



## Microemulsion Electrokinetic Chromatographic Separation and Determination of Three Benzoic Acid Compounds

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A novel microemulsion electrokinetic chromatography with photo diode array detection method was developed for separation and determination of 2-methoxybenzoic acid, 2-chlorobenzoic acid and 2-hydroxybenzoic acid. Under the conditions of microemulsion 10 % (v/v),  $\text{Na}_2\text{B}_4\text{O}_7$  20 mmol/L, pH 10.10 and separation voltage 12 kV, the separation was achieved. Good linear relationships between peak height and concentration were found in the tested range. Relative standard deviations ( $n = 5$ ) of migration times and peak heights were less than 1.95 and 3.26 %, respectively. The detection limits for 2-methoxybenzoic acid, 2-chlorobenzoic acid and 2-hydroxybenzoic acid were 14.1, 7.46 and 10.0 ng/L, respectively. The method was applied for the determination of the three compounds in lab waste water with recoveries of 94.2-112.4 %.

**Keywords:** Microemulsion electrokinetic chromatography, 2-Methoxybenzoic acid, 2-Chlorobenzoic acid, 2-Hydroxybenzoic acid.

### INTRODUCTION

Benzoic acid compounds are important chemical intermediates, which are the basic raw materials for medicines and dyes. 2-Methoxybenzoic acid is a type of organic synthesis intermediate, which is often applied to spices, medication, anticorrosion, *etc.* 2-Chlorobenzoic acid is an intermediate for dyes, medication or pesticides, to make preservatives and the raw materials of organic synthesis and antiseptic as well. 2-Hydroxybenzoic acid, also known as salicylic acid has contents of plants and is important fine chemical raw materials and in pharmaceutical industry a type of antiseptic with a wide usage and a kind of very important medicate intermediate. It was reported in the literature<sup>1</sup> that 2-hydroxybenzoic acid present in plant samples was determined by using formic acid as the interior standard and electrokinetic chromatography as separate mode.

Microemulsion electrokinetic chromatography (MEEKC) is a type of electrokinetic chromatographic separation mode, using microemulsion as back-ground electrolyte. In the specific application of MEEKC, there are many factors that affect the separation process, including all terms of surface active agent, cosurfactant, oil phase and aqueous phase, *etc.*<sup>2</sup>. Consequently it decides that MEEKC has some advantages such as better sample species diversity and bigger product capacity than other electrophoresis modes. Microemulsion electrokinetic chromatography is often used for the determination of oil-water

partition coefficient<sup>3</sup>, the separation and determination of substances<sup>4</sup>, the environmental analysis<sup>5,6</sup> and the separation of chiral compounds<sup>7</sup> and so on. But to our best of knowledge, there is no analytical methods (including no MEEKC method) reported for simultaneous determination of the three analytes above.

The microemulsion system in this experiment is made of sodium dodecyl sulfate (SDS), *n*-butyl alcohol, *n*-heptane and distilled water<sup>8</sup>. A novel MEEKC method based on it was then developed to separate and determine 2-methoxybenzoic acid, 2-chlorobenzoic acid and 2-hydroxybenzoic acid in the mixture.

### EXPERIMENTAL

Experiments were performed on a P/ACE™ MDQ capillary electrophoresis system from Beckman Coulter (Fullerton, CA, USA) equipped with a PDA detector using a 32 Karat software (Version 8.0 Beckman). Applied detection wavelength was 230 nm.

Chemicals are of analytical-reagent grade (AR) or chemical-pure grade (CP), including 2-methoxybenzoic acid (CP, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), 2-chlorobenzoic acid (AR, Guangfu Fine Chemical, Tianjin, China), 2-hydroxybenzoic acid (Tianjin Chemical Reagent, Tianjin, China), sodium tetraborate ( $\text{Na}_2\text{B}_4\text{O}_7$ , CP, Kaifeng No. 4 Chemical Plant, Kaifeng, China) and sodium dodecyl sulfate

(SDS, CP, Zhanyun Chemical Co., Ltd., Shanghai, China). All other chemicals were also made in China and of were analytical reagent grade. Distilled water was used throughout.

The stock microemulsion was prepared by mixing heptane (3.24 %, w/w), SDS (13.24 %, w/w), butanol (26.44 %, w/w) and distilled water (57.08 %, w/w). The buffers were prepared by mixing the stock microemulsion and 100 mM  $\text{Na}_2\text{B}_4\text{O}_7$  solution. The stock solution of 2 mg/mL 2-methoxybenzoic acid, or 2-chlorobenzoic acid, or 2-hydroxybenzoic acid solution is prepared in 1:1 ethanol-distilled water (v/v).

Lab wastewater was collected in a polypropylene plastic tube. After being shaken rigorously, blended and 20 min of 4000 rpm centrifuged and filtered through a 0.45  $\mu\text{m}$  membrane filter, supernatant fluid was selected for MEEKC analysis.

The separation was carried out on a 31.2 cm (21 cm to the detector)  $\times$  75  $\mu\text{m}$  id fused-silica capillary from Beckman Coulter (Fullerton, CA, USA). The capillary was treated prior to its first use by flushing with 1.0 M HCl for 20 min, 0.5 M NaOH for 20 min and distilled water for 10 min. At the beginning of each day, the capillary was rinsed with 0.5 M NaOH for 2 min and distilled water for 2 min, respectively. Between MEEKC runs, a rinse-cycle, 0.5 M NaOH for 0.5 min, distilled water for 0.5 min and run buffer for 0.5 min was used. At the end of each day, the capillary was rinsed with 0.5 M NaOH for 2 min and distilled water for 3 min. After optimization, the run buffer included 20 mmol/L  $\text{Na}_2\text{B}_4\text{O}_7$  and 10 % (v/v) stock microemulsion. The pH of run buffer was 10.10. The applied voltage was 12 kV. The capillary was maintained at 25  $^\circ\text{C}$ . Sample was injected by applying a pressure of 0.5 psi (1 psi = 6894.76 Pa) for 5 s.

## RESULTS AND DISCUSSION

The effect of the microemulsion concentration (expressed as volume percentage of the stock microemulsion added to the run buffer) was investigated in the range of 5-15 % while borax buffer solution was maintained 20 mmol/L. It was observed that the percentage was crucial for improving separation. When the ratio was less than or equal to 7.5 %, 2-chlorobenzoic acid peak was interfered by followed 2-methoxybenzoic acid peak. When the ratio was bigger than or equal to 12.5 %, there was big current led to large Joule heat and then low column efficiency and 2-chlorobenzoic acid peak was overlapped with 2-hydroxybenzoic acid peak. The final choice of microemulsion percentage was 10 %.

To improve the resolution of the three analytes, the effect of pH on the separation was investigated in the range of 9.30-10.30. It was observed that 2-chlorobenzoic acid peak was overlapped with 2-hydroxybenzoic acid peak with pH less than 9.70 and the resolutions increased when pH increased from 9.70-10.30. Also, the results indicated that the migration times

of the analytes increased and column efficiency became lower with the increasing of pH, so finally pH 10.10 was used for the separation.

The effect of applied voltage on the migration time of the analytes in this method was also investigated in the range 8-20 kV. The electropherograms showed that, with the increase in applied voltage, the migration time became shorter. However, the resolution between the peaks reduced. In this paper, 12 kV was applied for the final separation of the three analytes.

For evaluation of the quantitative applicability of the method, seven standard solutions of the analytes in the range of 400-4.0 mg/L were analyzed under optimized separation conditions. The linearity between the peak height and the concentration was investigated and the linear regression equations are illustrated in Table-1.

The reproducibility of the method was studied by measuring the migration times and peak heights of the three analytes at the top concentrations in the linear ranges. The relative standard deviations (RSD,  $n = 5$ ) of the migration time and peak height for the three analytes and the detection limits (based on signal-to-noise ratio  $S/N = 3$ ) were also shown in Table-1.

The method was applied to the analysis of the three analytes in the lab water waste. The typical electropherograms for the standard and the water waste are illustrated in Figs. 1 and 2, respectively. The determination recoveries are 108.7 % for 2-methoxybenzoic acid and 112.4 % for 2-chlorobenzoic acid and 94.2 % for 2-hydroxybenzoic acid, respectively.

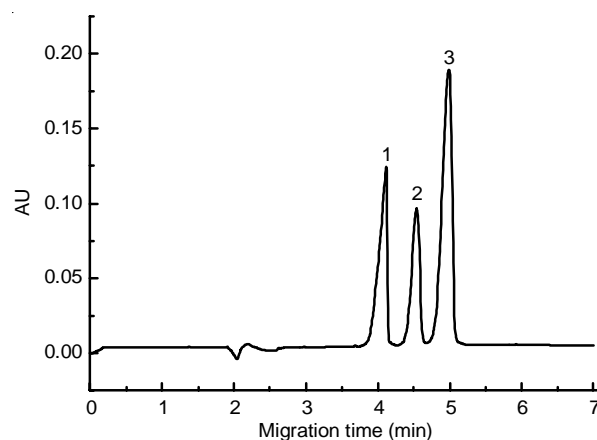


Fig. 1. Electropherogram for standard of the three analytes. Peak identification: 1, 2-methoxybenzoic acid; 2, 2-chlorobenzoic acid; 3, 2-hydroxybenzoic acid. HPCE conditions: 10 % microemulsion (v/v), sodium tetraborate 20 mmol/L, pH 10.10, separation voltage 12 kV

## Conclusion

A novel MEEKC with photo diode array detection method for separation and determination of 2-methoxybenzoic acid,

TABLE-1  
REGRESSION DATA AND DETECT LIMITS OF THE ANALYTES ( $n = 5$ )

Analyte	Regression equation*	Correlation coefficient	RSD (%)		Detection limit (ng/L)
			Time	Height	
2-Methoxybenzoic acid	$A = 0.00468 + 2.60078 \times 10^{-4}c$	0.9994	1.95	3.26	14.1
2-Chlorobenzoic acid	$A = 0.00182 + 7.38742 \times 10^{-4}c$	0.9991	1.85	2.81	7.46
2-Hydroxybenzoic acid	$A = 0.00374 + 4.34865 \times 10^{-4}c$	0.9985	1.35	3.01	10.0

\*A was peak height (Au); c was analyte concentration (mg/mL).

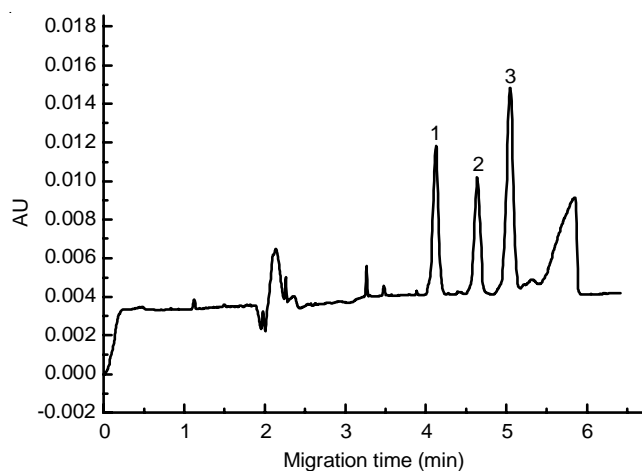


Fig. 2. Electrophorogram for lab water waste. Peak identification: 1, 2-methoxybenzoic acid; 2, 2-chlorobenzoic acid; 3, 2-hydroxybenzoic acid. For HPCE conditions see Fig. 1

2-chlorobenzoic acid and 2-hydroxybenzoic acid was developed with satisfactory results.

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