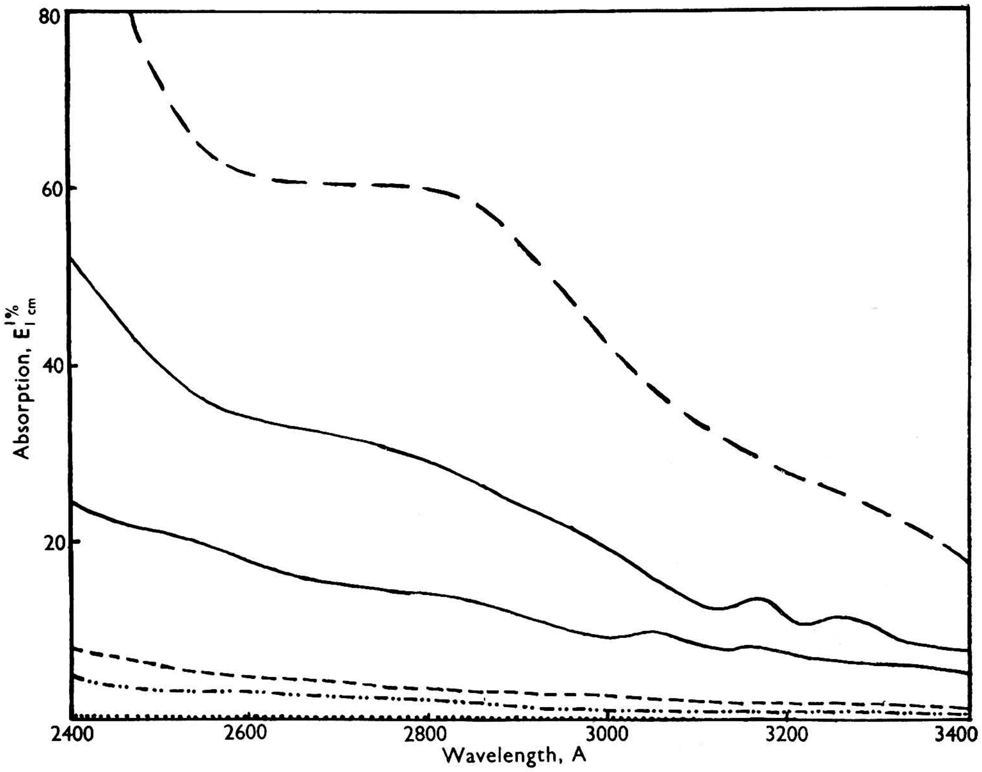

Fig. 1. Absorption spectra of (i) the unsaponifiable oils of breads made from flours of different extraction rate; (ii) the unsaponifiable oils from bread containing 0·26 per cent. of liquid paraffin, and of the chromatographed extract of this oil; and (iii) liquid paraffin—the basis for comparison.

— — Unsaponifiable oil from a wholemeal bread (highest flour extraction rate).
——— Unsaponifiable oils from National breads (lower flour extraction rates, 80 to 85 per cent.).
– – – – Unsaponifiable oil from National bread containing 0·26 per cent. of liquid paraffin.
–..–.. Chromatographed extract of the unsaponifiable oil from National bread containing 0·26 per cent. of liquid paraffin.
...... Liquid paraffin

A comparison of these absorption characteristics with those in Table I for the five mineral oils studied reveals that the light absorption in the region of the ultra-violet explored is much greater for these unsaponifiable bread-oils than for white mineral oils, and that these naturally occurring unsaponifiable oils have absorption intensities similar to those of some mineral oils that have not undergone the same degree of refining as white oils. As compared with liquid paraffin, B.P., these less refined oils show 100 to 10,000 times as much absorption. The variations in the intensity of absorption of unsaponifiable oil from different batches of flour might be attributed to the presence of various amounts of a highly absorbing component in the unsaponifiable part of the natural oil.

Fractionation of the natural unsaponifiable oil on a column of activated alumina has indicated the existence of several components, some of which, separated with light petroleum - benzene mixtures as eluants, absorb much more strongly than those shown in Fig. 1, *e.g.*, at 2600 A one fraction shows a maximum $E^{1\%}_{1\,\text{cm}}$ value of 480, and a second fraction crosses this peak and rises to an $E^{1\%}_{1\,\text{cm}}$ value of 750 at 2400 A. It is hoped that further details about this particular phenomenon will be published later.

UNSAPONIFIABLE OILS CONTAINING MINERAL OILS—

The maximum permitted quantity[2] of mineral oil that may be present in a food as a result of its use as a processing aid is 0·2 per cent. by weight of the food, but the desirable quality of the mineral oil is not legally defined.

The method of oil extraction described in the experimental section, when applied to a bread containing no mineral oil, yields approximately 0·4 per cent. of oil, expressed on the bread weight, and approximately 0·53 per cent. when a bread containing 0·2 per cent. of mineral oil is extracted. After saponification of these oils the remaining unsaponifiable oils are approximately 0·04 and 0·11 per cent., respectively, expressed on the bread weight, *i.e.*, about two-thirds of the latter is attributable to mineral oil. It is, therefore, in the light of the various spectra discussed, and having regard to the high intensity of the bread's natural unsaponifiable oil, clearly not to be expected that the quality of the mineral oil originally used as a lubricant can always be ascertained by merely examining the spectrum of the total unsaponifiable oil; the interference would be too great. The spectra in Fig. 1 of liquid paraffin and of the unsaponifiable oils from National breads with and without liquid paraffin, *i.e.*, the first, third and fifth lines from the abscissa, illustrate this point. However, when the mineral oil is of rather low quality, a detectable increase in absorption intensity of the combined unsaponifiable matter might be expected. This has been found to occur in practice, provided there is present about 0·2 per cent. of oil of a quality approximately equivalent to an acid and clay-treated Edeleanu extract of a lubricating oil distillation fraction, but if less than 0·1 per cent. is present, it is very difficult to detect.

A further factor to be taken into consideration is that the proportion of natural to mineral oil in the total unsaponifiable oil will not be constant, so that, in the absence of any characteristic band structure connected with the mineral oil in the combined oil, quantitative measurements would be difficult to apply in estimating the quality of the mineral oil.

It is seen, therefore, that because of the relatively large absorption and heterogeneity of the naturally occurring unsaponifiable matter, and also the variation in the proportion of mineral oils in the unsaponifiable oil, a separation of the mineral oil fraction of the total unsaponifiable oil must be achieved in order to appraise the quality of the mineral oil in the mixture.

THE ATTEMPTED SEPARATION OF MINERAL OIL FROM UNSAPONIFIABLE MATTER BY CHROMATOGRAPHIC METHODS—

The use of adsorption columns of activated alumina for the separation of hydrocarbons has been known for some time. This technique has been suggested as a way of treating the unsaponifiable fraction in a general method for determining mineral oil in saponifiable oils,[5] and particularly for determining mineral oil in bread.[6] However, it was reported in our paper[6] that a small fraction of the natural unsaponifiable matter was eluted immediately without being adsorbed on the separation column. If mineral oil is also present in the unsaponifiable material, it is possible that not only is the same natural non-adsorbed fraction (probably hydrocarbon in nature) eluted, but that further fractions of the natural oil may be eluted at the same time by a solvent action of the mineral oil.

It has been found that the eluates containing mineral oil obtained by this method are still sufficiently variable and intense in absorption to make difficult the discernment of the quality of the mineral oil. Although the absorption intensities of these chromatographed fractions are not so great as those exhibited by the total unsaponifiable matters containing mineral oils, they are still appreciably greater than those of the original mineral oils.

The second and third lines from the abscissa in Fig. 1 show typical results given by the unsaponifiable matter from bread oil mixed with liquid paraffin, B.P., before and after removal of some of the absorbing materials by chromatographic separation. The liquid paraffin was present at a concentration of 0·26 per cent. in the bread.

More efficient separation than that provided by this particular chromatographic technique would therefore be necessary to determine the original quality or degree of refining of a mineral oil that has been extracted from a loaf of bread. Although greater efficiency might be attained by varying the adsorbent in the chromatographic column and the eluting solvent, separation of the mineral fraction by sulphation appeared to hold more promise.

SEPARATION OF MINERAL OIL BY SULPHATION OF UNSAPONIFIABLE MATTER—

The A.O.A.C. method[7] for determining mineral oil in fats is based on heating the unsaponifiable fraction with concentrated sulphuric acid of not less than 94 per cent. w/w at 100° C for 30 minutes with occasional shaking. The apparatus used for this digestion is a Babcock milk-test bottle into which the fat is introduced in ether solution, the ether then being evaporated before the acid is added. After sulphation, further acid is added to the cooled digest until the liquid rises well into the graduated neck of the bottle, which is then centrifuged to bring the oil layer to the surface, where its volume is determined. This technique has been recommended[3] for determining mineral oil in the unsaponifiable matter from bread oil, but it was suggested that the results might be unreliable if a low grade of mineral oil was present, owing to removal of impurities in the oil.

In a preliminary experiment, the five mineral oils of various degrees of refining were treated as described above, and the ultra-violet absorption spectra of the sulphated oils were examined. The results in the fourth column of Table I indicate appreciable differences from the spectra of the corresponding materials before acid treatment.

Although the more impure oils showed considerable reaction, as evidenced by loss of colour and appreciable reduction in absorption intensity, the white oils exhibited an increased absorption, particularly in the wavelength region below 2600 A, the $E^{1\%}_{1\,\mathrm{cm}}$ values at 2400 A being 0·19 for liquid paraffin, B.P., and 0·9 for a technical white oil. The corresponding values for the non-sulphated oils were 0·05 and 0·65. The increase in absorption intensity may be caused by the formation of sulphonic acids and the dehydrogenation of reactive naphthenes by this intense treatment with sulphuric acid; an alcohol extraction and clay treatment would probably be necessary to remove these absorbing compounds.

Less drastic conditions of sulphation of the unsaponifiable matter from the total oil extracted from bread containing mineral oil were investigated. A temperature of 50° C was arbitrarily chosen for the reaction, and the acid concentration reduced until separation of the mineral oil either no longer occurred or was difficult. This was found to occur with 80 per cent. sulphuric acid. The use of 85 per cent. acid, however, permitted a separation of oil after the reaction. Under these conditions the less refined oils usually still retained some of their colour after treatment and this in itself allowed a rapid distinction to be drawn between them and white oils. If the layer above the acid in the Babcock bottle is solid or semi-solid in reasonable quantity and not a thin black sludge, as occurs when no mineral oil is present, it is safe to assume that a paraffin wax or petroleum jelly has been absorbed into the bread dough.

Fig. 2 shows the spectra given by separated mineral oils after sulphation of the total unsaponifiable matters from breads containing these oils with 85 per cent. w/w sulphuric acid at 50° C for 30 minutes. These results have been obtained in duplicate by operatives working in different laboratories, and in one case using a Uvispek absorption spectrophotometer.

The spectra of the extracted mineral oils after this sulphation treatment closely approximated to those of the original oils, except for some fall in the extinction values for the least refined oil. The main difficulty with this technique is that the spectra of the recovered two white oils are not readily distinguishable, although the figures in Table I show that in relation to each other measurable differences exist. The reason for this may, in part, lie in a reaction between sulphuric acid and quite highly refined oils, with the formation of substances having different absorption characteristics.

A further series of experiments was made with 90 per cent. instead of 85 per cent. sulphuric acid, and although the resultant spectra of the extracted oils still permitted distinction between oils of different purities, they were not as sensitive as those obtained after treatment with 85 per cent. acid.

The wavelength region between 2100 and 2400 A was examined in the hope that discrimination would be possible between two white oils of different quality after extraction from bread. Liquid paraffin and a technical white oil were examined, but the spectra of these oils after extraction from bread could not be readily distinguished at these lower wavelengths.

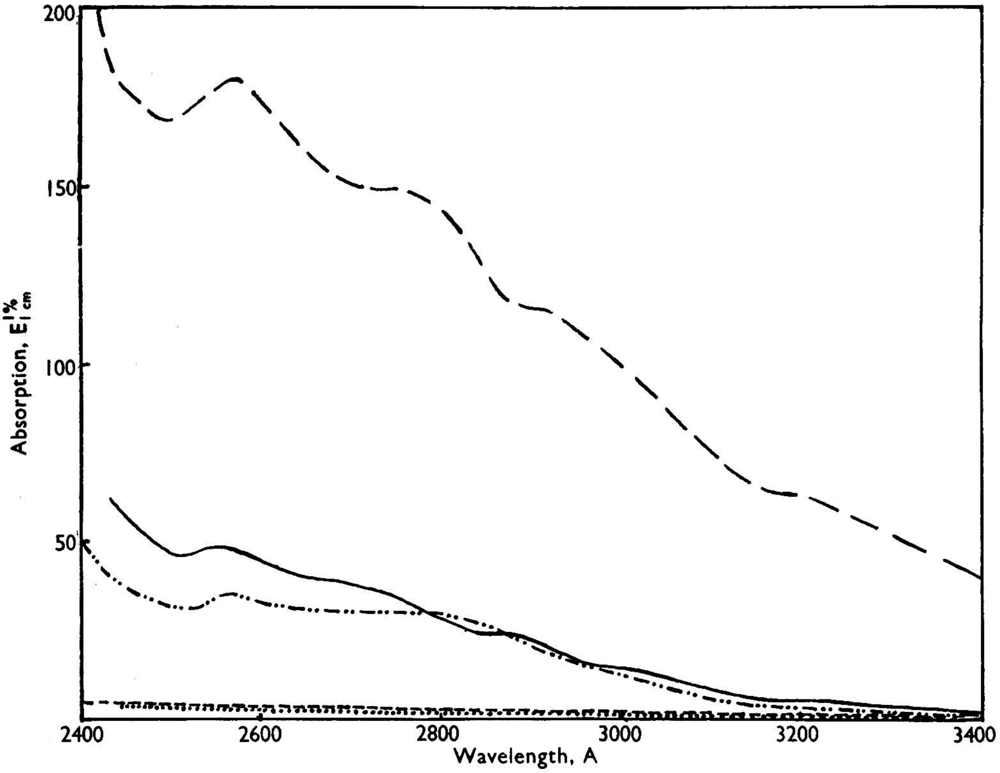

Fig. 2. Absorption spectra of mineral oils separated from the unsaponifiable matter of bread oils with 85 per cent. sulphuric acid at 50° C.

...... Liquid paraffin.
– – – – Technical white oil.
–··–·· Transformer oil.
——— An acid and clay treated Edeleanu extract of a lubricating oil distillation fraction.
— — Spindle oil

The results recorded and used for correlation purposes in the original quantitative work on mineral oils in bread[3] have not been amended by this new sulphation technique, as the re-determinations would be very tedious and would not significantly alter or increase the sensitivity of the quantitative method. None the less, the technique now described is preferable for routine use if a quality determination is also required.

CONCLUSIONS

For the lubricating oils used in bread-making, the question arises whether liquid paraffin, B.P., and some other white oils are so significantly different in physical characteristics that only the former, as has previously been maintained, should be used. It is suggested that Table II, which shows the $E^{1\%}_{1\,cm}$ values at 2600 A for the various oils examined before use and after extraction from bread, will supply the answer to this question. From the figures shown in this table, it is clear that medicinal liquid paraffin, B.P., and some other white oils, even though they may not be of B.P. quality, fall into a related group when compared with the less refined colour-bearing mineral oils (see Fig. 2). The absorption intensities of the mineral oils when compared with the natural unsaponifiable oils from bread, are (*i*) lower for the white oils, (*ii*) usually greater for the colour-bearing mineral oils and (*iii*) lower for all the mineral oils examined when compared with some of the components that have been isolated from natural unsaponifiable matter of the oil in bread. Mineral oils can be recovered from bread by the suggested procedure comparatively unchanged spectroscopically, and the relationship of their absorption intensities to that of the natural unsaponifiable oil of bread remains as summarised here.

TABLE II

Mineral oil	$E^{1\%}_{1\,\text{cm}}$ at 2600 A Before use	 Extracted from bread
Liquid paraffin, B.P.	0·030	2·6
Technical white oil	0·270	4·0
Transformer oil	37·0	33·0
An acid-treated Edeleanu extract of a lubricating-oil distillation fraction	49·0	44·0
Spindle oil	220·0	173·0

Provided, as is desirable on both pharmacological[8] and technical grounds, that the viscosity of the oil is not abnormally low, then, it seems to us for the above reasons that mineral oils for which the $E^{1\%}_{1\,\text{cm}}$ value at 2600 A does not exceed 0·5 before use, or 5·0 after extraction from bread by the procedure suggested above, could be regarded as satisfactory for the lubrication of plant used in bread production.

The authors express their thanks to Mr. W. F. Maddams, M.Sc., Miss P. M. Martin, M.Sc., and Mr. R. Mayoh, B.Sc., for their assistance with the spectrographic work.

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DISCUSSION

THE PRESIDENT, Dr. D. W. Kent-Jones, said he thought that many of the chemists who specialised in the examination of oils might have important observations to make, as they probably had experience of such matters in other connections.

As many analysts did not possess spectrophotometers, he wished to know if it was possible to use the ordinary Spekker absorptiometer with the ultra-violet light attachment. He was interested to hear that wholemeal bread, as opposed to white bread, apparently contained something in the unsaponifiable portion of the bread oil that had very high absorption in the ultra-violet spectrum, and he wondered if the authors had any information as to what these substances were.

MR. COOKSON replied that preliminary experiments indicated that mineral oils of various qualities could be differentiated with a Spekker absorptiometer adapted to measure the transmission of ultra-violet light through the oil. With suitable filters to exclude as much as possible of the visible light, increasingly large-scale deflections were obtained as the quality of the mineral oil decreased. However, more recent work had indicated that further experimentation was required before such a technique might be found to be successful.

The compounds naturally present in the unsaponifiable fraction of bread oils and responsible for high absorption in the ultra-violet spectrum had not as yet been identified. Adsorption chromatography had enabled a separation of some constituents of unsaponifiable flour and bread oils to be made, and the similarity between these oils from various sources had been shown by ultra-violet spectrophotometry. The reason for the greater absorption intensity of the unsaponifiable oil from wholemeal bread was unknown, but it might be due to the presence in this oil of compounds that are removed when flours are milled to a lower extraction rate. The very high extinction coefficients found at wavelengths below 3000 A were, however, of some interest.

DR. J. R. NICHOLLS said that white petroleum oils were prepared commercially by the somewhat drastic treatment of coloured oils with sulphuric acid, whereby many other impurities were removed with the colour. The better grades were used for pharmaceutical purposes and were required by the British Pharmacopoeia to conform to a maximum limit of carbonisable substances. From the absorption curves illustrated it appeared clear that technical white oils contained only slightly greater amounts of impurities than liquid paraffin, B.P., and that the proposed method was adequate for distinguishing white oils from cruder products.

The only difficulty in permitting all grades of white oils to be used for lubrication of plant in bread-making was the possibility that some grades might contain carcinogens. Bearing in mind the purification that all white oils had undergone and the maximum amount of oil that was allowed to be present in the finished bread, any danger appeared to be small. But it was desirable that adequate tests should be made.

MR. COOKSON said that extensive surveys of the literature and discussions with authorities on this subject had failed to produce the slightest evidence of carcinogenicity from the oils included in their specification.

DR. K. A. WILLIAMS said he understood that both white and medicinal oils were made from naphthenic-base stocks by treatment first with fuming sulphuric acid and then with alcohol. The hydrocarbons present included a fairly high proportion of *cyclo*paraffins mainly derived from *cyclo*pentane and *cyclo*hexane.

MR. N. L. ALLPORT referred to a sample of medicinal liquid paraffin sent to him because it had been reported against as failing the British Pharmacopoeia's sulphuric acid test. In point of fact it quite safely passed the B.P. requirement, and in the course of subsequent discussion it transpired that the first analyst had used concentrated sulphuric acid of 98 to 99 per cent., whereas the Pharmacopoeia specified a special reagent described in the First Appendix as consisting of 96 per cent. by weight of nitrogen-free sulphuric acid.

When presenting the paper Dr. Coppock had interposed that with certain unsaponifiable fractions the extinction coefficient as measured by a photo-electric type of spectrophotometer was different from that recorded by a photographic type of instrument. He asked if the authors could say which instrument gave the true value.

DR. SCHNURMANN replied that in the wavelength region between 2400 and 3000 A it did not matter whether a photographic plate instrument or a photo-electric instrument was used. With the same type of prism in either of these instruments, results were almost identical. Difficulties might arise, however, at wavelengths shorter than 2400 A when photo-electric instruments were used because at these shorter wavelengths the emission intensity of the hydrogen discharge tube fell off, the sensitivity of the photo-cell decreased, the reflectivity of the mirrors became poorer, and the proportion of scattered radiation increased; hence with photo-electric instruments the indicated absorption intensity might appear to decrease in the region between 2400 and 2000 A on approaching the shorter wavelength end even for samples for which it could be proved with the help of photographic plate instruments that the absorption intensity increased towards shorter wavelengths. The photographic plate instrument, in addition to supplying a permanent record, had the advantage that it could be safely used over the whole region over which the photographic plate was sensitive.

DR. W. W. MYDDLETON, in a written contribution, said he wondered what use was to be made of the results described in the paper, and asked whether there was to be a drive in favour of using medicinal paraffin or technical while oils and, if so, on what grounds.

He asked if it was claimed that poorly refined oils endowed the bread with an objectionable taste, smell or colour, because if so, the results of the spectrophotometric method could but confirm the evidence of the senses.

He said that at one point in the presentation of the paper they were left to infer that, because medicinal paraffin and a technical white oil could be heated above 200° C for some time without any effect on the ultra-violet absorption spectrum, a similar state of affairs would exist in the baking of the loaf. He found this inference difficult to draw, for the oil in the bread was probably in thin films and was in contact with many different components of the bread and, in addition, some of it was in contact with metallic and metallic oxide surfaces during baking. There was therefore a probability of catalysed reactions occurring and involving, perhaps, dehydrogenation, polymerisation or pyrolysis.

If after being baked the product was cleaned by extraction and controlled sulphation, it was doubtful what was in fact produced as a result of heating the mineral oil, or even medicinal paraffin. There was no evidence; it had not been looked for and, in fact, it may have been destroyed.

As an example of the variable behaviour of mineral oils under different conditions, Dr. Myddleton referred to his own experience of the changes that occurred in a technical white oil, similar to the one heated to 200° C by the authors, when exposed to sunlight for three months (*J. Inst. Pet.*, 1951, **37**, 57). The maximum temperature was about 62° C for about 1 hour on bright days. The minimum on the ultra-violet absorption curve was raised by 64 per cent. and the maximum by 14·5 per cent. The ratio of the maximum to the minimum was lowered from 1·63 to 1·18 and the position of the maximum was shifted from 2710 to 2650 A. He therefore felt diffident in accepting the assumption that even highly refined white oils remained unchanged in the process of bread baking.

DR. COPPOCK, in a written reply, said he wished to emphasise that the main purpose of the paper was to describe a method whereby the original quality of oils used for lubrication in bread-making could be determined after use. In reply to the first question raised by Dr. Myddleton, he pointed out that the Mineral Oil in Food Order restricted the quantity of mineral oils present in foods as a result of processing operations to not more than 0·2 per cent. by weight, but did not define the quality of oil regarded as desirable.

The authors had advocated previously that the quality of mineral oil used for lubricating bread-making machinery should be that of liquid paraffin, B.P., but in view of the spectrophotometric evidence presented in this paper they now suggested that certain white oils might also be used. The pharmacology of mineral oils had naturally been taken into account, and there was no evidence that the oils included in the proposed specification could in any way be harmful when used for this purpose.

Secondly, the smell, colour or taste of poorly refined oils could not be detected at levels of 0·2 per cent. in bread, and therefore more scientific methods would be required to show their presence. Thirdly, the heating of white oils to 200° C in air caused negligible change in the oils as determined by their ultra-violet absorption spectra; Dr. Myddleton's experience of the effect of sunlight on mineral oils would appear irrelevant to a consideration of the baking process, but the above conditions of heating were considerably more drastic than would be found in bread-baking, for which the temperature, except at the crust, did not exceed 100° C and the atmosphere was preponderantly carbon dioxide. Further, none of the components of bread would be expected to decompose mineral oil, even if the oil was dispersed in thin films. The possibility of decomposition at the baking-tin surface could not be ignored, but it was doubtful whether the conditions were sufficient for this to occur; it should also be noted that the commercial use of mineral oils for tin greasing was almost negligible. In any event, a vegetable oil could be just as prone, and possibly even more prone, to adverse reactions; *e.g.*, their breakdown products under certain conditions of heating were believed to be carcinogenic (see, *e.g.*, Peacock, P. R., and Beck, S., *Nature*, 1948, **162**, 252) and there were inherent dangers should vegetable oils become rancid (Frazer, A. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, 1952, **46**, 576).

Fourthly, it was extremely unlikely that the extraction procedure removed any decomposition products formed in mineral oils on baking in bread and, as just stated, the authors did not believe that any decomposition occurred. It should be remembered that white oils were refined by far more drastic sulphuric acid treatments than were used to separate the mineral oil from the natural unsaponifiable oil in bread, and the sulphation conditions were selected to be just sufficient to achieve this. The similarity of the ultra-violet absorption spectra of the mineral oils of different quality, before use and after recovery from bread, were striking, and did not suggest that any marked changes in the mineral oils had occurred, or that the controlled sulphation was sufficiently vigorous to destroy any decomposition products that may have formed. The much more pronounced absorption intensities of the natural unsaponifiable oils in bread compared with those of white oils should also be noted in this connection.

Finally, with regard to Dr. Myddleton's statement of the effect of daylight exposure of a technical white oil which by spectrophotometric evidence was not stable, experiments recorded elsewhere (Schnurmann, R., paper presented at the Réunion Internationale de Spectroscopie Moleculaire, Paris, July, 1953) have shown that medicinal liquid paraffins that obeyed the spectrophotometric quality and stability criterion showed no significant changes of their spectra after exposure to an ultra-violet light source *provided* that the oils were contained in a glass vessel.

The Spectrophotometric Determination of Long-chain Fatty Acids Containing Ketonic Groups

With particular reference to Licanic Acid

BY A. MENDELOWITZ AND J. P. RILEY

A spectrophotometric procedure, based on the alkaline 2:4-dinitrophenylhydrazone method of Lappin and Clark, is described for the determination of ketonic groups in long-chain compounds. The effect of a number of variables on the final intensity of the colour has been investigated. The method has been applied to the determination of licanic acid in the presence of other saturated and unsaturated fatty acids and shows a standard deviation of 0·8 per cent.

IN the course of a study[1] of oils containing licanic acid (4-oxo-octadeca-9:11:13-trienoic acid), a method was required for its determination in the presence of elaeostearic acid (octadeca-9:11:13-trienoic acid), with which it is usually associated. As the ultra-violet absorption spectra of the two acids are almost identical,[2] measurement of the absorption at 270·5 mμ gives only a measure of the total conjugated trienoic unsaturation. On the other hand, licanic acid can be estimated by determination of the ketone value.

Several volumetric procedures have been described for the estimation of ketonic groups in long-chain fatty-acid derivatives,[2,3,4,5] mainly adaptations of the hydroxylamine method originally introduced by Brochet and Cambrier,[6] or its modification in which pyridine is used to ensure complete reaction.[7] These methods suffer from the disadvantage that the end-point is always somewhat indistinct, particularly in the presence of pyridine.

A number of workers have used phenylhydrazine and its derivatives for the gravimetric[8] or volumetric[9,10,11] determination of ketonic compounds. These procedures depend on either the quantitative recovery of the phenylhydrazone of the ketone or the determination of unreacted phenylhydrazine. All were found to be inapplicable to long-chain fatty-acid derivatives owing to the difficulty of separating the phenylhydrazones in a pure state; for instance, attempts to prepare the 2:4-dinitrophenylhydrazone of methyl 12-oxostearate resulted in the production of an oily product containing much unreacted 2:4-dinitrophenylhydrazine, which could only be removed with great difficulty.

Observations by Bamberger[12] and Gnehm and Benda[13] that nitrophenylhydrazones form dark-coloured water-soluble compounds on treatment with alkali have been made the basis of colorimetric procedures for the estimation of ketonic compounds. The earlier applications of this method[14,15,16,17,18] were to water-soluble ketonic compounds, and they are therefore unsuitable for use with the higher fatty acids and their derivatives. Lappin and Clark[19] have reported an extension of the method to the determination of traces of aldehydes and ketones in water, organic solvents or organic reaction products. They reported that both the wavelength of maximum absorption and the molecular extinction were independent of the structure of the ketonic compound.

Poole and Kloose[20] have described a spectrophotometric procedure for the estimation of monoketonic compounds in rancid foods. The 2:4-dinitrophenylhydrazones of the ketones were formed in benzene solution in a column of activated alumina. Alcoholic potassium hydroxide was added to the eluate from the column and the intensity of the resultant red colour was determined at 435 mμ. The colour was not stable, however, and faded rather rapidly.

In view of the poor results that were attained with the hydroxylamine method for the determination of methyl 12-oxostearate, the procedure of Lappin and Clark seemed to offer the advantages of accuracy and simplicity. These workers[19] treated 1 ml of a methanolic solution of the ketone with 1 ml of a 0·06 per cent. solution of 2:4-dinitrophenylhydrazine in methanol and 1 drop of concentrated hydrochloric acid. This was heated in a loosely stoppered test tube at 100° C for 5 minutes and then 5 ml of 10 per cent. methanolic potassium hydroxide were added. The absorption of the solution was measured at 480 mμ against a blank determination made with 1 ml of methanol instead of the ketone solution.

Preliminary experiments made with *cyclo*hexanone and methyl 12-oxostearate revealed five defects in their procedure—

(*i*) Much evaporation took place during the heating at 100° C, and often no methanol remained at the end of the reaction period. In order to reduce evaporation to a minimum, a temperature of 60° C was used for the condensation in all subsequent work and ethanol was substituted for methanol.

(*ii*) Small variations in the amount of hydrochloric acid added as catalyst in the condensation reaction produced large differences in the intensity of the colour.

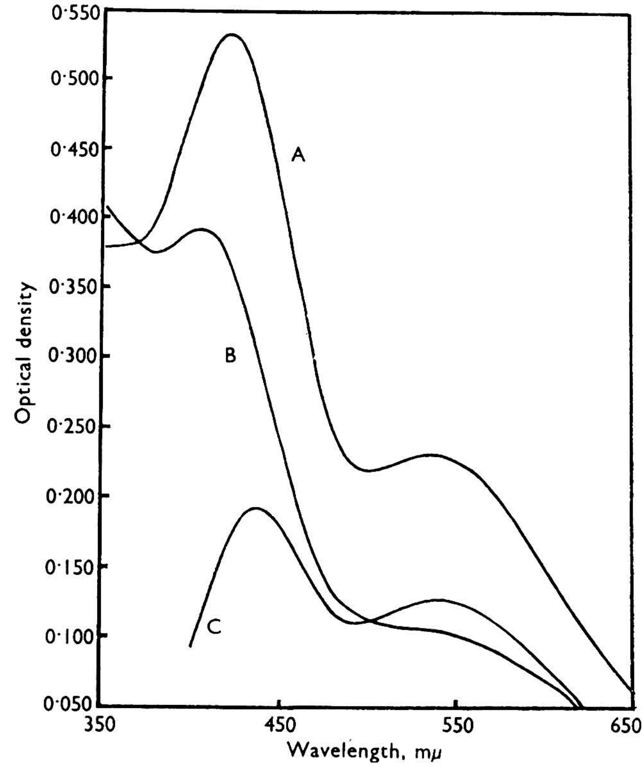

Fig. 1. Comparison of absorption curves of solution and blank solution. Curve A, methyl oxostearate; curve B, reagent blank; curve C, methyl oxostearate less reagent blank

(*iii*) Potassium chloride was precipitated after the addition of alcoholic potassium hydroxide and it was difficult to obtain absolutely clear solutions.

(*iv*) The wavelength of maximum absorption was not 480 mμ, but varied with the nature of the ketone. Long-chain aliphatic ketones and *cyclo*hexanone exhibited maximum absorption at 435 mμ (with a secondary maximum at 535 mμ, see Fig. 1), whereas with cinnamaldehyde the maximum absorption was at 485 mμ.

(*v*) Owing partly to the difference in structure of the dinitrophenylhydrazones and partly to the equilibrium nature of the condensation reactions, the molecular extinction at the wavelength of maximum absorption depended markedly on the nature of the ketonic compound. For example, *cyclo*hexanone and methyl oxostearate gave ϵ (at 435 mμ) of 17,900 and 11,400, respectively. A sample of pure *cyclo*hexanone-2:4-dinitrophenylhydrazone in the same strength of alcoholic potassium hydroxide had an ϵ of 19,530 at 435 mμ, showing that the condensation of *cyclo*hexanone with the reagent had only proceeded to the extent of about 92 per cent.

During preliminary work it was found that high reagent blanks resulted when measurements were made at 435 mμ. Fig. 1 shows the absorption curves for a determination with methyl oxostearate and for the reagent blank, both measured against water in the compensator cell. The rather high absorption of the reagent blank at 435 mμ was almost entirely due to the shoulder of the absorption band at about 395 mμ of alkaline 2:4-dinitrophenylhydrazine.[21] On the other hand, at the secondary absorption maximum at 535 mμ, the absorption of the blank was quite low and remained fairly constant over a considerable range of wavelengths; for these reasons all further measurements were made at this wavelength.

The effect of variation in hydrochloric acid concentration on the condensation reaction was examined. Some 1-ml samples of a 0·0065 per cent. ethanolic solution of methyl oxostearate were treated with 1-ml portions of dinitrophenylhydrazine reagent containing different strengths of acid (0·06 g of dinitrophenylhydrazine with 1 ml of hydrochloric acid made up to 100 ml with ethanol). The mixture was heated at 60° C for 50 minutes; then, after cooling, 5 ml of 10 per cent. alcoholic potassium hydroxide were added and the solution was diluted to 10 ml with water. The results are shown below—

Normality of acid	2	3	4·6	6
$E^{1\%}_{1\,cm}$ at 535 mμ*	227	344	352	361

* After deduction of reagent blank.

To minimise variations caused by changes of acid concentration, all subsequent work was performed with a reagent containing 1 ml of constant-boiling hydrochloric acid per 100 ml.

With this reagent, the time for attainment of equilibrium in the initial reaction was determined for licanic acid and oxostearic acid and its methyl ester, with the results shown in Table I. In each determination the condensation was complete within 50 minutes at 60° C.

TABLE I

RATE OF REACTION OF LONG-CHAIN KETONIC COMPOUNDS WITH 2:4-DINITROPHENYLHYDRAZINE

Time, minutes	$E^{1\%}_{1\,cm}$ at 535 mμ for oxostearic acid	methyl oxostearate	licanic acid
30	361	346	—
35	370	355	—
40	376	359	—
45	378	362	265
50	378	362	270
55	—	—	268
60	377	362	269

Small changes in the amount of alcoholic potassium hydroxide solution added produced little alteration in the intensity of the red colour; for instance, use of 7·5 ml of this reagent instead of 5 ml increased the final optical density by only 1·5 per cent. The substitution of water for alcohol in the dilution to the final volume had only a slight effect on the final colour and prevented the precipitation of potassium chloride that had been encountered when the method of Lappin and Clark was used.

With the modified conditions it was found that, although highly reproducible results were attained (for methyl oxostearate) if the same sample of alcohol was used throughout, fresh batches of alcohol gave rather different values. That this was due to the presence during the condensation reaction of variable amounts of water in the alcohol was shown by the addition of known amounts of water to the alcohol used as solvent for the ketonic compound.

Water in alcohol, per cent.	0	0·1	0·5	1·0
$E^{1\%}_{1\,cm}$ at 535 mμ	377	377	362	356

To overcome this difficulty a control test must be made simultaneously on the pure ketone being determined, or if this is not stable, on some ketonic compound of a similar nature that has been calibrated against the unstable ketonic compound. In the present work, oxostearic acid was used as a control for licanic acid and for methyl oxostearate, with the results shown in Table II. The constancy of the extinction coefficients of both methyl oxostearate and licanic acid, when related to an arbitrary fixed value of 360 for the extinction coefficient for oxostearic acid, is proof of the validity of this procedure.

TABLE II

$E^{1\%}_{1\,\mathrm{cm}}$ VALUES FOR SIMULTANEOUS RUNS OF OXOSTEARIC ACID WITH METHYL OXOSTEARATE AND WITH LICANIC ACID

	Methyl oxostearate			Licanic acid	
Oxostearic acid	found	calculated to oxostearic acid = 360	Oxostearic acid	found	calculated to oxostearic acid = 360
370	362	352	358	270	272
368	360	352	373	280	273
364	358	354	363	275	270
367	360	353			
		Mean 353			Mean 272

It will be noted that the value found for licanic acid is very much lower than those of either oxostearic acid or its methyl ester. This emphasises the fact that the method can only be applied to a particular ketonic compound after standardisation with that compound.

Unsuccessful attempts were made to replace the alcohol used in the condensation reaction by other solvents, such as benzene or *cyclo*hexane, which are more readily obtained free from ketonic compounds. The red colour, if it was produced at all, was very weak and ephemeral.

METHOD

STANDARD SUBSTANCES—

Methyl 12-*oxostearate*—Methyl ricinoleate (purity 99·4 per cent.)[22] was hydrogenated with Raney-nickel catalyst.[23] The resulting methyl hydroxystearate was treated with a 100 per cent. excess of a 10 per cent. solution of chromium trioxide in glacial acetic acid. After being allowed to stand at room temperature for 2 hours the mixture was poured into water and filtered. The precipitate was dissolved in ether and the ethereal solution was washed with 5 per cent. potassium carbonate solution and with water. The crude ester, recovered by evaporation of the solvent, was crystallised three times from acetone at 0° C. The purified methyl 12-oxostearate consisted of white plates, m.p. 46·8° to 47·5° C; previous workers have reported 45° to 46° C[24] and 44·5° to 45° C.[25]

12-*Oxostearic acid*—Crude methyl oxostearate was saponified with alcoholic potassium hydroxide. The recovered acid was crystallised three times from acetone at 0° C. The pure acid had m.p. 81·5° to 82° C; previously recorded values are 80° to 81° C[24] and 81° C.[25]

Licanic acid—The mixed acids obtained from a commercial oiticica oil were dissolved in 20 ml of boiling light petroleum, boiling range 40° to 60° C, per gram of mixed acids. The boiling solution was filtered through a warm funnel to remove small amounts of resinous oxidation products. On cooling, the filtrate deposited crude licanic acid, which was recrystallised five times from light petroleum. The purified licanic acid was pressed well on the filter and dried in a high vacuum for not longer than 10 minutes. The products from a number of preparations all melted in the range 74·5° to 75·5° C, and gave the following values of $E^{1\%}_{1\,\mathrm{cm}}$ at 270·5 mμ: 1711, 1703, 1724, 1702, 1725, 1729, mean 1716; Rose and Jamieson[2] record 74·5° to 75·5° C and 1784.

REAGENTS—

Ketone-free ethanol—Distil absolute alcohol from zinc dust and potassium hydroxide. Heat 1·5 litres of the distillate under reflux with 10 g of 2:4-dinitrophenylhydrazine and 10 ml of concentrated sulphuric acid. Distil the ketone-free ethanol after 2 hours and store it in a tightly stoppered bottle in the dark. The alcohol, which is slightly coloured yellow owing to the volatility of 2:4-dinitrophenylhydrazine, is stable for about a week.

2:4-*Dinitrophenylhydrazine reagent*—Add 1 ml of constant-boiling hydrochloric acid to 0·06 g of 2:4-dinitrophenylhydrazine, add 80 ml of ketone-free ethanol and warm until all is dissolved. Cool and dilute to 100 ml with ketone-free ethanol. The reagent is stable for at least 3 days if stored in the dark.

Alcoholic potassium hydroxide—Dissolve 10 g of potassium hydroxide in 20 ml of water and dilute to 100 ml with ethanol (which need not be ketone-free).

PROCEDURE—

Weigh about 0·1 g of the substance to be examined, dissolve it and make the solution up to 100 ml with ketone-free ethanol. Dilute this solution accurately with the same alcohol to give a solution approximately 0·001 per cent. with respect to the ketonic group ($>C=O$). Place 1 ml of the diluted solution in a 10-ml calibrated flask and add 1 ml of dinitrophenylhydrazine reagent. Stopper the flask loosely and heat it to 60° C in a thermostatically controlled bath. After 1 minute press the stopper home to minimise evaporation. After 50 minutes, cool the flask in water for 2 minutes. Add 5 ml of alcoholic potassium hydroxide rapidly and dilute to 10 ml with water. Measure the optical density of the solution in a 1-cm cell at the secondary absorption maximum (535 mμ for 12-oxostearic and licanic acids), using water in the compensating cell. Determine the reagent blank in the same manner with 1 ml of purified ethanol instead of the solution of the ketone. Carry out control estimations with the pure ketone simultaneously with the determination. If the ketonic compound to be determined is unstable, *e.g.*, licanic acid, make simultaneous calibration tests with a related, but stable, ketone, *e.g.*, oxostearic acid, and with the unstable compound. Use the stable ketone in place of the unstable compound as a control in all determinations.

CALCULATION FOR UNSTABLE KETONES—

Calculate the extinction coefficients ($E^{1\%}_{1\,cm}$) of the sample and standards from the optical densities of the solutions after deduction of the reagent blanks.

Let a and b be the extinction coefficients, determined simultaneously for the pure unstable ketone and the stable standard, respectively. If x and y are the extinction coefficients of the sample containing the unstable ketone and of the stable ketone respectively, then the percentage of the unstable ketone in the sample is—

$$\frac{b \times x \times 100.}{a \times y}$$

DETERMINATION OF KETONES IN THE PRESENCE OF FATTY ACIDS—

Known mixtures of ketones with non-ketonic compounds were analysed with the results shown in Table III. The non-ketonic compounds used included the mixed acids of tung oil, in order to ensure that such acids would not interfere in the determination of licanic acid in oils such as oiticica. Each result is the mean of four determinations showing an average standard deviation of 0·8 per cent.

TABLE III

ANALYSIS OF MIXTURES

Ketone	Non-ketonic compound	$E^{1\%}_{1\,cm}$ of mixture	$E^{1\%}_{1\,cm}$ of control	Ketone in mixture Found, %	Ketone in mixture Calculated, %
Methyl oxostearate	Methyl stearate	154	350*	44·0	43·6
Methyl oxostearate	Palmitic acid	183	350*	52·3	52·5
—	Palmitic acid	0	350*	0	0
—	Tung mixed acids	2·4	378†	0·8‡	0
Oxostearic acid	Tung mixed acids	176	378†	46·5	46·3
Licanic acid	Tung mixed acids	164	373†	58·6	58·1

* Methyl oxostearate. † Oxostearic acid.
‡ As licanic acid, but probably due to oxidation products.

CONCLUSIONS—

The method described is by no means limited to the fatty acids, but could be applied after suitable standardisation to many other types of ketonic compounds. It presents the advantages of rapidity and accuracy, and can be very readily adapted for use with micro-samples.

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Design and Operating Technique of a Vacuum Drying Oven

Part I. Design of the Oven

BY S. D. GARDINER

A symmetrical vacuum drying oven of circular plan, large heat capacity and uniform temperature distribution has been designed. It has a shallow, hollow but thick metal base, which holds six sample dishes with the minimum of free space. Controlled dry air bleed is incorporated.

A STANDARD reference method for determining water in sugar products, as distinct from quick routine methods, requires the use of a vacuum oven free from the many faults in design inherent in almost all of the ovens currently available, such as those mentioned in the *Proceedings of the International Commission for Uniform Methods of Sugar Analysis*, 1949. To overcome the difficulties in drying such labile products as sugar syrups and molasses containing fructose, the oven described here was designed and constructed in the Tate and Lyle Research Laboratories. Its use need not be limited to the products mentioned—it can be more generally utilised whenever exact and reproducible results of experiments on drying losses from many other materials are required.

The design incorporates features of the oven reported to the author by a colleague, who visited the National Gas Board Laboratories. Attention has also been paid to the recommendations made by Iles and Sharman,[1] although these refer to atmospheric pressure ovens.

GENERAL DESCRIPTION—

Fig. 1 shows that the oven consists primarily of a shallow, hollow, but thick, metal base, which holds six sample dishes with minimum free space. The lid is shown resting in the off position (at the rear of the figure), where it is out of the way and suspended so that it can be kept clean and undamaged. The vacuum seal is produced with the aid of a wing-nut that clamps down the heavy lid over the central control spigot, the raised annulus of the base sealing the oven by pressing on to the grease-free silicone-rubber ring, inserted into the complementary circular groove in the lid. At the same time, provided sufficient pressure is applied, good thermal contact is made with the base. Vacuum sealing at the spigot is ensured by a copper washer under the wing nut. The washer is held in position by a circular projection in the lid and is normally lubricated with a little grease. To conform with the shape of the oven the air flow has been arranged symmetrically. Fig. 2 shows the under-side of the oven with the air-distributing leads from the central supply connected by thick rubber tubes to six entry ports situated around the perimeter of the base of the oven. Fig. 3 shows that these ports, which are inclined at an angle of 45°, project the air up against the oven lid immediately over the sample dishes, from whence it passes over their surfaces to be drawn out through six exit ports arranged symmetrically in the lid near the centre. These exit ports inclined at 30° force the air upwards through the centre hole enclosing the spigot, and so out of the oven. The angles are chosen to facilitate removal of water vapour from above the sample. The air flow is adjustable to between 0·5 to 100 ml per minute by means of an Edwards needle-control valve (type LB1), after the air has been dried through activated alumina (6 to 8 mesh) and over barium oxide lumps. The alumina is arranged in a long narrow tube (Fig. 1) in order to reduce the water content of the air to 0·005 mg per litre; the

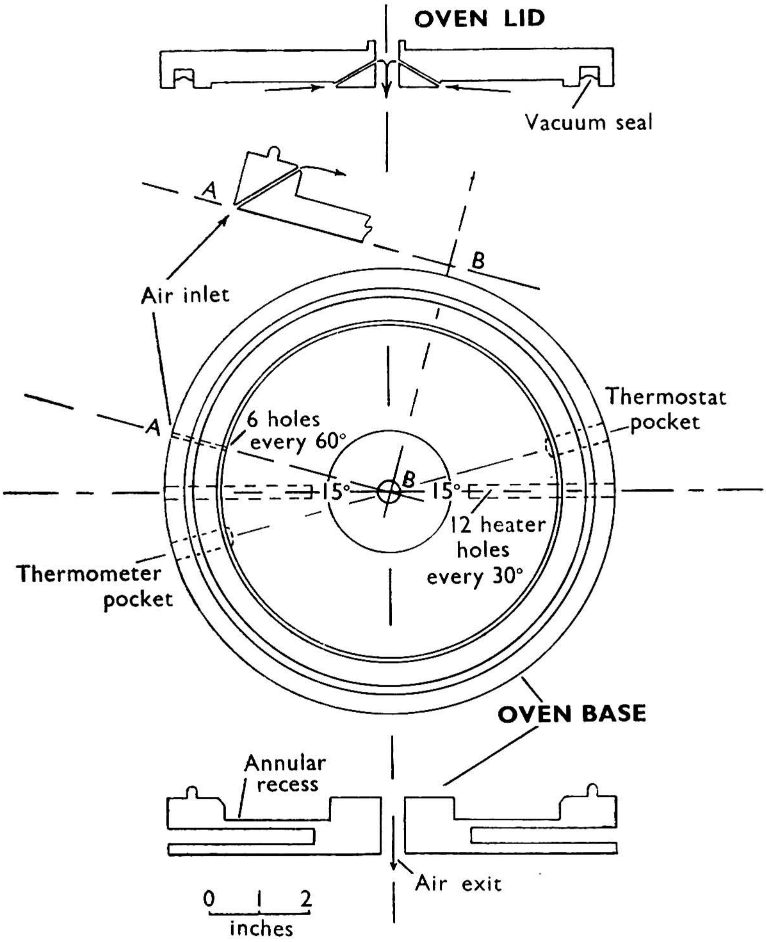

Fig. 3. Design of vacuum oven

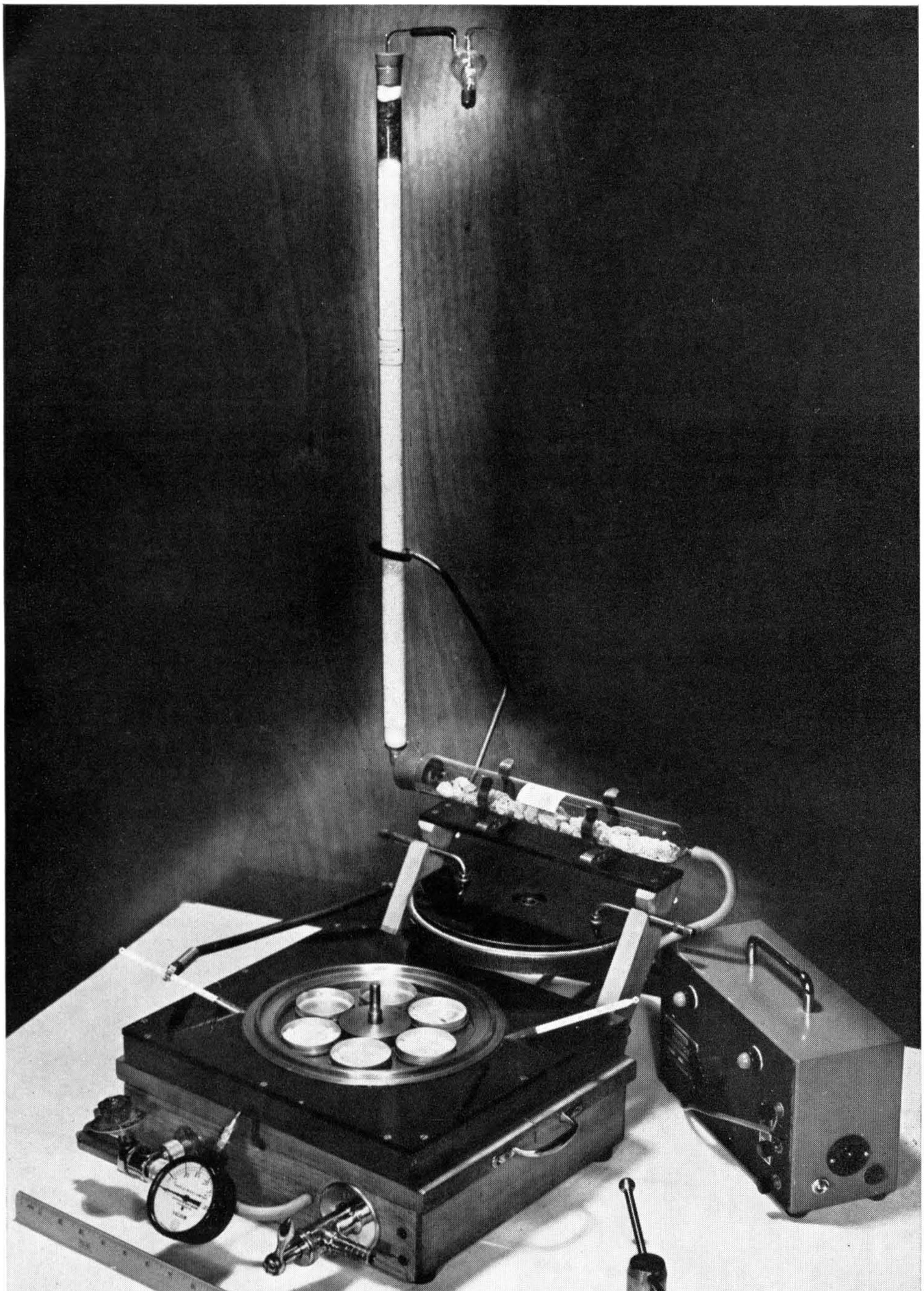

Fig. 1. General view of the oven

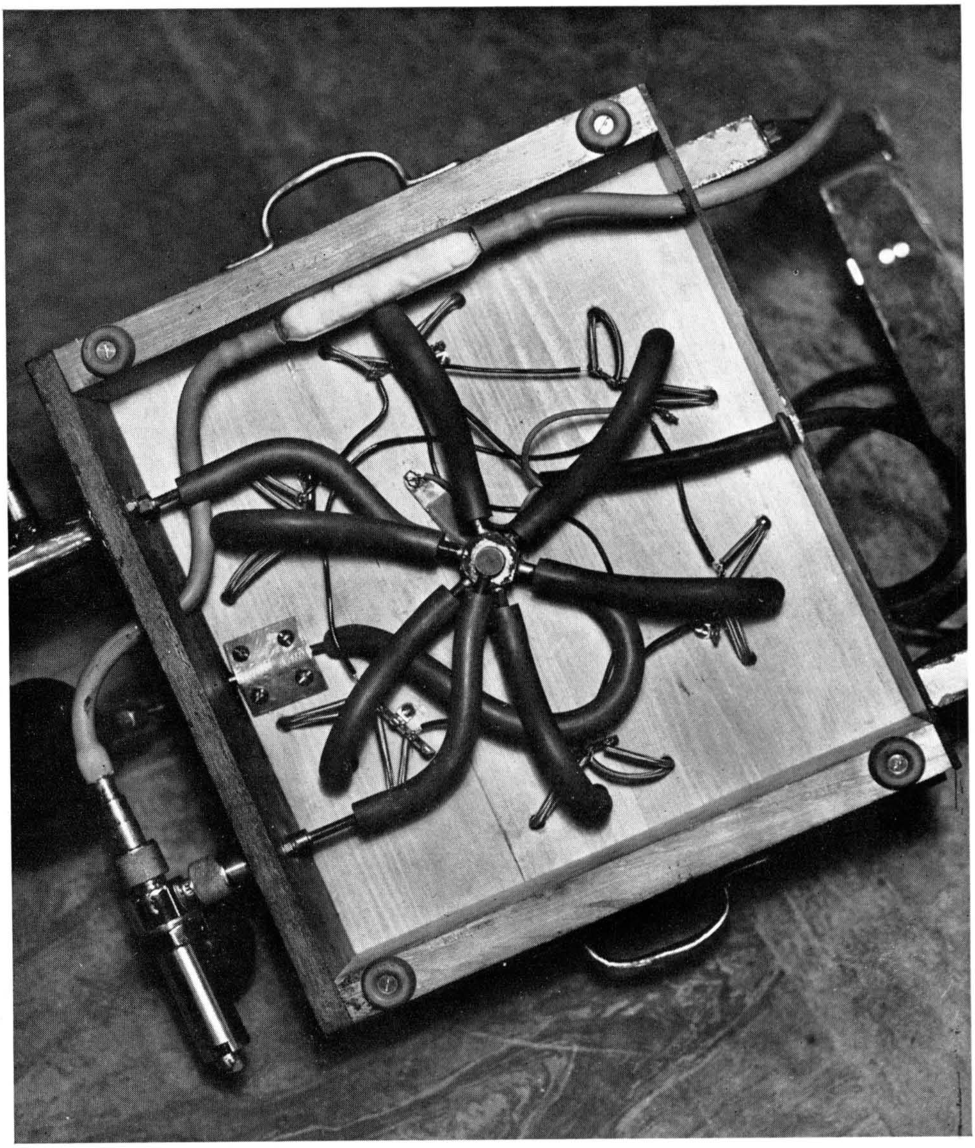

Fig. 2. View of the underside of the oven and the air distributing leads

lumps further reduce the water to 0·0007 mg per litre. The drying train is attached to the oven and moved with it. Iles and Sharman state that in the absence of the steaming effect (catalysis of glucose and amino-acid condensation) about 2 ml of air per minute per gram of water are required. The new vacuum oven is certainly free from this effect. Six samples each holding a maximum of 2·5 g of water (including added water) would have a maximum total of 15·0 g of water to be removed, which according to Iles and Sharman would need a minimum of 30 ml of air per minute, but as this amount is well within the limit of control by the needle valve, the steaming effect is safeguarded against on two counts. A simple liquid-detergent film air-flow meter may be suspended from the vertical drying tube.

Fryd and Kiff[2] found experimentally that the heat from the air flow contributes only slowly to the rise in temperature of the sample; conduction and radiation supply most of the heat required. The oven described here gives the same degree of heat transfer by radiation and conduction at all points in its interior. As the surface of the recess is ground flat, heat transfer is facilitated to such a degree that it is essential to commence heating at a low temperature and to heat to operational temperature by resetting the thermostatic control. This procedure assists in preventing crust formation. Uniform temperature distribution is attained by using twelve electric heaters inserted radially around the base and penetrating to near the centre. The total wattage is 80, each heater being deliberately run at one-tenth of its normal rating. An indicator lamp assists in setting the thermostatic control.

Two methods of temperature control were attempted. One oven was controlled to within ±0·5° C by a Sunvic adjustable bimetal thermostat (T.S.1) in an oil-filled pocket, in conjunction with a Sunvic hot-wire vacuum relay (F.102–3M). Another oven was regulated to within ±1·0° C by an electronic capacity-switch, operating off the 0° to 110° C thermometer. The former method is cheaper and, in the long run, simpler to maintain. Temperatures are read on an ordinary 0° to 110° C thermometer, inserted in an oil-filled pocket drilled into the base. Two thermometers diagonally situated give identical temperatures; these comply with the suggestion of Hayes[3] and show agreement with the surface temperature of the interior of the oven. Quadruplicate drying tests with cane molasses agree to within less than ±0·05 per cent. loss, and with sucrose solution to less than ±0·02 per cent. The oven is designed to operate efficiently from 50° to 90° C. For drying at atmospheric pressure the temperature may be raised to 105° C as long as the air flow is sufficient to prevent rusting. As an additional precaution the exposed metal surfaces can be phosphate treated.

Once an efficient oven has been constructed there remain other problems in the determination of water in thermally sensitive materials. Choice of absorbent, the effect of extra water, sample to absorbent ratio, particle size, deliberate formation of degradation products are all being investigated. Sugar syrups and molasses containing fructose and organic non-sugars require especial care.

Tests are being made in drying golden syrup and molasses, and the results are being utilised to find a correction for refractometer solids based on ash content, after correction has been made for the effect of invert sugar. The refractometer is so useful in the sugar industry that the oven recommended here is not intended to replace it for routine use, especially as the time required for drying is too long for this purpose, although it is considerably shorter than has been needed for other vacuum ovens of less suitable design. The results of the work, particularly on cane molasses, will be dealt with in further papers.

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The Determination of Total Phosphatide in Commercial Lecithin

By H. H. HUTT, H. WEATHERALL and T. CULSHAW

In a previous publication it has been suggested that the difference between acetone-insoluble matter and petrol-insoluble matter can be made the basis of a means for assessing the phosphatide content of commercial lecithin for works-control purposes. A modified method, which has improved precision, is now described for the determination of acetone-insoluble matter; it is compared experimentally with previously published methods. The ratio of phosphatide to non-phosphatide removed at various stages of washing is examined.

The difference between acetone-insoluble matter determined by the method proposed and petrol-insoluble matter is suggested as giving a suitable measure of phosphatide content.

Two general methods for the determination of the total phosphatide content of commercial lecithin[1] are (*i*) by determination of the phosphorus content, the found value being multiplied by an arbitrary factor (usually 26·31 for commercial purposes) and (*ii*) by determination of the difference between acetone-insoluble content and the amount of matter insoluble in petroleum spirit of boiling range 40° to 60° C.

The rapid works-control method for the determination of acetone-insoluble matter by direct elution previously described[1] was based mainly upon experience with commercial ground-nut lecithin. Examination of a large number of specimens from various botanical sources has subsequently afforded us the opportunity of modifying the method and of making it more acceptable as a means of comparatively assessing the total phosphatide content of all types of material, when this is defined as acetone-insoluble content minus petrol-insoluble content.

Results recorded in the literature for phosphatides of different botanical origins show that the phosphorus contents of carefully prepared petrol-soluble acetone-insoluble materials are lower than expected for di-oleyl glycerophosphoryl choline (3·94 per cent.) or di-oleyl glycerophosphoryl ethanolamine (4·16 per cent.). Typical specimens of total phosphatide mixtures from soya-bean lecithin have been reported[2] as containing 3·4 per cent. of phosphorus, and from cotton-seed lecithin,[3] 2·9 per cent. of phosphorus, and we have found 3·0 per cent. of phosphorus in total phosphatides of ground-nut lecithin prepared in the manner indicated.

The lower phosphorus content found in the oil-free preparations from the naturally occurring phosphatides, as compared with the theoretical contents of the chemical compounds mentioned above, is caused mainly by combined and closely associated carbohydrates.[2,3,4,5] As these carbohydrates appear always to be present in the natural substances and as it is probable that their hydroxyl groups have a function in most of the industrial purposes for which commercial phosphatides are used, it seems permissible to include them in any comparative assessments of total-phosphatides. They in no way constitute any deleterious addition to, or indicate any incomplete purification of, the material under examination. Moreover, phosphorus determination is a lengthy and somewhat expensive procedure. We therefore suggest that a method that will give reasonably reproducible results for matter insoluble in acetone can be used as a basis for assessing total phosphatide.

Of methods proposed for this determination, other than the one already mentioned, that of dissolving the material in light petroleum and precipitating and eluting with acetone[6] is probably the most widely used. The comparative merits of this method and the proposed modified method are reported upon below.

Error in the method formerly proposed for production control analysis[1] was largely caused by entrainment of insoluble matter during decantation—this was particularly noticeable with cotton-seed lecithin—and by the solubility of phosphatide in the oil-acetone solution on the first wash—observed especially with soya-bean lecithin. Preliminary centrifugation eliminates the error caused by entrained material and the solubility error can be minimised by first washing the sample in the cold.

METHOD

PROCEDURE—

Weigh accurately 3·0 ± 0·5 g of sample into a centrifuge tube and add 40 ml of acetone. Stir thoroughly, kneading the lecithin in the acetone, and then setting the tube aside for 10 minutes at room temperature. Centrifuge for 3 to 5 minutes at a relative centrifugal force of approximately 1000 *g* and decant the supernatant liquid. Extract twice more with boiling acetone and cool to 0° C before centrifuging. For soya-bean lecithin, only one of these further extractions is required. After completion of the washing, remove the bulk of the residual acetone from the insoluble material on a water-bath in a current of air and dry the residue in a vacuum oven at 100° C and at a pressure of approximately 40 cm of mercury.

EXPERIMENTAL

Acetone conforming to analytical reagent standards was used for all determinations.

Table I shows a comparison of results by the earlier method[1] with those by the same procedure corrected for matter recovered from the decanted solutions by centrifugation and with those found by the proposed modified method.

TABLE I

ACETONE-INSOLUBLE CONTENTS OF COMMERCIAL LECITHINS

	Acetone-insoluble material		
Type of lecithin	Former method,[1] %	Former method[1] corrected for material recovered by centrifugation, %	Proposed method, %
Unrefined ground-nut	50·7	53·4	53·5
	43·6	47·0	47·7
	48·3	50·3	50·7
	49·9	53·0	54·6
	53·6	56·2	56·8
Refined ground-nut	63·4	65·1	65·6
	66·1	67·6	68·4
	66·4	68·2	67·8
	66·0	66·3	67·5
	67·5	68·3	69·1
Soya bean	53·9	56·2	61·0
	61·5	63·5	66·6
	54·3	62·6	66·2
	45·2	62·6	66·1
	23·5	33·5	35·1
Cotton-seed	49·8	52·5	53·6
	49·4	51·2	52·5
	51·3	54·7	55·4
	47·6	52·6	52·2
	47·3	50·3	51·2

NOTE—Results are the means of duplicate determinations.

Table II compares results obtained by this modified elution method with those obtained by the method of precipitation and elution.[6]

TABLE II

COMPOSITION OF COMMERCIAL LECITHINS

	Acetone-insoluble content*				
Lecithin	Proposed method, %	A.O.C.S. method,[6] %	Moisture, %	Benzene-insoluble content, %	Petrol-insoluble content, %
Unrefined ground-nut (L 3) ..	57·8, 57·9	59·4, 60·0	2·50	0·29	0·47
Refined ground-nut (L 49)	69·8, 69·9	71·1, 71·6	0·73	0·39	0·76
Bleached soya bean (D 41) ..	66·1, 66·2	65·5, 65·7	1·30	trace	trace
Unbleached soya bean (D 84)..	66·2, 65·9	67·0, 66·6	0·32	trace	trace
Cotton-seed (L 50)	56·7, 56·8	60·9, 60·8	0·58	0·14	0·26

* No correction was made for petrol-insoluble or benzene-insoluble matter.

Phosphatides, which are known to be slightly soluble in acetone in the presence of fatty acids,[7] have been found to be slightly soluble in pure acetone (see Table IV).

To assess and compare the solubility errors, acetone-soluble matter was weighed separately in individual washes (see Table III) obtained by the proposed method and the A.O.C.S. method, phosphorus being determined in these individual extracts (see Table IV).

TABLE III

REMOVAL OF ACETONE-SOLUBLE MATTER BY SUCCESSIVE WASHING

		Amount of acetone-soluble material removed by wash number							
Lecithin	Method	1, %	2, %	3, %	4, %	5, %	6, %	7, %	8, %
Unrefined ground-nut (L 3)	Proposed	29·8	7·83	2·50	1·00	0·76	0·46	0·54	0·62
	A.O.C.S.	33·3	4·46	1·26	0·81	0·73	0·42	0·43	0·52
Refined ground-nut (L 49)	Proposed	22·7	5·30	1·45	1·14				
	A.O.C.S.	24·5	3·40	0·94	—				
Bleached soya bean (D 41)	Proposed	27·8	6·27	2·04	1·20	1·10	1·09	0·78	0·75
	A.O.C.S.	29·7	3·49	1·29	1·01	0·92	0·90	0·60	0·85
Unbleached soya bean (D 84)	Proposed	27·8	6·59	2·38	1·36				
	A.O.C.S.	30·2	2·69	1·48	—				
Cotton-seed (L 50)	Proposed	32·1	8·05	2·12	1·36				
	A.O.C.S.	34·5	4·00	1·05	—				

TABLE IV

PHOSPHATIDE IN ACETONE-SOLUBLE MATTER REMOVED BY SUCCESSIVE WASHING

		Amount of phosphatide* removed by wash number							
Lecithin	Method	1, %	2, %	3, %	4, %	5, %	6, %	7, %	8, %
Unrefined ground-nut (L 3)	Proposed	0·40	0·35	0·22	0·21	0·20	0·19	0·20	0·23
	A.O.C.S.	0·48	0·15	0·11	0·32	0·32	0·15	0·18	0·23
Refined ground-nut (L 49)	Proposed	0·41	0·36	0·25	0·26				
	A.O.C.S.	0·29	0·19	0·15	—				
Bleached soya bean (D 41)	Proposed	0·69	0·87	0·60	0·37	0·40	0·45	0·37	0·36
	A.O.C.S.	0·66	0·74	0·43	0·37	0·38	0·40	0·28	0·43
Unbleached soya bean (D 84)	Proposed	0·74	1·27	0·91	0·68				
	A.O.C.S.	1·31	0·35	0·32	—				
Cotton-seed (L 50)	Proposed	0·43	0·48	0·29	0·40				
	A.O.C.S.	0·69	0·16	0·09	—				

* Calculated by multiplying the figure found for phosphorus by the factor 26·31.

For the phosphorus determinations, the acetone-soluble matter was saponified and ignited to ash; the ash was dissolved in water and the phosphorus measured on a Spekker absorptiometer after development of a blue colour by an adaptation of Zinzadze's method,[8,9,10] by means of molybdic anhydride reduced with molybdenum metal.

The results shown in Table IV are expressed as the percentage of phosphatide. This was calculated by multiplying the figure for phosphorus by the arbitrary factor 26·31, which is at present used in the commercial assessment of phosphatide on the basis of phosphorus determination. As pointed out earlier, the factor necessary for converting phosphorus to phosphatide would be higher than this for the total mixed phosphatides and it is probably much higher for phosphatides removed in the fourth and subsequent washes. Nevertheless, this variation does not greatly alter the interpretation of the figures.

A more important consideration is whether or not the phosphorus-containing material removed during the acetone washing is definitely phosphatide. It is evident that it is because (*i*) the phosphorus content of the acetone washes can be suppressed by using acetone previously saturated with oil-free phosphatide (the addition being allowed for), (*ii*) the material removed in the later washes cannot be redissolved in a smaller volume of acetone and (*iii*) if larger amounts of acetone are used, correspondingly larger amounts of phosphorus-containing material are dissolved. Further, as only minor amounts of water-soluble phosphorus were present in the acetone washes, the acetone-soluble phosphorus derivatives could not be free phosphoric or glycerophosphoric acids.

There does not appear to be any other suitable fat solvent in which the phosphatides are less soluble generally than acetone. In attempts to find ways of suppressing the existing solubility, other than by temperature control, it was found that acetone saturated with phosphatide or magnesium chloride gives a lowering that is insufficient to warrant its use. Examination of the existing techniques was therefore continued. To assess the balance between phosphatide removed from the sample by acetone and oleaginous non-phosphatide left in, at given stages of the washing, the calculations summarised in Table V were made.

TABLE V

BALANCE OF ERRORS IN ESTIMATING ACETONE-INSOLUBLE MATERIAL

			Wash number						
Lecithin	Method	Factor*	2, %	3, %	4, %	5, %	6, %	7, %	8, %
Unrefined ground-nut (L 3)	Proposed	I	0·40	0·75	0·97	1·18	1·38	1·57	1·77
		II	7·48	2·28	0·79	0·56	0·27	0·34	0·39
		III	+7·08	+1·53	−0·18	−0·62	−1·11	−1·23	−1·38
Unrefined ground-nut (L 3)	A.O.C.S.	I	0·48	0·63	0·74	1·06	1·38	1·53	1·71
		II	4·31	1·15	0·49	0·41	0·27	0·25	0·29
		III	+3·83	+0·52	−0·25	−0·65	−1·11	−1·28	−1·42
Refined ground-nut (L 49)	Proposed	I	0·41	0·77	1·02				
		II	4·94	1·20	0·88				
		III	+4·53	+0·83	−0·14				
Refined ground-nut (L 49)	A.O.C.S.	I	0·29	0·48					
		II	3·21	0·79					
		III	+2·92	+0·31					
Bleached soya bean (D 41)	Proposed	I	0·69	1·56	2·16	2·53	2·93	3·38	3·75
		II	5·40	1·44	0·83	0·70	0·64	0·41	0·39
		III	+4·71	−0·12	−1·33	−1·83	−2·29	−2·97	−3·36
Bleached soya bean (D 41)	A.O.C.S.	I	0·66	1·40	1·83	2·20	2·58	2·98	3·16
		II	2·75	0·86	0·64	0·54	0·50	0·32	0·42
		III	+2·09	−0·54	−1·19	−1·66	−2·08	−2·66	−2·74
Unbleached soya bean (D 84)	Proposed	I	0·74	2·01	2·92				
		II	5·32	1·47	0·68				
		III	+4·58	−0·54	−2·24				
Unbleached soya bean (D 84)	A.O.C.S.	I	1·31	1·66					
		II	2·34	1·16					
		III	+1·03	−0·50					
Cotton-seed (L 50)	Proposed	I	0·43	0·91	1·20				
		II	7·57	1·83	0·96				
		III	+6·94	+0·92	−0·24				
Cotton-seed (L 50)	A.O.C.S.	I	0·69	0·85					
		II	3·84	0·96					
		III	+3·15	+0·11					

* I: Total phosphatide removed by preceding washes.
II: Non-phosphatide removed by wash enumerated.
III: Difference between I and II, after previous wash.

DISCUSSION OF RESULTS

The errors in these calculations, which are caused by using the factor 26·31 for converting phosphorus to phosphatide, are small compared with the amounts involved and, further, their direction is such as to contribute to the arguments in their favour. This being so, certain useful deductions can be made. The most important of these is that the least error is incurred in either method by washing no further after the second operation for soya-bean lecithin and after the third for the other lecithins.

If a higher conversion factor were used, the estimates of phosphatide removed (I) would be higher and of non-phosphatide removed (II) correspondingly lower. The increased disparity would be such as to require an earlier discontinuation of the washing. The diminishing amounts of oleaginous non-phosphatide removed, however, suggest that this would be undesirable.

The persistent removal of what appeared to be non-phosphatide in the fourth and subsequent washes is probably caused by a different type of phosphatide with a lower phosphorus content, but this, at present, is not certain. If, however, these substances were glycerides, the amount removed would diminish at a greater rate.

As already shown in Table I, centrifugation reduces the entrainment almost completely. It is also evident that a cold initial wash reduces the amount of phosphatide dissolved in the oil - acetone solution.

Statistical treatment of the duplicate analyses, of which averages are given in Table I, shows that the standard deviation of the modified method is significantly lower than that of the former method (see Table VI).

From a limited number of results (five pairs, representative of different types of lecithin—see Table II) it seems that the standard deviation (0·114) of the proposed method, calculated on the same range of duplicates, is significantly lower than that (0·286) of the elution method.

The improved precision in measuring acetone-insoluble matter is still reflected in the ultimate estimation of the difference between acetone-insoluble matter and petrol-insoluble matter when the standard deviation of the method of determining the latter (0·0186) is taken into account. The standard deviation of the difference is 0·164 for the proposed method compared with 0·321 for the earlier method,[1] even when the results for soya bean lecithin, which has been shown to have a significantly higher variance than other types, are excluded.

TABLE VI

PRECISION OF METHODS OF DETERMINING ACETONE-INSOLUBLE MATTER

Type of lecithin	Standard deviation Former method[1]	 Proposed method
Unrefined ground-nut	0·366	0·134
Refined ground-nut	0·265	0·190
Soya bean	0·975	0·118
Cotton-seed	0·326	0·195
(Over-all)	0·321*	0·163

* Excluding soya-bean lecithin, for which variance was shown to be significantly greater than it was for other lecithins.

In the light of the results presented and discussed, we suggest that the difference between acetone-insoluble matter and petrol-insoluble matter gives an adequate measure of the phosphatide content of commercial lecithin, acetone-insoluble matter being determined by the method described in this paper and petrol-insoluble matter being determined as described previously.[1]

Other criteria may need to be referred to occasionally, and, as the modified method gives higher results than the earlier procedure, standards will need to be revised for given qualities, when those standards have been based upon acetone-insoluble content.

We are indebted to other colleagues of our Analytical Department for considerable assistance, to Mr. J. W. Lord for statistical analysis of results and to J. Bibby & Sons, Ltd. for permission to publish this work.

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The Determination of Small Amounts of Potassium, Calcium and Magnesium in Sodium and its Compounds

By LOUIS SILVERMAN AND K. TREGO

Preliminary concentration is necessary before potassium can be determined in the presence of large amounts of sodium ion.

Metallic sodium, sodium hydroxide and sodium salts are converted to chloride. Measured, cold, saturated aqueous chloride solutions are treated with dry hydrogen chloride gas, which separates the bulk of the sodium chloride. The residual potassium, calcium and magnesium can then be determined by the usual methods.

Calcium and magnesium may also be determined with or without preliminary sodium chloride separation.

THE potassium content of commercial sodium metal is about 0·005 per cent. This is below the limits for direct quantitative determination of potassium in sodium[1,2,3,4] and necessitates preliminary separation of sodium or concentration of potassium.

In the preliminary separation as sodium chloride, the initial weight of test sample is reduced from 5 to 50 g of sodium chloride to 0·2 to 0·5 g of concentrate, containing all of the potassium, calcium and magnesium with incidental sodium chloride. This concentrate may contain 0·5 mg or more of potassium perchlorate, which can be conveniently determined by the ethyl acetate extraction procedure.[4]

This paper describes the precipitation of the bulk of the sodium chloride from aqueous solution by hydrogen chloride gas[5,6] and results are presented to demonstrate the effectiveness of the separation of the potassium from the bulk of the sodium chloride. Potassium (as well as calcium and magnesium) is not retained by the sodium chloride precipitate.

A method for the direct determination of calcium[7,8] in sodium chloride has been reported, but none has been described for magnesium.[9] These direct determinations may be used as check methods against the pre-concentration procedure for the determination of calcium and magnesium.

METHOD

REAGENTS—

Acetic acid solution of 8-hydroxyquinoline—Place 5 g of 8-hydroxyquinoline and 10 ml of glacial acetic acid in a beaker. Warm to 50° to 60° C for several minutes to complete solution and dilute to 200 ml with water. Discard after 2 weeks.

Alcoholic solution of 8-hydroxyquinoline—Dissolve 5 g of 8-hydroxyquinoline in 200 ml of ethyl or methyl alcohol. Discard after 2 weeks.

Ammonium oxalate solution—Dissolve 10 g of the salt in 100 ml of water.

Saturated hydrochloric acid solution—Saturate hydrochloric acid, sp.gr. 1·2, with dry hydrogen chloride gas, at 0° C.

Wash water, pH 10—Add ammonium hydroxide, sp.gr. 0·880, to distilled water until the pH is 10.

PROCEDURE FOR DIRECT DETERMINATION OF POTASSIUM—

Metallic sodium—Weigh the sodium metal plus container. Open the container and transfer it and the sample to a weighed glass beaker. Treat the sample with discrete portions of anhydrous methyl or ethyl alcohol until the metallic sodium has been converted to the alcoholate. Small portions of water may be added cautiously to accelerate the action. Remove the container and wash, dry and weigh it in order to find the amount of metallic sodium taken.

Add water to the alcoholate solution to increase its volume by a quarter and mix thoroughly. The sodium content if as low as 1 to 3 g may also be determined at this point by titration with *N* hydrochloric acid solution. Add concentrated hydrochloric acid to the alcoholate solution until the solution turns litmus paper red, and add about 5 ml of hydrochloric acid in excess. Evaporate the solution nearly to dryness, then bake the residue for 4 to 12 hours in an oven at 110° to 130° C. Cool and weigh. The gain in weight is equivalent to the amount of sodium chloride. This weight of sodium chloride may be used to calculate or check the weight of metallic sodium taken for analysis.

Divide the weight of sodium chloride in grams by 0·35 (the solubility of sodium chloride in water is 35 per cent.) and add this calculated amount of water to the beaker containing the sodium chloride. Warm the solution to dissolve the salt completely. Cool the beaker in the refrigerator to 0° C and weigh the beaker and contents cold.

Sodium hydroxide—Weigh the sodium hydroxide and transfer the sample to a beaker, weighed to the nearest tenth of a gram, containing five times as much water as sodium hydroxide by weight. Add a measured amount of hydrochloric acid to make the solution just acid. Cool. Calculate the weight of sodium chloride formed. Evaporate the solution until the beaker contains a saturated solution of sodium chloride.

Sodium chloride—Weigh the sodium chloride, transfer the sample to a weighed beaker and add sufficient water to make a saturated solution of the sodium chloride.

Separation of salt—Pass dry hydrogen chloride gas through a train into the cold solution. If a tank of gas is available, successively pass the gas through a wash bottle of sulphuric acid, a safety bottle and an inverted funnel or thistle tube into the aqueous sodium chloride solution. The funnel or thistle tube should be well below the surface of the salt solution. Set the cold sodium chloride solution in an ice-bath and maintain the ice in the bath throughout the action. Pass in dry hydrogen chloride gas slowly at first, then increase the flow of gas until bubbles pass through the sodium chloride solution. If the amount of sample is large the salt solution may become hot, but it will cool when the absorption of gas is complete. The volume will increase by about 30 per cent. When the absorption seems complete and the liquid is fuming, remove the beaker from the system and weigh it. The action is complete when the salt solution has increased in weight by 47 to 51 per cent.—roughly, twice the weight of the dry sodium chloride. Set the covered beaker in the refrigerator until it is ready for filtration.

Decant the cold fuming solution through a cold dry 500-ml sintered-glass crucible, transfer the solid to the crucible and wash it with 20 ml of cold hydrochloric acid saturated with hydrogen chloride. Discard the residue. Evaporate the filtrate to a small volume, transfer the solution to a 150-ml beaker weighed to the nearest hundredth of a gram and evaporate the liquid nearly to dryness; dry the residue in an oven at 110° to 130° C. Cool and weigh. The gain in weight (about 0·2 g) represents sample concentrate, and the amount of ethyl acetate to be used will depend on this weight.

Separation of potassium from sodium—Add 5 ml of nitric acid, sp.gr. 1·4, and 5 ml of 72 per cent. perchloric acid and evaporate nearly to dryness, leaving only sufficient perchloric acid to prevent formation of insoluble ferric oxide. Cool to room temperature. Add 6 ml of anhydrous ethyl acetate (5 ml will dissolve the equivalent of 0·3 g of sodium chloride) and heat. Complete the potassium determination by the procedure of Willard and Smith.[4]

PROCEDURE FOR SEQUENCE DETERMINATION OF CALCIUM, MAGNESIUM AND POTASSIUM—

Preliminary separation from sodium—Proceed as described for direct potassium determination with sodium metal, sodium hydroxide or sodium chloride to the point where the dried sample concentrate would be treated with nitric and perchloric acids. Instead add 50 ml of water to the 150-ml beaker containing the sample concentrate of sodium chloride. Warm to dissolve the salts.

Separation of calcium and magnesium from potassium and sodium—Add 15 ml of the alcoholic solution of 8-hydroxyquinoline and warm to 60° to 80° C. Slowly add ammonium hydroxide, sp.gr. 0·880, until the pH is raised to between 9·5 and 10·5. A pH meter or thymolphthalein indicator may be used. Heat to incipient boiling and then cool to room temperature. Check the pH reading, adding ammonium hydroxide, if necessary. Filter the solution through a 12·5-cm Whatman No. 40 filter-paper into a 250-ml beaker. Wash the beaker with an aqueous solution of ammonium hydroxide adjusted to a pH value of 10. Wash the precipitate and paper several times with the wash-water. Reserve the filtrate for potassium determination.[4]

Calcium determination—Return the paper and its contents to the beaker and add 15 ml of nitric acid, sp.gr. 1·4, and 8 ml of 72 per cent. perchloric acid. Mix and evaporate the solution to heavy fumes of perchloric acid. Cool. Dilute to 50 ml with water and boil for 1 minute. If the solution appears cloudy, filter, wash and discard the residue.

Precipitate the calcium as oxalate, filter, reserve the filtrate for magnesium determination, and complete the calcium determination by titrating with 0·02 *N* potassium permanganate solution in the usual manner.

Magnesium determination—Heat the filtrate from the calcium oxalate precipitation to boiling and slowly add 15 ml of the solution of 8-hydroxyquinoline in acetic acid. If iron is present a black precipitate forms and the supernatant liquid should be yellow. Additional reagent is added, if necessary, to make the solution yellow. Stir the solution until the black precipitate coagulates, then filter off the iron precipitate on a Whatman No. 40 filter-paper containing paper pulp and collect the filtrate in a 400-ml beaker. Do not allow the black particles to creep above the paper. Wash the paper and precipitate with 0·2 per cent. v/v acetic acid solution. Discard the paper and the iron precipitate.

If the solution is not yellow, add 5 to 10 ml of alcoholic 8-hydroxyquinoline reagent to the filtrate and then warm to 60° to 80° C.

Complete the magnesium determination by the procedure of Berg.[9]

Potassium determination—To the reserved potassium solution, add 15 ml of nitric acid, sp.gr. 1·4, and 8 ml of 72 per cent. perchloric acid, evaporate to heavy fumes of perchloric acid and continue heating until nearly all the perchloric acid has been evaporated. Heat the beaker over an uncovered bunsen burner flame to volatilise ammonium salts.

Complete the potassium determination by the procedure of Willard and Smith.[4]

PROCEDURE FOR DIRECT DETERMINATION OF APPRECIABLE AMOUNTS OF CALCIUM AND MAGNESIUM[8]—

Convert the metallic sodium, sodium hydroxide or other sodium samples to sodium chloride. Adjust the volume of solution so that the salt content is 20 to 25 per cent. and proceed according to the section on separating calcium and magnesium from potassium and sodium (p. 718).

DISCUSSION OF RESULTS

The effective separation of the bulk of the sodium chloride is shown in Table I. The first column shows the weight of sodium chloride (weighed or calculated), the second the

TABLE I

SEPARATION OF SODIUM CHLORIDE BY HYDROGEN CHLORIDE GAS

NaCl, g	Water added, ml	HCl absorbed, g	Gain in weight from HCl, %	Weight of sample concentrate, g
38·8	110·9	37·8	25	0·80
47·8	136·8	80·5	44	0·70
69·7	194·3	57·8	22	0·50
116·5	329·0	62·1	14	14·8
117·2	334·7	180·5	40	3·3
122·3	349·4	240·0	51	0·45
149·9	428·3	285·0	50	0·62
69·45	198·4	138·3	52	0·24
61·8	176·7	112·8	48	0·22
22·45	64·1	40·5	47	0·12
57·35	163·9	105·3	48	0·20
10·75	30·7	19·2	47	0·10
4·85	13·9	9·0	48	0·09
9·75	27·9	18·1	48	0·05
84·95	242·7	156·3	48	0·30
56·65	161·9	100·0	46	0·24
70·5	201·4	135·4	50	0·19
54·2	154·9	98·5	47	0·18
44·65	127·6	87·4	51	0·15
40·0*	114·0	72·2	47	0·15
40·0*	114·0	74·1	48	0·15
40·0*	114·0	71·9	47	0·14

* Synthetic sample.

amount of water added and the third the weight of hydrogen chloride gas absorbed. The fourth column shows the percentage gain in weight of the solution and the fifth shows that all but a small amount of salt is precipitated from solution.

It is evident from Table I that a sample weight of 15 to 20 g of sodium metal (40 g of sodium chloride) can be used, and that all but about 0·2 g of salt can be precipitated from solution when the salt solution has absorbed nearly 50 per cent. of its weight of hydrogen chloride.

For the results in Table II, a 40-g sample of sodium chloride was used, and blank determinations of potassium, calcium and magnesium were made. After this, known amounts of the three elements were added to 40-g samples of the same batch of C.P. sodium chloride, and the three elements were determined. It should be noted that the C.P. sodium chloride sample itself contains 0·002 per cent. of potassium, 0·0002 per cent. of calcium and 0·00016 per cent. of magnesium, and these are the blank values that must be subtracted, as mentioned in a footnote to Table II.

TABLE II

SYNTHETIC SOLUTIONS OF POTASSIUM, CALCIUM AND MAGNESIUM IN 40 g OF SODIUM CHLORIDE

	By preliminary hydrogen chloride separation								
	Potassium			Calcium			Magnesium		
Elements added	Added, mg	Found, mg	%	Added, mg	Found, mg	%	Added, mg	Found, mg	%
None. C.P. sample used for blank values	—	0·80	0·0020	—	0·082	0·00020	—	0·065	0·00016
	—	0·84*	0·0021	—	0·10	0·00025	—		
K	0·3	0·3†	0·00075						
K, Ca	0·30	0·40*†	0·0010	0·40	0·48†	0·0012			
Ca				0·30	0·25†	0·0006			
Ca, Mg							0·40	0·36†	0·0009
Ca, Mg							0·40	0·43†	0·0011

	By direct 8-hydroxyquinoline separation					
	Calcium			Magnesium		
Elements added	Added, mg	Found, mg	%	Added, mg	Found, mg	%
None. C.P. sample used for blank values	—	0·08	0·0002	—	0·05	0·00013
K						
K, Ca						
Ca						
Ca, Mg	0·20	0·20†	0·0005	0·40	0·35†	0·0009
Ca, Mg	0·40	0·35†	0·0009	0·40	0·35†	0·0009

* No potassium found in re-worked precipitate.
† Corrected for blank values.

The solubility of the potassium, calcium and magnesium in the mixture of cold saturated hydrochloric acid and sodium chloride solution is important. The calcium and magnesium form anionic complexes that are soluble. Only the potassium has limited solubility, but in the technique described no error need be expected. For example, consider the synthetic sample in Table I. For 40 g of sodium chloride, 114 ml of water are used, and after the addition of hydrogen chloride the volume has increased by 30 ml. From this precipitation 0·15 g of sample concentrate was left in solution. From the point of view of the common ion effect, this residuum could be divided as 80 per cent. of sodium and 20 per cent. of potassium, *i.e.*, 30 mg of potassium chloride (15 mg of potassium) would remain in solution. Since the obvious application of this analytical technique is to separate small amounts, or at the most 5 mg, of potassium from large amounts of sodium, the technique is well-founded.

It is also necessary to prove that no potassium is occluded by sodium chloride. This is shown experimentally in Table II. Here, the precipitated sodium chloride was filtered from the sample concentrate, dissolved in water, precipitated as before with hydrogen chloride gas and a second sample concentrate was collected. No potassium was found in this second sample concentrate, and it is then stated that no potassium is found in the re-worked precipitate.

TABLE III

DETERMINATION OF POTASSIUM IN SODIUM METAL

Number of experiment*	Type of metal	Amount of metal, g	Potassium found, mg	Potassium, %
1	As received	33·4	2·9	0·009
1	As received	39·8	4·7	0·012
1	As received	31·5	7·0	0·022
2	Distilled†	15·7	1·4	0·009
2	Distilled†	17·2	1·4	0·008
3	Treated‡	23·75	0·72	0·003
3	Treated‡	21·95	0·41	0·002
3	Treated‡	17·7	0·51	0·003
4	Treated‡	29·8	0·68	0·002
4	Treated‡	29·4	1·53	0·004
5	Treated‡	38·8	0·3	0·001
5	Treated‡	24·5	0·2	0·001
5	Treated‡	22·4	0·6	0·003

* Samples with the same number are considered as nearly duplicate samples.
†Loftness, Ruebsamen and Coultas.[10]
‡Ruebsamen.[11]

This procedure has particular application to the determination of small changes of potassium metal content in treated and untreated sodium metal (see Table III). Samples received from the manufacturer contain 0·01 to 0·02 per cent. of potassium. Vacuum distillation reduces the figure, but treatment with graphite chips[4] reduces the amount of potassium to about 0·001 per cent. The sets of treated samples are considered as duplicates from the same operation.

To check the results for calcium and magnesium the method described for direct 8-hydroxyquinoline separation (p. 719) was used, as shown in Table II. It was thought that the results obtained from the preliminary hydrogen chloride separation (p. 718) would be higher, but this was not so.

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An Isopiestic Method for the Micro-determination of Molecular Weights

By Miss J. E. MORTON, A. D. CAMPBELL* and T. S. MA

A simple method is described for the determination of the molecular weight of non-volatile compounds; it is based on Sinclair's isopiestic method for the determination of vapour pressures. A solution of the compound in a volatile solvent is rocked at constant temperature in a small desiccator with a solution, in the same solvent, of a substance of known molecular weight. When the two solutions are isopiestic, the molecular weight of the compound is calculated from the molecular weight of the standard substance and the concentrations (by weight) of the two solutions. For 3 to 7-mg samples concordant results have been obtained with a number of solvents.

In microchemical work there is a need for a simple yet accurate method for the determination of molecular weights on 3 to 7-mg samples. Both the ebullioscopic and cryoscopic methods at present in use require extremely careful control by a skilled worker before accuracy can be attained.

When two solutions containing different solutes are placed in a closed space, vapour will distil from the solution of higher vapour pressure (*i.e.*, the more dilute solution) and condense in the one having the lower vapour pressure until equality of vapour pressure is attained. This isopiestic principle has been used for the determination of approximate molecular weights by Barger,[1] who placed drops, separated by small air spaces, of the two solutions in a capillary tube. By observing the direction of the distillation of the solvent under a microscope he could determine which solution had the greater vapour pressure, and by varying the concentration of one of the solutions he was able to determine when the two solutions were isopiestic. From the concentrations by weight of the isopiestic solutions and the molecular weight of one solute, the molecular weight of the other could be determined. In spite of many variations by later workers the probable error is high, as it is not possible to avoid completely some contamination of one solution by the other. Higher accuracy was attained by Clark,[2] but his method required a 20-mg sample. The two solutions were placed one in each arm of an H-shaped apparatus, from which air was evacuated. The concentration of the two solutions was determined by inverting the apparatus so that the solutions ran into accurately calibrated measuring tubes.

Most isopiestic methods suffer from the disadvantage that equilibrium is attained only slowly, but this was overcome by Sinclair[3,4] who, when making extremely accurate vapour pressure determinations, placed the two solutions in gold-plated silver dishes in close contact on a copper block in an evacuated desiccator, which was rocked in a thermostat. In the method described here Sinclair's method has been adapted to the determination of the molecular weights of a number of organic compounds in various volatile solvents.

A more elaborate apparatus has been described by Claesson[5]; it was designed particularly for the determination of molecular weights between 1000 and 10,000. It was similar to the apparatus used by Scatchard[6] for the determination of activities, but had an additional attachment for closing the silver beakers containing the solutions before the apparatus was opened. Wright[7] described a very simple apparatus in which the solutions to be equilibrated were adsorbed on filter-papers in two aluminium trays that could be fitted together for equilibration, but the order of accuracy was not high.

METHOD

APPARATUS—

The solutions are contained in two platinum crucibles of 1·3 ml capacity that fit into two holes in a brass block weighing about 250 g placed inside a small vacuum desiccator of approximately 250 ml capacity (see Fig. 1). To prevent loss of volatile solvent, the platinum crucibles are weighed inside small weighing bottles with tightly fitting ground-glass stoppers.

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PROCEDURE—

Weigh the sample, which should be between 3 and 7 mg (a larger sample must be taken for compounds of very high molecular weight), in one of the platinum crucibles and weigh the standard substance in the other. If the approximate molecular weight of the sample is known, the attainment of equilibrium can be speeded by taking similar molar quantities of sample and standard. Add the solvent, *e.g.*, about 0·3 ml of chloroform, to each of the two crucibles and then weigh them inside the counterpoised weighing bottles fitted with ground-glass stoppers. Place the crucibles in the holes in the brass block inside the desiccator. Pour a small quantity of solvent (about 0·5 ml) on the brass block and then evacuate air from the desiccator under controlled conditions so that each crucible still contains 100 to 200 mg of solvent at the end of the experiment. This is achieved by connecting the desiccator through a large bottle, of capacity about 2½ litres, to a water pump. With the connection between the bottle and the desiccator closed, reduce the pressure in the bottle to 200 mm of mercury; then close the connection between the bottle and the pump and open that between the bottle and the desiccator and allow the pressure to equalise. Disconnect the desiccator and rock it at 10 strokes per minute through an angle of 30° in a water-bath at 25° C for 24 hours. At the end of this time open the desiccator and quickly place the crucibles inside the weighing bottles and weigh them. Then return the crucibles to the desiccator, place a few drops of solvent inside the jar and evacuate the apparatus as before. After a further 24 hours of rocking in the water-bath, again weigh the crucibles.

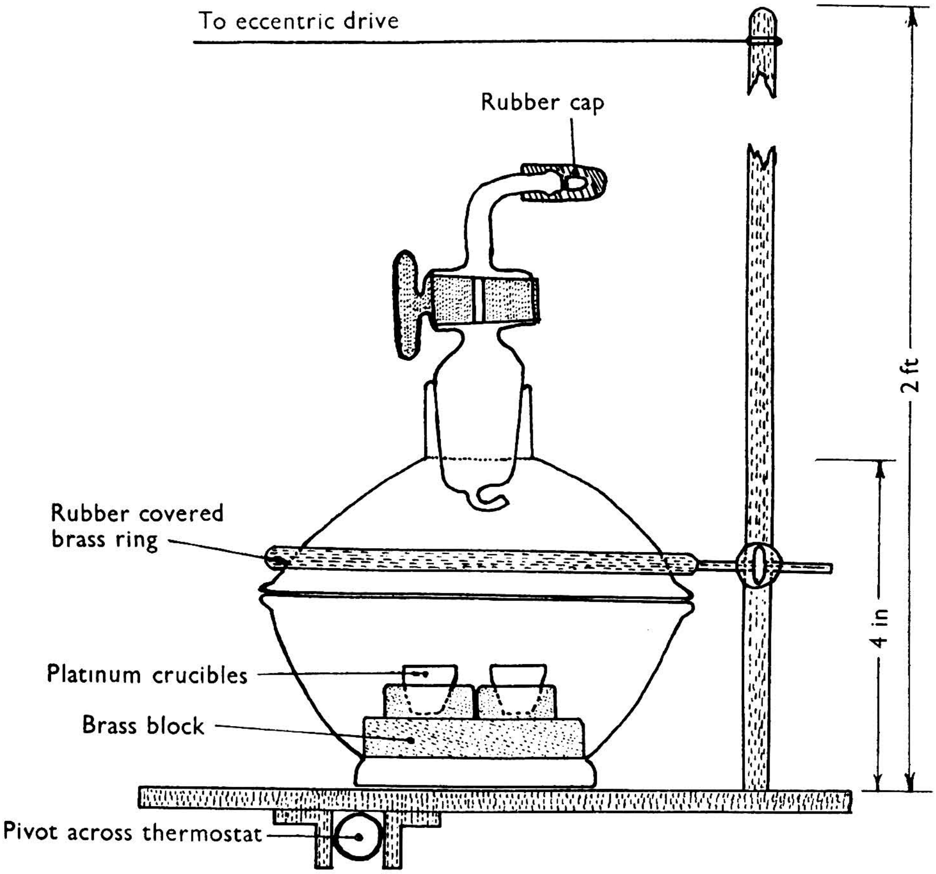

Fig. 1. Micro vacuum desiccator mounted in thermostatically controlled bath

The solutions are assumed to be isopiestic when the ratio of their concentrations (by weight) is constant for two consecutive readings at 24-hour intervals. The molecular weight of the sample is then calculated from the formula—

$$M_1 = \frac{W_1 S_2 M_2}{W_2 S_1},$$

where M_1 and M_2 are the molecular weights of the unknown and standard substance, respectively, W_1 and W_2 are the weights of the unknown and standard substance, and S_1 and S_2 are the weights of solvent in the crucible containing the unknown and standard substance, respectively.

The approach to equilibrium with time is shown by a comparison of the weight ratios of the standard and unknown solutions, *i.e.*, by determination of W_1/S_1 divided by W_2/S_2. When equilibrium is reached this ratio becomes constant. The approach to constant weight of the solution of the unknown cannot be used as a criterion of equilibrium because the breaking of the vacuum and subsequent re-evacuation of air alters the total amount of solvent in the system. In preliminary work the same solute, azobenzene, was used in both crucibles.

RESULTS AND DISCUSSION

The results shown in Table I indicate the high order of accuracy attainable by this method for a number of compounds and various volatile solvents.

TABLE I

MOLECULAR WEIGHT DETERMINATIONS

Compound	Weight of compound, mg	Solvent	Weight of solvent at equilibrium, mg	Molecular weight		Error, %
				Found	Theoretical	
p-Phenyl-phenacyl bromide	6·441	Chloroform	81·2	278	275	+1·1
Azobenzene (m.w. 182)	3·369		64·8			
p-Phenyl-phenacyl bromide	6·516	Chloroform	112·2	278	275	+1·1
Azobenzene (m.w. 182)	3·438		90·4			
2:4-Dinitrochlorobenzene	5·443	Benzene	90·6	194	192·5	+0·8
Phenyl benzoate (m.w. 198)	5·521		90·3			
Salicylic acid	3·040	Acetone	95·3	137	138	−0·7
Azobenzene (m.w. 182)	3·967		93·6			
Adipic acid	3·102	Acetone	113·4	144	146	−1·4
Azobenzene (m.w. 182)	3·874		112·3			
Phenacetin	5·020	Methanol	125·2	167	169	−1·2
Azobenzene (m.w. 182)	4·877		111·2			
Adipic acid	4·675	Methanol	122·6	143	146	−2·1
Phenacetin (m.w. 179)	5·248		116·4			
Salicylic acid	5·308	Ethanol	85·2	135	138	−2·2
Benzoic acid (m.w. 122)	4·855		85·90			
Salicylic acid	5·671	Ethanol	150·2	139	138	+0·7
Benzoic acid (m.w. 122)	4·957		149·3			
Methyl oleate	6·110	Chloroform	191·5	302	292	+3·4
Azobenzene (m.w. 182)	5·563		288·6			
Sesquiterpene nitrosate	6·922	Chloroform	114·6	679	684	−0·7
Azobenzene (m.w. 182)	4·671		288·6		or 342	
Sesquiterpene nitrosate	12·365	Benzene	102·5	716	684	+4·7
Phenyl benzoate (m.w. 198)	4·890		146·7		or 342	
Caffeine	4·342	Chloroform	108·5	194	196	−1·0
Azobenzene (m.w. 182)	4·846		128·7			
Standard deviation						±1·6

NOTE—The second compound of each pair was used as the reference substance of known molecular weight.

Solutions in chloroform, acetone and benzene attain equilibrium in 24 hours and therefore the first two determinations at 24-hour intervals are in agreement, but with ethanol and methanol a third determination is necessary before agreement is attained. The quantity of solvent placed in the desiccator and in the crucibles depends on the nature of the solvent used, the pressure to which the desiccator is evacuated and the size of the desiccator. The quantities of solvent and the pressure quoted in the procedure have been found suitable when chloroform is used as solvent. With chloroform and other very volatile solvents a correction may have to be made for loss of solvent from the weighing bottles during the weighing operation, but it is possible to grind the stoppers so that this loss is negligible. The choice of standard substance and solvent is governed by the solubility at room temperature, the stability and a consideration of the possibility of association in the solvent concerned. The limitations of the method are determined by the well-known deviations from Raoult's law.

As residual air in the apparatus has been found to slow up the equilibration,[6] the controlled evacuation should be repeated once or twice for solvents that are less volatile than chloroform.

MACRO-DETERMINATION OF MOLECULAR WEIGHTS

With readily available laboratory apparatus and an ordinary analytical balance, the isopiestic principle can be used to determine the molecular weights of compounds on a 0·2-g sample. This is no great disadvantage, as the sample is readily recovered from the solution at the end of the experiment.

The results in Table II were recorded with glass weighing bottles, of diameter 3 cm, as containers for the two solutions placed inside a glass jar of 500 ml capacity that was adapted for evacuation and was rocked in a thermostatically controlled bath. The jar was evacuated to a pressure of 50 mm of mercury by water pump, controlled evacuation being unnecessary on the macro scale.

TABLE II

MOLECULAR WEIGHT DETERMINATIONS ON A MACRO SCALE

Compound	Weight of compound, g	Solvent	Weight of solvent at equilibrium, g	Molecular weight Found	Molecular weight Theoretical	Error, %
Phenyl benzoate	0·2010	Chloroform	1·593	198	198	0·0
Azobenzene (m.w. 182) ..	0·1999		1·728			
Benzoic acid	0·2326	Acetone	1·604	123	122	+0·8
Azobenzene (m.w. 182) ..	0·3030		1·410			
Glutaric acid	0·2162	Methanol	1·8442	130	132	−1·5
Succinic acid (m.w. 118) ..	0·2186		2·0626			
Salicylic acid	0·2436	Methanol	1·1676	139	138	+0·7
Glutaric acid (m.w. 132) ..	0·2264		1·142			
Adipic acid	0·2498	Methanol	1·9282	145	146	−0·7
Succinic acid (m.w. 118) ..	0·2526		2·3910			
Standard deviation ..						±0·5

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Notes

THE LIPASE ACTIVITY OF CERTAIN CEREAL PRODUCTS

SOMETIMES off-flavours in finished baked products can be caused by the action of lipase originally present in the flour or cereal product used in their manufacture. This effect is well-known in baked oat products in which the oatmeal has not had sufficient treatment to destroy the lipase originally present in the grain. Methods of treatment designed to destroy lipase activity have been described by Moran.[1]

Hutchinson and Martin[2] have published a method for assessing lipase activity in oats and oatmeals, and a modification mentioned by these authors seemed the most promising as a means of assessing the activity of malt flours and other flours. The method was tried and the results may be of interest to analysts investigating off-flavours in foodstuffs.

The method has been used by us in a large number of investigations and the results have proved informative. As an example, the results of an examination of five samples of malted barley flour, a malted wheat flour and two wholemeal wheat flours are quoted below. Some of the samples were known to have been used in products about which complaints of off-flavour had been made. Comparison was also made with two fine and medium-ground oatmeals, known to have caused a soapy flavour in oatcakes.

TABLE I

LIPASE ACTIVITY OF SAMPLES OF FLOUR AND OATMEAL

	Amount of oil hydrolysed after incubation for				
Sample	2 hours, %	3 hours, %	4 hours, %	5 hours, %	6 hours, %
1. Barley malt flour	0·68	1·24	1·06	1·09	1·44
2. ,, ...	0·51	0·72	1·18	1·27	1·43
3. ,, ...	0·53	0·55	0·72	0·92	0·93
4. ,, ...	1·28	2·34	2·23	2·88	2·87
5. ,, ...	0·35	0·56	0·88	0·65	1·26
6. Wheat malt flour	0·64	0·78	0·95	0·94	1·76
7. Wholemeal flour	0·10	0·09	0·03	0·08	0·07
8. ,, ...	1·24	1·63	1·64	1·67	2·33
9. Oatmeal					29·08
10. ,, ...					41·49

The flours were defatted by washing with light petroleum as described by Hutchinson and Martin,[2] so that no complications from differences in substrate were involved.

Six portions of 0·5 g of the defatted malt flour were weighed and placed in 2-inch × ½-inch tubes, 7 drops (about 0·2 g) of olive oil and 0·2 ml of water were added to five of the tubes, and the time at which the water was added was noted. The mixture was stirred into a smooth dough with a glass rod and the tubes were tightly corked. The tubes were placed in a bath at 37° C and incubated for 2, 3, 4, 5 or 6 hours.

When incubation was completed for any tube, it was removed from the bath and the oil and free fatty acid were extracted from the dough by stirring vigorously with a glass rod with successive small portions of light petroleum (A.R. grade boiling between 40° and 60° C) until 25 ml had been used. The light petroleum extract was centrifuged to remove the solid matter and then decanted into a tared flask. The light petroleum was distilled off, and the oil and free fatty acid were dried for an hour in an air oven at 100° C and weighed.

The oil and free fatty acid were dissolved in 25 ml of a neutral (1 + 1) mixture of alcohol and benzene, brought to the boil on the water-bath and titrated with 0·02 *N* alcoholic sodium hydroxide, with phenolphthalein as indicator.

The experimental blank was determined on the sixth portion by adding 7 drops of olive oil, but no water, and then treating it exactly as the others but without incubation.

By assuming the free fatty acid to be oleic acid, the proportion of oil hydrolysed was calculated as a percentage of the total oil.

Each assessment was carried out three times and the means of the three results are shown in Table I. The amount of olive oil hydrolysed after incubation for 6 hours seemed to give the most useful indication of lipase activity, the differences between replicates being least at this period.

Two experiments with doughs of higher water content were made in an attempt to reduce the time required for the assessment, but the quantities of oil recovered from the mixtures were too small for a reliable test.

Finally, three 6-hour incubations were run simultaneously and "worked up" together. The results were of the same order as those that had been obtained separately.

It is evident that there was a distinct difference between samples 3 and 4, sample 4 being known from other experiments to have more lipase activity than is desirable in a malt flour. Sample 6, the wheat malt flour, was known to have an undesirably high lipase activity, having given a soapy taste to biscuits, and a higher percentage of olive oil was hydrolysed by this flour than by sample 5, which was not suspect. Of the two wholemeal flours, sample 7 had almost no lipase activity, whilst sample 8 had sufficient to impart distinctly adverse odours and flavours to the flour. Both the oatmeals were highly active, between thirty and forty times as active as any of the other flours examined. Oatcakes made with these meals had a very unpleasant taste.

From the results it would appear that a malt or wholemeal wheaten flour with sufficient lipase activity to hydrolyse rather more than 1·5 per cent. of olive oil in 6 hours might not be safe to use, but much depends on the proportion of malt flour in the goods and the time that elapses between mixing and baking.

Hard and fast conclusions cannot be drawn from the figures shown, but they give an indication of the state of the flour and whether or not it is responsible for the soapy taste in samples of baked goods.

We wish to express our appreciation and thanks to Mr. H. F. Martin of the Cereals Research Station, St. Albans, for his help in showing us the latest modification to his method, and we are indebted also to the Council of the Research Association for permission to publish this note.

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TESTS FOR NITRITE AND NITRATE APPLICABLE OVER WIDE CONCENTRATION RANGES

THE long-established method for identifying nitrite in solution by diazotisation has given rise to many investigations into the possibility of estimating very small amounts. The method of Ilosvay[1] makes use of sulphanilic acid and α-naphthylamine as the two reagents to be coupled; this reaction can be used for quantitative colorimetric analysis if certain precautions are observed.[2] Many other reagents have been suggested for attaining increased sensitivity and a more stable colour, *e.g.*, dimethyl-α-naphthylamine and sulphanilic acid,[3,4] dimethylaniline and sulphanilic acid,[5] and N-α-naphthylethylenediamine hydrochloride and sulphanilamide[6,7]; comparative tests have also been carried out.[8]

A good qualitative colorimetric test should give (*a*) a positive indication at any concentration likely to be encountered when the test is applied under standard conditions and (*b*) an approximate indication of the concentration of the test solution (nitrite in this instance) according to the intensity of the colour produced. It is never safe to assume that a test that is perfectly satisfactory for solutions of molarity 10^{-5} or less will be equally satisfactory when applied to solutions that may be molar. On this basis the Ilosvay test is clearly unsuitable for use in routine analysis, since it gives its maximum colour intensity with about 0·01 M nitrite (with the added objection that this colour is unstable and soon changes to a brown deposit); with concentrated solutions only a very pale yellow colour is produced. An excess of nitrite is capable of destroying the red colour even after it has been formed—presumably because the possibilities for resonance are much more restricted when the amine is converted into a diazonium salt. This latter difficulty can be avoided by the use of a tertiary, instead of a primary, amine as coupling agent; but aromatic tertiary amines readily give nitroso derivatives with free nitrous acid, and the nitrite test in concentrated solution gives a muddy coloured unstable precipitate.

For routine identification of nitrite, phenols in alkaline solution are preferable to amines in acid solution. Results are good with sulphanilic acid and α-naphthol (for which a concentration limit of 1 part in 10^7 was claimed[9] in 1887); the diazotisation is carried out in acetic acid solution and the product is poured into an alkaline solution of α-naphthol. Benford and Ingold[10] have recommended a somewhat similar procedure for the quantitative determination of traces.

METHOD FOR THE IDENTIFICATION OF NITRITE

PROCEDURE—

To 1 ml of the suspected nitrite solution, which should be neutral, add 0·5 ml of sulphanilic acid solution (1 per cent. w/v in 5 *N* acetic acid). Shake well, allowing at least 10 seconds for the diazotisation to proceed; then pour into 2 ml of a fresh solution of α-naphthol in 2 *N* sodium hydroxide or 2 *N* ammonium hydroxide. The concentration of this α-naphthol solution is not critical, but an approximately 0·5 per cent. w/v solution is recommended. A deep red colour, which is stable, and the intensity of which depends on the original concentration of nitrite, is produced. If traces are being sought, diazotisation should take about 10 minutes to reach completion; the solution is then made alkaline. The reagents used should all be of analytical reagent quality.

INTERFERING RADICALS—

Sulphate, nitrate, fluoride, chloride, bromide, silicate, borate, phosphate, arsenate and the common organic acids do not interfere. The slight colour found by Odekerken[11] in the Ilosvay test with fluoride, chloride, sulphate, nitrate and carbonate can almost certainly be ascribed to traces of nitrite present as impurity. Iodide, chromate, sulphite, thiosulphate and ferrocyanide all interfere to some extent; addition of lead acetate precipitates the corresponding lead salt, and there is no need to filter, as these precipitates are not appreciably soluble in the dilute acetic acid of the sulphanilic acid reagent and are redissolved from the diazotised solution when it is poured into the sodium hydroxide. Ferricyanide also interferes and can be removed similarly by the addition of zinc sulphate solution. Cyanide and thiocyanate interfere if present in large amounts; they can be precipitated by careful addition of silver sulphate solution (or nitrate, if this radical is not being sought) until a faint permanent precipitate is just produced for the cyanide, or until precipitation is complete for the thiocyanate. If the alkali used for making the α-naphthol solution is ammonium hydroxide, any excess of silver ion will be taken back into solution as the ammine. As the solubility of silver nitrite is only about 0·015 moles per litre at room temperature, any nitrite in excess of this value will be removed along with the interfering radicals. Coloured ions can be allowed for by the use of a comparison solution, or they can be removed by precipitation.

EXTENSION OF THE METHOD TO THE IDENTIFICATION OF NITRATE

The main problem in extending the method to nitrates is to find a suitable reducing agent for the reduction of nitrate to nitrite. The following are all unsuitable for various reasons: magnesium, zinc needles or wool, cadmium, iron, tin or mercury in acid solution; sodium or zinc amalgam; salts such as hypophosphite and organic reducing agents such as formaldehyde. Many textbooks recommend the addition of Ilosvay's reagent to the suspected nitrate, followed by reduction with zinc dust. This procedure is unsatisfactory, as the reduction easily goes much further than the nitrite stage in solutions of such high acidity—the efficiency may easily be much less than 1 per cent.—and the desired azo-compound is itself easily reduced by zinc dust in acetic acid. Consequently, no colour is produced unless the zinc dust is added with extreme caution. Odekerken[11] suggests that hydrogen peroxide stabilises the colour produced; it presumably also reduces the rate of reduction, but the method is open to the same objections in a lesser degree. The only satisfactory method is to control the acidity within the range pH 4 to 5, to use only a slight excess of zinc, and to remove this excess by filtration before testing for nitrite in the usual way.

PROCEDURE FOR THE IDENTIFICATION OF NITRATE—

To 1 ml of the suspected nitrate solution, which should be neutral, add 3 ml of acetate buffer (50 ml of 2 *N* acetic acid and 25 ml of 2 *N* sodium hydroxide diluted to 100 ml). Add 40 to 50 mg of zinc dust, shake the mixture for about 30 seconds and then filter. Test the filtrate for nitrite as before. Under these conditions, measurements with a Spekker photo-electric absorptiometer show that the efficiency of reduction varies from about 50 per cent. at 0·25 *M* to about 40 per cent. at 0·0001 *M*, *i.e.*, the reduction is efficient and nearly independent of the concentration.

PROCEDURE IF BOTH NITRITE AND NITRATE ARE PRESENT—

The usual method is to identify the nitrite, destroy it, and then to proceed to the identification of nitrate by reduction to nitrite. Of the three methods commonly used for the destruction of nitrite, Odekerken[11] recommends the use of sodium azide in acetic acid solution. Although this method is certainly more rapid than the use of urea in acid solution, both methods are objectionable in so far as a little nitrate is inevitably introduced into the solution during the destruction of the nitrite; consequently the interpretation of any subsequent test is rendered uncertain. Moreover, Odekerken omits to mention the need for removing the excess of azide after the destruction of the nitrite. Clearly this is important, as its reaction with nitrite is fairly rapid even in the cold; the excess of azide can be removed by precipitation with silver sulphate. The precipitated silver azide is not dangerous if thoroughly wet, but it should be destroyed chemically. One would hesitate to recommend such a test to inexperienced students.

Nitrite is best destroyed in *neutral* solution by boiling it with an equal volume of 4 M ammonium chloride solution. During the 10 minutes required any loss of nitrate is quite negligible (measurements on the Spekker absorptiometer showed that losses at 0·25 M and at 0·0001 M are not greater than 5 per cent. and may well be less). There is also the further advantage that any excess of ammonium chloride does not interfere with the subsequent test for nitrite, as this test is carried out in the cold.

University students, when using textbook methods for the detection of nitrite or nitrate, or both, often obtained erroneous results, and their attempts to judge relative amounts were generally unsound. The difficulties have been surmounted by the introduction of the tests described above, which have been found to give reliable indications in terms of such rough quantitative categories as large, small or trace.

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PREPARATION OF A MATERIAL RICH IN "CADION" SUITABLE FOR THE DETECTION OF CADMIUM

DWYER'S "cadion,"[1] *p*-nitrodiazoaminoazobenzene ($C_6H_5.N:N.C_6H_4.NH.N:N.C_6H_4.NO_2$), is probably the most satisfactory reagent for the detection of cadmium. Unfortunately, the use of cadion has been limited, because its preparation by Dwyer's procedure[1] or by that of van Nieuwenburg[2] is rather involved. This note describes a simplified method for preparing a satisfactory grade of cadion.

When synthesising cadion from *p*-nitrodiazoaminobenzene ($C_6H_5.NH.N:N.C_6H_4.NO_2$) by van Nieuwenburg's procedure,[2] we observed that crude *p*-nitrodiazoaminobenzene prepared in this laboratory gave a satisfactory spot test for cadmium. The sensitivity to cadmium of this crude *p*-nitrodiazoaminobenzene was essentially the same as that of cadion made by the procedures of Dwyer and van Nieuwenburg. Purification of the crude *p*-nitrodiazoaminobenzene by Dwyer's method[3,4] produced two fractions. One is composed of fairly pure *p*-nitrodiazoaminobenzene; it is inactive with respect to cadmium. The other fraction, consisting of impure cadion, gives an excellent spot test for cadmium. This indicates that cadion is the active principle in the crude *p*-nitrodiazoaminobenzene. Apparently, the high acid content of the preparation medium induces the formation of a large amount of cadion by Earl's reaction.[5,6,7]

PREPARATION OF CRUDE *p*-NITRODIAZOAMINOBENZENE RICH IN CADION—

This synthesis is based upon that of Nölting and Binder.[8] Dissolve 2·8 g of *p*-nitroaniline in 60 ml of diluted hydrochloric acid (1 + 1) by heating the mixture to 70° to 80° C. Cool the solution to room temperature, add 1·9 g of aniline and cool to 0° C. Some *p*-nitroaniline hydrochloride is usually precipitated on cooling, but it dissolves when nitrite solution is added. Stir the cold mixture while slowly adding a solution of 1·4 g of sodium nitrite in 25 ml of ice-water; keep the temperature of the solution below 6° C. Place the mixture in a bath of cold water for approximately 5 minutes. Then add an ice-cold solution of 32 g of anhydrous sodium acetate in 100 ml of water; this causes crude *p*-nitrodiazoaminobenzene that is rich in cadion to precipitate. Filter the cold slurry under suction. Wash the residue with approximately 500 ml of ice-water, and dry it in an oven at 35° to 40° C.

PREPARATION OF CADION SOLUTION—

Dissolve 40 mg of the crude *p*-nitrodiazoaminobenzene that is rich in cadion in 100 ml of 95·6 per cent. w/w alcohol. This solution remains stable for over six months.

SELECTION OF SPOT-TEST PAPER—

Filter-papers differ greatly in their suitability for use in this cadmium test. In general, semi-crepe qualitative papers are superior to other filter-papers; highly retentive quantitative papers are usually unsatisfactory.

PROCEDURE FOR DETECTION OF CADMIUM—

Place a drop of the test solution, which is either neutral or faintly acidic with acetic acid and which is free of interfering substances,[1,2] on a strip of filter-paper. Add a drop of cadion solution to the spot, and then a drop of 2 *M* potassium hydroxide solution. If cadmium is present, the spot becomes pink-orange when the alkali is added. In the absence of cadmium, the added alkali causes the spot to turn red-purple.

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ADSORPTION OF THIAMINE ON GLASSWARE

BIRD[1] has reported that, under certain conditions, there can be considerable adsorption of quinine sulphate from solutions of the sulphate in 0·1 *N* sulphuric acid solution on the glass walls of the containing vessel. This is very serious when the quinine sulphate solution is used as a reference standard in the determination of thiamine, but the phenomenon can be prevented if the glassware is first treated for 1 minute with boiling 30 per cent. sodium hydroxide solution.

When a non-linear graph was obtained for a series of standard thiamine solutions, it seemed probable that, in spite of Bird's claim that thiochrome is not adsorbed, thiamine might be, under circumstances similar to the quinine sulphate adsorption. This has been found to be true.

EXPERIMENTAL

Thirty millilitres of thiamine solution containing 0·1 µg of thiamine hydrochloride per ml were swirled for 20 seconds in each of a number of glass vessels in succession. From the last vessel in the series, three 5-ml aliquots were removed by means of a pipette for the thiamine assay by the thiochrome procedure; 0·1 per cent. potassium ferricyanide solution was used as the oxidising agent and *n*-butyl alcohol as the extractant. The butyl alcohol phase was clarified with ethyl alcohol before the fluorescence was read on a Coleman, Model 12A, photofluorimeter.

The loss of thiamine was expressed as a percentage of the thiamine present in the untreated stock solution.

In Table I results are set out for a number of glass vessels. All, with the exception of the 100-ml calibrated flasks (series 8), were from normal laboratory stocks and had been in general use. The 50-ml Erlenmeyer flasks had served many times to hold the solutions of thiochrome in butyl alcohol immediately before the fluorescence was measured in the final stage of the thiamine assay. The 100-ml calibrated flasks had never been used before. All glassware was used only once in these and in most of the other experiments carried out.

TABLE I

ADSORPTION OF THIAMINE BY GLASSWARE

Series No.	Kind of glassware	No. in Series	Loss of thiamine, %
1	50-ml Erlenmeyer flasks	7	22
2	50-ml ,,	5	34
3	50-ml ,,	5	31
4	50-ml ,,	6	42
5	250-ml calibrated cylinders	5	21
6	500-ml calibrated flasks	5	25
7	25-ml stoppered cylinders	6	44
8	100-ml calibrated flasks	3	34
9	100-ml Erlenmeyer flasks	7	27

That this loss was due largely to adsorption of thiamine on the glass was shown by the recovery of 75 per cent. of it by washing the glassware with alkaline ferricyanide solution.

Thiochrome was not adsorbed either from aqueous solutions or from the mixture of butyl and ethyl alcohols used in making the final reading. On the other hand, fluorescence, shown by treatment with nitric acid and by irradiation in ultra-violet light not to be thiochrome, was picked up from the glass by the mixture of butyl and ethyl alcohols.

Of special interest in these laboratories are assays on yeast extract, in which solutions containing 0·10 to 0·15 g of yeast extract per 100 ml are acidified to pH 1 with concentrated hydrochloric acid and washed by thorough shaking with butyl alcohol to remove interfering fluorescence before the thiamine is assayed. Another series of experiments showed that under these conditions of high acidity thiamine is not adsorbed on glass.

Bird[1] claims that when glassware showing quinine sulphate adsorption is boiled for 1 minute in 30 per cent. sodium hydroxide solution, it can be then used without fear of further adsorption. Solutions rinsed successively through five alkali-treated flasks showed, within an experimental error of ±5 per cent., no loss of thiamine. Further experimental work showed that Bird's procedure prevented adsorption of thiamine, and, in addition, prevented fluorescent material from being removed from the glass by the mixed alcohol solutions.

It was found that the preventive action of the alkali treatment is not permanent, adsorption causing losses of thiamine greater than experimental error after two or three uses. Nevertheless, it must be remembered that the adsorption is exaggerated by swilling the test solutions through six flasks before assaying, and, in addition, that it is almost completely avoided in untreated flasks if strongly acidified solutions are used.

The elution of the fluorescent material from glass could lead to higher results, and the errors involved could be well outside experimental error. This is more serious than the loss of thiamine by adsorption, for although the latter can be prevented in strongly acidic solutions, or recovered by oxidation to thiochrome, pick-up of foreign fluorescence from the glass can only be prevented by previous treatment of the glassware. In the authors' laboratory, acidified solutions are always used, and the glassware is treated with alkali every 3 months.

This work has mainly been on glassware from one maker, *viz.*, Crown Crystal Glass Pty. Ltd., Sydney. We have yet to prove whether the same phenomena would be observed with glassware from other manufacturers, although a few flasks made elsewhere have behaved similarly.

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K. T. H. FARRER
W. C. J. HOLLENBERG

KRAFT FOODS LIMITED
MELBOURNE, AUSTRALIA

May 6th, 1953

THE DETERMINATION OF ACETYLENE IN AIR

IMPROVEMENTS have been made to the method of Purser.[1] A standard solution of acetylene is used as a reference sample for each batch of reagents. Other analytical details have been amended as a result of a long series of experiments.

CALIBRATION—

Slight differences in the technique of preparing the cuprous chloride reagent can produce slightly different shades of colour when the reagent is added to the acetylene solution and this may cause the calibration to vary for each batch of reagent. Various ways of standardising the technique were tried without success and it was found necessary to prepare a reference solution of acetylene for each batch of reagent, as follows.

Fill a small calibrated gas-sampling tube of approximately 10 ml capacity with acetylene. Connect this tube to a bead bubbler, as used in the procedure, containing 40 ml of acetone and cooled in solid carbon dioxide. Absorb the acetylene by drawing air through the tube into the bubbler for a few minutes at a rate of 50 ml per minute. Analyse aliquots from this bubbler by the procedure described. Satisfactory readings are given by 0·5, 1·0, 1·5 and 2·0-ml aliquots of this bubbler solution in a 1-cm cell. A calibration factor can be calculated if the temperature and pressure at the time of filling the sampling tube are known.

PROCEDURE—

Store all glass vessels in a bath of ice-water before use. Keep the pipettes cool by storing them in a glass cylinder cooled in an ice-bath.

Empty the contents of the bubbler into a suitable vessel, such as a 25-ml cylinder, kept in the ice-water bath and quickly add exactly 10 ml of this into a cooled 50-ml cylinder already charged with 10 ml of acetone. Add 20 ml of cooled water, then 2 ml of reagent and mix the whole by means of a glass-rod plunger. Place this cylinder in a water-bath at 20° C for 5 minutes and measure the resulting colour in a Spekker absorptiometer using the appropriate cell (4 or 1-cm) to suit the depth of colour.

If the colour appears to be too deep for the 1-cm cell, quickly add a smaller aliquot by means of a pipette and repeat the determination in another cooled 50-ml cylinder filled with sufficient acetone to bring the volume to 20 ml after the aliquot has been added. When small aliquots are used, allow the end of the pipette to dip beneath the layer of acetone in the 50-ml cylinder.

Arrange to have the reagent added to the correct aliquot of acetylene solution no later than 2 minutes after the removal of the bubbler from the solid carbon dioxide.

The author thanks the Chief Scientist, Ministry of Supply, for permission to publish this note.

REFERENCE

1. Purser, B. J., *Analyst*, 1949, **74**, 237.

MINISTRY OF SUPPLY
CHEMICAL DEFENCE EXPERIMENTAL ESTABLISHMENT
PORTON, WILTS.

B. J. PURSER
May 18th, 1953

A QUALITATIVE TEST FOR MONOSACCHARIDES

WHEN glucose is heated for a few minutes at 100° C in diluted sulphuric acid (3 + 1), a pink colour develops.[1] The colour has been used for the estimation of glucose in blood[2] and of glycogen in tissue.[3] It was recently shown[4] that the colour was due to condensation of 5-hydroxymethyl furfural, a dehydration product of glucose, with the groups of carbon atoms 2 and 3 in the sugar molecule. A more intense pink colour was produced when pure 5-hydroxymethyl furfural was added to the glucose before heating. Differences in the resulting colours produced by other sugars with added 5-hydroxymethyl furfural have made possible a simple procedure for their identification.

METHOD

REAGENTS—

Sulphuric acid—AnalaR grade, sp.gr. 1·840.

5-Hydroxymethyl furfural—Prepare by the method of Haworth and Jones[5] and twice distil at a pressure of 0·1 mm of mercury. Make up the reagent to contain 1 mg per ml in distilled water.

PROCEDURE—

After showing by the conventional colour tests that pentoses, polysaccharides and keto-hexoses are absent, distinguish the three aldohexoses as follows.

Place 1 ml of 5-hydroxymethyl furfural solution in a 6-inch × ¾-inch test tube by means of a pipette and add 1 ml of the sugar solution. The concentration of the sugar should be within the range 0·2 to 4·0 mg per ml. Place the tube in a basin of running cold water or, better, in ice-water. Add about 2 ml of sulphuric acid from a burette with constant swirling, and continue to swirl the tube until it has cooled. Add a further 4 ml of acid, swirling the tube in the cold bath all the time.

Examine the solution immediately the operation is complete. If it is coloured pink, the sugar is mannose. If it is colourless, immerse the tube for about 3 minutes in a bath of boiling water and then cool. If the solution is coloured pink, the sugar is glucose, if brown, it is galactose.

OTHER SUGARS—

Fructose and sorbose exhibit a pale yellow colour immediately the sulphuric acid is added. On heating as above, fructose becomes yellow-pink and sorbose becomes brown.

Both the pentoses, ribose and lyxose give pink colours in the cold, which are visible at concentrations above 0·3 and 0·2 mg per ml, respectively, and which deepen on standing. Xylose shows no colour immediately after the acid is added, apart from a slight darkening at concentrations above 5 mg per ml; after 30 minutes at room temperature, a slight pink colour develops. Arabinose shows no colour at all, even after heating.

Oligosaccharides and polysaccharides behave like mixtures of the individual sugars, so the reaction is unsatisfactory for their characterisation.

Sorbitol and mannitol produce a slight pinkish colour and galacturonic acid becomes deep pink on heating; gluconic acid and glucosamine show no colour at all, apart from the slight colour of the 5-hydroxymethyl furfural. Rhamnose becomes greenish-brown on heating.

Proteins interfere with the reaction, but if trichloroacetic acid is added, the protein-free filtrate reacts satisfactorily.

CONCLUSIONS—

The reaction is useful when only small amounts of hexose are available, *e.g.*, in eluates from chromatograms. Much less sugar is required than in the osazone test, and mannose can be distinguished from glucose. The colours produced by pentoses can often be used as confirmatory tests, although alone they are insufficiently specific to identify the sugars with certainty.

The work described in this paper was carried out as part of the programme of the Food Investigation Organisation of the Department of Scientific and Industrial Research.

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3. Van der Kleij, B. J., *Biochim. Biophys. Acta*, 1951, **7**, 481.
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DEPARTMENT OF SCIENTIFIC AND
INDUSTRIAL RESEARCH (FOOD INVESTIGATION)
TORRY RESEARCH STATION
ABERDEEN

R. M. LOVE
June 15th, 1953

THE DETERMINATION OF ZINC BY TITRATION WITH DI-SODIUM ETHYLENEDIAMINETETRA-ACETATE

A RECENT paper[1] describes the amperometric titration of small amounts of zinc with di-sodium ethylenediaminetetra-acetate after appropriate removal or masking of interfering cations.

The object of the present note is to draw attention to the fact that the same micro-scale titration can be carried out with at least the same accuracy, and much more simply and expeditiously, to a visual end-point by the method of Biedermann and Schwarzenbach,[2] who used the dyestuff Colour Index No. 203 (Society of Dyers and Colourists), obtainable commercially as Solochrome Black WDFA (I.C.I. Ltd.), as indicator. Biedermann and Schwarzenbach showed that the end-point with zinc is particularly sharp, being better than with magnesium and calcium.

Experiments in this laboratory have indicated, for example, that 0·2 mg of zinc ions in a volume of 10 to 15 ml can be titrated with 0·01 *M* di-sodium ethylenediaminetetra-acetate in presence of an ammonium hydroxide and ammonium chloride buffer solution, by means of a 0·5-ml micrometer syringe burette, to an accuracy of within ±2 µg. As Pickles and Washbrook claim, the presence of calcium can be masked completely by the addition of sodium fluoride.

REFERENCES

1. Pickles, D., and Washbrook, C. C., *Analyst*, 1953, **78**, 304.
2. Biedermann, W., and Schwarzenbach, G., *Chimia*, 1948, **2**, 56.

DIVISION ANALYTICAL DEPARTMENT
IMPERIAL CHEMICAL INDUSTRIES LIMITED
DYESTUFFS DIVISION
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N. STRAFFORD
June 8th, 1953

Apparatus

A MODIFIED ALL-GLASS APPARATUS FOR THE DETERMINATION OF NITROGEN BY THE MICRO-KJELDAHL METHOD

KJELDAHL'S[1] original apparatus for the determination of total nitrogen in body fluids, tissues and excretions, has undergone many changes since its inception in 1883. These modifications have been largely concerned with devising an efficient method for the distillation of the ammonia. Of the various distillation methods, Pregl's steam distillation technique is undoubtedly the safest and smoothest, and the most reliable, especially when considerable amounts of concentrated sulphuric acid have to be used for the digestion process.

In 1942, Markham[2] introduced an all-glass steam distillation apparatus for the distillation of volatile fatty acids. It has also proved successful in the determination of nitrogen by the micro-Kjeldahl method. The apparatus, although different in some structural details from Pregl's, is basically similar in its method of distillation and of generating the steam. As the apparatus is essentially for steam distillation, the digestion process is carried out in a separate Kjeldahl flask or tube. The digest is then transferred and washed into the distillation apparatus, as completely as possible, with distilled water through a cup-shaped container that is also used for the sodium hydroxide. When similar determinations are made with the new apparatus, this procedure is unnecessary. As a result, no material is lost through transference and the amount of liquid in the Kjeldahl flask is reduced to a minimum (approximately 25 ml) before distillation of the ammonia.

EXPERIMENTAL—

Preliminary chemical tests and trials indicated that superheated steam, which is "on tap" in most clinical laboratories, might be used for this purpose. More extensive experiments proved that there was no difference between the superheated steam "blank," and the blank value obtained by the more orthodox procedure. Although an iron steam-pipe corrodes to a slight extent, it was found that any iron contaminating the steam does not interfere with the Kjeldahl determinations.

There are many advantages in using steam under pressure, provided it is clean and free from chemical impurities. It accelerates the rate of distillation, aids in the decomposition of amino or ammino-complexes that may be formed, requires no preparation (the conventional method does) and occupies little space. Moreover, it is much safer, easier to control and manipulate, requires little or no attention, and is always available for use. It is possible, moreover, to make any number of determinations simultaneously, the only limiting factor being the ability of the individual to regulate the rate of distillation, which is controlled by a steam-release valve connected to the main steam-pipe.

The condensed water in the superheated steam is trapped in a wide-mouthed bolt-head flask that is also used as a steam reservoir. Thus only dry steam, *i.e.*, steam unmixed with fine drops of water, is used for the distillation process. A simple siphoning system fitted to the bolt-head flask keeps the flask free from water. The incoming steam produces the necessary force to automatically siphon off any water that otherwise collects in the flask. The steam from the steam reservoir can be cut off from the distillation apparatus by a Pyrex-glass tap as necessary. This device is especially useful when distillation is finished, as it eliminates any interference with the main steam supply. It also helps to maintain a constant head of pressure, and so minimises slight fluctuations in steam pressure.

APPARATUS—

The apparatus is made of hard glass and is constructed in four sections (A, B, C and D in Fig. 1). Each section is detachable and can be easily replaced or repaired.

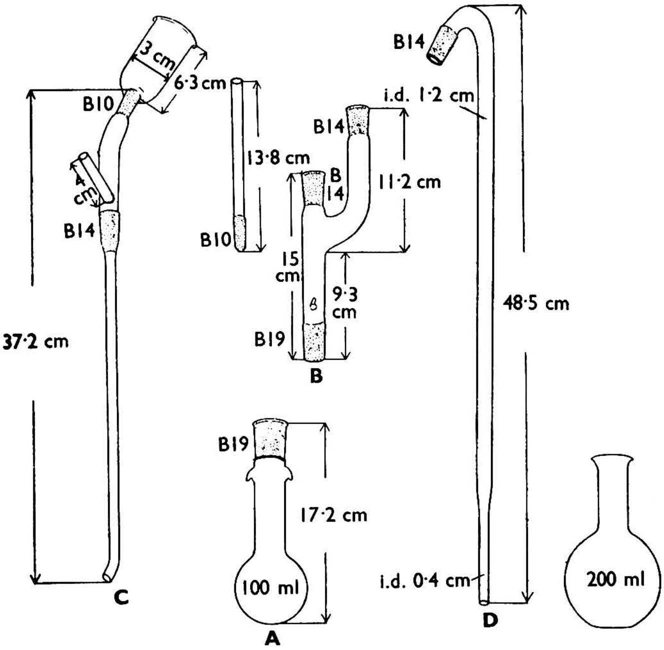

Fig. 1. View of individual section of apparatus. A, 100-ml Kjeldahl flask; B, "Claisen-like" still head; C, reservoir and steam inlet tube; D, air condenser and flask

For the distillation a 100-ml Kjeldahl flask, A, the neck of which carries a B19 standard joint, is attached to a B19 cone on the "Claisen-like" still head, the main arm of which is used to support the whole apparatus. This arm is clamped at an angle, as shown in Fig. 2, to prevent the strongly alkaline solution in the flask from being splashed into the entrance of the air condenser, and so being blown over mechanically into the condenser. This design allows the flask to be taken off or attached quite freely, without interference to any other part of the apparatus. The Kjeldahl flask is firmly held in position by a spring clip, which attaches it by means of hooks to the still-head.

The "Claisen-like" still-head, B, besides being used as an attachment for the Kjeldahl flask, the air condenser by a B14 standard joint, and the reservoir and steam inlet tube, also serves as an efficient splash-head. Any spray is trapped in the side-arm of the still-head and returned as a steady stream of liquid to the Kjeldahl flask. It also offers the minimum obstruction to the flow of the steam and does not trap pools of distillate.

Into the B14 socket of the main arm of the "Claisen-like" still-head a long glass tube, C, reaching to the bottom of the Kjeldahl flask, is fitted by a B14 cone. Above the B14 cone this glass tube branches off to a steam delivery tube and a cup-shaped reservoir for the 40 per cent. sodium hydroxide solution. The cup-shaped reservoir is plugged by a closed B10 standard cone.

A long air condenser, D, made of relatively wide bore glass tubing, has its lower end drawn out to the thickness of an ordinary glass-tubing, internal diameter 4 mm, and to approximately 9 cm long. It dips under the standard acid and is constructed to prevent any disastrous suck-back.

Recovery experiments proved that it is unnecessary to use a water condenser, as the air condenser, provided it is long enough to dip below the surface of the acid, is just as efficient. The air condenser is in many ways preferable, since it considerably simplifies the apparatus and prevents the steam from condensing on the sides of the condenser. For the collection of the ammonia, a 200-ml flask is used, which stands on a wooden block 6 inches high and 4 inches wide. A half-inch groove in this wooden block accommodates the flask and holds it firmly in position. This facilitates the easy withdrawal of the flask, which is necessary for subsequent manipulations.

In twenty-four different determinations on solutions of specially purified ammonium sulphate containing 4·71 mg per 5 ml (1 mg of nitrogen per 5 ml) a recovery of 99 to 100 per cent. was obtained in 2 minutes. For use with the micro-method, 10 ml of N/70 sulphuric acid was back-titrated with N/70 sodium hydroxide solution, a mixed indicator being used (3 parts 0·1 per cent. of bromocresol green in alcohol, and 1 part 0·2 per cent. of methyl red in alcohol). This covers the range of 0 to 2 mg of nitrogen.

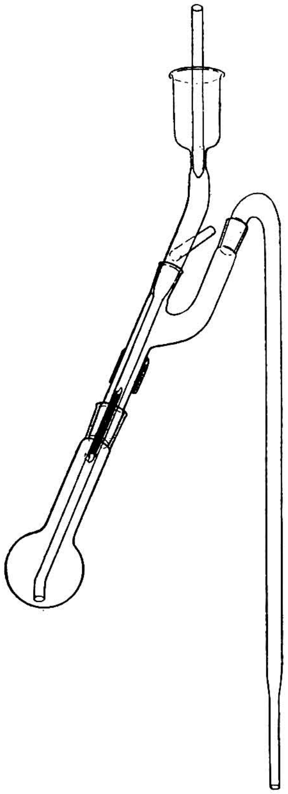

Fig. 2. View of assembly of apparatus

ADVANTAGES AND OTHER USES—

No rubber connections, which are always a potential source of trouble and error, are used in its assembly, and it can be mounted on a single retort stand requiring only one clamp to keep it in position at an angle of approximately 20° to the air condenser.

The digestion and distillation processes are done in one Kjeldahl flask, which is always much safer than the transfer of the digest to another distillation flask, especially when small quantities of nitrogen are to be determined. This also saves time and effort.

The apparatus can also be used for the purification of many water-insoluble compounds, both solid and liquid, provided that the compound is volatile and the impurities non-volatile. Further, it can be used for the separation and determination of volatile poisons and for allied procedures. It could also be used, without making any structural alterations to the apparatus, for the determination of nitrogen by the macro-Kjeldahl method by substituting a much larger flask (not necessarily a Kjeldahl flask) carrying a B19 standard joint, in the digestion and distillation procedures.

I wish to express my thanks to Mr. D. MacDonald whose glass-blowing skill and co-operation made possible the new apparatus.

The apparatus can now be obtained from Messrs. Quickfit & Quartz Ltd.

REFERENCES

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2. Markham, R., *Biochem. J.*, 1942, **36**, 790.

DEPARTMENT OF CLINICAL CHEMISTRY
UNIVERSITY OF EDINBURGH
ROYAL INFIRMARY, EDINBURGH

F. J. SCANDRETT
June 24th, 1953

Ministry of Food

STATUTORY INSTRUMENTS*

1953—No. 1609. The Public Health (Condensed Milk) (Amendment) Regulations, 1953. Price 2d.

These Regulations, which came into operation on November 11*th,* 1953, *amend the Public Health (Condensed Milk) Regulations,* 1923 (*S.R. & O.*, 1923, *No.* 509; *Analyst,* 1923, **48**, 272), *as amended, by permitting for a limited period the sale of full cream unsweetened condensed milk, imported by the Minister of Food, containing not less than* 7·8 *per cent. of milk-fat and not less than* 25·5 *per cent. of all milk-solids including fat.*

1953—No. 1610. The Public Health (Preservatives etc. in Food) (Amendment) Regulations, 1953. Price 2d.

These Regulations, which came into operation on November 11*th,* 1953, *amend the Public Health (Preservatives etc. in Food) Regulations,* 1925 (*S.R. & O.*, 1925, *No.* 775; *Analyst,* 1925, **50**, 455), *as amended. They—*

(*i*) *Provide for the sale and importation of certain specified dehydrated vegetables that contain sulphur dioxide not exceeding prescribed limits, as follows—*

Food	Preservative	Parts per million
Dehydrated Vegetables:		
(*a*) Potatoes	Sulphur dioxide	550
(*b*) Runner beans, turnips, spinach, swedes, peas, parsnips, carrots, mixed vegetables.	Sulphur dioxide	2,000
(*c*) Cabbage	Sulphur dioxide	3,000

(*ii*) *Increase the maximum permitted sulphur dioxide content of jam from* 40 *to* 100 *parts per million;*

(*iii*) *Permit the presence of diphenyl in citrus fruit imported in wrappers treated with diphenyl; and*

(*iv*) *Permit, for a limited period, the addition of borax to margarine, the amount of boron preservative (expressed as boric acid) not to exceed* 2,500 *parts per million.*

1953—No. 1618. The Public Health (Condensed Milk) (Scotland) Amendment Regulations, 1953. Price 2d.

These Regulations, which came into operation on November 16*th,* 1953, *amend the Public Health (Condensed Milk) Regulations (Scotland),* 1931, *as amended, in the same manner as S.I.,* 1953, *No.* 1609 (*see above*) *amends the corresponding Regulation for England.*

CIRCULAR MF 20/53

This circular (price 2*d.*), *dated November* 11*th,* 1953, *draws attention to S.I.,* 1953, *Nos.* 1609 *and* 1610 (*see above*). *It states that these amending Regulations re-enact substantially the provisions of Regulation* 60 CAA *of the Defence (General) Regulations, which will be allowed to lapse on December* 10*th,* 1953, *unless revoked at an earlier date. The provision whereby meat may contain sulphur dioxide is no longer necessary and is not re-enacted.*

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

British Standards Institution

DRAFT SPECIFICATIONS

A FEW copies of the following draft specifications, issued for comment only, are available to members of the Society, and can be obtained from the Secretary, Society of Public Analysts and Other Analytical Chemists, 7–8, Idol Lane, London, E.C.3.

Draft Specification prepared by Sub-Committee LBC/1/1—Dean and Stark and Ubbelohde Apparatus.

CR(LBC)6796—Draft B.S. for Ubbelohde Apparatus for Flow and Drop Points. (Revision of B.S. 894.)

Draft Specification prepared by Technical Committee LBC/12—Dairy Apparatus.

CR(LBC)6270—Draft B.S. for Centrifuge Tubes and Sedimentation Vessels for the Determination of Visible Dirt in Milk. (Revision of B.S. 736.)

Book Reviews

INORGANIC THERMOGRAVIMETRIC ANALYSIS. By CLÉMENT DUVAL. Pp. xvi + 531. Amsterdam, London and New York: Elsevier Publishing Co. Ltd. London: Cleaver-Hume Press Ltd. 1953. Price 60s.

The many papers on this subject published by Professor Duval and his collaborators over the last five years in *Analytica Chimica Acta* have now been collected in this single volume. It is noted in the preface that "this preliminary work" has so stimulated the interest of analysts that nearly two thousand requests for reprints of the papers have been received. One must indeed admire the industry that has gone to the recording of such a vast amount of potentially useful data comprising as it does "the pyrolysis curves of 967 precipitates proposed up to July, 1951, for inorganic gravimetric analysis."

For any who may be unfamiliar with this work it should be explained that a "pyrolysis curve" records the constancy, loss or gain in weight of a substance during a heating cycle in a furnace, the crucible containing the substance under observation being permanently attached to a continuously recording sensitive balance. For these studies, Professor Duval has used the Chevenard thermobalance, of which a detailed description is given in Chapter 2 of the book; a short historical review of thermobalances is given in the first chapter.

Chapter 3 deals with "Applications of the Thermobalance," Chapter 4 with "Automatic Gravimetric Analysis" and, save for a one-page "Postscript," the remainder of the book is given over to "The Thermogravimetric Investigation of Analytical Precipitates." In this, the main section, are recorded data on compounds of 62 elements, from lithium to uranium. The pattern is similar throughout—a number (sometimes as many as 25) of precipitants for the element in question are listed; the conditions of each precipitation, or the appropriate references, are given; the pyrolysis curves are reproduced; a tabular summary is given showing the precipitating reagent, form weighed and temperature limits; and finally, there is a list of references.

This specific information is potentially of very great value, and the techniques by which it has been obtained are clearly elegant, but it is doubtful if the author is justified in presenting these pyrolysis curves as absolute data. The impression is given that the weight of a precipitate is solely a function of temperature; the possible influence of rate of increase of temperature (an arbitrary figure governed by the gearing of the rheostat) and of the many other variables appear to be ignored. In the categorical acceptance or rejection of a particular precipitant, the thermogravimetric data are interpreted on the assumption that the thermobalance can and will be used. For normal methods of ignition and weighing, other factors, not disclosed by the thermogravimetric studies, must be taken into account.

That Professor Duval has not done this derives no doubt from his expressed opinions of analytical practice. He contends that in most analytical laboratories precipitates are heated for too long and at too high a temperature; this is probably true, but does he seriously believe that competent inorganic analysts have not improved on the techniques of Berzelius and Rose and on those of Winkler and Treadwell, singled out for criticism on p. 41? It is unfortunate that the author has sought, unnecessarily, to justify his work by arguments that are invalid because they are out of date.

On the interpretation of the pyrolysis curves, the reviewer would have found some of the deductions more convincing had they been backed by complementary information from X-ray diffraction studies of the pyrolysis products. This view is expressed in the knowledge that some of the deductions are at complete variance with established X-ray data. For example, in the table on p. 116, minimum ignition temperatures are given for the precipitates obtained by treating solutions of aluminium with 24 different precipitants. Only two of these temperatures are higher than 950° C, and one is as low as 280° C. The most recent studies show that when hydrated alumina is heated to a temperature of 400° C, the alumina is wholly in the form of the alpha or beta monohydrate, while the gamma anhydrous form, which is strongly hygroscopic, is likely to persist up to at least 950° C. The pyrolysis curve for hydrargillite (a trihydrate) on p. 117 is in such disagreement with published verifiable crystallographic data as to raise some doubts as to the exact meaning of these curves. Again, although the curve for the zinc - oxine complex is in reasonable agreement with the reviewer's published X-ray studies, that for the magnesium complex is not. It may be that the explanation lies in the author's own comment that "the form of curve obtained with this substance depends very much on the method of precipitation and on the rate of heating."

In the tabular summaries at the end of each chapter, the methods suitable for the automatic determination of the element concerned are indicated by means of an asterisk placed before the precipitating reagent. It is this concept of "automatic gravimetric analysis" and the philosophy behind it that calls for most comment. Techniques for single substances and for binary mixtures are described in Chapter 4. In the first, the weight of the precipitate is obtained by subsequent linear measurement of the height of the "horizontal" (the measuring scale is graduated in half millimetres) and by comparison with a calibrated "horizontal," say from a 50-mg weight. It is frankly difficult to see in what way this becomes usefully more automatic than by a normal weighing on the latest type of rider-loaded air-damped balance.

A mixture of calcium and magnesium oxalates is used to illustrate the application to binary mixtures. Suffice it to say that by recording the levels at 500° C and 900° C and comparing the heights above the calibration line, "two first degree equations can be set up with the unknowns x and y as the weights of calcium and magnesium and with m and n as the known weights of the mixtures present at 500° C and 900° C"!

So much for the technique, but is it properly named? Surely, it is only the ignition and weighing that can be considered automatic. In gravimetric analysis, the major and most difficult part comprises the separations that must precede the final determination. It is not sufficiently realised, even now, that in applied analysis those operations that make the determination possible are often more important than those that attend the determination itself.

As to the philosophy: as before, Professor Duval uses arguments to justify the techniques that suggest that he is out of touch both with the methods and the problems of the industrial inorganic analyst.

It would seem that we have only two choices open to us: to pay for a thermobalance plus the salary of "a non-specialised laboratory technician who is capable of changing the substance to be determined every 15 minutes" or "to pay the emoluments of a graduate chemist who with inexpensive equipment carries out evaporations, separations, precipitations, filtrations through paper, ignitions of paper, who heats crucibles at ill-defined temperatures for indeterminate periods of time which are generally too long, who weighs, re-heats, re-weighs and risks making mistakes in the weight, and who, having done all this, has no evidence of any kind as to whether in the event of the determination being incorrect the mistake has been voluntary or involuntary."

It seems odd that in the next (and shorter) sentence the author should feel able to declare that "the role of the modern graduate chemist is to create and not to perform throughout his whole career the same analytical operations more or less with distaste" and, it is pertinent to ask if the ability to carry out the simpler and repetitive operations of analytical chemistry really well is a handicap in later essays in creation or, to use a popular word, research.

Fortunately, these misapprehensions will not deter the discerning reader from looking for the real merit in the author's work. The extent and nature of the reviewer's comments and reservations are not intended to detract from the large amount of potentially valuable data, which should be of interest to all analysts. It is to be hoped that Professor Duval will extend his work to a more fundamental and detailed study of the promising compounds he has examined in this preliminary survey.

It remains to add that the translation by Mr. N. G. Bissett is well done, only the word "docimasy" seems out of context, and that the book is extremely well produced.

R. C. CHIRNSIDE

POLYSACCHARIDE CHEMISTRY. By R. L. WHISTLER and C. L. SMART. Pp. xvi + 493. New York: Academic Press Inc. 1953. Price $10.80; 86s. 6d.

Until relatively recently a book under the present title would have dealt almost exclusively with cellulose, starch and pectins. Current investigations in carbohydrate chemistry have greatly extended our knowledge of polymolecular structures based on sugars and allied substances. We now know that polysaccharides constitute approximately three-quarters of the dry weight of the higher plants and sea weeds. They form the basis of the structural material of plants, the exoskeleton of insects and crustaceans and the protective sheaths and capsular material of bacteria; they contribute largely to fuel reserves stored as cell inclusions; and they protect surfaces against desiccation and they seal wounds.

Plant tissue is a matrix of giant polysaccharide molecules, sometimes parallel to each other as in rigid structural tissue and sometimes weaving and entangling together as in thickening material, the whole fabric being permeated and held together by many other substances, including lipids, proteins, salts and colouring matter. This close association, with many secondary valence links, makes the separation and purification of molecules of one type very difficult.

Glycogen used to be considered almost the sole animal polysaccharide, but hyaluronic acid is important as intercellular cement and chitin as a structural support for lower animals. Specific polysaccharides play a significant part in synovial and other body fluids, contribute to blood group specificity and immunological reactions, and have protective and lubricative functions on mucous surfaces.

The first two chapters of the present volume deal with occurrence, nomenclature and classification and with methods used in general characterisation and proof of structure. Then follow 33 chapters each dealing with a particular type of polysaccharide. Cellulose, starch and pectins each occupy about 10 per cent. of the space, an amount that is admitted to be insufficient to cover the whole chemistry of such important substances, but adequate for the particular purposes of this book. A complete literature survey is claimed for all the other polysaccharides, and the only omission that the reviewer has noticed is under lignin, where no indication is given of its nature or even if it is a polysaccharide.

The book is a valuable compendium on the occurrence, structure and properties of the lesser-known polysaccharides. Their analytical chemistry is not in a very advanced state, so that the few methods of analysis given are largely of academic interest. The information should stimulate analysts to devise methods for the determination of these important substances.

J. R. NICHOLLS

Publications Received

THE IDENTIFICATION OF TEXTILE FIBRES. QUALITATIVE AND QUANTITATIVE ANALYSIS OF FIBRE BLENDS. By BRUNO LUNIAK. Pp. xiv + 177. London: Sir Isaac Pitman & Sons Ltd. 1953. Price 45s.

STANDARD X-RAY DIFFRACTION POWDER PATTERNS. Volume I. National Bureau of Standards Circular 539. By HOWARD E. SWANSON and ELEANOR TATGE. Pp. ii + 95. Washington: U.S. Government Printing Office. 1953. Price 45 cents.

STANDARD X-RAY DIFFRACTION POWDER PATTERNS. Volume II. National Bureau of Standards Circular 539. By HOWARD E. SWANSON and RUTH K. FUYAT. Pp. ii + 65. Washington: U.S. Government Printing Office. 1953. Price 45 cents.

MISES AU POINT DE CHIMIE ANALYTIQUE PURE ET APPLIQUÉE ET D'ANALYSE BROMATOLOGIQUE. Publiées sous la direction de J.-A. GAUTIER. Pp. iv + 172. Paris: Masson et Cie. 1953. Price 1400 fr.

ION EXCHANGE RESINS. Pp. 36. Poole, Dorset: The British Drug Houses Ltd. 1953. Gratis.

THE CHEMICAL INDUSTRY: PAST AND PRESENT. By T. I. WILLIAMS, M.A., B.Sc., D.Phil., F.R.I.C. London: Penguin Books. 1953. Price 2s.

CHEMISTRY OF THE LANTHANONS. By R. C. VICKERY, D.Sc., Ph.D. Pp. viii + 296. London: Butterworths Scientific Publications. 1953. Price 35s.

SOAP MANUFACTURE. Volume I. By J. DAVIDSOHN, E. J. BETTER and A. DAVIDSOHN. Pp. xii + 525. London and New York: Interscience Publishers Inc. 1953. Price $12.50; 95s.

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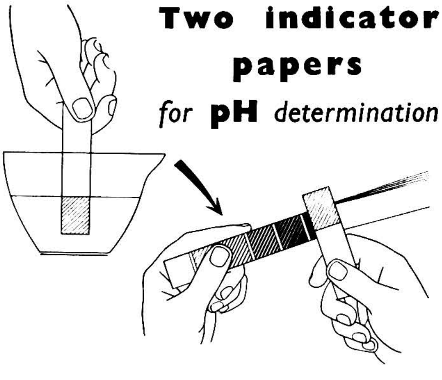

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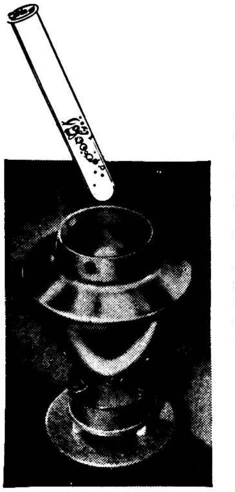

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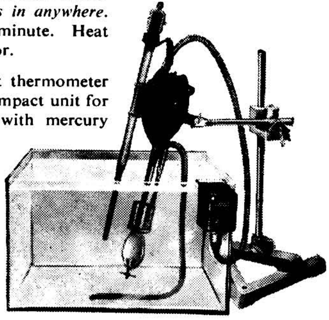

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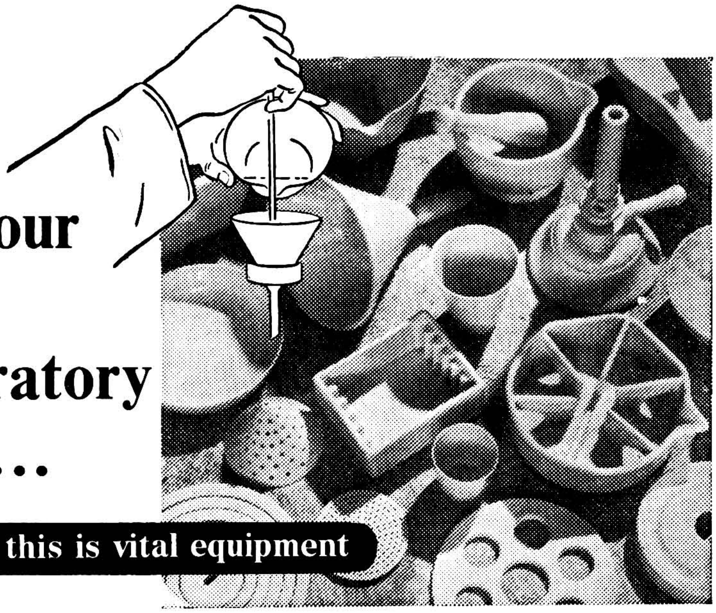

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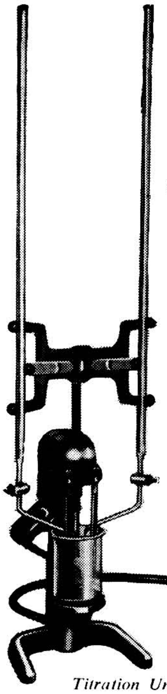

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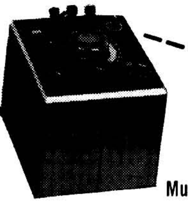

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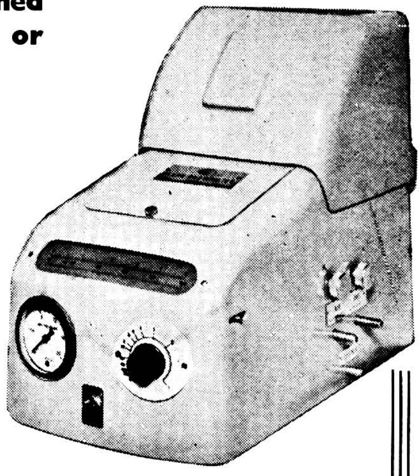

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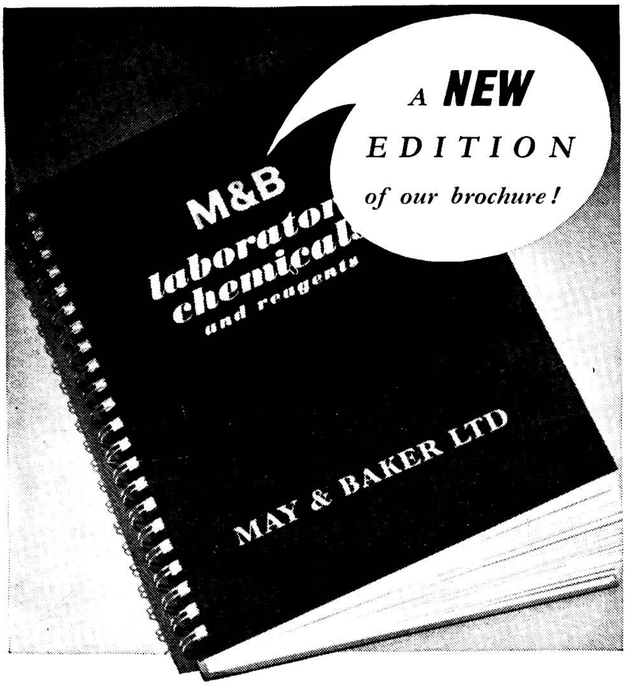

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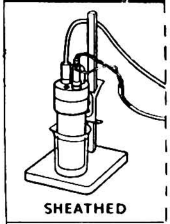

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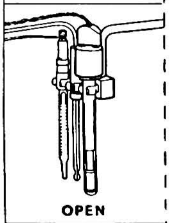

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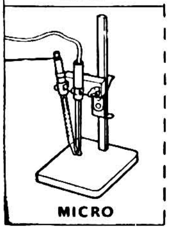

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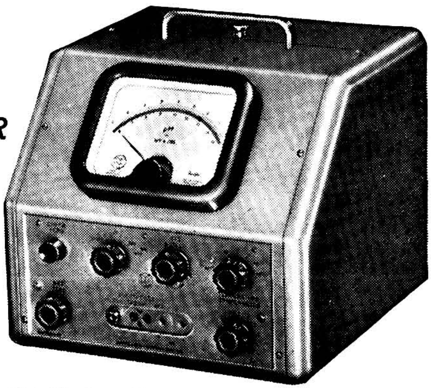

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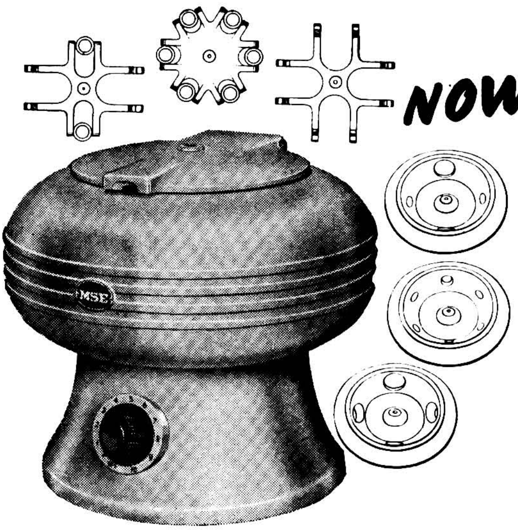

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REF: *1. Higgins M., Monthly Bull. Min. of Health & Pub. Health Lab. Service. Feb. 1950 p. 49.*
2. Higgins M. & Hobbs B. ibid p.38.

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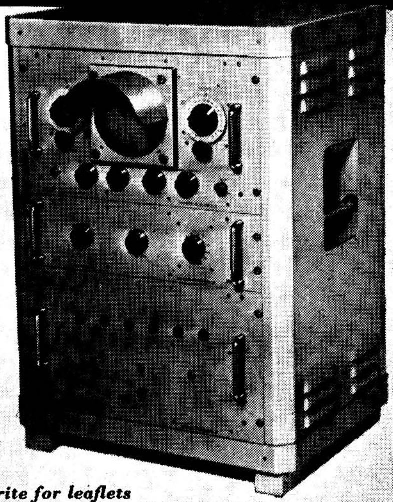

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No. 14 October, 1953

THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

BULLETIN

FORTHCOMING MEETINGS

Ordinary Meeting of the Society, October 23rd, 1953

An Ordinary Meeting of the Society will be held in the Lecture Hall of **Southampton** University on Friday, October 23rd, 1953, at 5 p.m., and will be preceded by a visit to the Esso Refinery at Fawley. This meeting has been organised by the Physical Methods Group.

The subject of the meeting will be "Paper Electrophoresis," and the following papers will be presented—

"The Analysis of Inorganic Compounds by Electromigration and Electrochromatography," by F. H. Pollard, B.Sc., Ph.D.

"The Use of Paper Electrophoresis in the Study of Nucleic Acids," by Roy Markham, M.A., Ph.D.

"Paper-Strip Electrophoresis of Serum Proteins," by A. L. Latner, M.Sc., M.D., F.R.I.C.

Ordinary Meeting of the Society, November 4th, 1953

An Ordinary Meeting of the Society will be held at 7 p.m. on Wednesday, November 4th, 1953, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

The subject of the meeting will be "The Determination of Niobium in Minerals and Mineral Dressing Products," and the following papers will be presented—

"The Absorptiometric Determination of Niobium in Some African Low-grade Minerals and Mineral Dressing Products," by G. W. C. Milner, B.Sc., F.R.I.C., A.Inst.P., and A. A. Smales, B.Sc., F.R.I.C. (A.E.R.E., Harwell).

"The Absorptiometric Determination of Niobium in Some African Low-grade Ores," by A. E. O. Marzys (Uganda Development Corporation, Ltd.).

"Spectrographic Determination of Niobium and Tantalum in Sukulu-type Soils," by C. S. Campbell, M.A., and D. Nicholas (Fulmer Research Institute, Ltd.).

"Inorganic Chromatography on Cellulose. Part XIV. A Shortened Method for the Determination of Niobium and Tantalum in Minerals and Ores," by R. A. Mercer and R. A. Wells, B.Sc., A.R.I.C. (Chemical Research Laboratory, Teddington).

"The Colorimetric Estimation of Niobium and Tantalum with Pyrogallol," by E. C. Hunt, B.Sc., A.R.I.C., and R. A. Wells, B.Sc., A.R.I.C. (Chemical Research Laboratory, Teddington).

"Inorganic Chromatography on Cellulose. Part XV. A Rapid Method for the Determination of Niobium in Low-grade Ores," by E. C. Hunt, B.S., A.R.I.C. (Chemical Research Laboratory, Teddington).

Annual General Meeting of the Society, March 3rd, 1954

THE Annual General Meeting of the Society will be held at 4.30 p.m. on **Wednesday**, March 3rd, 1954,* in the Meeting Room of the Royal Society, Burlington House, London, W.1.

This will be followed at 5 p.m. by the Bernard Dyer Memorial Lecture given by E. B. Hughes, D.Sc., F.R.I.C.

Meeting of the North of England Section, November 28th, 1953

AN Ordinary Meeting of the North of England Section will be held at 2 p.m. on Saturday, November 28th, 1953, at the City Laboratories, Liverpool.

At this meeting a paper on "Chromatography" will be presented by Dr. R. L. M. Synge, F.R.S.

Meeting of the Scottish Section, November 10th, 1953

AN Ordinary Meeting of the Scottish Section will be held at 7.15 p.m. on Tuesday, November 10th, 1953, at the Central Station Hotel, Glasgow.

The following papers will be presented—

"Rapid Determination of Glycerol in Fermentation Solutions: a New Chromatographic Procedure," by A. F. Williams, B.Sc., F.R.I.C.

"Field Analysis in Connection with Water Treatment Problems," by I. A. Heald.

Annual General Meeting of the Physical Methods Group, November 24th, 1953

THE Ninth Annual General Meeting of the Physical Methods Group will be held at 6 p.m. on Tuesday, November 24th, 1953, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

The Business Meeting will be followed by a Lecture by the retiring Chairman, J. Haslam, D.Sc., F.R.I.C.

PAPERS ACCEPTED FOR PUBLICATION IN *THE ANALYST*

THE following papers have been accepted for publication in *The Analyst*, and are expected to appear in the near future. It is not possible to enter into correspondence about any of them.

"A Simple Volumetric Method for the Routine Determination of Glycerol," by J. W. B. Erskine, C. R. N. Strouts, G. Walley and W. Lazarus.

> Glycerol is oxidised at room temperature by a solution of sodium metaperiodate to give formaldehyde and formic acid. The formic acid, after the excess of periodate has been destroyed with ethylene glycol, is titrated with standard alkali, with phenol red as indicator, to give a measure of the glycerol present. Carbon dioxide must be excluded, and the oxidation must be carried out in the dark to minimise side reactions between sodium metaperiodate and the formaldehyde and formic acid. A number of other polyhydric alcohols and sugars interfere, but these are not likely to be present in the glycerol-containing products of soap-making and fat-splitting, such as crude glycerine, lye and soap. The most likely organic impurities in these products are trimethylene glycol, which does not react, and polyglycerols, which do not yield formic acid; these substances, therefore, do not interfere as they do in the acetin and dichromate methods. Glycols with adjacent hydroxyl groups, like ethylene glycol, react, but produce only formaldehyde.
>
> The method has been checked against distilled glycerine, made-up soap-lye crude glycerine, soap lyes and soaps containing known added amounts of glycerol, and also on commercial crudes, soap lyes and soaps by comparison with the acetin and dichromate methods.

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* The date of this meeting has been altered since the Programme of Meetings for the Session was printed.

"The Determination of *iso*Butyraldehyde in *n*-Butyraldehyde," by G. R. Primavesi.

*iso*Butyraldehyde and *n*-butyraldehyde are reduced to the corresponding alcohols by sodium borohydride. The *iso*butanol is then estimated absorptiometrically by means of a modified Komarowsky reaction for higher alcohols, of improved specificity, carried out under controlled conditions.

"The Quantitative Separation of Copper, Lead and Tin by Cathodic Deposition," by G. H. Aylward and A. Bryson.

A method is described for the quantitative separation of copper, lead and tin by cathodic deposition from phosphoric acid solution. The tin forms an anionic complex that is not reduced at the cathode and the copper is separated from the lead by deposition at a controlled potential. The lead is deposited in a metallic form and the tin is determined volumetrically. The difficulties associated with the quantitative cathodic deposition of lead have been investigated and optimum working conditions have been established to avoid these difficulties.

"Diethylammonium Diethyldithiocarbamate for the Separation and Determination of Small Amounts of Metals," by P. F. Wyatt.

A scheme for the successive separation and absorptiometric determination of copper, manganese and iron is described, whereby iron is isolated as cupferrate, and copper and manganese separately as diethyldithiocarbamates. Methods are described for the absorptiometric determination of all three elements as their coloured diethyldithiocarbamate complexes in chloroform, and alternative absorptiometric procedures are indicated.

Tests on random mixtures of the three metals over the ranges 20 to 1000 μg of iron, 5 to 70 μg of copper and 10 to 200 μg of manganese show good recoveries.

"The Photometric Determination of Phosphorus in Copper-Based Alloys Containing Tin," by H. K. Lutwak.

The method of the A.S.T.M. (E62–50T) for the determination of phosphorus in copper-based alloys has several shortcomings. An investigation has been made to obviate them and to simplify the method.

Calibration graphs that obeyed Beer's law were obtained by (*a*) determining the sensitivity of the phosphovanadomolybdate complex to acidity and dilution changes and choosing the most favourable conditions, and (*b*) increasing the rate of colour development by heating the coloured solution before dilution. Colour intensities that reach their final value at room temperature are stable; colours obtained on heating fade slowly on standing.

Separation of metastannic acid is prevented by the use of a suitable mixture and amount of nitric and hydrochloric acids in the final solution. The conditions chosen also provide optimum acidity for colour development.

The same procedure is recommended for development of calibration graphs for alloys of both low and high phosphorus contents.

"The Indirect Polarographic Determination of Calcium by Chloranilic Acid," by B. Breyer and J. McPhillips.

Calcium reacts quantitatively with chloranilic acid, precipitating calcium chloranilate. The concentration of the calcium can be determined, without removing the precipitate, by measuring the polarographic diffusion current of the residual chloranilic acid and referring the value obtained to a calibration graph. Calcium can thus be determined within a concentration range of $1{\cdot}3 \times 10^{-4}\,M$ to $1{\cdot}2 \times 10^{-2}\,M$ with a maximum error of ± 3 per cent.

The determination cannot be carried out in the presence of cobalt, zinc, lead, manganese, cadmium, nickel, aluminium, copper and silver ions, because they precipitate the reagent. Sodium, potassium, ammonium, magnesium, iron and mercury ions do not cause precipitation, but ferric and mercuric ions interfere if their concentrations are greater than $4{\cdot}8 \times 10^{-4}\,M$ and $5{\cdot}9 \times 10^{-4}\,M$, respectively. Magnesium does not interfere if present at concentrations of less than $2{\cdot}4 \times 10^{-3}\,M$, nor do lithium, barium and chromium when their concentrations are below $5{\cdot}9 \times 10^{-4}\,M$. Strontium does not affect the determination at concentrations up to $3{\cdot}6 \times 10^{-4}\,M$.

Calcium has been determined in blood serum and in milk.

"The Determination of Small Amounts of Potassium, Calcium and Magnesium in Sodium and its Compounds," by Louis Silverman and K. Trego.

Preliminary concentration is necessary before potassium can be determined in the presence of large amounts of sodium ion.

Metallic sodium, sodium hydroxide and sodium salts are converted to chloride. Measured, cold, saturated aqueous chloride solutions are treated with dry hydrogen chloride gas, which separates the bulk of the sodium chloride. The residual potassium, calcium and magnesium can then be determined by the usual methods.

Calcium and magnesium may also be determined with or without preliminary sodium chloride separation.

"Determination of Borate in Presence of Silver," by S. Z. Haider.

A modification of the usual volumetric procedure for the determination of boric acid in presence of silver ions is described. The interfering effect of the silver ion is first nullified by masking it as a soluble thiosulphate complex, after which the usual procedure is applicable. The proportions of free acid and thiosulphate have to be carefully controlled.

The investigation covered a range of boric acid from 0·0805 to 0·2480 g and sodium pyroborate from 0·0560 to 0·1505 g in mixtures with different proportions of silver nitrate. Results were found to vary within an error of ±0·7 per cent.

"The Analysis of Acid Chlorides," by C. C. T. Chinnick and P. A. Lincoln.

A method is described for the analysis of samples of organic acid chlorides containing as impurities free organic acid, anhydride and hydrochloric acid.

The procedure consists in (*i*) plotting a titration graph with a pH meter, from which the points corresponding to the neutralisation of hydrochloric acid and organic acid are read and (*ii*) determining the ester by hydrolysis with an excess of standard alkali.

Special provision is made for determining anhydride in the presence of hydrochloric acid and details of an alternative, but less convenient, titration procedure, by means of a double indicator, are also given.

Formulae for the calculation of results are included in the paper.

"Micro-analysis of Silicate Rocks," by Christina C. Miller and Robert A. Chalmers.

A new procedure is prescribed for the separation and direct determination of alumina in 5-mg samples of silicate rocks. Silica is volatilised by heating with hydrofluoric and sulphuric acids, and the residue is fused with potassium bisulphate and extracted with N hydrochloric acid. Iron, titanium, vanadium and zirconium are removed together by precipitation with cupferron and extraction with *o*-dichlorobenzene. Acetylacetone is added to the aqueous phase, and aluminium and beryllium acetylacetonates are extracted from the buffered solution at a pH of 6 to 7 by means of diethyl ether. From the ether extract aluminium and beryllium are withdrawn into 6 N hydrochloric acid, and the aluminium alone is precipitated and weighed as aluminium 8-hydroxyquinolinate.

The method has been applied in the presence of all the elements commonly found in silicate rocks. In the analysis of rocks, the results attained are relatively 1 per cent. lower than the corresponding ones based on the classical procedure.

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THE ANALYST

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ERRATA:

VOL. 77, 1952:

Sept., p. 463. The equation on line 4: $x(y - \text{titration})/5$ is applicable only when $x = 50$, as described in the procedure. The general expression should be: $500(y - \text{titration})/x$.

VOL. 78, 1953:

p. 219. The paper by Rây should bear the date "August 14th, 1952."

p. 309. The equations on lines 33 and 35 should read:—

$$Na_2H_2V_e + Zn^{\cdot\cdot} \rightarrow ZnNa_2V_e + 2H^{\cdot}$$

where V_e is

$$[(COO.CH_2)_2.N.CH_2.CH_2.N(CH_2COO)_2]''''.$$

p. 551, title and lines 13 and 17 of synopsis; p. 552, line 9. *For* "esters" *read* "ethers."

INDEX TO VOLUME 78

INDEX TO NAMES

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

INDEX TO SUBJECTS

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