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Short communication

Enantioselective separation of phenylglycidates by capillary electrophoresis employing sulfated β-cyclodextrin as chiral selector

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Abstract

A capillary electrophoresis (CE) method for the enantioseparation of phenylglycidates has been developed. Successful enantioseparation was achieved using sulfated β-cyclodextrin as chiral selector in a phosphate buffer. The effects of varying pH, sulfated β-cyclodextrin concentration and electrophoresis voltage were systematically investigated and the optimized separation conditions were thus obtained. When the migration time was set at the threshold value, it was found that the best enantioseparation was obtained at 10 kV with 3% (w/v) sulfated β-cyclodextrin at pH 6.5. A range of substituted phenylglycidates were successfully separated using the method and the results shown to be superior to those obtained using gas chromatography (GC).

Keywords: Enantioseparation; Sulfated β-cyclodextrin; Phenylglycidates

1. Introduction

Optically active phenylglycidates, which serve as the chiral building blocks of diltiazem [1] and taxol [2], have attracted considerable attention in the past decade. Development of effective techniques for the separation of their enantiomers is therefore of significant interest and the use of high performance liquid chromatography (HPLC) [3] and gas chromatography (GC) methods [4] has previously been reported in the literature. Although capillary electrophoresis (CE) has been shown to be an effective technique for enantioseparation of a range of biochemically important species [5–14], there have been no reports of the use of this method for the separation of phenylglycidates. In CE, cyclodextrins (CDs) are among the most commonly used chiral selectors [5], in addition to proteins [6], crown ethers [7] and macrocyclic antibiotics [8–12]. Derivatized CDs such as dimethyl-β-cyclodextrin or sulfated β-cyclodextrin are usually employed, since they have higher solubility in aqueous solutions than native CDs [5]. Recently, several reports of the use of charged derivatives such as sulfated, sulfobutylether, glutamated and carboxymethylated CDs as chiral selectors have been published. These CDs have been successfully employed in the enantioseparation of both basic and acidic chiral compounds such as hydrobenzoin, propranolol [13] and warfarin [14]. The most significant potential applications of such charged CDs are in the enantioseparation of neutral compounds. In previous work, we successfully developed a method to separate enantiomers of some epoxides in low pH buffer using sulfated β-cyclodextrin as the chiral selector [15]. Although phenylglycidates are epoxides themselves, it was found that the method could not be used for practical separation of their enantiomers because of the long migration times involved. In this paper, we describe an alternative method for the efficient enantioseparation of phenylglycidates.

2. Experimental

2.1. Chemicals

Sulfated β-cyclodextrin (average degree of substitution, 14) was purchased from Sigma–Aldrich Corporation (USA). Phosphoric acid and triethanolamine were purchased from Merck & Co. Inc. (USA). All phenylglycidates were synthesized [16] in our laboratory and their structures were confirmed by high

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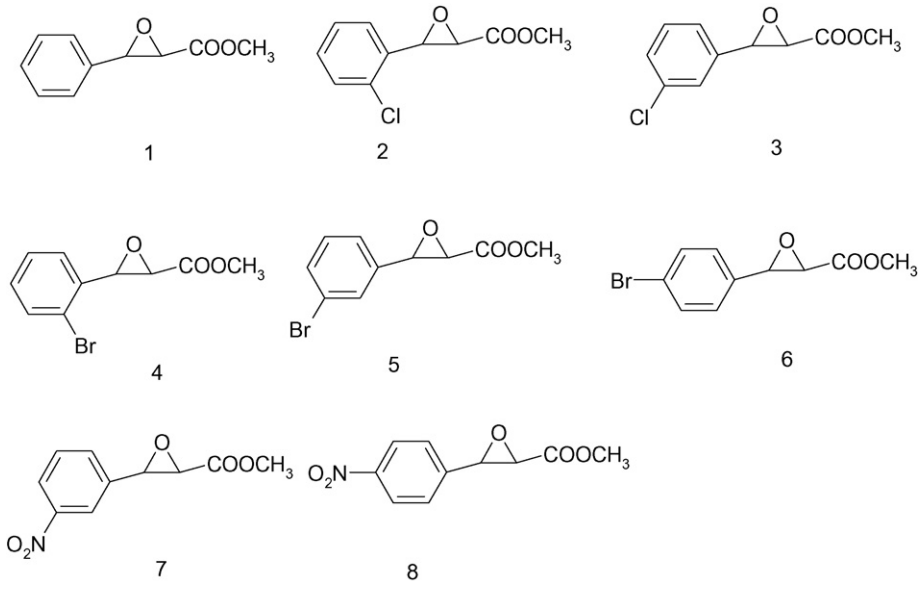

Fig. 1. Structure of phenylglycidates separated in this study.

resolution ^{1}H nuclear magnetic resonance spectroscopy which showed that all of the samples were *trans* isomers.

2.2. Instruments and electrophoresis procedure

CE was performed using a P/ACE MDQ capillary electrophoresis instrument (Beckman, Fullerton CA, USA) equipped with an online UV detector. The silica capillary tube (length 37 cm, effective length 30 cm, I.D. 75 μm) was purchased from Yongnian Optical Fiber Company (Hebei, China). The capillary was successively flushed with 0.1 mol/L NaOH for 2 min, and then with double deionized water for 2 min, followed by running of the buffer for 2 min. Samples were dissolved in methanol with concentration of 0.1 mg/mL and introduced hydrodynamically into the sample injection port at a rate of 0.1 psi for 1 s. The capillary temperature was set at 15 °C and analytes were detected by UV absorption at 214 nm.

An HP 5890 instrument (Hewlett-Packard, USA) with FID detector, equipped with a 10 m Chiraldex G-TA type column (Advanced Separation Ltd., USA) and nitrogen used as carrier gas was employed in chiral GC separation. Parameters were set as follows: column temperature 105 °C; injection temperature, 160 °C; detector temperature, 220 °C; flow rate, 1 mL/min.

2.3. Data acquisition

P/ACE system MDQ software Version 1.5 was used for data acquisition together with an HP3365 series II Chemstation Version A.03.21.

3. Results and discussion

In order to optimize the enantioseparation of phenylgycidate (compound 1 in Fig. 1), the effects of varying key experimental parameters were investigated. The value of pH was varied over the range 2.5–8.0 using phosphate-trihydroxyethylamine buffers (pH 2.5–5.5) and phosphate buffers (pH 6.0–8.0) as background electrolyte (BGE). As shown in Fig. 2, the best enantioseparation was obtained at low pH, with a maximum value of resolution (Rs 7.9) at pH 2.5 with 9 mmol/L added triethanolmine. At low pH however, the observed migration times (almost 30 min per analysis) were too long to be practical for handling a large number of samples. By taking into account both the migration time and the Rs value, an intermediate value of pH 6.5 was chosen for further study.

The effect of varying the concentration of sulfated β-cyclodextrin on enantioselectivity was investigated over the range 0.1–5.0% (w/v). As shown in Fig. 3, the enantioseparation was enhanced by increasing the concentration of sulfated β-cyclodextrin; the migration time was also extended, however

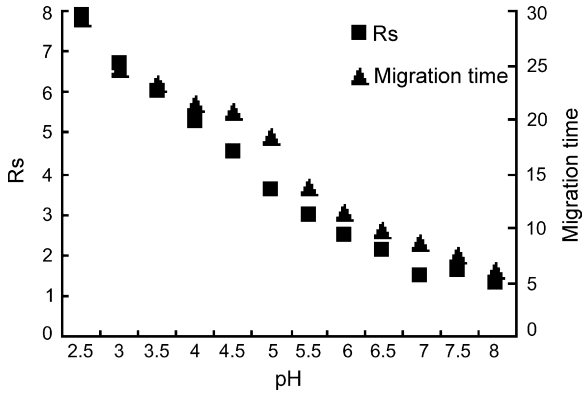

Fig. 2. Influence of pH value of buffer phosphate-trihydroxyethylamine buffer was used as BGE when pH value was adjusted from pH 2.5 to pH 5.5 (10 mmol/L phosphate 9 mmol/L trihydroxyethylamine), while the 10 mmol/L phosphate buffer was used as BGE when pH value was adjusted from pH 6.0 to pH 8, sulfated β-cyclodextrin 3% (w/v); voltage −10 kV (phosphate-trihydroxyethylamine buffer); +10 kV (phosphate buffer).

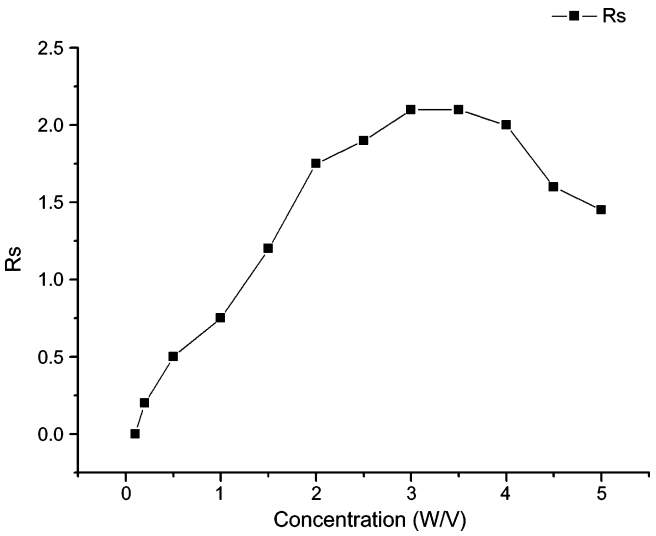

Fig. 3. Influence of concentration of sulfated β-cyclodextrin (pH 6.5, 10 mmol/L phosphate buffer, +10 kV) on enantioseparation.

(data not shown). In addition, sulfated β-cyclodextrin can induce high currents that give rise to excessive Joule heating which leads to a decrease in the Rs value, and the voltage had to be adjusted when larger concentrations of sulfated β-cyclodextrin were added to the BGE. It was therefore found necessary to optimize the voltage for each value of the concentration of sulfated β-cyclodextrin.

Overall, using both the Rs value of phenylglycidate enantiomers and the migration time of the first peak as criteria, it was concluded that when the migration time was set at the threshold value, the optimum enantioseparation was obtained at 10 kV with 3% (w/v) sulfated β-cyclodextrin (see Fig. 4).

The above method was used to separate a range of substituted phenylglycidates (see Fig. 1). Most of them could be baseline separated without adjustment of the analysis parameters (Table 1). The extent enantioseparation did not show any significant correlation with the position of the subsistent.

The above results compare favorably with those obtained by GC analysis as shown in Table 1. In the GC method, the observed

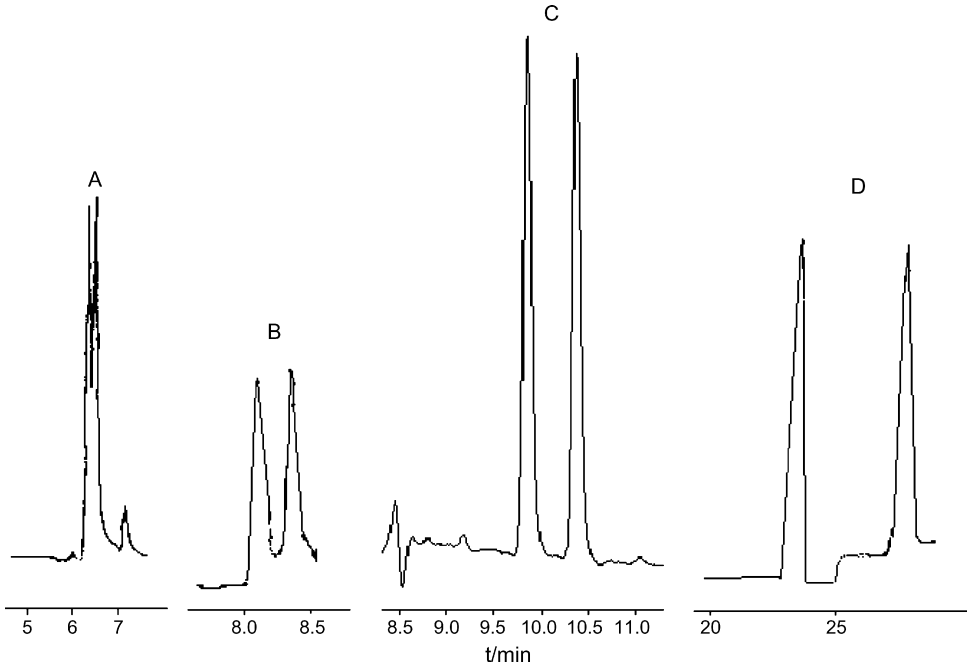

Fig. 4. Electrophoresis of methyl phenylglycidate when electrolytes consisted of 3% (w/v) sulfated β-cyclodextrin in pH 6.5 10 mmol/L phosphate buffer at +15 kV (A); in pH 7.0 10 mmol/L phosphate buffer at +10 kV (B); in pH 6.5 10 mmol/L phosphate buffer at +10 kV (C); in pH 2.5 10 mmol/L phosphate-trihydroxyethylamine buffer at −10 kV (D).

Table 1
Enantioseparation of phenylglycidates

Compound	CE analysis		GC analysis	
	Rs	Unit of migration time (min)[a]	Rs	Unit of migration time (min)[a]
1	2.17	9.8	1.70	9.9
2	1.99	15.7	1.20	21.8
3	1.90	12.7	1.60	21.6
4	2.90	21.4	2.00	77.3
5	2.01	17.0	1.70	70.7
6	2.30	25.1	1.40	27.7
7	1.99	18.7	1.43	80.2
8	3.60	23.3	1.5	50.3

[a] The first migrated peak of enanotimers.

Rs values of the substituted phenylglycidates ranged from 1.2 to 2.0, and most of the enantiomers were not baseline separated. Furthermore most migration times exceeded 25 min and times of up to 70 min were observed.

4. Conclusion

A CE method for the separation of phenylglycidate enantiomers using sulfated β-cyclodextrin as the chiral selector has been developed. Under optimal conditions, phenylglycidates and a variety of its substituted derivatives can be baseline separated. The method is more effective than previously reported GC methods.

Acknowledgments

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