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Migration order reversal of enantiomers in capillary electrophoretic separation

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Abstract

Optical resolution of (*R,S*)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]methylpiperidine (E2020) was achieved by cyclodextrin-modified capillary zone electrophoresis (CD-CZE) and cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC). We intend to use the CD-MEKC mode to reverse the migration order of an enantiomer in the CD-CZE mode. For the (*S*)-enantiomer sample, including a varying amount of the (*R*)-enantiomer, the limit of quantitation (LOQ) was 3.0% in the CD-CZE mode, where the (*S*)-enantiomer migrated faster than the (*R*)-enantiomer, while it was 0.8% in the CD-MEKC mode, where the migration order of these enantiomers was reversed. It is quite useful to choose these two modes properly in order to obtain the precise optical purity. This technique is expected to be applied to optical purity tests of other chiral pharmaceuticals.

Keywords: Enantiomer separation; Enzyme inhibitors; Cyclodextrins

1. Introduction

Recently, unfavorable enantiomers have been regarded as impurities, because a large number of racemic pharmaceutical substances have different biological activities within the enantiomers. It is quite dangerous to administer the racemic mixtures without separating these enantiomers, because some enantiomers are toxicoids. Hence, it is indispensable to develop optical resolution techniques for pharmaceutical research.

An acetylcholine esterase inhibitor, (*R,S*)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]methylpiperidine hydrochloride (E2020) was developed as a novel cure for Alzheimer's disease [1]. The chemical structure of E2020 is shown in Fig. 1. Optical resolution of E2020 was achieved by HPLC methods with protein bonded columns, using avidin [2] or ovomucoid [3]. However, protein bonded columns are expensive and have low numbers of theoretical plates.

In contrast, optical resolution by capillary electrophoresis (CE) has been developed, with its ultra-high resolution (100 000–10 000 000 theoretical plates), high speed analysis and nano-scale sample volumes. In CE, cyclodextrins (CD) and their derivatives [4–6], ovomucoid [7], avidin [8], crown ethers [9,10], macrocyclic antibiotics [11,12], bile salt [13], saponin [14], polysaccharides [15], etc., have been used as chiral selectors. In this study, 2,6-di-O-methyl-β-cyclodextrin (DMCD) was used as a chiral selector for E2020.

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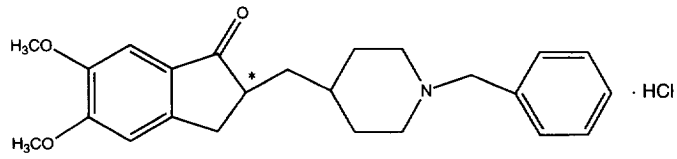

Fig. 1. Chemical structure of E2020.

For the enantiomeric separations, it is necessary to detect a minor component before the major component, in cases where the migration times of these components are not very different. This is because the basic compounds tend to exhibit tailing, due to the interaction between positively charged analytes and the negatively charged silica wall of a capillary [16]. Therefore, it is worth establishing techniques to control the migration order of the enantiomers for optical purity tests.

In CE, several possible principles of migration order reversal have been proposed or discussed: (i) Suppress or reverse the direction of electroosmotic flow by using a coated capillary, (ii) use the chiral selectors that have the opposite chiral recognition ability [17,18], (iii) change the mobility or the direction of the chiral selectors by using the modified chiral selector [19–21], (iv) change the pH and the concentration of the chiral selector, based on the theory of ionoselective and duoselective separations [22–25], (v) reverse the electrophoretic mobility of the analytes themselves [26].

In this study, we intend to reverse the migration order of the enantiomers using principle v. So far, principle v has been applied only to zwitterionic compounds. We developed a method to control the electrophoretic mobility of the analytes using sodium dodecyl sulfate (SDS). The migration order of the enantiomers was reversed when the cationic analyte E2020 was included in the SDS micelle. This simple and convenient method can easily be applied to optical purity tests for pharmaceutical research.

2. Experimental

2.1. Apparatus

CE was performed with a Beckman P/ACE System 2100 (Beckman Instruments, Fullerton, CA, USA). Detection was performed by measuring the UV absorbance at 214 nm. An uncoated fused-silica capillary (J&W Scientific, Folsom, CA, USA) of 36.2 cm (29.5 cm effective length)×50 μm I.D. was used for optimization of the concentration of DMCD in CD-CZE and CD-MEKC. A coated DB-1 capillary (J&W Scientific) of 47.0 cm (40.0 cm effective length)×50 μm I.D. and an uncoated fused-silica capillary (J&W Scientific) of 46.8 cm (40.0 cm effective length)×50 μm I.D. were used to obtain baseline separation in CD-CZE and CD-MEKC, respectively. The capillaries were thermostatted at 25°C using a liquid coolant. The applied voltages were 15 kV for CD optimization, 5 kV for the measurement of the critical micelle concentration (CMC) and 13 kV for the validation of the optical purity test.

2.2. Reagents and materials

The (*R*)-, (*S*)- and (*R,S*)-forms of E2020 were synthesized at Eisai Chemical (Hasaki, Ibaraki, Japan). DMCD, from Nacalai tesque (Kyoto, Japan), was used as a chiral selector. Sodium dihydrogenphosphate dihydrate, phosphoric acid and 3,6-bis-(dimethylamino)-10-dodecylacridinium bromide (AO-10-DB) were from Wako (Osaka, Japan). SDS was from Sigma (St. Louis, MO, USA).

2.3. Procedure

A phosphate buffer (pH 3.0) was prepared by mixing 50 m*M* sodium dihydrogenphosphate dihydrate and 50 m*M* phosphoric acid. All buffer solutions were passed through a 0.45-μm membrane filter before analyses. Capillaries were washed with water and the employed buffer (3 min each) after every run. The sample was dissolved in 10 m*M* phosphate buffer (pH 3.0) at a concentration of 0.1 mg/ml and was injected for 2 s using pressure (0.5

p.s.i.; 1 p.s.i.=6894.76 Pa). Analyses were performed in triplicate. The average mobility difference between the enantiomers was calculated from the mobility difference in each analysis. The CMC was determined from the current value of various SDS concentrations. The limits of quantitation (LOQs) were obtained using the accuracy ranges from 85 to 115%.

3. Results and discussion

3.1. Reversal of the migration order using SDS

At first, the migration order of the enantiomers was confirmed in both modes by injection of (*R*)-rich samples. The conditions were exactly the same between the two modes, except for the addition of 40 m*M* SDS in the CD-MEKC mode. The electropherograms of these separations are shown in Fig. 2. The (*S*)-enantiomer migrated before the (*R*)-enantiomer in CD-CZE, whereas the (*R*)-enantiomer migrated before the (*S*)-enantiomer in CD-MEKC.

This phenomenon could be explained from the theory based on the following equation [27]

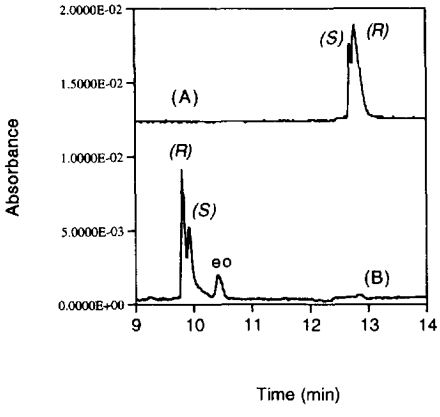

Fig. 2. Optical resolution of an (*R*)-rich sample in (A) CD-CZE and (B) CD-MEKC. Conditions: capillary, uncoated, 36.2 cm (29.5 cm to the detector)×50 μm I.D.; 50 m*M* phosphate buffer (pH 3.0) containing 65 m*M* DMCD for CD-CZE and with the further addition of 40 m*M* SDS for CD-MEKC; applied voltage, 15 kV. A mixture containing 0.1 mg/ml (*R*)-E2020 and 0.05 mg/ml (*S*)-E2020 was injected.

$$\Delta\mu_e = \mu_S - \mu_R = \frac{[C](\mu_f - \mu_c)(K_R - K_S)}{1 + [C](K_S + K_R) + K_S K_R [C]^2} \quad (1)$$

where [C] is the CD concentration, μ_f is the electrophoretic mobility of the analyte in free solution, μ_c is the electrophoretic mobility of the analyte–chiral selector complex and K_S and K_R are binding constants. The migration order was reversed because $\mu_f > 0$ was changed to $\mu_f < 0$ by the addition of the SDS micelle. To confirm the change in μ_f, MEKC was performed using the buffer containing 40 m*M* SDS without DMCD. AO-10-DB [28] was used as a micelle tracer. The result was that the tracer peak overlapped with the analyte peak. It can be said from this result that analytes in the aqueous phase were mostly included in the micellar phase in CD-MEKC. The analyte–CD complex migrates faster than the analyte–micelle complex in CD-MEKC, whereas the analyte–CD complex migrates more slowly than the free analyte in aqueous phase in CD-CZE.

Fig. 3 shows the $\Delta\mu_e$ as a function of the DMCD concentration and of the SDS concentration. When more than 10 m*M* SDS was added to the buffer containing 60 or 90 m*M* DMCD, $\Delta\mu_e > 0$ was converted to $\Delta\mu_e < 0$. The value of $\Delta\mu_e$ decreased up to 40 m*M* and finally increased to zero again, upon further addition. The maximum $|\Delta\mu_e|$ in CD-MEKC was larger than the maximum $|\Delta\mu_e|$ in CD-CZE.

3.2. The interaction between the CD and SDS

It may be considered that K_S and K_R as well as μ_f could be affected by SDS. Nishi and Fukuyama [29] reported that the monomeric surfactant can be included by the CD because of the lipophilic hydrocarbon chain [29]. In order to evaluate the interaction between SDS and CD, the relationship between the SDS concentration, DMCD concentration and the current value was studied (Fig. 4). In a curve without the addition of DMCD, a slope changing point was observed in 2–3 m*M* SDS and this region is considered to be the CMC. On the other hand, two slope changing points were observed at 2–3 and 5–7 m*M* SDS for curves obtained on addition of DMCD. The interactions between analytes and DMCD found to be more complicated in the presence of SDS. It is

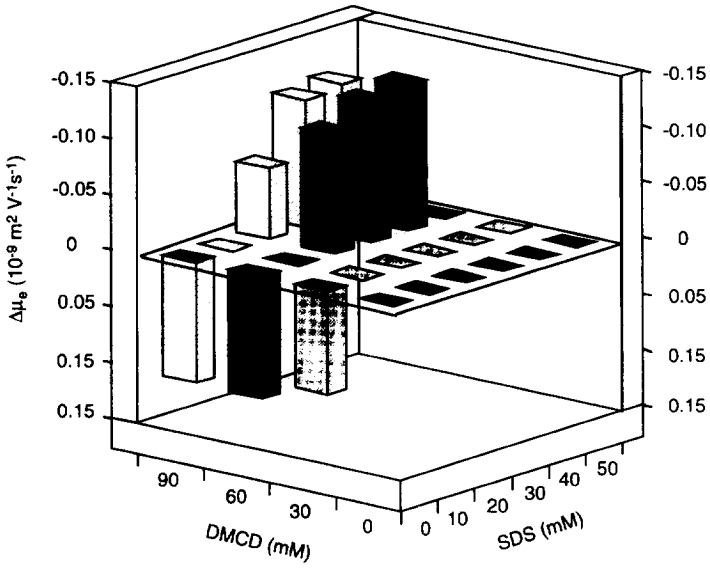

Fig. 3. Three-dimensional graph of $\Delta\mu_e$ as a function of the concentrations of DMCD and SDS. Conditions were as described in the legend to Fig. 2.

suggested that reciprocal action between the analytes, CD and SDS increased $|\mu_f - \mu_c|$ and/or $|K_R - K_S|$ and, as a result, increased $|\Delta\mu_e|$ in CD-MEKC.

The quantitative evaluation of the change in binding constant in CD-MEKC could not be discussed from these preliminary results. However, these results show the effectiveness of CD-MEKC, not only in reversing the migration order of the enantiomers, but also in increasing $\Delta\mu_e$.

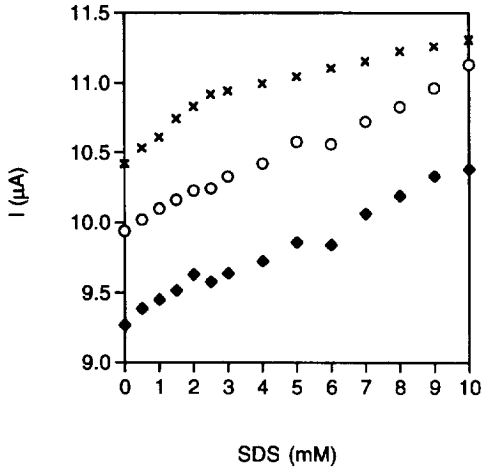

Fig. 4. Measurement of the CMC at various CD concentrations. Conditions: capillary, uncoated, 36.2 cm (29.5 cm to the detector)×50 μm I.D.; 50 m*M* phosphate buffer (pH 3.0) containing various concentrations of DMCD; applied voltage, 5 kV. Symbols: × = 0 m*M* CD; ○ = 30 m*M* CD; ♦ = 60 m*M* CD.

3.3. Application of the reversal of migration order to the optical purity test

The technique of reversal of the migration order was applied to an optical purity test. A coated capillary was used to obtain baseline separation in CD-CZE. The baseline separations of the (*S*)-enantiomer with 3% of the (*R*)-enantiomer in CD-CZE and CD-MEKC are shown in Fig. 5. The main peak of the (*S*)-enantiomer overlapped with the minor peak of the (*R*)-enantiomer, because of the tailing in CD-CZE. In contrast, the minor peak of the (*R*)-enantiomer was not affected by tailing when the migration order was reversed in CD-MEKC.

This migration order technique is expected to be applied to the quantitation of the minor enantiomer, which is regarded as an impurity. The validation data in the determination of the (*R*)-enantiomer in the (*S*)-enantiomer is shown in Table 1. In CD-CZE, a sharp increase in the accuracy and the R.S.D. was observed when the amount of the (*R*)-enantiomer

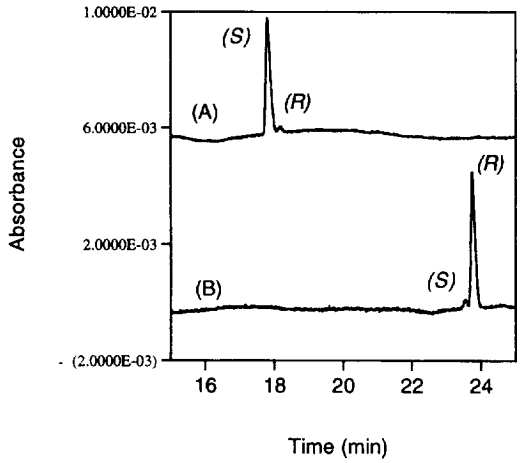

Fig. 5. Baseline separation in (*S*)-E2020 with 3% (*R*)-E2020 in (A) CD-CZE and (B) CD-MEKC. Conditions: (A) capillary, coated, 47.0 cm (40.0 cm to the detector)×50 μm I.D.; 50 m*M* phosphate buffer (pH 3.0) containing 5 m*M* DMCD; applied voltage, 15 kV; (B) uncoated, 46.8 cm (40.0 cm to the detector)× 50 μm I.D.; 50 m*M* phosphate buffer (pH 3.0) containing 65 m*M* DMCD and 40 m*M* SDS; applied voltage, 13 kV.

added was decreased to 2.0%, and this was improved to 0.5% when the migration order of the enantiomer was reversed in CD-MEKC. From this result, the LOQ values were determined to be 3.0 and 0.8% in CD-CZE and CD-MEKC, respectively. In addition, in CD-CZE, there is a tendency to recover less of the (*R*)-enantiomer than was added. This result can be attributed to the error in the integration of the peak area, which is caused by tailing. Therefore, determination of the enantiomeric purity should be performed in the mode that can detect the minor enantiomer before the major enantiomer. We should select these two modes properly in order to perform the optical purity test with good reproducibility.

4. Conclusions

This study has shown that reversal of the migration order of the enantiomers, by changing the mode from CD-CZE to CD-MEKC, is an excellent method for performing an optical purity test. The accuracy and the LOQ were improved by selecting the mode that allows the minor peak of one enantiomer to migrate before the major peak of the other enantiomer.

The mechanism for determining the migration order was not as simple as we expected. It was suggested that we should take the interaction between SDS and DMCD into account. The next task is to predict the optimum concentrations of CD and SDS using the theoretical calculation in CD-MEKC, so that reversal of the migration order could be performed much more easily.

This was the first case of principle (v) being used for compounds that were not zwitterionic. Therefore, it is expected to be applicable to other chiral pharmaceuticals.

Table 1
Validation in the determination of the amount of (*R*)-E2020 in (*S*)-E2020[a]

Amount of (*R*)-E2020 added (%)	Amount found (ng/ml)		Accuracy[b] (%)		R.S.D.[c] (%)	
	CD-CZE	CD-MEKC	CD-CZE	CD-MEKC	CD-CZE	CD-MEKC
0.5	0.67	0.62	135.41	125.21	30.22	29.76
0.8	0.12	0.79	150.39	99.92	9.73	2.28
1.0	1.42	1.00	142.50	100.53	11.00	2.15
2.0	2.86	1.99	143.22	99.95	14.30	0.62
3.0	2.94	3.02	98.24	100.88	4.39	1.48
5.0	4.68	4.97	93.67	99.59	2.22	1.11
10.0	9.76	9.97	97.67	99.75	2.57	0.66
20.0	19.81	20.00	99.09	100.01	0.61	0.51

[a] The concentration of the (*S*)-E2020 is 0.1 mg/ml.
[b] Accuracy (%)=(amount found)/(amount added)×100.
[c] $n=3$.
Conditions: as in Fig. 5.

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