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High-Efficiency On-Line Concentration Technique of Capillary Electrochromatography

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With the coexistence of the mobile phase, the stationary phase, and the electric field in capillary electrochromatography, the chromatographic zone-sharpening effect and field-enhanced sample-stacking technique were utilized to improve detection sensitivity. By the former means, with less organic modifier in the sample solution compared to that in the mobile phase, the concentration factors of neutral solutes benzoin and mephenytion were 134 and 219, respectively. Through the latter one, without electrolyte in the sample solution, the detection sensitivity of propatenene with positive charge was improved by 1600 times. While with the combination of these two methods, improvement of over 17 000 times for the sensitivity of propatenene was obtained. By the combined means, the analysis of basic pharmaceutical compounds at concentrations of nanograms per milliliter by UV detection was realized. In addition, parameters that might affect the efficiency of on-line concentration were studied and equations that described the on-line concentration procedure were deduced.

Capillary electrochromatography (CEC), as a hybrid of high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), has the advantages of high resolution, high efficiency, low consumption of solvents and samples, and so on. Accordingly, it has been regarded as one of the most promising separation techniques and has been applied in different fields.[1−8]

However, similar to other CE techniques, the relatively low detection sensitivity is one of the most serious problems that CEC has to solve. Although many methods to decrease the limit of detection of CE have been proposed, such as using laser-induced fluorescence detector,[9−11] modifying the structure of the detection cell,[12−14] and taking advantage of the existence of micelles in micellar electrokinetic capillary chromatography (MECC),[15] not much work in this aspect of CEC has been carried out and the improvement of detection sensitivity was only about 1−2 orders.[16−21]

The chromatographic zone-sharpening effect and field-enhanced sample-stacking effect have already been used in HPLC and CE to lower the detection limit of neutral and charged solutes. Recently, research on the high-efficiency on-line concentration of CEC by either one or the combination of these two techniques was carried out in our group. The detection sensitivity in analysis of neutral and positively charged samples has been improved by 2−4 orders, which are much more than those previously reported.

EXPERIMENTAL SECTION.

Materials. Hypersil ODS (3 μm) was bought from Shandon. Benzoin (from Sigma Chemical Co., St. Louis, MO), mephenytoin, and propatenene (both kindly donated by Dr. Jianzhong Rui of the Capital Hospital of Nanjing Army, China) were of analytical grade. Acetonitrile (ACN), purchased from Yuwang Chemical Plant (Shandong, China), was of chromatographic grade. Tris(hydroxylmethyl)aminomethane (Tris) (Shanghai No.1. Chemical Plant, China) was analytical grade. Triethylamine (TEA) (Shenyang Chemical Plant, China) was of analytical grade. Double deionized water was purified by Milli-Q (Millipore, Waltham, MA). Capillary with 75 μm inner and 365 μm outer diameters was purchased from Yongnian Optic Fiber Plant (Hebei, China).

Procedure. CEC columns were prepared by the slurry method on a HPLC pump (Spectra-physics Inc., San Jose, CA).

Experiments were performed on a Beckman P/ACE 5010 (Beckman Inc.) instrument with the column temperature at 20 °C, UV detection wavelength of 214 nm, and separation voltage of 10 kV. The concentration of the samples was 5 μg/mL.

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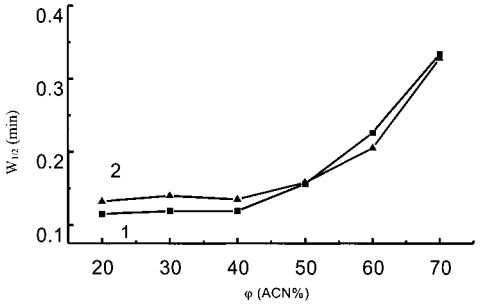

Figure 1. Effect of the volume fraction of ACN (φ) in the sample solution on the peak width at half-height of neutral solutes. Experimental conditions: stationary phase, 3 μm Hypersil ODS, packed/total column length 20/27 cm; mobile phase, V_{ACN}:V_{H_2O} = 70/30, 2 mM Tris, pH 7.60; injection, 10 kV 20 s. Samples: 1, benzoin; 2, mephenytoin.

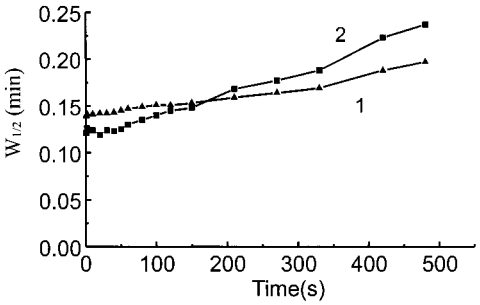

Figure 2. Effect of injection time on the peak width at half-height of neutral solutes. Experimental conditions: sample solution, V_{ACN}:V_{H_2O} = 30/70; others were the same as shown in Figure 1. Samples: 1, benzoin; 2, mephenytoin.

RESULTS AND DISCUSSION

Because of the coexistence of the mobile and stationary phases in CEC, neutral solutes injected for a relatively long time on the column could be retained on the stationary phase because of the distribution between the two phases. Once they were eluted by a mobile phase with stronger elution power compared to the sample solution, the sample zone would be sharpened. Accordingly, the detection sensitivity of compounds might be improved. However, an important factor—the loadability of samples—must be taken into consideration. Otherwise, the overload of samples might lead to the loss of column efficiency, resulting in poor resolution. Accordingly, to decrease the detection limit of CEC, the injection procedure of samples must be optimized.

In a certain mobile-phase system, the effects of organic modifier concentration in the sample solution on peak width at half-height of neutral solutes ($W_{1/2}$) were studied systematically, as shown in Figure 1. It could be seen that when the concentration of ACN in the mobile phase was fixed at 70% (volume percentage), with less than 40% ACN in the sample solution, a relatively long injection time had little effect on $W_{1/2}$, while with a further increase of ACN concentration in the sample solution, $W_{1/2}$ increased greatly, resulting in the obvious decrease of column efficiency. Considering that too much water in the sample solution might lead to easy formation of bubbles in the column system because of the hydrophobic surface of the stationary phase, we chose 30% ACN as the optimum organic modifier concentration of the sample solution in our experiments.

Injection time was another factor that affected the quantity of the samples injected onto the column. From Figure 2 it could be seen that injection times up to 500 s had not great effect on $W_{1/2}$ of compounds with 10 kV as the injection voltage. Under such conditions, the more samples injected into the column, the higher the improvement of detection sensitivity. By study of the linear ranges between injection time and peak height, 330 and 480 s at 10 kV were chosen as the optimum injection conditions for mephenytoin and benzoin, respectively.

On the basis of the optimum conditions discussed above, with 30% ACN in the sample solution and 70% ACN in the mobile phase,

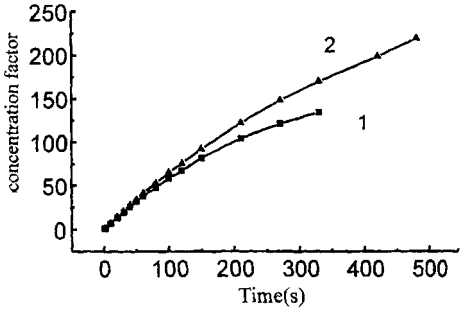

Figure 3. Effect of injection time on the concentration factor of neutral compounds. Experimental conditions: the same as shown in Figure 2. Samples: 1, benzoin; 2, mephenytoin.

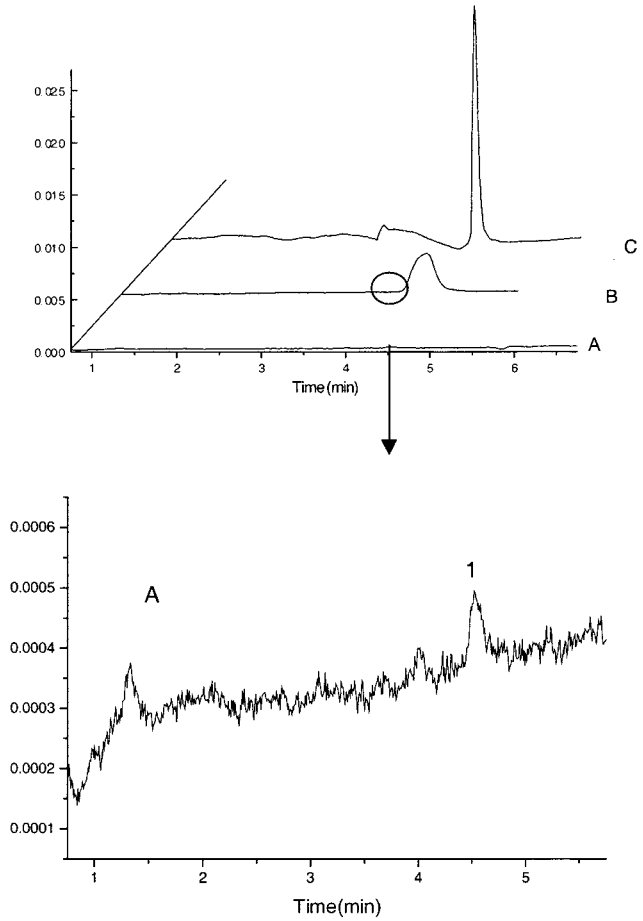

Figure 4. Electrochromatograms of propatenene with normal injection and on-line concentration by the field-enhanced sample-stacking technique. Experimental conditions: mobile phase, V_{ACN}:V_{H_2O} = 85/15, 2 mM Tris, 0.6 mM TEA; sample solutions, (A) and (B) the same as the mobile phase, (C) V_{ACN}:V_{H_2O} = 85/15; injections (A) and (B) 10 kV, 1 and 10 s, respectively, (C) 10 kV, 20 s; others were the same as shown in Figure 1. Propatenene concentration: (A) and (B) 5 μg mL^{-1}; (C) 0.5 μg mL^{-1}.

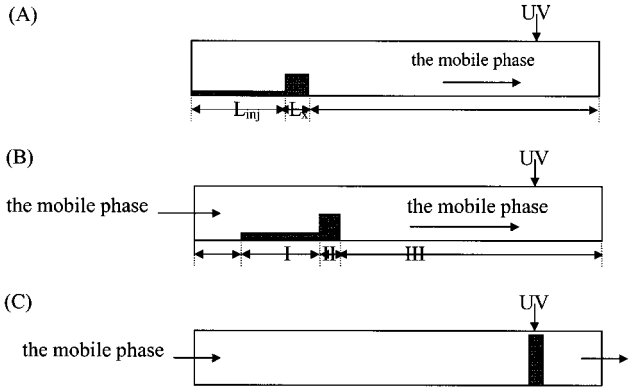

Figure 5. Procedure for the on-line concentration of charged solutes in CEC with the combined methodelectrokinetic injection of sample solution with low elution strength and low electrolyte concentration; analyte eluted by mobile phase with high elution strength and high electrolyte concentration condensed sample zone passing through the detection window.

under the injection conditions of 330 and 480 s with 10 kV voltage, the concentration factors for mephenytoin and benzoin, which were defined as the ratios of peak heights of solutes after and before the concentration procedure, were calculated to be 134 and 219 in two individual runs (shown in Figure 3).

Besides the mobile and stationary phases, an electric field also existed in CEC. Accordingly, a field-enhanced sample-stacking technique could also be utilized to make the detection limit low in analysis of charged samples. Once the concentration of electrolyte in the sample zone was lower than that in the mobile phase, the electric field added on it would be higher than that on the mobile-phase zone. Consequently, the charged analytes migrated fast in the sample zone. Once the analytes moved to the boundary of the sample zone and the mobile phase, the velocity was reduced and the sample zone was condensed.

Considering the interaction between positively charged samples and negatively charged surface of the stationary phase, TEA was added into the mobile phase. Figure 4 showed the electrochromatograms of propatenene resolved in the mobile-phase solution with and without electrolyte. It could be seen that, without electrolyte in the sample solution, high detection sensitivity could be obtained for compounds with low concentration by increasing the injection times. According to eq 1, defined for the concentration factor of propatenene, 1626 times improvement of the detection sensitivity was obtained, which demonstrated that the field-enhanced sample-stacking technique was quite efficient to decrease the detection limit of the charged samples in CEC.

$$\chi = \frac{h/C}{h_0/C_0} \qquad (1)$$

Here h and h_0 were peak heights of the compound under final and initial conditions; C and C_0 were the concentrations of the compound in the two statements, respectively.

As mentioned above, the improvements of detection sensitivity were 2−3 orders by either the chromatographic zone-sharpening effect or field-enhanced sample-stacking effect. Because of the coexistence of both the mobile and stationary phases as well as the electric field in CEC, these two methods could be combined

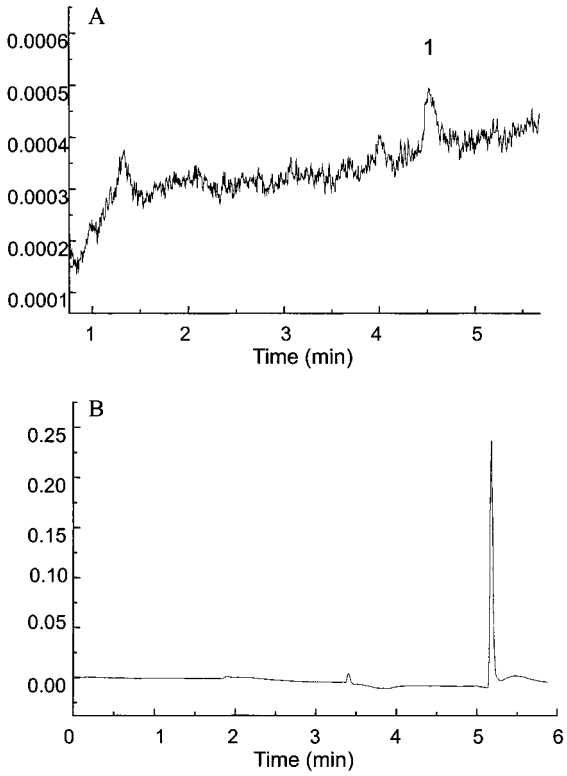

Figure 6. Electrochromatograms of propatenene with normal injection and on-line concentration with the combination of chromatographic zone-sharpening effect and field-enhanced sample-stacking technique. Experimental conditions: mobile phase, V_{ACN}:V_{H_2O} = 85/15, 2 mM Tris, 0.6 mM TEA, pH 7.60; sample solutions, (A) the same as the mobile phase, (B) V_{ACN}:V_{H_2O} = 40/60; injections, (A) 10 kV, 1 s, (B) 10 kV, 120 s; others were the same as shown in Figure 3. Propatenene concentrations: (A) 5 μg/mL; (B) 0.5 μg/mL.

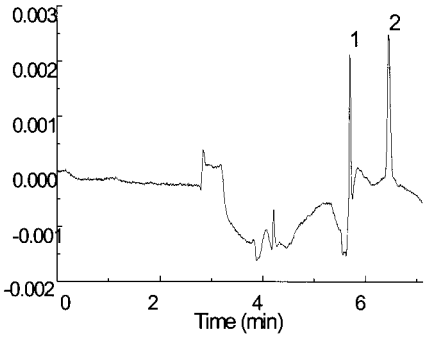

Figure 7. Electrochromatogram of positively charged solutes with a high-efficiency on-line concentration technique. Experimental condition: mobile phase, V_{ACN}:V_{H_2O} = 85/15, 2 mM Tris, 0.6 mM TEA, pH 7.60; sample solution, V_{ACN}:V_{H_2O} = 40/60; injection, 10 kV 120 s; others conditions were the same as shown in Figure 3. Sample: (1) 2 ng/mL verapamil; (2) 5 ng/mL norverapamil.

to improve the detection sensitivity of charged samples further. Such a procedure was described in Figure 5. The charged analyte was prepared in a solution with lower elution strength and lower concentration of electrolyte compared to the mobile phase. Once it was injected onto the column, the sample zone was composed of two parts with different concentrations. The higher part was caused by the field-enhanced sample-stacking effect and the lower part was the same as that in the sample solution, as shown in Figure 5A. With the elution of the mobile phase, the sample zone was further condensed by the chromatographic process since the

elution power of the mobile phase was strong, as shown in Figure 5B. When the sample passed the detection window, the detection sensitivity was improved greatly by the combination of these two procedures, as shown in Figure 5C.

On the basis of our previous theoretic work, the concentration factor of charged samples in CEC (χ) could be described by the following equations,[22]

$$\chi = \sqrt{\frac{\left(\frac{u_1}{u_2}\right)^2\left(\frac{L_I}{L_{inj}}\right)^2\left[1+\frac{L_x}{L_{inj}}\delta\right]^2}{1+\left(\frac{u_1}{u_2}\right)^2\left(\frac{L_x}{L_{inj}}\right)^4\delta^2+6\left(\frac{u_1}{u_2}\right)\left(\frac{L_x}{L_{inj}}\right)^2\delta+4\delta\left(\frac{L_x}{L_{inj}}\right)^2}} \quad (2)$$

$$u_1/u_2 = \gamma(1+k_2')/(1+k_1') \quad (3)$$

$$L_I = u_2 t_{inj} \quad (4)$$

$$L_{inj} = \bar{u}_{eo1} t_{inj} \quad (5)$$

$$L_x = \bar{u}_2 t_{inj} \quad (6)$$

$$\delta = (1+\beta k_1')/\gamma(1+\beta k_2') \quad (7)$$

where u_1 and u_2 were the migration velocities of the analyte in parts 1 and 2, respectively; L_{inj}, L_x, and L_I were the length of injection in the column, condensed length by the field-amplifying sample-stacking effect, and part I, respectively; γ was the ratio of the electric field strength in the sample zone and the mobile phase; β was the phase ratio; k_1' and k_2' were the capacity factors of analytes in the sample zone and the mobile phase; $\bar{u}_{eo1}$ was the average electroosmotic velocity in the sample plug during injection; and $\bar{u}_2$ was the average migration velocity of analyte in the mobile phase during injection.

(22) Zhang, W.; Xu, G.; Zhang, Y. Proceedings of 11th National Chromatography Conference, Dalian, China, August 1997; p 705.

From these equations, it could be seen that the higher difference between capacity factors of the analytes in the sample zone and the mobile phase and longer injection time were helpful to increase the detection sensitivity. In addition, an optimum value existed to obtain the highest concentration efficiency. In our experiment, the concentration factor of protatenene could reach ~17 000 under the optimum separation conditions, as shown in Figure 6, which was much higher than other published papers in this field of CEC.

In addition, by such a combined method, two basic compounds, verapamil and norverapamil, at concentrations of 2 and 5 ng/mL, were separated with high efficiency, high resolution, and high detection sensitivity (as shown in Figure 7), which demonstrated that such an on-line concentration technique could make the UV detection of trace charged sample at concentrations on the order of nanograms per millileter possible.

CONCLUSION

All these results mentioned above demonstrated that on-line concentration techniques of CEC with the chromatographic zone-sharpening effect, field-enhanced sample-stacking technique, and the combination of them could improve the detection sensitivity of neutral samples by 2 orders and that of positively charged analytes over 4 orders. These techniques could be useful to broaden the application of CEC in the trace analysis of pharmaceutical, environmental, and biological samples. Further work on the quantitative prediction of the concentration factor of analytes and the on-line concentration of negatively charged solutes is being carried out.

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