A CONDUCTIMETRIC METHOD FOR THE ESTIMATION OF SMALL QUANTITIES OF AMMONIA

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(*Received 22 July* 1957)

INTRODUCTION

Measurements of the ammonia content of fluids are frequently required in biological investigations. The need for these measurements arises not only in studies of the role of ammonia in nitrogen excretion, and in other metabolic processes, but also in connexion with work on many other nitrogenous compounds of biological interest. This is because the analytical procedures for estimating the concentration of many of these substances call for the quantitative analysis of ammonia in the final stage of the technique. The Kjeldahl method for the estimation of total nitrogen can be cited as perhaps the most familiar example of this method of analysis, but similar methods are also widely used for the evaluation of the concentration of urea, amide-N, amino-N and others.

Of the techniques which have been evolved for measuring small quantities of ammonia, that in which the ammonia contained in the sample is liberated as a gas and subsequently recovered in a solution of standard acid is to be preferred because of its ease and high accuracy. Of the various ways in which this has been performed Conway's diffusion method (Conway & Byrne, 1933; Conway, 1933, 1950) has proved the most versatile and adaptable. This method is simple and can be used for measuring quantities of ammonia down to about 1 μg. ammonia-N. Small versions of the diffusion unit, together with ultra-micro titration methods, have pushed the lower limit down to about 0·1 μg. ammonia-N (Glick, 1949; Shaw & Beadle, 1949; Conway, 1950; Kirk, 1950).

In considering the estimation of even smaller quantities of ammonia there is no reason to believe that the present level represents the lower limit of the diffusion method. The limit is set solely by the difficulties of the volumetric procedure for the titration of the absorbing acid. Thus, for the measurement of 1 μg. ammonia-N only 7 μl. of a 0·01 N solution are used for the back titration. To increase the volume of titrating fluid the standard solution can, of course, be diluted, but this leads to a greatly increased difficulty in end-point detection. The difficulties of end-point detection have been partially overcome by the use of a glass electrode assembly for the continuous measurement of the pH of the standard acid solution during titration (Borsook & Dubnoff, 1939), but this arrangement is rather cumbersome and not very suitable for measurements on very small drops.

Although many improvements could, no doubt, be made in titration techniques, it seems that any attempt to reduce the level of estimation to below 0·01 μg. ammonia-N, using the titration method, will come up against grave manipulative difficulties. One solution to the problem is to avoid titration of the standard acid and to use a colorimetric method for the estimation of the absorbed ammonia. This method has been used in micro-ammonia estimations by Borsook (1935) who utilized the blue colour produced by ammonia and an alkaline solution of phenol and hypochlorite. Kirk (1950, p. 284) outlines a procedure using micro-cells and a spectrophotometer, whereby the same colour reaction can be used for ultra-micro measurements with a lower practical limit of 0·01 μg. ammonia-N.

The method described in this paper solves the problem in an entirely different way. It is a conductimetric method which is both simple and reliable, and with the present apparatus allows accurate measurements to be made rapidly down to a level of about 1 mμg. ammonia-N (10^{-9} g.).

PRINCIPLE OF THE METHOD

The fact that the electrical conductance of a standard acid solution decreases as ammonia gas is absorbed forms the principle of the method. The conductance of the acid solution is made up of the separate contributions from hydrogen ions and from the acid anions. Free hydrogen ions are replaced by ammonium ions as ammonia is absorbed by the acid, and the decrease in conductance is due to the difference in mobility between these ions. Thus, for a standard solution of sulphuric acid at 25° C., the mobilities of H^+ and $\frac{1}{2}SO_4^{2-}$ are 350 and 79, respectively, so that the equivalent conductance at infinite dilution is 429 ohms^{-1} cm.^{2}. Ignoring hydrolysis, if the whole of the acid is neutralized by ammonia gas then all free hydrogen ions will be replaced by ammonium ions of mobility 74·5, and the equivalent conductance will fall to 135·5 ohms^{-1} cm.^{2}. The ratio of the conductance of the free acid to the neutralized acid is 2·79. If only 50% of the acid is neutralized then the conductance is the arithmetical mean of the two, and the relationship between conductance and percentage of acid neutralized is a linear one. Conductance measurements are sensitive and can be made with an accuracy at least equal to that of a volumetric procedure. In practice, no manipulations are required other than the transfer of the standard acid solution to the conductivity cell for measurement.

Before turning to the practical details of the method there are certain features of the behaviour of ions in solution which have been overlooked in the simple treatment given above. These are (*a*) that at finite dilutions the equivalent conductances of both acid and salt are somewhat reduced; (*b*) that ion impurities in the water containing the standard acid may make a significant contribution to the measured conductance, and that this may vary with the concentration of free hydrogen ions in the solution; and (*c*) that hydrolysis of the salt produced by neutralization may lead to high values for the conductance. Any of these three factors could affect the linearity of the relationship between measured conductance and the percentage

of the acid neutralized. The influence of each, therefore, will be considered in turn.

(*a*) Table 1 shows some values for the equivalent conductances of ammonium sulphate, and sulphuric acid solutions at given concentrations (from *International Critical Tables*). The conductances decrease in both cases as the concentration is increased, but the ratio of conductances remains constant (within the limits of experimental error) over the range 0–1·0 m-equiv./l. Thus, any dilution of acid within this range can be used. Further, although the concentration of hydrogen ions falls during the absorption of ammonia, it is replaced by an equivalent concentration of ammonium ions and the anion concentration is unaffected—thus the total ionic strength is maintained during absorption and there is no change in equivalent conductance due to this cause.

Table 1. *The equivalent conductances of sulphuric acid and ammonium sulphate solutions*

Concentration (m.-equiv./l.)	Equivalent conductances ($ohms^{-1}$ $cm.^2$)			Conductance ratio
	$\frac{1}{2}(NH_4)_2SO_4$		$\frac{1}{2}H_2SO_4$ 25° C.	
	18° C.	25° C.		
0·1	130	(150·2)	420	2·79
0·2	128	(147·8)	417·9	2 82
0·5	127	(146·3)	413·1	2·82
1 0	124·5	143·4	399·5	2·78
2·0	122	141·5	390·5	2·76

The figures are taken from *International Critical Tables* and those in brackets are calculated from the temperature coefficients.

(*b*) The chief source of ion impurity in the water is carbon dioxide absorbed from the atmosphere. Distilled water can be specially prepared (for example, by passing the first batch of water through a mixed ion exchange resin) with a conductance which is insignificant compared with that of the standard acid but, unless special precautions are taken, this rapidly increases to an equilibrium value due to the uptake of atmospheric carbon dioxide. The contribution of dissolved carbon dioxide, in equilibrium with the atmosphere, to the conductance of the water can easily be calculated from its concentration in the water ($1·7 \times 10^{-5}$ M; see Conway, 1950, p. 317), its dissociation constant ($3·0 \times 10^{-7}$) and the equivalent conductance (399·5). The specific conductance of the dissolved CO^2 is $0·85 \times 10^{-6}$ $ohms^{-1}/cm$. In acid solution the ionization of the carbon dioxide will be suppressed and hence its conductance greatly reduced. In Table 2 the specific conductances of some standard, CO^2-free sulphuric acid solutions are given together with similar figures for solutions partially and completely neutralized with ammonia. By the side of each is a value for the additional specific conductance due to dissolved CO^2, in equilibrium with the atmosphere, calculated in each case with due regard to the pH of the acid solution. The table shows that for an acid concentration of 1 m-equiv./l. the conductance due to CO^2 is for all practical purposes negligible. An acid solution of

0·1 m-equiv./l. can also be used providing that it does not absorb more than about 90% of ammonia necessary for complete neutralization. This is the practical lower limit for the dilution of the standard acid. At concentrations below this the conductance due to CO^2 is marked, and such solutions cannot be used unless special precautions are taken to use CO^2-free distilled water and to work in a CO^2-free atmosphere.

Table 2. *The specific conductances (ohms^{-1}/cm. $\times 10^{-6}$) of CO_2-free mixtures of sulphuric acid and ammonium sulphate solutions of the same concentration together with values for the additional specific conductance due to dissolved carbon dioxide.*

Ratio $\frac{H_2SO_4}{H_2SO_4+(NH_4)_2SO_4}$ (%)	Concentration of H_2SO_4 and $(NH_4)_2SO_4$ mixtures (m-equiv./l.)					
	1·0	CO_2 conductance	0·1	CO_2 conductance	0 01	CO_2 conductance
100	399 5	0·002	42·0	0·02	4·29	0·20
70	322·7	0·004	33 9	0·04	3·46	0 27
40	235 8	0·006	25·8	0·06	2·63	0·41
10	169 0	0·02	17·7	0·20	1·81	0·70
0	143·4	0·85	15·0	0·85	1 53	0·85

(*c*) The effect of hydrolysis of the ammonium salt on the measured conductance can be readily calculated. At a salt concentration of 0·1 m-equiv./l. the specific conductance is increased from 15 to $15{\cdot}07 \times 10^{-6}$ ohms^{-1}/cm. in the absence of dissolved CO_2. If CO_2 is present, as is generally the case, then the hydrolysis is greatly reduced by mutual interaction and its effect on the total conductance can be safely ignored.

The principles outlined above were tested. Standard solutions of sulphuric acid and ammonium sulphate were prepared in the same concentrations. The solutions were mixed in known proportions to simulate acid solutions in which known amounts of ammonia had been absorbed. The conductance of each mixture was measured in a small conductivity cell (this cell had a volume of 10 μl. and its construction is described below) and plotted against the proportion of H^+ to total cations in the mixture. The cell constant was measured in the usual way with standard potassium chloride solutions. The results for two different concentration (1·0 and 0·1 m-equiv./l.) are shown in Fig. 1. For the 1·0 m-equiv./l. solutions very good linearity was found and the ratio of conductances of acid and salt (2·75) agrees well with that predicted from theory (2·78; see Table 1). In the more dilute solutions the relationship is also linear until the acid concentration falls to about 0·02 m-equiv./l.; below this the effect of CO_2 increases the conductance in the manner predicted above. The slope of the straight part of the line corresponds to an acid/salt ratio of 2·78; again in excellent agreement with the predicted value of 2·79. In both cases the specific conductances approximate to the values expected from Table 2.

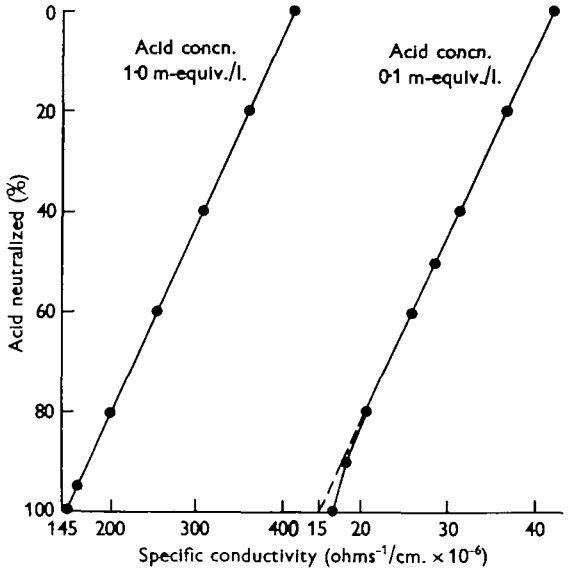

Fig. 1. The relation between specific conductivity and percentage of acid neutralized with ammonia in two standard sulphuric acid solutions.

PRACTICAL DETAILS

Ammonia liberated from the sample by alkali in a small diffusion chamber is absorbed in a small drop of standard acid. At the end of the diffusion period the acid drop is transferred to a small conductivity cell maintained at constant temperature and made up to a mark with distilled water. The conductance of the solution is measured and the percentage of the acid neutralized is calculated by reference to a calibration curve.

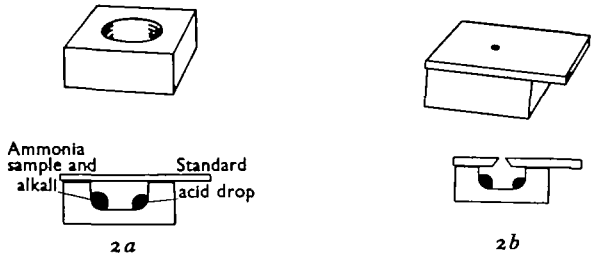

Fig. 2. The diffusion chamber. (*a*) The normal chamber together with a sectional view to show the position of the reagent drops. (*b*) The modified chamber for use at low ammonia levels together with a sectional view.

(*a*) *The diffusion procedure*

The diffusion apparatus (Fig. 2*a*) consists of a brass chamber, approximately 8 mm. in internal diameter and 3·5 mm. deep, and a glass lid which projects in one direction beyond the chamber, the projection forming a handle for ease of manipulation. The chamber is coated internally with paraffin wax.

The sample drop and the drop of strong alkali (3–5 μl. of half-saturated potassium metaborate solution) are placed close to each other in the bottom of the chamber on one side, the standard acid drop (1–3 μl. of a standard sulphuric acid solution, e.g. 0·001 N), from an ultra-micro pipette, is placed on the other. Next, the lid is sealed in position by means of a paraffin wax/liquid paraffin fixative (Conway, 1950, p. 93), and the alkali and sample drops mixed by tapping the chamber. The chamber is now left until complete absorption of ammonia has occurred.

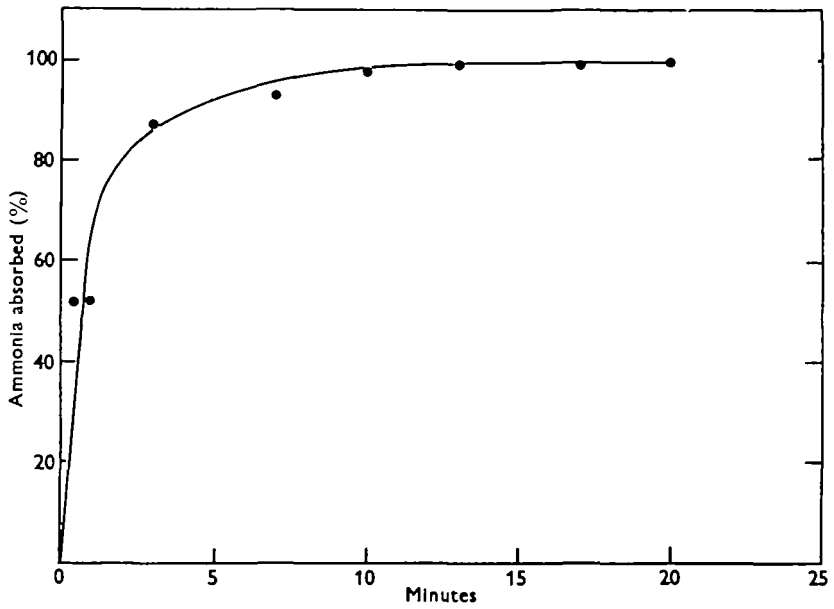

Fig. 3. The time course for the diffusion of ammonia (8·3 mμg. ammonia-N) into 1·48 μl. of 0·5 m.-equiv./l. H_2SO_4.

The progress of a typical diffusion at 20° C. is shown in Fig. 3. Diffusion is quite rapid. In the case illustrated it is complete in 20 min. For most conditions 30 min. can be regarded as a suitable period of time for diffusion.

In many laboratories ammonia may be present in the air in quantities sufficient to create an appreciable error in the measurement of the smallest quantities of ammonia (1–10 mμg.). The presence of extraneous ammonia can be revealed if a normal diffusion is performed without an ammonia-containing sample; a fall in the conductance of the standard acid drop indicates the uptake of ammonia. If detectable quantities of ammonia are present it is necessary to employ a slightly modified version of the diffusion apparatus (Fig. 2*b*), the interior of which can be cleared of ammonia before commencing an actual diffusion. The chamber remains unchanged, but the lid, now made of Perspex, has a small hole drilled in the centre. Prior to diffusion, alkali and a drop of standard acid are introduced into the chamber, and the lid then sealed into position (an alternative, which has been found very satisfactory in practice, is to seal the lid into position with wax, by applying to the

chamber when the coating wax is still molten, and later to introduce all solutions through the hole in the lid; the wax seal is completely effective and easily broken when necessary). The small hole in the lid is temporarily covered with a microscope cover-slip. The apparatus is left for a period of 30 min. during which time any extraneous ammonia in the chamber is absorbed by the standard acid drop. At the end of this period the acid drop is removed by inserting a pipette through the small hole, and the sample and another drop of standard acid introduced. Between the introduction or removal of solutions the cover-slip remains over the aperture. Finally, the cover slip is removed, the small hole immediately sealed with a small blob of hot wax, and the sample and alkali drops mixed. The diffusion process now proceeds in the normal way.

(*b*) *Conductance measurement*

The conductivity cell is made in Perspex and consists of three pieces bolted together in the form of a sandwich, the middle piece being drilled to make the cell (see Fig. 4). On the inner faces of the outer pieces small circles of bright platinum foil (cleaned in chromic acid and very well washed) just larger than the diameter of the cell are cemented. These form the two electrodes and thin strips from them are brought out through the lower bolt holes, the bolts of which are used as the electrode terminals. A narrow hole in the edge of the middle piece and at right angles to the cell serves as a neck for filling the cell. The cell is filled to a mark about half-way up the neck. The edges of the block are polished so that the cell and its contents can be viewed from front and back and also from the sides. The dimensions of the cell depend on the range of ammonia estimations being made. Thus, in the lowest range the cell diameter is $\frac{1}{16}$ in. and length also $\frac{1}{16}$ in. giving a volume, including the neck region, of about 5 μl. By increasing the diameter of the cell, or by increasing the thickness of the Perspex forming the middle piece, greater volumes can be accommodated.

When in use the block containing the cell is bolted to a long narrow piece of Perspex which serves as a handle for inserting the cell into a tube immersed in a thermostatically controlled water-bath.

The cell electrodes can be connected to any suitable conductivity bridge—a Mullard type E7566 can be used if it is provided with additional standard resistances so that the readings can always be brought to the middle part of the potentiometer scale.

The cell is thoroughly washed with distilled water before introducing the standard for estimation. Transfer of the standard is effected by means of a capillary pipette of capacity approximately four times greater than that of the cell. Before sucking in the standard, the pipette is half-filled with distilled water. The point of the pipette is introduced down the neck to the bottom of the cell, and then the acid sample is blown out together with sufficient water to fill the cell on the withdrawal of the pipette. The distilled water remaining in the pipette serves as a barrier preventing exhaled CO_2 from contaminating the cell contents.

If the sample volume is small compared with the volume of the neck the cell need

never be emptied of fluid; an advantage if it shows a propensity to trap small air bubbles beneath the shoulders against the surface of the electrodes. The cell is flushed with water and drained to the base of the neck only.

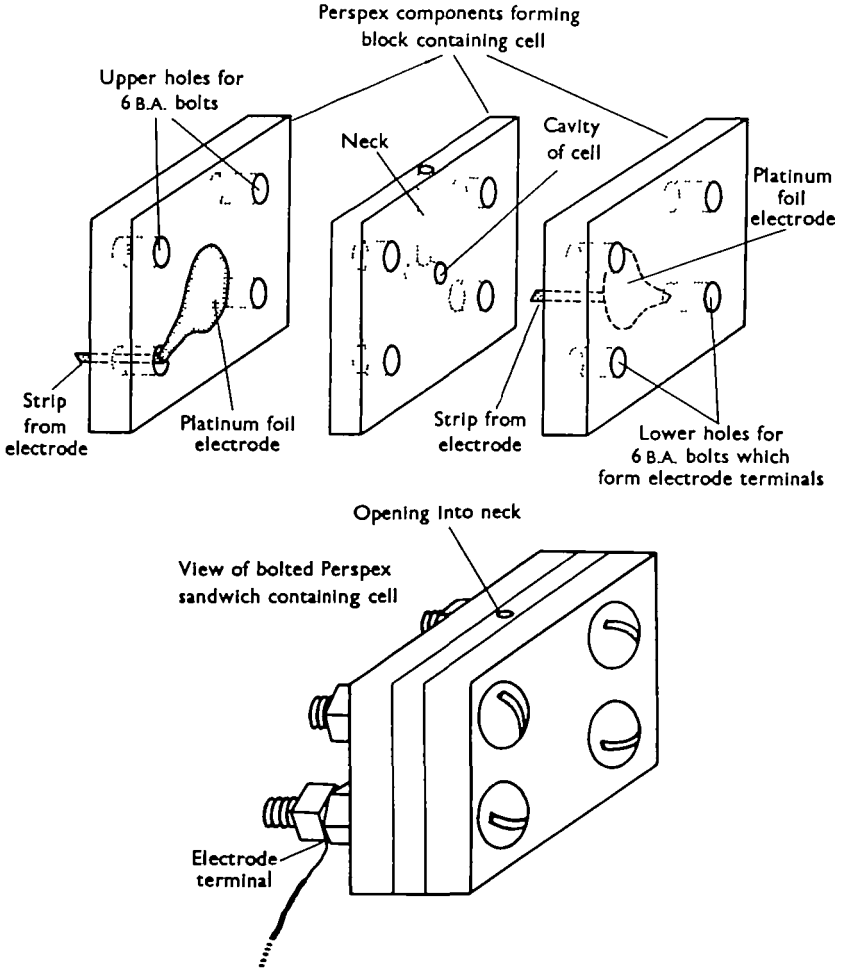

Fig. 4. Details of construction of a conductivity cell.

The cell, when filled, is lowered by means of the Perspex handle to the bottom of a Perspex (or thin glass) tube which is immersed, almost to the top, in a temperature-controlled water-bath. (Any delay experienced waiting for temperature equilibrium can be avoided by washing and filling the cell *in situ*, but this calls for greater care.)

The fluid in the cell is now thoroughly stirred for a period of $\frac{1}{2}$ min. The stirrer consists of a thin polythene rod attached to a narrow glass rod which acts as a handle. Stirring is accomplished by introducing the polythene rod through the neck of the cell and spinning the glass handle between the forefinger and thumb of one hand.

When a cell is used for the first time a calibration curve must be produced. For this purpose a series of mixtures are prepared from standard sulphuric acid and ammonium sulphate solutions of the same concentration. The primary standards, 0·1 N-H_2SO_4 and 0·1 N-$(NH_4)_2SO_4$, are stored in polythene bottles. These primary standards are mixed in different proportions and suitably diluted when required for the preparation of the calibration curve. The conductance of each mixture is determined and the results obtained plotted against the proportion of hydrogen ions to total cations.

With new cells the first readings on acid solutions are sometimes too low so that the cell should be washed out several times with the solution until a consistent reading is obtained. Low readings may also occur if a cell has been left for a period without use. Prior to a diffusion analysis, therefore, it is advisable to charge the cell several times with a volume and concentration of standard acid identical with that which will be employed in the diffusion chamber until a constant value for the conductance is obtained. This value should be checked against the value obtained in the original calibration.

RESULTS

Over a hundred measurements on standard ammonium sulphate solutions ranging in content from 2 mμg. to 12 μg. N are summarized in Table 3. Conductivity cells of different sizes were used for the various ranges. A standard commercial cell, 2 ml. in volume, was found to be satisfactory for estimating quantities of ammonia ranging from 0·5 to 12 μg. N. For all other ranges Perspex cells were used. The recovery of ammonia was invariably complete although sometimes a little in excess, particularly with respect to the smallest quantities estimated in certain ranges.

Measurements were made on samples containing respectively 20 and 80% of that quantity of ammonia required to neutralize the standard acid fully. These quantities approach the limits of the range. From Table 3 it is evident that measurements can

Table 3. *Ammonium sulphate solutions*

Range nmonia-N (μg.)	Ammonia-N expected (μg.)	Standard acid neutralized (%)	Ammonia-N found (μg.)	% recovery	No. of measure-ments	Standard deviation (μg. ammonia-N)	Standard deviation (%)
2·5–12	11·92	80	11·96	100·3	11	0·11	0·92
	2·98	20	3·15	105·7	18	0·09	2·86
0·5–2·5	2·38	80	2·40	100·8	7	0 02	0·83
	0·60	20	0·60	100·0	7	0·01	1·67
(mμg.)	(mμg.)		(mμg)			(mμg. ammonia-N)	
30–150	143·3	80	143	99·8	9	0·6	0·42
	35·8	20	36	100·6	10	1·2	3·33
7–32	30·24	80	30·4	100·6	19	0·4	1·32
	30·24	80	30·3	100·1	8	0·4	1·32
	7·56	20	7·8	103·2	10	0·3	3·85
	7·56	20	8·0	105·8	8	0·4	5·0
1–9	8·28	80	8·24	99·5	8	0·06	0·73
	2·07	20	2·14	103·0	9	0 08	3·74

be made in any range with an error $< \pm 4\%$ (standard deviation); with an error $< \pm 1\%$ provided that 80% of the standard acid is neutralized by ammonia. The percentage error of measurements in the range 7–32 mμg. ammonia-N is somewhat higher but this, no doubt, can be reduced. Mid-range quantities of ammonia, neutralizing 50% of the standard acid, can be expected to show a standard deviation, of ± 1–2% in all ranges. The method can, therefore, measure quantities down to 5 mμg. ammonia-N with an error $< \pm 2\%$, provided that at least 50% of the acid is neutralized during diffusion. Measurements on samples containing 1 mμg. ammonia-N can be expected to show a standard deviation of ± 6–8%.

Table 4. *Biological fluids*

Species	Material	Vol. of sample (μl.)	No. of measurements	Ammonia-N (mμg.)	Standard deviation (mμg.)	Conc. of ammonia-N (mg. N/100 ml
Aeshna cyanea, nymph	Haemolymph	0·90	6	1 5	0 07	0·17
		0·58	6	3·1	0·07	0·53
		0·58	7	1 3	0·07	0·22
Sialis lutaria, larva	Haemolymph	2·22	3	18 0	—	0·81
		2·22	3	5·6	—	0·25
	Rectal fluid	0·10	3	132	—	132
		0·06	3	66	—	110
Carcinus maenas, adult	Haemolymph	2 99	22 (from 6 animals)	—	—	0·32
	Single muscle fibres	—	16 (from 4 animals)	—	—	3·93

Some measurements on biological materials are listed in Table 4. The measurements on haemolymph and excretory fluid hardly require comment, except that there was no evidence of ammonia production in the shed haemolymph of either *Aeshna*, *Sialis* or *Carcinus*, and that little more than 0·001 μl. would have sufficed for a single estimation on the rectal fluid of *Sialis*, in which the concentration of ammonia frequently exceeds 100 mg. N/100 ml. The measurements obtained on single muscle fibres from *Carcinus maenas* illustrate the utility of the method in determining the ammonia content of large cells. The dissected fibre, placed directly in the diffusion chamber, is disrupted by the strong alkali and the free ammonia liberated is collected in the usual manner.

Sufficient estimations were made on the blood of *Aeshna* nymphs to enable standard deviations to be calculated. The standard deviations obtained were of the same order as standard deviations calculated from measurements on solutions of ammonium sulphate.

No attempt has been made to reduce the level of ammonia estimation below 1 mμg. N, although there seems no doubt that this can be done if necessary. The smallest conductivity cell employed in this investigation had a volume of 5 μl., but smaller cells can be made. Bayliss & Walker (1930) used for their studies on the

amphibian glomerular fluid a cell with a volume as small as 0·5 μl. Although their cell had a number of defects the principle of using the cut-ends of pieces of platinum wire as electrodes could, no doubt, be used to make successful micro-cells.

SUMMARY

1. A method is described for the estimation of small quantities of ammonia down to 0·001 μg. N.

2. The principle of the method is that ammonia is liberated from the sample and recovered in a solution of standard acid by Conway's diffusion method; the electrical conductance of the acid falls as ammonia is absorbed, due to the difference in the mobility of hydrogen and ammonium ions.

3. The results of estimations on standard ammonium sulphate solutions and biological fluids are tabulated. The method can deal with quantities down to 0·005 μg. ammonia-N with an error $< \pm 2\%$ (standard deviation), 0·001 μg. ammonia-N with an error ± 6–8%.

4. An account is given of the construction of small diffusion chambers and small conductivity cells.

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