108. A GRAVIMETRIC METHOD FOR THE ESTIMATION OF BROMIDE IN ORGANIC MATERIAL¹

By SIMON BEHRMAN, DENIS BRINTON
AND R. G. L. WALLER

From the National Heart Hospital, Queen Square, and St Mary's Hospital, London

(Received 9 June 1941)

DURING the past decade interest in the problem of bromide intoxication has steadily increased and numerous methods of estimating bromide in biological fluids have been described. Three main types can be distinguished, colorimetric, volumetric and electrolytic. The first two are based on reactions of extreme delicacy which are not easily controlled. The last is not well suited to clinical investigation because large amounts of fluid as well as expensive and delicate apparatus are required. If information on the subject of bromism has not kept pace with the interest it has evoked, the inaccuracies of the present clinical methods can alone be blamed. The advantages of the method to be described are that it is based on a simple chemical reaction which for practical purposes is irreversible. The analysis cannot therefore be disturbed by slight departures from the standard conditions of the experiment, such as differences in colour of the materials to be analysed, temperature, concentration of the reagents and pH of the fluids. Moreover, the amounts of bromide and chloride in the material to be estimated are determined in one experiment.

In principle the method depends upon the difference between the weights of AgBr and AgCl. Both halides are precipitated at the outset with excess of a measured quantity of AgNO₃ and the precipitate is removed by filtration and its weight (w) is estimated. The excess of AgNO₃ is then precipitated with NaCl, and after filtration its weight (p) is determined. If c represents the weight of AgCl obtainable from the amount of standard solution of AgNO₃ taken and if x represents the amount of AgCl and y the amount of AgBr obtained from the unknown material, then

$$x + y = w, \quad (i)$$

$$x + \frac{143}{188}y + p = c, \quad (ii)$$

From (i) and (ii)

$$w - y = c - p - \frac{143}{188}y, \quad (iii)$$

and from (iii)

$$\frac{188}{45}(w + p - c) = y. \quad (iv)$$

But if y' represents the weight of NaBr in the unknown material, then

$$y' = \frac{103}{188}y, \quad (v)$$

and from (iv) and (v)

$$y' = \frac{103}{45}(w + p - c).$$

¹ Work undertaken on behalf of the Medical Research Council while Dr Brinton held a Halley Stewart Research Fellowship.

Special apparatus required

(1) Boiling tube of 50 mm. diameter and 260 ml. capacity. The neck of the boiling tube should be accurately ground to take a reflux condenser.

(2) Reflux condenser ground to fit boiling tube (1) and extraction tube (6).

(3) Rubber ring, round in section and of a size suitable for stretching over sintered glass filter (4).

(4) Two sintered glass filters (Schott und Gen., Jena), graded as having a porosity of 'less than 7' (No. 10 G 4). These are subsequently referred to in the text as 'filter-cups'. They are prepared in the manner described below.

(5) Special funnel into which filter-cups are placed for the purpose of filtration under pressure. The wide portion of the funnel is so shaped as to allow the free insertion of a filter-cup. The rubber ring stretched round the filter-cup forms an airtight joint between the funnel and the cup. The narrow lower portion of the funnel is inserted into a rubber stopper placed in a pressure flask of a suitable size. When negative pressure is applied the filter-cup is prevented from sinking too far by means of three equally spaced projections within the funnel. These projections are part of the funnel itself, being produced by indentations on its outer surface (see Fig. 1).

Fig. 1.

Fig. 1. Section of filter-cup and funnel. Position (A) before and (B) after application of negative pressure to pressure flask. 1, filter-cup. 2, rubber ring. 3, tape. 4, funnel. 5, rubber stopper.

Fig. 2.

Fig. 2. Apparatus for extraction. 1, reflux condenser. 2, alcohol reservoir. 3, glass rods. 4, filter-cup. 5, absolute alcohol. 6, indentations.

(6) Special extraction tube of 50 mm. diameter and approximately 270 ml. capacity. About 2 in. from its bottom it bears three indentations in its outer wall; these support the filter-cup in position. The neck of the extraction tube should be ground to fit the reflux condenser (2).

(7) 'Alcohol reservoir', represented in Fig. 2. It is about 1½ in. in depth and only a little narrower than the outer extraction tube (6). Alcohol which collects

in it during the process of extraction can escape from its lower end through a narrow capillary tube. The alcohol reservoir is maintained in position on the top of the filter-cup by three radiating glass rods. These are fused to a point near to the origin of the capillary tube. The latter should be long enough to reach nearly to the bottom of the filter-cup when the apparatus is assembled.

Reagents

(i) For all filtrations and washings only water which has been doubly distilled in glass is used.

(ii) *The standard silver solution.* It has been found that at least 15 ml. of concentrated acid are required to complete the digestion of 10 ml. of blood to a stage when all the fatty material becomes soluble in alcohol. Therefore the *N/20* standard solution used is prepared by dissolving the requisite amount of pure AgNO_3 in a minimal quantity of doubly distilled water and making up the solution to 1 l. with pure conc. HNO_3 . If larger quantities of blood are used proportionately more standard solution must be taken.

(iii) *An approximately N/20 solution of pure NaCl* is prepared for precipitation of the excess of the standard silver solution present in the first filtrate. About 20 ml. of this are required.

Preparation of sintered glass filter cups

The required number of cups can be cleaned simultaneously in an ordinary wide-necked Erlenmeyer flask of 2 l. capacity.

The cups are gently lowered into the flask and need not be removed until all stages of preparation are completed. First a 50 % solution of commercial conc. HNO_3 in tap water is added and the flask placed in a boiling water bath for 1 hr. At the conclusion of this stage the contents are removed by suction and the cups are washed several times in clean tap water.

Sufficient absolute alcohol to cover the cups is then placed in the flask, which is again set in the boiling water bath for 1 hr. The remaining alcohol is removed and the cups freely washed with doubly distilled water. Finally the flask is replaced on the water bath and the cups are heated there for 1 hr. in distilled water and washed again several times. They are then ready for drying to a constant weight.

If the cups have previously contained silver halides, it is wise as a preliminary to run a strong solution of commercial ammonia through them in the reverse direction to the usual flow. They are treated subsequently in the way already described.

DESCRIPTION OF METHOD

Stage of digestion

A measured quantity of oxalated blood (approximately 10 ml.) is carefully transferred to the special boiling tube (1). To the blood in the tube are gradually added exactly 20 ml. of the special standard solution of AgNO_3 in conc. HNO_3 . Eisenman [1929] has found that no dissociation of halides can occur provided that the solution of strong acid and silver is added sufficiently slowly. The boiling tube is now fitted with the reflux condenser (2) and is then placed in a constant-level boiling water bath. Digestion is allowed to proceed for fully 12 hr. and after its completion the tube is found to contain a straw-coloured fluid on the surface of which are small flakes of fatty material. The white halide precipitate has sunk to the bottom.

Stage of filtration

A washed rubber ring (3) is stretched by means of three tapes passed through it. The stretched ring is lowered over a weighed filter-cup (4) which is secured by allowing the tension to relax; the tapes are left in position. The filter-cup may then be lifted by the rubber ring which grips it, and placed in the funnel (5) of a pressure flask. It is important that no contamination should occur on the sides of the filter-cups, and with this technique they are not touched at all.

The ring forms an air-tight joint between the funnel and the filter-cup. The filtration is done under a strong negative pressure. When nearly all the fluid from the boiling tube has been transferred, an assistant directs a fine jet of doubly distilled water under pressure upon the precipitate which is thus broken up into small particles. The tube is then again inclined and the contents decanted, whilst the assistant continues to wash the tube free of all particles. This is continued until no more precipitate can be seen, but as a further precaution more water is added and the tube examined against a dark background to exclude the presence of minute floating particles. With a little practice this procedure can be carried out with absolute assurance.

After emptying the tube it is further washed to remove all traces of AgNO_3 solution and the glass rod and cup are similarly treated.

A clean watch-glass is next placed over the top of the filter-cup; by means of gentle digital pressure on the watch-glass a sudden and violent release of the filter-cup can be prevented when the pressure-pump is disconnected. The filter-cup is now held over the funnel by the tapes of the ring to enable the assistant to wash its under surface thoroughly. The washings are carefully collected into the flask containing the filtrate. Next the filter-cup is released from the ring on to a desiccator plate, where its outer surface is further sprayed as an additional precaution. The funnel is then washed down several times to free it from all traces of AgNO_3 . It is disconnected from the pressure-flask, which is put aside after all the AgNO_3 has been precipitated with excess of NaCl .

Stages of extraction

In addition to halide precipitate the cup contains flaky fatty material. This has now to be removed completely by continuous extraction with absolute alcohol in a special piece of apparatus (see Fig. 2) which consists of an extraction tube (6), an alcohol reservoir (7) and a reflux condenser (2).

The filter-cup is gently lowered by means of a long pair of forceps into the extraction tube, which contains some 30–40 ml. of absolute alcohol. The alcohol reservoir is then similarly placed in its position and the reflux condenser attached.

Extraction is allowed to proceed on a gently boiling water bath for 1–2 hr. The purpose of the alcohol reservoir is threefold. Firstly, it collects the pure alcohol as it condenses; secondly, it directs the flow of alcohol to the bottom of the cup where the fat-containing precipitate is situated; thirdly, its capillary tube exit compels a gentle flow which prevents any disturbance of the precipitate. The level of the alcohol rises in the cup and overflows, carrying the fatty material with it in solution.

The extraction is completed by allowing the water in the bath to cool, but no attempt is made to remove the filter-cup until all alcohol has left it, for fear of disturbing the precipitate. Usually 1–2 hr. are required for this to be accomplished.

The condenser is now disconnected and with the same long pair of forceps the reservoir and cup are removed. A crucible cover is then placed over the filter-cup, which is conveniently put aside on a clean desiccator plate.

Estimation of excess AgNO₃

The pressure-flask, containing the excess Ag which was precipitated immediately after filtration, is placed in a boiling water bath. After about an hour's heating the precipitate of AgCl will be found to have consolidated and to be in a suitable state for filtration through another specially prepared and weighed filter-cup. The procedure is exactly similar to the first filtration, except that the pressure-flask now takes the place of the digestion tube.

The two cups containing the first and second precipitates are then dried to constant weight in a hot-air oven at a temperature not exceeding 150°. On removal from the oven the cups are allowed to cool in a desiccator over phosphorus pentoxide before weighing.

Possible sources of error of the method

(a) *Loss of halides during the state of digestion.* Contrary to the opinion of Morris & Morris [1930], even prolonged heating of a simple halide precipitate in the presence of conc. HNO₃ does not cause any appreciable loss, as the following experiment shows.

A series of six tubes were put up, each containing 10 ml. of a standard solution of AgNO₃ to which had been added excess of NaCl solution and conc. HNO₃. Three of these tubes were filtered after 40 min. on a boiling water bath, but the heating in the case of the other three was prolonged for 12 hr. The weight of AgCl in the several tubes was as follows:

Duration of heating	Wt. of AgCl, g.		
	0-1416	0-1417	0-1418
40 min.			
12 hr.	0-1416	0-1419	0-1419

Similar conclusions were reached by Norris & Ampt [1933] who aspirated chlorine-free air through a flask containing AgCl in the presence of conc. HNO₃ at 100° and were unable to detect any trace of Cl in the fumes. Sunderman & Williams [1933], using a somewhat similar procedure, were also unable to find more than negligible amounts of halides. Further, Comey & Hahn [1921] have shown that AgBr is not decomposed by boiling with strong HNO₃.

(b) *Fat as an interfering constituent in halide analysis.* Sunderman & Williams [1931] have attributed a diminished recovery of chlorides to the presence of fat. These low figures were only observed when the analysis was carried out on dried, fat-containing material and with digestions by HNO₃ lasting at most 6 hr. In the present work all digestions were done wet and were allowed to proceed for at least 12 hr. In addition, it was found that the fat which resisted digestion yielded no halides on being dissolved in alcohol.

(c) *Technical difficulties related to the transfer of the halide precipitate to the filter-cup.* With sufficient practice this transfer can be done with great accuracy. The difficulties are therefore more apparent than real and are not a source of appreciable error. In the course of the present work many experiments have been performed to prove this, of which a small series is quoted below. The figures refer to the weight of silver halide recovered in two series of 4 and 3 parallel estimations.

Exp.	Wt., g.	Exp.	Wt., g.
1	0-0898	1	0-0936
2	0-0898	2	0-0936
3	0-0898	3	0-0936
4	0-0898		

(d) *Suitability of sintered glass filters used.* Personal experience has fully confirmed the claims of the makers (Schott und Gen., Jena) that the filters graded as having a porosity of 'less than 7 (No. 10 G 4)' are impermeable to silver halide precipitates, and that the less fine grades allow a proportion of the precipitate to escape through the filter.

If the cups are prepared exactly as is described above before each estimation, it appears that they retain their efficiency for a great many experiments.

(e) *Choice of solvent for blood-fats.* After many different solvents had been tried it was proved that with absolute alcohol it was possible to remove all fatty materials left after heating blood with HNO_3 , provided that this digestion had been allowed to proceed sufficiently long. The following experiment illustrates this point. 10 ml. of blood were digested with 20 ml. of conc. HNO_3 for 12 hr., and when cool the resulting digest was filtered through a clean cup of known weight. The cup was then extracted with absolute alcohol for 1 hr. and weighed again after drying. Figures for four such experiments show that the change of weight is within the limits of experimental error.

Wt. of cup empty, g.	Wt. of cup after extraction g.
13.2053	13.2052
15.1296	15.1293
12.7384	12.7386
11.5047	11.5046

(f) *Solubility of silver halides in alcohol.* From the solubilities of the silver halides in alcohol at room temperature, established by Neustadt [1910], it is evident that errors from this cause are negligible.

(g) *Mechanical disturbance of precipitate.* During the extraction the solvent is entering the cup from above, filling it and flowing over its sides and, theoretically, it might carry with it solid particles, thus upsetting the final weight of the precipitate. In practice this criticism is not valid for several reasons. First, the capillary tube of the alcohol reservoir is made to extend nearly to the bottom of the cup and to allow a slow escape of alcohol, so that splashing is avoided. Secondly, the solvent used is of low specific gravity, whereas the silver precipitate is heavy, and even if very small particles are disturbed they immediately gravitate to the bottom. Thirdly, our own experiments, with extractions up to 12 hr. duration, prove conclusively that there is no appreciable loss. This is shown in the accompanying table.

Exp.	Nett gain or loss in wt. (g.) following extraction
1	+0.0001
2	-0.0001
3	-0.0001
4	No change
5	+0.0001
6	-0.0001
7	+0.0001

(h) *Solubility of silver halides in water and conc. HNO_3 .* On consideration of the solubilities of silver halides in these solvents, it is evident that the amounts lost thereby must be minute.

20 ml. of conc. HNO_3 at room temperature dissolve precipitated AgCl to the extent of 0.04 mg. [Thorpe, 1872]. Similar data for AgBr are not available, but the error here is undoubtedly smaller if the relative solubilities of the two halides are taken into account.

At room temperature 100 ml. of water dissolve 0.0001462 g. of AgCl [Thorpe, 1926] and 0.0000107 g. of AgBr [Kohlrausch, 1908; Kohlrausch & Dolezalek, 1901]. Similar data for these solubilities are given by Hill [1908] and in the *International Critical Tables*. The total washings in each experiment do not exceed 200 ml.

Sample analysis

It has been found in a number of experiments, of which the following are examples, that consistent results can be obtained by this method.

Example 1. Five boiling-tubes were put up. Two of these contained 10 ml. of AgNO₃ solution, 10 ml. conc. HNO₃ and excess of NaCl, as controls for standardization of the AgNO₃ used. The three others contained 10 ml. of AgNO₃ solution, 10 ml. conc. HNO₃ and 10 ml. of a mixed solution of NaCl and NaBr. The halides in the mixture were then estimated by the method described. The amount of AgCl in each of the two control tubes was exactly 0.1379 g. The amounts of Cl and Br are given in the following table.

Exp.	Wt. of halide ppt., g.	
	NaCl	NaBr
1	0.0202	0.0221
2	0.0209	0.0213
3	0.0202	0.0221

Example 2. Three samples of blood were taken from a patient who had been under close observation for some weeks, and who was known not to have been taking any bromide. The total halide content of each of these was estimated with the following results:

Exp.	Wt. of halide ppt., g./100 g. blood	
	NaCl	Calculated figure for NaBr
1	0.4228	0.0006
2	0.4347	0.0002
3	0.4224	0.0008

SUMMARY

1. A gravimetric method of estimating bromides and chlorides in organic materials has been described.
2. The possible sources of error have been considered.
3. It is claimed that the method is simple and accurate and is not open to many of the objections to which colorimetric and volumetric procedures are subject.

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