[Chem. Pharm. Bull.
33(12)5375–5379(1985)]

Spectrophotometric Determination of Basic Carbodiimide Perchlorates by the Use of Ferric Benzohydroxamate Formation

YASUHIKO KASAI,* SHIGETAKA HOSHINO, YASUKO SUZUKI,
and HIROSHI YAMADA

*Kanto Chemical Co., Inc., 3-7, Honcho Nihonbashi,
Chuo-ku, Tokyo 103, Japan*

(Received March 8, 1985)

A method was developed for the determination of 1-ethyl-, 1-isopropyl-, and 1-cyclohexyl-3-(3-dimethylaminopropyl)carbodiimide perchlorates. Benzoic acid and hydroxylamine perchlorate were coupled at 20 °C for 40 min in ethanolic medium by using the above carbodiimide perchlorates (0.25 to 7.5 mM) at pH 5.5. Ferric perchlorate in ethanolic perchloric acid was added to the benzohydroxamic acid formed. The absorbance of the resultant ferric benzohydroxamate was measured at 550 nm *vs.* the blank solution. This method is more sensitive than that using hydroxamate formation *via* carboxylic acid anhydride, or than the oxalic acid or cyanide method, and is simpler than the method using aniline or barbiturates or aconitic acid.

Keywords——carbodiimide determination; spectrophotometric determination; 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate; 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate; 1-cyclohexyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate; hydroxylamine perchlorate; ferric benzohydroxamate; 1-ethyl-3-(3-dimethylaminopropyl)urea perchlorate

Carbodiimides are widely used for dehydrative reactions in chemical syntheses and analyses.[1] 1,3-Dicyclohexylcarbodiimide is the most important reagent, but this and the corresponding urea formed as a reaction product are less soluble in aqueous medium, frequently rendering separation of the products from the reaction mixture difficult. For this reason, Sheehan *et al.*[2] have introduced acid- and water-soluble carbodiimides, and among them, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (**1**) hydrochloride and 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate are now commercially available. However, the former is extremely hygroscopic, and the latter, though non-hygroscopic, requires a longer reaction time in some cases.[2a]

Previously, we first reported[3] the preparation of several basic carbodiimide perchlorates, which are non-hygroscopic and have moderate solubility in aqueous and organic media. In order to evaluate the stability of the compounds in various media in detail, it was necessary to develop a simple, highly sensitive, and reproducible assay method. Current methods for carbodiimide assay are based on the principles that carbodiimides react quantitatively with either oxalic acid,[4–7] evolving carbon monoxide and carbon dioxide, or cyanide,[8] producing an addition compound, and the carbodiimide can be determined by either trapping of evolved carbon dioxide[4] or back-titrating the excess reagents.[5–8]

Recently, chromophore formation was utilized for the carbodiimide assay, that is, reaction with aniline,[9] pyridine and 1,2-diaminoethane or piperadine,[10] barbiturates,[11] and measurement of Fe(III)-chelated acetohydroxamic acid[9] which is derived from hydroxylamine and acetic anhydride formed by dehydration of acetic acid mediated by carbodiimide. Fluorophore formation with *trans*-aconitic acid[12] was also developed.

Except for the methods using chromophore formation with pyridine and 1,2-diaminoethane or piperadine, and ferric acetohydroxamate, these methods are highly sensitive but require rigorous conditions to be reproducible, *e.g.*, measurement at precisely 230 nm in 0.01 M aniline hydrochloride,[9] measurement at an exact time during chromophore formation,[10] choosing suitable assay conditions for a variety of sample concentrations,[11] or anhydrous conditions.[12]

Carbodiimides mediate the coupling reaction of carboxylic acid to hydroxylamine *via* a single step,[13,14] forming hydroxamic acid, which could be determined spectrophotometrically as ferric hydroxamate. We utilized this reaction and established a simple, highly sensitive, and reproducible procedure using **1**, 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide (**2**), and 1-cyclohexyl-3-(3-dimethylaminopropyl)carbodiimide (**3**) perchlorates.

Experimental

Materials——**1**, **2**, and **3** were prepared by dehydration of the corresponding ureas according to the method of Sheehan and Cruickshank,[15] and identified by comparing the boiling points with those in the literature.[2b] Then, metathesis with pyridine perchlorate was carried out to form **1**, **2**, and **3** perchlorates.[3]

A stirred solution of 15.5 g (0.10 mol) of **1** in 200 ml of methylene chloride in an ice water-cooled bath was treated portionwise with 18.0 g (0.10 mol) of pyridine perchlorate. The smell of pyridine became perceptible as the reaction proceeded. After the addition, the mixture was allowed to stand for 30 min, then filtered if turbid. Addition of 200 ml of anhydrous diethyl ether precipitated the product. The crystalline product was collected by filtration. Recrystallization from methylene chloride–diethyl ether gave 24.5 g of perchlorate. Two other compounds, **2**, and **3** perchlorates, were prepared in the same manner as **1** perchlorate. The data for the three compounds are summarized in Table I.

The infrared (IR) spectra of the crystalline solid **1**, **2**, and **3** perchlorates (KBr) were in agreement with the literature,[2b] showing strong absorptions at 3250 (NH) and 1700 cm^{-1} (C=N). The IR spectra in chloroform solution showed the 2130 cm^{-1} band (N=C=N).

1-Ethyl-3-(3-dimethylaminopropyl)urea perchlorate was derived from **1** perchlorate without addition of any catalytic agent. **1** perchlorate (20 g, 0.078 mol) was incubated in 200 ml of distilled water for 24 h at 60 °C until the carbodiimide color reaction (assay procedure mentioned below) no longer appeared. Water was evaporated off under reduced pressure (bath temperature below 60 °C). The pale yellow oily residue was triturated with 100 ml of hexane. The resulting white crystalline product was collected by filtration, washed with a small portion of hexane and dried. Yield 10 g (47%), mp 70.4—73 °C. *Anal.* Calcd for $C_8H_{20}ClN_3O_5$: C, 35.10; H, 7.37; Cl, 12.95; N, 15.35. Found: C, 34.71; H, 7.34; Cl, 13.19; N, 15.55. The IR spectrum showed a band at 1640 cm^{-1} (C=O).

Hydroxylamine perchlorate[16] used was hydroxylamine perchlorate solution[18] diluted with 99.5% ethanol to the prescribed concentration.

Benzoic acid–hydroxylamine perchlorate solution was prepared by adding benzoic acid and hydroxylamine perchlorate to 99.5% ethanol so as to give 75 and 50 mM concentrations, respectively, and the pH was adjusted to 5.5 (glass electrode pH meter) with triethylamine.

0.01 M Fe(III) solution: Ferric perchlorate, $Fe(ClO_4)_3 \cdot 6H_2O$, was dissoved in 2.0 M perchloric acid—70% perchloric acid was diluted with 99.5% ethanol (exothermic!)—to the prescribed concentration.

Other reagents were of analytical grade and were used without further purification.

Instrument——A Hitachi 320 spectrophotometer was used for spectrophotometric measurement.

TABLE I. Basic Carbodiimide Perchlorates

Perchlorate	Yield[a] (%)	mp (°C)[b]	Formula	Analysis (%) Calcd (Found)			
				C	H	Cl	N
1	96	97—98	$C_8H_{18}ClN_3O_4$	37.58	7.10	13.87	16.43
				(37.52	7.11	14.23	16.40)
2	98	88—90	$C_9H_{20}ClN_3O_4$	40.08	7.48	13.14	15.58
				(39.70	7.60	13.29	15.43)
3	98	90—91	$C_{12}H_{24}ClN_3O_4$	46.53	7.81	11.44	13.56
				(46.59	8.01	11.55	13.52)

a) From carbodiimide. *b*) Uncorrected.

Determination Procedure——To 1 ml of ethanolic solution containing 0.25 to 7.5 μmol of carbodiimide perchlorate add 2 ml of benzoic acid–hydroxylamine perchlorate solution and incubate for 40 min at 20 °C. Then, add 2 ml of 0.01 M Fe(III) solution to the mixture and allow to stand for 5 min. Absorbance is measured at 550 nm *vs.* a reagent blank solution.

Results and Discussion

Carbodiimide perchlorates were prepared from the corresponding basic carbodiimides by using non-hygroscopic pyridine perchlorate in high yields. The hydration of **1** perchlorate to the urea derivative was performed non-catalytically with a longer incubation period to obtain a product uncontaminated by catalytic agent, though with alkaline- or acid-catalyzed hydration was in general.[2b,19] The effect of pH on hydroxamate formation was similar to that described in previous papers;[13,14] maximum yield of hydroxamate was obtained between pH 4.0 and 6.0 (Fig. 1). Therefore, pH 5.5 was chosen in subsequent assays.

The effect of temperature varied from case to case (Fig. 2); that is, with **1** perchlorate the yield was consistently high over the temperature surveyed, whereas with **2** perchlorate the curve of hydroxamate formation was convex and with **3** perchlorate it was concave. These results imply differences of reactivity for mediating the coupling of benzoic acid to hydroxylamine among the three. It is possible that in the **2**, and **3** perchlorate assays, side-reaction(s) may increase with temperature. Determination was therefore done at 20 °C.

Effect of Molar Ratio of Reagents

The highest yield was obtained at 25 to 40 molar ratio of benzoic acid and hydroxylamine to carbodiimide, and when the molar ratio of benzoic acid to hydroxylamine was 1.0 to 2.0. A preliminary experiment showed that Fe(III) was sufficient at 2.0 molar excess over carbodiimide.

Therefore, in the assay procedure for 0.25 to 7.5 μmol of carbodiimide, 150 μmol of benzoic acid, 100 μmol of hydroxylamine and 20 μmol of Fe(III) were used.

Effect of Reaction Time

All three compounds yielded benzohydroxamic acid maximally at a reaction time of

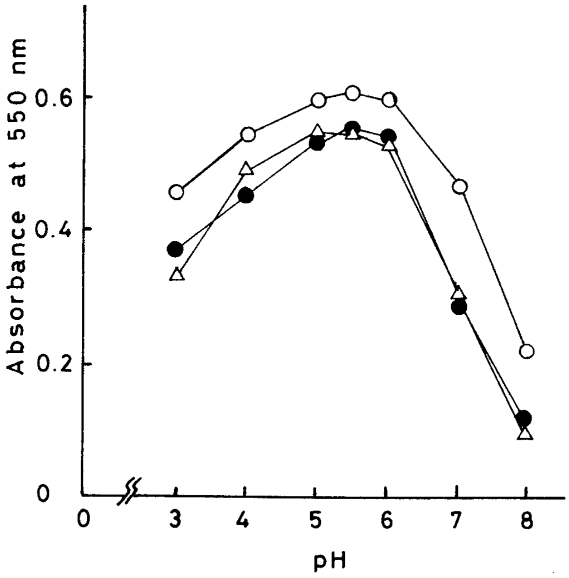

Fig. 1. Effects of pH on the Hydroxamate Formation

The absorbance was measured according to the procedure in the text except for pH adjustment. Amount: 2.5 μmol each of **1** (○), **2** (●), and **3** (△) perchlorates.

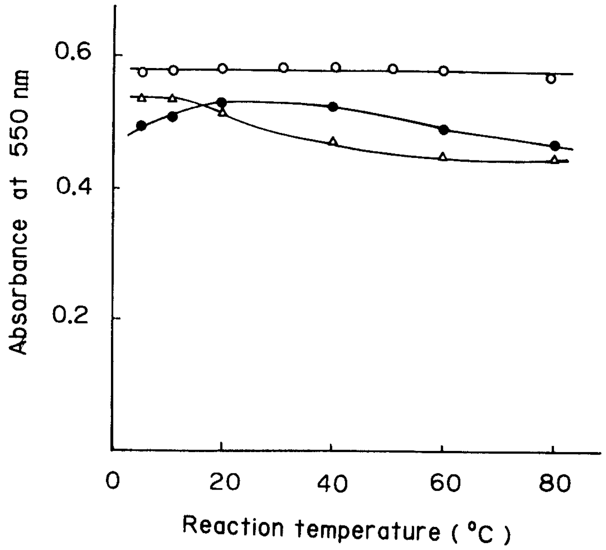

Fig. 2. Effects of Temperature on the Hydroxamate Formation

Perchlorates of ○, **1**; ●, **2**; △, **3** (each 2.5 μmol). Conditions were the same as in the text except for the temperature.

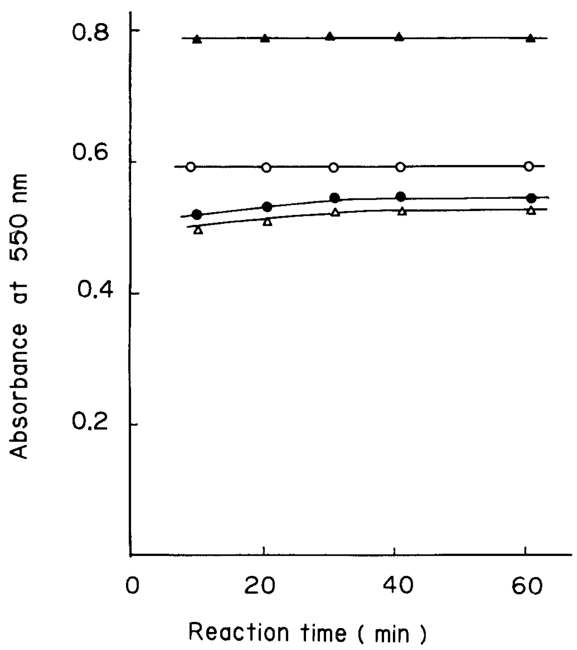

Fig. 3. Rate of Hydroxamate Formation at Various Reaction Times

Carbodiimide : benzoic acid : hydroxylamine = 1 : 150 : 100 molar ratio. Other conditions are the same as in the text.

○, **1**; ●, **2**; △, **3** perchlorates, and ▲, authentic benzohydroxamic acid treated in the same way.

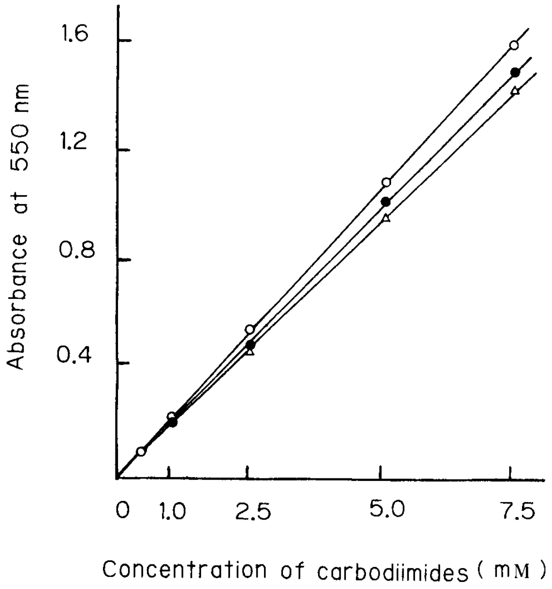

Fig. 4. Calibration Curves of Basic Carbodiimide Perchlorates

○, **1**; ●, **2**; △, **3** perchlorates.

more than 30 min, and the reaction curves displayed plateaus thereafter (Fig. 3). Consequently in the determination procedure, the reaction was carried out for 40 min.

The percent yields of benzohydroxamate formed by **1**, **2**, and **3** perchlorates were 75, 70 and 68%, respectively, calculated from equimolar authentic benzohydroxamic acid and carbodiimide, under the same conditions as in the assay procedure. The absorbance of the ferric benzohydroxamate formed changed little during 60 min under the assay conditions.

As mentioned in the literature,[1,13)] carbodiimides react with carboxylic acid and form *O*-acylisourea, which can rearrange spontaneously to an *N*-acylurea unreactive to hydroxylamine. On the other hand, carbodiimides also react with hydroxylamine to form inert products. Accordingly, benzoic acid and hydroxylamine were mixed prior to the reaction with carbodiimide. The corresponding urea did not affect the hydroxamate formation in **1** perchlorate determination even when the urea was added at twofold molar excess over **1** perchlorate.

In the determination, hydroxamate formation was linear with respect to the amount of carbodiimides in the concentration range of 0.25 to 7.5 mM (Fig. 4).

In this study, benzoic acid was used because of ease of handling and its availability in pure form; further, its hydroxamate has been well studied.[20)] The present method is simpler than the other highly sensitive methods as mentioned earlier, and has sufficient sensitivity. This method is applicable to water-soluble and water-insoluble carbodiimides. We are now attempting to extend the scope of this method.

Acknowledgement The authers wish to thank the staff of the Microanalytical Center, the Institute of Physical and Chemical Research, Japan, for the elemental analyses. Thanks are also due to Mr. Kamada for his assistance in the experimental work.

References and Notes

1) *a*) H. G. Khorana, *Chem. Rev.*, **53**, 145 (1958); *b*) F. Kurzer and K. Douraghi-Zadeh, *ibid.*, **67**, 107 (1967); *c*) A. Williams and I. T. Ibrahim, *ibid.*, **81**, 589 (1981); *d*) M. Mikolajczyk and P. Kielbasinski, *Tetrahedron*, **37**, 233 (1981).
2) *a*) J. C. Sheehan and J. J. Hlavka, *J. Org. Chem.*, **21**, 439 (1956); *b*) J. C. Sheehan, P. A. Cruickshank, and G. L. Boshart, *ibid.*, **26**, 2525 (1961).
3) Kanto Chemical Co., Inc., Japan. Patent 29519 (1975) [*Chem. Abstr.*, **83**, 58082*h* (1975)].
4) F. Zetzsche and A. Fredrich, *Ber.*, **72**, 363 (1939).
5) J. E. Zarembo and M. M. Watts, *Microchem. J. Symp. Ser.*, **2**, 591 (1962).
6) W. Adam and F. Yany, *Anal. Chem.*, **49**, 676 (1977).
7) Y. Sakai and Y. Kasai, *Bunseki Kagaku*, **30**, 550 (1981).
8) S. Hünig, H. Lehmann, and G. Grimmer, *Justus Liebigs Ann. Chem.*, **579**, 77 (1953).
9) A. Williams, S. V. Hill, and I. T. Ibrahim, *Anal. Biochem.*, **114**, 173 (1981).
10) B. S. Jacobson and K. R. Fairman, *Anal. Biochem.*, **106**, 114 (1980).
11) M. Wilcheck, T. Miron, and J. Kohn, *Anal. Biochem.*, **114**, 419 (1981).
12) S.-C. Chen, *Anal. Biochem.*, **132**, 272 (1983).
13) Y. Kasai, T. Tanimura, and Z. Tamura, *Anal. Chem.*, **47**, 34 (1975).
14) T. Takeuchi, R. Horikawa, and T. Tanimura, *Anal. Lett.*, **13A**, 603 (1980).
15) J. C. Sheehan and P. A. Cruickshank, "Organic Syntheses," Coll. Vol. V, ed. by A. H. Blatt, John Wiley and Sons, Inc., New York, 1973, p. 555.
16) Hydroxylamine perchlorate can be prepared by reaction of hydroxylamine sulfate and barium perchlorate.17)
17) J. H. Robson, U. S. Patent 2768874 (1956) [*Chem. Abstr.*, **51**, 3146*c* (1957)].
18) A. 13.4% (w/v) ethanolic solution of hydroxylamine perchlorate, manufactured by Kanto Chemical Co., Inc., Tokyo (Cat. No. 56661).
19) S. Hünig, H. Lehmann, and G. Grimmer, *Justus Liebigs Ann. Chem.*, **579**, 87 (1953); I. K. Korobeinicheva, E. D. Krivousova, and O. A. Migorodskaya, *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk*, **1969**, 115 [*Chem. Abstr.*, **71**, 60317 (1969)].
20) R. F. Goddu, N. F. LeBlank, and C. M. Wright, *Anal. Chem.*, **27**, 1251 (1955).