The Spectrophotometric Determination of Ethylene Diamine with Phthalaldehyde and 2-Mercaptoethanol

Goro Hihara,* Hiroshi Miyamae, and Miharu Nagata
Department of Chemistry, Faculty of Science, Josai University, Sakado, Saitama 350
(Received January 16, 1981)

A simple spectrophotometric method has been developed for the determination of ethylenediamine in an aqueous or ethanolic solution. This method is based on the reaction of ethylenediamine with phthalaldehyde and 2-mercaptoethanol, and on the measurement of the absorbance of the yellow-developed solution at 430 nm. Beer's law holds for 0.5—10 μg of ethylenediamine per 1 ml. Secondary and tertiary amines do not interfere with theestimation. Two of the primary amines, diethylenetriamine and triethylenetetramine, interfere with the estimation when present in quantities of more than 10 μg per ml of the sample solutions. 1,3-Propanediamine interferes seriously. The colored compound was found to be composed of ethylenediamine, phthalaldehyde, and 2-mercaptoethanol in a molar ratio of 1:2:1.

Several spectrophotometric methods for the determination of ethylenediamine have already been reported.[1-4] These methods, however, have low sensitivities. Roth[5] developed a highly sensitive fluorophotometric method for amino acids, in which the amino acids were combined with phthalaldehyde and 2-mercaptoethanol in an alkaline medium to grow fluorescent materials. Roth[6] and Svedas *et al.*[7] improved this method so that these products could be detected by a method of spectrophotometric estimation. Simons and Johnson[8] made it clear that this fluorogenic compound was 1-alkylthio-2-alkyl-substituted isoindole by a reaction of propylamine with phthalaldehyde and 2-mercaptoethanol. Moreover, they found that phthalaldehyde reacts only primary amines and not with secondary amines.

The objective of this paper is to examine the effects of various factors on the coloration and to present a spectrophotometric method for ethylenediamine in an aqueous or ethanolic solution.

Experimental

Apparatus. A Hitachi 101 spectrophotometer was used for the measurement of the absorbance, while a Hitachi EPS-3 spectrophotometer was used to estimate the absorption spectra with 10-mm glass cells. For the adjustment of the pH of the buffer solutions, a Toa Denpa HM-5A pH meter was used.

Reagents. Phthalaldehyde of a biochemical grade and ethylenediamine with a 99.5% purity were obtained from Wako Pure Chemical Industries, Ltd. 2-Mercaptoethanol was also obtained with a purity of 98%. The other reagents, except for the secondary and tertiary amines, were of a guaranteed grade.

Buffered Reagent for Coloration. A 0.1 M† solution of sodium tetraborate was adjusted to pH 9.5 with a concentrated NaOH solution. Into 100 ml of this borate buffer solution, 1 ml of an ethanol solution containing 20 mg of phthalaldehyde and 1 ml of an ethanolic solution of 70 mg of 2-mercaptoethanol were then mixed. This reagent was stable for at least two days at room temperature.

Procedure A for the Measurement of Ethylenediamine in an Aqueous Solution. 1.0 ml of an aqueous sample solution containing 0.5—10 μg of ethylenediamine was mixed with 1 ml of ethanol and 3 ml of the buffered reagent, after which the mixture was allowed to stand for 30 min at 26±0.5 °C in a thermostat. Then the absorbance of the solution was measured at 430 nm against the reagent blank.

Procedure B for the Measurement of Ethylenediamine in an Ethanolic Solution. The procedure was the same as in Procedure A, except that 1 ml of pure water was used in place of 1 ml of ethanol.

† 1 M = 1 mol dm^{-3}.

Results and Discussion

Influence of the pH of the Buffered Reagent. One-ml portions of an aqueous solution containing 4.68 μg of ethylenediamine were mixed with 1 ml of distilled water and 3 ml of a buffered reagent, the pH of which was varied in the range of 5—11, the absorption spectrum was then examined. As is shown in Fig. 1, the absorption maximum in every spectrum shifted toward lower wavelength, as the pH of the solution became higher, converging on 430 nm in the pH range of 9—11. The intensity of the absorbance also varied with the change in the pH. It seemed to become highest in the 9—10 pH range. Figure 2

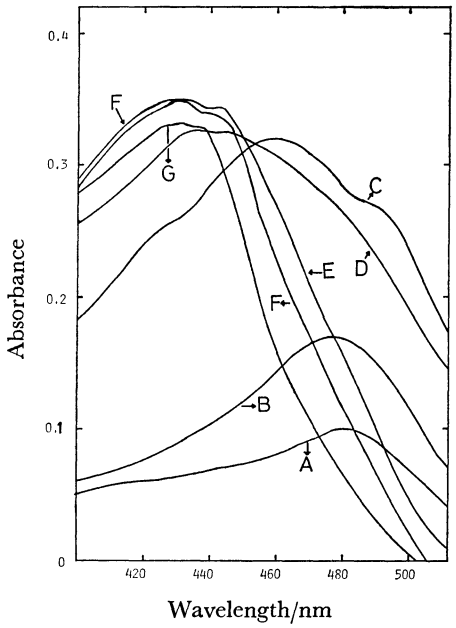


Fig. 1. The absorption curves at the various values of pH.
A: pH 5, B: pH 6, C: pH 7, D: pH 8, E: pH 9, F: pH 10, G: pH 11. KH_2PO_4–Na_2HPO_4 buffer solutions were used in pH 5—8, HCl–$Na_2B_4O_7$ in pH9, and NaOH–$Na_2B_4O_7$ in pH 10 and 11.

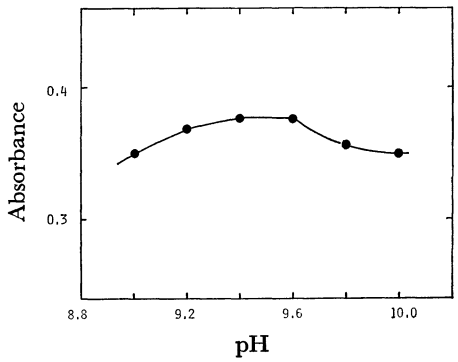


Fig. 2. Effect of pH on the absorbance at 430 nm between pH 9.0 and 10.0. 1 ml of an aqueous solution of ethylenediamine (4.68 μg/ml) was used.

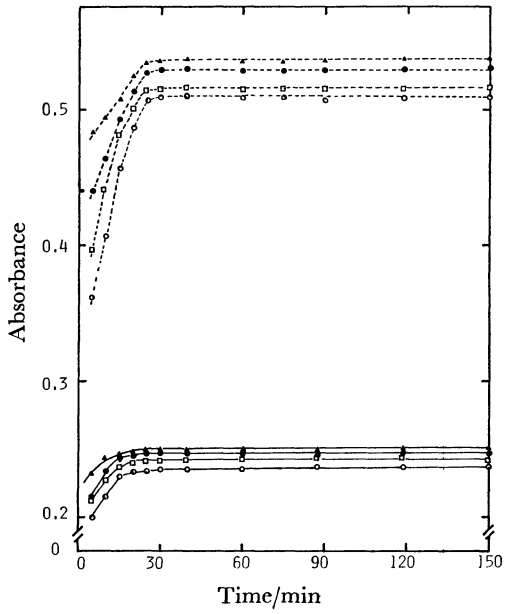


Fig. 3. Influence of temperature and time-dependance of the coloration.
——: Concentration of ethylenediamine 1.56 μg/ml, ···: 3.12 μg/ml, ○: 20 °C, □: 23 °C, ▲: 26 °C, ●: 30 °C.

shows that the absorbance measured at 430 nm attained its maximum value at the pH values of 9.4 and 9.6. Thus, the borate solution of pH 9.5 was used as a buffer in the color reaction throughout this work, and the absorbance was measured at 430 nm.

Influence of Time Delay and Reaction Temperature. After 2-ml portions of the ethylenediamine solution (1.56 and 3.12 μg/ml) had been mixed with 3 ml of the buffered reagent, they were held in a thermostat at various temperatures between 20—30 °C, while the absorbances of the solution were measured at regular time intervals. The results are shown in Fig. 3. The absorbances of every solution attained constancy within 30 min, and thereafter remained unchanged for 150 min regardless of the reaction temperatures. However, the intensities of the absorption were slightly affected by the reaction temperature and showed their maximum at 26 °C.

Influence of Addition of Ethanol and Other Organic Solvents. When 1 ml of distilled water was added instead of 1 ml of ethanol in Procedure A, Beer's law was adopted for the relation of the absorbance *vs.*

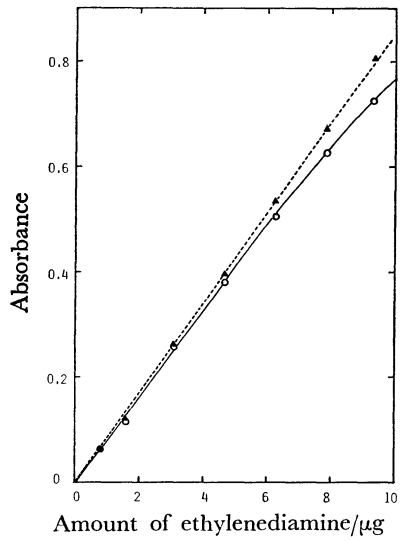


Fig. 4. Calibration curves in the presence and absence of ethanol.
···▲···: Procedure A, —○—: in the absence of ethanol.

Table 1. Effect of coexisting compounds and ions on the absorbance

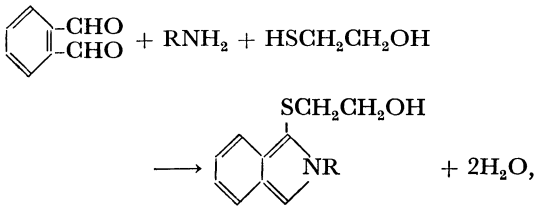
Compound	Amount taken/μg	Relative value[a] of the absorbance
Propylamine	100	0.92
	10	1.01
Dimethylamine	500	1.01
Triethylamine	1000	1.00
1,3-Propanediamine	80	ppt[b]
	10	1.40
Aniline	500	ppt
	50	0.95
N-Methylaniline	500	1.00
Dien[c]	100	ppt
	10	1.02
Trien[c]	100	ppt
	10	1.02
HMTA[c]	600	0.86
	60	0.99
Pyridine	100	1.00
Pyrazine	500	1.00
Piperazine	600	1.01
Acetonitrile	5000	1.01
Urea	500	1.01
Thiourea	500	1.00
Pb^{2+} (from $Pb(NO_3)_2$)	100	1.02
I^- (from KI)	1000	1.02
NH^+_4 (from NH_4Cl)	10	1.03

a) This value is the ratio of the absorbance with and without the compound listed in the left colum. A 1-ml portion of the ethylenediamine solution(4.68 μg/ml) was chosen. b) ppt means that the precipitate formed when the buffered reagent was added. c) dien, trien, and HMTA represent diethylenetriamine, triethylenetetramine, and hexamethylenetetramine respectively.

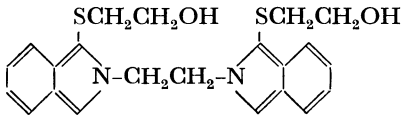
the ethylenediamine concentration only in the range of 0.5—5.0 μg of ethylenediamine contents, the absorbance showed a negative deviation from Beer's law at larger concentrations of ethylenediamine as is shown in Fig. 4. In order to improve the linearity in the relation of the absorbance *vs.* the ethylenediamine content, the addition of organic solvents miscible with water was tested. The addition of acetone remarkably reduced the coloration. Methanol and ethanol increased the absorbance, with no fluctuation in the absorption maximum in wavelength. With the addition of 1 ml of ethanol, the linearity was extended up to an ethylenediamine content of 10 μg, as is shown in Fig. 4.

Influence of Active Compounds and Ions. The influence of the addition of several compounds and ions which may interfere with the coloration in the present method was also examined. A 1-ml portion of an aqueous solution of each compound listed in Table 1 was mixed with 1 ml of an aqueous solution of ethylenediamine and 3 ml of the buffered reagent, and the absorbance was estimated at 430 nm. The results of the examination are shown in Table 1. Dimethylamine and trimethylamine did not interfere, and propylamine reduced the absorbance only slightly. This corresponds to the conclusion suggested by Simons *et al.* that phthalaldehyde reacts only with primary amines and not with secondary and tertiary amines. Among the tested compounds, only 1,3-propanediamine interferes significantly.

The Composition of the Product. Simons *et al.* offered the following equation for the reaction of primary amines with phthalaldehyde and 2-mercaptoethanol:



where R is an alkyl group. When ethylenediamine reacts on phthalaldehyde with 2-mercaptoethanol, the product may be supposed to have the structure shown as (I).



(I)

In order to elucidate the structure of the product, 0.06 g of ethylenediamine (1 mmol) was added to 50 ml of a borate buffer solution (pH 9.5) containing 0.27 g of phthalaldehyde (2 mmol) and 0.16 g of 2-mercaptoethanol (2 mmol). A yellow precipitate formed very soon, it had turned brown within one or two hours after it was separated from the solution. Therefore, the composition of the product only was determined as follows: to 1-ml portions of ethylenediamine solutions (3.12, 6.24, and 9.36 μg/ml), various amounts of a 2-mercaptoethanol aqueous solution (10.9 μg/ml) were added, and then the containers were filled up to 3 ml with pure water. Then 2 ml of a borate-buffer solution (pH 9.5) containing phthalaldehyde (0.2 mg/ml) was added, and the absorbance at 430 nm was estimated after 30 min. The relationships are shown in Fig. 5 between the absorbances and the amounts of 2-mercaptoethanol added. A similar relation is given in Fig. 6, where ethylenedi-

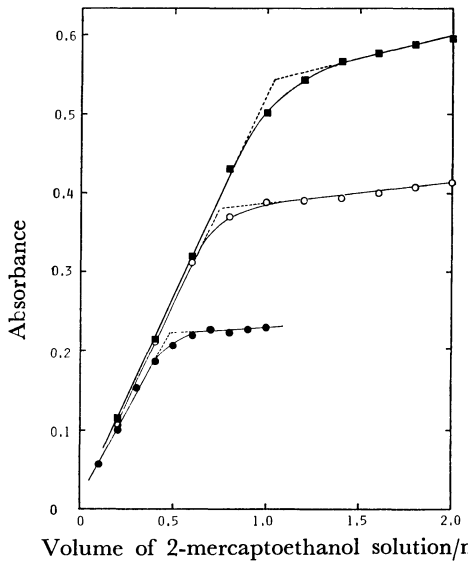


Fig. 5. Relationships between the absorbance and the added amounts of 2-mercaptoethanol solution (10.9 μg/ml) in the presence of excess phthalaldehyde. The solid curves are of the experimental estimation and the dotted lines are the prolongations of the tangential lines. ●: Ethylenediamine 3.12 μg/ml, ○: 6.24 μg/ml, □: 9.36 μg/ml.

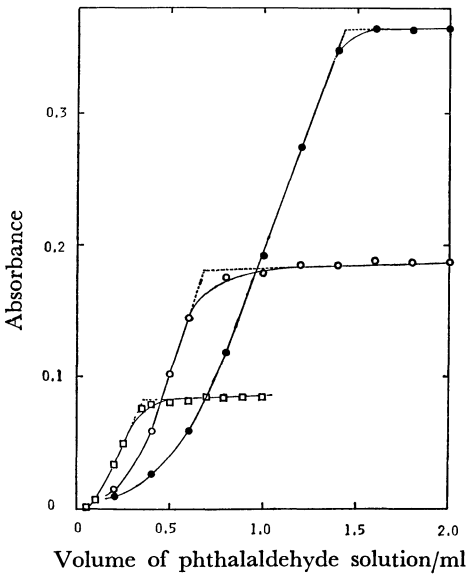


Fig. 6. Relationships between the absorbance and the added volume of an aqueous solution of phthalaldehyde (20.0 μg/ml) in the presence of excess of 2-mercaptoethanol.
Borate buffer solution (pH 9.5) was used containing 2-mercaptoethanol (0.7 mg/ml). □: Ethylenediamine 1.56 μg/ml, ○: ethylenediamine 3.12 μg/ml, ●: ethylenediamine 6.24 μg/ml.

amine reacts with phthalaldehyde in the presence of an excess amount of 2-mercaptoethanol. The absorbances were nearly equal, regardless of the amount of ethylenediamine present, and increased proportionally to a certain point as the amount of 2-mercaptoethanol increased when there was a large excess of phthalaldehyde. On the other hand, in the presence of a large excess of 2-mercaptoethanol, the absorbances increased only a little as the content of phthalaldehyde increased. They began to increase proportionally to the amount of phthalaldehyde after the molar ratio of phthalaldehyde/ethylenediamine became nearly 1:1. This can be explained by assuming that phthalaldehyde reacts with ethylenediamine to give an intermediate product consisting of 1:1 (=phthalaldehyde: ethylenediamine), which does not have the absorption near 430 nm. The intermediate then reacts with one more mole of ethylenediamine to give a final product having its absorption peak at 430 nm. Two tangential lines are drawn per curve in Figs. 5 and 6, one in the range where the absorbances increase linearly with the amount of 2-mercaptoethanol or phthalaldehyde added, and the other in the plateau range where the absorbances become almost constant. These lines were then extended, and the point of intersection was found. These points were 0.35, 0.68, and 1.43 ml of the phthalaldehyde solution when the concentrations of ethylenediamine were 1.56, 3.12, and 6.24 μg/ml respectively. The molar ratios of phthalaldehyde/ethylenediamine were calculated to be 2.01, 1.96, and 2.06 respectively. In Fig. 5 the points of intersection are 0.43, 0.74, and 1.04 ml of a 2-mercaptoethanol solution when the concentrations of ethylenediamine are 3.12, 6.24, and 9.36 μg/ml, and 1.16, 0.99, and 0.93 respectively, in terms of the molar ratio of 2-mercaptoethanol/ethylenediamine. The composition of the product was found to be 1:2:1(ethylenediamine: phthalaldehyde : 2-mercaptoethanol).

Precision. The precision of Procedure A was checked with a sample containing a known amount of ethylenediamine. Based on six determinations, the relative standard deviations were 1.2% at the level of 1.56 μg and 1.0% at 9.36 μg.

This method is less sensitive than the fluorophotometric one reported by Benson and Hare[10] who determined primary amines in the range of picomole. This method is very simple and convenient for use assaying ethylenediamine in an aqueous or ethanolic solution, even in the presence of secondary and tertiary amines.

References

1) J. S. Hanker, M. D. Sulkin, M. Gilman, and A. M. Seligman, *Anal. Chim. Acta*, **28**, 150 (1963).
2) E. I. Kas'yanenko and I. V. Samborskii, *Teoriya i Prakt. Ionnogo Obmena, Akad. Nauk Kaz. SSR, Tr. Resp. Soveshch*, **1962**, 100; *Chem. Abstr.*, **62**, 2242 (1965).
3) T. S. Al-Ghabsha, S. A. Rahim, and A. Townshend, *Anal. Chim. Acta*, **1976**, 85, 189.
4) G. N. Smith and M. G. Swank, *Anal. Chem.*, **32**, 978 (1960).
5) M. Roth, *Anal. Chem.*, **43** 880 (1971).
6) M. Roth, Ger. Offen., 2 102 985(Cl.G0ln), 02 Sep. 1971; Swiss Appl. 17 Feb. 1970; *Chem. Abstr.*, **75** 136846 m (1971).
7) V. Svedas, I. Yu. Galaev, and I. V. Berezin, *Bioorg. Khim.*, **1978**, 4, 19.
8) S. S. Simons, Jr., and D. F. Johnson, *J. Am. Chem. Soc.*, **98**, 7098 (1976).
9) S. S. Simons, Jr., and D. F. Johnson, *J. Chem. Soc., Chem. Commun.*, **1977**, 374.
10) J. R. Benson, and P. E. Hare, *Proc. Natl. Acad. Sci. U.S.A.*, **1975**, 72, 619.