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Short communication

Direct chiral resolution of pantothenic acid using 2-hydroxypropyl-β-cyclodextrin in capillary electrophoresis

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Abstract

Chiral resolution of native DL-pantothenic acid was performed by capillary electrophoresis using 2-hydroxypropyl-β-cyclodextrin as a chiral selector. Various factors affecting chiral resolution and migration time of pantothenic acid were studied. The optimum running conditions for separation of pantothenic acid were found to be 60 m*M* phosphate buffer (pH 7.0) containing 60 m*M* 2-hydroxypropyl-β-cyclodextrin and 10% (v/v) methanol with an effective voltage of 20 kV at 15°C, using direct detection at 200 nm. With this system, pantothenic acid in a soft drink was analyzed successfully.

Keywords: Enantiomer separation; Soft drinks; Food analysis; Pantothenic acid; Vitamins

1. Introduction

Pantothenic acid [$HOCH_2–C(CH_3)_2–CH(OH)–CO–NH–CH_2–CH_2–COOH$] is a member of the B complex vitamins. Several methods have been reported for the determination of pantothenic acid, using microbiological techniques [1], colorimetry [2], and high-performance liquid chromatography (HPLC) [3]. It is well known that D-(+)-pantothenic acid is a precursor of the biologically important coenzyme A, but the L-(−) isomer is inactive. Recently, optical resolution of pantothenic acid by HPLC has been reported [4–6]. Capillary electrophoresis (CE) is a recently developed powerful analytical technique with a wide range of applications. The availability of many chiral selectors makes CE an important tool for chiral analysis. As described in several reviews [7–10], cyclodextrins (CDs) and their derivatives have been widely applied in CE for the separation of enantiomers of many compounds. Most compounds described in these reviews have aromatic rings. In the inclusion complexation mechanism, the hydrophobic interaction between the cavity of the native or derivatized CDs and the hydrophobic part, such as an aromatic ring, of the compounds plays an important role in the stereoselective interaction. On the other hand, little has been reported on the chiral resolution of hydrophilic-group rich compounds that have no aromatic rings, such as pantothenic acid, by CE techniques without derivatization.

Of several CDs that were examined for their ability to separate racemic pantothenic acid by CE, only one, 2-hydroxypropyl-β-CD (2HP-β-CD), was found to be effective. Various parameters influencing the resolution and the migration time of pantothenic

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acid were investigated. Chiral analysis of pantothenic acid in a soft drink was also studied.

2. Experimental

2.1. Chemicals

Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin and 2-HP-β-CD (average degree of substitution: 7) were obtained from Sigma (St. Louis, MO, USA). α-CD, β-CD, γ-CD, 2,3,6-tri-*O*-methyl-β-cyclodextrin, calcium (+)-pantothenate, and other chemicals (guaranteed grade) were purchased from Wako (Osaka, Japan). DL-Pantothenic acid calcium salt was purchased from Tokyo Kasei (Toyko, Japan).

2.2. Apparatus for CE

Electrophoretic experiments were carried out using a HP^{3D} capillary electrophoresis system (Hewlett-Packard, Palo Alto, CA, USA). The injection of the samples was done by pressure (50 hPa, for 4 s). The separations were performed in a fused-silica bubble cell capillary of 56 cm×75 μm I.D. (Hewlett-Packard). The capillary was kept at 20°C. The analytes were detected at 200 nm. The power supply was operated in the constant-voltage mode, at 20 kV, and the substances migrated towards the negative pole.

2.3. Buffer and sample preparation

The background electrolyte (BGE) in the electrophoretic experiments, unless stated otherwise, was 60 m*M* phosphate buffer (pH 7.0) containing 60 m*M* 2HP-β-CD was filtered with a 0.22-μm filter before use.

Deionized water was prepared using a Yamato Auto Still Model WA-52G (Tokyo, Japan).

Stock solutions of 100 m*M* DL-pantothenic acid and 100 m*M* D-pantothenic acid were prepared in deionized water, stocked at 4°C and diluted to 0.2 m*M* and 0.1 m*M*, respectively, before use.

A soft drink containing pantothenic acid was decarbonized in an ultrasonic bath, diluted threefold with deionized water and then analyzed by CE.

2.4. Calculation of resolution

The resolution (R_s) of the enantiomer was calculated by using the following equation:

$$R_s = 2(t_2 - t_1)/(w_2 + w_1) \quad (1)$$

where t is the migration time, and w is the width of the peak at the baseline.

3. Results and discussion

3.1. Factors affecting chiral separation

3.1.1. Cyclodextrin type and concentration

DL-Pantothenic acid was analyzed by CE using a BGE containing separately 50 m*M* α-CD, γ-CD, 2,6-di-*O*-methyl-β-CD, 2,3,6-tri-*O*-methyl-β-CD or 2HP-β-CD. In the case of β-CD, the above analysis was carried out at the concentration of 15 m*M* owing to its low solubility. Of these CDs, only 2HP-β-CD was found to be effective for the resolution of pantothenate enantiomers. In the BGE at pH 7, the carboxyl group of pantothenic acid is dissociated and thus the analyte electrophoretically migrates as an anion, i.e., away from the detector. However, the electroosmotic flow (EOF) was sufficient to reverse the direction of the apparent mobility. By the analysis of the mixture of DL- and D-pantothenic acids, it was found that the D isomer moved faster than the L isomer. Thus, it was indicated that D-pantothenic acid formed a stronger diastereomer complex with 2HP-β-CD than did the L isomer, because neutral 2HP-β-CD migrates faster than anionic DL-pantothenic acid. The average molar substitution of the 2HP-β-CD that was used was seven. The degree of the substitution has been reported to significantly influence the enantiomers [11]. Therefore, different degrees of substitution of 2HP-β-CD may result in different resolution.

The effect of 2HP-β-CD concentration on the resolution and the migration time of pantothenic acid was studied (Fig. 1). It was reported that high CD concentrations resulted in dimerization of CDs, leading to a loss of selectivity and shorter migration times [12]. The resolution and the migration time,

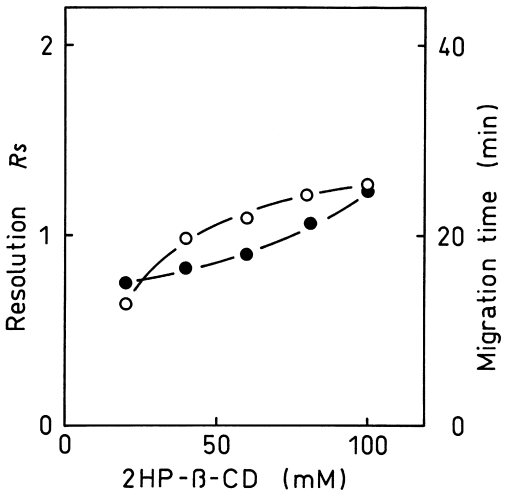

Fig. 1. Effect of 2HP-β-CD concentration on the enantiomeric resolution and migration time of pantothenic acid. Racemic pantothenic acid (0.2 m*M*) was analyzed by CE. The BGE was composed of various concentrations of 2HP-β-CD containing 60 m*M* phosphate buffer (pH 7.0). (○) Resolution (R_s), (●) migration time of D-pantothenic acid.

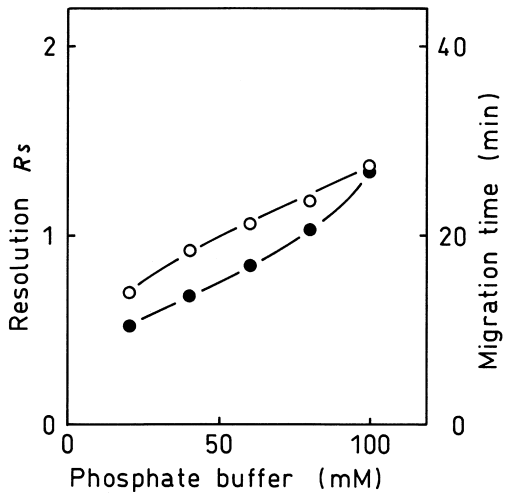

Fig. 2. Effect of phosphate buffer concentration on the enantiomeric resolution and migration time of pantothenic acid. Racemic pantothenic acid (0.2 m*M*) was analyzed by CE. The BGE was composed of various concentrations of phospate buffer (pH 7.0) containing 60 m*M* 2HP-β-CD. (○) Resolution (R_s), (●) migration time of D-pantothenic acid.

however, increased with increasing amounts of 2HP-β-CD.

3.1.2. Temperature

The effects of temperature on selectivity has been observed in CE as well as in other types of chromatography. Thus, the effect of capillary temperature on the resolution and the migration time of pantothenic acid was studied. In the present study, it was found that a lower capillary temperature caused increases in both the resolution and the migration time. According to Heuermann and Blaschke [12], the increase in the R_s value with a decrease in temperature might be explained by a decrease in rotational and/or vibrational energy, increasing the fixation of the enantiomers inside or at the rim of CD and thus, increasing the enantioselectivity.

3.1.3. Buffer conditions

An increase in the concentration of phosphate buffer brought about increases in the resolution and the migration time of pantothenic acid (Fig. 2). Fig. 3 shows the effect of the pH of the BGE on the resolution and the migration time of pantothenic acid. In this range of pH 5.5–8.0, the carboxyl group

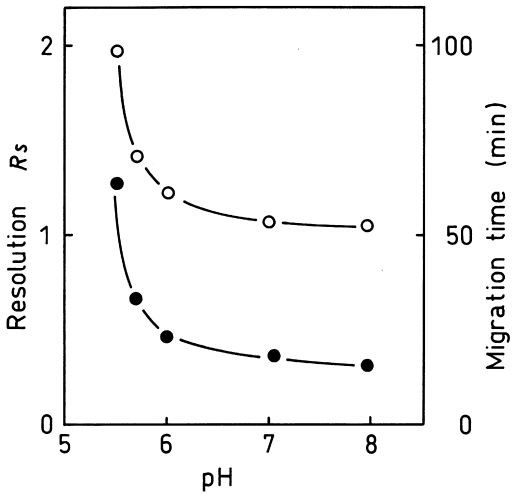

Fig. 3. Effect of pH of buffer solution on the enantiomeric resolution and migration time of pantothenic acid. Racemic pantothenic acid (0.2 m*M*) was analyzed by CE. The BGE was composed of 60 m*M* of phospate buffer (pH 5.5–8.0) containing 60 m*M* 2HP-β-CD. (○) Resolution (R_s), (●) migration time of D-pantothenic acid.

of the analyte is dissociated. Both of the resolution and the migration time increased significantly as the pH decreased from 6.0 to 5.5. At pH 5.0 it was not possible to detect pantothenic acid, because pantothenic acid was moving too slowly. It appears that the longer migration time at pH lower than 6 reduces the EOF and this increase of enantioselectivity is ascribed to the longer time spent by the analytes into the CD cavity. It has been reported that organic acids have to be in the ionic form in order for their enantiomers to be separated with uncharged CDs [11]. We tried to resolve the enantiomers of pantothenic acid by CE at pH 3.0, but were unable to do so. Therefore, it could be suggested that the dissociation of carboxyl group of pantothenic acid played an important role in its chiral resolution in this CE system.

3.1.4. Addition of methanol

It has been reported that the addition of an organic solvent to the BGE can affect the inclusion-complex formation constants, resulting in either enhancement or degradation of the separation depending on the solvent [13]. The effect of the addition of methanol to the BGE (0, 5, 10 and 20%, v/v) was studied. The resolution and migration time of the enantiomers increased with increasing methanol concentration. Furthermore, it was found that the addition of methanol caused a decrease in the baseline noise in this CE system.

The above results suggested that the analytical conditions that resulted in longer migration times gave higher resolution. Therefore, the optimum BGE conditions for the higher resolution and the shorter migration time were found to be 60 m*M* 2HP-β-CD in 60 m*M* phosphate buffer (pH 7.0) containing 10% methanol with an effective voltage of 20 kV at 15°C. Fig. 4A shows the electropherogram of DL-pantothenic acid analyzed under the above conditions. The resolution (R_s) was 1.18.

3.2. Analysis of DL-pantothenic acid

Racemic pantothenic acid (6–600 μ*M*) was applied to the CE system using the above optimum conditions. Linearity ($r > 0.9999$) was demonstrated in the range 3–300 μ*M* by standard curves of each D- and L-pantothenic acid. The precision of five consecutive determinations was evaluated at 200 μ*M* for racemic pantothenic acid. High reproducibilities of peak areas (R.S.D. 0.7%) and migration times (R.S.D. 1.2%) of both D and L isomers were obtained. In addition, the resolution was also high reproducible (R.S.D. 0.3%). D-Pantothenic acid and racemic mixture of pantothenic acid were mixed at various ratios and the mixture was analyzed by the CE system. It was found that the L isomer could be as small as 3% in total pantothenic acid and still be detected.

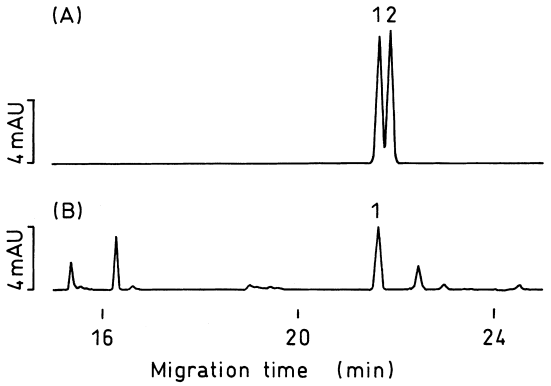

Fig. 4. Electropherogram of racemic pantothenic acid (A) and a soft drink (B). 1=D-Pantothenic acid, 2=L-pantothenic acid.

The CE analysis of a soft drink showed the presence of only D-pantothenic acid (Fig. 4B). The analyzed value (131 μ*M*) of D-pantothenic acid in the sample was almost the same as its indicated value. Racemic pantothenic acid (80 μ*M*) was added to the sample. The recoveries of D- and L-pantothenic acid were 100.1% and 100.4%, respectively.

4. Conclusions

We analyzed racemic pantothenic acid by CE using several CDs and found that the chiral resolution of pantothenate enantiomers was achieved by using only 2HP-β-CD. It was shown that the analytical conditions that resulted in longer migration times gave higher resolution. Generally, it seems that this increase in enantioselectivity can be ascribed to the longer time spent by the analytes in the CD cavity. This CE method was found to be very

reproducible and useful for quantitative chiral analysis of soft drinks containing pantothenic acid.

There are many reports on the direct chiral resolution of various compounds, having one or more aromatic rings, by CE using CDs and their derivatives. Using this CE method with 2HP-β-CD, however, the chiral separation of DL-pantothenic acid without its derivatization was successfully achieved. Therefore, the direct chiral resolution of various organic acid having no aromatic rings may become possible with CE using native and derivatized CDs.

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