XL. MICRO-DETERMINATION OF AMMONIA IN PRESENCE OF ALIPHATIC AMINES

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(Received 29 December 1936)

It has been found [Pugh & Quastel, 1937] that a number of aliphatic amines are broken down by slices of certain tissues with the production of ammonia. For the quantitative investigation of this phenomenon it has been essential to devise a method for the accurate estimation of ammonia in presence of the amines.

The aliphatic amines under investigation are volatile, and therefore aeration methods depending on the transference of the ammonia into standard acid solutions and back-titration are obviously out of the question. The direct use of the Nessler reagent is also impracticable, except in a qualitative manner, owing to the fact that amines give precipitates with the Nessler reagent which interfere seriously with the coloration produced by the traces of ammonia present.

It has been found possible to estimate the small quantities of ammonia produced in the metabolism of aliphatic amines by making use of the fact that yellow mercuric oxide will effect a sharp separation of ammonia and amines by taking up ammonia and not amines. François [1907] first used this property of mercuric oxide for freeing aliphatic amines from the ammonia with which they are usually contaminated. His method was to shake the mixture of amines and ammonia with mercuric oxide in alkaline solution; the filtrate was then free from ammonia. François also used the method for the estimation of both amines and ammonia, the latter being liberated from mercuric oxide combination by treatment with potassium iodide and subsequent distillation. François, however, worked with far larger quantities of ammonia and amines than those dealt with in the present work, and our method, though similar to his in principle, differs from it considerably in detail.

The present method consists essentially of three stages: (1) the transference of volatile amine and ammonia, by aeration in presence of K_2CO_3 and at the temperature of the boiling water-bath, into standard acid solution; (2) the treatment of the latter solution with fresh mercuric oxide, centrifuging and washing; (3) the decomposition of the mercuric oxide-ammonia complex with strong NaOH, and transference of the ammonia by aeration into acid solution. The ammonia is then estimated colorimetrically by means of the Nessler reagent.

Apparatus

A special apparatus¹ (Fig. 1) is used in which the amount of rubber present is reduced to a minimum. The glass vessel (A), capacity 50 ml., in which absorption of ammonia and amine into standard acid solution takes place in stage (1) is used as the centrifuge tube in stage (2), and finally as the tube in which decomposition of the mercuric oxide-ammonia complex is brought about

¹ Prepared for us by Messrs Quickfit and Quartz, Ltd., Birmingham.

by alkali and the ammonia removed by aeration in stage (3). The use of the one tube for the operations described considerably decreases the experimental error. The tube has a ground glass neck and is provided with a glass lid (*B*) having inlet and outlet tubes (*I* and *O*). The ground glass joint between the lid

Fig. 1.

and the neck of the tube becomes perfectly airtight when moistened with water. All tubes and lids are interchangeable. The inlet tube attached to the glass lid is of such a length that, when fitted, the end is within $\frac{1}{4}$ in. of the bottom of the centrifuge tube. It has sealed to it, about half-way up, a circular plate of glass whose diameter is a little smaller than that of the top of the centrifuge tube; this plate breaks up frothing and thus prevents mercuric oxide or caustic alkali from being carried over during the final aeration in stage (3).

Details of procedure

The solution containing the volatile amine and ammonia, from which, in the present investigation, tissue slices have been removed, is placed in the distillation tube. 1 ml. pure paraffin and 2.5 ml. saturated K_2CO_3 are added. The lid is fitted and connected to an absorption tube, similar to the distillation tube, containing 10 ml. $N/10$ H_2SO_4 . The distillation tube is placed in a boiling water-bath. Slow aeration is carried out, the air being previously well washed by passing through acid solution. After 15 min. the aeration is quickened, and transference of the ammonia (together with that of most of the volatile amine) is completed by blowing over rapidly for a few minutes at the end of an hour.

After washing the lead-in tube, the volume of liquid in the absorption and centrifuge tube is about 20 ml. To this are added 1 ml. N $NaOH$ to neutralize the H_2SO_4 , 3 ml. $M/5$ phosphate buffer solution pH 7.4 and finally 0.5 g. mercuric oxide. The mixture is heated in a water-bath to a moderate temperature (about 70°) for 15 min. with constant stirring. The most convenient stirrer for the heavy mercuric oxide is a strip of glass curved at one end to fit the bottom of the centrifuge tube.

The mixture is now centrifuged for 4–5 min. at 2500 r.p.m., the clear supernatant liquid is decanted, and the residual mercuric oxide is stirred with 10 ml. water and the mixture again centrifuged. The washing is carried out twice. The

residue, after the final centrifuging, is stirred with a few drops of water and the mercuric oxide is washed off the stirrer and loosened from the sides of the tube with the aid of a rubber-tipped rod. The mercuric oxide deposit contains the ammonia complex and is now free from aliphatic amine. 1 ml. pure paraffin and 2.5 ml. 5*N* NaOH are added and the distillation of ammonia is carried out as before in a good current of air for an hour in the boiling water-bath. The ammonia is absorbed into 10 ml. *N*/10 H₂SO₄ solution, and estimated by nesslerization.

Technical details. In the last stage of the process described above, strong NaOH is necessary to decompose the mercuric oxide-ammonia complex. If rubber stoppers are used, instead of an all-glass apparatus, erratic results may be obtained. Sometimes they appear to contribute ammonia, and sometimes there may be loss of ammonia owing to leakage when the rubber stoppers harden after prolonged usage.

The mercuric oxide used in this work should be free from ammonia. This may be secured by preliminary treatment with caustic alkali solution and subsequent washing with ammonia-free water. The oxide should be kept in a well-stoppered bottle, preferably in the concentrated H₂SO₄ desiccator, since the oxide easily picks up traces of ammonia from the air. In the present work, it has been found sufficient, for complete absorption of ammonia by the oxide, to warm the solution with the mercuric oxide for 15 min. with constant stirring. Without warming, absorption of ammonia is incomplete in that period. The *pH* of the mixture is kept at 7.4.

For quantities of ammonia corresponding to 0.5 mg. of NH₄Cl at least 0.5 g. mercuric oxide must be used. Smaller quantities of mercuric oxide give low results. This is probably due to the fact that a film of mercuric oxide is lost when decantation takes place after each centrifuging. If the amount of mercuric oxide used is relatively large, the loss is negligible. Excessive quantities of the oxide cannot be used since these inevitably introduce too large a blank. A medium quantity, such as that used here, seems to work quite satisfactorily.

Under the conditions of the present experiments it has been found that the ammonia is completely removed by mercuric oxide from admixture with the following amines: methylamine, ethylamine, propylamine, butylamine, amylamine, isoamylamine and heptylamine.

Necessity for pre-distillation with K₂CO₃. This process, stage (1), is carried out to avoid a complication which arises when mercuric oxide is added directly to the fluid in which the tissue slices have been allowed to metabolize for the duration of the experiment. Some substance appears to be liberated from the tissue slices which either forms ammonia in presence of mercuric oxide or which forms a complex with it which subsequently breaks down, liberating ammonia. This substance appears to be unaffected by the treatment with potassium carbonate, normal figures for ammonia production by the tissues (in absence of added amine) being then obtained.

Typical results

A. Recovery of NH₃ in stage (1)—distillation with potassium carbonate solution.

	mg.		mg.
NH ₄ Cl present	0.25	NH ₄ Cl recovered	0.25
„	0.50	„	0.49

B. Recovery of NH₃ after treatment with HgO (with and without warming) and NaOH.

	mg.		mg.
NH ₄ Cl present	0.25	NH ₄ Cl recovered	0.25 (with warming)
„	0.25	„	0.30 („)
„	0.25	„	0.15 (in the cold)
„	0.25	„	0.20 („)

C. *Recovery of NH_3 from a mixture of NH_4Cl and butylamine hydrochloride.*Amount of butylamine present = 0.5 ml. *M*/10 solution.

Treatment with HgO and NaOH.

	mg.		mg.
NH_4Cl present	0.1	NH_4Cl recovered	0.13
„	0.2	„	0.21
„	0.5	„	0.50

Blank, for HgO freshly treated with 5*N* NaOH, 0.05 mg.D. *Recovery of NH_3 from a mixture of NH_4Cl and butylamine hydrochloride.*Amount of butylamine present = 0.5 ml. *M*/10 solution.Distillation with K_2CO_3 , followed by treatment with HgO and NaOH.

	mg.		mg.
NH_4Cl present	0.25	NH_4Cl recovered	0.28
„	0.50	„	0.50

Blank, for HgO freshly treated with 5*N* NaOH, 0.06 mg.

SUMMARY

A method is described for the estimation of small quantities of ammonia in presence of volatile aliphatic amines. The method depends on the specific absorption of ammonia by yellow mercuric oxide.

One of us (C. E. M. P.) is much indebted to the Medical Research Council for a whole time assistance grant.

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