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References

- BRUCE, H. E. 1969. The role of dissolved amino acids as a nitrogen source for marine phytoplankton in an estuarine environment in southeastern Alaska. Ph.D. thesis, Oregon State Univ. 124 p.
- CHAN, Y. K., AND J. P. RILEY. 1966. The determination of amino acids in sea-water. *Deep-Sea Res.* **13**: 1115-1125.
- HEAD, P. C. 1971. An automated phenolhypochlorite method for the determination of ammonia in sea-water. *Deep-Sea Res.* **18**: 531-532.
- HELLEBUST, J. A., AND R. R. L. GUILLARD. 1967. Uptake specificity for organic substrates by the marine diatom *Melosira nummuloides*. *J. Phycol.* **3**: 132-136.
- SHELL, D. M. 1971. Uptake and regeneration of dissolved nitrogen in southeastern Alaskan marine waters. Ph.D. thesis, Univ. Alaska. 142 p.
- SIEGEL, A., AND E. T. DEGENS. 1966. Concentration of dissolved amino acids from saline waters by ligand-exchange chromatography. *Science* **151**: 1098-1101.
- TECHNICON INSTRUMENTS CORPORATION. 1970. Revised operation manual for the Technicon amino acid analyzer system model No. NC-1. Tech. Publ. TAO-0155-00. Terrytown, N.Y.
- YEMM, E. W., AND E. C. COCKING. 1954. The determination of amino acids with ninhydrin. *Analyst* **80**: 209-213.

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The determination of ammonia in seawater

Abstract—A simple phenolhypochlorite method for the estimation of ammonia in seawater obeys Beer's Law over the concentration range 0-20 $\mu\text{g-atoms NH}_3\text{-N liter}^{-1}$; the standard deviation on a set of samples containing 4 $\mu\text{g-atoms NH}_3\text{-N liter}^{-1}$ is 0.04.

The estimation of ammonia in seawater has always proved difficult and several methods have been tried in the Plymouth Laboratory with varying degrees of success. Solórzano has published a phenolhypochlorite method (Solórzano 1969) which has since been used by many workers, some of whom have reported difficulties (McCarthy and Kamykowski 1972). We also obtained anomalous results and after a series of experiments have modified the method. This modified procedure has been in use for several months and has given us consistent results.

Initially we found that the blank absorbancies were high and erratic. By substituting potassium ferrocyanide in place of sodium nitroprusside as the catalyst, we got stable reproducible blanks of about

0.050 absorbance, the same level as the one given by Solórzano, in a 10-cm cuvette. To make certain that the blank could be applied to seawater in our method, we checked it against aged irradiated seawater samples. Commercial hypochlorite solutions too were a source of variation because of their instability, making necessary a daily check on the concentration of available chlorine. Sodium dichloroisocyanurate has been used as an alternative hypochlorite donor by Grasshoff and Johannsen (1972) and also by Benesch and Mangelsdorf (1972) in their ammonia procedures. This salt has the advantage of being a stable solid, and the yield of hypochlorite on hydrolysis is both rapid and quantitative.

Even with these modifications the intensity of the blue indophenol color still varied from day to day, being almost twice as strong on a bright sunny day as on a dull overcast one. Various sources of artificial light were therefore investigated to find the optimum conditions for color development. The most efficient source was an ultraviolet lamp. We used a Hytek mer-

cury longwave ultraviolet lamp, type MBW, with maximum energy output at 365 nm. One reason for using this particular lamp was that being low power it could be used in the laboratory without great inconvenience; other ultraviolet sources may well prove to be just as efficient. Initially we placed a reflector behind the lamp but subsequently we placed the lamp in a specially constructed aluminum box. The all round reflecting surface thus obtained made possible the color development of greater numbers of samples at the same time. Attempts to use tungsten or fluorescent lamps in place of the ultraviolet lamp were unsuccessful, the time taken for color development being considerably longer and the actual number of hours required varying with the particular lamp involved.

Reagents—1. De-ionized water: Prepare immediately before use by passing distilled water through a freshly charged cation exchange resin in the hydrogen form.

2. Phenol-alcohol reagent: Dissolve 10 g of Analar phenol in 100 ml of Analar 95% v/v ethyl alcohol.

3. Oxidizing solution: Dissolve 0.2 g of sodium dichloroisocyanurate in a solution of 1.6 g of Analar sodium hydroxide in 40 ml of de-ionized water. To this add a solution of 20 g of Analar trisodium citrate in 40 ml of de-ionized water. Adjust volume to 100 ml with de-ionized water.

4. Catalyst: Dissolve 0.5 g of Analar potassium ferrocyanide in 100 ml of de-ionized water. Transfer to an amber bottle.

Reagents 2 and 3 are stable for 24 hr when stored in a refrigerator, but the catalyst must be prepared daily.

To a 50-ml sample in a 100-ml Pyrex conical flask add 2 ml of phenol-alcohol reagent, 5 ml of oxidizing solution, and 2 ml of catalyst, mixing between each addition. Cover the tops of the flasks with glass or plastic caps and place within 0.5 m of a Hytek mercury lamp fitted with a reflector such that each flask receives maximum en-

ergy. Color development is complete in 40 min over a temperature range of 22–27°C. Measure the adsorption at 640 nm in a spectrophotometer against distilled water with 10-cm cuvettes.

A calibration should be made with each batch of samples using spiked seawater.

As with all methods for the estimation of low concentrations of ammonia, the risks of contamination are high. The work should be carried out in an ammonia-free laboratory with acid washed glassware.

In seawater the factor relating absorbance to concentration (in $\mu\text{g-atoms NH}_3\text{-N liter}^{-1}$) is 6.5 while in freshwater it is 5.8. On sets of samples containing 1 and 4 $\mu\text{g-atoms NH}_3\text{-N liter}^{-1}$ the standard deviations were 0.02 and 0.04 respectively. Beer's Law was obeyed over the concentration range of 0–20 $\mu\text{g-atoms NH}_3\text{-N liter}^{-1}$.

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References

- BENESCH, R., AND P. MANGELSDORF. 1972. Eine Methode zur colorimetrischen Bestimmung von Ammoniak in Meerwasser. *Helgol. Wiss. Meeresunters.* **23**: 365–375.
- GRASSHOFF, K., AND H. JOHANNSEN. 1972. A new sensitive and direct method for the automatic determination of ammonia in sea water. *J. Cons., Cons. Int. Explor. Mer* **34**: 516–521.
- MCCARTHY, J. J., AND D. KAMYKOWSKI. 1972. Urea and other nitrogenous nutrients in La Jolla Bay during February, March and April, 1970. *Fish. Bull.* **70**: 1261–1274.
- SOLÓRZANO, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* **14**: 799–801.

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