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ISOTACHOPHORETIC SEPARATION AND BEHAVIOUR OF CATECHOL DERIVATIVES

SHUNITZ TANAKA, TAKASHI KANETA and HITOSHI YOSHIDA*
Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060 (Japan)
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SUMMARY

The separation and migration behaviour of ten catechol derivatives containing electrically neutral species were investigated by capillary tube isotachophoresis. In the usual migration system at pH 7.5 consisting of Tris buffer as the leading electrolyte and histidine as the terminating electrolyte, six of the catechols were separated but the other four with small acid dissociation constants could not be detected. However, by using boric acid as the terminating electrolyte and converting the catechols into anionic catechol–borate complexes in the migrating capillary, all ten catechols could be detected and nine of them could be separated even at pH 7.5.

INTRODUCTION

Catechol and its derivatives form primary structure of dopamine, epinephrine, etc., which act as neurotransmitters and are physiologically important substances[1]. These substances participate in an oxidation–reduction system with *o*-quinone and play important roles in the electron transport system *in vivo*. Analytical separations of these substances have been performed using various types of chromatography[2,3] and flow-injection analysis[4]. Capillary tube isotachophoresis (CITP) is also an excellent separation method but there have been few reports of its application. For the separation of catechols by CITP it is necessary to convert them into ionic species. The hydroxyl group of catechols can dissociate in the alkaline operating system of CITP. However, catechols are oxidized by air in alkaline media to form *o*-quinone and consequently the zones of catechols suffer interferences in the alkaline operating system of CITP. At pH below *ca.* 7, catechols that have small acid dissociation constants cannot migrate because they exist as electrically neutral species.

An efficient method for changing the mobilities of species under isotachophoretic investigation is utilization of complex-forming equilibria. The behaviour of complexes under such conditions was dealt with in detail both theoretically and experimentally by Gebauer *et al.*[5]. We have also reported a new operating system involving reaction with the terminating ion[6]. In this method, sample molecules were converted into negatively charged species with the terminating ion and migrated as a result of complex-forming equilibria. Compared with the conventional method using the com-

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plex-forming equilibria between the sample ions and the counter ion in the leading electrolyte[8,9], this method has the important advantage that the effective mobilities of the sample species become larger according to the strength of the interaction with the terminating ion. Therefore, this method can be applied to the migration of species with low mobilities and electrically neutral species. In a previous paper[10] we demonstrated the probability that electrically neutral catechols can be made to migrate as borate complexes by using borate as the terminating ion.

In this paper, the isotachophoretic behaviour and separation of ten catechol derivatives containing electrically neutral species are described. The order of migration of the catechols was correlated with the acid dissociation constants obtained by potentiometric measurement using acid–base titration. In addition, using boric acid as the terminating electrolyte, the migration system for catechols as borate complexes was investigated. Nine catechols, including some with small acid dissociation constants, could be separated at neutral pH (7.5) in this system.

EXPERIMENTAL

Apparatus

A Model IP-1B capillary tube isotachophoretic analyser equipped with a potential gradient detector (Shimadzu, Kyoto, Japan) was used. The PTFE tube for separation consisted of a main column (150 mm × 0.5 mm I.D.) and a precolumn (40 mm × 1.0 mm I.D.). The capillary tube was filled with electrolyte by pressure of nitrogen gas. In this study the R_E value was used as the index of identification, where R_E is the ratio of the potential gradient of the sample zone to that of the leading zone, *i.e.*, corresponding to the ratio of the mobility of the leading ion to that of the sample ion.

A Model M-8L pH meter (Horiba, Tokyo, Japan) was used to detect neutralization in potentiometric titration.

A Model 174A polarographic analyser (Princeton Applied Research, Princeton, NJ, U.S.A.) was used for measuring cyclic voltammograms. The working electrode was a Metrohm Model E 410 hanging mercury drop electrode (HMDE) and the counter electrode was a glassy-carbon rod. A saturated calomel electrode with a diaphragm tube containing 1 *M* potassium nitrate solution was used as the reference electrode.

Reagent

All reagents were of analytical-reagent grade and solutions were prepared by dissolution in doubly distilled, deionized water. The solutions of catechols were stored in a refrigerator.

2,3-Dihydroxybenzoic acid (2,3-DBA), 3,4-dihydroxybenzoic acid (3,4-DBA), 2,3-dihydroxybenzaldehyde (2,3-DBAL), 3,4-dihydroxybenzaldehyde (3,4-DBAL), 2,3-dihydroxynaphthalene (DN), 4-chlorocatechol (CC) and 4-methylcatechol (MC) were obtained from Tokyo Kasei (Tokyo, Japan), pyrocatechol (PC) from Kanto (Tokyo, Japan) and pyrogallol (PG), hydroxyhydroquinone (HHQ), hydroquinone (HQ) and others chemicals from Wako (Osaka, Japan).

The leading electrolyte was prepared by adding tris(hydroxymethyl)aminomethane to 10 m*M* hydrochloric acid containing $1.25 \cdot 10^{-3}$% poly(vinyl alcohol) to

TABLE I

OPERATING SYSTEM

Parameter	*Leading electrolyte*	*Terminating electrolyte*
Anion	Cl^-	System 1: histidine System 2: germanate System 3: borate
Counter ion	$TrisH^+$	Ba^{2+}
Concentration of anion	10 m*M*	10 m*M*
Additive	$1.25 \cdot 10^{-3}$% poly(vinyl alcohol)	5 m*M* $Ba(OH)_2$
pH	System 1: 7.5 System 2: 8.0 System 3: 7.5	10

adjust the pH. Terminating electrolytes were prepared by adding barium hydroxide to histidine or germanic acid or boric acid solution (pH 10).

The operating systems are summarized in Table I. System 1 consisted of a leading electrolyte of pH 7.5 and a terminating electrolyte of 10 m*M* histidine, system 2 consisted of a leading electrolyte of pH 8.0 and a terminating electrolyte of 10 m*M* germanic acid and system 3 consisted of a leading electrolyte of pH 7.5 and a terminating electrolyte of 10 m*M* boric acid. All terminating electrolytes contained 5 m*M* barium hydroxide.

Acid–base titration

Potentiometric acid–base titration was performed in order to determine the acid dissociation constants of catechols. The dissociation constants were determined

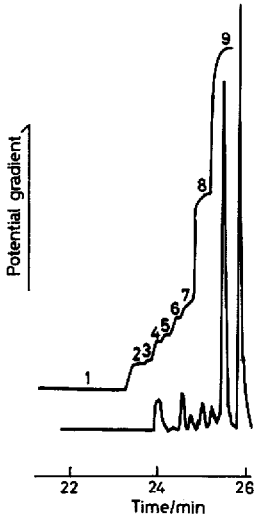


Fig. 1. Isotachopherogram of catechols in system 1. 1 = Chloride; 2 = hydrogencarbonate; 3 = HHQ; 4 = 2,3-DBA; 5 = 3,4-DBA; 6 = 3,4-DBAL; 7 = 2,3-DBAL; 8 = CC; 9 = histidine. Leading electrolyte, 10 m*M* HCl + Tris, $1.25 \cdot 10^{-3}$% PVA, pH 7.5. Sample, 10 μl of a mixed solution of 1 m*M*. Driving current, 100 μA.

by measuring the pH of the mid-point of the titration curve. A 5 or 10 m*M* solution of the sample in 50 m*M* potassium nitrate solution was used as the titrand and 50 m*M* sodium hydroxide solution as the titrant.

RESULTS AND DISCUSSION

Migration of catechol derivatives

The isotachopherogram of the ten catechols obtained by using system 1 in Table I is shown in Fig. 1. Six of the catechols could be detected and separated but the other four were not detected. The effective mobilities of the catechols in this migration system were determined from the acid dissociation constants and the pH of the leading electrolyte. The acid dissociation constants were determined by potentiometric acid–base titration and are given in Table II.

In order of the dissociation constants, 2,3-DBA, 3,4-DBA, 3,4-DBAL, 2,3-DBAL, and CC migrated, 2,3-DBA and 3,4-DBA owing to the dissociation of the carboxyl group and 3,4-DBAL, 2,3-DBAL and CC owing to the dissociation of the hydroxyl group. HHQ had large mobility but its dissociation constant could not be determined. The migration behaviour of HHQ will be described in detail later. The other four catechols, DN, PC, PG and MC, did not migrate because they have small acid dissociation constants and are present as electrically neutral species in this operating system. The mobilities of the catechols increased in alkaline systems such as HCl–NH_3 and HCl–arginine buffer, but the differences in their mobilities decreased and the separation became difficult. Also, some catechols gave unstable zones because they are oxidized by dissolved oxygen in alkaline media.

Migration of catechols as complexes

It is known that polyols, such as catechols and carbohydrates, are complexed with oxy acids such as germanic and boric acid[11,12] and the resulting complexes act as stronger acids than the original germanic and boric acid. These reactions have been used in the titration of boric acid[11], zone electrophoresis of carbohydrates[13,14] and chromatography of carbohydrates[15,16]. In order to utilize this reaction in isotachophoretic separations, we designed a new migrating system using an oxy acid as the terminating electrolyte. In this system, injected catechols might be converted into catechol–oxy acid complexes by reaction with terminating ion and then migrate. In this method it is necessary to satisfy the following requirements for an oxy acid as the terminating electrolyte: (1) the oxy acid should form a stable terminating zone where

TABLE II

ACID DISSOCIATION CONSTANTS (pK_a) OF CATECHOL DERIVATIVES

Compound	pK_a	*Compound*	pK_a
2,3-DBA	3.2	DN	8.4
3,4-DBA	4.5	MC	8.9
3,4-DBAL	7.1	PC	9.1
2,3-DBAL	7.6	PG	8.7
CC	8.4	HHQ	–

the effective mobility is sufficiently different from that of the leading ion, and (2) the catechol–oxy acid complexes formed should have higher effective mobilities than that of the oxy acid.

Germanate and borate were selected as terminating ions and the migration behaviour of catechols in the migration systems shown in Table I was investigated.

Migration as germanate complexes. Fig. 2. shows the isotachopherogram of ten catechols obtained by using germanic acid as the terminating electrolyte (system 2 in Table I). They had high mobilities but except for 2,3-DBA and HHQ they had the mobilities were very similar and the compounds could not be separated. It seems that the germanate–catechol complexes are too stable to be separated using complex–forming equilibria with the terminating ion.

Migration as borate complexes. Fig. 3. shows the isotachopherogram of catechols obtained by using borate as the terminating ion (System 3 in Table I). Some of the catechols that did not migrate in the system 1, could be detected and nine of the catechols could be separated. It seems that the injected catechols form complexes with the boric acid and the complexes act as stronger acids than boric acid in this operating system, as shown by following equations:

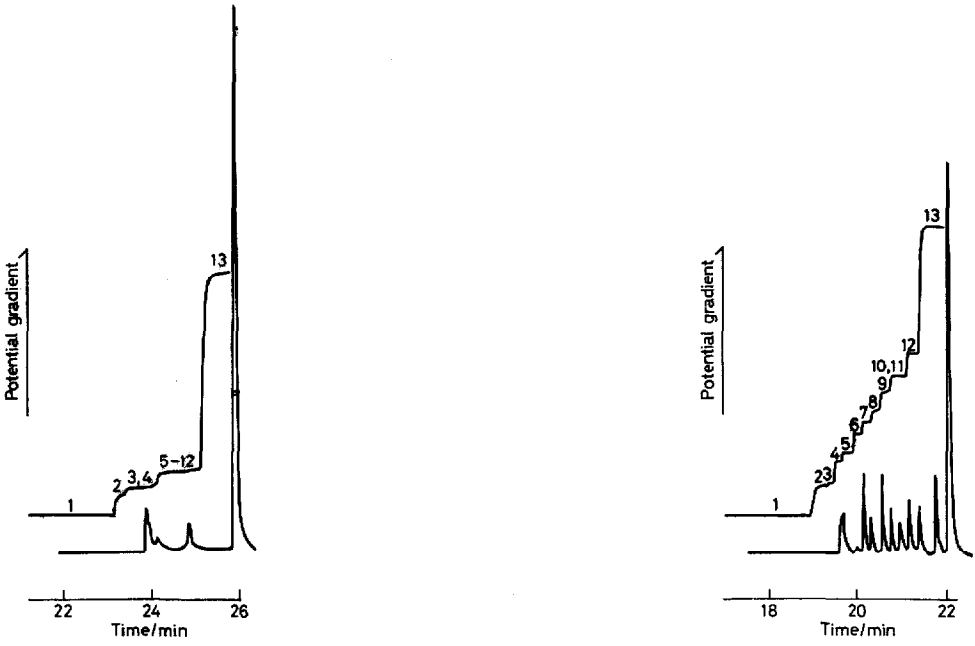


Fig. 2. Isotachopherogram of catechols in system 2. 1 = Chloride; 2 = 2,3-DBA; 3 = hydrogencarbonate; 4 = HHQ; 5 = 3,4-DBA; 6 = 3,4-DBAL; 7 = 2,3-DBAL; 8 = DN; 9 = CC; 10 = PC; 11 = PG; 12 = MC; 13 = germanate. Leading electrolyte, 10 m*M* HCl + Tris, 1.25 · 10^{-3}% PVA, pH 8.0. Other conditions as in Fig. 1.

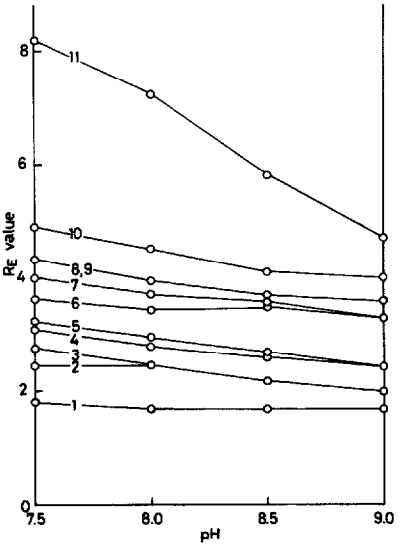
Fig. 3. Isotachopherogram of catechols in system 3. 1 = Chloride; 2 = 2,3-DBA; 3 = hydrogencarbonate; 4 = HHQ; 5 = 3,4-DBA; 6 = 3,4-DBAL; 7 = 2,3-DBAL; 8 = DN; 9 = CC; 10 = PC; 11 = PG; 12 = MC; 13 = borate. Leading electrolyte, 10 m*M* HCl + Tris, 1.25 · 10^{-3}% PVA, pH 7.5. Other conditions as in Fig. 1.

$$C + B \rightleftharpoons CB$$
$$CB + H_2O \rightleftharpoons CBOH^- + H^+$$

where C represents catechols and B represents boric acid. Consequently, the effective mobilities of the catechols become higher than that of the terminating ion. Hydroquinone and resorcinol, which are isomers of pyrocatechol, did not migrate even in this system. It was considered that their borate complexes would have very small stability constants owing to their structures. In operating system 3, only compounds having the catechol structure migrated selectively. Fig. 4 shows the effect of the pH of the leading electrolyte on the R_E values of catechols when boric acid was used as the terminating electrolyte. With increasing pH the effective mobilities became larger but the differences between the effective mobilities became smaller. The operating system adjusted to pH 7.5 was suitable for separating the catechols. The separation of PC and PG, however, could not be achieved in the pH range 7.5–9.0

Behaviour of hydroxyhydroquinone (HHQ)

HHQ has a larger effective mobility than that expected from its structure. The acid dissociation constant of HHQ could not be determined by acid–base titration because the titration curve did not have a well defined end-point. We considered that HHQ was oxidized and the oxide had a high mobility. To confirm this, cyclic voltammetry with HMDE as the working electrode was performed on HQ and HHQ in KH_2PO_4–K_2HPO_4 buffer. Both HQ and HHQ showed the reversible cyclic voltammograms. Fig. 5 shows the relationships between pH and peak potential of the oxidation waves of HQ and HHQ. The slopes for HQ and HHQ were 57 and 86 mV/pH,



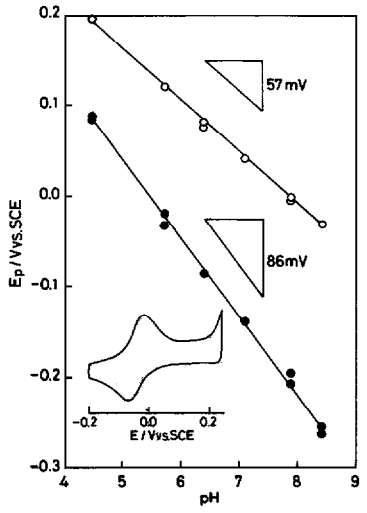
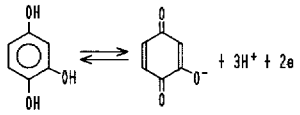


Fig. 4. Effect of pH of the leading electroltyte on R_E values of catechol–borate complexes. 1 = HHQ; 2 = 2,3-DBA; 3 = 3,4-DBA; 4 = 3,4-DBAL; 5 = 2,3-DBAL; 6 = DN; 7 = CC; 8 = PC; 9 = PG; 10 = HC; 11 = borate. Leading electrolyte, 10 m*M* HCl + Tris, $1.25 \cdot 10^{-3}$% PVA. Driving current, 100 μA.

Fig. 5. Relationship between pH and peak potential. ○ = HQ; ● = HHQ. Buffer, 0.1 *M* phosphate.

respectively. It is known that two electrons and two protons participate in the oxidation reaction of HQ. The slope expected theoretically is 59 mV/pH, *i.e.*, in agreement with the experimental value. The slope for HHQ 86 mV/pH, suggested that two electrons and three protons participate in the oxidation reaction of HHQ. That is, the third proton of HHQ is easily dissociated when HHQ is oxidized to form *o*-quinone as follows:



Consequently, the oxidation product of HHQ has a high mobility.

Using boric acid as the terminating electrolyte, nine of the catechols with either large or small mobilities could be separated simultaneously. The utility of complex-forming equilibria between the sample ion and terminating ion was thus confirmed.

REFERENCES

1 W. C. McMurray, *Essentials of Human Metabolism—The Relationship of Biochemistry to Human Physiology and Disease*, Harper & Row, New York, 1983; translated by M. Saito and Y Yojima, *Jintai-no-taisho*, Tokyo Kagaku Dohjin, Tokyo, 1987.
2 T. Ishimitsu and S. Hirose, *Talanta*, 32 (1985) 865.
3 H. Nohta, A. Mitsui, Y. Umegae and Y. Ohkura, *Anal. Sci.*, 2 (1986) 303.
4 H. Satake, Y. Kohri and S. Ikeda, *Nippon Kagaku Kaishi*, (1986) 42.
5 P. Gebauer, P. Boček, M. Deml and J. Janak, *J. Chromatogr.*, 199 (1980) 81.
6 Y. Hirama and H. Yoshida, *J. Chromatogr.*, 322 (1985) 139.
7 S. Tanaka, T. Kaneta and H. Yoshida, *J. Chromatogr.*, 477 (1988) 383.
8 P. Boček, I. Miedziak, M. Deml and J. Janák, *J. Chromatogr.*, 137 (1977) 83.
9 I. Nukatsuka, M. Taga and H. Yoshida, *J. Chromatogr.*, 205 (1981) 95.
10 S. Tanaka, T. Kaneta and H. Yoshida, *Anal. Sci.*, 5 (1989) 217.
11 R.S. Braman, in I.M. Kolthoff and P.J. Elving (Editors), *Treatise on Analytical Chemistry*, Vol. 10, Part II, Wiley, New York, 1975, pp. 37–58.
12 J. Boeseken, *Adv. Carbohydr. Chem.*, 4 (1949) 189.
13 K. W. Fuller and D. H. Northcote, *Biochem. J.*, 64 (1956) 657.
14 R. Piras and E. Cabib, *J. Chromatogr.*, 8 (1962) 63.
15 R. S. Ersser and J. D. Mitchell, *J. Chromatogr.*, 307 (1984) 393.
16 T. Okada and T. Kuwamoto, *Anal. Chem.*, 58 (1986) 1375.