Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Chromatography A, 1121 (2006) 274–279

JOURNAL OF
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Separation of hydrophobic solutes by organic-solvent-based micellar electrokinetic chromatography using cation surfactants

Suxuan Gong [a], Fujia Liu [b], Wei Li [a], Fei Gao [a], Chunjin Gao [b],
Yiping Liao [a], Huwei Liu [a,*]

[a] *The Key Lab of Bioorganic Chemistry and Molecular Engineering, Ministry of Education, Institute of Analytical Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China*
[b] *Beijing Chaoyang Hospital, Beijing 100020, China*

Received 12 January 2006; received in revised form 23 March 2006; accepted 5 April 2006
Available online 2 June 2006

Abstract

In this study, the separation of 13 homologous stick-like hydrophobic solutes, i.e., biphenyl nitrile derivatives, by organic-solvent-based micellar electrokinetic chromatography (MEKC) was investigated in terms of separation medium composition, species and concentration of surfactant, other additives, separation voltage and temperature. The results showed that the 13 strong hydrophobic compounds were baseline separated in 25 min with a repeatability of less than 1.3% (RSD) for migration time. The separation medium was a mixture of methanol, 2-propanol and water (58.5:10:31.5), containing 150 mM cetyltrimethylammonium bromide (CTAB) and 20 mM sodium borate. Variety of solvent composition, temperature and applied voltage all showed remarkable effect on the separation. The organic-solvent-based MEKC method proved to be superior to the aqueous MEKC and microemulsion electrokinetic chromatography (MEEKC) methods for the separation of strongly hydrophobic compounds.

Keywords: Micellar electrokinetic chromatography; Cation surfactant biphenyl nitrile derivatives; Organic solvent

1. Introduction

Since micellar electrokinetic chromatography (MEKC) method was firstly introduced as an important mode of capillary electrophoresis (CE) [1], it has shown powerful separation ability for separation of neutral compounds as well as ionized ones. Different surfactants including sodium dodecyl sulfate (SDS), diethylhexyl sodium sulfosuccinate, taurodexycholic acid sodium salt [2], sodium cholate [3] and tetradecyltrimethylammonium bromide (TTAB) [4–6] have been utilized to separate hydrophobic solutes in MEKC. As to cation surfactant, which could reverse the electroosmotic flow and form micelle simultaneously, the major study focused on the application of TTAB in the analysis of polycyclic aromatic hydrocarbons (PAHs) [4,6], fat-soluble vitamins [7], ointment components [5] and other compounds [8]. The existence of surfactant helps to extend the migration window and increase the hydrophobic interactions between solutes and additives. Some other polymers were also used as pseudostationary phase to enhance the selectivity of the analytes due to their special hydrophobic characters, which have great effect on the retention of the analytes in micelle [9–11]. In some chiral separation cases, the length of different hydrophobic chains and degree of polymerization of molecular micelles also resulted in various separation behaviors [12,13].

The separation of neutral analytes, especially for some strong hydrophobic compounds, often encountered many difficulties in aqueous CE, such as long migration time, poor resolution and low efficiency, basically due to the apolar property and the poor solubility of analytes in water. It can be further explained by the simplex property of water and the uniformly high solute–micelle bonding constant, reducing the separation selectivity for hydrophobic compounds. In order to improve the separation, organic reagents were usually applied as additives to alter the separation selectivity, which provides superiorities over the aqueous CE. The expanded array of solvent parameters such as dielectric constant, viscosity, polarity and autoprotolysis has made MEKC to be a powerful analytical method. Through

---

* Corresponding author. Tel.: +86 10 62754976; fax: +86 10 62751708.
E-mail address: hwliu@pku.edu.cn (H. Liu).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.04.091

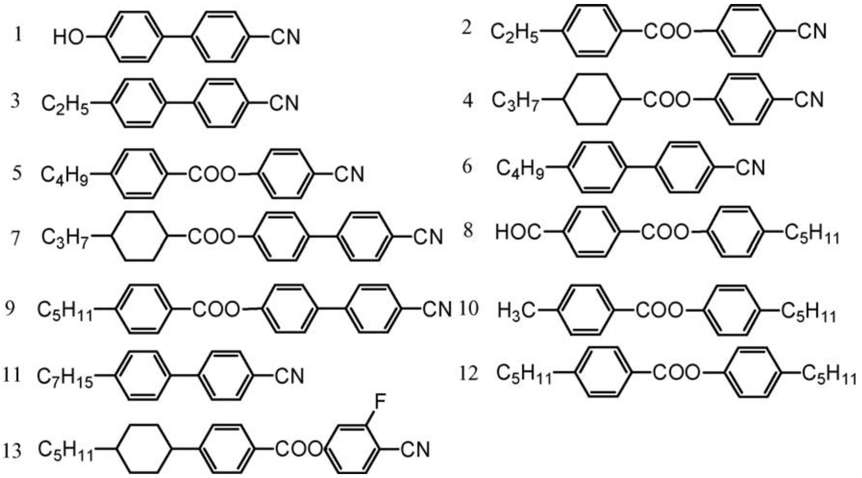

Fig. 1. Structures of 13 compounds studied.

varying the type and content of organic solvents in buffer, dramatic changes in migration order could be achieved [14–17]. In addition, organic separation media provides higher solubility for hydrophobic compounds, leading to less aggregation of hydrophobic analytes, lower current and less peak broadening, thus higher electrolytes concentration is permitted in running buffers.

Biphenyl nitrile derivatives are a series of strong hydrophobic compounds, which are stick-like neutral molecules used to synthesize liquid crystals [18], and the analysis of these compounds and their analogues is required to ensure the quality of products consisted of related liquid crystals. These strongly hydrophobic analytes provide the possibility to study the separation mechanism of highly water-insoluble compounds. In this paper, the effect of background electrolyte (BGE) composition in MEKC based on high concentration of organic solvent was investigated by using 13 biphenyl nitrile derivatives (Fig. 1) as model compounds. It was demonstrated that organic BGEs provided higher solubility of surfactant, resulting in wider migration window, and that the addition of water in organic BGEs had remarkable effect on the selectivity. Furthermore, the influence of surfactant chain length in MEKC was also investigated through five congeneric surfactants. Finally the 13 stick-like hydrophobic compounds were separated in a ternary system using an organic-solvent-based MEKC method, which showed comparable advantages over the aqueous MEKC, MEEKC [18] method in the separation of strong hydrophobic compounds.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade methanol, acetonitrile and 2-propanol were purchased from Burdick & Jackson. The other reagents were of analysis-grade. Butanol, sodium hydroxide, hydrochloric acid, sodium tetraborate, octyltrimethylammonium bromide, dodecyltrimethylammonium bromide (DTAB), TTAB and octadecyltrimethylammonium bromide (OTAB) were from Beijing Chemical Factory (Beijing, China). CTAB and sodium cholate (SC) were purchased from Sigma. The water used for the preparation of solutions was purified by Aquapro Reverse Osmosis system (Aquapro, Chongqing, China). Thirteen analytes (as shown in Fig. 1) were kindly presented by Professor L. Huang at our college.

2.2. BGE and sample solutions

BGE solutions were prepared by adding CTAB and sodium tetraborate in methanol or mixed organic solvents, and then filtered through the 0.45 μm membrane and sonicated for 1 min before use. Stock solutions of analytes (biphenyl nitrile compounds and analogs) were individually prepared at a concentration of 1 mg/ml in methanol. The final sample consisting of all the 13 solutes was prepared by dilution with methanol.

2.3. Instrumentation and conditions

All experiments were performed with a HP ^{3D}CE system with air-cooling and a diode-array detector (Agilent, CA, USA), and an uncoated fused-silica capillary, 50 μm I.D. × 375 um O.D. (Xinnuo, Hebei, China), with a total length of 48.5 cm (40.0 cm effective) was utilized. The applied voltage was −30 kV and the capillary temperature was maintained at 10 °C. UV detection was carried out at both 225 nm and 240 nm.

New capillary was rinsed with 0.1 M NaOH for 20 min, then with re-distilled water for 5 min, and finally with methanol for 5 min. The capillary was conditioned daily by methanol wash for 5 min and maintained in methanol/water mixture overnight. Between consecutive analysis, the capillary was flushed with 0.1 M NaOH for 1 min, water for 2 min, methanol for 2 min and then BGE for 3 min. After every five runs, the capillary was flushed with 0.1 M hydrochloric acid for 3 min.

3. Results and discussion

3.1. Selection of organic media and surfactants

Methanol, ethanol, acetonitrile, 2-propanol, formamide, tetrahydrofuran (THF), dimethyl sulfoxide (DMSO) and their