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Flow Injection Analysis with Spectrophotometry for Ammonium ion with 1-Naphthol and Dichloroisocyanurate

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The proposed FIA is based on a modified Berthelot reaction of ammonium ion with 1-naphthol and dichloroisocyanurate to form an indophenol blue derivative. We study to improve the sensitivity of the modified Berthelot reaction, and propose the addition of acetone. The FIA consists of two line flow system. Reagent solution 1 is an alkaline (14.5 g L^{-1} of NaOH) - sodium dichloroisocyanurate (1.2 g L^{-1}) solution containing of trisodium citrate dehydrate (4 g L^{-1}). Reagent solution 1 plays a sample carrier role in the FIA. Reagent solution 2 is 1-napthol (24 g L^{-1}) solution (water : acetone : ethanol = 4 : 1 : 5). The interference of foreign ions in the sample is studied and removed by the addition of the complexing reagent, such as citrate. A linear range of the FIA is 0 to 4.0 μg mL^{-1} of ammonium ion. The relative standard deviation is 0.6% for 2.0 μg mL^{-1} of ammonium ion (n = 10) and the detection limit is 0.013 μg mL^{-1} of ammonium ion. The FIA is applied to environmental water samples, such as river, lake, and sea water.

Keywords ammonium ion, 1-naphthol, dichloroisocyanurate, Berthelot reaction, spectrophotometry, Flow injection analysis (FIA)

1. Introduction

Nowadays, the monitoring of nitrogenous chemical species is very important to prevent the eutrophication of environmental water [1, 2]. Ammonium ion (NH_4^+) is important nitrogen species in a water ecosystem [3].

To determine ammonium ion, many methods have been described. For example, spectrophotometry [4-16] fluorometry [17-19] conductometry [20, 21], and potentiometry [22] have been reported. Spectrophotometry with hypochlorite and phenol reagents has been commonly used on a manual and flow analysis because of a simple and convenient method [4-12].

Ammonium ion reacts with hypochlorite and phenolic compounds to form an indophenol blue derivative. The reaction is called Berthelot reaction. Phenol [9, 23], sodium salicylate [11, 24, 25], thymol [4, 7, 26], *o*-phenylphenol [27], and 1-naphthol [5] have been used as phenolic compounds on Berthelot reaction. Sodium hypochlorite and sodium dichloroisocyanurate (DIC) have been used as chlorine compounds [28]. It has been also reported that the addition of nitroprusside improved the sensitivity of Berthelot reaction [28].

The toxicity of phenol and nitroprusside has been well known. These toxic reagents should be replaced by less toxic reagents. Sodium salicylate has been reported on Berthelot reaction. Salicylate is less toxicity than phenol. However, nitroprusside is necessary to form the indophenol blue derivative using salicylate. It has been also reported another modified Berthelot reaction using 1-naphthol without nitroprusside [5, 29-31].

In this work, we focused to a FIA based on a modified Berthelot reaction using 1-naphthol and DIC, which had many advantage than phenol and sodium hypochlorite.

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2. Experimental

2.1 Reagents

All reagents used were of analytical reagent grade. Distilled water was used through. A stock standard solution of ammonium ion (1000 mg-NH_4^+ L^{-1}) was prepared by dissolving 2.97 g of ammonium chloride in 1000 mL of water. A working standard solution of ammonium ion was prepared by the appropriate dilution of the stock standard solution before use. Regent solution 1 (RS1) was prepared by dissolving 1.2 g of DIC, 14.5 g of sodium hydroxide, and 4 g trisodium citrate dihydrate (citrate) in 1000 mL of water. Regent solution 2 (RS2) was prepared by dissolving 24 g of 1-naphthol in 1000 mL of mixed solvent (water : acetone : ethanol = 4 : 1 : 5). RS2 was degassed to use ultrasonic bath for 15 minutes.

2.2 Apparatus

A FIA system for ammonium ion determination is shown in Fig. 1.

The FIA system was composed of a double plunger pump (Dual Pump KP-11; FLOM Corporation), a six-port switching valve (A-VALVE; FIA Instrument), a double beam spectrophotometer (optical path length was 10 mm of the flow cell, Model S-3250; SOMA Optics), a thermostatic bath (Column oven Model 502, FIA Instrument), and a recorder (R-21, RIKADENKI). All line tube was made of PTFE.

A double beam spectrophotometer (UV-1950-PC, SHIMADZU) was used for the recording spectra and the measurements of absorbance in glass cell of 10 mm light-path length on a manual procedure.

2.3 Manual procedure of screening tests for an improvement on a sensitivity of the modified Berthelot reaction

A sample (20 mL) containing 40 μg of ammonium ion was taken into a 25 mL volumetric flask. Then, 1 mL of alkaline (5 g L^{-1} as NaOH) DIC solution (1.72 g L^{-1}) and 1 mL of 1-naphthol ethanol solution (16 g L^{-1}) were added. Next, acetone or manganese sulfate solution, or sodium tetraborate solution was added. The mixed solution was diluted to 25 mL with water. The solution was stood for 60 min at 25°C in water bath. The absorbance of the solution was measured.

2.4 FIA method

The FIA of Fig. 1 was used for the study of optimized conditions on the FIA using 1-naphthol and the determination of ammonium ion in an environment sample.

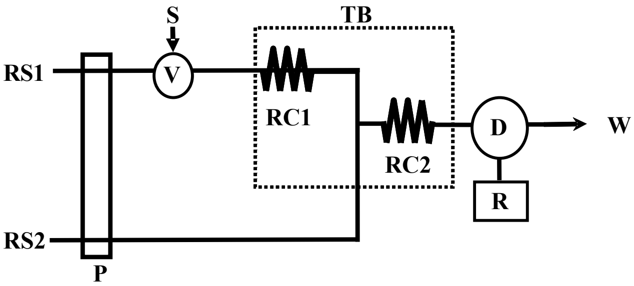

Fig. 1 FIA system for ammonium ion determination.
RS1: 1.2 g L^{-1} of DIC, 14.5 g L^{-1} of NaOH, and 4 g L^{-1} of trisodium citrate dihydrate, RS2: 24 g L^{-1} of 1-naphthol in mixed solvent (water : acetone : ethanol = 4 : 1 : 5), P: double plunger pump (each flow rate: 0.5 mL min^{-1}), S: sample (70 μL), V: six-port switching valve, RC1: reaction coil 1 (0.5 mm i.d. × 2 m), RC2: reaction coil 2 (0.5 mm i.d. × 6 m), TB: thermostatic bath (40°C), D: detector (735 nm), R: recorder, W: waste.

3. Results and Discussion

3.1 Selection of reagents for Berthelot reaction

Many reagents for Berthelot reaction have been reported. In this work, 1-naphthol was chosen because it reacted with ammonium ion to form the indophenol blue derivative without nitroprusside and was lower toxicity than phenol.

We examined acetone [5, 28, 32], manganese (Mn^{2+}) [24, 33], and sodium tetraborate decahydrate [12, 34], which have been reported as catalyst or coupling reagent on Berthelot reaction to improve the sensitivity. The results are shown in Table 1. Acetone was found to show the largest effect. Thus, acetone was chosen.

The role of these reagents has not still been elucidated on Berthelot reaction. On the screening test, acetone interfered with the first step of Berthelot reaction, in which ammonium ion reacted with DIC to form monochloramine. Acetone should not be added before the addition of DIC. On Berthelot reaction by using salicylate, acetone could not be used as substitute for nitroprusside. Thus, salicylate was not chosen in this paper.

In stead of sodium hypochlorite, DIC was used as an oxidation reagent because it was a stable powder reagent.

3.2 Effects of concentration of reagents for FIA

3.2.1 Effects of 1-naphthol and NaOH concentrations

The indophenol blue derivative is formed under alkaline [35], while, 1-naphthol is acid. As concentration of 1-naphthol increases, pH decreases. In order to the lower pH, the indophenol blue derivative is not sufficiently formed.

At first, we examined an effect of NaOH concentration in RS1 under a constant concentration of 1-naphthol in RS2. Fig. 2 shows the effect of NaOH concentration under 24 g L^{-1} of 1-naphthol. Suitable concentration of NaOH was 14.5 g L^{-1} at this 1-naphthol concentration in RS2. We also examined the effects of NaOH concentration in RS1 under the 4 - 48 g L^{-1} concentration of 1-naphthol in RS2 in the same way. Next, we examined an effect of 1-naphthol concentration under the suitable concentration of NaOH obtained. As the results Fig. 3, the suitable concentration of 1-naphthol was 24 g L^{-1}.

Table 1 Effect of the addition of some reagents on Berthelot reaction using 1-naphthol.

Added	Concentration	Absorbance
Non		0.024[a]
$Na_2B_4O_7 \cdot 10H_2O$	2.1×10^{-3} M	0.007[a]
	4.2×10^{-3} M	0.007[a]
$MnSO_4$	1.0×10^{-5} M	0.027[a]
	2.0×10^{-5} M	0.015[a]
Acetone	4.0%	0.396[b]
	16%	0.753[b]

Ammonium ion concentration: 1.6 μg mL^{-1},
a: 720 nm, b: 735 nm

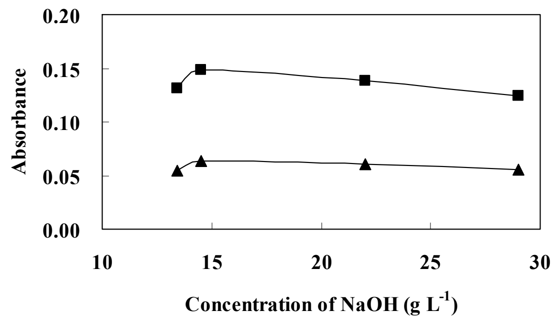

Fig. 2 Effect of NaOH concentration in RS1.
Ammonium ion concentration: (▲) 0.4 μg mL^{-1}, (■) 1.0 μg mL^{-1}, RS1: 1.2 g L^{-1} of DIC and NaOH, RS2: 24 g L^{-1} of 1-naphthol in mixed solvent (water : acetone : ethanol = 4 : 1 : 5), RC1: 3 m, RC2: 7 m, reaction temperature: 30°C, each flow rate: 0.4 mL min^{-1}.

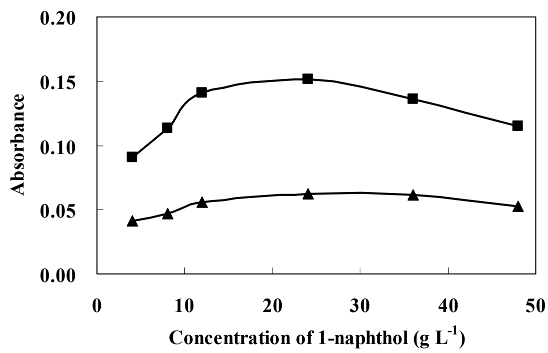

Fig. 3 Effect of 1-naphthol concentration in RS2.
Ammonium ion concentration: (▲) 0.4 μg mL^{-1}, (■) 1.0 μg mL^{-1}, RS1: 1.2 g L^{-1} of DIC and 8.9 to 21.1 g L^{-1} of NaOH, RS2: 1-naphthol in mixed solvent (water : acetone : ethanol = 4 : 1 : 5), RC1: 3 m, RC2: 7 m, reaction temperature: 30°C, each flow rate: 0.4 mL min^{-1}.

3.2.2 Effect of acetone concentration

Acetone concentration in RS2 was varied from 0 to 60%(v/v).

Mixed solvent (40%(v/v) of water and 60%(v/v) of ethanol + acetone) as RS2 solvent was used to prevent the deposition of 1-naphthol because 1-naphthol is high hydrophobicity. As changing the acetone concentration in RS2, the ethanol concentration was changed. For example, the ethanol concentration was 50%(v/v) when the acetone concentration was 10%(v/v). As shown in Fig. 4, the constant and maximum absorbance was obtained over 5%(v/v) of acetone. Suitable concentration of acetone was 10%(v/v). Thus, mixed solvent (water : acetone : ethanol = 4 : 1 : 5) was used as RS2 solvent.

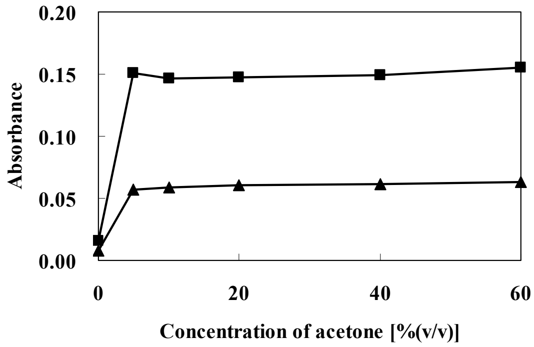

Fig. 4 Effect of acetone concentration in RS2.
Ammonium ion concentration: (▲) 0.4 μg mL^{-1}, (■) 1.0μg mL^{-1}, RS1: 1.2 g L^{-1} of DIC and 14.5 g L^{-1} of NaOH, RS2: 24 g L^{-1} of 1-naphthol in mixed solvent (water : acetone : ethanol), RC1: 3 m, RC2: 7 m, reaction temperature: 30°C, each flow rate: 0.4 mL min^{-1}.

3.2.3 Effect of DIC concentration

DIC concentration in RS1 was studied was varied from 0.4 to 2.0 g L^{-1}. Suitable concentration of DIC in RS1 was 1.2 g L^{-1} as shown in Fig. 5.

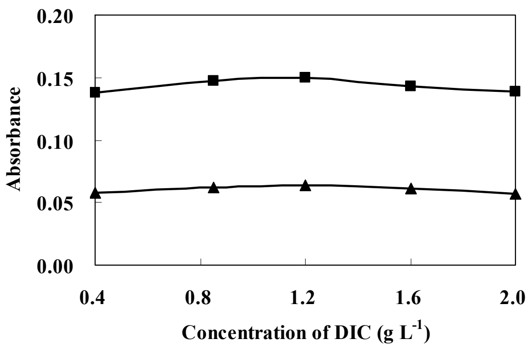

Fig. 5 Effect of DIC concentration in RS1.
Ammonium ion concentration: (▲) 0.4 μg mL^{-1}, (■) 1.0 μg mL^{-1}, RS1: DIC and 14.5 g L^{-1} of NaOH, RS2: 24 g L^{-1} of 1-naphthol in mixed solvent (water : acetone : ethanol = 4 : 1 :5), RC1: 3 m, RC2: 7 m, reaction temperature: 30°C, each flow rate: 0.4 mL min^{-1}.

3.3 Effect of reaction temperature

The effect of reaction temperature was varied from 20 to 60°C. The absorbance increased with increasing the temperature up to 40°C as shown in Fig. 6. However, the absorbance decreased at a temperature above 50°C. Suitable reaction temperature was 40°C.

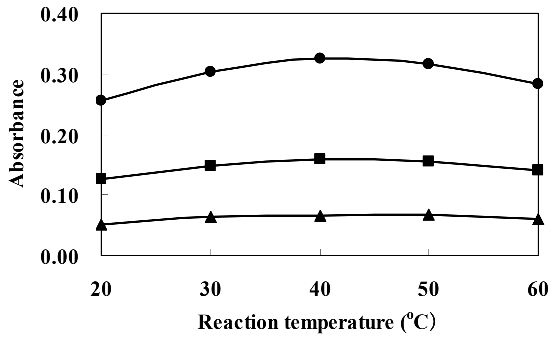

Fig. 6 Effect of reaction temperature.
Ammonium ion concentration: (▲) 0.4 μg mL^{-1}, (■) 1.0 μg mL^{-1}, (●) 2.0 μg mL^{-1}, RS1: 1.2 g L^{-1} of DIC and 14.5 g L^{-1} of NaOH, RS2: 24 g L^{-1} of 1-naphthol in mixed solvent (water : acetone : ethanol = 4 : 1 :5), RC1: 3 m, RC2: 7 m, each flow rate: 0.4 mL min^{-1}.

3.4 Effect of reaction coil length

3.4.1 Effect of the reaction coil 1 length

In reaction coil 1, ammonium ion reacts with DIC to form a monochloramine. The reaction coil 1 length was varied from 0.5 to 6.5 m. As shown in Fig. 7, the constant and maximum absorbance was obtained when length of reaction coil 1 was from 1.5 to 2.5 m. Suitable length of reaction coil 1 was 2 m.

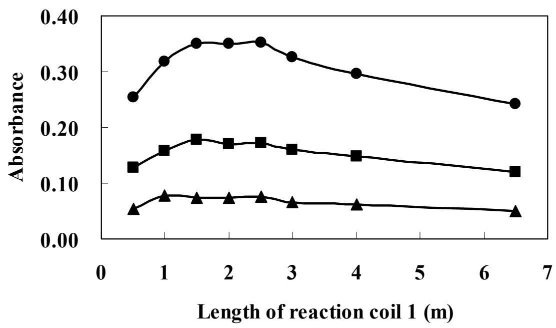

Fig. 7 Effect of reaction coil 1 length.
Ammonium ion concentration: (▲) 0.4 μg mL^{-1}, (■) 1.0 μg mL^{-1}, (●) 2.0 μg mL^{-1}, RS1: 1.2 g L^{-1} of DIC and 14.5 g L^{-1} of NaOH, RS2: 24 g L^{-1} of 1-naphthol in mixed solvent (water : acetone : ethanol = 4 : 1 :5), RC2: 7 m, reaction temperature: 40°C, each flow rate: 0.4 mL min^{-1}.

3.4.2 Effect of reaction coil 2 length

In reaction coil 2, the monochloramine reacts with 1-naphthol to form the indophenol blue derivative. The reaction coil 2 length was varied from 5 to 9 m. As shown in Fig. 8, the constant and maximum absorbance was obtained when length of reaction coil 2 was from 4 to 9 m. Suitable length of reaction coil 2 was 6 m.

3.5 Effects of sample volume and flow rate

The sample volumes of 50, 60, 70, 80, 90, 100, 200 μL were tested by changing the length of the sample loop. When the sample volume was above 80 μL, split peak was obtained. In this manifold, sample was injected into RS1 stream. When the sample loop was too long, the sample and RS1 were not completely mixed. However, as lower sample volume, the absorbance decreased. Thus, suitable sample volume was 70 μL.

The flow rates from 0.3 to 0.7 mL min^{-1} were studied. The constant and maximum absorbance was obtained when the flow rate was 0.4 and 0.5 mL min^{-1}. The slower flow rate increased analysis time. The flow rate of 0.5 mL min^{-1} was adapted.

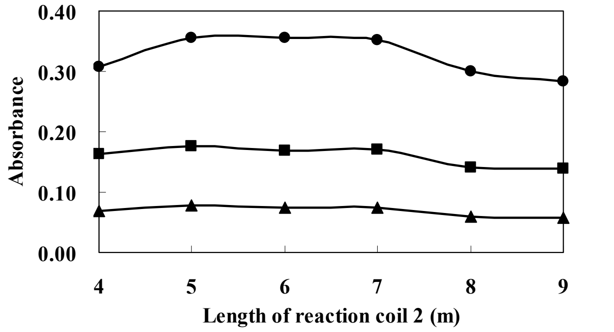

Fig. 8 Effect of reaction coil 2 length.
Ammonium ion concentration: (▲) 0.4 µg mL^{-1}, (■) 1.0 µg mL^{-1}, (●) 2.0 µg mL^{-1}, RS1: 1.2 g L^{-1} of DIC and 14.5 g L^{-1} of NaOH, RS2: 24 g L^{-1} of 1-naphthol in mixed solvent (water : acetone : ethanol = 4 : 1 :5), RC1: 2 m, reaction temperature: 40°C, each flow rate: 0.4 mL min^{-1}.

3.6 Effects of foreign ions

The indophenol blue derivative with Berthleot reaction is formed under high alkaline solution. Therefore, complexing reagent has been added to the sample to prevent the formation of metal hydroxides on the ammonium ion determination of an environmental water sample.

Effects of foreign ions were studied on the determination of ammonium ion by the proposed FIA (Fig. 1). As shown in Table 2, the results revealed that there was no serious interference from most foreign ions, except for Cu^{2+}. When RS1 contained 40 g L^{-1} of citrate was used, a good recovery was obtained at 10 mg L^{-1} of Cu^{2+}.

Table 2 Effects of foreign ions on the determination of ammonium ion using the proposed FIA.

Foreign ions	Concentration (mg L^{-1})	Added as	Recovery (%)
Na^+	30000	Na_2SO_4	99.2
Cl^-	30000	KCl	99.2
NO_3^-	10	KNO_3	100.3
NO_2^-	10	$NaNO_2$	98.5
SO_4^{2-}	60000	Na_2SO_4	99.2
K^+	30000	KCl	99.2
Al^{3+}	10	$Al(NO_3)_3$	95.4
Ca^{2+}	10	$CaCl_2$	97.0
Fe^{3+}	10	$FeCl_3$	99.7
Cu^{2+}	10	$CuSO_4$	79.5
			92.4[c]
Mg^{2+}	10	$MgCl_2$	96.8

Ammonium ion concentration: 2.0 µg mL^{-1}, c: 40 g L^{-1} of trisodium citrate dehydrate in RS1

3.7 Analytical characteristics

On the proposed FIA, the calibration curve was prepared using the working standard solutions of ammonium ion at the concentration range of 0 to 4.0 µg mL^{-1} as shown in Fig. 9. The relative standard deviation was 0.6% for 2.0 µg mL^{-1} of ammonium ion (n = 10) and the detection limit, as defined as the concentration giving an absorbance equal to three times the standard deviation of the blank, was 0.013 µg mL^{-1} of ammonium ion (n = 10). The sampling rate was *ca*. 26 injection h^{-1}.

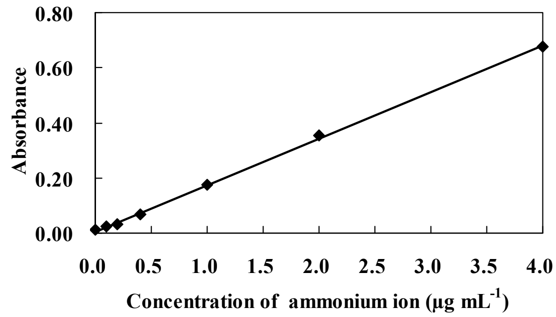

Fig. 9 Calibration curve by the proposed FIA for determination of ammonium ion.

3.8 Application to the environmental water sample

3.8.1 Comparison of complexing reagents

Many complexing reagents have been used for manual and automated determination of ammonium ion with Berthelot reaction [28,35].

Citrate, tartrate (sodium potassium tartrate tetrahydrate), and EDTA (tetrasodium ethylenediaminetetraacetate dihydrate) were studied. As shown in Table 3, there was no major difference from complelxing reagents. Using high concentration of complexing reagents such as 40 g L^{-1} of citrate and tartrate, sensitivity slightly decreases. However, there was no serious problem. If necessary, high concentration of complexing reagents can be used.

3.8.2 Application to the environmental water sample

The proposed FIA was applied to the analysis of environmental water samples. The results are shown in Table 4. Good recoveries were obtained on the ammonium ion analysis of river, lake, and sea water sample.

4. Conclusion

The proposed FIA was based on the modified Berthelot reaction with 1-napthol and DIC. These reagents are commonly purchased as powder. The FIA did not require toxic reagent, like nitroprusside. Acetone was the best sensitivity improvement in this investigation. We recommended using 1-naphthol, DIC, and acetone for the modified Berthelot reaction.

Under optimized conditions, the linear relationship was obtained between the absorbance and the concentration of ammonium ion in the range 0 – 4 µg mL^{-1}. Foreign ions did not interfere with the determination of ammonium ion using the complexing reagent. The FIA was very simple system and could be applied to determine ammonium ion in the environmental water sample.

Table 3 Comparison of complexing reagents.

Complexing reagents	Concentration in RS1 (g L^{-1})	Calibration curve	R^2	Recovery of ammonium ion added to water samples (%)	
				river water	sea water
Citrate	4	y = 0.1687x + 0.0058	0.9993	97.5	94.9
	40	y = 0.1568x + 0.0171	0.9996	96.4	95.4
Tartrate	40	y = 0.1563x + 0.0146	0.9996	96.5	93.9
EDTA	1	y = 0.1670x - 0.0051	0.9993	96.0	92.9

Table 4 Recovery of ammonium ion added to water samples.

Water sample	NH_4^+ added (μg mL^{-1})	NH_4^+ found (μg mL^{-1})	Recovery (%)
River water	0.4	0.46	
	1.0	1.04	97.1
	2.0	2.02	97.9
Lake water	0.4	0.41	
	1.0	1.01	99.8
	2.0	1.99	98.7
Sea water (1)	0.4	0.42	
	1.0	0.98	92.5
	2.0	1.98	97.3
Sea water (2)	0.4	0.41	
	1.0	0.99	98.9
	2.0	1.98	99.0

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