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Short Communication

Separation of chiral isomers of *p*-nitrophenyl-2-amino-3-hydroxypropanone by capillary zone electrophoresis using cyclodextrins as chiral selector

Junling Gu, Ruonong Fu*

Department of Chemical Engineering, Beijing Institute of Technology, Beijing 100081, China

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Abstract

Chiral isomers of *p*-nitrophenyl-2-amino-3-hydroxypropanone (NPAP) were separated by capillary zone electrophoresis in a background electrolyte containing β-cyclodextrin as stereospecific selector. In order to improve the resolution, three soluble cellulose derivatives as modifiers of the background electrolyte were studied. The operating conditions of capillary zone electrophoresis, such as concentrations of β-cyclodextrin and Tris, pH of buffer solution and applied voltage, were investigated. Under the optimized condition the NPAP optical isomers could be successfully separated.

1. Introduction

In recent years capillary zone electrophoresis (CZE) has become a powerful technique for the separation of a variety of complex mixtures. Micellar electrokinetic capillary chromatography (MECC) [1], which combines the advantages of HPLC and CZE, permits the separation of neutral compounds. One important area of CZE application is enantiomer separations, owing to the high separation power and the use of some chiral selectors. Cyclodextrins (CDs) and their derivatives have been successfully used in HPLC as stationary phases or mobile phase additives [2] for many years and more recently in capillary GC as stationary phases [3]. There are many examples of the use of CDs and their derivatives as chiral selectors for the separation of optical isomers, such as drugs, amino acids and plant growth regulators [4–8].

p-Nitrophenyl-2-amino-3-hydroxypropanone (NPAP) (Fig. 1) is one of the metabolites of chloramphenicol (CAP) in human blood and may play a significant role in CAP-induced hematotoxicity [9–11]. CAP metabolites have been separated by HPLC but the separation of NPAP optical isomers has not been reported. The L-isomer of NPAP is a raw material for producing L-CAP and therefore the separation of the optical isomers of NPAP is an important task.

In this work, a CZE method was developed for the separation of NPAP optical isomers. The operating conditions are discussed.

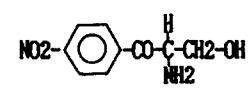

Fig. 1. Structure of NPAP.

* Corresponding author.

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2. Experimental

2.1. Apparatus

The capillary used for electrophoresis was a fused-silica capillary tube of 60 cm × 50 μm I.D. with a 40-cm effective length (Hebei Yongnian Optical Fibre Factory, Hebei, China). The experiments were carried out on a high-performance capillary electrophoresis apparatus obtained from Beijing Institute of New Technology Application, with UV detection at 254 nm.

2.2. Reagents

Standard D- and L-*p*-nitrophenyl-2-amino-3-hydroxypropanone (NPAP) were donated by the Centre of Material Science, Beijing Institute of Technology. β-Cyclodextrin (β-CD) was purchased from Guangdong Yunang Glutamate Factory (Guangdong Yunang, China) and recrystallized several times. Tris(hydroxymethyl)aminomethane (Tris) was obtained from Sichuan Chengdu Chemicals Factory (Sichuan Chengdu, China). Methylcellulose (MC) hydroxypropylmethylcellulose (HPMC), and carboxymethylcellulose (CMC) were provided by the Sichuan Luzhou Chemical Factory (Sichuan Luzhou, China). Citric acid obtained from Beijing Chemicals Factory (Beijing, China) was of analytical-reagent grade. Other chemicals used were of reagent grade. The buffer solution was prepared from citric acid and Tris. The samples were injected by the hydrodynamic or electrodynamic method.

3. Results and discussion

3.1. Effect of CD concentration on the separation of optical isomers

Optical isomers are not easily separated from each other by common methods. CDs are cyclic oligosaccharides consisting of six, seven or eight glucopyranose units corresponding to α-, β- and γ-cyclodextrin. These compounds have been successfully used as chiral separation selectors.

Table 1
Effect of β-CD concentration on the resolution of NPAP optical isomers

Parameter	Isomer	β-CD concentration (m*M*)		
		10	20	30
R_h (%)[a]	–	22.3	59.0	76.1
Migration time (min s)	L	6 03	6 24	6 50
	D	6 08	6 29	6 55

Operating conditions: buffer, 20 m*M* Tris–citric acid; pH, 3.5; applied voltage, 25 kV; current, 12–13 μA.
[a] $R_h = (H - H')/H$, where H and H' are the heights of the lower isomer peak and of the valley between the two peaks, respectively.

In this work, β-CD was used as a chiral selector for the separation of NPAP optical isomers. The separation data are given in Table 1, where it can be seen that the resolution of the optical isomers increases with increasing β-CD concentration. This relationship has been reported by other workers [12]. The optimum concentration of β-CD is 30 m*M*, at which the migration time of isomers is longer than that at lower β-CD concentrations. Because the solubility of β-CD in water is lower, it is difficult to use concentrations of β-CD higher than 40 m*M*.

3.2. Effect of cellulose derivatives on the separation of optical isomers

It is clear that even though β-CD can improve the separation of NPAP optical isomers, baseline resolution was not obtained. Snopek *et al.* [13] used soluble alkylhydroxycellulose derivatives to modify the background electrolytes, improving the separation of chloramphenicol drug optical isomers. Therefore, three cellulose derivatives were added to the buffer in order to improve the separation of NPAP optical isomers (Fig. 2). Of the three cellulose derivatives tried, hydroxypropylmethylcellulose (HPMC) is the best one for modifying the background electrolyte for the separation of NPAP optical isomers. In this instance, complete baseline separation of the optical isomers was achieved, because HPMC in the buffer decreases the electroosmotic flow,

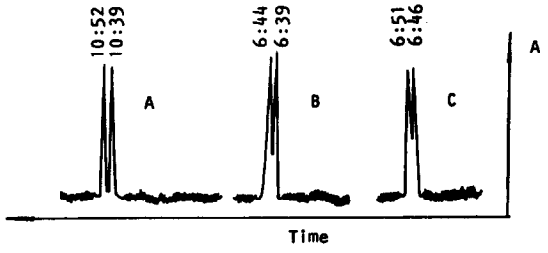

Fig. 2. Influence of the type of methylcellulose on the separation of NPAP optical isomers. Conditions as in Table 1. (A) background electrolyte (BE) + 0.1% HPMC, (B) BE + 0.1% MC, (C) BE + 0.1% CMC. Time at peaks indicated in min:s.

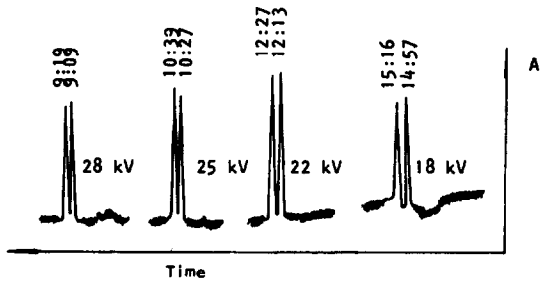

Fig. 3. Influence of applied voltage on the separation of NPAP optical isomers. Conditions as in Fig. 2A. Time at peaks indicated in min:s.

giving more time for the interaction between β-CD and the optical isomers investigated. Fig. 2 shows that the migration time of D-NPAP using HPMC as additive is 10 min 52 s, whereas using the others the migration times are less than 7 min. Table 2 gives the electroosmotic flow-rates (EOF) in 20 m*M* Tris–citric acid and 20 m*M* β-CD solution containing 0.1% cellulose derivatives, using dimethyl sulphoxide (DMSO) as a tracer. The data clearly show that HPMC possesses the highest EOF and CMC the lowest; CMC does not assist the separation.

3.3. Effect of applied voltage on the separation

In general, the higher the applied voltage over the two sides of capillary tube for electrophoresis, the higher is the efficiency obtained. On the other hand, the migration time of a solute is inversely proportional to the applied voltage. For these reasons, under a higher applied voltage the high column efficiency will provide a separation, but it can shorten the migration time, which lowers the resolution, as mentioned above. Therefore, the applied voltage does not notably influence the separation, as shown in Fig. 3.

Table 2
Effect of type of methylcellulose derivatives on u_{EOF} (time over the 60 cm length)

Type of MC derivatives	u_{EOF} (min s)
None	20 54
HPMC	>70
CMC	19 15
MC	30 04

3.4. Effect of acidity on the separation

In CZE, pH is an important parameter for the separation, with a large influence on the EOF and the migration times of solutes. Fig. 4 illustrates the influence of the pH of the background electrolyte on the separation of NPAP optical isomers. It shows that at pH 3.5–6 the separation of isomers is perfect. In this pH range the EOF is lower and NPAP possesses a positive charge.

3.5. Effect of ionic strength on the separation

In order to test the influence of the ionic strength of the buffer solution on the separation,

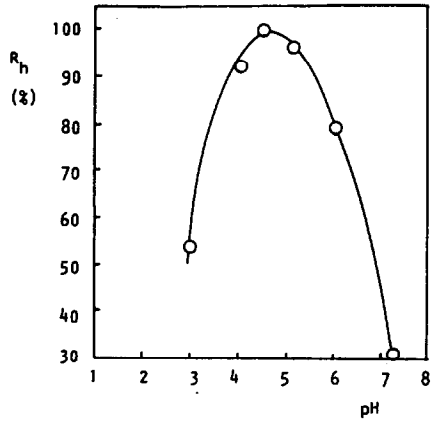

Fig. 4. Influence of pH of background electrolyte on the separation of NPAP optical isomers. Conditions as in Fig. 2A, except pH of buffer.

Table 3
Effect of ionic strength of buffer on the separation of NPAP optical isomers

Parameter	Ionic strength (m*M* Tris)			
	10	20	30	40
R_h (%)[a]	81.5	100	100	100
Migration time (min)	10.17 10.28	10.22 10.38	10.40 10.62	10.18 10.37

[a] See Table 1.

concentrations of 10–40 m*M* Tris were added to the background electrolytes. The separation of NPAP optical isomers is depicted in Table 3. The results clearly show that with concentrations of Tris above 20 m*M* a perfect separation can be achieved.

4. Conclusions

Chiral isomers of *p*-nitrophenyl-2-amino-3-hydroxypropanone (NPAP) can be separated by CZE. The background electrolyte contains β-CD as a stereospecific selector and hydropropylmethylcellulose as an electroosmotic flow reducer. The optimized operating conditions are as follows: background electrolyte containing 0.2–0.4 m*M* Tris, 0.2 m*M* citric acid, 0.2–0.3 m*M* β-CD and 0.1% HPMC; applied voltage, *ca.* 25 kV; and pH, 3.5–6.

5. Acknowledgement

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