364. *Submicro-methods for the Analysis of Organic Compounds. Part XX.* The Determination of Phosphorus and Arsenic*

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Methods are described for the determination of phosphorus or arsenic in 30—60 μg. samples of organic compounds. The samples are burned by a modified oxygen-flask method. Phosphorus is determined by precipitation as quinoline phosphomolybdate and alkalimetric titration of the precipitate, or spectrophotometrically as molybdenum blue with iron(II) ammonium sulphate as the reductant. Arsenic is determined spectrophotometrically by a similar method with hydrazine sulphate as reductant.

Classical methods for the determination of phosphorus and arsenic in organic compounds generally depend on wet combustion in a sealed tube with fuming nitric acid. Attempts to determine phosphorus in this way on the microgram scale were unsuccessful; very low results were obtained and it was suspected that some adsorption took place on the surface of the tube. Similar results were obtained when pure potassium dihydrogen phosphate was subjected to the same treatment. Accordingly, a different decomposition procedure was sought. The oxygen-flask method of decomposition has already been applied on the microgram scale to the determination of halogens and sulphur (see Parts X, XIV, XV, and XVII), and this method was therefore examined further. Many Papers have been published on the determination of phosphorus and arsenic by flask methods on other scales of working.

Determination of Phosphorus.—When combustions were done in the special 25 ml. flask with the sample support described previously,[1] the results obtained were consistently 0·5—1% low. When the original platinum-gauze hinge was formed into a cup so that less heat was conducted away from the combustion zone and the sample did not come into immediate contact with the support during the combustion, the recoveries were much better. Polythene foil was used as the sample wrapper and contained no significant blank. The fuse material was filter paper, because the linen thread previously recommended caused low recoveries (cf. ref. 2). When the spectrophotometric finish was applied, 25 ml. round-bottomed flasks could be used instead of the specially designed flasks, because there was no need to collect the solution in a suitable compartment for titration.

An absorption solution of alkaline sodium hypobromite as recommended on the milligram scale[3] was found satisfactory. Because it was necessary to keep the volume low (*ca.* 0·1 ml.) on the microgram scale the absorption time had to be increased to avoid low recoveries.

For the final determination of phosphate produced in the reaction, precipitation as quinoline phosphomolybdate[4] was first examined. This is undoubtedly the best of all

* Part XIX, R. Belcher, and B. Fleet, *J.*, 1965, 1740.

[1] R. Belcher, P. Gouverneur, and A. M. G. Macdonald, *J.*, 1962, 1938.
[2] R. Belcher, A. D. Campbell, P. Gouverneur, and A. M. G. Macdonald, *J.*, 1962, 3033.
[3] R. Belcher and A. M. G. Macdonald, *Talanta*, 1958, **1**, 185.
[4] H. N. Wilson, *Analyst*, 1951, **76**, 65.

the methods in which phosphomolybdic acid is precipitated by a base and has been applied in organic microanalysis after combustion in an oxygen flask.[3] Satisfactory results were obtained by a scaled-down procedure when pure phosphate solutions were analysed on the microgram scale. When organic compounds were tested, the interference of silicon, released from the wall of the flask during the combustion, was found to be quite serious. Citric acid was added to overcome the interference [3,4] but the amount to be added was rather critical, for the solubility of quinoline phosphomolybdate increases when citric acid is present. The amount specified in the Experimental section is correct for normal usage. However, when new flasks were used, excessive amounts of silica were produced and it was best to condition the flasks by burning about 6 unweighed samples before actual determinations were carried out. A number of organic compounds containing phosphorus was analysed by the recommended procedure; representative results are shown in Table 1.

TABLE 1

Determination of phosphorus

Compound	No. of detns.	Phosphorus (%) Calc.	Found	Mean error (%)	Range of errors (%)
Diphenylcyclohexylamine phosphate (*a*)	11	9·35	9·23	−0·12	−0·40—+0·27
(*b*)	4		9·16	−0·19	−0·48—+0·43
Diphenylphenetidine phosphonate	4	8·39	8·68	+0·29	+0·13—+0·70
Diphenylbenzylamine phosphonate	4	9·13	9·47	+0·34	+0·13—+0·49
Sodium glycerol phosphate	4	9·86	9·85	−0·01	−0·38—+0·55
Triphenylphosphine (*a*)	5	11·81	11·72	−0·09	−0·20—+0·01
(*b*)	3		11·64	−0·17	−0·51—+0·15
Triphenyl phosphate (*a*)	10	9·52	9·57	+0·05	−0·22—+0·25
(*b*)	7		9·48	−0·04	−0·35—+0·45
N-Methyldiphenylamine phosphonate ...	6	11·80	11·80	0·00	−0·36—+0·29
Tris[(trifluoromethylphenyl)-(diethyl)] copper phosphonium iodide	6	10·41	10·56	+0·15	−0·18—+0·92

(*a*) Titrimetric procedure. (*b*) Spectrophotometric procedure.

Only one compound containing fluorine as well as phosphorus was available; satisfactory results were obtained when boric acid was added immediately after the flask had been opened.[3]

The method involving precipitation of quinoline phosphomolybdate gave excellent results but the technique required for the high accuracy attainable was undoubtedly demanding. It was therefore considered desirable to have an alternative simpler method and spectrophotometric procedures were examined. Many methods are available, most of which depend on the formation of ammonium phosphovanadomolybdate or of molybdenum blue. The latter type of method is by far the more sensitive and was therefore selected. A bewildering variety of reductants has been proposed for the formation of molybdenum blue; in the present work, the iron(II) ammonium sulphate reductant described by Chalmers and Thomson [5] was preferred. By suitable modification satisfactory results were obtained; the calibration curve was a straight line passing through the origin, and Beer's law was obeyed up to 10 μg. of phosphorus per 25 ml. Representative results obtained by this method are also shown in Table 1. Generally speaking, there was little difference in the accuracy attainable by the two methods, but the results obtained by the spectrophotometric method were less precise.

Determination of Arsenic.—Arsenic compounds were also decomposed by an oxygen-flask method; because arsenic forms an alloy with platinum under the conditions of the combustion,[6] a silica spiral was used as the sample support instead of platinum gauze. A small crystal of potassium nitrate was added to the sample to assist complete combustion. Arsenic(III) is mainly formed in the combustion [6] and an oxidising absorbent

[5] R. A. Chalmers and D. A. Thomson, *Analyt. Chim. Acta*, 1958, **18**, 575.
[6] R. Belcher, A. M. G. Macdonald, and T. S. West, *Talanta*, 1958, **1**, 408.

was therefore necessary; the same absorbent as for the determination of phosphorus was found satisfactory.

There are very few suitable classical methods for the determination of arsenic(v). It is usually preferable to reduce to arsenic(III) and then titrate with a suitable oxidising solution. Unfortunately, the few reagents which can effect this reduction did not seem promising for work on the microgram scale; distillation of arsenic trichloride was not considered for the same reason. The successful determination of arsenic(v) by precipitation as quinoline arsenomolybdate has been reported,[7] but attempts to modify this method for the microgram-scale were abortive. In the presence of the minimum amount of citric acid necessary to prevent interference by silica, appreciable dissolution of the precipitate occurred; the problem was further aggravated by the necessity of using the silica spiral as sample holder, so that the silica contamination was much greater than in the case of phosphorus. The precipitation of some other base arsenomolybdates was examined briefly but eventually attempts to find a suitable classical determination were abandoned and attention was concentrated on spectrophotometry.

The method chosen was based on that described by Morris and Calvery [8] in which hydrazine sulphate was used as the reductant. A straight-line calibration graph passing through the origin and obeying Beer's law up to 20 μg. of arsenic per 10 ml. of solution was obtained. Satisfactory results were obtained for a variety of organic compounds as shown in Table 2.

TABLE 2

Spectrophotometric determination of arsenic

Compound	No. of detns.	Arsenic (%) Calc.	Found	Mean error (%)	Range of errors (%)
o-Arsanilic acid	12	34·52	34·42	−0·10	−0·88—+0·82
4-Chloro-4-nitrophenylarsonic acid	3	26·62	26·74	+0·12	−0·37—+0·54
o-Nitrophenylarsonic acid	5	30·33	30·36	+0·03	−0·61—+0·61
Sodium 1-*o*-arsonophenylazonaphthol-3,6-disulphonate	4	12·52	12·41	−0·11	−0·52—+0·28
p-Dimethylaminobenzeneazo-*p*-phenylarsonic acid	4	21·45	21·44	−0·01	−0·41—+0·69
Triphenylmethylarsonium iodide	3	16·72	16·88	+0·16	−0·01—+0·40

EXPERIMENTAL

Apparatus.—The microgram balance and its accessories, the titration apparatus, oxygen line, and Polythene sheet were as described previously.[1] Optical densities were measured in a Unicam S.P. 600 spectrophotometer. Unless otherwise specified, reagents were added from droppers, the tips of which had been drawn out to a capillary capable of delivering drops of reasonably constant size (*ca.* 0·01 ml.). The size of the drop was calibrated.

Flasks for Combustion.—For the titrimetric determination specially shaped flasks [1] were used. For the spectrophotometric determinations 25 ml. round-bottomed flasks could be used instead. In the determination of phosphorus, the sample support of platinum gauze was shaped into a cup to hold the wrapped sample. In the determination of arsenic, a silica spiral was made from a 4—5 cm. length of 1 mm. diameter tubing; the spiral had 3—4 turns and was tapered at the lower end. It was sealed to a supporting rod from the usual ground-glass stopper, so that it was situated in the centre of the flask. The flasks were cleaned thoroughly with " Teepol " and then steamed [1] between determinations.

Determination of Phosphorus. (a) *Titrimetric method. Reagents. Sodium molybdate solution.* 15 g. of AnalaR molybdenum trioxide and 3 g. of AnalaR sodium hydroxide were dissolved in 50 ml. of distilled water by heating for 30 min. The solution was filtered and 46 ml. of concentrated hydrochloric acid was added followed by a drop of 100-vol. M.A.R. hydrogen peroxide to clear the green-blue colour.

[7] S. Meyer and O. G. Koch, *Z. analyt. Chem.*, 1958, **160**, 253.
[8] H. J. Morris and H. O. Calvery, ***Ind. Eng. Chem. Analyt.***, 1937, **9**, 447.

Quinoline hydrochloride solution. 2·8 ml. of redistilled quinoline was dissolved in 60 ml. of 1 : 1 hydrochloric acid.

Both these solutions were stored in Polythene vessels.

All other reagents were of M.A.R. or A.R. grade and glass-distilled water was used throughout.

Procedure. A 30—70 μg. sample was weighed on to a Polythene square and wrapped as described previously.[1] A 1·0 × 0·2 cm. piece of ashless filter paper served as the fuse. About 0·1 ml. of 0·5N-sodium hydroxide and 0·1 ml. of saturated bromine water were placed in the flask which was then flushed with oxygen. The combustion was done in the usual way.[1] Immediately after the combustion, the flask was rotated so that the absorption solution covered the lower half of the wall; it was allowed to drain for 30 min., rotated again, and then allowed to drain for 1 hr.

The flask was opened and the stopper, the sample support, and the wall of the flask were rinsed with a total of 1·5 ml. of water from a hypodermic syringe, the technique being similar to that described earlier.[1,2] A drainage time of 15 min. was allowed. If the compound contained fluorine, 0·015 ml. of saturated aqueous boric acid solution was added. The solution was then heated to boiling and 0·1 ml. of N-hydrochloric acid was added through the gauze sample holder, the gauze being rinsed afterwards with a drop of water. The solution was then boiled gently on a water-bath to remove most of the bromine.

A freshly prepared aqueous 5% solution of citric acid (0·1 ml.) was added and the solution (with a total volume of *ca.* 1·5 ml.) was heated to 80—90° on the water-bath during the addition of 0·06 ml. of sodium molybdate solution and 0·04 ml. of quinoline solution. The flask was swirled quickly and the precipitate digested for 1 min. before being allowed to cool to room temperature. The precipitate was then filtered on a paper-pulp pad [9] supported by a tiny perforated Polythene disk. The flask was rinsed thrice with 1—2 ml. portions of water from a fine-tipped wash-bottle and the precipitate was then washed with small portions of water until the washings were neutral. The pad and precipitate were transferred quantitatively to the original vessel with a little water and the vessel was placed on the titration apparatus. A stream of nitrogen was introduced through a drawn-out tube and maintained during subsequent operations. An excess of standard 0·02N-sodium hydroxide was added from an Agla burette; usually 400 μl. sufficed. (The sodium hydroxide was previously standardised against potassium hydrogen phthalate.) The solution was stirred magnetically until complete dissolution was achieved. The flask wall was then rinsed with a little water and the excess of alkali was titrated with standard 0·02N-hydrochloric acid from an Agla burette in the presence of phenolphthalein indicator. Blank determinations must be done very carefully; it is simpler to calculate a blank from a series of standard determinations.

(b) *Spectrophotometric procedure. Reagents. Acid molybdate solution.* 50 g. of ammonium molybdate were dissolved in 500 ml. of water, and added to a cold mixture of 125 ml. of M.A.R. concentrated sulphuric acid and 350 ml. of water. This was diluted to 1 litre.

Iron(II) ammonium sulphate solution. 10 g. of iron(II) ammonium sulphate were dissolved in 100 ml. of water containing about 15 ml. of 4N-sulphuric acid. The solution was prepared daily.

Standard phosphate solution. 4·394 g. of potassium dihydrogen phosphate were dissolved in water and diluted accurately to 1 litre. The solution contains 1 mg. of phosphorus per ml. The above solution was diluted twenty times in order to obtain a stock solution of 5 μg. of phosphorus per 100 μl. All solutions were made slightly acidic (*ca.* 0·002N) to minimise adsorption of phosphate on glass.

Procedure. The combustion and standing period were as described above. The stopper and the sample support were then rinsed with *ca.* 0·3 ml. of water from a hypodermic syringe. The solution was heated to boiling and the bromine was removed by gentle boiling after addition of 0·1 ml. of N-sulphuric acid added through the gauze followed by a few drops of water. The solution was then transferred to a 25 ml. volumetric flask with four 2 ml. portions of water, and 2 ml. of 1·5N-sulphuric acid, 5 ml. of the acid molybdate solution, and 2·5 ml. of the iron(II) solution were added from pipettes. The solution was diluted to the mark, thoroughly mixed, and left aside for 30 min. for full colour development. The optical density was then measured in a 4-cm. cell at 750 mμ against a reagent blank.

[9] R. Belcher, R. A. Shah, and T. S. West, *J.*, 1958, 2998.

A calibration graph was prepared for the range 1—10 μg. of phosphorus by following the above procedure from the addition of 1·5N-sulphuric acid.

Determination of Arsenic.—Reagents. Ammonium molybdate solution. 5 g. of ammonium molybdate were dissolved in 1 litre of 1 : 19 sulphuric acid : water.

Hydrazine sulphate. An aqueous 0·025% solution was prepared daily.

Standard arsenic solution. 0·132 g. of arsenic trioxide was dissolved in 2 ml. of N-sodium hydroxide. The solution was diluted with water and made slightly acid with hydrochloric acid, before dilution to 100 ml. This solution was diluted 10 times to obtain a stock solution containing 10 μg. of arsenic per 100 μl.

Procedure. The combustion and absorption were as described above, except that a small crystal of potassium nitrate was added to the sample before it was wrapped and placed in the silica spiral. The flask was opened, the absorption solution was acidified with 0·1 ml. of N-sulphuric acid, and the solution was boiled gently to drive off bromine. The spiral and the walls of the flask were then rinsed with 4 ml. of water, and 2 ml. of the ammonium molybdate solution and 2·5 of the hydrazine sulphate solution were added from pipettes. The solution was thoroughly mixed and heated on a water-bath (90—100°) for 10 min. to develop maximum colour. The solution was cooled and transferred to a 10 ml. volumetric flask, the combustion flask being rinsed with sufficient water (*ca.* 2 ml.) to dilute the solution to the mark. The optical density, which remained constant for at least 24 hr., was measured in a 1 cm. cell at 840 mμ against a reagent blank.

Calibration curve. Suitable aliquot portions of the standard arsenic solution (2—20 μg. As) were transferred to combustion flasks containing 0·1 ml. of 0·5N-sodium hydroxide and 0·1 ml. of saturated bromine water. Samples of sucrose or benzoic acid were burned as described above and then the above procedure was followed exactly.

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