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DETERMINATION OF AMMONIA IN SEA WATER AS RUBAZOIC ACID

by

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Summary

The method of Procházková for the determination of ammonia as rubazoic acid has been modified for sea water. Full directions for the determination are described. The sensitivity of the proposed method has been assessed and the "salt-error" was found to be 0.60 relative to distilled water. There are no likely sources of interfering ions in non-polluted sea water and the method is not temperature sensitive below 20°C.

Knowledge of the nutrients required by marine phytoplankton is much less complete than might be inferred from examples given in the usual text-books and from numerous accounts of certain nutrients in small and often specialized areas. Comprehensive descriptions of the levels of the important nutrients in large sea areas are sadly lacking. Amidst this rather gloomy picture ammonia (more precisely but less familiarly "ammonium ion") stands out as the major nutrient about which least is known.

The analytical problem has not escaped attention but many of the existing solutions are either excessively unpleasant and troublesome (the pyridine-bispyrazolone method of Strickland and Austin, 1959) or non-specific (such as methods involving the bleaching of dyestuffs, e.g. Koroleff, 1960) or unworkable (certain chelating methods). Recently Lidmila Procházková (1964) described the determination of ammonia as rubazoic acid and explored the background chemistry. In this paper a variant of this method is suggested for ammonia in sea water which has proved convenient and reliable.

The determination involves four stages. The ammonia first reacts with chloramine-T to form dichloramine which is then coupled with bis-pyrazolone to yield rubazoic acid in the alkaline medium. A side product, Pyrazolone Blue, is decolorised with pyrazolone and the rubazoic acid is extracted into an organic solvent. Full details are given by Procházková.

Procházková found that pH 6 was the optimum for the reaction (stage 1) of ammonia with chloramine-T, and this was substantiated for 5 µg-at. NH_4-N per litre in sea water. The suggested phosphate-citrate buffer cannot be used because of precipitation at a later stage and a simple citric acid-citrate buffer was substituted for use with sea water. The reaction time and the concentration of chloramine-T used, both affect the final colour yield and are both closely interrelated. A full investigation has not been attempted but in Fig. 1 is shown the effect of concentration of chloramine-T for a 15 minute reaction period. However, on selecting the optimum concentration (0.75%) it was subsequently found that greater colour yield results after only 2½ minutes reaction (Fig. 2). Since dichloramine is not the final stable end-product of the reaction it is essential to observe a strict time schedule. Procházková has shown the reaction not to be sensitive to temperature over the range 0-20°C but the yield is greatly decreased at 30°.

In the reaction (stage 2) between dichloramine and bis-pyrazolone, the reaction time and the reagent concentration again influence the eventual yield of rubazoic acid. Using the same ammonia solution in sea water and a reaction time of 5 minutes the effect of the bis- and mono-reagent concentration is found as in Fig. 3. Although the relationship is rather complex the addition of 8 mg "bis" per sample appears to be optimal and the time dependence of the reaction at this concentration has been evaluated as in Fig. 4. The reaction is seen to be quite rapid and the product is fairly stable with optimum colour at 11 or 12 minutes. It was found that the optimum pH 10 ± 0.1 found by Procházková could not be attained without precipitation using sea water and the sodium carbonate concentration was therefore reduced to give pH 9.3 and provide a small margin before precipitation set in.

In the third stage an excess of pyrazolone reagent is necessary to decolorize the pyrazolone blue and 100% excess appears to yield the optimum final colour (see Fig. 5).

In the final stage the rubazoic acid (blue in aqueous solution) is extracted into carbon tetrachloride to give a yellow solution whose absorbance is measured at 450 mµ. It was found unnecessary to acidify with hydrochloric acid before extraction. The yellow extract was found to be stable over periods up to 2 hours and possibly longer.

Experimental Procedure

Apparatus

Spectrophotometric measurements are made at 450 mµ using a 2 cm stoppered glass cuvette with carbon tetrachloride in the reference path. The Unicam SP600 is suitable.

Reagents

It cannot be overemphasized that the organic reagents for this determination are insufficiently pure as received from laboratory suppliers. Purification is simple and if large batches are done at a time the effort is not unduly time-consuming. Each organic chemical must be purified as described by Strickland (1958) making sure that excessive heating is avoided. The citric acid-citrate buffer solution and the distilled water should be checked for traces of ammonia. Fresh solutions have been preferred in this work but the keeping qualities of the reagents as indicated by Procházková (1964) and Strickland and Austin (1959) are quoted for guidance.

Reagents

Buffer

Mix together 24 ml 0.2 M citric acid and 976 ml 0.2 M sodium citrate solutions prepared from analytical grade chemicals. If the buffer is to be kept, a few drops of 1:1 toluene-carbon tetrachloride mixture reduces the risk of bacterial growths.

Chloramine-T

Dissolve 0.75 g of recrystallised chloramine-T in distilled water, warming if necessary, and make up to 100 ml. The solution is stable for several days.

"Bis" reagent

Prepare 1 litre 0.15 M sodium carbonate solution and use 400 ml to dissolve 1.6 g of 3,3' dimethyl-5, 5'-dioxo- 1, 1'-diphenyl-(4, 4'-bi-2-pyrazoline) warming to 90°C or below. Cool. Dilute the solution to 1 l with 0.15 M Na_2CO_3 solution. Procházková suggests that storage for at least 10 days is obtained under nitrogen at 0°C.

"Mono" reagent

Dissolve 15 g of recrystallised 3-methyl-1-phenyl-5-pyrazolone in 1 litre of distilled water. The solution is stable for at least a month.

Carbon tetrachloride

The reagent grade solvent is suitable without further treatment.

Procedure

To each 50.0 ml. sea water sample is added 5 ml buffer solution followed, after mixing, by 2 ml chloramine-T reagent. Mix at once and allow the reaction to proceed at room temperature (20°C or less) for exactly 2½ minutes then add 5 ml "bis" reagent and shake to mix the solutions thoroughly. After 8 minutes add 10 ml "mono" reagent and 5 minutes later extract the rubazoic acid into 10 ml carbon tetrachloride by shaking vigorously for 30 seconds and again shake after a further 3 minutes. A single extraction is sufficient and the extract may then be separated and dried by passing through a separating funnel containing a roll of filter paper in the stem. Measure the absorbance of the carbon tetrachloride extract at 450 mμ using 2 cm stoppered cuvettes.

Conclusion

This modification of the method of Procházková is convenient and reliable and avoids the pyridine reagent which is so unpleasant in Strickland's method. The sensitivities of the methods may be compared from the values of the absorbance for 50 ml samples containing 10 μg NH_4-N per sample based on a 0.5 cm cell. Strickland and Austin gave 0.25, Procházková 0.81 and the present method 0.48. The apparent salt error is 0.60 compared with 0.62 found by Strickland. All sea water samples were close to 35‰ salinity and the effect of salinity variation (cf. Strickland) has not yet been re-examined. As mentioned by Procházková and by Strickland, interference from other substances is unlikely to be serious at their natural levels of occurrence in sea water.

Results from the analysis of sea water samples are as yet incomplete and will be reviewed later when more worthwhile quantities of data are secured.

References

Koroleff, K. (Unpublished communication) Colorimetric determination of ammonia in sea water. I.C.E.S. C.M. 1960. Hydrographical Committee, Paper No. 330.

Procházková, L. Spectrophotometric determination of ammonia as rubazoic acid with bis-pyrazolone reagent. Analyt. Chem., 36, 865-71, 1964.

Strickland, J.D.H. Standard methods of sea water analysis. Ms Rep. Ser., Fish. Res. Bd Can., (1-0) No. 18, Vol. II, 49-58, 1958.

Strickland, J.D.H. and Austin, K. The direct estimation of ammonia in sea water. J. Cons. perm. int. Explor. Mer, 24, 446-8, (1959).

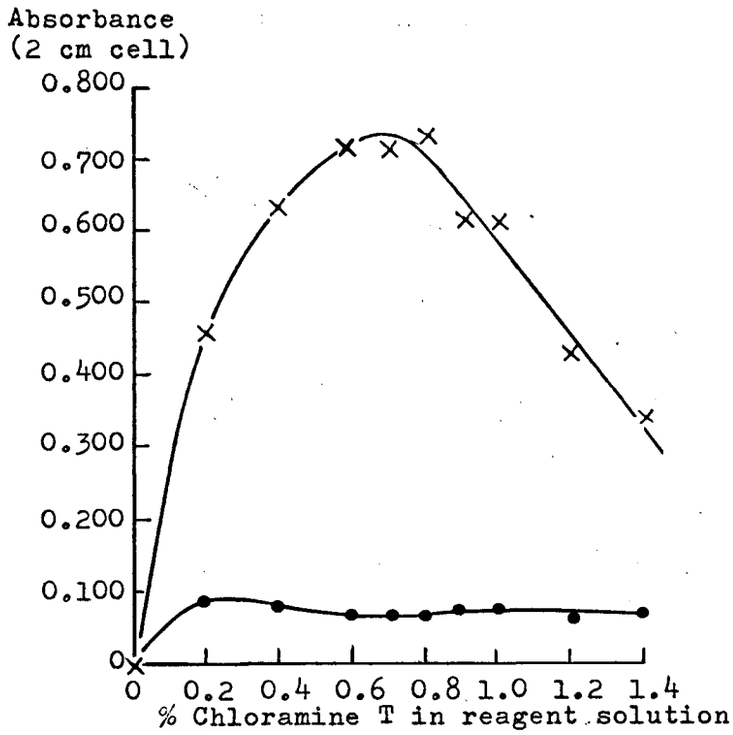

Fig. 1. Absorbance as a function of chloramine-T concentration, time 15 mins.

x=S.W. + 5×10^{-6}M ammonia.

•=S.W. ("blank").

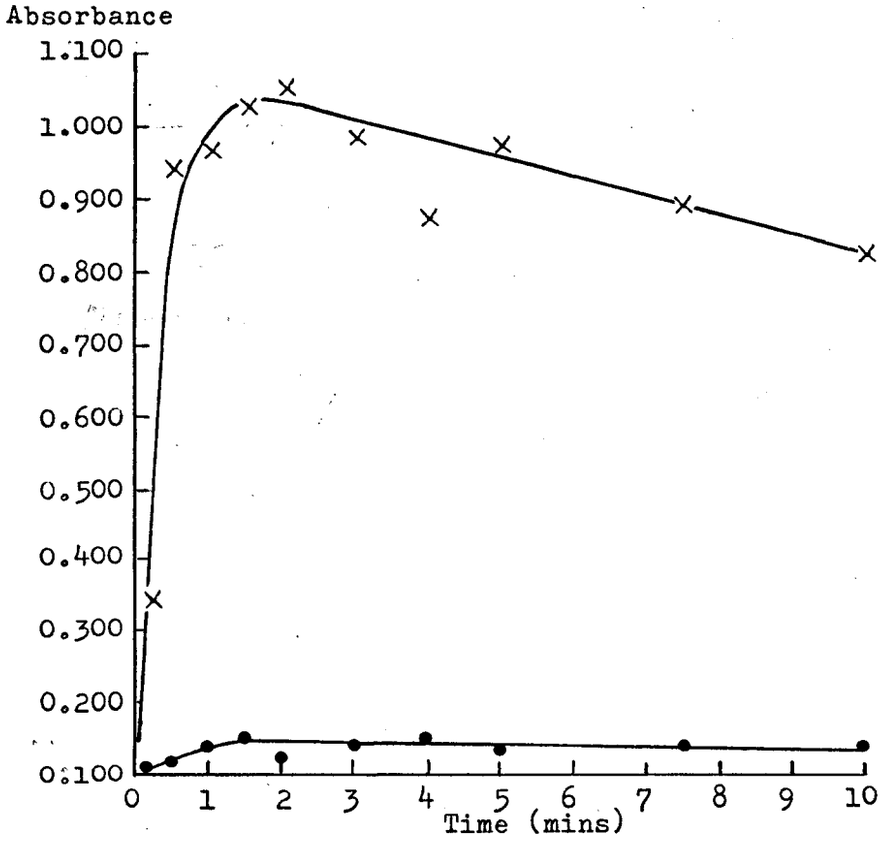

Fig. 2. Absorbance as a function of reaction time with chloramine-T, concn. = 0.75%.

x=S.W. + 5×10^{-6}M ammonia.

•=S.W. ("blank").

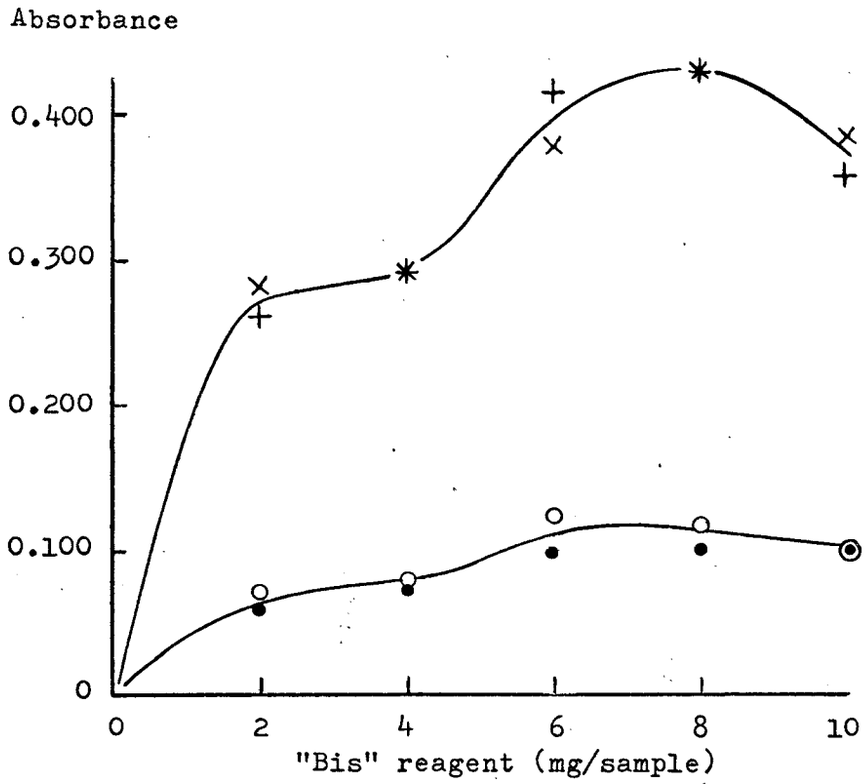

Fig. 3. Absorbance as a function of "bis" and "mono" reagents, time 5 mins.

x=S.W. + 2×10^{-6}M, usual "mono" reagent.
+=S.W. + 2×10^{-6}M, double strength "mono" reagent.
•=S.W. "blank", usual "mono" reagent.
o=S.W. "blank", double strength "mono" reagent.

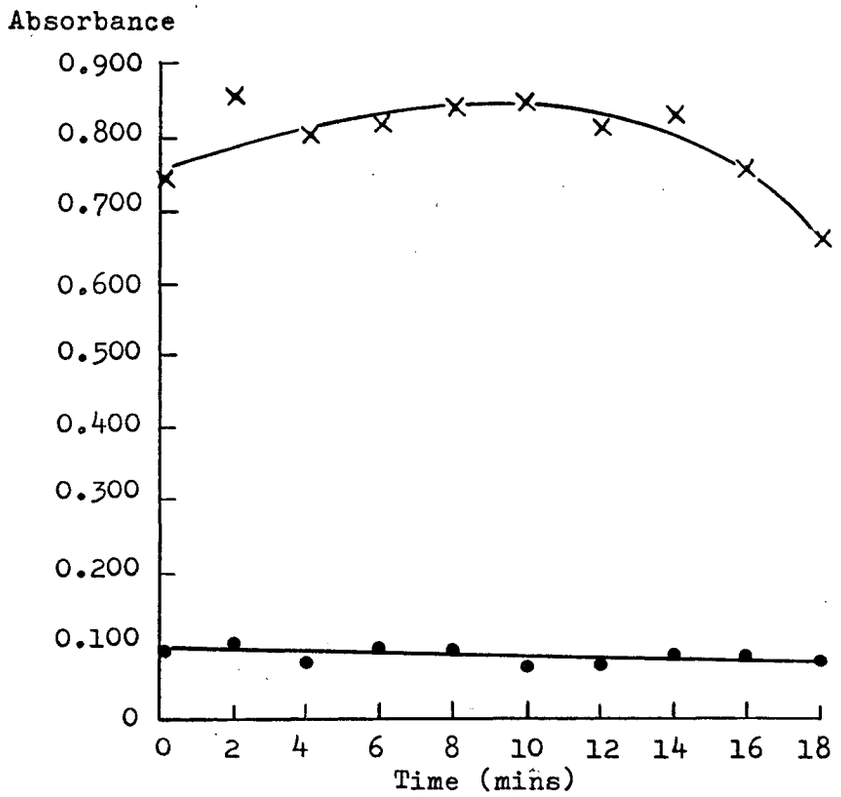

Fig. 4. The effect of the "bis" reaction period on absorbance.
x=S.W. + 5×10^{-6}M ammonia.
●=S.W. ("blank").
(Usual strength "mono" reagent.)

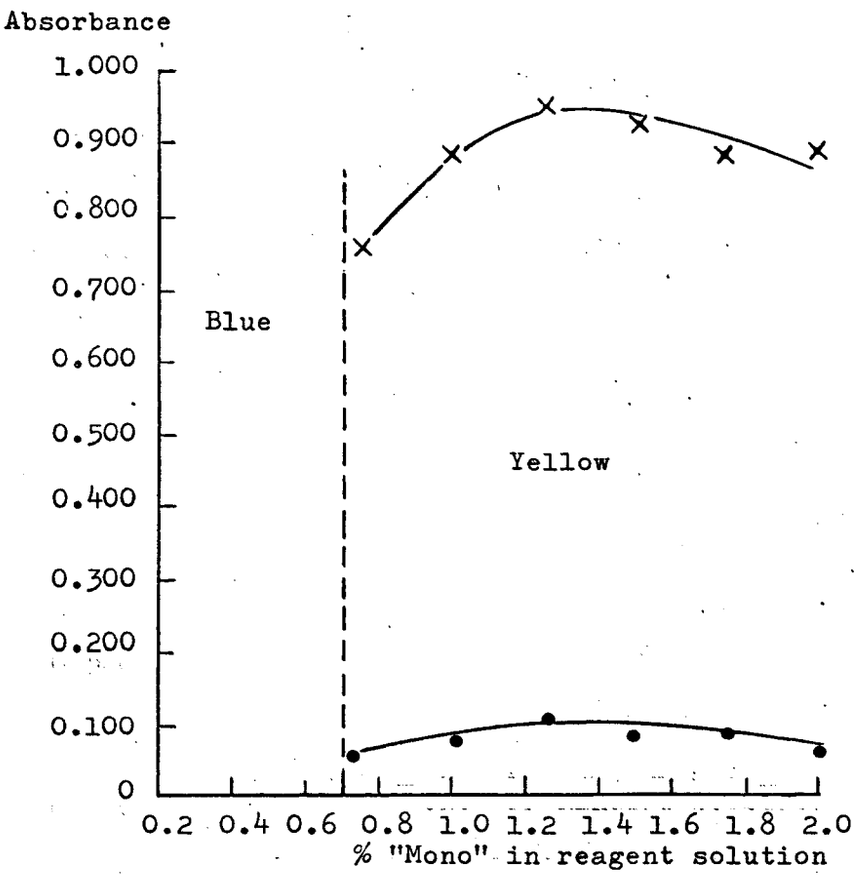

Fig. 5. The reaction between "mono" reagent and Pyrazolone Blue in presence of 5×10^{-6}M ammonia. time 8 mins.