Determination of Ammonia in Estuary*1

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(Received September 21, 1979)

In order to determine ammonia in estuaries or bays influenced by fresh water, we examined the effects of temperature and salt concentration on the development of the blue color of indophenol.

It is found that the reaction temperature and the salt concentration of samples have complex effects on the time required for the completion of the color development and the absorbance in completion. However salt error was less than 5% when the reaction was performed for 30 min at 40°C.

Ammonia in estuary is often determined by many investigators, especially in studies on eutrophication. The determination of ammonia in seawater is performed mostly by the method of SOLORZANO¹⁾ or its modified one²⁾, however it displays salt error²⁾, contrary to the author's report, and is not suitable for the determination in the sea in which salt concentration changes largely.

We examined the development of the blue color of indophenol under several conditions, and report a condition not displaying salt error.

Material and Method

Reagents

The method by SOLORZANO¹⁾ was modified as follows.

1) Phenol-alcohol solution. Dissolve 10 g of reagent grade phenol in 100 ml of 95% v/v ethyl alcohol.

2) Oxidizing solution. Dissolve 0.5 g of potassium dichloroisocyanurate in a solution of 5 g of sodium hydroxide in 100 ml of de-ionized water. To this add a solution of 50 g of trisodium citrate in 100 ml of de-ionized water. Adjust volume to 250 ml with de-ionized water.

3) Sodium nitroprusside solution. Dissolve 0.5 g of sodium nitroprusside in 100 ml of de-ionized water.

To a 25 ml sample in a 100 ml conical flask add 1 ml of phenol-alcohol solution, 2.5 ml of oxidizing solution and 1 ml of sodium nitroprusside solution, mixing between each addition.

Development of the Blue Color of Indophenol in

Several Conditions

The sample of known salt concentration was prepared using a filtered seawater. The sample mixed with reagents was transferred to a cuvette kept at different temperatures (22°-34°C), and the development of the color was recorded at 640 nm on a self-recording spectrophotometer (Hitachi EPS3T). In another case (temperature above 40°C), the sample was placed in a water bath at a known temperature and successively the sample was taken out at an appropriate interval and the absorbance of the color developed was read at 640 nm with a spectrophotometer (Hitachi 101).

Result and Discussion

Effects of the Salt Concentration on the Color Development

As shown in Fig. 1, the absorption maximum of the blue color is at 645 nm in the sample of fresh water and at 635 nm in seawater (chlorinity of

Fig. 1. Absorption curve of the color developed as described in Material and Method. FW: Fresh water, SW: Seawater. Chlorinity of SW is 19‰.

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Fig. 2. Effects of the salt concentration (chlorinity) on the development at 28°C.

19‰). The absorbance of the color developed was read at 640 nm in the following experiments as mentioned in Material and Method. When we examined the effects of various salt concentrations at room temperature (28°C), the time required for the completion of the color development was different one another, and is shorter in lower salt concentration as shown in Fig. 2. The absorbance in completion was also different one another, it increased in the sample of 9.5‰ of chlorinity and then decreased as the salt concentration increased.

Effects of the Reaction Temperature on the Color Development

The reaction temperature had influence on both the time required for the completion of the color development and the absorbance in completion. As shown in Fig. 3, the higher the temperature, the faster the reaction completes in both freshwater and seawater. When the temperature increased, the absorbance in completion decreased in freshwater, however it decreased at 28°C and increased again in higher temperature in seawater. The effects of the salt concentration and the reaction temperature are summarized in Tables 1 and 2.

Table 1. Effects of the salt concentration on the color development (ammonia concentration: 40 μ M)

Cl (‰)	Time required for completion (min)	Ab ₆₄₀ in completion
0	15	0.600
4.5	15	0.575
9.5	25	0.610
15.5	50	0.592
16.0	50	0.572
17.0	50	0.543
18.0	60	0.505
19.0	90	0.500

Table 2. Effects of the reaction temperature on the color development (ammonia concentration: 40 μ M)

Cl (‰)	T (°C)	Time required for completion (min)	Ab ₆₄₀ in completion
0	20	40	0.670
	28	15	0.600
	34	10	0.575
	40	10	0.577
19	20	140	0.550
	28	90	0.500
	34	60	0.545
	40	30	0.564

Suitable Condition not Displaying Salt Error

Salt error was found at 20°C, as shown in Table 2, namely, the absorbance was 0.670 in freshwater and 0.550 in seawater, but the difference becomes small as the reaction temperature is higher as shown in Table 2. Then, we examined the color development at 40°C and 50°C. In the reaction at 40°C, as shown in Fig. 4, the color development is complete in 30 min even in seawater and the

Fig. 3. Effects of the reaction temperature on the color development in samples of various salt concentrations.

Fig. 4. Time-absorbance curves of fresh- and sea-water kept at 40°C.

Fig. 5. Time-absorbance curves of fresh- and sea-water kept at 50°C.

Fig. 6. Relationships of the absorbance and the concentration of ammonia in the fresh- and sea-water reacted for 30 min at 40°C.

difference of the absorbance is small, but in the reaction at 50°C, as shown in Fig. 5, the color development is not complete even in 80 min although the difference is small. The relationship of the absorbance after the reaction completed in 30 min and the concentration of ammonia is shown in Fig. 6 and the factor relating absorbance to concentration (in $\mu\text{g atoms N/liter}$) with 1 cm cuvettes was about 65 and the salt error was less than 5%. In the determination of ammonia in samples of various salt concentrations, therefore we propose that the reaction is performed for 30 min at 40°C and the absorbance of the color developed is read at 640 nm.

Discussion

Although SOLORZANO¹⁾ described that the sensitivity of her method is identical in freshwater and seawater, LIDDICOAT *et al.* reported that the factor relating absorbance to concentration (in $\mu\text{g atoms N/liter}$) with 10 cm cuvette was 6.5 in seawater in freshwater it was 5.8.

The results described here also showed the salt error, namely the factor with 1 cm cuvette was 60 in freshwater while it was 73 in seawater in the reaction at 20°C, however the factor was nearly equal both in freshwater and seawater when the reaction was performed for 30 min at 40°C. The reason why the color development of indophenol was affected by the reaction temperature and the salt concentration of the sample is not clear to us, but it is thought to have a practical meaning that the factor is nearly equal in freshwater and seawater.

References

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