Spectrophotometric Determination of 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride by Flow Injection Analysis

Kunihiko Seno,[†] Kazuki Matumura, Mitsuko Oshima, and Shoji Motomizu

Department of Chemistry, Faculty of Science, Graduate School of Natural Sciences and Technology, Okayama University, 3-1-1 Tsushimanaka, Okayama 700-8530, Japan

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) is a very useful agent to form amide bonds (peptide bonds) in an aqueous medium. A simple and fast detection system was developed using the reaction with pyridine and ethylenediamine in acidic aqueous solution and spectrophotometric flow injection analysis. The absorbances were measured at 400 nm and the reaction was accelerated at 40°C. The calibration graph showed good linearity from 0 to 10% of EDC·HCl solutions: the regression equation was $y = 3.15 \times 10^4 x$ (y, peak area; x, % concentration of EDC·HCl). The RSD was under 1.0%. Sample throughput was 15 h^{-1}. This method was applied to monitoring the EDC·HCl concentration that remained after the anhydration of phthalic acid in water, esterification of acetic acid in methanol or dehydration condensation of malonic acid and ethylenediamine in water.

Introduction

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and dicyclohexylcarbodiimide (DCC) are very useful agents to form nucleic acid[1-4] and compounds with amide bonds (peptide bonds);[5-8] they are called carbodiimide reagents. Carbodiimide reagents are also effectively used in polynucleotide synthesis,[9,10] anhydroxydation,[11,12] lactonization,[13] esterification,[14] and elsewhere. To get the target compounds, one adds carbodiimide reagents after mixing a carboxylic acid and an amine or one mixes them with a carboxylic acid before addition of an amine. After the reaction was finished, the carbodiimide reagent has changed to the corresponding urea. DC-urea produced by using DCC is hard to dissolve into almost all solvents. Therefore, precipitated DC-urea should be separate from the reaction products. However, in spite of this demerit, DCC is widely used in the manufacture because of its cost. On the other hand, EDC-urea generated by using EDC·HCl is easy to dissolve into almost all solvents. Recently, demands for the synthesis of macromolecules, high performance reagents and medicines are increased. In these cases, EDC·HCl is very useful because of its solubility in solvents, especially in water. One more merit is that EDC·HCl has less toxicity to health than DCC. Furthermore, EDC·HCl is a smooth powdery crystal and very easy to treat, whereas DCC is an oily solid. It is expected that EDC·HCl will come to be used more and more in the near future.

However, there are few effective and simple determination methods for EDC·HCl. EDC·HCl crystal was neutralized with a base and gas chromatography to determine EDC dissolved in the base was tried. However, neutralization did not quantitatively proceed and this method could not give accurate values. A direct method for the determination of carbodiimide reagents was application of titration of residual carboxylic acid after esterification, but this method needs extra skillfulness. Jacobson *et al.* have reported a spectrophotometric method for carbodiimide reagents,[15-17] but the reaction time was long, several hours, and thus not practical. When this method is applied to the determination of EDC·HCl, the reaction rate was much slower than DCC. The delay was caused by the structure of EDC·HCl:EDC·HCl, has the interesting property to be a ring structure in water,[18-20] whereas DCC is a straight chain and is insoluble in water. The ring structure of EDC·HCl can easily be confirmed by IR spectrum. It is stable in aqueous medium; therefore, it takes much time to react. We developed a simple and quick determination method, applying the flow injection analysis (FIA) which can determine even if the reaction rate is low before the reaction reaches equilibrium. The proposed method was able to measure 1 sample within 4 min and could be applied to the determination of residual EDC·HCl used in some amide bond formation reactions.

Experimental

Reagents and chemicals

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) was obtained from Osaka Synthetic Chemical Laboratories Inc., Hyogo. Hydrochloric acid, pyridine and ethylenediamine were obtained from Wako Pure Chemical Industries, Ltd., Osaka. Ethylenediamine solution (0.25 mol/L) was prepared by dissolving 15 g of ethylenediamine in 500 ml of water; the solution was adjusted to pH 7 with hydrochloric acid and diluted to 1000 ml with water. After 40 g of pyridine was dissolved in 500 ml water, adjusted to pH 7 with hydrochloric acid and was diluted to 1000 ml with water (0.5 mol/L). As a carrier solution (CS), 0.1 mol/L HCl was used. For a reagent solution (RS), 0.25 mol/L ethylenediamine and 0.5

[†] To whom correspondence should be addressed.
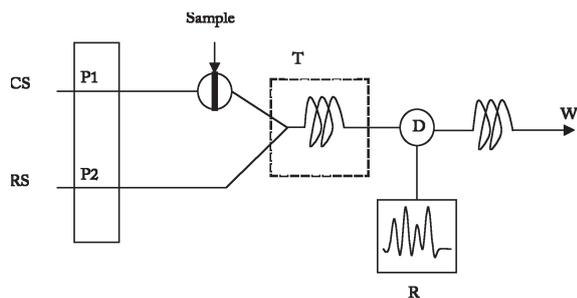E-mail: k.senou@tec-osc.co.jp

Fig. 1   Flow diagram for the determination of EDC·HCl. CS, carrier stream, 0.1 mol/L HCl; RS, reagent stream, 0.25 mol/L ethylenediamine + 0.5 mol/L pyridine; sample, 20 μL of EDC·HCl aqueous solution; P1, P2, pump, each flow rate, 0.25 ml/min; T, 40°C; R, recorder; W, waste.
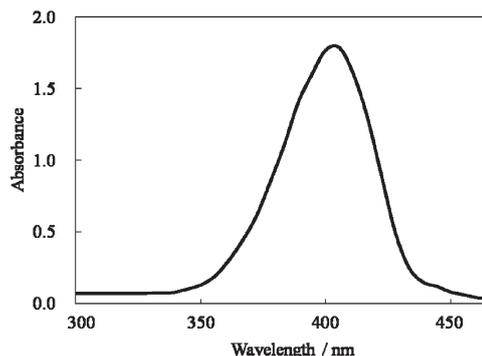

Fig. 2   Absorption spectrum of the reaction product of this reaction system. The mixed solution of 0.25 mol/L ethylenediamine, 0.5 mol/L pyridine, and 0.25 mol/L EDC·HCl, at pH 7 in water.

mol/L pyridine solutions were mixed 1 to 1 by volume: final concentrations were 0.125 mol/L ethylenediamine and 0.25 mol/L pyridine in RS.

Apparatus

A flow diagram of the FIA system is shown in Fig. 1. The system consisted of a double plunger-type pump (RX703T, Sanuki Kogyo Co. Ltd., Tokyo), a sample injector valve (7725i, Rheodine Co. Ltd., USA), and a UV-detector (LC-10AD, Shimadzu Corporation, Kyoto) with a 10-mm path length flow cell. The absorbance was measured at 400 nm. Flow rates of RS and CS in Fig. 1 were both 0.25 ml/min. The sample volume was 20 μL. Temperature was controlled at 40°C with an air bath (U-620, Sugai Chemical Ind. Co. Ltd., Wakayama). The peak area was obtained by an integrator (C-R6A, Shimadzu Corporation). Peak areas are shown in units of mV *versus* second.

Procedure

About 1 g of EDC·HCl was weighed correctly, and dissolved in water using a 10-mL volumetric flask; the volume was adjusted to the mark with water. The sample volume of 20 μL (contained W1 g of EDC·HCl) was injected into CS stream with the sample injector. The peak area (S1) was used for the calculation of the concentration of EDC·HCl. The standard EDC·HCl solution (contained W2 g of EDC·HCl) was weighed similarly as the sample solution, and the peak area (S2) was obtained. The concentration in the sample solution was calculated by the following equation.

Sample (%) = {(S1 × W2)/(S2 × W1)} × 100

Results and Discussion

The absorption spectra

The absorption spectrum of the reaction product is shown in Fig. 2. The maximum absorption wavelength was 400 nm and both ethylenediamine and pyridine had no absorption around this wavelength. Therefore, the measurement was done at 400 nm.

Effect of sample volume

Effects of the sample volume were tested up to 600 μL. The peak area increased linearly with increase in the sample volume. In this case, the concentration of the sample solution was relatively high (0.1 – 10%), so the sample volume was selected
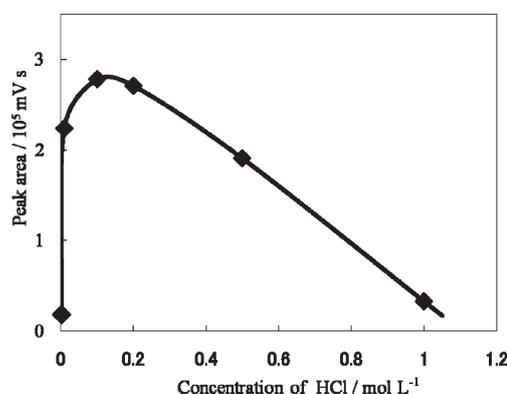
Fig. 3   Effect of the concentration of HCl in the carrier stream. The acid concentration in the carrier stream has been changed in the range 0.0 – 1.0 mol L⁻¹. Other conditions are the same as in Fig. 1.

to be 20 μL for low waste volume and good linearity of the calibration graph.

Effect of flow rate and reaction coil length

The total flow rate was examined in the range 0.2 – 1.5 mL min⁻¹: the slower the flow rate, the larger the peak area. The analytical concentration range of this case was 0.1 to 10% of EDC·HCl; we selected 0.5 mL min⁻¹ because of the adequate peak areas, analytical time and reagent consumption. The reaction coil length was varied from 3 to 13 m: the peak areas gradually increased with increase in the length, but the overall result was not so effective. We used 5 m coil length.

Effect of reagent concentrations

The selected reaction proceeds under the acidic condition. At higher acid concentration, ethylenediamine and pyridine are protonated and the reaction does not proceed. The acid concentration in the carrier stream affected to peak area: the effect of the hydrochloric acid concentration is shown in Fig. 3. At around 0.1 mol/L the peak area became largest: 0.1 mol/L was selected. At concentration lower than 0.1 mol/L HCl, EDC·HCl changes in a ring structure and the reaction does not occur. The effects of ethylenediamine and pyridine concentrations were examined. When ethylenediamine concentration was higher than 0.25 mol/L, the baseline noise became larger. At concentrations lower than 0.25 mol/L, the sensitivity was decreased, so the ethylenediamine concentration was fixed at

Table 1   Reproducibility test

Concentration of EDC·HCl, %	Average (area) mV × time/s	Standard deviation[a]	Relative standard deviation, %
2.5	7.5×10^4	4.2×10^2	0.75
5.0	1.6×10^5	8.9×10^2	0.56
10.3	3.3×10^5	1.1×10^3	0.56

a. $n = 8$.

0.25 mol/L and the pyridine concentration was changed from 0.25 to 0.5 mol/L. The peak area at 0.5 mol/L was higher than 0.25 mol/L and showed enough sensitivity: we thus selected 0.25 mol/L of ethylenediamine and 0.5 mol/L of pyridine as stable baseline and reasonable reagent consumption.

Effect of reaction temperature

The reaction proceeds relatively slowly, so the effect of temperature was tested from 30 to 60°C to accelerate the reaction efficiency. The peak areas were moderately and linearly increased with increase in the temperature. We selected 40°C, because the acceleration effect was not so much as expected.

Linearity of the calibration graph and reproducibility

The calibration graph showed good linearity from 0 to 10% of EDC·HCl solutions. At higher concentrations than those, the graph was curved upward. The regression equation is $y = 3.15 \times 10^4 x$ (y, peak area; x, % concentration of EDC·HCl; when x is mol/L unit: $y = 1.64 \times 10^2 x$). The detection limit ($3s/n$) was 0.1%. The reproducibility was tested with 2.5, 5.0, and 10.0% of EDC·HCl solution ($n = 8$); RSDs were 0.75, 0.56, and 0.56%, respectively (Table 1). The sample throughput was 15 h^{-1}.

Application to condensation reactions

These reactions with ethylenediamine and pyridine are specific to carbodiimide reagents. The interferences from other alkyldiamides, such as 1,3-diaminopropane, 1,4-diaminobutane and 1,5-diaminopentane, or amino acids, such as glycine, alanine and serine, were checked but no interferences were found. Other carbodiimides, such as diisopropylcarbodiimide (DIC) and dicyclohexylcarbodiimide (DCC), react with the same mechanism as this reaction, but two or more of carbodiimides will not be used in the same reaction systems. This method was applied to the next 3 anhydration reactions.

Anhydration of phthalic acid in water

The consumption rate of EDC·HCl in anhydration of phthalic acid in water was examined. Phthalic acid was dissolved in water, and an equivalent mole amount of EDC·HCl was added. Immediately, the solution became turbid and the crystal was produced. The crystal was identified as a phthalic anhydride by its IR spectrum. In this case, EDC·HCl was consumed within 5 min, as shown in Fig. 4. Because phthalic acid is a dibasic acid and the intramolecular hydrogen bond is formed in aqueous solution, dehydration of phthalic acid is structurally smooth and the reaction finished soon.

Esterification of acetic acid in methanol

EDC·HCl concentration was monitored in esterification of acetic acid in methanol. The product was confirmed to be methylacetate by GC. The results are shown in Fig. 5. In this reaction, EDC·HCl was consumed comparatively slowly. This reaction seems to be slow because OH of acetic acid and H of
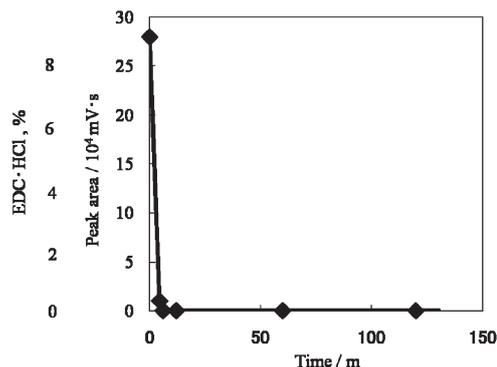

Fig. 4   EDC·HCl consumption rate in anhydration of phtharic acid in water. The measurement was started immediately after mixing the 50 mL aqueous solutions of 4.26 g potassium hydrogen phthalate and 4.00 g of EDC·HCl and each 20 μl sample solution was picked up.
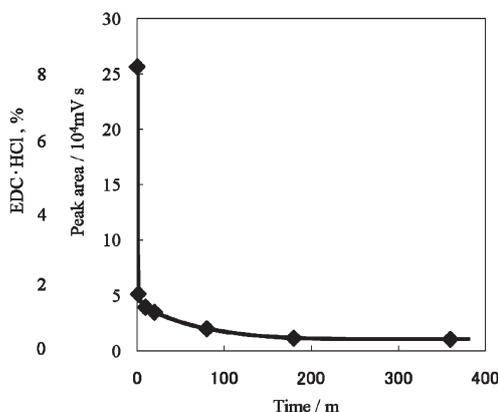

Fig. 5   EDC·HCl consumption rate in esterification of acetic acid in methanol. The sample solution was a mixture of 2.51 g acetic acid methanol solution and 4.00 g of EDC·HCl. Other conditions were the same as in Fig. 4.

methanol do not form an intramolecular hydrogen bond as in the case of phthalic acid.

Dehydration condensation of malonic acid and ethylenediamine in water

Dehydration condensation reactions of malonic acid and ethylenediamine in water were examined: the results are shown in Fig. 6. In Fig. 6, the results obtained by measuring the absorbance of the reaction product at 262 nm are also shown. The product was increased with increasing in time and corresponded to EDC·HCl consumption. EDC·HCl was spent proportionally to molar amounts of malonic acid and ethylenediamine. By monitoring the concentration of EDC·HCl, we could estimate the reaction rate.

Conclusions

A spectrophotometric method for determination of EDC·HCl in an aqueous medium, which can be measured in a short time and with a simple flow system, was developed. Analysis time for 1 sample was less than 4 min, and application to monitoring the dehydration condensation reaction rate could be accomplished successfully by measuring the EDC·HCl concentration.
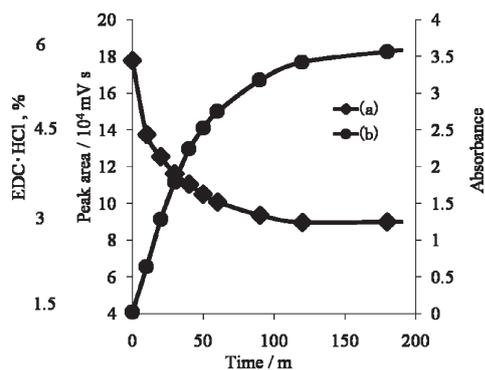
Fig. 6 EDC·HCl consumption rate in dehydration condensation of malonic acid and ethylenediamine in water. The 100 mL sample solution was a mixture of 2.4 g malonic acid, 1.4 g ethylenediamine, and 9.2 g of EDC·HCl; (a) detected at 400 nm (this method). Other conditions were the same as in Fig. 6; (b) detected at 262 nm, the sample was diluted by the solution of (a) to 1250 times.

References

1. H. G. Khorana, *Chem. Rev.*, **1953**, *53*, 145.
2. H. G. Khorana and R. A. Todd, *J. Chem. Soc.*, **1953**, 2257.
3. C. A. Dekker and H. G. Khorana, *J. Am. Chem. Soc.*, **1954**, *76*, 3522.
4. H. G. Khorana and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **1961**, *83*, 675.
5. J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **1955**, *77*, 1067.
6. E. Wünsch and F. Deimer, *Z. Physiol. Chem.*, **1972**, *353*, 1246.
7. E. Wünsch and F. Deimer, *Ber.*, **1966**, *99*, 110.
8. W. König and R. Geiger, *Ber.*, **1970**, *103*, 788.
9. G. M. Tener, *J. Am. Chem. Soc.*, **1961**, *83*, 159.
10. E. Ohtsuka, M. Ubasawa, and M. Ikehara, *J. Am. Chem. Soc.*, **1970**, *92*, 5507.
11. E. Vowinkel and C. Walft, *Ber.*, **1974**, *107*, 907.
12. E. Vowinkel and I. Büthe, *Ber.*, **1974**, *107*, 1353.
13. W. S. Johnson, V. J. Bauer, M. A. Frisch, L. H. Dreger, and W. H. Hubbard, *J. Am. Chem. Soc.*, **1961**, *83*, 606.
14. B. Neises and W. Steglech, *Angew. Chem.*, *Int. Ed.*, **1978**, *17*, 522.
15. B. S. Jacobson and K. R. Fairman, *Anal. Biochem.*, **1980**, *106*, 114.
16. M. Wilchek, T. Miron, and J. Kohn, *Anal. Biochem.*, **1981**, *114*, 419.
17. S. H. Chen, *Anal. Biochem.*, **1983**, *132*, 272.
18. T. Ibrahim and A. Williams, *J. Am. Chem. Soc.*, **1978**, *100*, 7420.
19. J. C. Sheehan, P. A. Cruickshank, and G. L. Boshart, *J. Org. Chem.*, **1961**, *26*, 2525.
20. A. Williams and I. T. Ibrahim, *J. Am. Chem. Soc.*, **1981**, *103*, 7090.