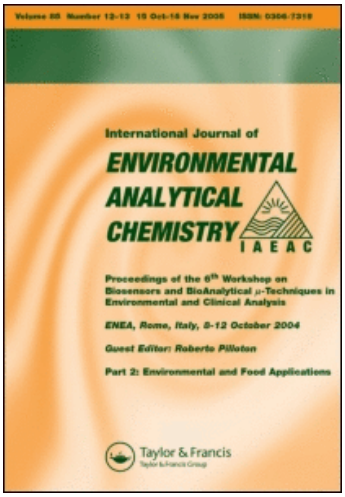

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S. Hashimoto[a]; H. Y. Sun[a]; A. Otsuki[a]; S. Kawakami[b]; F. Murata[b]; M. Yokomizo[b]
[a] Department of Marine Science and Technology, Tokyo University of Fisheries, Minato-ku, Tokyo, Japan [b] Olympus Optical Co. Ltd., Hachiooji-shi, Tokyo, Japan

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ENZYMATIC DETERMINATION OF AMMONIA IN LAKE WATER USING A SEMI-AUTOMATIC ANALYSER

S. HASHIMOTO*, H. Y. SUN and A. OTSUKI

Department of Marine Science and Technology, Tokyo University of Fisheries, 4-5-7 Konan, Minato-ku, Tokyo 108, Japan

S. KAWAKAMI, F. MURATA and M. YOKOMIZO

Olympus Optical Co. Ltd., 2-3 Kuboyama-chou, Hachiooji-shi, Tokyo 192, Japan.

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An enzymatic method was developed for the determination of ammonia concentrations in lake water. Lake water samples containing ammonia were mixed with a glutamate dehydrogenase (GlDH), reduced nicotinamide adenine dinucleotide phosphate (NADPH) and 2-oxoglutarate. The decrease in the absorbance intensity caused by the disappearance of NADPH by this reaction was measured at 340 nm. There was a linear relationship ($r = 0.9997$) between peak height and ammonia concentration over the range 0–29 μM. The detection limit was 0.29 μM for a sample volume of 250 μl. Interference of amino acids and urea at concentrations of 50 mg l^{-1} was negligible. Good agreement was found between the enzymatic method and indophenol blue colorimetry.

KEY WORDS: Ammonia determination, enzymatic method, semi-automatic analyser, lake water, glutamate dehydrogenase.

INTRODUCTION

Nitrogen availability depends on its chemical forms and influences the rate of primary production in marine systems.[1] Because ammonia is one of the most important nitrogen nutrient forms in supporting production and is the main first product of mineralization of organic N compounds, measurement of ammonia in natural samples is essential to study the dynamics of nitrogen in the environment.

At present, the determination of ammonia† in aqueous samples is commonly based on indophenol blue colorimetry.[2–4] This method is used for determining ammonia in many natural samples such as seawater,[5–7] lake water,[8,9] and sediments.[10] However, ammonia determination is complicated by the use of an alkaline

* To whom all correspondence should be addressed.

† Throughout this paper, the term ammonia refers to the total concentration of ammonia gas plus ammonium ions present, i.e., total ammonia nitrogen concentration.

reagent which may hydrolyze ammonia from the labile amide groups of amino acids. The positive interferences due to the release of ammonia from urea and some common aminoacids such as glycine and glutamic acid have been found when the determination of ammonia was done with alkaline reagents.[11] These compounds would give positive interferences within the range of 3 to 19% at 100 μg N l^{-1} level.[11] Thus the concentration of ammonia in natural waters determined by colorimetry may not be the true concentration.

The enzyme glutamate dehydrogenase (GlDH) catalyzes the following reaction:

$$\text{2-Oxoglutarate} + NH_4^+ + \text{NADPH} \overset{\text{GlDH}}{\rightleftharpoons} \text{L-glutamate} + H_2O + NADP^+$$

where $NADP^+$ is nicotinamide adenine dinucleotide phosphate. By measuring the decrease in the absorbance of the NADPH at 340 nm the concentration of ammonia is determined.

The enzymatic determination of ammonia is usually specific, simple and sensitive which makes it the most suitable for ammonia measurement[12] among a number of methods such as the gas-phase selective electrode,[13] an optical ammonia sensor[14], and that using HPLC[15].

This enzymatic method has been used in medical studies[16,17] but there is no application for environmental samples. One of the merits of the enzymatic method is the fact that phenol is not used and the harmful compound such as *o*-chlorophenol is not produced during the analysis[18]. Furthermore, the measurements are carried out under mild buffer conditions (pH 7.65, 37°C) and so the possibility of hydrolysis reactions which liberate additional ammonia is reduced.

We report here the application of the enzymatic method to determine ammonia in lake water and compare it with the indophenol blue colorimetry.

EXPERIMENTAL

Reagents

For the enzymatic analysis, the following reagents were obtained from Olympus Optical Co. Ltd., Japan. 2-Oxoglutarate (2.667 mM), NADPH (0.064 mM), GIDH (266.7 unit) and certain stabilizers were contained in reagent A (Olympus MR1149). 2-Oxoglutarate (3.08 mM), tris buffer (46.7 mM, pH 7.65) and certain surface active agents were contained in reagent B (Olympus MR 1150). The enzyme glutamate dehydrogenase, from *E. coli*, was used to catalyze the reaction. Reagents A and B were mixed immediately prior to use (reagent C).

Reagents used in indophenol blue colorimetry were as follows. Stock complexing solution: 16 g of trans-1,2-cyclohexanediamine-tetraacetic acid (CyDTA), 5 g of NaOH, and 5 g of Na_2CO_3 were dissolved in distilled, de-ionized water (Milli-Q water, Nihon Millipore Kogyo K. K.). After adjustment with HCl to pH 10.0, stock was diluted to 1,000 ml with Milli-Q water.

Nitroprusside solution: 0.12 g of sodium pentacyanonitrosylferrate (III) dihydrate was dissolved in 200 ml of Milli-Q water.

Complexing reagent (reagent 1): 200 ml of stock complexing solution, 50 ml of nitroprusside solution, and 0.5 ml of 40% Triton X-100 were mixed on the day of analysis.

Alkaline phenol reagent (reagent 2): 47.5 g of phenol and 19 g of NaOH were dissolved in Milli-Q water.

Sodium hypochlorite reagent (reagent 3): A solution from commercially available reagent was freshly prepared to contain about 1.5% available chlorine.

A stock solution of 0.05 M NH_4Cl was prepared by dissolving in Milli-Q water. Working solutions were prepared fresh daily. All other chemicals used in these experiments were of analytical reagent grade.

Sampling sites

Lake Kasumigaura is located in the eastern part of the Kanto Plain, the second largest lake in Japan, with a surface area of 171 km^2, a mean depth of 4 m, and a maximum depth of 7.4 m. This lake was already eutrophic in the early twentieth century and the eutrophication process has accelerated since then.

The lake water was collected at 10 different sampling stations, including the lake center, once a month from January to December in 1990. The samples were taken at a 0–2 m water depth by a 2 m long column sampling bottle with 60 mm ID. The collected lake water was homogenized and stored at −20°C until analysis after GF/C filtration.

Instrumentation

The semi-automatic analyzer system used for ammonia determination (both enzymatic method and colorimetry) was a KL 500 (Olympus Optical Co. Ltd.) instrument equipped with a spectrophotometer. This instrument has been developed for a medical use. The reaction vessel was of disposable plastic with a 10 mm path-length. The sample solution and the three kinds of reagents are delivered automatically to the reaction vessel. The output signal was analyzed with a data processor. The reaction reagents were kept at 4°C. All glassware was soaked in 0.1 N HCl before use.

Determination of ammonia by enzymatic method

The enzymatic analysis was carried out in the following way. 50-μl of reagent C were mixed with 250-μl of sample in the reaction vessel kept at 37°C. The decrease in the absorbance intensity caused by the disappearance of NADPH was measured at 340 nm. The reaction time was 9.2 min. Ammonia concentrations were estimated by subtracting the initial concentration from the final concentration of NADPH. The reagent blank was measured with distilled water.

Determination of ammonia by colorimetry

The procedure for indophenol blue colorimetry was as given below: The first, 250-μl sample, 50-μl reagent 1, and 50-μl reagent 2 were added to the reaction vessel kept

at 37°C and finally 50-μl of reagent 3 was added. The increase in the absorbance intensity was measured at 600 nm for about 5 min. The reagent blank was measured with distilled water.

RESULTS AND DISCUSSION

Optimization of analytical conditions

The conditions for the determination of ammonia were optimized by studying the effect of some parameters such as sample volume, reagent volume and the length of reaction time. The sensitivity increased with sample volume in the range from 50 to 250 μl. Therefore, a 250-μl sample volume was used in all subsequent experiments. The reaction was followed by measuring the change in absorbance every 36 seconds. Figure 1 shows the changes in absorbance by the enzymatic method and indophenol blue colorimetry. From the Figure it is observed that the reaction attained equilibrium when the ammonia concentration was analyzed by both methods.

All analyses of ammonia by these methods were repeated four times. Standard solution was measured with every 10 samples. Glasswares soaked on 0.1 N HCl were used and ammonia determinations were made within 10 min to prevent contamination of ammonia from air.

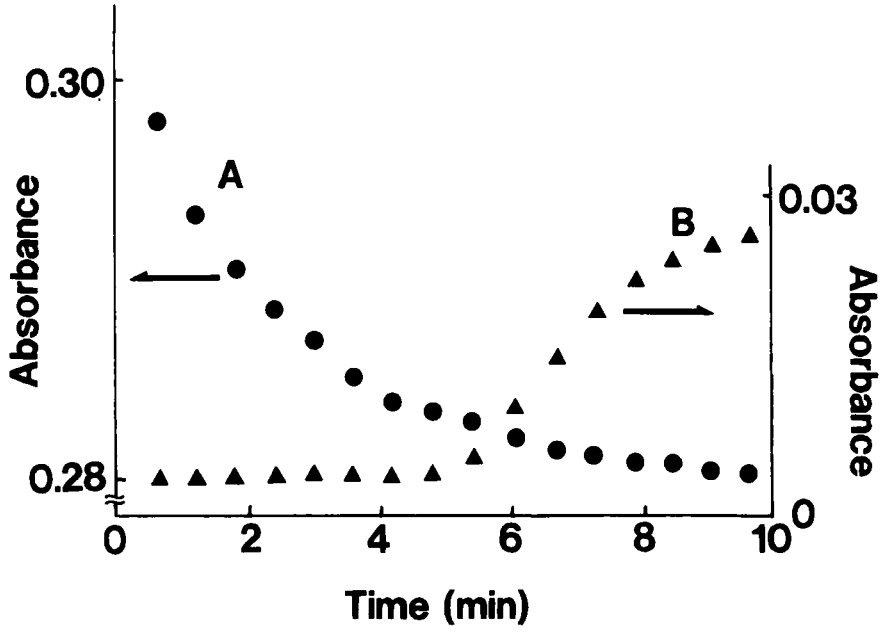

Figure 1 Time course of absorbance observed in enzymatic method and indophenol blue colorimetry at 2.9-μM ammonia concentration. (A) enzymatic method, (B) indophenol blue colorimetry.

Calibration curve and detection limit

Ammonia was determined by the enzymatic method with small sample volume (50–250 μl). The detection limit was 0.29 μM defined as the concentration corresponding to twice the standard deviation in the blank signal (distilled water) for a sample volume of 250 μl using a 1-cm vessel by the enzymatic method. The detection limit was 0.22 μM by indophenol blue colorimetry. There was a linear relationship ($r = 0.9997$, 4-point calibration) between peak height and ammonia concentration over the range 0–29 μM. The concentrations of ammonia of 400 samples were measured within one hour and the reproducibility of the enzymatic method was 5% for 6 successive measurements at 1.5 μM levels.

Effect of potentially interfering compounds on measurement of ammonia

The interferences of urea, ascorbic acid and amino acids such as glycine, arginine and glutamic acid were studied. The concentration of 50 mg l^{-1} of these compounds were added to 50 μg l^{-1} of ammonia standard solution and these were then measured by the enzymatic method. The results show there was no interference caused by these compounds at all interference experiments (Table 1). There was no peak detected in the reagent blanks by the enzymatic method.

Comparison between enzymatic method and indophenol blue colorimetry for ammonia

To make sure that the enzymatic method is applicable to the lake water sample, ammonia in samples was measured by both enzymatic and colorimetric methods. Figure 2 compares the analytical results of the enzymatic method with indophenol blue colorimetry. As shown in Figure 2, correlation was quite acceptable between both methods, and the results clearly demonstrate that the enzymatic method proves to be useful for natural water ammonia measurements.

The concentrations of dissolved free amino acids in seawater during the morning and early afternoon were about 50–200 nM and this implies that only minor

Table 1 Effect of potentially interfering compounds on the measurement of ammonia

Interfering compound	*Measured concentration of ammonia ($\mu g\ l^{-1}$)*	
	Mean ($n = 12$)	*Standard deviation*
None	47.67	3.95
Urea	46.43	4.20
Ascorbic acid	55.41	8.20
Glycine	45.28	6.44
Arginine	52.74	18.25
Glutamic acid	43.57	3.35

The concentration of 50 mg l^{-1} of interfering compounds were added to 50 μg l^{-1} of ammonia standard solution.

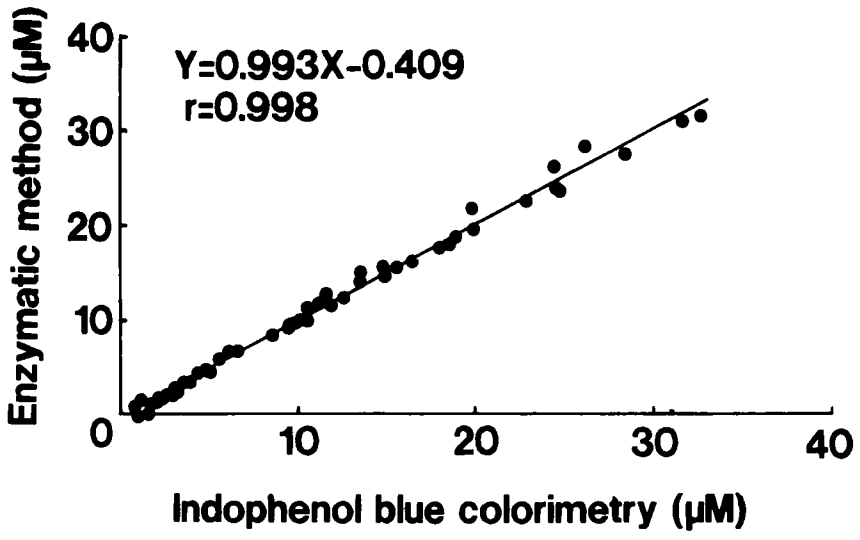

Figure 2 Comparison between the enzymatic method and indophenol blue colorimetry for ammonia. Samples were collected in Lake Kasumigaura.

quantities of free amino acids are released during photosynthesis.[19] If amino acids are present at high concentrations in eutrophic lake water, the rapid uptake of them may bring the equilibrium in which the concentration of free amino acids are as low as the available concentration for the organisms. The intercept value of Figure 2, 0.409, may imply that the constant low level of amino acids are always present in lake water and the hydrolyzed ammonia from their labile amino groups by indophenol blue method makes this intercept negative.

CONCLUSION

We have shown in this study an analytical application of the enzymatic method for the ammonia determination in lake water. The method has several merits over the current indophenol blue colorimetry. In view of the specificity, we are confident that this enzymatic procedure may be a desirable selection.

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