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The development of extraction photometry for the determination of ammonia by use of easily-solidifying solvent

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Abstract

The proposed method for the determination of ammonia comprises three unique techniques, namely, utilization of easily-solidifying solvent, phase separation by cooling centrifugation, and the extraction of ion association complex. In concrete terms, 1.2.4-trichlorobenzene was mainly used as extracting solvent among the potential easily-solidifying solvents, and phases were separated by the cooling centrifugation at −3℃ and 3000 rpm for 30min after shaking, and ammonia was extracted in the form of ion association complex of indothymol and Capriquat. Under the optimum condition, linear relationship between absorbance of organic phase and ammonia concentration was obtained in the concentration range of 0.25 to 25 ppb NH_4-N with the correlation coefficients of 0.999_6 for 20 mL of indothymol solution.

Keywords: Extraction photometry, Easily-solidifying solvent, Cooling centrifuge, Indothymol, and Ion association complex

Introduction

The solvent extraction is venerable and sophisticated separation method, but if pressed phase separation is tedious procedure to perform. If it is possible to solidify the organic phase, phases can be simply separated by decanting aqueous phase. Based on this idea, one of the authors proposed the usage of easily-solidifying solvent as an extracting solvent. An easily-solidifying solvent possesses a melting point lying in the vicinity of room temperature, and becomes a solid by centrifugation cooling below its melting point after equilibration with aqueous phase. After phase separation by decanting an aqueous phase, an organic phase would reliquefy by leaving it for a while under the room temperature. This simplification in the phase separation brings about another advantage, the concentration. That is to say, it is possible to enlarge the volume ratio of aqueous phase to organic one greater than that used in the usual extraction methods.

The authors had already reported the utilization of easily-solidifying solvent to the metal extraction[1]. The present study was undertaken to confirm whether easily-solidifying solvent could be successfully applied to the extraction of non-metal species or not and ammonia was selected as an analyte because of its importance in the environmental science. First, easily-solidifying solvent alone and the mixture of easily-solidifying solvent and polar solvent such as acetonitrile were examined to determine the suitable solvent for the extraction of dissociated-form indothymol, however, none could extract it perfectly. Then, 8 kinds of counter cations were arbitrarily selected and were examined with their performances for the ion association extraction with indothymol. It was found that tetradecyldimethylbenzyl ammonium chloride and tri-*n*-octylmethylammonium chloride gave the satisfactory results and their extractabilities were discussed by use of cation value[2-4].

Experimental

Reagents and apparatus All reagents used in this study were of analytical grade and were used without further purification. A Hitachi CF7D cooling centrifuge was used for phase separation, and Nihonbunko V-560DS spectrophotometer for absorbance measurements.

Extraction procedure Color development of ammonia to indothymol was carried out by the conventional spectrophotometric method[5]. That is,

Table 1 Effects of pH and the constituent of organic phase on the extraction of indothymol

Organic solvent	pH 4.5	pH 11.6	pH 12	pH 13
1.0 mL Acetophenone	U-IPS	U-IE	U-IE	U-PE
1.0 mL Acetophenone + 1.0 mL acetonitrile	U-IPS	U-IPS	U-IPS	D-IE-IPS
1.0 mL Acetophenone + 2.0 mL acetonitrile	U-PE	U-IPS	U-IPS	D-IE-IPS
1.0 mL Acetophenone + 2.0 mL tetrahydrofuran	U-PE	U-PE	U-PE	U-IE
1.0 mL Acetophenone + 2.0 mL dimethylsulfoxide	U-PE	U-H	U-H	D-IE
1.0 mL Acetophenone + 2.0 mL dimethylformamide	U-PE	U-IPS	PE	U-IE
2.5 mL Benzylbenzoate	-	-	U-PE	U-IE
2.0 mL Diphenylether	U-IE	U-IPS	U-IPS	U-IE
1.0 mL 1,2,4-Trichlorobenzene	U-IPS	U-IE-IPS	U-IE-IPS	U-IE

Aqueous phase: 10 mL of 2 ppm NH_4-N solution. PE : Perfect extraction, IE : incomplete extraction, D : dissociated species, U : undissociated species, IPS : imperfect phase separation, H : phases dissolved mutually to form homogeneous solution, - : not examined

ammonium ion in a solution was reacted with sodium hypochlorite containing 3% available chlorine to form monochloramine at pH 10 adjusted with sodium carbonate-sodium hydrogen carbonate buffer solution and was reacted with thymol at pH 12 adjusted with 0.55 M sodium hydroxide, followed by being diluted to 50 mL exactly. An aliquot of indothymol solution prepared by the above mentioned method was transferred to 30mL extraction vial and was mixed with 1 wt/v% counter cation aqueous solution and 2 mL of easily-solidifying solvent. The mixture was shook for 10 min vigorously and was centrifuged at –3℃ and 3000 rpm for 30 min, followed by being subjected to absorbance measurement.

Results and Discussion

Effect of pH and the constituent of organic phase on the extraction of indothymol A dissociated form indothymol that is blue has greater molar absorptivity than an undissociated one that is pink, so that it is desirable to extract a dissociated form indothymol in terms of sensitivity. At first, acetophenone(m.p. 20℃), benzylbenzoate(m.p. 19℃), diphenylether(m.p. 27℃), and 1.2.4-trichlorobenzene(m.p. 17-18℃) were selected as the promising easily-solidifying solvent[6], and the extractability of dissociated form indothymol was examined with these solvents individually as well as the mixed solvent containing acetophenone and polar solvent

Table 2 Effect of the secondary solvent added to easily-solidifying solvent on the extraction

An annex to easily-solidifying solvent	Easily-solidifying solvent[1)] *p*-Xylene	1,2,4-Trichlorobenzene
1.0 mL Dioxane	U-PE	U-PE
3.0 mL Dioxane	U-IE	U-IE
4.0 mL Dioxane	U-IE	U-IE
5.0 mL Dioxane	U-IE	U-IE
6.0 mL Dioxane	U-IE	U-IE
1.0 mL Dimethylsulfoxide	U-PE	U-PE
2.0 mL Dimethylsulfoxide	U-IE	U-IE
3.0 mL Dimethylsulfoxide	U-IE	U-IE
4.0 mL Dimethylsulfoxide	-	U-IE
5.0 mL Dimethylsulfoxide	U-IE	U-IE
1.0 mL Acetonitrile	U-PE	U-PE
2.0 mL Acetonitrile	U-IE	U-IE
3.0 mL Acetonitrile	U-IE	U-IE
4.0 mL Acetonitrile	D-IE	U-IE
5.0 mL Acetonitrile	D-IE	D-IE

1) Used volume of solvent was 1.0mL. Aqueous phase: 10 mL of 2 ppm NH_4-N solution. PE : Perfect extraction, IE : incomplete extraction, D : dissociated species, U : undissociated species, IPS : imperfect phase separation, H : phases dissolved mutually to form homogeneous solution, - : not examined.

such as acetonitrile, tetrahydrofuran, dimethylsulfoxide, and dimethylformamide, at different pH values (Table 1). Although the mixed solvents containing acetophenone and acetonitrile or dimethylsulfoxide partly extracted dissociated form indothymol, most solvents could not extract it. Acetophenone, however, did not always solidify, that is, acetophenone sometimes did not solidify. The reason of this phenomenon may be ascribable to the solubility of acetophenone in water and hence acetophenone was not adopted as an easily-solidifying solvent in this study. Then, *p*-xylene and 1,2,4-trichlorobenzene were selected as easily-solidifying solvent in the respect of the stability of solidifying behavior and it was attempted to improve their polarity by adding the polar solvent such as dioxane, dimethylsulfoxide, and acetonitrile, since they were nonpolar and were considered to be difficult to extract polar species singly. As can be seen from Table 2, only the mixed solvent containing a large amount of acetonitrile could extract dissociated form indothymol somewhat. But enlargement of acetonitrile content brought about harmful effect on the solidifying behavior of the mixed solvent.

Effect of counter cation Next, it was attempted to extract indothymol in the form of ion association complex, since it was difficult to extract dissociated form indothymol alone. The following 8 compounds was arbitrarily selected as a counter cation; Rhodamine B, Malachite Green, Pararoseaniline, Methylene Blue, 1-methyl-4-(4-diethylaminophenylazo)pyridinium iodide (MDEPAP), benzyldimethyltetradecylammonium chloride (Zephiramine), 1-hexadecylpyridinium chloride, and tri-n-octylmethylammonium chloride (Capriquat). The results obtained with 1,2,4-trichlorobenzene for blank solution and standard solution containing 200 ng NH_4-N were summarized in Table 3, and were almost similar to those obtained with *p*-xylene which were not shown here in order to avoid repetition. Rhodamine B, Malachite Green, Pararoseaniline, Methylene Blue, and MDEPAP are cationic dyes and were selected expecting that the absorbance of ion association complex would be

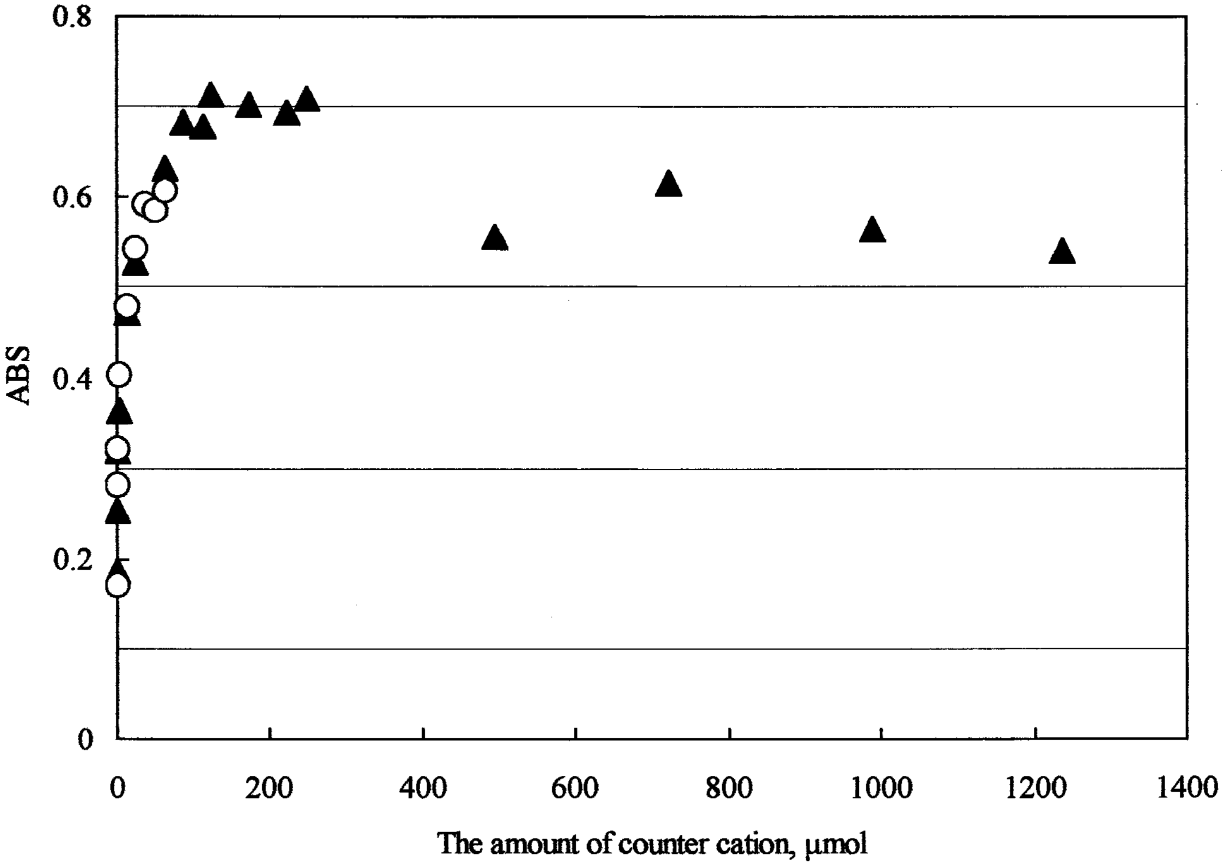
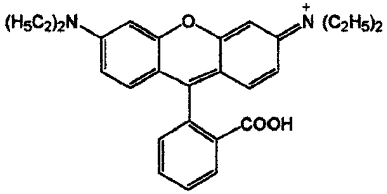
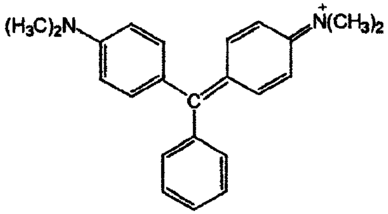
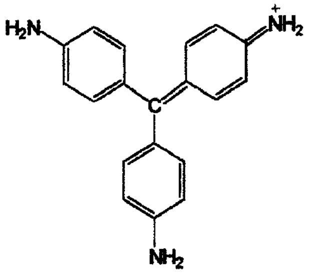
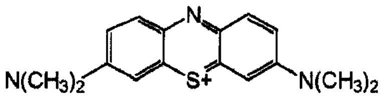
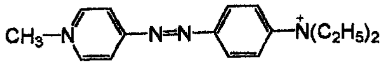
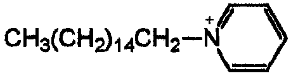
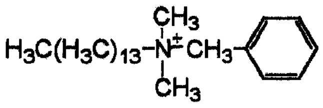


Fig. 1. Effect of the amount of counter cation on the absorbance of extracted ion association complex.
Organic phase: 2 mL 1,2,4-trichlorobenzene, aqueous phase: 20 mL aqueous solution containing 200 ng NH_4-N.
○: Zephiramine, ▲: Capriquat.

Table 3 Spectral data of ion-pair complexes of indothymol with various counter cations

Counter cation	Structural formula and C value of counter cation	Spectral data of a blank solution			Spectral date of 200ng NH_4^+-N containing solution			
		Wavelength(nm) at absorption maximum	Absorbance at absorption maximum	Absorbance at 660nm	Wavelength (nm) at absorption maximum	Absorbance at absorption maximum	Absorbance at 660nm	Corrected absorbance at 660nm
Rhodamine B	 —	544	0.420	0.092	515	0.413	0.179	0.087
Malachite Green	 11.20	630 427	0.440 0.118	0.167	497	0.126	0.031	0.000
Pararoseaniline	 6.69	496	0.059	0.001	516	0.190	0.011	0.010

Methylene Blue	 8.16	481	0.366	0.304	548	1.405	0.532	0.228
1-Methyl-4-(4-diethylamino phenylazo) pyridinium iodide (MDEPAP)	 8.21	563	1.532	0.026	576	3.158	0.136	0.110
1-Hexadecyl pyridinium chloride	 7.24	400	0.495	0.007	647	0.226	0.219	0.212
Benzyldimethyl tetradecyl ammonium chloride (Zephiramine)	 12.76	660	0.036	0.035	647	0.404	0.311	0.276
Tri-n-octyl methyl ammonium chloride (Capriquat)	$[CH_3(CH_2)_6CH_2]_3\overset{+}{N}CH_3$ 13.55	669	0.012	0.010	648	0.320	0.314	0.304

Aqueous phase: 20 mL aqueous solution containing 1 μ mol counter cation and 200 ng NH_4-N. Organic phase: 2 mL of 1.2.4-trichlorobenzene.

increased compared to that of free indothymol and the absorption maximum of ion association complex would be shifted to longer wavelength (bathochromic shift), however, these cationic dyes dissolved in 1,2,4-trichlorobenzene to gave a fairly large absorbance for blank solution and the absorption maximum of ion association complex was shifted to shorter wavelength (hyperchromic shift). Therefore, these cationic dyes were not suitable counter cations for the extraction photometry of indothymol, although ion association complexes with Methylene Blue and MDEPAP gave considerably large absorbance for NH_4-N standard solution.

Motomizu proposed special measures, C value for cation and A value for anion, for ion association extraction. The extractability of the ion association complex, K_{ex}, is represented by the following equation.

$$\log K_{ex} = C + A$$

C values for cation used in this study are cited in Table 3. A value for dissociated form indothymol is not known yet, however, it is constant. The above equation implies that the extractability is governed by C value. Table 3 clearly demonstrates that cation of which C value is greater than 12 can extract the dissociated form indothymol, namely, Zephiramine and Capriquat. 1-Hexadecylpyridinium chloride also could extract quantitatively dissociated form indothymol irrespective of small C value, however, the absorbance of the ion association complex with 1-hexadecylpyridinium chloride was significantly small compared to that with Zephiramine or Capriquat. Therefore, this discrepancy could be neglected.

As mentioned above, 1,2,4-trichlorobenzene and *p*-xylene gave the almost same results, however, *p*-xylene sometimes did not solidify under the experimental condition, especially in summer, because of its low melting point(13.263℃). Finally, 1,2,4-trichlorobenzene was mainly used in this study.

Effect of the amount of counter cation Figure 1 shows that the effect of the amount of counter cation on the absorbance of extracted ion association complex in 1,2,4-trichlorobenzene. In case of Zephiramine, when the added amount of Zephiramine exceeded 61.9 μ mol, the solution became turbid and absorbance of 1,2,4-trichlorobenzene solution could not be determined exactly, although the absorbance increased with increasing the added amount of Zephiramine till 61.9 μ mol. On the other hand, Capriquat did not cause 1,2,4-trichlorobenzene solution turbid even though more than 1 mmol of Capriquat was added. Since the maximum and constant absorbance was obtained in the range of added amount of Capriquat 150 to 250 μ mol, 200 μ mol of Capriquat was added as a counter cation hereafter.

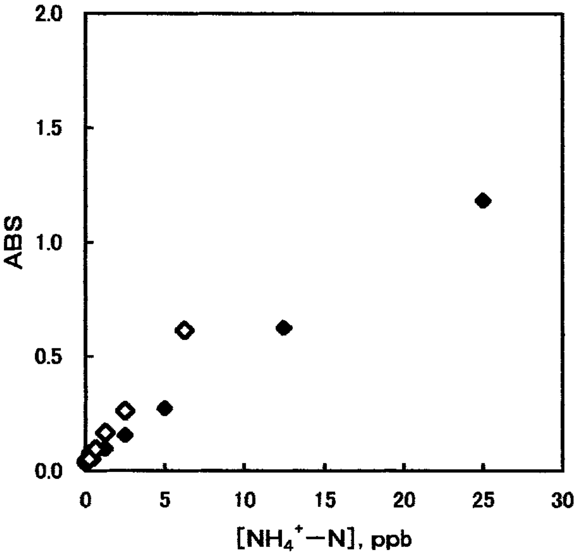


Fig.2-a. Relationship between absorbance and the concentration of NH_4-N obtained by the discontinuous extraction procedure.
Organic phase: 1 mL of 1,2,4-trichlorobenzene, aqueous phase: ◆; 20 mL, ◇; 40 mL.

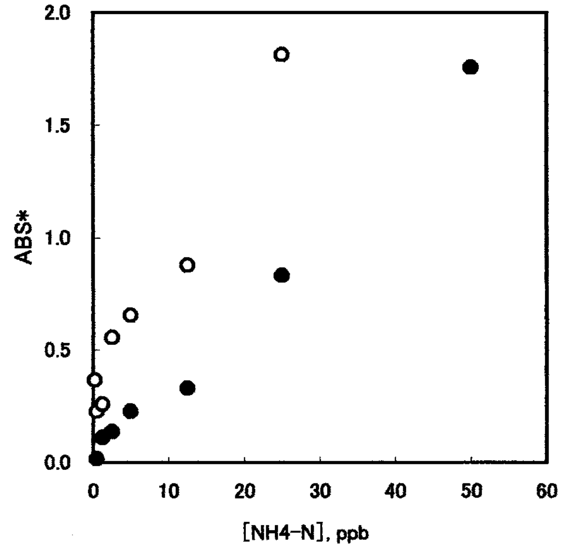


Fig.2-b. Relationship between corrected absorbance by reducing blank absorbance and the concentration of NH_4-N obtained by the continuous extraction procedure.
Organic phase: 1mL of 1,2,4-trichlorobenzene, aqueous phase: ●; 20 mL, ○; 40 mL.

Calibration curve The calibration graphs were shown in Figs 2-a and -b. The graph 2-a was prepared by the discontinuous extraction procedure as follows; Reaction between ammonia and thymol was carried out in a volumetric flask and 20 mL or 40 mL of produced indothymol solution was transferred to the extraction vial, followed by extracting indothymol-Capriquat ion association complex into 1 mL of 1,2,4-trichlorobenzene. The graph 2-b was obtained by the continuous extraction procedure, that is, the color development and the extraction of ammonia was carried out successively in the same extraction vial. As can be seen from these figures, the correlation coefficients of calibration curves obtained by the former were $r = 0.999_6$ for 20 mL of indothymol solution and $r = 0.998_8$ for 40 mL one and were much better than those obtained by the latter($r = 0.995$ for 20 mL of ammonia solution and $r = 0.981$ for 40 mL one). Moreover, the absorbances for the blank solutions obtained by the former procedure were settled in the range of 0.03 to 0.05 and were also obviously smaller than those obtained by the latter method(0.2 to 0.3). The worse precision of the latter method could be mainly attributed to the difficulty of avoiding the contamination of ammonia from environment for the extraction vial. The latter extraction method is simple and convenient but necessitates the greatest care to controlling the contamination.

Finally, according to the present method, ammonia can be determined precisely and more than 20 times sensitively compared to the conventional spectrophotometric method, if special care was taken to handling a sample solution by use of a volumetric flask.

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