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STUDIES ON THE ANALYTICAL METHOD OF AMMONIA AND ORGANIC NITROGEN IN SEA WATER

By

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Methods for the estimation of the ammonia and the organic nitrogen in sea water have developed by many investigators during the past thirty years. The Nessler's direct colorimetric analysis of ammonia has been verified to be satisfactory for use in most fresh waters. For the sea water, however, a difficulty arises that the Nessler's reagent which is strongly alkaline makes the magnesium and calcium compounds in the sea water precipitate and as a result, the samples become so turbid that the colorimetry is made impossible in spite of the precaution taken (3).

On the other hand, the estimation of the organic nitrogen in sea water involves elaborate procedures and about 250 cc of samples are necessary for successful results and it requires at least 2 or 3 hours for the complete wet combustion of organic matter and the distillation of ammonia.

Recently, another method with regard to the determination of ammonia based on a different principle has been developed by M. Buljan (1). This method depends upon the reaction of the ammonia to sodium hypobromite in an alkaline medium, when



If the excess amount of hypobromite for the ammonia is added to the sea water, the amount of ammonia in sea water can be measured from the consumption of hypobromite.

M. Buljan has estimated the hypobromite colorimetrically by adding Bordeaux B dye solution. Similarly, H. W. Harvey (2) estimated it with a titration by using the so-called 'iodine method'. In the present work, Harvey's method is modified to a colorimetric method for the analysis of ammonia and organic nitrogen in sea water in which the color formation is based on the reaction of starch-dextrin mixture for the iodine evolved by the hypobromite from the potassium iodide in acid medium.

I. Reagents

- i) Sodium hypobromite 0.005 *N*, made by diluting 0.1 *N* stock solution, which is prepared by dissolving 2.5 g NaOH and 1.25 cc fluid bromine in 500 cc of distilled water. This 0.005 *N* NaBrO is made alkaline by adding 1 per cent of 2 *N* Na₂CO₃.
- ii) Alkaline buffer solution. 40 g of potassium hydroxide, 6 g of boric acid and 10 g of sodium carbonate are dissolved in 100 cc of distilled water. This solution is boiled until the ammonia in the solution is removed.
- iii) Acetic acid. Three hundred cc of glacial acetic acid are diluted to 1000 cc with distilled water.
- iv) Potassium iodide crystals.
- v) Starch-dextrin mixture, freshly prepared. This mixture is made by mixing 2 per cent starch solution and 2.5 per cent dextrin solution in the same volume.
- vi) 30 per cent H₂O₂.
- vii) 50 per cent sulphuric acid.
- viii) Standard ammonium chloride solution. 0.535 g of ammonium chloride is dissolved in 1000 cc of ammonia free distilled water. One cubic centimeter contains 10 μ g-atoms N.
- iv) Thymolphthalein indicator. 0.04 g thymolphthalein is dissolved in 20 cc of alcohol and made up to 100 cc with distilled water.

II. Procedure

1. *The determination of ammonia in sea water.*

Exactly 25 cc of the sea water sample is transferred to a 50 cc flask by a pipette. One cubic centimeter of 0.005 *N* sodium hypobromite and one drop of alkaline buffer solution are added. After the ammonia in the sample has reacted with the sodium hypobromite, a piece of potassium iodide crystal, one cc of acetic acid and two cc of starch-dextrin mixture are added respectively. The extinction of blue color formed by iodine-starch reaction is estimated with a spectrophotometer at a wave length of 580 $m\mu$ using a 10 mm cuvette.

A series of standard is made up from the standard ammonium chloride solution. The blue color is developed by the same procedure as the treatment of the sample, and the intensity of color is measured. A standardization curve of ammonia thus obtained is shown in Fig. 1.

2. *The determination of organic nitrogen in sea water.*

Exactly 25 cc of the sea water sample is transferred to a 50 cc Kjeldahl

flask by a pipette. Two cubic centimeters of 50 per cent sulphuric acid and two drops of H_2O_2 are added. The flask is heated on an electric heater until the organic matter in the sample is completely incinerated. The heating is put in practice slowly to protect the bubbling of the sample and it is continued until copious fumes of sulphuric acid are given off. After cooling the flask until it can be held in the hand, the fluid in the flask is diluted to 50 cc with ammonia free water and this acid solution is supplied to the analysis of the ammonia.

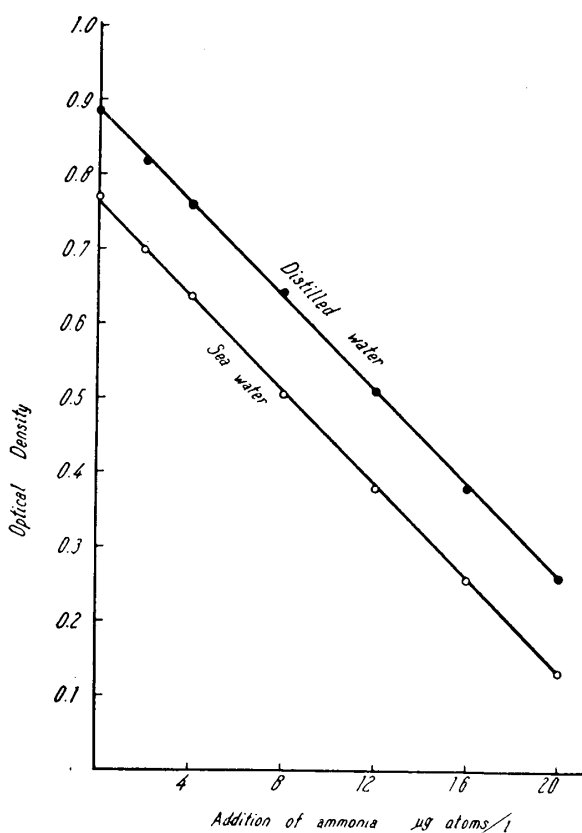


Fig.1 Relationship between concentrations of ammonia and optical densities of hypobromite at a wave length of $580m\mu$.

One cubic centimeter of 0.005 N sodium hypobromite solution is added to 25 cc of the acid solution by using a micro-burette and immediately, the requisite amount of the alkaline buffer solution for neutralising the solution to the pH of 10.5 is added. This amount of the alkaline buffer solution is determined by titrating another part of the acid solution using thymolphthalein as an indicator. After one minute a piece of potassium iodide crystal, 2 cc of acetic acid and 2 cc starch-dextrin mixture are added. The extinction of blue colour is measured with a spectrophotometer at a wave length of $580 m\mu$ using a 10 mm cuvette.

A series of nitrogen standard, 0, 8, 16, 24, 32 and 40 μg -atoms/l is prepared from a standard ammonium chloride solution. The analysis of the nitrogen standard is made in the same manner as the sample and a standardization curve of nitrogen as shown in Fig. 2 is obtained. The nitrogen of the samples calculated from this standardization curve gives the total nitrogen which does not include nitrite and nitrate nitrogen and therefore the organic nitrogen in the samples is obtained by subtracting the free ammonia nitrogen as determined by the direct method from the total nitrogen.

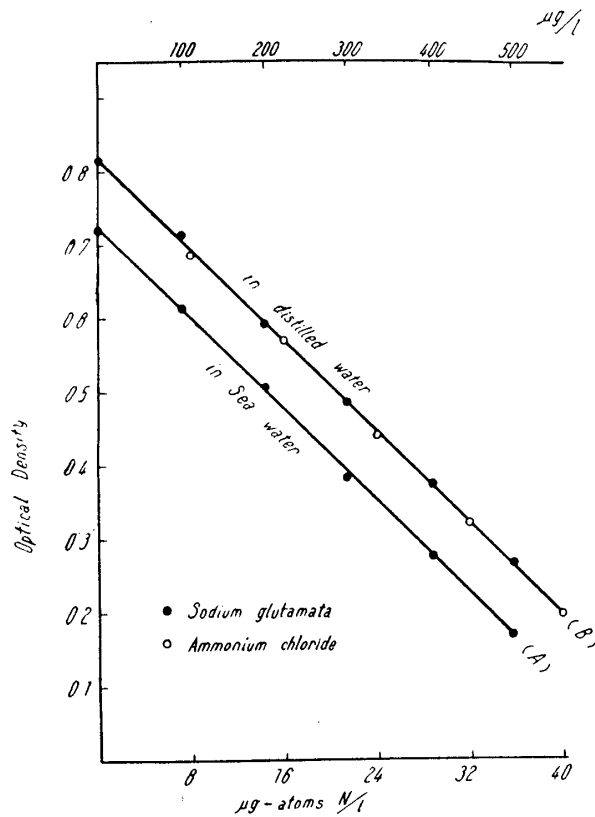


Fig. 2. Relationship between the concentrations of nitrogen and optical densities of hypobromite at a wave length of 580m μ .

III. Experimental notes

1. *Maximum light absorption of the blue color formed by iodine-starch reaction.*

As a preliminary experiment, the optical density-absorbency or extinction of iodine-starch blue color was measured with a spectrophotometer at the range of wave length from 360 m μ to 700 m μ with a 10 mm cuvette. The results of the measurement is shown in Fig. 3. From the figure it is evident that the maximum absorption of iodine-starch blue color takes place at a wave length of 580 m μ . Therefore, all measurements of the optical density have been made

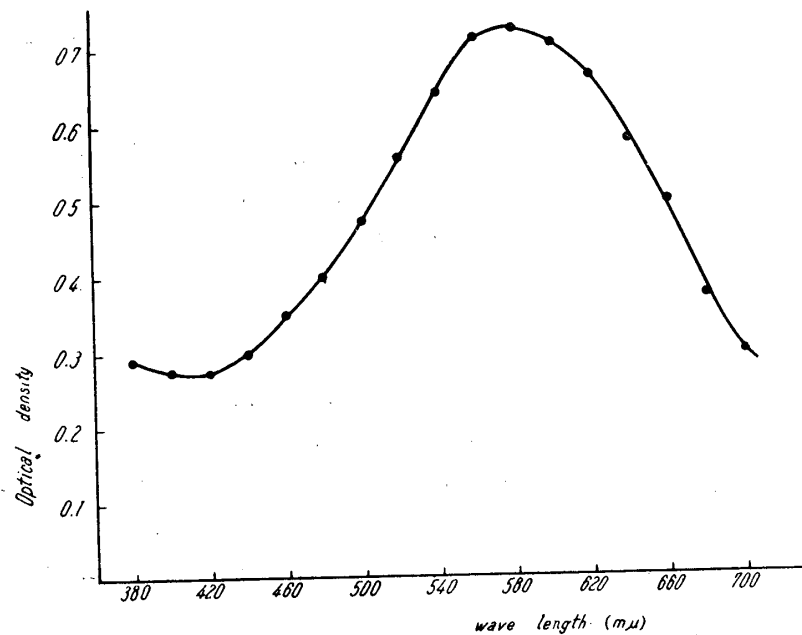


Fig. 3. Absorption curve of the blue colour formed by iodine-starch reaction.

at a wave length of 580 $m\mu$ with a 10 mm cuvette.

2. *The effect of the pH on the reaction of ammonia to the hypobromite.*

Fig. 4 shows the relationship between the pH and the corresponding optical density in the ammonia solution containing 10 μg -atoms N/l. The pH in the solution has been regulated with acetic acid, sodium carbonate and sodium hydroxide. The minimum optical density of blue color after the reaction takes place at pH 10.5. Therefore, the reaction of ammonia with the sodium hypobromite is considered as most favorable at the pH of 10.5.

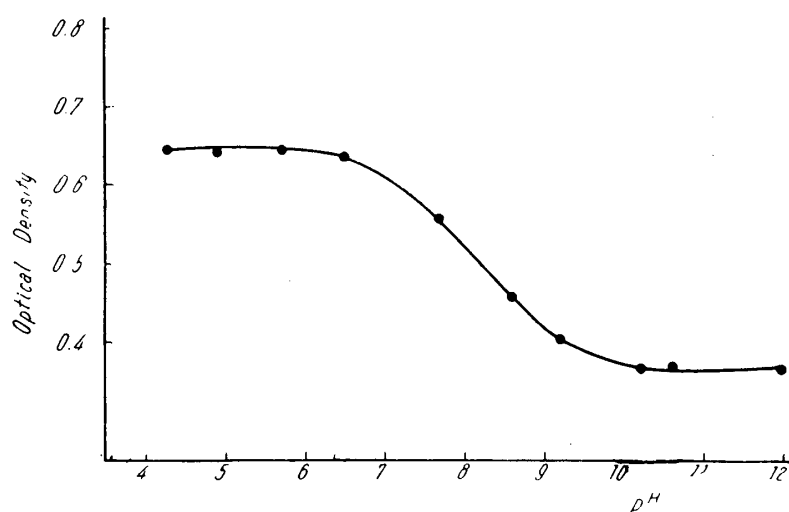


Fig. 4. The effect of pH on the reaction of sodium hypobromite with the ammonia.

When one cc of 0.005 *N* sodium hypobromite is added to 25 cc of the distilled water, the pH of the medium shows about 10.5, while in the case of sea water it shows about 9.3. In the ammonia determination of sea water, it is necessary to add the alkaline buffer solution. The quantity of the alkaline buffer solution required for the regulation of the pH in 25 cc of sea-water is about one drop.

3. *The determination of sodium hypobromite.*

As the determination of ammonia shown in this paper depends upon the reaction of ammonia to sodium hypobromite in an alkaline medium, the hypobromite in the solution must be determined perfectly. From this point of view the following experiments have been carried out.

The distilled water and the sea water are set free from ammonia by boiling after making alkaline and variable quantities of sodium hypobromite are added respectively to them. From each sample, 25 cc are transferred into 50 cc flasks by a pipette. A piece of potassium iodide crystal and one cc of acetic acid are added. The iodine is evolved in the quantity equivalent to

the sodium hypobromite in each sample. And then, two cc of starch-dextrin mixture are added. The iodine-starch blue color formed by these treatments has been determined with a spectrophotometer at a wave length of 580 $m\mu$. The result of measurements are shown in Fig. 5. From this experiment, it is found that the sodium hypobromite added to the distilled water and the sea water can be estimated perfectly, until a concentration as low as 4×10^{-5} normal.

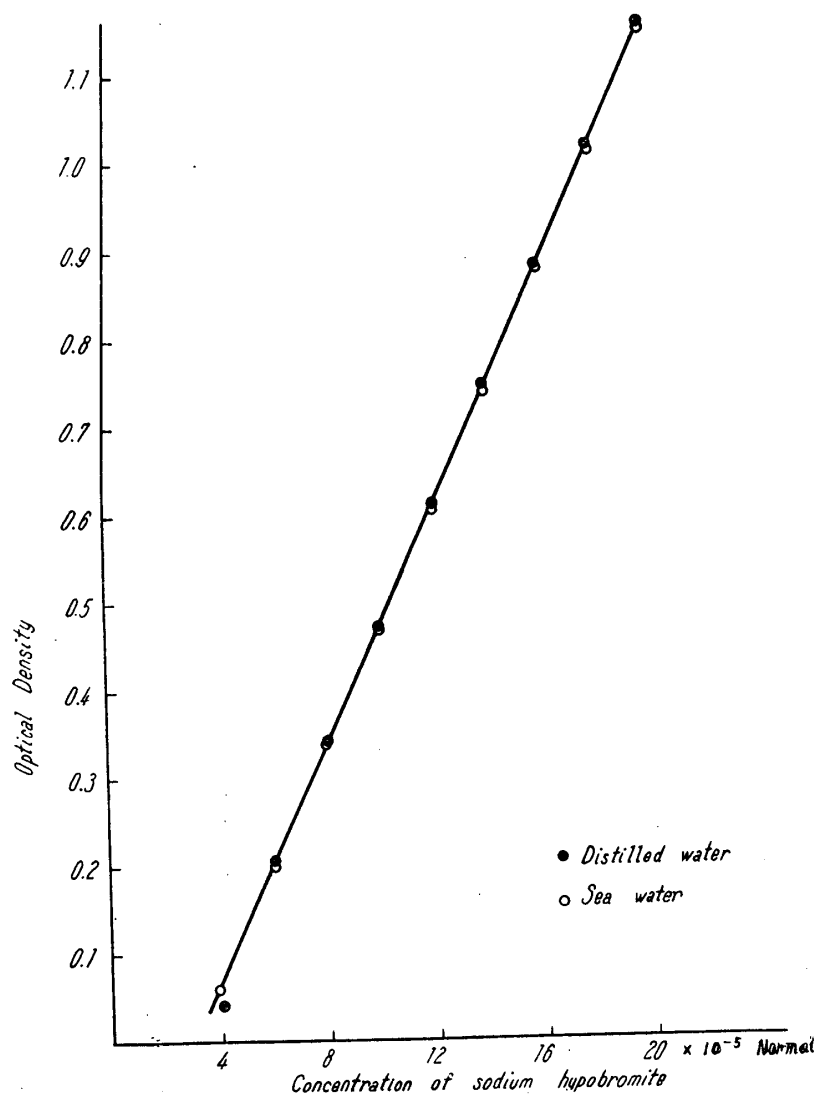


Fig. 5. Calibration graph of sodium hypobromite for distilled water and sea water.

In the determination of organic nitrogen in sea water, the organic matter in sea water is incinerated with sulphuric acid. Accordingly, the organic nitrogen in the sample is given as the ammonium sulphate in acid medium. As the reaction of ammonia with sodium hypobromite takes place in the

alkaline medium, the strong acid sample should be neutralized by adding the alkaline buffer solution. On the other hand, the ammonia in the alkaline solution is likely to evaporate in the air at room temperature. Therefore, a method has been devised so as to exclude so far as possible the evaporation of ammonia by adding the sodium hypobromite before the neutralization of the sample. The result is shown in Fig. 2.

4. *On the reaction of sodium hypobromite with other nitrogenous substances.*

a) Reaction of sodium hypobromite with nitrite-nitrogen.

A series of the standard of sodium nitrite, containing 0 – 16 μg -atoms N/l, are prepared. The nitrite standard has been treated with the same procedure as the ammonia determination. The result of the measurements is shown in Table I. From the Table I it is found that the nitrite-nitrogen does not react with hypobromite in the course of analysis. In Table II is shown the effect of the reaction time after the addition of hypobromite to the nitrite-nitrogen solution containing 7.5 μg -atoms N/l. It can be stated also from the Table II that the nitrite in the solution does not react with hypobromite even after a reaction time for 25 minutes at room temperature.

Table I. Reaction of sodium hypobromite with nitrite-nitrogen.

$\text{NO}_2\text{-N}$ $\mu\text{g-atom/l}$	Optical density
0	0.580
3.2	0.592
6.4	0.582
9.6	0.590
12.8	0.605
16.0	0.585

Table II. Changes of hypobromite-nitrite reaction with reaction time.

Reaction time in minutes	Optical density
0	0.545
5	0.565
10	0.517
15	0.510
20	0.515
25	0.495

b) Reaction of sodium hypobromite with amino acids.

The amino acid used on the experiments is glycocoll and sodium glutamat. The two series of standards of glycocoll and sodium glutamate, containing 0–20 μg -atoms N/l are prepared. The relationship between the amino-nitrogen concentration and the optical density has been obtained by the same procedure as the ammonia determination. The result of the measurements is shown in Fig. 6. From the figure it is obvious that the glycocoll in the alkaline medium can be perfectly determined by the same method as the ammonia determination. In the glutamate, however, the relationship between the amino nitrogen and the optical density is as shown by the curve B in Fig. 6.

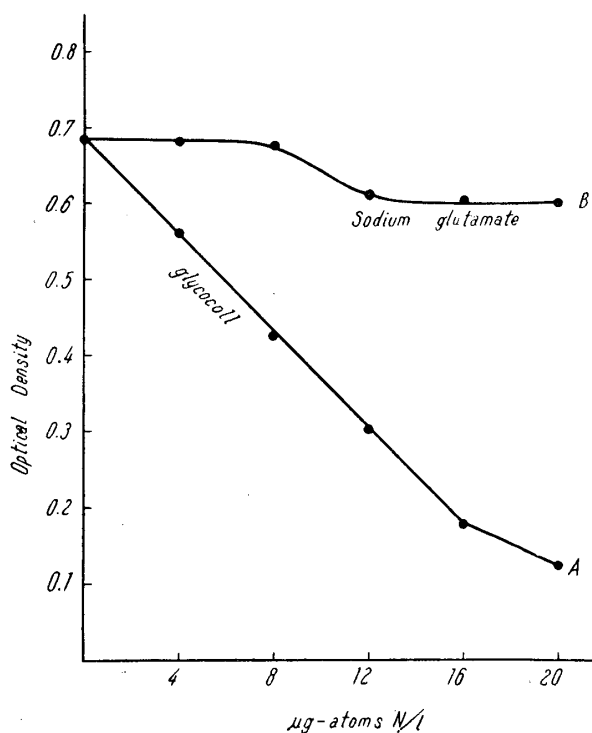


Fig. 6. Reaction of sodium hypobromite with amino acids.

Table III. The effect of copper on the determination.

Cu $\mu\text{g/l}$	Optical density
0	0.398
160	0.382
320	0.372
480	0.382
640	0.387
800	0.395

determination by hypobromite.

6. *The recovery of organic nitrogen in the sea water and the distilled water.*

The sodium glutamate standard solution containing 0, 100, 200, 300, 400 and 500 $\mu\text{g N/l}$ is prepared with the ammonia free natural sea water and distilled water by dissolving the stock solution of sodium glutamate. The organic nitrogen of this standard solution has been determined by the already mentioned method. Similarly, the standardization curve has been obtained by using the standard ammonium chloride solution. The result of the measurements is shown in Fig. 2 and Table IV. Line A in Fig. 2 shows the analytical

In both cases, the amino-nitrogen has reacted with the sodium hypobromite. Accordingly, the ammonia nitrogen estimated by this method may include some amino-nitrogens.

5. *On the effect of heavy metals.*

As the copper and other heavy metals isolate the iodine from the potassium iodide in the acid medium, their existence in a great quantity may interfere with the analysis. The copper and the other heavy metals in sea water are very scarce but sometimes the sea water collected by Nansen's sampling bottle contains 5–500 $\mu\text{g/l}$ of copper. Therefore, the following experiment has been carried out.

The samples as shown in Table III have been prepared and the effect of copper has been examined. The result of the measurements is shown in the table. From the table it is evident that the quantity of copper in the range of the experiment have no influence on the ammonia

Table IV. The recovery of sodium glutamate in the distilled water.

Addition of Glutamate N $\mu\text{g}/\text{l}$	Optical density	Theoretical optical density	Recovery in $\mu\text{g}/\text{l}$	Recovery in %
0	0.814	0.814		
100	0.712	0.703	95.2	95.2
200	0.600	0.597	206	103
300	0.490	0.485	302.4	100.8
400	0.375	0.375	410	102.5
500	0.275	0.274	503	100.6
				average 100.4%

result of the samples and Line B the standardization curve. From Table IV it is found that the recovery of amino-nitrogen added to the distilled water is 100.6 per cent in this analytical method. From Fig. 2 it is obvious that the organic nitrogen added to the sea water is estimated perfectly since Line A is parallel to Line B. On the other hand, from the difference of optical density between Lines A and B the concentration of organic nitrogen contained in sea water can be calculated. In this sea water, it is 6.1 μg -atoms N/l.

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