

## THE DETERMINATION OF AMMONIA AND TOTAL IONIC INORGANIC NITROGEN IN SEA WATER

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(Text-figs. 1 and 2)

Combined inorganic nitrogen occurs in sea water principally as nitrate, nitrite and ammonium ions, the concentrations of which lie in the ranges 1-600 mg  $\text{NO}_3^-$ -N/m<sup>3</sup>, 0.1-50 mg  $\text{NO}_2^-$ -N/m<sup>3</sup> and 5-50 mg  $\text{NH}_4^+$ -N/m<sup>3</sup> respectively. Nitrogen occurring in any of these forms is readily assimilable by marine organisms, and its exhaustion in sea water is frequently a growth-limiting factor in the water. This paper describes a method for the determination of the total ionic inorganic nitrogen in sea water, based on preliminary reduction to ammonia followed by separation and estimation of the latter colorimetrically.

Riley (1953) has discussed the determination of ammonia in sea water, and has concluded that ammonia is best separated from the water, adjusted to pH 9.2, by distillation under reduced pressure in a current of air as described by Krogh (1934). The distillation process is rather time-consuming and requires a special apparatus. Experiments were therefore carried out to separate the ammonia by diffusion. It was found that the capacity of the concentric microdiffusion cells employed by Conway (1950, p. 8) was too small for the volume of sea water which was necessary if low concentrations of ammonia were to be determined. When diffusion was carried out in the flasks (Fig. 1) described by Cavett (1937) for the microdetermination of alcohol in blood, reproducible recoveries of approximately 73% of added ammonium salt were obtained from 50 ml. of sea water after diffusion at 70° C for 24 h at pH 9.2 (Table 1).

Much of the earliest work on the determination of nitrate in sea water was based on its reduction to ammonia, which was separated by distillation and determined by Nessler's method. Few of the reducing agents (magnesium, Raben, 1905*a*, sodium amalgam, Raben, 1905*b*; aluminium amalgam, Raben, 1910, 1914; Devarda's alloy, Brandt, 1927; iron-zinc couple, Thorpe & Morton, 1871; aluminium and sulphuric acid, Gad-Andresen, 1923) which have been used for this purpose are satisfactory if the ammonia is to be separated by diffusion, owing to the evolution of hydrogen which causes pressure to develop in the Cavett flask. Attempts to carry out the reduction using ferrous hydroxide and titanous salts gave very poor recoveries of ammonia.

Raney nickel (semi-colloidal nickel prepared by the action of strong alkali on nickel-aluminium alloy) has been used by Van Dalen (1951) as a catalyst in the hydrogenation of macro amounts of nitrate to ammonia. Since the catalytic nickel contains up to 120 ml. H/g (Mozingo, Wolf, Harris & Folkes, 1943) it was thought that microgram quantities of nitrate could be reduced to

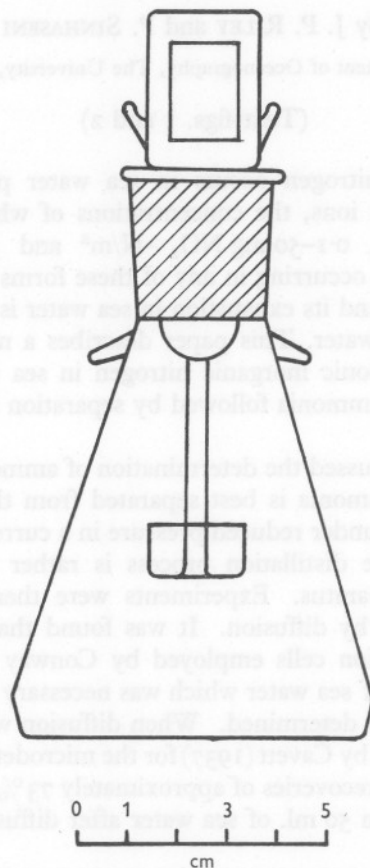


Fig. 1. A Cavett flask as used for the microdiffusion of ammonia.

TABLE 1. RECOVERY OF AMMONIA FROM 50 ML. OF SEA WATER CONTAINING ADDED AMMONIUM SULPHATE AND 10 ML. OF METABORATE BUFFER DIFFUSED AT 70° C FOR 20 H.

Added	$\mu\text{g NH}_4^+-\text{N}$		Mean recovery (%)
	Found		
0.0	0.0		—
2.5	1.7	1.7 1.8	69
5.0	3.5	3.6 3.6 3.7	72
10.0	7.2	7.5	73

ammonia by the action of its adsorbed hydrogen. This proved to be the case. It was found that the reduction proceeded much more rapidly in the presence of ethylenediamine-tetraacetic acid (EDTA) which also served to reduce the quantity of magnesium and calcium salts precipitated from the sea water at the pH of 10.5 used in the reduction and diffusion.

#### COLORIMETRIC DETERMINATION OF AMMONIA

The advantages of the phenate-hypochlorite method over the Nessler method for the colorimetric determination of ammonia have been enumerated by Riley (1953). Crowther & Large (1956) have found that the indophenol-blue colour develops rapidly at room temperature if the sodium phenate reagent is made up in an aqueous mixture of acetone and methyl alcohol instead of water.

TABLE 2. EFFECT OF VARIATION OF VOLUMES OF SODIUM PHENATE AND SODIUM HYPOCHLORITE REAGENTS ON OPTICAL DENSITIES OF SOLUTIONS MEASURED AT  $625 \text{ M}\mu$  IN A 1 CM CELL ( $10 \mu\text{G NH}_4^+-\text{N}$  IN FINAL VOLUME OF 10 ML.)

ml. of hypochlorite*	0.5	1.0	1.5	2.0	2.5	3.0
0.5 ml. of phenate†	0.387	0.340	0.280	0.234	0.185	0.165
1.0 ml. of phenate†	0.372	0.380	0.373	0.370	0.350	0.328
1.5 ml. of phenate†	0.273	0.320	0.337	0.370	0.365	0.352
2.0 ml. of phenate†	0.163	0.280	0.310	0.340	0.355	—

\* 0.9% available chlorine.

† In methanol containing acetone.

In order to find the optimum conditions for the determination of ammonia using Crowther's reagent, determinations were carried out using  $10 \mu\text{G NH}_4^+-\text{N}$  and varying the amounts of both sodium phenate (prepared according to Crowther & Large, 1956) and sodium hypochlorite added. The optical densities of the solutions were measured at  $625 \text{ m}\mu$  after 1 h. The results, which are given in Table 2, indicated that 1 ml. of sodium phenate and 1.5 ml. of sodium hypochlorite were the most suitable volumes. Under these conditions, which were adopted for all subsequent work, the maximum optical density was attained after 45 min at  $20^\circ \text{C}$ , and remained constant for a further 60 min, after which time slow fading occurred.

A calibration curve was prepared using known amounts of ammonium salt (Fig. 2); it indicated that Beer's law is obeyed up to a concentration of  $1.5 \mu\text{G NH}_4^+-\text{N}/\text{ml}$ . (in a final volume of 10 ml.). Above this concentration the change in optical density per unit weight of  $\text{NH}_4^+-\text{N}$  is considerably reduced. Replicate determinations (6) carried out with 5 and  $10 \mu\text{G NH}_4^+-\text{N}$  gave mean optical densities (less blank) of  $0.190 \pm 0.001$  and  $0.377 \pm 0.002$ .

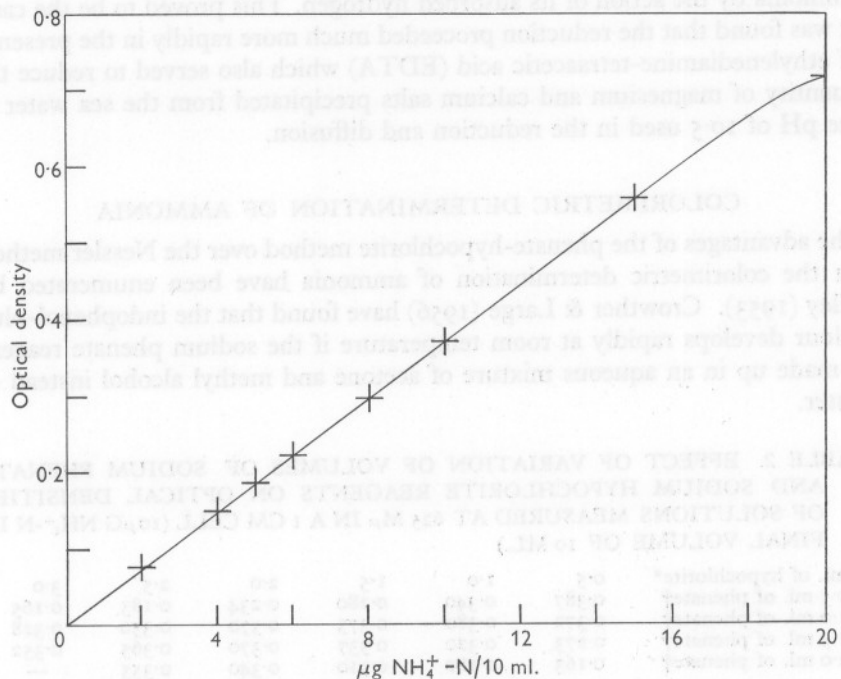


Fig. 2. Calibration chart of the method for the determination of ammonia in sea water, prepared by using known amounts of ammonia. Optical density at  $625 \text{ m}\mu$  using a 1 cm cell.

#### METHOD

All measurements of optical density were made with a Unicam S.P. 500 spectrophotometer using 1 cm glass cells, against distilled water in the compensating cell.

#### REAGENTS

All reagents and solutions must be made up with water which has been freshly distilled from dilute sulphuric acid in an all-glass still.

##### 1. Metaborate buffer

Dissolve 2.55 g of sodium metaborate and 2.0 g of sodium hydroxide in distilled water, dilute to 100 ml.

##### 2. Raney nickel

Weigh out *c.* 5 g of powdered nickel-aluminium alloy, (50/50) and add it gradually to a 250 ml. beaker containing a solution of 10 g of sodium hydroxide in 70 ml. of distilled water. After the addition has been completed, heat the covered beaker on a hot plate for 30 min, when all effervescence should have

ceased. Wash the finely divided nickel with hot water by decantation, until the washings are free from alkali. Preserve it under water and do not allow it to become dry. Fresh Raney nickel should be prepared at least weekly.

Caution: care should be taken when disposing of waste Raney nickel as it is pyrophoric when dry.

### 3. EDTA reagent

Dissolve 4 g of ethylenediamine-tetraacetic acid (disodium salt) in 100 ml. of distilled water.

### 4. Sodium hydroxide (2N)

Gently boil a solution of 8 g of sodium hydroxide in 60 ml. of water for a few minutes. When cool, dilute to 100 ml.

### 5. Hydrochloric acid (0.02N)

Dilute 2 ml. of concentrated hydrochloric acid to 1000 ml.

### 6. Sodium phenate reagent

(a) Phenol solution (solution A). Dissolve 62.5 g of phenol B.P. in methyl alcohol, add 20 ml. of acetone and dilute to 100 ml. with methyl alcohol. The reagent, which should be prepared each week, must be kept in a refrigerator.

(b) Sodium hydroxide (solution B). Dissolve 27 g of sodium hydroxide A.R. in c. 60 ml. of distilled water, boil gently for a few minutes to remove ammonia, cool and dilute to 100 ml.

For use mix 20 ml. portions of solutions A and B and dilute to 100 ml. The mixed reagent should be prepared freshly as required.

### 7. Sodium hypochlorite reagent

The reagent solution is prepared by dilution of commercial sodium hypochlorite solution and contains 0.9 g of available chlorine per 100 ml. The strength of the stock solution should be checked periodically.

## STANDARD SOLUTIONS

### 1. Ammonium sulphate

Weigh out 0.4716 g of ammonium sulphate A.R., dissolve in water and dilute to 1000 ml. This solution, which contains  $100 \mu\text{g NH}_4^+\text{-N/ml}$ . is used for the preparation of the working solution containing  $2.5 \mu\text{g. NH}_4^+\text{-N/ml}$ .

### 2. Potassium nitrate

Prepare a solution of potassium nitrate containing  $10 \mu\text{g NO}_3^-\text{-N/ml}$ . by dissolving 0.0722 g of the A.R. grade salt in water and diluting to 1000 ml. Prepare from this a working solution containing  $2.5 \mu\text{g NO}_3^-\text{-N/ml}$ .

### 3. *Sodium nitrite*

Dissolve 0.1099 g of recrystallized silver nitrite in about 10 ml. of boiling water, and add while still hot *c.* 0.10 g of sodium chloride. Shake until the silver chloride flocculates, set aside in the dark until the solution has cleared. Dilute to 1000 ml. From this solution, which contains  $10 \mu\text{g NO}_2^- \text{-N/ml.}$ , prepare a working solution containing  $2.5 \mu\text{g. NO}_2^- \text{-N/ml.}$

#### TREATMENT OF FLASKS

Wash all Cavett flasks and graduated flasks with concentrated hydrochloric acid. Rinse with distilled water, then with 0.1N sodium hydroxide and again with water, allow them to drain. Lubricate the upper part of the ground surface of the Cavett flask stoppers with a mixture of equal weights of paraffin wax (congealing point  $49^\circ \text{C}$ ) and medicinal paraffin.

#### DETERMINATION OF AMMONIA IN SEA WATER

Pipette 50 ml. of filtered sea water into a 150 ml. conical flask fitted with a B24 socket, add 2 ml. of 4% EDTA reagent and raise to boiling on the hot plate. Remove from the heater and add *c.* 2 ml. of sodium metaborate buffer from a fast running pipette. Immediately close the flask with a lubricated Cavett stopper (Quickfit and Quartz Catalogue No. BC<sub>3</sub>CH), the cup of which contains 1 ml. of 0.02N hydrochloric acid. Gently shake the stoppered flask and then heat in an oven at  $70^\circ \text{C}$ . After 24 h remove the flask from the oven and allow to cool for a few minutes. Transfer the hydrochloric acid in the cup to a 10 ml. graduated flask by means of a drawn-out glass tube fitted with a rubber teat. Wash the cup well with distilled water and transfer the washings to the graduated flask. To the combined solutions add 1 ml. of sodium phenate reagent and 1.5 ml. of sodium hypochlorite solution, dilute to volume and mix thoroughly. After 45–60 min measure the optical density at  $625 \text{ m}\mu$ . Run a reagent blank on 10 ml. of distilled water in the same manner. Calibrate the method by carrying out a determination on 50 ml. of the same sea water to which  $5 \mu\text{g NH}_4^+ \text{-N}$  has been added.

#### DETERMINATION OF TOTAL IONIC INORGANIC NITROGEN IN SEA WATER

Pipette 50 ml. of sea water into a 150 ml. conical flask fitted with a B24 socket, add from a small scoop *c.* 0.2 g of Raney nickel and 1 ml. of 4% EDTA reagent. Heat the solution on a hot plate and allow to boil gently for 15 min. Remove from the heater and add rapidly 2 ml. of 2N sodium hydroxide. Immediately close the flask with a well-lubricated Cavett stopper, the cup of which contains 1 ml. of 0.02N hydrochloric acid. Place in an oven at  $70^\circ \text{C}$  to allow the ammonia to diffuse. After 24 h determine the ammonia as described above.

Carry out a blank determination on 10 ml. of distilled water and a calibration run on the same sea water, to which has been added  $5 \mu\text{g NO}_3^-$  or  $\text{NO}_2^-$ -N.

Great care must be taken to avoid contamination by extraneous nitrogen compounds during the determination; in particular, the apparatus should not be handled more than is absolutely necessary.

It is important also to take approximately the same amount of Raney nickel in each determination and in the blank, since it always yields small amounts of ammonia during the diffusion.

### RESULTS

In order to test the recovery of nitrogen from sea water, samples of sea water very low in inorganic nitrogen were enriched by the addition of known amounts of nitrate, nitrite, or ammonium-nitrogen. These samples were then analyzed for total nitrogen as described on p. 166. The results are shown in Table 3. They show that the reduction is complete since a recovery of  $73 \pm 4\%$

TABLE 3. RECOVERY OF INORGANIC NITROGEN FROM SEA WATER

Nitrogen added	Nitrogen recovered ( $\mu\text{g N}$ )							Average recovery	Percentage recovery
Sea water only	0							0	—
$2.5 \mu\text{g NH}_4^+$ -N	1.9	1.9	1.7	1.8	1.7	2.0	2.0	1.86	74
$5.0 \mu\text{g NH}_4^+$ -N	3.5	3.4	3.3	3.6	3.5	3.6		3.48	70
$10.0 \mu\text{g NH}_4^+$ -N	7.6	7.6	7.5	7.5	7.3	7.7		7.53	75
$2.5 \mu\text{g NO}_3^-$ -N	2.0	1.7	1.8	2.1	2.1	1.9		1.92	77
$5.0 \mu\text{g NO}_3^-$ -N	3.5	3.7	3.7	3.6	3.7			3.64	73
$10.0 \mu\text{g NO}_3^-$ -N	7.1	7.3	7.5	7.7	7.6	7.7	7.2	7.42	74
$2.5 \mu\text{g NO}_2^-$ -N	1.5	1.7	1.8					1.69	68
$5.0 \mu\text{g NO}_2^-$ -N	3.8	3.9	3.3	3.7	3.4	3.4	3.9	3.62	72
$10.0 \mu\text{g NO}_2^-$ -N	7.5	7.3	7.6	7.9				7.58	76
								Mean	73%

of nitrate, nitrite and ammonium-nitrogen can be obtained using a diffusion time of 24 h. Higher recoveries can be obtained with longer diffusion periods, but the results are less reproducible. Using the recommended conditions, replicate determinations (7) were carried out on a sample of Irish Sea water; they showed that the water contained  $240 \mu\text{g N/l.}$  with a coefficient of variation of 2.5%.

In order to investigate the possible interference which might be caused by the breakdown of organic nitrogen compounds during the diffusion, the determination was carried out in the presence of a number of typical organic nitrogen compounds. No interference was experienced using  $20 \mu\text{g}$  of DL- $\alpha$ -alanine, L-arginine hydrochloride, cystine, glutamic acid, urea, or choline chloride.

## SUMMARY

Microdiffusion using a Cavett flask has been employed instead of vacuum distillation for the separation of ammonia from sea water; diffusion is approximately 75% complete after 24 h at 70° C. The recovered ammonia is determined by a modification of the phenate-hypochlorite method.

Raney nickel in the presence of ethylenediamine-tetraacetic acid has been used for the reduction of nitrate and nitrite to ammonia. The latter is separated by microdiffusion and determined colorimetrically. The method showed a coefficient of variation of 2.5% on a sample of sea water containing

$$240 \mu\text{g NO}_2^- + \text{NO}_3^- + \text{NH}_4^+ - \text{N/l.}$$

No interference was encountered from a variety of organic nitrogen compounds.

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