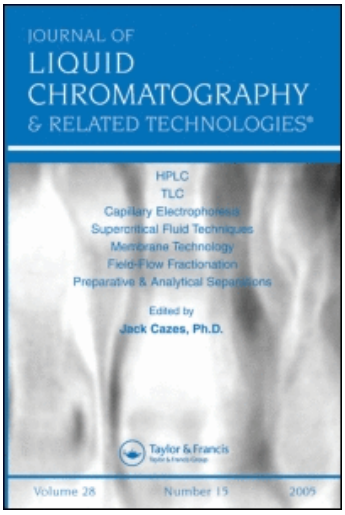

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Comparison of Different Capillary Electrophoresis Modes and HPLC for the Separation of Xanthones

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ABSTRACT

Nine xanthones from a Chinese traditional medicine, *Securidaca inappendiculata* Hassk, were separated by different capillary electrophoresis (CE) modes, including capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), microemulsion electrokinetic capillary chromatography (MEEKC), and capillary electrochromatography (CEC). The comparison of separation selectivity and efficiency for xanthones by using these CE methods indicated that different CE modes greatly varied in the separation selectivity and efficiency for xanthones; their advantages and disadvantages are discussed. Moreover, compared with traditional chromatographic methods, e.g., high performance liquid

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chromatography (HPLC), CE shows higher separation ability and analytical speed for these xanthones.

Key Words: Xanthone; Capillary electrophoresis; HPLC; Comparison.

INTRODUCTION

Xanthones are a class of active constituents from *Securidaca inappendiculata* Hassk, which is a traditional Chinese herbal medicine belonging to the Polygalaceae family, mainly distributed in the south of China and other tropical regions of Asia. The roots and stems are used as anti-inflammatory, antibacterial, and anti-rheumatism agents under the names "Chan yi teng" and "Wu wei teng" in the south of China.[1] Pharmacological investigations have shown that the xanthones, as main components accumulated in *S. inappendiculata*, have many bioactivities including monoamine oxidase (MAO) inhibition, cytotoxicity, anti-inflammatory properties, antitumor, antibacterial, antifungal, antioxidant activities, and tuberculoatatic properties.[2] Therefore, a simple and rapid method has been highly desired to quantitatively determine these bioactive components in related herbal medicines. On the other hand, the development of capillary electrophoresis (CE) has been reviewed many times[3–7] and, clearly, it continuous to be a very active area for research in separation science since this technique often provides higher resolving power, shorter analysis time, and lower operating cost than traditional chromatographic techniques such as high performance liquid chromatography (HPLC), especially for the analysis of natural products like traditional Chinese medicines. In recent years, some research groups have been devoting themselves to the analysis of traditional Chinese medicine by CE.[8–17]

In our previous work, Capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC)[18–20] separations for xanthones have been developed. Recently, microemulsion electrokinetic capillary chromatography (MEEKC)[21–22] and capillary electrochromatography (CEC),[23] which are two very attractive fields in CE separation, were investigated in our laboratory. Although the studies described above were published or have been submitted for publication, they were independently or partly described, and mainly dealt with the optimization process for CE separation, but the comparison of different CE modes for xanthone separation has not been involved. As is well known, the CE method includes several separation modes, including CZE, MEKC, MEEKC, and CEC, which serve as main approaches applied to the analysis of the active compounds in traditional Chinese medicines. Each different CE mode has a different separation mechanism, showing different separation efficiency and selectivity for analytes. Hence, it is

very important, for understanding the CE separation process, to compare the different CE separation modes, especially by using this class of xanthones as analytes, which have extremely similar and a diversity structures. In this paper, the parameters of different CE modes, analytical time, theoretical plate number, symmetry factor, and overall resolution are compared and discussed. Meanwhile, compared with HPLC, CE shows higher separation ability and analytical speed for the studied xanthones.

EXPERIMENTAL

Apparatus and Conditions

All CE separations were performed with an Agilent 3D CE system with air-cooling and a diode array detector (Agilent Technologies, Waldbronn, Germany). For CZE and MEKC, a 58.5 cm × 50 μm I.D. fused silica capillary (Yongnian Optical Fiber Factory, Hebei, China) was utilized with an effective length of 50 cm. For MEEKC, a 48.5 cm × 50 μm I.D. fused silica capillary was used with an effective length of 40 cm. For CEC, a 100 μm I.D × 350 μm OD CEC-Hypersil C_{18} column (Agilent Technologies, Waldbronn, Germany) with a packed bed length of 40 cm (3 μm particle size) was used. The total column length (48.5 cm) was the length of the packed bed plus 8.5 cm of polyimide-coated fused-silica tubing. For CZE, MEKC, and MEEKC, sample injection was at 50 mbar for 10 second. For CEC, both inlet and outlet were pressurized at 5 bar during conditioning and analysis and electrokinetic injection was used (20 kV for 10 seconds). For all CE separations, detection was performed at a wavelength of 265 nm.

High performance liquid chromatography separation was conducted on an Agilent 1100 HPLC system consisting of a vacuum degasser, quaternary pump, autosampler, thermostatted column compartment, and a diode array detector (Agilent Technologies, CA). A Zorbax RX-C_8 (4.6 mm × 25 cm, 5 μm) (Agilent Technologies, CA) was used. UV detection was set at 265 nm.

Chemicals

The xanthones (see Fig. 1 for their chemical structures) were kindly presented by the Institute of Medicinal Plant Development (Beijing, P.R. China). All chemicals were of analytical-reagent grade, purchased from Beijing Chemical Factory (Beijing, P.R. China); pure water prepared by Milli-Q system (Millipore, Bedford, MA, USA) was used for all buffer solutions. All cyclodextrins (CDs) were kindly presented by Bioanalytical System Inc. (West Lafayette, IN, USA).

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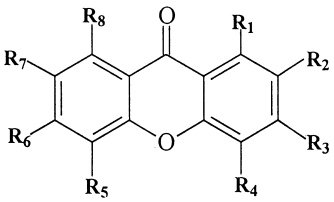

No	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1	HO	MeO	MeO	H	H	H	HO	H
2	MeO	H	H	MeO	H	H	HO	H
3	MeO	HO	H	H	H	H	MeO	H
4	MeO	HO	H	H	H	H	HO	H
5	HO	H	H	MeO	H	H	HO	H
6	HO	H	HO	MeO	H	H	HO	H
7	HO	MeO	HO	H	H	H	HO	H
8	HO	H	HO	H	H	H	HO	H
9	HO	MeO	MeO	H	H	HO	HO	H

Figure 1. The chemical structures of 9 xanthones.

Procedures

All buffers for CE separations were filtered through a 0.45 μm membrane filter and degassed by ultrosonication for approximately 10 min before use. A mixed standard solution of xanthones was prepared in methanol, filtered, and degassed with the same procedure as used for buffer solutions.

For CZE, MEKC, and MEEKC, the capillary was conditioned daily by washing first with 0.5 M sodium hydroxide (10 min), then with water (10 min), and, finally, with the running buffer (15 min). Between consecutive analyses, the capillary was flushed with 0.5 M sodium hydroxide (1 min), then with water (2 min) and, finally, with the running buffer (3 min) in order to improve the migration time and peak-shape reproducibility.

For CEC, equilibration of a new capillary column is required for successful operation. The column was purged with the buffer containing 5 mM (hydroxymethyl)-aminomethane (Tris) (adjusting the pH to 8 with 30% hydrochloric acid) with 80% acetonitrile for approximately 90 min. This was accomplished by applying 12 bar pressure on the inlet side and stepping up the voltage from 5 to 25 kV in 5 kV, 10 min steps. Next, we applied 12 bar pressure on both vials and the voltage (25 kV) for about 30 min and waited until the current and detector baseline were stable. The column was conditioned with every new background electrolyte (BGE) for at least 30 min before any samples were injected. Between consecutive analyses, the capillary was electroconditioned with BGE for 10 min.

Optimal Buffer Conditions for Capillary Electrophoresis and Chromatographic Conditions for High Performance Liquid Chromatography

Table 1 lists separation conditions for different CE modes used for the separation, based on our previous studies.

For HPLC, the mobile phase was composed of two solvents, water and acetonitrile, and a linear gradient program was developed. The volume

Table 1. Separation conditions for different CE modes.

CE Modes	Running buffer system	Voltage	Temperature
CZE	200 mM borate buffer (pH = 9.5) containing 10 mM sulfated β-CD	30 kV	40°C
MEKC	100 mM borate buffer (pH 10.5) containing 60 mM SDS and 5 mM β-CD	20 kV	35°C
MEEKC	50 mM borate buffer (pH 9.5) containing 80 mM *n*-heptane, 120 mM sodium dodecyl sulfate (SDS), 10% (v/v) *n*-butanol	20 kV	35°C
CEC	25 mM acetic acid buffer solution (pH 4) with 50% acetonitrile	25 kV	25°C

percentage of acetonitrile was linearly changed from 5% to 95% within 30 min, and then 95% acetonitrile was maintained for 5 min. The flow-rate and the column temperature were set at 1.0 mL/min and 25°C, respectively.

RESULTS AND DISCUSSION

Separation Selectivity

As shown in Fig. 2, there exist great differences in separation selectivity and analysis times for xanthones when adopting different CE modes. Microemulsion electrokinetic capillary chromatography showed the longest analysis time and the largest detection window scale (analysis time scale from first emerging peak to last one) compared with others. Baseline separation of nine xanthones can be successfully completed by CZE and MEEKC. Especially for CZE, the fastest analysis was achieved within 12.5 min. As to MEKC and CEC, however, baseline separation cannot be achieved. Moreover, for CEC, a poor overall resolution for xanthones was observed. The study also demonstrated that the peak order was greatly changed when using different CE modes, presumably due to different separation mechanisms. It should be noted that MEEKC, a variant of MEKC, showed much stronger separation ability for xanthones than MEKC. However, MEEKC, for the separation of natural products, has been very limited up to now. All in all, CZE showed the most powerful separation and highest-speed of analysis among all CE modes.

From Fig. 2, it can be seen that peak order fluctuated between different separation modes. The reason is the separation mechanisms are more or less different from each other. In CZE separation, the difference in xanthone mobilities resulted from the dissociation constants discrepancy of phenolic hydroxyl groups and the difference in binding constants between xanthones and sulfate β-CD.[19] Therefore, xanthones 6–9 with three hydroxyl groups were more negatively charged than xanthones 1–5 in the running buffer and, thus, were eluted last. Whereas, in MEKC and MEEKC, hydrophobicity, which can be represented by the logarithms of octanol–water partition coefficient ($\log P$), plays essential role in the separation. Log P of xanthones was calculated by CS Chem. 3D Pro 5.0 software (Cambridge Soft 1999) using Broto's method, as indicated in Table 2. In addition, the ionization of analyte also has an important impact on the separation. In the micellar or microemulsion system, solutes partition between the oil droplet or micelle and the aqueous buffer phases. Water-insoluble compounds will favor inclusion into the oil droplet or micelle rather than into the buffer phase,

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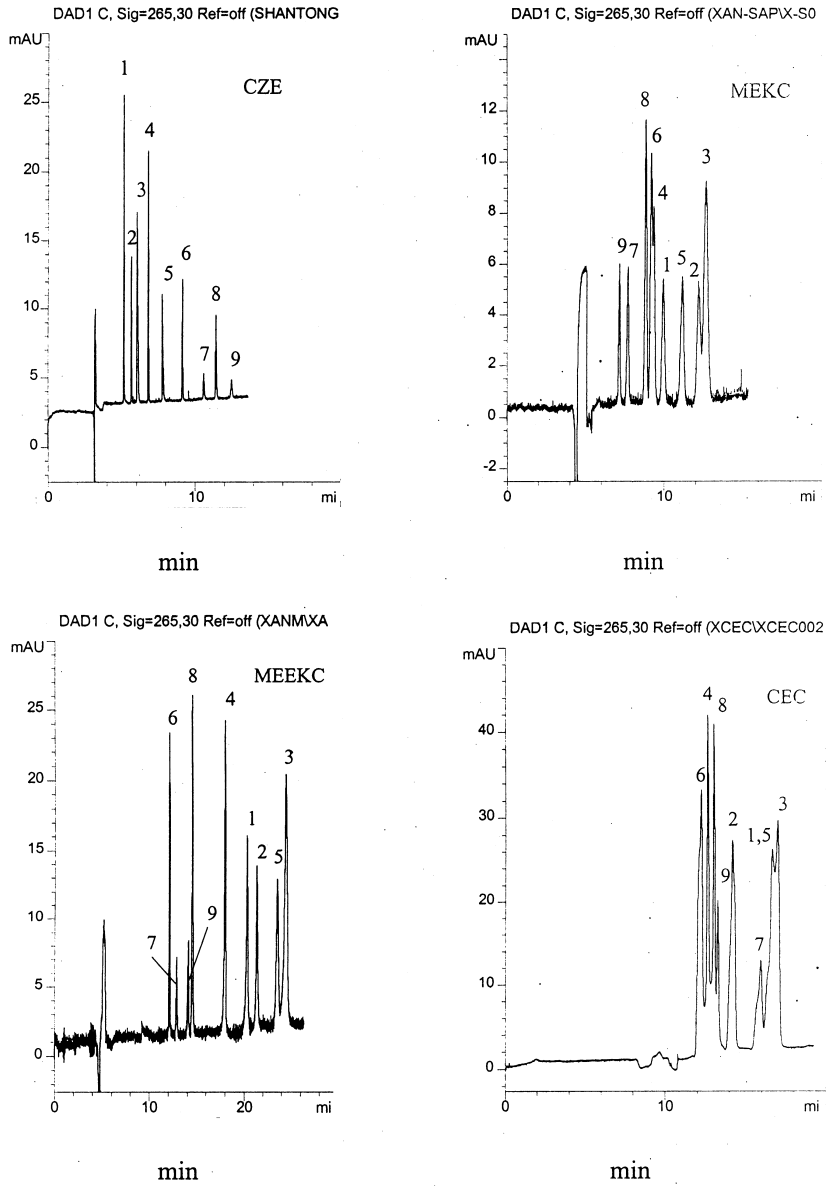

Figure 2. Separations by different CE modes for 9 xanthones. See Experimental for separation conditions and Fig. 1 for peak identification.

Table 2. Log *P* values of 9 xanthones.

Xanthones	1	2	3	4	5	6	7	8	9
Log *P*	2.30	2.69	2.69	2.17	2.17	1.78	1.78	1.65	1.91

making the partition of the solute possible in a chromatographic manner. The xanthones can be divided into two groups according to their log *P* values. The xanthones 1–3 and 5, with higher log *P* values had slower mobilities due to their stronger affinity to the oil droplet or micelle. In contrast, the xanthones 4, 6, and 7–9, with lower log *P* values, eluted faster because of less reaction with the oil droplet or micelle. Microemulsion electrokinetic capillary chromatography showed better separation ability for xanthones than MEKC, especially for those with similar hydrophobicities. It can be readily explained that the surface of the droplet was much more easily penetrated by solutes than the surface of a micelle, since the latter is more rigid than the former, allowing MEEKC to be applied to a wider range of solutes than MEKC.

Capillary electrochromatography is a recently developed technique that combines, in principle, advantages of HPLC and CE. Unfortunately, CEC did not show better separation for xanthones, compared with other CE modes. The reason is probably that C_{18} packing material and mobile phase used were not suitable for the separation of xanthones. In fact, these analytes were well separated on a C_8 column with gradient elution; this will be discussed in a later section.

Separation Efficiency

Theoretical plate number and symmetry factor are two essential parameters for evaluating CE separation efficiency. In this study, the differences in theoretical plate number and symmetry factor, using different CE modes for xanthone separation, were compared, as listed in Table 3. The investigation indicated that CEC showed the highest theoretical plate number, but yielded the poorest symmetry factor, compared with the other modes. Micellar electrokinetic chromatography demonstrated the worst separation efficiency with lowest theoretical plate number. Originated from MEKC, MEEKC showed higher theoretical plate number, similar to CZE, and a better symmetry factor. However, as the combination of CE and HPLC, CEC has not shown any advantage with respect to separation efficiency.

Table 3. The theoretical plate number (m^{-1}) and symmetry factor of 9 xanthones by using different CE methods.

Xanthones	CZE	MEKC	MEEKC	CEC
1	300700(0.29)[a]	29014(1.16)	210658(1.30)	—
2	208392(0.22)	25698(0.68)	223113(0.72)	26578(0.92)
3	73676(0.12)	21274(1.12)	81820(1.31)	—
4	307802(0.29)	54134(0.71)	187565(0.90)	140088(0.94)
5	132989(0.14)	26904(0.94)	125463(0.89)	—
6	397038(0.97)	39095(1.12)	252080(0.99)	22588(2.03)
7	231604(0.76)	50749(1.25)	193655(0.97)	31785(2.14)
8	325794(0.82)	49642(1.45)	228473(0.79)	152898(1.26)
9	195496(1.01)	59222(1.34)	192090(1.47)	146633(0.89)

[a]The data in the parentheses donate symmetry factor.

Repeatability of Migration or Retention Time

The repeatability of migration or retention time in CE separation was tested by using a pair of BGE vials for consecutive analysis. The investigation demonstrated that CZE showed excellent relative standard deviation (RSD), below 1%. Micellar electrokinetic chromatography, MEEKC, and CEC also demonstrated good RSD (<7%).

Comparison Between Capillary Electrophoresis and High Performance Liquid Chromatography

High performance liquid chromatography, for xanthone separation, was established for comparing HPLC and CE methods. As shown in Fig. 3, the baseline separation of xanthones was achieved, using a linear gradient program, within 40 min, which has much longer analysis time, high cost due to using acetonitrile as elution agent, and longer column equilibration time than that by CE. The study demonstrated that CE is a more powerful separation tool than HPLC for the separation of studied xanthones.

CONCLUSIONS

As a class of natural products with strong pharmacological effects, separation of xanthones is a challenging work due to their similar and diverse chemical structures. Based on our previous study, the different CE modes, including CZE, MEKC, MEEKC, and CEC were developed for the separation of xanthones and compared with regard to separation efficiency and

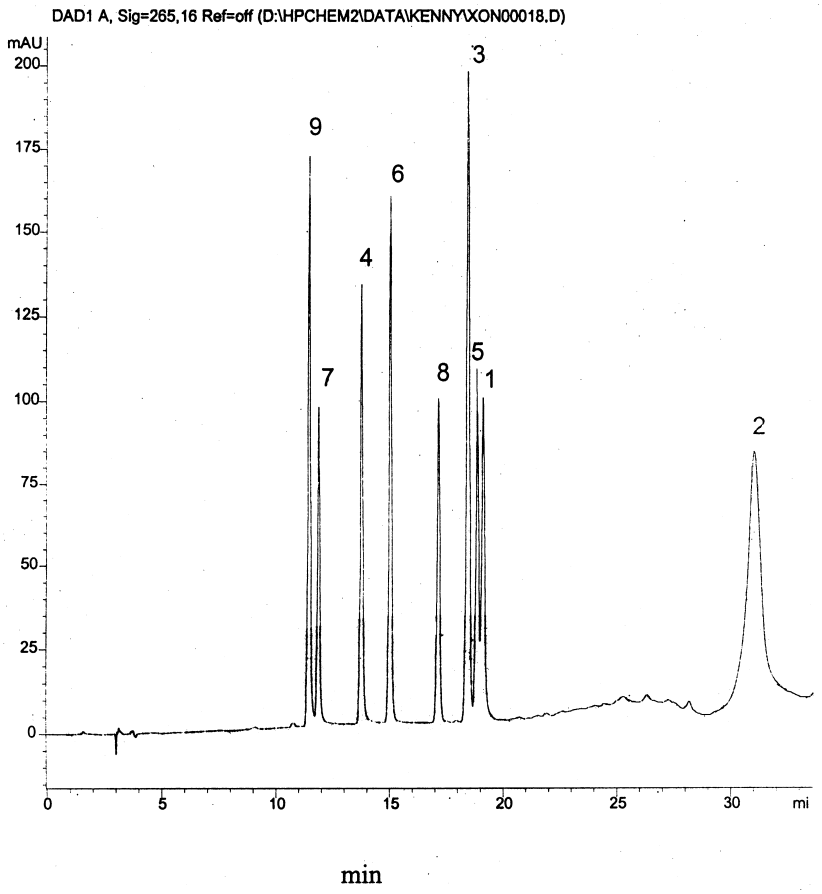

Figure 3. High performance liquid chromatography chromatogram for 9 xanthones. See Experimental for separation conditions and Fig. 1 for peak identification.

selectivity. Moreover, the comparison between CE and HPLC shows that CE is a more powerful separation tool for xanthones than is HPLC.

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