Fluorometric Determination of Ethylenediamine Using a Beryllium-Schiff Base Complex

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Ethylenediamine (en) reacts with salicylaldehyde and beryllium(II) in a weakly basic solution to form a fluorescent beryllium(II)-Schiff base complex. A fluorometric method for the determination of en by using the beryllium(II) complex has been established. Ethylenediamine could be determined in 2×10^{-7}—2×10^{-5} mol dm^{-3} within a relative standard deviation of 3%. The detection limit of en was 8×10^{-9} mol dm^{-3}. Ammonia, diethylamine, and triethylamine does not interfere with the determination of en. The present method has been applied to the analysis of en in aminophylline tablets. The fluorometric method for the determination of trace amount of amino acid by using an aluminum(III)-Schiff base complex has also been investigated.

Several methods for the determination of ethylenediamine (en) have already been reported.[1-10] Gas chromatography[1-3] and high-performance liquid chromatography[4-6] are usually used for the determination of en in the presence of various chemical substances, especially other primary amines. Though spectrophotometric[7-9] and titrimetric[10] methods can be used without separations for simpler samples, they have both low sensitivity and low selectivity. A few sensitive spectrophotometric[11] and chemiluminescence[12] methods have been reported, but they require strict controll of the analytical conditions.

Ethylenediamine reacts quantitatively with salicylaldehyde and beryllium(II) in a weakly basic solution to form a Schiff base complex, which has a strong blue fluorescence upon ultraviolet excitation.[13,14] This reaction is selective for en since most of the amines and amino acids form Schiff base complexes only in strongly basic solutions. In the present study, the fluorometric determination of en by using the beryllium(II) complex was investigated in order to establish a more sensitive and selective method than the conventional ones. Moreover, amino acids give fluorescence reactions by using aluminum(III) instead of beryllium(II), while most of amines, in this case, show no fluorescence. The method for the determination of trace amounts of amino acid by using the complex formed with aluminum(III) was also investigated.

Experimental

Reagents. A standard solution of en was prepared by dissolving en dihydrochloride with 99.5% purity (Wako Pure Chemical Industries, Ltd.) in distilled water. Standard solutions of the other diamines and amino acids were prepared by using diamines or their dihydrochloride and amino acids of analytical grade in the same way. Quinine solutions as reference standard were prepared by dissolving quinine hydrogen sulfate in a 0.05 M (1 M=1 mol dm^{-3}) sulfuric acid. All other reagents were of analytical grade.

Apparatus. The fluorescence spectra and the intensity were measured with a Hitachi Model 204 fluorescence spectrophotometer fitted with a 150 W Xenon lamp. A Toa pH Meter, Model HM-20E, was used for the pH measurements.

Determination Procedure: (A) Determination Procedure of en. (a) Fluorometric Reagent Solution. To 50 cm^3 of a 1×10^{-2} M beryllium sulfate tetrahydrate aqueous solution was added 100 cm^3 of a 5×10^{-3} M salicylaldehyde methanolic solution. The mixture was diluted to 200 cm^3 with water. This solution was kept in a dark place.

(b) Buffer Solution. To 7 cm^3 of a 1 M sodium hydroxide aqueous solution was added 4.8 g of HEPES [4-(2-hydroxyethyl)-piperazineethanesulfonic acid]. The solution was diluted to 100 cm^3 with water.

(c) Determination Procedure. To 1 cm^3 of a sample solution containing from 5×10^{-9} mol to 5×10^{-7} mol of en, were added 2 cm^3 of the fluorometric reagent solution and 1 cm^3 of the buffer solution. The mixture was left standing for 10 min at room temperature, and then diluted to 25 cm^3 with water. After standing for an additional 10 min, the fluorescence intensity was measured at 425 nm with an excitation wavelength of 345 nm by using (0.05—5) μg cm^{-3} of the standard quinine solution. Calibration curves for en were obtained by using 1 cm^3 of the standard en solutions containing various amounts of en. When analyses of samples solution containing $(1—5)\times10^{-9}$ mol cm^{-3} of en were required, 5 cm^3 of each sample solution was taken. The calibration curves were then determined under the same condition.

(B) Determination Procedure of Amino Acids. (a) Fluorometric Reagent Solution. To 60 cm^3 of a 3 M sodium acetate aqueous solution was added 100 cm^3 of a 2×10^{-2} M salicylaldehyde methanolic solution. The pH of the solution was adjusted to 6.3 with acetic acid. To the mixture was added 50 cm^3 of a 4×10^{-2} M aluminum nitrate nonahydrate aqueous solution; this solution was then diluted to about 250 cm^3 with water. The pH of the solution was adjusted to 6.3 with a 6 M sodium hydroxide aqueous solution and then diluted to 300 cm^3 with water. This solution was kept in a dark place.

(b) Determination Procedure. To 1 cm^3 of a sample solution of amino acid was added 3 cm^3 of the fluorometric

reagent solution. The mixture was warmed at 50 °C for 20 min and then diluted to 25 cm^3 with water. The fluorescence intensity was measured at 430 nm with an excitation wavelength of 360 nm against the standard quinine solution.

Results and Discussion

Excitation and Emission Spectra. The excitation and emission spectra of the beryllium(II) complex with the Schiff base (*N*-salicylideneethylenediamine) are shown in Fig. 1. The excitation and emission maxima of the complex occur at 345 nm and 425 (corrected value 430) nm, respectively. Fluorescence of a reagent blank appeared from a beryllium (II) complex with salicylaldehyde.

The excitation and emission spectra of aluminum(III)–*N*-salicylideneamino acid were similar to those of the beryllium(II) complex. The optimum excitation and emission wavelengths of the aluminum(III) complexes formed by using 12 kinds of amino acids were obtained in the ranges 350—360 nm and 430—440 nm, respectively. In this study, glycine was mainly used for the investigation of the optimum analytical conditions of amino acids.

Effect of Standing Time. The fluorescence intensity of the beryllium(II) complex required 120 min to reach its maximum, when the sample solution was diluted to 25 cm^3 with water immediately after a buffer solution added. Upon standing for 10 min after addition of the buffer solution, the fluorescence of the complex developed fully immediately after dilution. Though the intensity decreased by about 10% within 60 min, highly reproducible results were obtained as long as measurements were made between 10 and 15 min after dilution.

The maximum complex formation of amino acid with salicylaldehyde and aluminum(III) required heating at 50 °C for 20 min before dilution. The fluorescence intensity of the complex was stable for at least 2 h after diluting to 25 cm^3.

Effect of Volume of Sample Solution. Various volumes of en aqeous solutions were added to mixtures of the fluorometric reagent and buffer solutions, and the changes in the fluorescence intensities were measured. The intensity decreased as the volume of the en solution was increased. However, linear relationships were observed between the concentration of en and the fluorescence intensity when a constant volume of the en solution was used. In this study the volume of the sample solution of en was fixed at 1 cm^3. Similarly, the volume of sample solutions of amino acid was fixed to 1 cm^3.

Effect of pH. The effect of pH on the fluorescence intensity of the beryllium(II) complex was investigated. The pH was measured for the final solution after diluting to 25 cm^3. As shown in Fig. 2, a maximum fluorescence intensity was obtained at pH 7.7—7.9. A sample solution of en in the pH range 2—12 can be directly applied to the proposed method. The maximum fluorescence intensity of the aluminum(III) complex was obtained at nearly pH 6. Amino acid in sample solution at pH 4—8 could be determined directly without any pH adjustment.

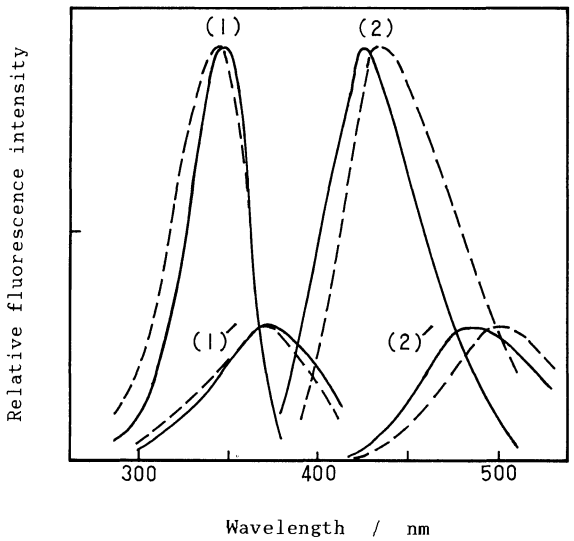


Fig. 1. Excitation (1) and emission (2) spectra of en reacted with salicylaldehyde and beryllium(II). en: 4×10^{-6} mol dm^{-3}, Salicylaldehyde: 2×10^{-4} mol dm^{-3}, Be(II): 2×10^{-4} mol dm^{-3}, pH: 7.8. —: apparent spectra. ---: corrected spectra. (1)′ and (2)′: excitation (1)′ and emission (2)′ spectra of reagent blank.

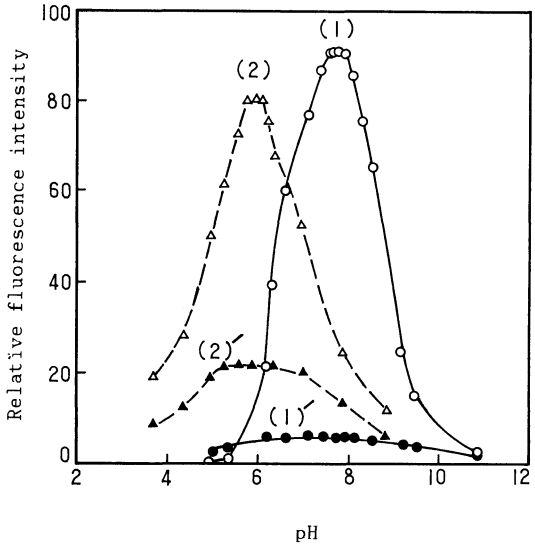


Fig. 2. Effects of pH on RFI of en (1) and glycine (2). (1) en: 4×10^{-6} mol dm^{-3}, Salicylaldehyde: 2×10^{-4} mol dm^{-3}, Be(II): 4×10^{-4} mol dm^{-3}, Wavelength: 345—425 nm, 45 div vs. 0.1 μg cm^{-3} quinine solution. (2) Glycine: 4×10^{-6} mol dm^{-3}, Salicylaldehyde: 8×10^{-4} mol dm^{-3}, Al(III): 8×10^{-4} mol dm^{-3}, Wavelength: 360—430 nm, 50 div vs. 0.1 μg cm^{-3} quinine solution. (1)′ and (2)′: reagent blank of (1) and (2).

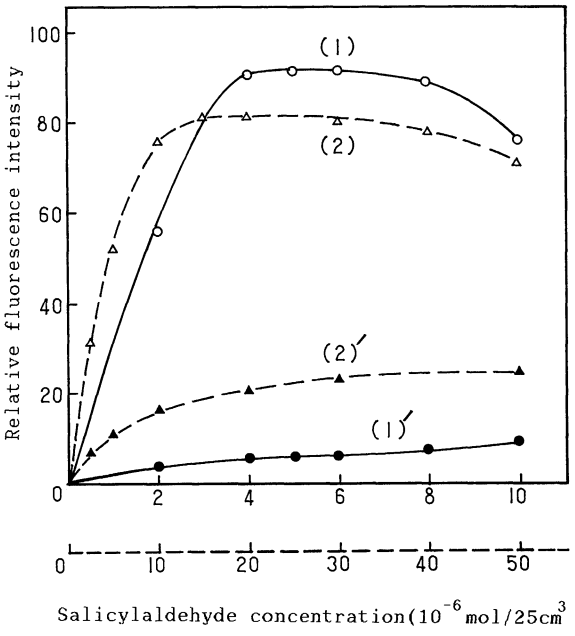


Fig. 3. Effect of concentration of salicylaldehyde on RFI of en (1) and glycine (2). (1) en: 4×10^{-6} mol dm^{-3}, Be(II): 2×10^{-4} mol dm^{-3}, pH: 7.8. (2) Glycine: 4×10^{-6} mol dm^{-3}, Al(III): 8×10^{-4} mol dm^{-3}, pH: 5.8. Other conditions: reference to Fig. 2. (1)′ and (2)′: reagent blank of (1) and (2).

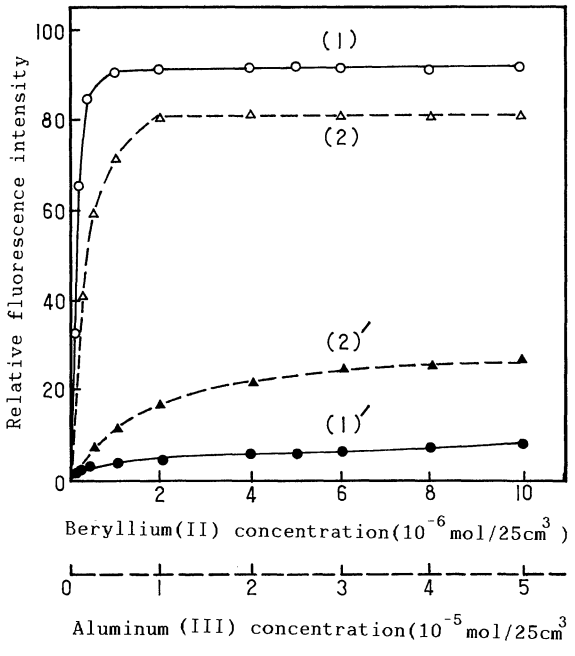


Fig. 4. Effect of concentration of beryllium(II) (1) and aluminum(III) (2) on RFI of en (1) and glycine (2). (1) en: 4×10^{-6} mol dm^{-3}, Salicylaldehyde: 5×10^{-6} mol dm^{-3}, pH: 7.8. (2) Glycine: 4×10^{-6} mol dm^{-3}, Salicylaldehyde: 8×10^{-4} mol dm^{-3}, pH: 5.8. Other conditions: reference to Fig. 2. (1)′ and (2)′: reagent blank of (1) and (2).

Effects of Concentrations of Salicylaldehyde and Beryllium(II). The effect of the concentration of salicylaldehyde is shown in Fig. 3. The maximum fluorescence intensity was obtained in the range (1.6—3.2)$\times10^{-4}$ M of salicylaldehyde. Figure 4 shows the effect of the concentration of beryllium(II). The maximum intensity was obtained for more than 4×10^{-5} M of beryllium(II). In the determination procedure of en, 2×10^{-4} M of both salicylaldehyde beryllium(II) was suitable. This concentration was achieved by adding 2 cm^3 of the fluorometric reagent solution described in Determination Procedure to a sample solution. Figures 3 and 4 indicated that 8×10^{-4} M of both salicylaldehyde and aluminum (III) was adequate for the determination of 4×10^{-6} M glycine.

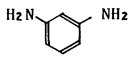
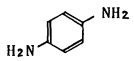
Calibration Curve and Detection Limit. According to Determination Procedure, en can be determined in the range (2×10^{-7}—2×10^{-5}) M within a relative standard deviation (r.s.d.) of 3%. Calibration curves are linear over this range. The r.s.d. for 4×10^{-6} M and 4×10^{-8} M of en were 1.8 and 8.2% (n=5) respectively. The lower detection limit of en was 8×10^{-9} M. In the case of amino acid, glycine could be determined in the range (4×10^{-7}—2×10^{-5}) M within a r.s.d. of 3%. The detection limit of glycine was 8×10^{-8} M.

Fluorescence Intensities of Various Diamines and Amino Acids. The fluorescence intensities of 11 kinds of diamines and triamine were examined. The results obtained under their optimum conditions are shown in Table 1. The fluorescence of 1,2-propanediamine at pH 8.5 was as intense as that of en, and the intensity of diethylenetriamine at pH 6.5 was about twice that of en. These amines reacted with salicylaldehyde to form the tridentate (*O,N,N*) or tetradentate (*O,N,N,N*) Schiff bases. On the other hand, diamines having four or more methylene groups between two amino groups acted as the bidentate (*O,N*) Schiff bases after reacting with salicylaldehyde; they reacted slightly near pH 11 with beryllium(II) to emit only weak fluorescence.

The fluorescence intensities of the 13 kinds of amino acids examined are listed in Table 2. Most of the α-amino acids were detected as sensitively as glycine, while β-alanine was not sensitive. The aluminum(III)–Schiff base complex in a weakly acidic solution was formed by the template mechanism via a mixed ligands complex formed with salicylaldehyde, amino acid and aluminum(III).[16] Zinc (II) also formed a fluorescent Schiff base complex with salicylaldehyde and amino acid,[15] but aluminum(III) was more selective for the determination of amino acid because most of amines do not form a mixed ligands complex with aluminum(III).

Effects of Other Amines. The effects of foreign amines on the determination of en (4×10^{-6} M) were investigated. 1,2-Propanediamine and 1,3-propanedi-

Table 1. Relative Fluorescence Intensity of Diamines

Amine	pH	RFI		Wavelength[a]/nm	
		Net	Blank	Ex.	Em.
$H_2NCH_2CH_2NH_2$	7.8	91.4	6.6	345	425
$H_2NCH(CH_3)CH_2NH_2$	8.5	92.2	4.0	340	425
$H_2N(CH_2)_3NH_2$	9.0	11.0	9.8	335	430
$H_2N(CH_2)_4NH_2$	10.5	2.0	3.6	340	430
$H_2N(CH_2)_5NH_2$	11.0	2.9	4.1	340	430
$H_2N(CH_2)_6NH_2$	11.0	2.0	4.1	340	430
$(H_2NCH_2CH_2)_2NH$	6.5	196.3	9.2	350	430
	8.5	13.8	5.0	345	430
	9.0	3.0	4.0	340	420
	—	0	—	—	—
	—	0	—	—	—

Amine: 4×10^{-6} M, Salicylaldehyde: 2×10^{-4} M, Be(II): 2×10^{-4} M, 45 div vs. 0.1 μg cm^{-3} quinine solution.
a) Apparent value.

Table 2. Relative Fluorescence Intensity of Amino Acids

$$H_2NCHCOOH$$
$$\quad\quad|$$
$$\quad\quad R$$

Amino acid	RFI (Net)[a]	Amino acid	RFI (Net)[a]
R: H	81.0	R: $CH_2CH_2CONH_2$	95.5
CH_3	66.7	$CH_2(CH_2)_3NH_2$	96.6
$CH(CH_3)_2$	55.1	CH_2SH	84.9
CH_2OH	90.8	CH_2CH_2SH	102.2
CH_2COOH	68.5		191.0
CH_2CH_2COOH	79.8		
CH_2CONH_2	82.4	$H_2NCH_2CH_2COOH$	6.2

Amino acid: 4×10^{-6} M, Salicylaldehyde: 8×10^{-4} M, Al(III): 8×10^{-4} M, pH: 5.8, Wavelength: 360 nm/430 nm, 50 div vs. 0.1 μg cm^{-3} quinine solution. a) RFI of reagent blank: 22.5.

Table 3. Analytical Results of en in Aminophylline Tablet[a]

Sample taken/g	Dilution/cm^3	Sample solution taken/cm^3	Replicate number of measurement	en found in the tablet/%	
				Each value (r.s.d., %)	Average value
By the proposed method					
0.0911[c]	1000	1	5	6.37, 6.55, 6.43, 6.48, 6.46 (0.92)	6.46
0.0503[c]	1000	1	5	6.76, 6.64, 6.75, 6.62, 6.79 (1.02)	6.71
By the titrimetric method[b]					
7.2178[d]	200	25	5	6.55, 6.57, 6.58, 6.58, 6.58 (0.18)	6.57
3.6143[e]	100	25	3	6.66, 6.70, 6.69 (0.25)	6.68

a) Commercially available, Nominal content of aminophylline: 100 mg in a tablet. Mean weight of one tablet: 0.2165 g (r.s.d. 1.42%, n=100). b) Titration with 0.1 M hydrochloric acid.[17] c) Sample taken from one tablet. d) Sample taken from 36 tablets. e) Sample taken from 18 tablets.

amine gave positive errors in an equivalent amount of en. 1,4-Butanediamine, 1,5-pentanediamine and 1,6-hexanediamine gave slightly positive errors in 10-fold excess of en. 10-Fold methylamine 100-fold aniline, and 1000-fold ammonia, diethylamine and triethylamime did not interfere. In the determination of amino acid (glycine; 4×10^{-6} M), 10-fold en compared to glycine concentration, 100-fold aniline, and ammonia, methylamine, diethylamine and triethylamine did not interfere at all. *o*-Aminophenol[13] did not interfere in an equivalent amount of amino acid, but gave a negative error in 10-fold excess.

Analysis of en in Aminophylline Tablet. The present method for the determination of en was applied to the analysis of en in commercial aminophylline ($C_{14}H_{16}N_8O_4\cdot en\cdot xH_2O$) tablet. The sample solutions used for analyses were prepared according to the reported procedure.[9] The results (Table 3) were compared with those obtained by titration with 0.1 M hydrochloric acid.[9,17] The titrimetric method, however, required many tablets. Good agreement was observed between the results obtained by the present method and the titrimetric method. The present method is highly sensitive and is useful for the determination of en in a small amount of sample. However, the effect of the matrix of the sample must be examined carefully with a recovery test before analysis.

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