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Synthesis of a New Metallofluorescent Indicator (*N*-(2-Naphthyl)ethylenediamine-*N*,*N'*,*N'*-triacetic Acid) and Its Application

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A new metallofluorescent indicator (*N*-(2-naphthyl)ethylenediamine-*N*,*N'*,*N'*-triacetic acid, (NEDTA)) was synthesized. Its possible applications are discussed.

NEDTA was synthesized in a high yield from 2-naphthol, and was stable in $NaHCO_3$ alkaline solution. The acid dissociation constants of NEDTA determined by paper electrophoresis and potentiometry, were 2.2 ± 0.1 (pK_{a1}), 3.95 ± 0.1 (pK_{a2}), and 9.21 ± 0.01 (pK_{a3}). The fluorescence of NEDTA was quenched by various metal ions and NEDTA was proved to be useful as an indicator for fluorophotometric titration. Using NEDTA as a metallofluorescent indicator, 5 μmol of Cd, Cu(II), Fe(III), Hg(II), Pb(II) or Zn at pH 4 to 6 or 5 μmol of Cd, Cu(II), Hg(II), Ni(II) or Zn at pH 9 to 10 was titrated with ethylenediaminetetraacetic acid (EDTA), and back titration with Zn was performed for 5 μmol of Al, Ni(II) or Th(VI) at pH 4.6. In every titration, the coefficient of variation and the error were within 0.6%.

Keywords metallofluorescent indicator; *N*-(2-naphthyl)ethylenediamine-*N*,*N'*,*N'*-triacetic acid; fluorophotometric titration; chelatometric titration; heavy metal ion

Most metallofluorescent indicators are quenched only by Cu(II), Co(II) or Ni (II), but not by Hg(II) or Pb(II), though back titration with a Cu(II) standard solution can be performed for chelatometric titration of heavy metal ions using a metallofluorescent indicator.[1]

It was suggested by den Boef[2] that lower limits of determination than 10^{-6} M should be possible for fluorimetric titrations, as an increase in the intensity of the light source decreases the lower limit of detection of fluorescent indicators. Nevertheless, micro chelatometric titration using a fluorescent indicator has not been achieved yet, because, in the back titration with Cu(II), the presence of colored Cu(II)–ethylenediaminetetraacetic acid (EDTA) makes the fluorescence change at the end point indistinct, and metallofluorescent indicators were not quenched by Hg, Pb or Zn, which form transparent complexes with EDTA.

We previously reported 2-naphthylamine-*N*,*N*-diacetic acid derivatives.[3] Like other metallofluorescent indicators, that are quenched only by Cu(II), Co(II), or Ni, and the stability constants are as low as about 10^5.

In this report, we present a synthesis of *N*-(2-naphthyl)-ethylenediamine-*N*,*N'*,*N'*-triacetic acid (NEDTA) with the aim of obtaining a higher stability constant with a reagent which can be quenched not only by Cu(II) but also by Fe(III), Hg(II) and Pb(II). This reagent should be a useful metallofluorescent indicator for heavy metal ions.

Results and Discussion

N-(2-Naphthyl)ethylenediamine hydrochloride was synthesized by the reaction of 2-naphthylamine with *N*-(2-bromoethyl)phthalimide. Alternatively, it could be obtained by reaction of 2-naphthol with ethylenediamine and ethylenediamine dihydrochloride in a sealed tube. NEDTA was prepared by the reaction of *N*-(2-naphthyl)ethylenediamine hydrochloride with chloroacetic acid under alkaline conditions (Na_2CO_3).

Decomposition of NEDTA was not observed in $NaHCO_3$ alkaline solution during storage for a month in a cold, dark place. NEDTA is very stable when compared to *o*-dianisidine-*N*,*N*,*N'*,*N'*-tetraacetic acid.[4] The fluorescence of NEDTA was quenched by Cu(II), Fe(III) and Hg(II) at pH 4.6, suggesting its potential usefulness as a metallofluorescent indicator.

The absorption and fluorescence spectra of NEDTA are shown in Fig. 1. The maximum absorption wavelength was 340 nm at pH 1.8. As the pH was increased, the maximum shifted to 357 nm at pH 12, and the absorbance increased. The fluorescence maximum wavelength was 395 nm at pH 1.8 and shifted to 410 nm at pH 12, and the fluorescence intensity increased with pH. Figure 2 shows a plot of the

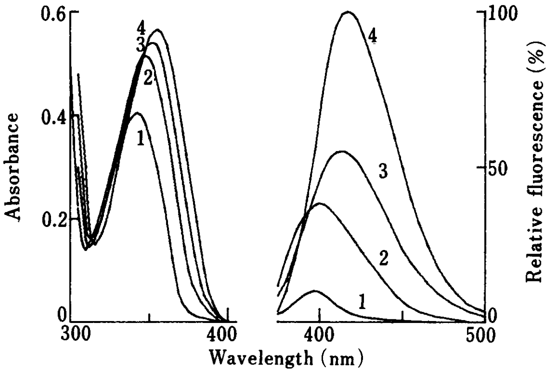

Fig. 1. Absorption and Fluorescence Spectra at Various pH

1) pH 1.8, 2) pH 3.5, 3) pH 7.8, 4) pH 13. Absorption spectra, 2×10^{-4} M NEDTA. Fluorescence spectra, 1×10^{-6} M NEDTA; excitation wavelength, absorption maximum wavelength at each pH.

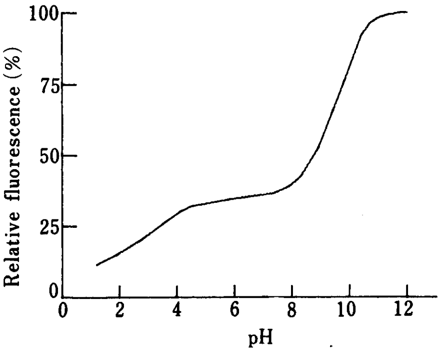

Fig. 2. Influence of pH on Fluorescence Intensity

Excitation, 365 nm (Hg-lamp with UV-2D filter, Toshiba); emission, Y-42 filter (Toshiba); NEDTA, 1×10^{-6} M.

fluorescence intensity against pH. The values of the acid dissociation constants of NEDTA were estimated as 4 and 9 from Fig. 2.

The acid dissociation constants of NEDTA were determined electrophoretically and potentiometrically. The pK_{a1} (relating to a carboxyl group) was determined by paper electrophoresis[5] because of the low solubility of NEDTA. The pK_{a1} was 2.2 ± 0.1. Since NEDTA did not dissolve in water until the addition of KOH up to 1.5 equivalent with respect to NEDTA, the titration for pK_{a2} was performed in 20—50% aqueous dioxane solutions, and the determined pK_{a2} was extrapolated to 0% dioxane. The pK_{a2} was determined to be 3.95 ± 0.1. The pK_{a3} was determined in water to be 9.21 ± 0.01. The pK_{a2} was considered to be due to the dissociation of aromatic ammonium because of the large shift of the absorption spectrum at pH 3 to 5 in Fig. 3, and the pK_{a3} was considered to be due to the dissociation of aliphatic ammonium. The purity of NEDTA in this experiment was found to be 98.33% from titration data.

The quenching effects were investigated with 12 kinds of metal ions (Al, Ca, Cd, Co(II), Cu(II), Fe(II), Fe(III), Hg(II), Ni(II), Pb(II), Th(IV) and Zn). In an acetate buffer (pH 5), Al Ca, Fe(II) or Th(IV) did not quench the fluorescence of NEDTA, but the other 8 ions did. In an ammonia/ammonium nitrate buffer (pH 10), the fluorescence was quenched by Cd, Co(II), Cu(II), Hg(II), Ni(II) and Zn, but no change in the fluorescence intensity was observed with Ca.

TABLE I. Fluorophotometric Titration of Some Metals with 0.01 M EDTA

Metal	pH	Taken (mg)	Found (mg)	Yield (%)	C.V. (%)	*n*	Titration curve in Fig. 3
Cd(II)	5.6	0.657_5	0.657_9	100.07	0.31	6	a
Cu(II)	5.6	0.3187	0.3197	100.33	0.25	5	a
Fe(III)	4.0	0.2820	0.2816	99.89	0.28	6	a
Hg(II)	4.0	1.118_3	1.118_3	100.00	0.06	5	a
Pb(II)	4.0	0.958_7	0.958_4	99.97	0.11	5	c
Zn(II)	4.6	0.3553	0.3568	100.42	0.22	5	a
Cd(II)	9.6	0.657_5	0.657_3	99.97	0.27	6	a
Cu(II)	9.6	0.3187	0.3206	100.61	0.17	5	a
Hg(II)	10	1.118_3	1.114_0	99.62	0.57	5	c
Ni(II)	10	0.2936	0.2953	100.58	0.36	6	b
Zn(II)	10	0.3553	0.3566	100.37	0.29	6	a
Al(III)[a)]	4.0	0.1358	0.1364	100.41	0.13	5	a′
Th(IV)[a)]	4.0	1.195_0	1.195_2	100.02	0.14	6	a′
Ni(II)[a)]	4.6	0.2936	0.2934	99.93	0.48	5	c′

C.V., coefficient of variation; *n*, number of titrations. *a*) Back titration with 0.01 M Zn; a′ and c′, upside-down shapes of a and c; 1.25×10^{-7} M NEDTA as an indicator; 40 ml titrated volume.

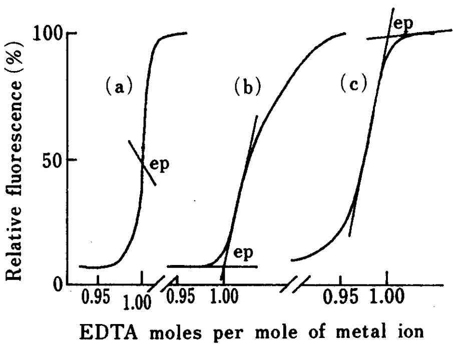

Fig. 3. Typical Titration Curves at the End-Point
Metal ion, 5 μmol/40 ml; NEDTA, 10^{-7} M; ep, end-point.

No metallofluorescent indicator which is quenched by Fe(III), Pb(II) or Zn has previously been reported. NEDTA is a quite new and characteristic metallofluorescent indicator.

The metal ions which showed quenching as mentioned above, were fluorophotometrically titrated with EDTA using NEDTA as an indicator. Each of Fe(III), Hg(II), Pb(II) and Zn (5 μmol) in a pH 4 to 4.6 acetate buffer, and each of Cd and Cu(II) (5 μmol) in a pH 5.6 hexamine/nitric acid buffer, was titrated and gave a good result. However, increasing fluorescence was observed in Co(II) solution at pH 5.6 before the equivalence point, and in the case of Ni(II) at pH 5.6, the fluorescence gradually increased after the equivalence point, so these metal ions could not be titrated. At pH 10, good results were obtained with 5 μmol of Cd, Cu(II), Hg(II) and Ni(II). Back titration with Zn was performed for 5 μmol of Al, Ni(II) or Th(IV) at pH 4.6, with good results (Table I).

The fluorophotometric titration curves around the end points could be grouped into 3 types (Fig. 3), and titrant volume was determined accordingly. In the titration of 10^{-4} M metal ions, a sharp change of fluorescence at the end point (such as in Fig. 3a) suggested that the NEDTA metal chelates have large formation constants. In the back titration with Zn, the titration curves around the end point are upside-down versions of the above curves (a′ and c′ in Table I). In all titrations, the coefficient of variation and the error were equal to or less than 0.6%.

Conclusion

NEDTA was synthesized in a high yield from 2-naphthol. It was stable in $NaHCO_3$ alkaline solution, and its fluorescence was quenched by various heavy metal ions. Accordingly, its application as a metallofluorescent indicator was examined. The fluorophotometric titration of Al, Cd, Cu(II), Fe(III), Hg(II), Ni(II), Pb(II), Th(IV) or Zn (5 μmol) was performed and each gave a good result.

NEDTA should be a useful indicator for microchelatometric titration. Further investigation of the titrating conditions and the use of masking agents should lead to applications for the fluorophotometric titrations of many metal ions.

Experimental

Equipment Spectrophotometer: Hitachi type 124. Spectrofluorophotometer: Hitachi type 204 (without correction). Fluorophotometric titrator: A TO-2 photometric and turbidometric titrator (Yanagimoto, Ltd.) was modified by replacing the tungsten lamp with a medium-pressure mercury lamp (Toshiba SHL-100 UV), and using a photoelectric tube with a photomultiplier HTV-106 (Hamamatu Co., Ltd.). Toshiba UV-2D and Y-42 filters were used as the excitation and emission filters, respectively, and a Potentiograph E-336 (Metrohm, Ltd.) was used with a 1 ml burette and recorder. Potentiometric titrator: A Potentiograph E-336 (Metrohm, Ltd.) was used with a 1 ml burette and a glass electrode (6028-10T, Horiba Co., Ltd.).

Reagents NEDTA Solution: NEDTA (30 mg) was dissolved in water with a small amount of $NaHCO_3$ and made up to a total volume of 100 ml with water (10^{-3} M). This solution was stored in a dark, cold place and used after diluting appropriately. A 0.01 M Zn solution: About 0.65 g of JIS standard reagent Zn was weighed precisely and dissolved in 30 ml of 10% aqueous HCl in the usual way, and the solution was made up to 1 l accurately. A 0.01 M EDTA solution: This was prepared according to JIS K-8001, and standardized by titration with 0.01 M Zn solution. Other metal solutions: Copper [$CuSO_4 \cdot 5H_2O$], mercury [$Hg(AcO)_2$], aluminum [$Al(NH_4)(SO_4)_2 \cdot 12H_2O$], calcium [$CaCO_3$], nickel [$NiSO_4$], cobalt [$CoSO_4$], iron [$Fe(NH_4)(SO_4)_2 \cdot 24H_2O$] and [$Fe_2(NH_4)_2(SO_4)_3 \cdot 6H_2O$] or thorium [$Th(NO_3)_4 \cdot 4H_2O$] of special reagent grade was used to prepare 0.01 M metal solution, and the metal solutions were standardized by chelatometric titration.[1)] Each solution was appropriately diluted before use. A 0.1 N KOH solution: A carbonate-free 0.1 N KOH solution was prepared with $Ba(OH)_2$,[6)] then standardized against standard reagent grade sulfamic acid by potentiometric titration. All the other materials used here were of special reagent grade.

***N*-(2-Naphthyl)ethylenediamine Hydrochloride** Method A: 2-Naphthylamine (8 g) and *N*-(2-bromoethyl)phthalimide (10 g) were dissolved in 30 ml of xylene, then the solution was refluxed for 15 h. When the reaction was completed, xylene was distilled off and the residue was washed with methanol. Next 1.5 ml of hydrazine hydrate and 20 ml of isopropyl alcohol were added to the residue and the mixture was refluxed for 5 h. Isopropyl alcohol was distilled off and 100 ml of 10% aqueous HCl was added, then the solution was concentrated under reduced pressure. The resulting mixture was allowed to stand overnight under ice-cooling, and precipitated *N*-(2-naphthyl)ethylenediamine hydrochloride was collected by filtration, and recrystallized from water. Yield, 4.3 g (49%), colorless flaky crystals, mp 243—245 °C (dec.) lit[7)] mp 230 °C (dec.). *Anal.* Calcd for $C_{12}H_{14}N_2 \cdot HCl$: C, 65.0; H, 6.4; N, 12.6. Found: C, 64.7; H, 6.7; N, 12.5. IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm}^{-1}$: 3100 ($NH_2$), 3000 ($NH^+$), 1610 (CH=CH), 800 (2-naphthyl). MS *m/z*: 186 ($M^+ - HCl$). Method B: A mixture of 10 g of 2-naphthol, 10 g of ethylenediamine dihydrochloride and 10 ml of ethylenediamine anhydride was heated in a sealed tube at 150—160 °C for 4 h. Then 300 ml of 10% aqueous HCl was added, and the reaction mixture was heated on boiling water bath. The mixture was filtered warm. The filtrate was concentrated under reduced pressure and allowed to stand overnight in ice, then precipitated *N*-(2-naphthyl)ethylenediamine hydrochloride was collected by filtration. Yield, 11.3 g (85%).

***N*-(2-Naphthyl)ethylenediamine-*N*,*N′*,*N′*-triacetic Acid (NEDTA)** *N*-(2-Naphthyl)ethylendiamine hydrochloride (7 g) was suspended in 100 ml of 25% aqueous Na_2CO_3 solution, and 30 g of chloroacetic acid in 100 ml of water was added dropwise to the stirred suspension on a boiling water bath.[4)] The reaction was continued until paper electrophoresis of a portion of the reaction mixture at pH 7 (phosphate buffer) showed only a spot of −2 charge. The reaction mixture was filtered. The filtrate was adjusted to pH 2 to 3 with concentrated HCl and cooled to obtain a precipitate of crude NEDTA. The crude NEDTA was dissolved in 100 ml of water with a small amount of $NaHCO_3$, and the solution was adjusted to pH 3 with concentrated HCl, then cooled to form a precipitate. The precipitate was collected by filtration, washed with acetone, and dried under a vacuum. Yield, 5.7 g (43%), colorless powdery crystals, mp 181—183 °C (dec.). *Anal.* Calcd for $C_{18}H_{20}N_2O_6$: C, 60.0; H, 5.6; N, 7.8. Found: C, 60.0; H, 5.6; N, 7.8. IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm}^{-1}$: 3030 ($COO^-$), 1600 (CH=CH), 800 (2-naphthyl).

pK_{a1} by Paper Electrophoresis The procedure was similar to that reported.[5)]

pK_{a2} and pK_{a3} by the Potentiometric Method Approximately 15 mg of NEDTA was weighed precisely and dissolved in 7.0 ml of 20—50% aqueous dioxane for pK_{a2} or water for pK_{a3}, then potentiometric titration with 0.1 N KOH was performed under nitrogen flow at 25 ± 0.5 °C. A set of 10 plots was used to determine each dissociation constant according to the following equation.

$$\mathrm{p}K_a = \mathrm{pH} + \log \frac{[A^-] + \{H^+\} - \{OH^-\}}{[HA] - \{H^+\} + \{OH^-\}}$$

Wherein [HA] is the concentration of the molecular form and is obtained as the difference between the total concentration of acid and the concentration of added KOH, and [A^-] is the concentration of the ionic form and is obtained as the concentration of KOH which was added, while {H^+} and {OH^-} are activities of hydrogen ion and hydroxy ion, and are obtained from the pH.

Fluorophotometric Titration A metal ion (5×10^{-6} mol) was added to the titration vessel (*ca.* 50 ml), and 0.1 ml of 5×10^{-5} M NEDTA solution as an indicator, 0.5 ml of 0.2 M buffer and water were added to give a total volume of about 40 ml, then the solution was fluorophotometrically titrated with 0.01 M EDTA.

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