The Effects of Micelles Standing Time and Concentration on the Peak Splitting in MEKC

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ABSTRACT

The effects of sample injection time, pH of the buffer solution, micelles concentration and micelles standing time on the peak splitting in MEKC were explored respectively in the research. 4- methyl phenethyl alcohol, 4- methoxy phenethyl alcohol, 4- nitro phenethyl alcohol, benzyl alcohol and phenethyl alcohol were selected as analytes and were separated by CE in a 50 μm i.d. × 60 cm (effective length 45 cm) fused-silica capillary at 20.0 kV. The buffer solution in MEKC consisted of 15.0 mM Na2B4O7, 10.0 mM NaH2PO4 and specific concentration of SDS. Suggestions to avoid the peak splitting were given.

INTRODUCTION

Micellar electro-kinetic chromatography(MEKC) is an efficient separation technique, in which the surfactant is added into the buffer solution to form micelles, the micelles move to the detection side under the effects of electrophoresis and electro-osmosis in the electric filed [1-2]. The analytes are constantly distributed between the aqueous phase and the pseudo stationary phase, simultaneously migrate in the role of electro osmotic flow and the electrophoresis in the capillary [3], then the analytes are separated according to the difference of distribution and electrophoretic mobility. Therefore, both neutral and electrification analytes can be separated due to distribution and electrophoretic effects [4-5].

Peak splitting phenomenon in MEKC has been reported [6]. Obvious peak splitting phenomenon was observed by Tomas [7-8] when the methylated flavone aglycones was analyzed in MEKC. There are many reasons leading to the appearance of peak splitting phenomenon. Ermakov [9] believes peak splitting phenomenon will occur when the sample was with the same element composition but different charge states. Stevenson [10-11] attributed the peak splitting phenomenon to the deterioration of the buffer solution. Some other researchers argued that peak splitting phenomenon is caused by the stable products of chemical reaction between samples and surfactant [12]. The peak-splitting phenomenon has a
The detrimental effect on analyses in MEKC in some cases, so it is important to find out effective ways to avoid peak splitting phenomenon [13].

The influence of the sample injection time, pH of the buffer solution, micelles concentration and especially micelles standing time on the peak splitting were explored respectively in MEKC. The purpose of the study is to propose suggestions to avoid peak splitting.

MATERIALS AND METHODS

Instruments and Materials

The five samples were operated with a model TH-3100 CE system (Baoding Tianhui Institute of Separation Science, Hebei province, China) with a tunable wavelength UV detector. The power supply was conducted in voltage-controlled mode at 20.0 kV. In CE separation, a 60 cm×50 μm id fused silica capillary (Yongnian Optical Fiber, Hebei province, China) was used. The detective wavelength was 254 nm by UV - 6000 PC UV-Vis spectro-photometer (Instrument of Yuanxi company., LTD, shanghai, China). The detective sensitivity is 0.005. Data collection and processing was accomplished by CXTH-3100 software (Jinan, Shandong province, China). The ACCULAB analytical balance was purchased from Beijing Sartorius Instrument System co., Ltd (Beijing, China).

Chemicals and Buffers

All the reagents in the experiment were at least of the analytical level except the SDS. The five samples including 4- methyl phenethyl alcohol, 4- methoxy phenethyl alcohol, 4- nitro phenethyl alcohol, benzyl alcohol and phenethyl alcohol were purchased from Shanghai Chemical Technology( Co Ltd Shanghai D B, shanghai, China). The SDS was provided by (Shanghai Reagent Co., China National Pharmaceutical Group Co., Shanghai, China). Doubly de-ionized water (DDW) was purified by double distillation apparatus (Yitong Electrical Ltd. Co., Jiangsu province, China). Buffer solution consisted of 15.0 mM Na2B4O7(Tianjin Chemicals Industry, Tianjin, China), 10.0 mM NaH2PO4(Tianjin Chemicals Industry, Tianjin, China) and specific concentration of SDS. The diameter of the membrane to filter buffer solution was 0.45 μm. The pH of the buffer solution was adjusted to about 9.0 with 0.1 M HCL and 0.1 M NaOH.

General Procedure

The new capillary was flushed with methanol 10.0 min, 1.0 M HCl 10.0 min, H2O 5.0 min, 1.0 M NaOH 20.0 min, H2O 5.0 min. Between two runs, the capillary was rinsed with 1.0 M NaOH for 3.0 min and buffer solution for 2.0 min to ensure reproducibility of the separations.

RESULTS AND DISCUSSION

Effect of the Injection Time

The resolution was used to characterize the efficiency of the separation in this research. Injection time represents the injection volume of samples, the resolution
varied with the change of the sample volume. Therefore injection time affects the resolution of samples uncommonly. In a certain range, longer injection time led to the increase of the resolution, however when the injection time exceeded a certain value, the resolution would decrease with the injection time. Only at a specific injection time, the resolution enjoyed a maximum.

In Figure 1, when the injection time did not reach 21 s, the resolution of 4-methoxy phenethyl alcohol showed a rising trend and was less than 0.83. When the injection time exceeded 21 s, the resolution decreased obviously and as the injection time reached 30 s, the resolution was reduced to 0.68. Only at a specific injection time 21 s, the resolution reached the maximum of 0.87.

![Figure 1. Effect of the injection time on peak splitting.](image)

(1) 4- methyl phenethyl alcohol, (2) 4- methoxy phenethyl alcohol, (3) 4- nitro phenethyl alcohol, (4) benzyl alcohol, (5) phenethyl alcohol at 254 nm using 15.0 mM Na$_2$B$_4$O$_7$, 10.0 mM NaH$_2$PO$_4$ and 100.0 mM SDS as buffer solution(pH = 9.0).

Effect of the Concentration of the SDS

The effect of micelle concentration on separation is critical in MEKC. As was shown in the Figure 2(a), resolution of the samples showed good regularity and the regularities are similar among the different samples. In specific concentration range, resolution of the samples increased with the increasing of the SDS concentration. However, when the resolution reached the maximum, the high concentration of the SDS contributed largely to the decrease of the resolution. When SDS concentration increased from 60.0 mM to 100.0 mM, the resolution of phenethyl alcohol enjoyed an explosive growth before reaching the maximum of 0.78. After the SDS concentration exceeded 100.0 mM and reached to 140 mM, the resolution declined to 0.59.

Figure 2(b) is the electro-phoretogram of phenethyl alcohol. It can be seen that with the ascending of SDS concentration, the peak splitting phenomenon became conspicuous when SDS concentration was from 60 mM to 100 mM, therefore the SDS concentration really has a critical influence on the peak splitting in MEKC.

Peak splitting would not occur when the SDS concentration was below the CMC(9.33 mM). In addition, high concentration of SDS also made the peak splitting not obvious because of the thermal effect of the current.
Effect of the concentration of the SDS on peak splitting. (1) 4- methyl phenethyl alcohol, (2) 4- methoxy phenethyl alcohol, (3) 4- nitro phenethyl alcohol, (4) benzyl alcohol, (5) phenethyl alcohol at 254 nm using 15.0 mM Na2B4O7, 10.0 mM NaH2PO4 and SDS of different concentrations as buffer solution (pH = 9.0) at the optimal injection time respectively.

Figure 2(a).

Effect of the pH of the Buffer Solution

The pH is also one of the factors affecting the peak splitting in MEKC. It can not only affect the effective mobility and electroosmotic flow in MEKC but also the distribution between the aqueous phase and the micellar phase. Figure 3(a) showed that peak splitting did not appear until the pH was adjusted above 7.0. When the pH was 7.5, the resolution of the samples reached the maximum, however, severe peak broadening phenomenon would occur. After reaching the maximum, the resolution did not vary that much with the ascending pH. In the Figure 3(b), the migration time of the same samples was similar in different batches of experiments, which indicated that the reproducibility of the experiment was desirable.
Effect of the Standing Time of the SDS

The standing time of SDS has a noticeable impact on the peak splitting for it affects the formation of the micelle groups in the buffer solution. In principle, only when the micelle concentration in the buffer exceeds the critical micelle concentration (CMC), peak splitting phenomenon will occur. The effect of the standing time of the SDS was investigated when the injection time, concentration of the SDS and the pH of the buffer solution were determined, SDS used in the experiment was held still for a span of time.

Figure 4(a). Effect of the standing time of the SDS on peak splitting. (1) 4- methyl phenethyl alcohol, (2) 4- methoxy phenethyl alcohol, (3) 4- nitro phenethyl alcohol, (4) benzyl alcohol, (5) phenethyl alcohol at 254 nm using 15.0 mM Na₂B₄O₇, 10.0 mM NaH₂PO₄ and SDS (80.0 mM for 4- methoxy phenethyl alcohol, 120.0 mM for 4- methyl phenethyl alcohol, 100.0 mM for other three samples) as buffer solution (pH = 9.5).

Figure 4(b). The infrared spectra of the SDS mixing phenethyl alcohol (2 mg/L phenethyl alcohol in the buffer solution), the buffer solution consisted of 15.0 mM Na₂B₄O₇, 10.0 mM NaH₂PO₄ and 100 mM SDS (pH = 9.5).
In the Figure 4(a), the resolution of the five samples reached the peak value when the standing time was at 2 or 4 days (2 days for 4-nitro phenethyl alcohol and benzyl alcohol, 4 days for the other three samples). Before reaching the maximum of 0.78, the resolution of phenethyl alcohol enjoyed an explosive growth with the increase of the standing time. After reaching the peak value, the resolution of the five samples did not change significantly and basically remained stable. It is worth noticing that the peak splitting phenomenon will occur when the standing time is only 0 d long. When the standing time of the SDS was only several hours, the resolution of the 4-nitro phenethyl alcohol was 0.55, when the SDS solution was held still for 4 days the resolution of the sample was 0.75. Figure 4(b) showed the infrared spectrum of a mixture of phenethyl alcohol and SDS of different standing time. With the increase of the standing time of the SDS the vibration peak at 2471 cm\(^{-1}\) also changed gradually, which strongly proved that the micellar standing time did have a great influence on the peak splitting. Figure 4(c) is the electro-phoretogram of 4-nitro phenethyl alcohol. It could be seen that with the standing time of SDS growing longer, the peak splitting phenomenon became obvious when the standing time was less than 4 days, therefore the standing time of SDS really has a critical influence on the peak splitting in MEKC.

![Figure 4(c). Effect of the SDS standing time on the 4-nitro phenethyl alcohol buffer solution consisted of 15.0 mM Na\(_2\)B\(_4\)O\(_7\), 10.0 mM NaH\(_2\)PO\(_4\) and 100mM SDS(pH=9.5) at 254 nm.](image)

**CONCLUSION**

This paper explored several important factors leading to the peak splitting in MEKC: injection time, the concentration of the SDS, pH of the buffer solution and standing time of the SDS. It was discovered that longer sample injection time, higher concentration of the SDS, specific standing time of the SDS and higher pH the buffer solution caused peak splitting easily. Therefore, reducing sample injection time and concentration of the SDS, choosing appropriate standing time, maintaining a lower pH value could avoid peak splitting effectively. However, high concentration of SDS was necessary to provide micellar groups while low concentration of SDS failed to do such work. Besides, the influence of the pH of the buffer solution on the resolution was not significant enough. Therefore, the most practical methods to avoid peak splitting are choosing the appropriate injection time, SDS concentration and the standing time of the SDS.
REFERENCES