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Separation of aromatic acids by reversed electroosmotic flow capillary electrophoresis

Ying-Mei Liu, Shuenn-Jyi Sheu*

Department of Chemistry, National Taiwan Normal University, Taipei, Taiwan

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Abstract

A reversed electroosmotic flow (EOF) capillary electrophoretic method for the separation of eight aromatic acids was developed. A carrier composed of aqueous buffer solution (10 m*M* lauryltrimethylammonium chloride, 8 m*M* sodium borate and 2 m*M* sodium dihydrogenphosphate)–acetonitrile (7:3) was found to be the most suitable electrolyte for this separation. The analysis time (5 min) was shorter than that of standard capillary zone electrophoresis (21 min). The effects of pH, EOF modifier concentration and organic modifier (acetonitrile) concentration of the carrier on the migration behaviour of the solutes were also studied.

1. Introduction

Capillary zone electrophoresis (CZE) is a powerful separation method, generally applicable to the determination of charged components. Jorgenson and Lukacs [1] reported that the electroosmotic flow (EOF) generated in capillary tubes was strong, and its direction was towards the cathode. Although the velocity of the flow could be affected by ionic species, salt concentration and the pH of the carrier, and also the material of construction of the capillary [1–4], its direction was generally the same. In order to obtain a reversed EOF, several methods have been used including the use of coating capillaries [5,6] and adding a cationic detergent to the carrier [7–11]. In reversed EOF and anionic-mode detection, the order of migrating species will be anions, neutral molecules and cations. Therefore, reversed EOF should be advantageous in the separation of anions, especially those derived from carboxylic acids and phenolic compounds. However, no work has been reported on the analysis of aromatic acids using reversed EOF.

In this work, the separations of eight aromatic acids which are common to herbs (Fig. 1) by standard CZE and reversed EOF CZE were examined. In addition, the effects of pH, EOF modifier concentration and organic modifier (acetonitrile) concentration of the carrier on the migration behaviour of the eight aromatic acids were investigated.

2. Experimental

2.1. Apparatus

The elecrophoretic experiments were carried out on a BioFocus 3000 capillary electrophoresis system equipped with a UV detector set at 210 nm and a 60 cm × 75 μm I.D. fused-silica capil-

* Corresponding author.

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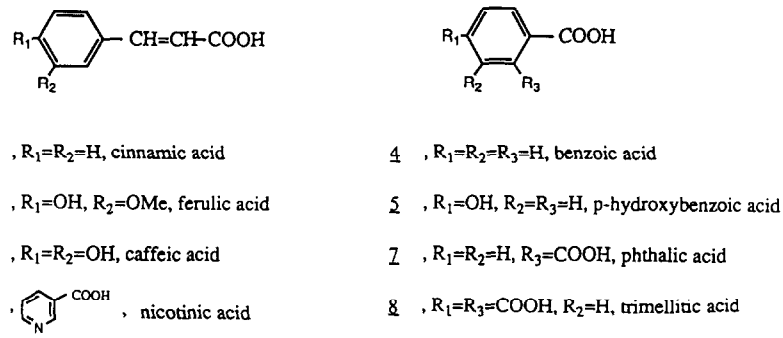

Fig. 1. Structures of the eight aromatic acids.

lary tube (Polymicro Technologies, Phoenix, AZ, USA) with the detection window placed at 55.4 cm. The conditions were as follows: injection mode, pressure 1 p.s.i. s (1 p.s.i. = 6894.76 Pa); cartridge temperature, 20°C; carousel temperature, 25°C; and applied voltage, +30 kV (constant voltage, positive to negative polarity) for the standard CZE method and −30 kV (constant voltage, negative to positive polarity) for reversed EOF CZE.

2.2. Reagents

Deionized water was provided by a Milli-Q Plus water-purification system (Millipore, Bedford, MA, USA). Lauryltrimethylammonium chloride (LTAC) was obtained from Nacalai Tesque (Kyoto, Japan), sodium borate from Wako (Osaka, Japan) and sodium dihydrogenphosphate from Kanto Chemicals (Kyoto, Japan). Nicotinic acid (**3**), ferulic acid (**2**) and caffeic acid (**6**) were purchased from Sigma (St. Louis, MO, USA) and benzoic acid (**4**), *p*-hydroxybenzoic acid (**5**), *trans*-cinnamic acid (**1**), phthalic acid (**7**) and trimellitic acid (**8**) from Aldrich (Milwaukee, WI, USA). Acetonitrile was of HPLC grade. All other reagents were of analytical-reagent grade and were used as received.

2.3. Standard solution

The standard solution used for electrophoretic experiments was prepared by dissolving the eight standard aromatic acids in water–methanol (1:1, v/v) and the concentration of each compound was 0.1 mg/ml.

3. Results and discussion

3.1. Standard CZE method

It is very common to assay aromatic acids by high-performance liquid chromatography (HPLC) [12,13]. In 1986, Fujiwara and Honda [14] separated cinnamic acid and its analogues by standard CZE: carrier, 0.025 *M* phosphate buffer (pH 9.2); voltage, 7.5 kV; run time, about 25 min. With our system, the best carrier for the eight aromatic acids was a mixture of 70% of borate–phosphate buffer (8 m*M* $Na_2B_4O_7$–2 m*M* NaH_2PO_4, pH 9.63) and 30% of acetonitrile. Fig. 2 shows the separation of eight aromatic acids by the standard CZE method with the following migration times: *trans*-cinnamic acid, 6.5 min; ferulic acid, 6.6 min; nicotinic acid, 7.1 min; benzoic acid, 7.4 min; *p*-hydroxybenzoic acid, 7.7 min; caffeic acid, 9.0 min; phthalic acid, 13.3 min; and trimellitic acid, 19.5 min (plate numbers: 19 000–160 000). The migration time of each compound became longer as the pH value of the buffer was increased, in accordance with the report of Fujiwara and Honda [14]. When the pH of the buffer was below 9.6, **1** and **2** were overlapped, as were **3**, **4** and **5**. As the acetonitrile concentration of the carrier was decreased, the migration times of the solutes became shorter, but **1** and **3** were not separated from **2** and **4**, respectively. Fig. 2 is the

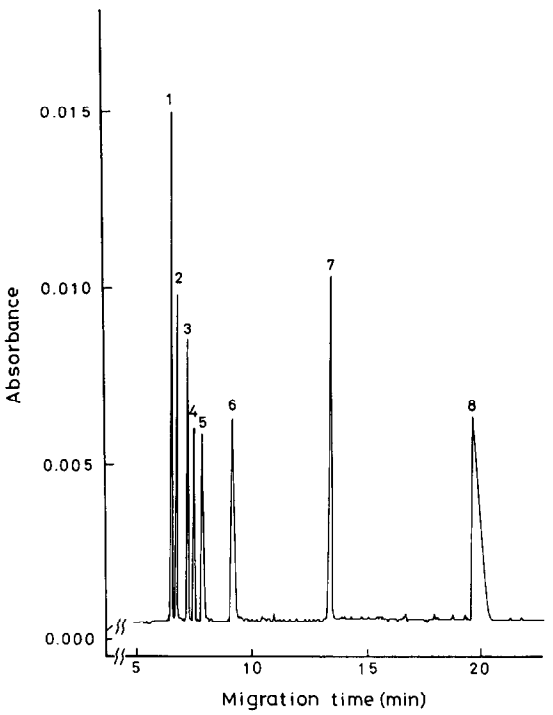

Fig. 2. Capillary electropherogram of the eight aromatic acids separated by standard CZE method: capillary, fused silica (60 cm × 75 μm I.D.); carrier, borate–phosphate buffer (8 m*M* $Na_2B_4O_7$–2 m*M* NaH_2PO_4, pH 9.63)–acetonitrile (7:3); applied voltage, +30 kV; wavelength for detection, 210 nm; injection, 1 p.s.i. s of a water–methanol solution containing 0.1 mg/ml each of the compounds. Peak assignment: 1 = *trans*-cinnamic acid; 2 = ferulic acid; 3 = nicotinic acid; 4 = benzoic acid; 5 = *p*-hydroxybenzoic acid; 6 = caffeic acid; 7 = phthalic acid; 8 = trimellitic acid.

best electropherogram that we could obtain and gives a good resolution for compounds **1–6**. However, for separating more negatively charged components (**7** and **8**), this standard CZE method was not good enough owing to the long migration times and broad peak widths.

3.2. Reversed EOF CZE

All eight aromatic acids were successfully separated in a single run by reversed EOF CZE under suitable conditions. The separation was achieved by optimizing the pH value, EOF modifier concentration and organic modifier (acetonitrile) concentration of the carrier. In reversed EOF with anionic-mode detection, the higher the negative charge the ion the shorter is the migration time.

Several electrolyte systems containing 70% of 10 m*M* EOF modifier (lauryltrimethylammonium chloride) at different pH values ranging from 7.47 to 9.77 (consisting of $Na_2B_4O_7$ and NaH_2PO_4, respective concentrations 2, 8 m*M*; 4, 6 m*M*; 6, 4 m*M*; 8, 2 m*M*; and 10, 0 m*M*) and 30% of acetonitrile were used in order to study the effect of pH on the selectivity of the separation. In Fig. 3, the migration times for the aromatic acids at different pH values are shown. The pH dependence of the migration time of each compound was similar and as the pH increased, the migration time became shorter. However, the migration orders of the compounds with hydroxyl groups (**2**, **5** and **6**) were

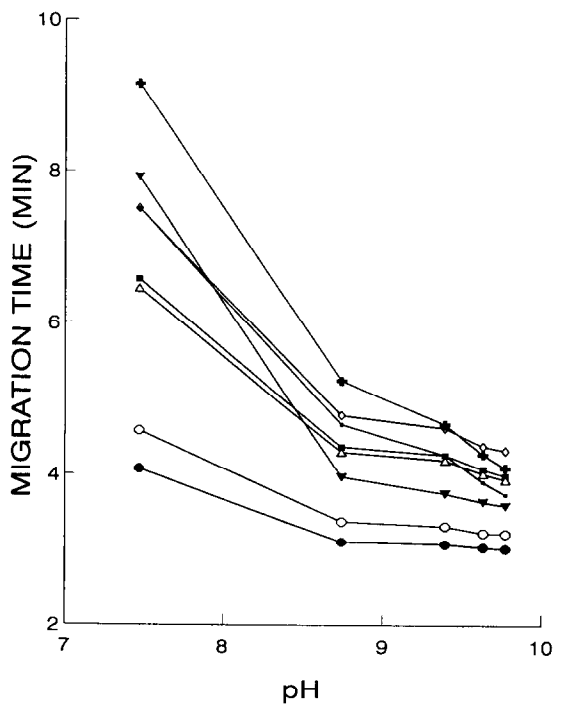

Fig. 3. Effect of pH on migration time. All these experiments were conducted at a voltage of −30 kV across the separating tube filled with 70% of borate–phosphate buffers of different pH values containing 10 m*M* LTAC and 30% of acetonitrile. Other conditions as in Fig. 2. ● = Trimellitic acid; ○ = phthalic acid; ▼ = caffeic acid; ▪ = *p*-hydroxybenzoic acid; △ = benzoic acid; ■ = nicotinic acid; + = ferulic acid; ◇ = *trans*-cinnamic acid.

changed as the pH increased. As higher pH, the carboxyl and phenolic hydroxyl groups were dissociated to form the carboxylate–phenolate divalent anions, which exhibited increased mobility. From the results, buffer solutions of pH 9.77 and 9.63 gave the best separations.

Fig. 4 shows the effect of EOF modifier concentration on the selectivity of the separation. The migration time of all the compounds became shorter as the electroosmotic flow increased with increasing EOF modifier concentration. The separation at 5 and 10 m*M* were good enough, but the latter has a shorter analysis time.

Fig. 5 shows the effect of organic modifier concentration on the selectivity of the separation. As the acetonitrile concentration increased, the migration times of all eight compounds became longer. This was because the EOF decreased as the acetonitrile concentration increased. When acetonitrile was not added or was present at a low concentration, many compounds were not separated. At 20% acetonitrile, most peaks were well separated but **3** and **4** overlapped. Analysis at either 30% or 40% acetonitrile could offer good separations but with a shorter run time at 30%. In addition, the response signals of the compounds became larger as the acetonitrile concentration increased.

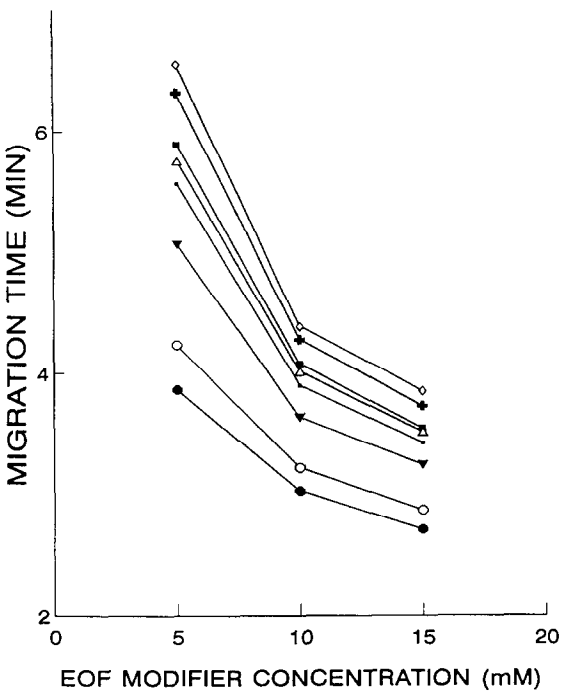

Fig. 4. Effect of EOF modifier concentration on migration time. The carriers were 70% of borate–phosphate buffer (8 m*M* $Na_2B_4O_7$–2 m*M* NaH_2PO_4, pH 9.63) containing 5–15 m*M* LTAC and 30% of acetonitrile. Other conditions and symbols as in Fig. 3.

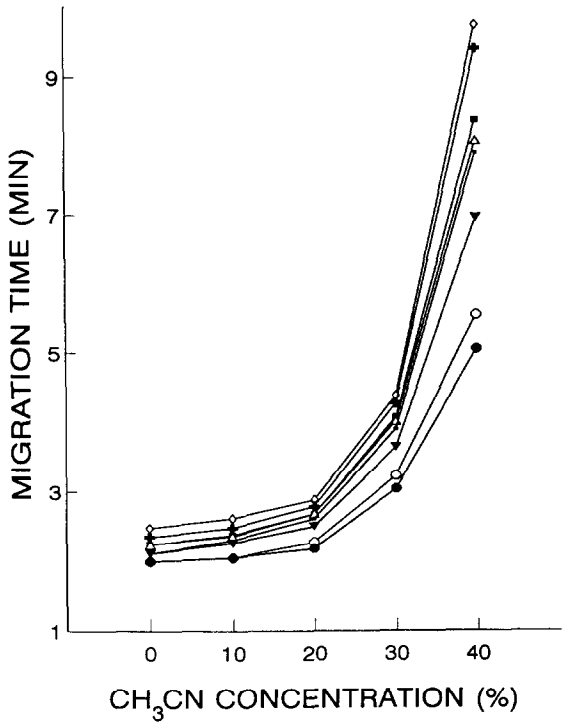

Fig. 5. Effect of acetonitrile concentration on migration time. The carriers were buffer solutions (10 m*M* LTAC–8 m*M* $Na_2B_4O_7$–2 m*M* NaH_2PO_4) mixed with different amounts of acetonitrile. Other conditions and symbols as in Fig. 3.

From the above results, the best resolution was obtained with an electrolyte containing 70% of buffer solution (10 m*M* LTAC, 8 m*M* $Na_2B_4O_7$ and 2 m*M* NaH_2PO_4) and 30% of acetonitrile. Fig. 6 is an electropherogram showing the separation of the eight aromatic acids with the following migration times: trimellitic acid, 3.04 min; phthalic acid, 3.23 min; caffeic acid, 3.64 min; *p*-hydroxybenzoic acid, 3.90 min; benzoic acid, 4.01 min; nicotinic acid, 4.07 min;

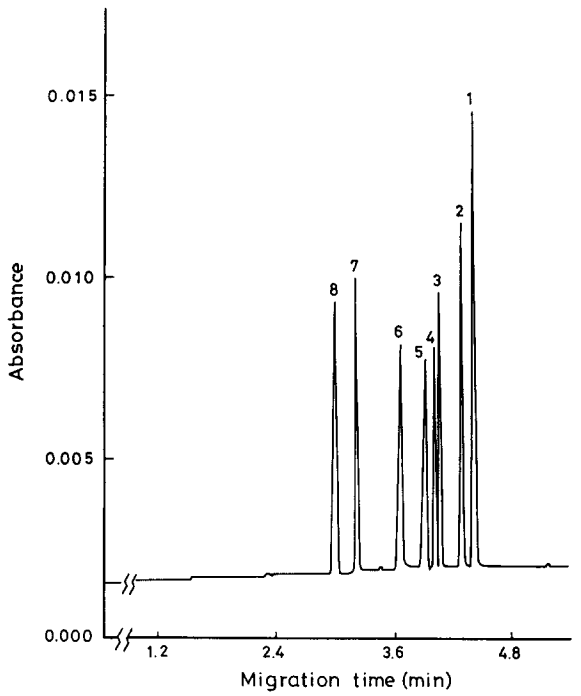

Fig. 6. Capillary electropherogram of the eight aromatic acids separated by reversed EOF CZE. Carrier, buffer solution (10 m*M* LTAC–8 m*M* $Na_2B_4O_7$–2 m*M* NaH_2PO_4)–acetonitrile (7:3); applied voltage, −30 kV; other conditions and peak numbers as in Fig. 2.

ferulic acid, 4.27 min; and *trans*-cinnamic acid, 4.38 min (plate numbers: 46 000–186 000).

In conclusion, by optimizing the pH value, EOF modifier and organic modifier concentration of the carrier, the separation of eight aromatic acids by reversed EOF CZE could be achieved within 5 min with a much smoother baseline. Compared with the standard CZE method, this method is more attractive, especially for those compounds with more than one net negative charge. Application of this technique to natural products and Chinese herb medicines is being studied.

4. Acknowledgement

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5. References

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