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Studies on Benzothiazole Derivatives as Chelating Agents. II.[1) The Fluorometric Determination of Zinc with N-Salicylidene-4-aminobenzothiazole

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N-Salicylidene-4-aminobenzothiazole forms fluorescent complexes with zinc, cadmium and magnesium, which are extractable with isoamylalcohol and the optimum pH for the formation and the extraction of zinc, cadmium and magnesium complexes are 8.9, 10.7, and 8.7, respectively. Especially the fluorescence of the zinc complex is very strong and it is possible to utilize N-salicylidene-4-aminobenzothiazole for the fluorometric determination of zinc in the range of 1.6×10^{-6}M to 0.5×10^{-8}M. Interfering ions except cadmium are suppressed by simple addition of masking agents.

Although the fluorescent characteristics of many zinc complexes have been investigated, only a few fluorometric methods for the determination of zinc have been reported.[3)

We reported previously the syntheses of benzothiazole derivatives and their color and fluorescence reactions with metal ions.[1) Among them, N-salicylidene-4-aminobenzothiazole (NS4ABT) reacts with zinc, cadmium and magnesium in weak alkaline solution to give fluorescence. Especially the fluorescence of the zinc complex is very strong and it is extractable with organic solvents. Using these reactions of NS4ABT, the fluorometric determination of zinc was attempted and good results were obtained.

Experimental

Apparatus——Fluorescence measurements were carried out with a Shimadzu Fluorospectrophotometer type GF–16 equipped with a Xenon arc lamp (Ushio Co. UXL–500W) using 10 mm square quartz cells. Measurements of pH were carried out with a Hitachi-Horiba pH meter type M–4.

Reagent Solution——A reagent solution containing 0.5 μ mole of NS4ABT per ml was made fresh daily by dissolving 6.36 mg of the reagent in 50 ml of iso-amylalcohol. This reagent solution can not be used after 2 days because of the hydrolysis.

Zinc Stock Solution——The solution (1.02×10^{-1}M) was prepared by dissolving 1.6700 g of pure zinc metal (99.999%, Mitsuwa Kagaku Yakuhin Co.) in a minimum volume of diluted hydrochloric acid and diluting the solution to 250 ml with de-ionized water.

Buffer Solutions——A series of buffer solutions was prepared to cover the pH ranges of pH 6.5—12.8 with sodium borate/hydrochloric acid or sodium borate/sodium hydroxide.

Iso-amylalcohol and all other reagents were of guaranteed grade.

Procedure——To the sample solution containing 3.33×10^{-3} to 8.8×10^{-1} μg of zinc, about 2 ml of buffer solution of pH 8.6 and masking agents, if required, were added and the volume was made to 10 ml with de-ionized water. The resulting aqueous solution was extracted with 10 ml of the reagent solution. The fluorescence intensity of the extracted iso-amylalcohol solution was measured at 505 mμ (excitation: 428 mμ) against the solution of reagent. The calibration curves were obtained by the method above mentioned.

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1) Part I: N. Shimidzu and T. Uno, *Chem. Pharm. Bull.* (Tokyo), **21**, 184 (1973).

2) Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*

3) L.L. Merritt, Jr., *Anal. Chem.*, **16**, 758 (1944); R. Trenholm and D.E. Ryan, *Anal. Chim. Acta*, **32**, 317 (1965); R.E. Jensen and R.T. Pflaum, *Anal. Chem.*, **38**, 1268 (1966); D.T. Haworth and R.H. Boeckeler, *Microchem. J.*, **13**, 158 (1968).

Result and discussion

Characteristics of Reagent

The reagent, NS4ABT, is slightly soluble in organic solvents and is insoluble in water. The reagent in iso-amylalcohol exhibits a very weak fluorescence(Fig. 1). The reagent reacts with many divalent metal ions and forms yellow products. A remarkable fluorescence is exhibited by the zinc, cadmium and magnesium complexes. All of the reaction products are extractable with common organic solvents.

The reagent was not hydrolysed in organic solvents, but was hydrolysed to some extent in aqueous solution. Accordingly, the solvent extraction which was scarcely influenced by the hydrolysis was employed for the determination of traces of zinc.

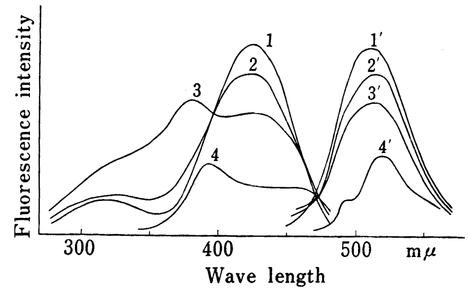

Fig. 1. Excitation and Emission Spectra of Zinc (1,1'), Cadmium (2,2') and Magnesium (3,3') Complexes with NS4ABT and of NS4ABT alone (4,4') in Isoamylalcohol

Spectral Characteristics

The excitation and emission spectra of the zinc, cadmium and magnesium complexes and of the reagent alone are shown in Fig. 1. They are uncorrected for the variation in spectral response of the source and detector system. The fluorescence intensities of cadmium and magnesium complexes are approximately one third and one sixth of that of the zinc complex, respectively, at the most suitable condition. The fluorescence intensity of NS4ABT alone is negligible compared with that of the zinc complex.

Effect of pH

The effect of pH on the formation and the extraction of zinc, cadmium and magnesium complexes are shown in Fig. 2. The maximum fluorescences of the zinc, cadmium and magnesium complexes in the extracted iso-amylalcohol solution occur at pH 8.9, 10.7 and 8.7, respectively. The scale of the ordinate of each curve is chosen properly to give a reasonable height. The fluorescence of the reagent is almost constant over the pH range of 7.2 to 11.3.

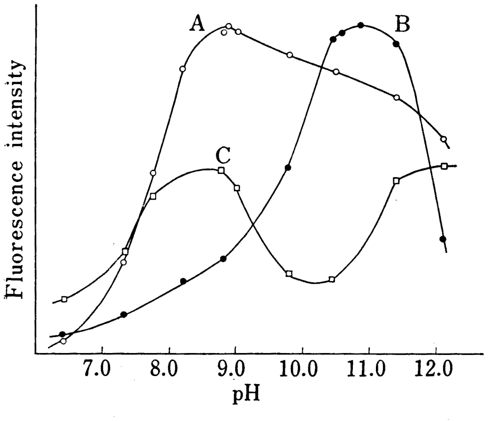

Fig. 2. Effect of pH on Formation and Extraction of Zinc(A), Cadmium(B) and Magnesium(C) Complexes with NS4ABT

NS4ABT: 1×10^{-4}M
zinc, cadmium and magnesium: 2×10^{-6}M

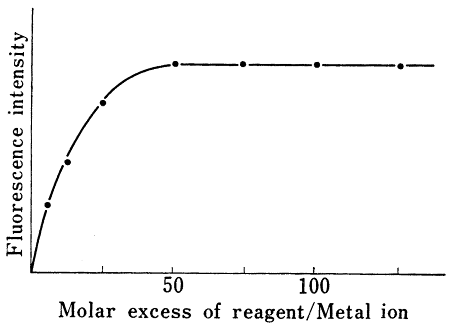

Fig. 3. Effect of Excess of Reagent

zinc concentration: 4×10^{-6}M

Effect of Reagent Concentration

The effect of variation of the reagent concentration on the formation and the extraction of the zinc complex is shown in Fig. 3. The fluorescence of the zinc complex reaches maximum with a *ca.* 50-fold molar excess of the reagent to 4×10^{-6}M of the metal. At lower concentration of zinc, it is necessary to use larger excess of the reagent.

Influence of Time

In Fig. 4 the fluorescence intensity is plotted against the time after the extraction. The relative fluorescence intensity of each extract at the time of 10 minutes after the extraction is taken to be 50. The fluorescence intensity decreases slowly and this is due probably to the hydrolysis of the complex.

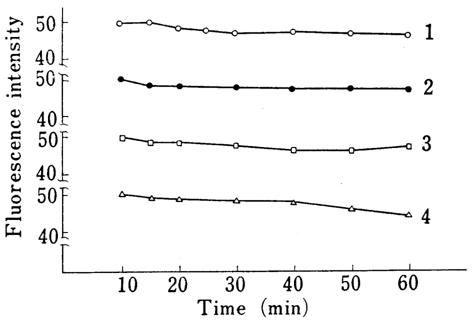

Fig. 4. Variation of Fluorescence with Time

zinc concentration: 4×10^{-6}M(1), 4×10^{-7}M(2), 4×10^{-8}M(3) and 5×10^{-9}M(4)

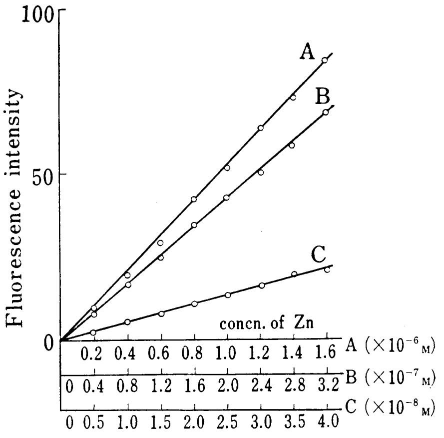

Fig. 5. Calibration Curves

Table I. Effect of Diverce Ions on Fluorescence Intensity of the Zinc Complex Zinc concentration = 1.02×10^{-6}M

Ion	Concentration 10^{-6}M	Source	Relative error, %[a]
Ca^{2+}	100	$CaCl_2$	0
Mn^{2+}	101	$MnCl_2$	0
Cu^{2+}	103	$CuCl_2$	−48.1
Ni^{2+}	100	$Ni(ClO_4)_2$	−19.2
Hg^{2+}	100	$HgCl_2$	0
Co^{2+}	102	$CoCl_2$	−80.4
Sr^{2+}	100	$Sr(CH_3CO_2)_2$	0
UO_2^{2+}	101	$UO_2(NO_3)_2$	−11.0
Be^{2+}	98	$BeCl_2$	−53.6
Cd^{2+}	100	$CdCl_2$	$>+100$
Mg^{2+}	100	$MgCl_2$	$>+100$
Fe^{3+}	101	$Fe(ClO_4)_3$	−84.2
Al^{3+}	101	$AlCl_3$	−55.6
Bi^{3+}	91	$Bi(NO_3)_3$	0
Ag^{+}	101	$AgNO_3$	ppt. in aq. phase
NH_4^{+}	102	NH_4Cl	0
PO_4^{3-}	102	KH_2PO_4	0
CN^{-}	101	KCN	−19.0
$S_2O_3^{2-}$	118	$Na_2S_2O_3$	−4.8
I^{-}	100	KI	0
F^{-}	101	KF	0
$C_2O_4^{2-}$	108	COOH·COOH	0

a) Rel. error = $\frac{I_{mix.}-I_{zinc}}{I_{zinc}}$

Sensitivity

The zinc-NS4ABT complex displayed a linear relationship between the concentration and the fluorescence intensity over the range of 0.5×10^{-8}M to 1.6×10^{-6}M(3.33×10^{-3} to 8.8×10^{-1} μg) zinc ion(Fig. 5). The linearity probably extends above this range.

Effect of Foreign Ions

The effect of *ca.* 100-fold molar excesses of diverse ions on the formation, the extraction and the measurement of the zinc complex are presented in Table I. Relative errors were calculated as indicated. Copper(II), nickel, cobalt, uranyl and iron(III) ions form colored complexes with the reagent. Although these complexes give no fluorescence, they are extracted into iso-amylalcohol and absorb the excitation radiation. Nickel could be tolerated in a 10-fold molar excess without the use of masking agents. Ten-fold molar excesses of copper(II) and cobalt could be tolerated on the addition of about five-fold excess of potassium cyanide against the interfering ions. However, the high concentrations of potassium cyanide, sufficient to mask the interference of 100-fold molar excesses of copper(II) and cobalt, served to destroy the zinc complex also. Aluminium and beryllium gave low result. The interference of 100-fold molar excesses of aluminium and beryllium were completely suppressed by the addition of large excess of potassium fluoride. Cadmium and magnesium act on the reagent and their complexes are extracted into iso-amylalcohol and show a fluorescence as the zinc complex. Ten-fold molar excesses of iron(III) and magnesium could be tolerated on the addition of *ca.* 100-fold excess of oxalate ion against the interfering ions, but the high concentration of oxalate ion weakened the fluorescence of the zinc complex, conversely. Potassium iodide, sodium thiosulfate and sodium diethyldithiocarbamate proved ineffective as masking agents for the interference of cadmium.