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Short Communication

Capillary tube isotachophoretic separation of catecholamines using cyclodextrin in the leading electrolyte

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ABSTRACT

The isotachophoretic separation of catecholamines based on inclusion complex formation with β-cyclodextrin (β-CD) was investigated. Separability was improved with increasing concentration of β-CD in the leading electrolyte. It was found that a neutral surfactant, added to suppress the electroosmotic flow and to form sharp zone boundaries, affects the resolution. Six catecholamines were separated by using complex formation with β-CD.

INTRODUCTION

Capillary isotachophoresis (CITP) is widely applied in separations of many ionic species. It is important to maximize the differences in the effective mobilities of the sample ions because the separation mechanism is based on the differential mobilities between analytes [1]. The use of complex-forming equilibria can be used to control the effective mobilities of analyte ions in CITP, and the optimum migration system can easily be established so that this technique has broad utility. Typically, a charged ligand or a metal ion is added to the leading electrolyte as a complexing counter ion in this method [2,3]. In recent years, methods utilizing the interaction between sample ions and a neutral ligand in the leading electrolyte have been developed. Especially utilizing cyclodextrin (CD) as the neutral ligand, optical isomers and structural isomers can be separated [4–7]. This technique will be able to improve the separation of many species in CITP.

The separation of catecholamines has been investigated by many techniques, such as high-performance liquid chromatography [8,9], capillary electrophoresis [10–13] and micellar electrokinetic capillary chromatography [14,15]. Although CITP is also an excellent separation technique, the separation of catecholamines by CITP is difficult because of the similarity of their structures and characteristics. We therefore attempted the separation of catecholamines by CITP using β-CD in the leading electrolyte.

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EXPERIMENTAL

Apparatus

A Model IP-3A capillary tube isotachophoretic analyser (Shimadzu, Kyoto, Japan), equipped with a potential gradient detector and a column system (Shimadzu) consisting of a PTFE preseparation capillary (80 × 0.7 mm I.D.) and a fused-silica analytical capillary column (170 × 0.2 mm I.D.), was used. The current was kept constant at 10 μA after migration at 360 μA for 4 min. The capillary tube was filled with the leading and terminating electrolytes using a peristaltic pump.

The R_E value, which is the ratio of the potential gradient of the sample zone to that of the leading zone, is used as an index of effective mobility.

Reagents

Potassium acetate, acetic acid, β-alanine, hydrochloric acid, Triton *X*-100 and poly(vinyl alcohol) (PVA) were of analytical-reagent grade from Wako (Osaka, Japan) and used without further purification, and α-β-cyclodextrins were purchased from Tokyo Kasei Kogyo (Tokyo, Japan).

Stock solutions of catecholamines were prepared by dissolving dopamine hydrochloride, *dl*-normetanephrine hydrochloride, *dl*-metanephrine hydrochloride (Nacalai, Tesque, Kyoto, Japan), 3,4-dihydroxybenzylamine, (+)-norepinephrine (Aldrich, Milwaukee, WI, USA), *dl*-epinephrine and *dl*-isoproterenol hydrochloride (Wako) in water.

Electrolytes

The operating systems used are given in Table I. The leading electrolyte was prepared by diluting a stock solution of 1 *M* potassium acetate and 5% PVA or 10% Triton X-100 and adjusting the pH to 5.0 by adding acetic acid. The terminating electrolyte was prepared by dissolving β-alanine and adjusting the pH to 1.7 by adding hydrochloric acid.

TABLE I

OPERATING SYSTEMS

Parameter	Leading electolyte	Terminating electrolyte
Cation	K^+	β-Alanine
Counter ion	CH_3COO^-	Cl^-
Concentration of cation	5 m*M*	10 m*M*
Additive	β-CD	None
Surfactant	0.05% PVA or 0.10% Triton X-100	None
pH	5.0	1.7

RESULTS AND DISCUSSION

The interaction between CD and catecholamines is a host–guest interaction, the intensity of which depends on the sizes of the CD cavity and of a guest molecule. First, we investigated the effect of adding α-CD. When α-CD was added to the leading electrolyte, no change in the separability of catecholamines was found. The cavity of α-CD accommodates molecules the size of benzene, but the catecholamines investigated here have two hydroxyl groups or one hydroxyl and one methoxy group, and are larger than benzene. We therefore studied the effect of β-CD.

Effect of β-CD on effective mobilities of catecholamines

The effective mobilities of catecholamines were dramatically changed on adding β-CD to the leading electrolyte. Fig. 1 shows the relationship be-

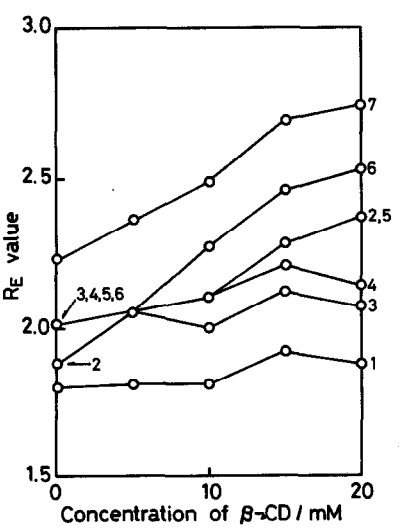

Fig. 1. Effect of β-CD concentration on R_E values of catecholamines. 1 = 3,4-Dihydroxybenzylamine; 2 = dopamine; 3 = normetanephrine; 4 = metanephrine; 5 = norepinephrine; 6 = epinephrine; 7 = isoproterenol.

tween the concentration of β-CD in a leading electrolyte containing 0.05% PVA and the R_E values of catecholamines. The R_E values increase and the effective mobilities decrease with increase in the β-CD concentration. This decrease in the effective mobilities is due to the increase in the size of the migrating species. As catecholamines complexed with β-CD are larger than the free catecholamines, the effective mobilities will decrease. The intensities of interaction of metanephrine and normetanephrine are smaller than those of the others because the decreases in their effective mobilities are very small.

In Fig. 1, the order of the decrease in the effective mobilities of catecholamines (*i.e.*, the order of the association constants of catecholamines with β-CD) is epinephrine > norepinephrine > metanephrine > normetanephrine, which indicates that the cavity of β-CD fits epinephrine and norepinephrine better than metanephrine and normetanephrine. Epinephrine and norepinephrine have two hydroxyl groups, whereas metanephrine and normetanephrine have one hydroxyl and one methoxy group. The structures of the aminoalkyl groups in metanephrine and normetanephrine are the same as those in epinephrine and norepinephrine, respectively. The methoxy group is larger than the hydroxyl group so that metanephrine and normetanephrine will not be able to interact with the β-CD cavity, and also the side of the catecholamines molecule bearing hydroxyl groups interacts with the cavity of β-CD.

Effect of surfactant

In CITP, an electrically neutral surfactant is usually added to the leading electrolyte in order to form a sharp zone boundary. We investigated two surfactants, PVA and Triton X-100. Table II gives the R_E values of catecholamines obtained utilizing two leading electrolytes containing PVA and Triton X-100. Metanephrine and norepinephrine can be separated in PVA solution, whereas they form a mixed zone in Triton X-100 slution. This may be due to phenyl groups existing in Triton X-100. As β-CD interacts with the phenyl groups of Triton X-100, the concentration of the free form CD in the leading electrolyte, that is, the concentration of β-CD interacting with catecholamines, decreases:

$$CD + Triton \rightleftharpoons CD(Triton)$$

TABLE II

EFFECT OF SURFACTANTS ON R_E VALUES OF CATECHOLAMINES

β-CD concentration is 20 m*M*.

Sample	R_E	
	0.05% PVA	0.10% Triton X-100
3,4-Dihydroxybenzylamine	1.88	1.93
Dopamine	2.37	2.30
Normetanephrine	2.07	2.17
Metanephrine	2.14	2.30
Norepinephrine	2.37	2.30
Epinephrine	2.53	2.48
Isoproterenol	2.74	2.72

Further, in the presence of Triton X-100, ligand-exchange equilibria may be achieved as follows:

$$CD(Triton) + CA \rightleftharpoons CD(CA) + Triton$$

where CA is catecholamine. In these instances, because the interaction between β-CD and catecholamines becomes weak, the differences in the effective mobilities of the catecholamines are also small. On the other hand, PVA will hardly interact with β-CD because of its linear structure and catecholamines can be sufficiently complexed with β-CD. As shown in Table II, PVA is preferred to Triton X-100 from the point of view of the separability.

An isotachopherogram of catecholamines using 0.05% PVA as a surfactant in the leading electrolyte is shown in Fig. 2. The concentration of β-CD

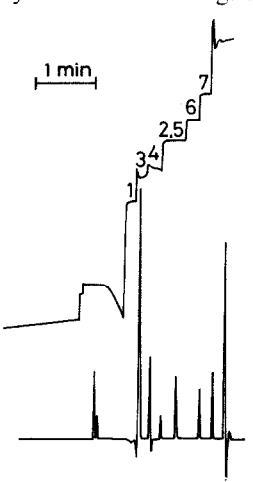

Fig. 2. Isotachopherogram of catecholamines. The β-CD concentration is 20 m*M*. Symbols as in Fig. 1.

is 20 m*M*. Six catecholamines were separated, the order of migration being 3,4-dihydroxybenzylamine > normetanephrine > metanephrine > dopamine = norepinephrine > epinephrine > isoproterenol.

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