

Some Improvements in the Salicylate-Dichloroisocyanurate Method for Determining Ammonia in Seawater*

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Abstract: An automated indophenol blue method using low toxic salicylate and stable dichloroisocyanurate are applied for determining ammonia in seawater. Light treated and non-light treated potassium hexacyanoferrate were examined as catalysts and the sensitivity were compared with nitroprusside. A low reaction temperature of 45°C which does not cause ammonia formation from amino acids can give sufficient sensitivity for the determination.

1. Introduction

Phenol-hypochlorite method of SOLORZANO (1969) is widely used to measure the concentration of ammonia in seawater in both manual and automated analyses. This method is based on indophenol reaction in which ammonia reacts with phenol and sodium hypochlorite in the presence of sodium nitroprusside to produce an indophenol blue. However, phenol is not easy to deal with because of its toxicity, stimulative smell and corrosiveness, especially in a laboratory where living organisms are handled. Furthermore, a noxious, highly volatile *o*-chlorophenol is formed during measurement. It penetrates human skin rapidly, and is nearly 3 times more toxic than phenol to fish (see BOWER and HOLM-HANSEN, 1980).

OTSUKI and SEKIGUHI (1983) applied salicylate-dichloroisocyanurate (DIC) method to an automated analysis of ammonia in freshwater. In this method, the salicylate has low toxicity and DIC is stable in the condition of powder and DIC is also more stable than hypochlorite in solution. They used potassium hexacyanoferrate (CF) as a catalyst which are known to give a blank lower than nitroprusside. A heating temperature of 55 °C were used in their study to get enough sensitivity, but they also suggested the temperature could cause interferences due to the

release of the ammonia from some amino acids and urea in the water. LIDDICOAT *et al.* (1975) and HAMPSON (1977) applied the ultraviolet light irradiation for activating CF as a catalyst to get higher sensitivity.

In the present study, we applied the salicylate-DIC method to automated determination of ammonia in seawater. CF activation by fluorescent lamp also has been tested to get high sensitivity and lower reaction temperature.

2. Experimental

Apparatus A Technicon-type autoanalyzer which is composed of a MHRE 22 proportioning pump (Watson-Marlow Co.) and a PP-1 colorimeter (Hirama Inst.) has been used in this study. The schematic layout of the manifold is shown in Fig. 1.

Reagents Ultra pure water which were obtained from Milli-Q system (Millipore Co.) was used for the preparation of reagents. Offshore seawater without detectable concentration of ammonia was used for the preparation of standard ammonia solutions. Reagents were prepared according to the previous method by OTSUKI and SEKIGUCHI (1983), except the sodium nitroprusside solution.

1) Buffer-catalyst reagent; 0.8g of sodium hydroxide, 20g of trisodium citrate and 4g of CF or 0.02-0.08g of sodium nitroprusside were dissolved and made up to 200ml.

2) Salicylate reagent; 80g of sodium salicylate was dissolved and made up to 200ml.

3) Chlorine donating reagent; 4g of sodium

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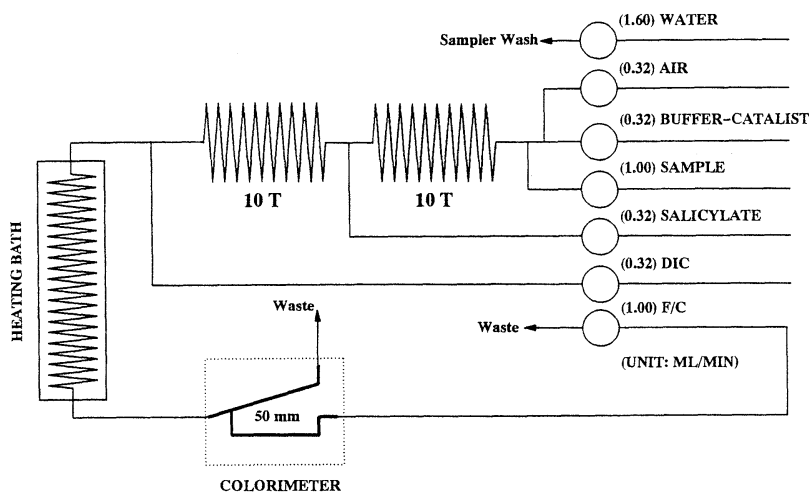


Fig. 1. Schematic diagram of manifold for the automated determination of ammonia in seawater.

Table 1. Influence of salinity.

Concentration of ammonia (μM)	Absorbance of color-developed sample			
	Freshwater		Seawater	
	I	II	I	II
10	0.185	0.189	0.189	0.190
50	0.483	0.485	0.490	0.492
100	0.650	—	0.645	—

hydroxide and 2g of DIC were dissolved and made up to 200ml.

4) Standards; The stock standard solution was prepared by dissolving 0.6607g of ammonium sulphate to 1000ml of water. The working standard solutions were prepared by diluting stock standard solution.

0.1ml of Brij-35 was added to each reagent.

Light activation of hexacyanoferrate by fluorescent lamp The buffer-catalyst reagents were irradiated by a fluorescent lamp (Toshiba NEOLINE, 10W) at a distance of 0.1m before adding to the sample, when CF was used as a catalyst. After keeping the buffer-catalyst reagent in the dark for 3 hours, the blank experiment was performed for the comparison with activated reagent.

3. Results and Discussion

Influence of salinity We applied the same

buffer and coloring reagent concentration as those which is used in OTSUKI and SEKIGUCHI's method (1983). To assess the effect of salinity on absorbance, two groups of standards were tested by using CF as the catalyst. Although differences in salinity caused fluctuation in absorbance in the flow cell of the colorimeter at the beginning of the mixing, identical height in absorbance was obtained with standards in freshwater and seawater (Table 1). According to KOROLEFF (see SEARLE, 1984) the "salt effect" is directly related to the differences in the reaction pH and precipitates formed with seawater. Therefore, the concentration of trisodium buffer and pH in the OTSUKI and SEKIGUCHI (1983) are adequate for seawater analysis.

Absorption spectrum The maximum absorption wave length of indophenols depends on the constituent of phenolic compound. They also have minor changes depending on the reagent concentrations, pH and the presence of an organic solvent. The maximum absorption wave length of indophenol obtained from the present method (Fig. 2) suggests that a range of wave length between 626 nm and 668 nm is suitable for the present automated method. The wave length of maximum absorption is similar to the result of acetone-catalyzed manual *o*-chlorophenol method, rather than that of sodium salicylate in manual methods (SEARLE, 1984).

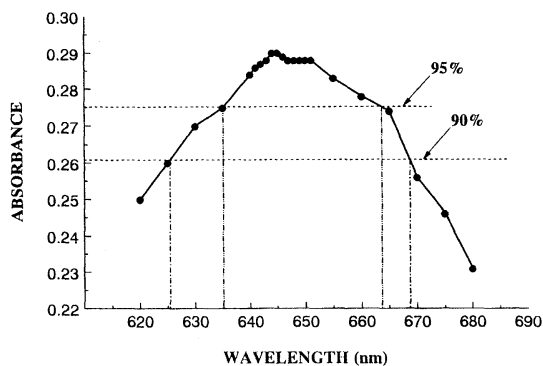


Fig. 2. Relationship between wavelength of colorimeter and absorbance of color-developed sample solution.

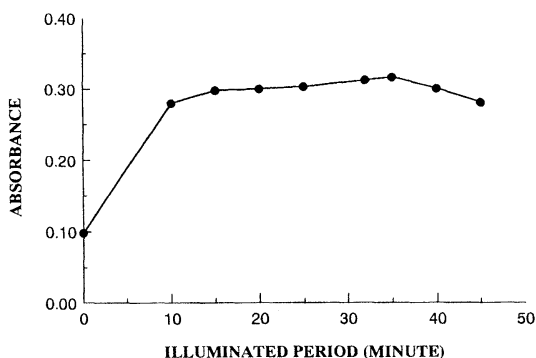


Fig. 3. Relationship between illuminated period of catalyst and absorbance of color-developed sample solution.

Catalyst Fluorescent lamp was applied instead of ultraviolet lamp, when CF was used as the catalyst. Absorbance increased about 3 times higher than that of untreated CF (Fig. 3), when an irradiation more than 10 minutes was used. Although the actual mechanism is not completely understood (SEARLE, 1984), it has been found that ultraviolet light irradiation is suitable for the formation of aquapentacyanoferrate which is considered to be the catalyzing ion. KROM (1980) summarized the reaction mechanism and concluded that aquapentacyanoferrate can be produced from nitroprusside via oxidation and also from hexacyanoferrate via ultraviolet light irradiation. Fluorescent lamp can also be used for the activation of CF.

OTSUKI and SEKIGUCHI (1983) raised the reaction temperature to 55 °C, for the purpose of increasing the sensitivity. We tested the light

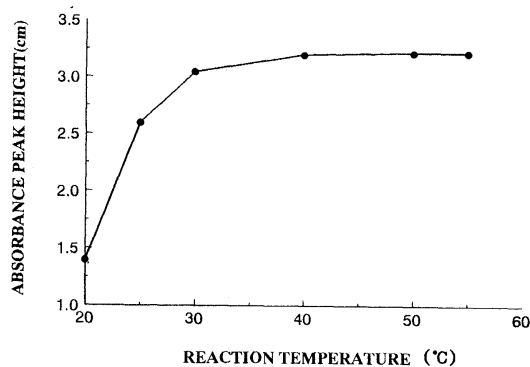


Fig. 4. Relationship between reaction temperature and absorbance peak height of color-developed sample solution.

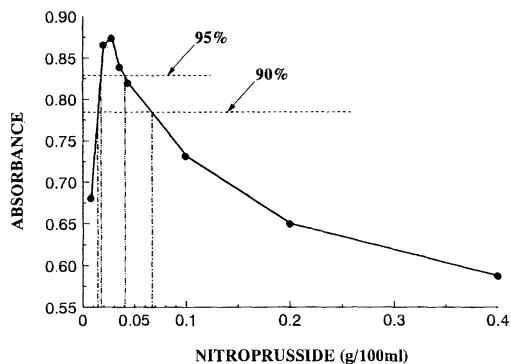


Fig. 5. Relationship between nitroprusside concentration and absorbance of color-developed sample solution.

activated catalyst reagent at various temperature between 20 and 55 °C (Fig. 4). The activity saturated when temperatures higher than 40 °C are applied. OTSUKI and SEKIGUCHI (1983) showed that with raising temperature absorbance peak was increased exponentially from 37 to 55 °C. If non-activated CF was used as the catalyst, higher reaction temperatures may be necessary to obtain higher sensitivity, because the formation of aquapentacyanoferrate may also be temperature dependent. Amino acids in seawater are reported to decompose and release ammonia at a temperature higher than 50 °C. Therefore 45 °C may adequate as reaction temperature, especially for the analysis in productive seawaters including high concentrations of amino acids.

To compare the sensitivity of salicylate-CF method and the salicylate-nitroprusside method, the optimum concentration of nitroprusside was

Table 2. Comparison between salicylate-nitroprusside method and salicylate-CF method on blank absorbances and sensitivities.

Concentration	Blank Abs.	10 μ M Abs.
Hexacyanoferrate	0.042	0.312
Nitroprusside	0.076	0.333

determined. Optimum concentrations of nitroprusside were between 0.02 to 0.04g/100 ml (Fig. 5). Higher concentration more than 0.4g/100 ml decreased the absorption value about 30 % of the optimum. The result is inconsistent to HARWOOD and HUYSER (1970) in which increasing concentration of nitroprusside over the optimum did not affect the sensitivity, if other reagents were kept constant. The final concentration to give highest sensitivity was 47 mg/l which is almost the half of the Technicon method (88 mg/l) (Technicon method, 1978). The salicylate-nitroprusside method has a slightly higher sensitivity than the salicylate-CF method, but it has higher blank (Table 2).

4. Conclusion

Salicylate-DIC method of OTSUKI and SEKIGUCHI (1983) can be applied for the determination of ammonia in seawater. Salicylate-CF method has lower blanks, but needs light irradiation; salicylate-nitroprusside method is more convenient, but has high blank values. We suggest the use of salicylate-nitroprusside

method for usual measurement, but salicylate-CF method with light activation for the determination of low concentration of ammonia which needs a low blank.

References

- BOWERE C.E., and T. HOLM-HANSEN (1980) : A salicylate-hypochlorite method for determining ammonia in seawater. *Can. J. Fish. Aquat. Sci.*, **37**, 794-798.
- KROM, M.D. (1980) : Spectrophotometric determination of ammonia: A study of a modified Berthelot reaction using salicylate and dichloroisocyanurate. *Analyst*, **105**, 305-316.
- LIDDICOAT, M. I., S. TIBBITTS and E.I. BUTLER (1975) : The determination of ammonia in sea water. *Limnol. Oceanogr.*, **20**, 131-132.
- HAMPSON, B.L. (1977) : The analysis of ammonia in polluted sea water. *Water Res.*, **11**, 305-308.
- HARWOOD, J.E. and D.J. HUYSER (1970) : Automated analysis of ammonia in water. *Water Res.*, **4**, 695-704.
- OTSUKI, A. and K. SEKIGUCHI (1983) : Automated determination of ammonia in natural freshwaters using salicylate-hexacyanoferrate-deichloroisocyanurate system. *Analytical letters*, **16** (A13), 979-985.
- SOLORZANO, L. (1969) : Determination of ammonia in natural waters by the phenol hypochlorite method. *Limnol. Oceanogr.*, **14**, 799-801.
- SEARLE, P.L. (1984) : The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. *Analyst*, vol. **109**, 549-568.
- Technicon method (1978) : No. 154-71W/B.

サリチル酸-Dichloroisocyanurate法による 海水中のアンモニアの測定

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要旨：海水中のアンモニアの自動分析は従来フェノール-次亜塩素酸ナトリウム法によって行われてきたが、フェノール及び中間生成物の *o*-chlorophenol は極めて毒性が高いため、生物を取り扱う研究室での使用には適さない。本研究では、フェノールの代わりに毒性の低いサリチル酸ナトリウムを、また不安定で保存しにくい次亜塩素酸ナトリウムの代わりに Dichloroisocyanurate を用いる方法を海水の自動分析に適用した。触媒としてはニトロプルシドとブランクの低い Hexacyanoferrate を用いる方法を比較した。Hexacyanoferrate を蛍光灯照射して活性化することにより、高い感度を得ることができた。これにより、反応温度をアミノ酸分解によるアンモニア生成の起こらない45℃以下に抑えることができた。