Chinese Chemical Letters Vol. 13, No. 9, pp 877 – 880, 2002 877
http://www.imm.ac.cn/journal/ccl/html

Optimized Separation of Nine Xanthones by Microemulsion Electrokinetic Chromatography

Tao BO, Hu Wei LIU, Ke An LI*

Chemistry Department, Peking University, Beijing 100871

Abstract: A microemulsion electrokinetic chromatography method has been firstly used for the separation of the therapeutically important xanthones from *Securidaca inappendiculata*. The separation of the nine xanthones was systematically optimized with respect to pH, composition of microemulsion, addition of cyclodextrins, applied voltage and column temperature. Baseline separation was successfully achieved for the nine xanthones, which was also compared with that by micellar electrokinetic chromatography.

Keywords: Separation, xanthones, *Securidaca inappendiculata*, microemulsion electrokinetic chromatography,.micellar electrokinetic chromatography.

Securidaca inappendiculata Hassk. is a traditional Chinese herbal medicine. Pharmacological investigations have shown that the xanthones, as main components accumulated in *S. inappendiculata* have many bioactivities, such as monoamine oxidase inhibition, antitumor activity, cytotoxicity, antibacterial activity, antifungal activity, anti-inflammatory properties, antioxidant activity and tuberculoatatic activity, *etc*.[1]. In our previous work, the xanthones have been separated successfully by capillary zone electrophoresis (CZE) [2], and the separation mechanism by CZE has been described [3].

Microemulsion electrokinetic chromatography (MEEKC) is a special variant of electrokinetic chromatography (EKC), whose separation principle is based on the different partition of solutes between the pseudostationary phase and buffers, and MEEKC shows a high solubilization capacity and separation efficiency [4, 5]. In this work, nine xanthones were firstly resolved by MEEKC, which gave very satisfactory results. The MEEKC separation was compared with that by MEKC.

Experimental

All separations were performed on an Agilent 3D CE system with air-cooling and a diode array detector (Agilent Technologies, Waldbronn, Germany). A 40 cm×50 μm I.D. fused silica capillary (Ruifeng Inc., Hebei, China) was used. The UV detection wavelength was set at 265 nm. Samples were injected into the capillary at 5000 Pa for 10 seconds.

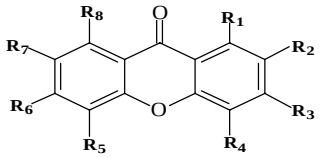
*E-mail: likn@chem.pku.edu.cn

The capillary was conditioned daily by washing first with 0.5 mol/L sodium hydroxide (10 minutes), then with water (10 minutes) and finally with the running buffer (15 minutes). Between consecutive analysis, the capillary was flushed with 0.5 mol/L sodium hydroxide (1 minute), then with water (2 minutes) and finally with the running buffer (3 minutes) in order to improve the migration time and peak-shape reproducibility.

The xanthones (for their structural formula, refer to **Table 1**) were provided by Institute of Medicinal Plant Development (Beijing, China). Pure water prepared by Milli-Q system (Millipore, Bedford, MA, USA) was used for all buffer solutions. Sulfated β-CD and β-CD were kindly presented by Bioanalytical System Inc (West Lafayette, USA). All other chemicals were of analytical-reagent grade.

The running buffers for MEEKC analysis were adjusted to the desired pH with 1 mol/L NaOH. All the buffers were filtered through a 0.45 μm membrane filter and degassed by ultrasonication for approximately 10 minutes before use. A standard solution of *ca.* 20 ppm of each xanthone was prepared in methanol, degassed in an ultrasonic bath and filtered through a 0.45 μm membrane filter.

Table 1 Structural formula of the xanthones



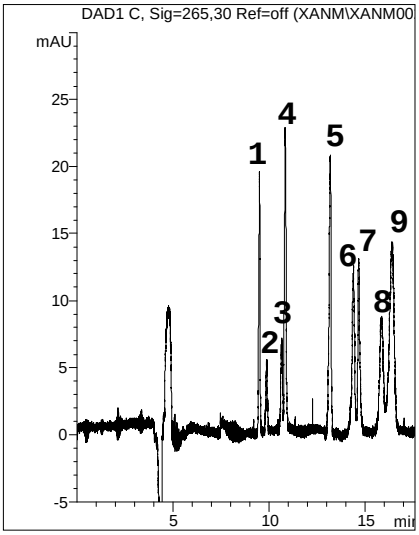
	Substituted groups							
Compd.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1	HO	H	HO	MeO	H	H	HO	H
2	HO	MeO	HO	H	H	H	HO	H
3	HO	MeO	MeO	H	H	HO	HO	H
4	HO	H	HO	H	H	H	HO	H
5	MeO	HO	H	H	H	H	HO	H
6	HO	MeO	MeO	H	H	H	HO	H
7	MeO	H	H	MeO	H	H	HO	H
8	HO	H	H	MeO	H	H	HO	H
9	H	H	MeO	HO	H	H	MeO	H

Results and Discussion

The pH, borate concentration and composition of microemulsion in the MEEKC analysis were optimized, and the optimum pH and borate concentration were 9.5 and 50 mmol/L, respectively, under 20 kV applied voltage and 35ºC temperature. The composition of microemulsion was 80 mmol/L heptane, 120 mmol/L sodium dodecyl sulfate (SDS) and 10% (v/v) *n*-butanol, respectively. The separation is indicated in **Figure 1**.

Five millimoles perliter β-CD and 6 mmol/L sulfated β-CD were evaluated as additives for optimizing separation, the effect of which on CE selectivity was studied at the conditions described above. The experiment shows that sulfated β-CD has better selectivity than β-CD for the MEEKC separation, as indicated in **Figures 2** and **3**. Sulfated β-CD is a counter-migrating complexing agent and combines the properties of CD and a surfactant by providing a hydrophobic cavity and a negative charge. Analytes will form inclusion complexes with sulfated β-CD whose electrophoretic mobilities are in direction opposite to the electro-osmotic flow. What is more, surfactant can result in electric and hydrogen bond effects with the phenolic hydroxyl groups of the xanthones. The inclusive effects are different because of the different amount and position of the phenolic hydroxyl groups of the xanthones, which induces different electric and hydrogen bond effect with sulfated β-CD. So adding sulfated β-CD results in different mobilities of the xanthones.

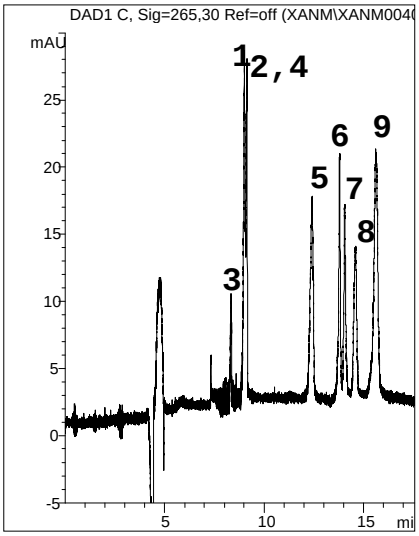
Figure 1 Chromatogram of nine xanthones by MEEKC without the addition of CD



Minute

Conditions see Experimental

Figure 2 Chromatogram of nine xanthones by β-CD modified MEEKC



Minute

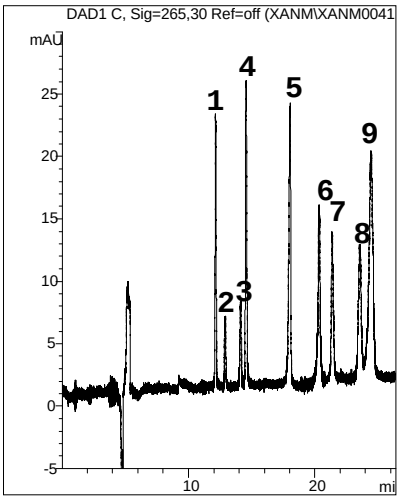
Conditions see Experimental

Finally, the effects of applied voltage (15-30 kV) and temperature (30-45ºC) on the separation were also studied in the optimum running buffer above. The optimum voltage and temperature were found to be 20 kV and 35ºC, respectively, which combined sufficient resolution with a moderate analysis time.

The repeatability of the migration time and the peak area of each xanthone under the optimum condition was very good with relatively standard deviation of less than 5%, and the theoretical plate number is in the range of 90120-252108 m^{-1}. The experiment indicated that the MEEKC analysis has better selectivity than MEKC. The MEKC

separation is shown in **Figure 4** (condition: 100 mmol/L borate, 60 mmol/L SDS, 5 mmol/L β-CD, pH 10.5, 265 nm UV detection, 20 kV applied voltage and 35ºC column temperture).

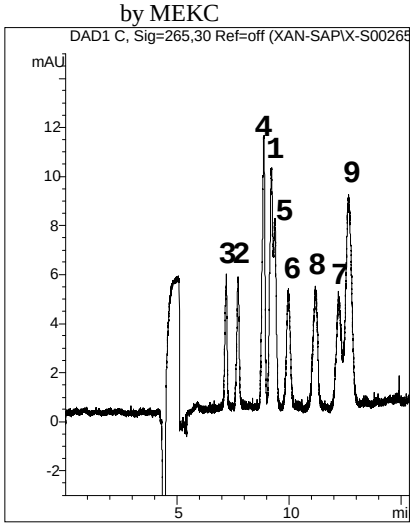
Figure 3 Chromatogram of nine xanthones by sulfated β-CD modified MEEKC



Minute

Conditions see Experimental.

Figure 4 Chromatogram of nine xanthones by MEKC



Minute

Conditions see Experimental.

Conclusion

This work demonstrates that the nine xanthones can be baseline separated by CE with satisfactory migration time and peak area repeatability. Compared with MEKC method, MEEKC has higher separation efficiency and selectivity.

Acknowledgments

The authors would like to acknowledge Agilent Technologies for providing the capillary electrophoresis system and Bioanalytical System for the present of sulfated β-CD. The study is supported by NSFC, Grant No. 29875001.

References

1. A. Marston, M. Hamburger, I. Sordat-Diserens, *et al.*, *Phytochemistry*, **1993**, *33* (4), 809.
2. T. Bo, X.D. Yang, F. Gao, *et al.*, *Chin. Chem. Lett.*, in press.
3. T. Bo, J. Ren, X.D. Yang, *et al.*, *Anal. Chem.*, submitted.
4. Kevin D. Altria, *J. Chromatogr. A*, **2000**, *892*, 171.
5. J.D. Lu, W. Yuan, X.Y. Fu, *Chin. Chem. Lett.*, **2001**, *12 (2)*, 155.

Received 24 December, 2001