

## Separation of Biphenyl Nitrile Compounds by Microemulsion Electrokinetic Chromatography with Mixed Surfactants

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**Abstract:** A mixture of nine biphenyl nitrile compounds with high hydrophobicity and similar structures was successfully separated by microemulsion electrokinetic chromatography (MEEKC) within 30 min. The buffer system contained 100 mmol/L sodium dodecyl sulfate (SDS), 80 mmol/L sodium cholate (SC), 0.81% heptane, 7.5% *n*-butanol, 10% acetonitrile and 10 mmol/L borate. The addition of SC, organic modifiers, sample preparation and temperature all showed remarkable effect on the separation. Meanwhile, the MEEKC method was briefly compared with micellar electrokinetic chromatography (MEKC) method.

**Keywords:** Microemulsion electrokinetic chromatography, separation, mixed surfactant, biphenyl nitrile compounds, micellar electrokinetic chromatography .

Biphenyl nitrile compounds, as intermediates for synthesis of liquid crystal, are very difficult to be separated by capillary zone electrophoresis (CZE) for their high hydrophobicity (see **Figure 1** for their structures). Microemulsion electrokinetic chromatography (MEEKC) has been successfully applied to the analysis of very hydrophobic substances<sup>1,2</sup>. In this paper, a MEEKC method for the separation of nine biphenyl nitrile compounds was established employing a mixed microemulsion system. The components of microemulsion were systematically optimized. Meanwhile, the effects of organic additives, sample preparation and temperature on the separation were also studied comprehensively. Finally, the results of MEEKC were compared with those from micellar electrokinetic chromatography (MEKC).

### Experimental

All experiments were performed on a HP<sup>3D</sup> CE system with air-cooling and a diode-array detector (Agilent Technologies, CA, USA). A 40.0 cm × 50 μm I.D. uncoated fused silica capillary, (Ruifeng Inc., Hebei, China) was utilized and maintained at 35°C. The applied voltage was 25 kV, and the UV detection was set at 254 nm.

The new capillary was flushed with 1.0 mol/L sodium hydroxide (20 min), and then

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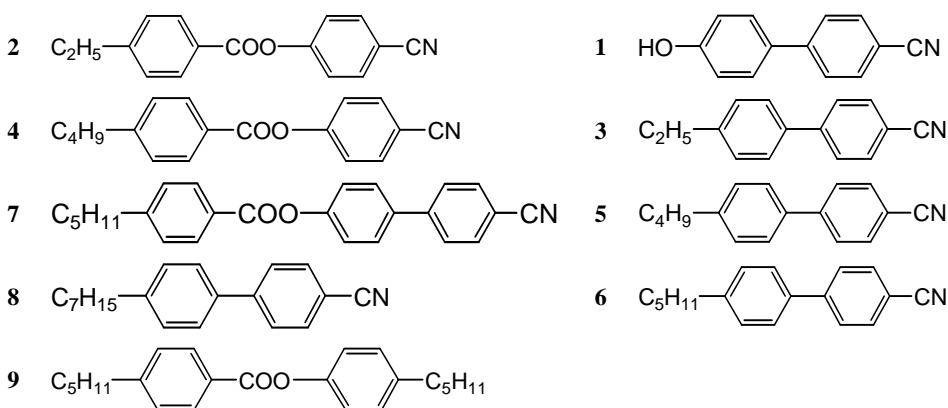
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redistilled water (10 min). The capillary was conditioned daily with 1.0 mol/L sodium hydroxide (5 min). Between consecutive analysis, the capillary was flushed with 0.5 mol/L sodium hydroxide (2 min), then with water (2 min) and finally with the running buffer (3 min) in order to improve repeatability.

SDS, CTAB and SC were purchased from Fluka, acetonitrile and methanol from Fisher. The other reagents were obtained from Beijing Chemical Factory (Beijing, China). Redistilled water was used for the preparation of solutions. Nine biphenyl nitrile compounds (as shown in **Figure 1**) were kindly presented by Department of Polymer Science and Engineering of Peking University. All reagents were of analytical-reagent grade except for HPLC grade acetonitrile and methanol.

The microemulsion system was prepared by sonicating a mixture of SDS, SC, *n*-butanol, heptane and 10 mmol/L sodium tetraborate buffer for 30 minutes, and then filtered through a 0.45  $\mu\text{m}$  filter before use. The studied solutes were dissolved in microemulsion solution and also filtrated through a 0.45  $\mu\text{m}$  filter before analysis.

**Figure 1** Structures of biphenyl nitrile compounds studied



## Results and Discussion

Surfactant is a key parameter in MEEKC separation. Both SDS and CTAB, when used individually, were not effective to separate these biphenyl nitrile compounds, although SDS showed a better resolution than CTAB. Considering the strong hydrophobicity of analytes, SC, which has both hydrophobic and hydrophilic region<sup>3,4</sup> was used to form a mixed microemulsion system with SDS, resulting in much lower partitioning coefficient between oil and aqueous phase for the solutes. A good separation of nine analytes was obtained when 120 mmol/L SDS and 100 mmol/L SC were utilized, but the elution time was very long (about 50 min), and the current was also high (180  $\mu\text{A}$ ) due to the high ionic strength of the microemulsion solution.

Organic solvents are usually added into the buffer to improve the separation, as demonstrated by Altria and his coworkers<sup>5</sup>. Methanol, acetonitrile, isopropanol, tetrahydrofuran and ethyl acetate were individually added to the microemulsion solution to study the effect of organic modifiers. Isopropanol, tetrahydrofuran and ethyl acetate had

a negative effect on the separation, while methanol and acetonitrile showed significant improvement in the peak shape and resolution. Consequently, acetonitrile and methanol were both evaluated as the modifier.

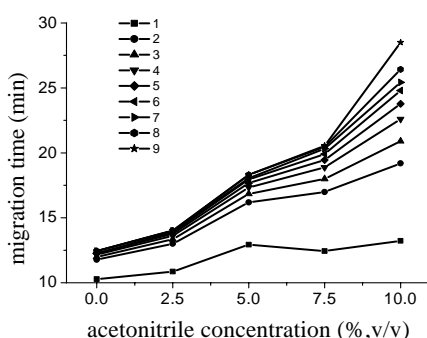
Acetonitrile with concentration from 2.5 % (v/v) to 12.5 % (v/v) (see **Figure 2**) and methanol from 2.5 % (v/v) to 15 % (v/v) were investigated. It was observed that the addition of 12.5 % (v/v) acetonitrile or 15% (v/v) methanol caused deemulsification. The reason is probably that the high surfactant concentration used in our study may limit the addition of acetonitrile with higher percentage.

When 10% (v/v) of acetonitrile or 10% (v/v) of methanol was added, the nine solutes were baseline separated, and the total analysis time with methanol was 35 min, while 28 min with acetonitrile. Besides, the theoretic plate number and the peak shape with methanol as a modifier is not as good as with acetonitrile. As a result, acetonitrile was selected as the most suitable modifier for further study. **Figure 3** illustrates a typical electrophoregram of the nine analytes when 10% acetonitrile was added.

The investigation of capillary temperature in a range of 25°C to 40°C demonstrated that the capacity factor  $k$  did not change much for most solutes, and 35°C was proved to be most suitable. In addition, acetonitrile and microemulsion solution were utilized to prepare the sample solutions, showing that much better resolution was obtained when the sample was prepared in microemulsion, and the theoretical plate numbers for the analytes were at least doubled, even increased by four times.

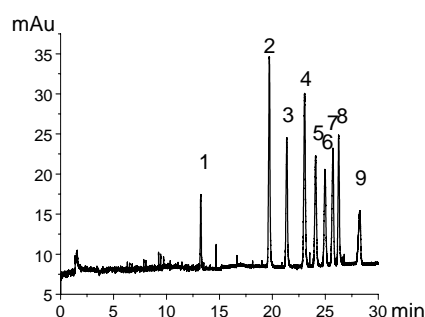
After all the optimizations, nine biphenyl nitrile compound were baseline separated (as shown in **Figure 3**) by using a mixed surfactant microemulsion system containing 100 mmol/L SDS, 80 mmol/L SC, 0.81% (w/w) heptane, 7.5% (w/w) *n*-butanol, 10% (v/v) acetonitrile and 10 mmol/L pH 8.9 borate buffer. The relative standard deviation of migration time for five runs was below 1.2%, and for peak area was below 4.8%, showing that the repeatability is well acceptable.

**Figure 2** Effect of acetonitrile concentration on the migration time of solutes



Separation conditions see Experimental and the curve number is correspondent to the compound number in **Figure 1**

**Figure 3** Electrophoregram of nine objects



Separation conditions see Experimental and the peak number is correspondent to the compound number in **Figure 1**

MEKC was initially applied in this separation and further optimized due to its advantages in the analysis of neutral solutes over CZE. A pH range from 8.0 to 10.5, borate concentration from 10 mmol/L to 50 mmol/L and SDS concentration from 30 mmol/L to 110 mmol/L were optimized with no obvious separation tendency. Considering the great improvement in MEEKC separation by SC and organic modifiers, SC and organic solvent were both added to the SDS micellar system. Unfortunately, after all these attempts, the nine compounds could not be baseline resolved. Therefore, it can be concluded that MEEKC showed advantages over MEKC in the analysis of these highly hydrophobic compounds. The reasons are properly the larger relative volume of pseudo-phase to aqueous phase in MEEKC, resulting in easier penetrating of analytes into the oil drops<sup>1</sup>, and the greatly extended migration window by increasing the surfactant concentration in MEEKC. In our study, the migration window for MEKC was only 6 min at most, while 55 min under the optimized MEEKC conditions.

### Conclusion

This work demonstrated that nine biphenyl nitrile compounds with strong hydrophobicity were successfully separated employing MEEKC method with a mixed surfactant system, and repeatabilities of migration time and peak area are satisfactory. MEEKC showed obvious advantages over MEKC for the separation of these water-insoluble compounds.

### Acknowledgment

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