Investigation on False Peak Phenomena in On-line Sweeping Technique in MEKC

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In this paper, several factors that could lead to the appearance of false peak were investigated by using on-line sample sweeping technique under different experimental conditions. The tested analytes were buflomedil hydrochloride, ephedrine hydrochloride, benzyl alcohol, vanillin, *p*-hydroxybenzaldehyde and *m*-methylphenol. Results showed that among the six compounds, three of them, *i.e.*, buflomedil hydrochloride, ephedrine hydrochloride and benzyl alcohol will cause false peaks to appear when sample injection time is long, sodium dodecyl sulfate (SDS) concentration is high and there is pH gradient between cathode and anode. In order to avoid the appearance of false peak, the pH gradient should be avoided.

Keywords false peak, MEKC, on-line sweeping technique

Introduction

Micellar electrokinetic chromatography (MEKC) was invented to extend the utility of capillary electrophoresis (CE) and permitted the separation and analysis of neutral compounds. But just like other modes of CE, MEKC are hampered by the low concentration sensitivity of the ultraviolet (UV) detector. In order to improve the concentration detection limits of MEKC, many methods such as off-line sample concentration (*e.g.*, solid-phase extraction), transient isotachophoresis,[1] increasing the path-length for photometric detector,[2-4] using high sensitivity detector[5-9] and on-line concentration have been used.

On-line sweeping is one of on-line concentration approachs which is defined as the picking and accumulating of analyte molecules by the pseudo-stationary phase that enters and fills the sample zone upon application of voltage.[10] 5000-Fold improvement have been reported,[10,11] which is greater than that by any other reported concentration techniques. The sample sweeping is much efficient and independent of the electroosmosis flow (EOF), thus it is becoming an almighty technique. In addition it works for not only charged solutes but also uncharged solutes.

However, despite its above advantages, this method suffers some limitations in practical applications. The false peak phenomenon is one of the limitations because of its giving false information which would lead to wrong result.

False peak phenomena were ever discussed in capillary electrophoresis. Maria *et al.*[12] reported that when separating methylated flavone aglycones using sodium dodecyl sulfate micellar as pseudo-stationary phase in MEKC, the doublelet and triplelet phenomena for some components were discovered. However, the mechanism of the phenomena has not been investigated deeply yet.

Just like MEKC, the false peak phenomenon also exist in on-line sample sweeping. The present work was to investigate the false peak phenomena appearing in on-line sample concentration in MEKC and the factors that would lead to the appearance of false peak by using on-line sample sweeping technique under different experimental conditions.

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Experimental

Apparatus

The experiment was performed on a Waters Quanta 4000E capillary electrophoresis system (Milford, MA, USA) with a built-in 0—30 kV high voltage power supply, a fixed wavelength UV detector near the cathodic end and a forced-air cooling system. Uncoated fused capillary (75 μm I.D., total length is 60 cm and the effective length is 52 cm) was from Yong Nian Optical Fiber Factory, Hebei Province, China. The UV detector wavelength was 214 nm. The temperature remained constant at 25 ℃. Samples were introduced to the capillary by using gravity injection and the injection height was 12 cm. Data processing was carried out with a Waters Millennium 2010 chromatography system. The sensitivity of the detector was set at 0.005.

Reagents and solutions

Buflomedil hydrochloride was provided by Bohai Pharmaceutical Factory. Sodium dodecyl sulphate (SDS) was purchased from Zhongxi Chemical Plant. The other chemical reagents are all of analytical grade. Stock solutions of buflomedil hydrochloride (3.0 mg/mL), ephedrine hydrochloride (3.0 mg/mL), benzyl alkohol (3.0 mg/mL), anillin (3.0 mg/mL), *p*-hydroxybenzaldehyde (3.0 mg/mL), *m*-methylphenol (3.0 mg/mL) were prepared with double-distilled water at pH 2.5.

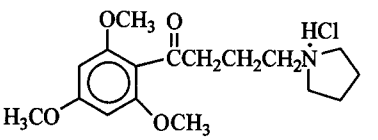
All of the backgrounds (BGs) were prepared by mixing stock solutions of 200 mmol/L SDS, 100 mmol/L sodium borate and 200 mmol/L NaH_2PO_4 with double-distilled water and filtered through 0.45 μm filters (Ruili Separation Instrument Factory, Shanghai, China) prior to use.

Procedures

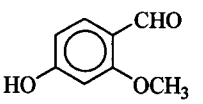
The capillary was purged with 1 mol/L NaOH (20 min), followed by methanol (20 min), 0.1 mol/L NaOH (20 min), double-distilled water (20 min) and the BGs (5 min) before use. In order to ensure repeatability, the capillary was purged between consecutive analysis with 0.5 mol/L NaOH (4 min), purified water (3 min) and BGs (3 min).

Results and discussion

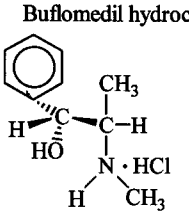
In order to investigate the appearance of false peaks, four kinds of factors including injection time, SDS concentration, buffer solution pH value and pH gradient of buffer have been studied. The molecular structures of buflomedil hydrochloride, ephedrine hydrochloride and vanillin are showed as follows:



Buflomedil hydrochloride



Vanillin



Ephedrine hydrochloride

Effect of the injection time

In the experiment, when the buffer solution [SDS (95 mmol/L) + $Na_2B_4O_7$ (15 mmol/L) + NaH_2PO_4 (14 mmol/L), pH = 9.05] and the pH value of the sample solutions (pH = 2.5) were maintained unchanged, the effect of different injection time on the appearance of false peaks was investigated in the range from 5 s to 120 s. For buflomedil hydrochloride, it was shown that the peak was split into two peaks of unequal height when the injection time was over 70 s (Fig. 1). Resolution of the two peaks increased and the peak area ratio of them was instable with the increasing of injection time. For benzyl alcohol, a false peak appeared when the injection time was above 60 s (Fig. 2). For ephedrine hydrochloridel, false peak also appeared when the injection time was over 60 s (Fig. 3) and more false peaks could be found as the injection time increased. For Vanillin, *p*-hydroxybenzaldehyde, *m*-methylphenol, false peaks could not be found even the injection time was above 120 s.

Effect of SDS *concentration*

In this experiment, the effect of SDS concentrations ranging from 10 mmol/L to 100 mmol/L was investigated when the injection time was kept at 120 s and other experiment condition were the same as the above. For bu-

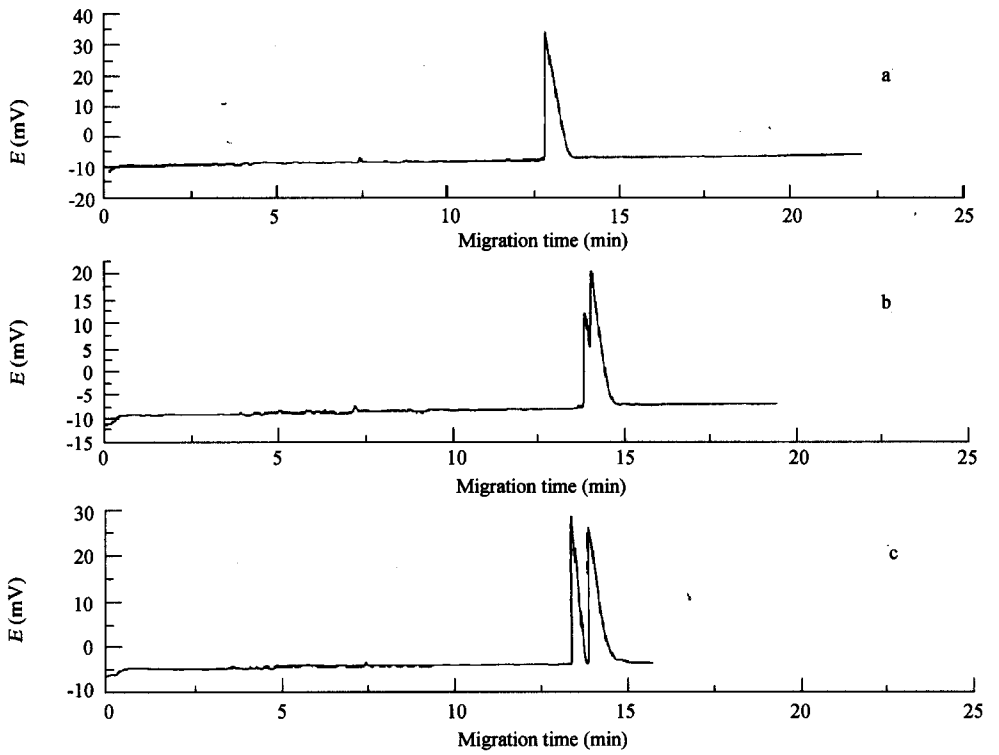


Fig. 1 Effect of the injection time on the false electropherograms for buflomedil hydrochloride. Buffer, $Na_2B_4O_7$ (15 mmol/L) + NaH_2PO_4 (14 mmol/L) + SDS (95 mmol/L) at pH = 9.05. Column, 75 μm I.D. × 60 cm (effective length 52 cm). Applied voltage, 18 kV. UV detector wavelength, 214 nm, injection time. (a) 55 s, (b) 70 s and (c) 120 s. Sample pH = 2.0, sample concentration 0.50 mg/mL.

flomedil hy drochloride, false peak appeared when SDS concentration was above 85 mmol/L and the resolution of the two peaks increased with the increase of SDS concentration (Fig. 4). From the experiment, it was shown that the ratio of peak area and peak height of the two splitting peaks of buflomedil hydrochloride maintained unchanged. For ephedrine hydrochloride and benzyl alcohol, false peaks could also be found when SDS concentration was above 90 mmol/L.

Effect of buffer solution pH *value*

It was reported that sweeping technique was possible in the presence or in the absence of EOF.[2,3] In this paper, the effect of buffer pH value on the appearance of false peaks was investigated when the other conditions were not changed [buffer: SDS (95 mmol/L) + $Na_2B_4O_7$ (15 mmol/L) + NaH_2PO_4 (14 mmol/L), pH = 9.05, injection time 120 s]. From the experiment it was found that when the pH value was higher than 8.5, false peak appeared for buflomedil hydrochloride, ephedrine hydrochloride and benzyl alcohol. When the pH value was lower than 8.0, the retention time of these compounds became too long to detect the peak because of low EOF.

Effect of pH *gradient of buffer*

As a matter of fact, all of the effects on false peak were investigated under pH gradient existing. The pH gradient was achieved by maintaining the pH in the cathode vial at 9.05 but decreasing the pH in the anode vial so that the pH differences of the two vials were changed from 0.3 to 0.5. However, when there was not pH gradient, for all the six kinds of compounds there appear no false peaks.

Mechanism discussion

From above experiments, it can be seen that false peak phenomena can show up under different conditions. Although the reasons are not very clear for the false peak's showup, the following might be an explanation for

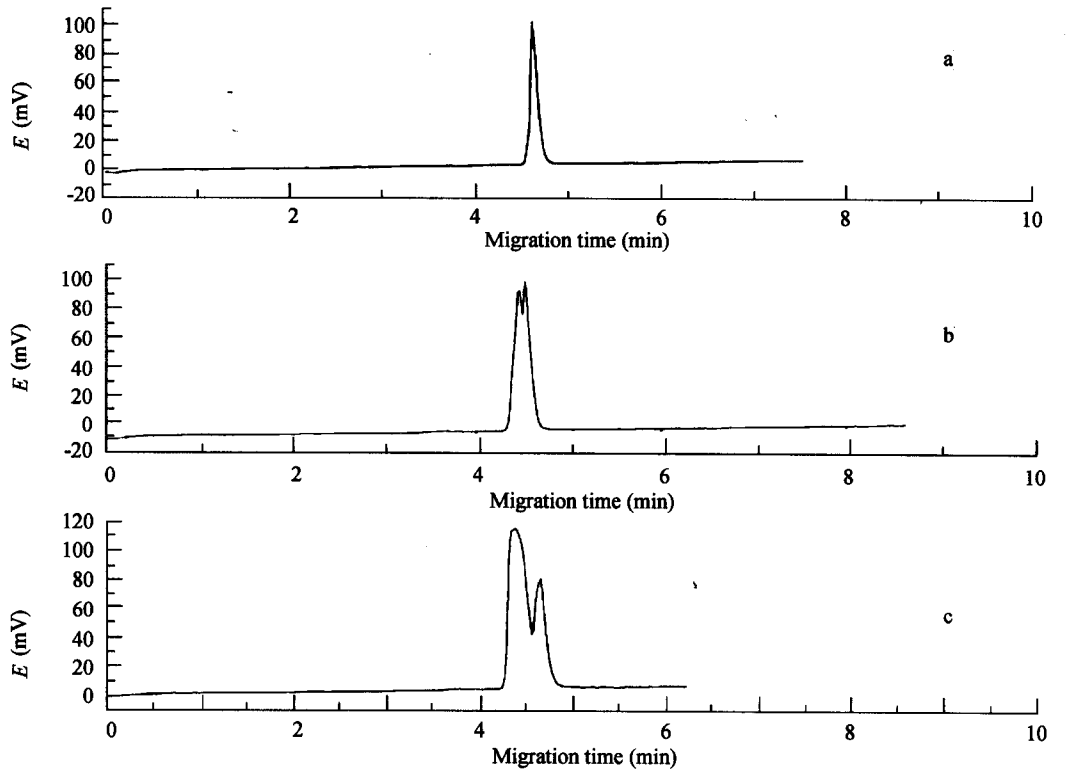


Fig. 2 Effect of the injection time on the false peaks for benzalcohol. Injection time (a) 30 s, (b) 60 s and (c) 120 s. Sample solution pH = 2.0, sample concentration 0.75 mg/mL. The other conditions are the same as those in Fig. 1.

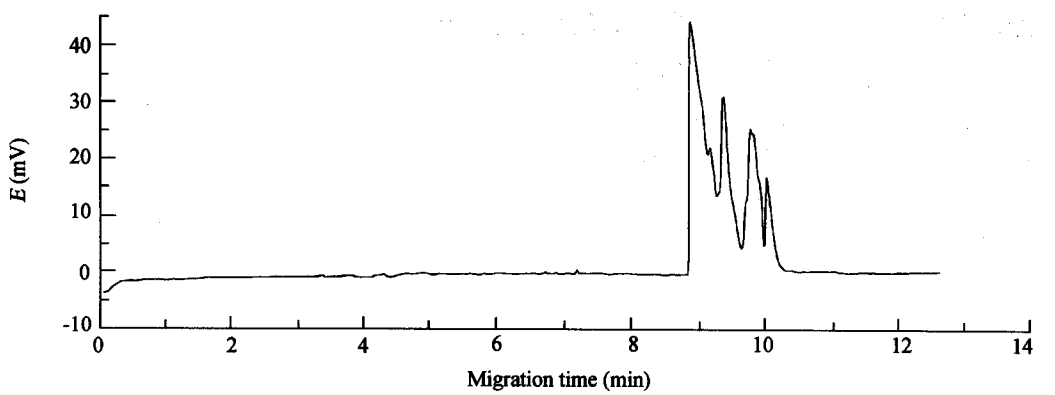


Fig. 3 False peaks of ephedrine hydrochloride. Injection time 120 s. Sample solution pH = 2.0, sample concentration 0.5 mg/mL. The other conditions are the same as those in Fig. 1.

the false peak's appearance in on-line sample concentration by sweeping technique.

Injection time

From the experiment, it was shown that when the injection time was short (such as 5 s), false peak would not appear for these analytes. The effect of injection time on false peak might be that when the injection time is short, such as 5 s, the sample solution will be mingled with the buffer instantly, and no sweeping occurs. Only when the injection time is long enough, the extruding of two sides of the sample zone will become obvious and the false peak would appear.

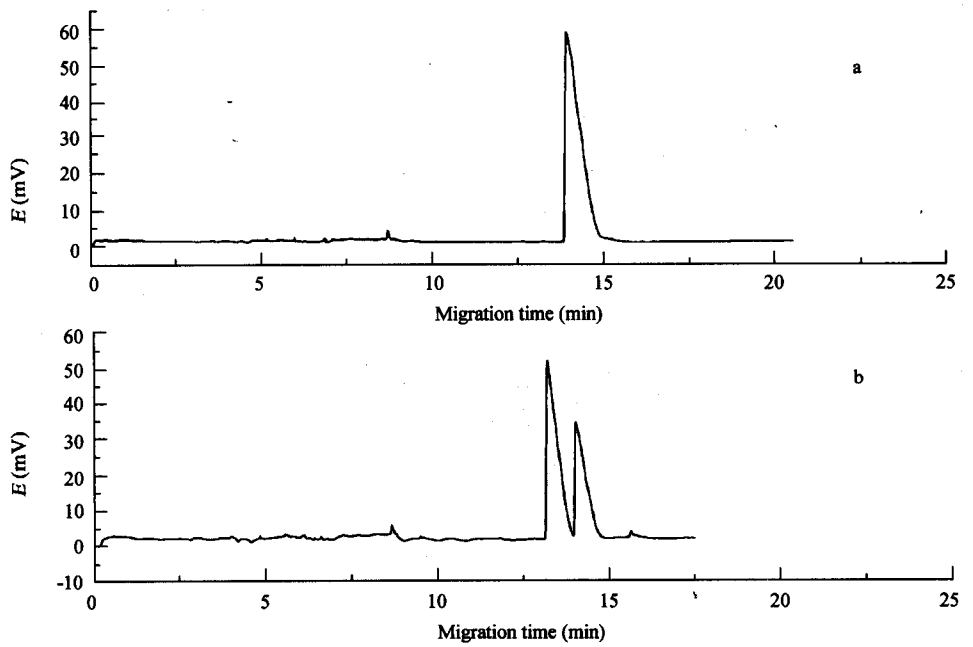


Fig. 4 Effect of the concentration on the false peaks for buflomedil hydrocholride. Buffer, $Na_2B_4O_7$ (15 mmol/L) + NaH_2PO_4 (14 mmol/L) + 70 mmol/L SDS (a) and 100 mmol/L SDS (b) at pH = 9.05. Sample pH = 2.0, sample concentration 0.50 mg/mL and the other conditions are the same as Fig. 1.

Molecular structure

From the experiment it can be seen that the appearance of false peak was influenced by molecular structure also. Of the six compounds investigated, vanillin, *p*-hydroxybenzaldehyde and *m*-methylphenol did not show any false peak under the above experiment conditions. Compared with other three compounds, they are more structurally stable.

The above only gives some possible explanation for these phenomena. In fact pH gradient is a very important factor, but the reason is not clear. Further study is needed to get an overall understanding of the theoretical mechanism.

Conclusion

Sweeping is a kind of good on-line sample concentration technique. In order to avoid the appearance of false peak and acquire right information, the choice of the electrophoresis condition must be careful. But first of all, the pH gradient should not exist.

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