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# Effect of various modes of pressurization on the separation in capillary electrochromatography

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## Abstract

In capillary electrophoresis and capillary electrochromatography, the driving factor of the separation is electroosmotic flow (EOF). Pressurized capillary electrochromatography, in which the separation is controlled by EOF as well as the pressure, becomes more and more attractive. We studied the influence of various pressures on capillary electrochromatographic separation. The results reveal that in pressurized capillary electrochromatography, which was performed by EOF combined with the forward and reverse pressure, the main driving factor is still the EOF. It was also found that, when reverse pressure was applied in capillary electrochromatography, the repeatability of the capillary electrochromatographic separation was increased dramatically. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Electrochromatography; Pressurized electrochromatography; Thiourea; Phenylenediamine; Toluene

## 1. Introduction

Pressurized capillary electrochromatography (pCEC) is a novel technique, which involves the application of pressure in the electrochromatographic separation by using a HPLC pump [1]. Though the application of the pressure will change the flow type and lead to a decrease of the column efficiency, the pump pressure could be applied as another driving factor to increase the flow-rate and shorten the separation time. This will benefit the separation especially when the electroosmotic (EOF) of the column is too low. Another advantage is that the application of pressure in capillary electrochromatography could avoid the formation of bubbles during the separation, so that the repeatability is

increased. Through adjusting the ratio of the pump pressure and the separation voltage, the resolution of the separation could be increased [2]. Recent research on pCEC mainly focused on the following three aspects: to develop new pCEC instruments [3,4] and new packing materials used in pCEC columns [5–7], to study the effect of various driving factors on the separation mechanism of pCEC [8,9], and to widen the application field of pCEC [10,11]. We tested the effect of various pressures on the pCEC separation, and found that reversed pressure could increase the repeatability of pCEC. The results obtained here may be a useful addition to the pioneer research on pCEC [12–20].

## 2. Experimental

### 2.1. Reagents and materials

Thiourea, toluene, phenylenediamine, sodium bo-

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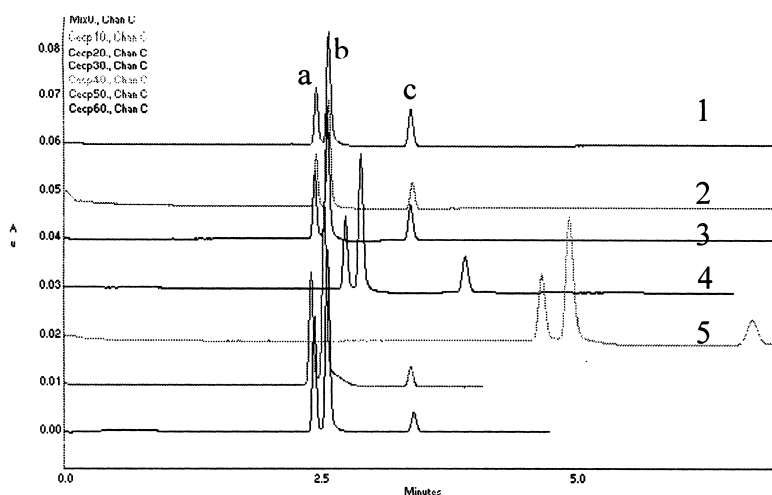


Fig. 1. Influence of the forward pressure on the separation of pCEC. The separation voltage was 25 kV. Traces: 1=without forward pressure, 2–7=with the application of the forward pressure (10, 20, 30, 40, 50, 60 p.s.i., respectively) on the outlet of the column. Peaks: a=thiourea, b=phenylenediamine, c=toluene. Other conditions as in Section 2.2.

rate are analytical grade reagents from Beijing Chemical Factory (Beijing, China) and Tris in an analytical grade reagent from The First Chemical Factory (Shanghai, China). Acetonitrile is a HPLC grade reagent from Fisher Scientific (NJ, USA).

The mobile phase is prepared with distilled water. It contains 4 mmol/l borax (pH 9.0)–acetonitrile (3:7 v/v). Prior to the separation, the mobile phase was degassed in an ultrasonic cell for 20 min. Before use, the pCEC column should be equilibrated with fresh buffer for 30 min at least.

## 2.2. Instruments and methods

All experiments were performed on a Beckman P/ACE MDQ system (Fullerton, CA, USA). The reversed-phase ODS column [27 cm (effective length 20 cm)×100  $\mu\text{m}$ , 3  $\mu\text{m}$ ] was provided by Unimicro Technologies (Pleasanton, CA, USA).

The sample was electrophoretically injected at 1 kV for 1 s. The separation voltage was 10–30 kV. During the separation, 0–40  $\text{kg}/\text{cm}^2$  (0–60 p.s.i.) pressure was applied from the two ends of the capillary column. The temperature of the column was kept at 15°C, and the wavelength of the UV detection was 214 nm.

## 3. Results and discussions

### 3.1. Influence of the forward pressure on the separation of pCEC

Fig. 1 shows the separation result of pCEC, in which 25 kV was applied while the pressure of 10–60 p.s.i. was applied to the inlet of the column. It was found that, at first, with increasing pressure, the migration times of the components increased too;

Table 1

The electrophoretic mobility in pCEC with the application of different forward pressures (1 p.s.i.=6894.76 Pa)

Mobility ( $\text{mm}^2/\text{s}\cdot\text{V}$ )	Pressure (p.s.i.)						
	0	10	20	30	40	50	60
Thiourea	0.016	0.016	0.016	0.015	0.009	0.016	0.016
Phenylenediamine	0.016	0.16	0.016	0.015	0.008	0.016	0.016
Toluene	0.012	0.012	0.012	0.010	0.006	0.012	0.012

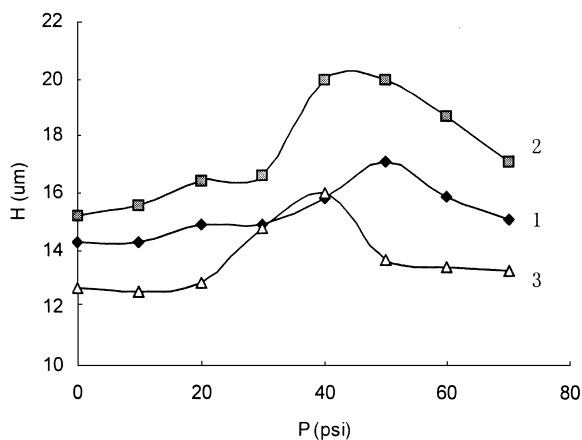


Fig. 2. Relationship between the theoretical plate height and the forward pressure. Curves: 1=thiourea, 2=phenylenediamine, 3=toluene. Other conditions as in Fig. 1.

then, after the critical value, the migration times of the components decreased. Table 1 shows the statistics to the mobility of the components under different forward pressures, and Fig. 2 shows the relationship between the theoretical plate height and the forward pressure.

Under the same forward pressure, the influence of the applied voltage on the separation was determined

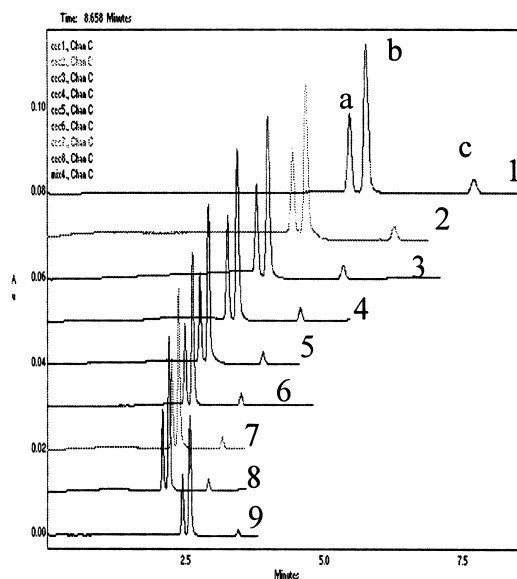


Fig. 4. Influence of the voltage on the separation of CEC. Traces 1–8: the separation voltage was 12.5, 15.0, 17.5, 20.0, 22.5, 25.0, 27.5, and 30.0 kV orderly, respectively; curve 9 was the repeat of 25.0 kV. Peaks: a=thiourea, b=phenylenediamine, c=toluene. Other conditions as in Fig. 1.

(shown in Fig. 3). Compared with the result obtained by capillary electrochromatography (shown in Fig. 4), it was found that the relationship between the

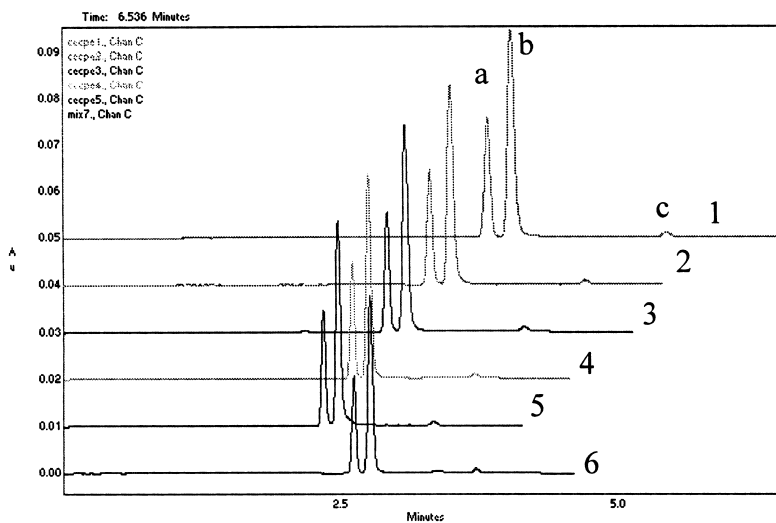


Fig. 3. Influence of the voltage on the separation of pCEC with the application of forward pressure. The forward pressure was 30 p.s.i. Traces 1–5: the separation voltage was 17.5, 20.0, 22.5, 25.0, and 27.5 kV, respectively; curve 6 was the normal capillary electrochromatography separation under 25 kV. Peaks: a=thiourea, b=phenylenediamine, c=toluene. Other conditions as in Fig. 1.

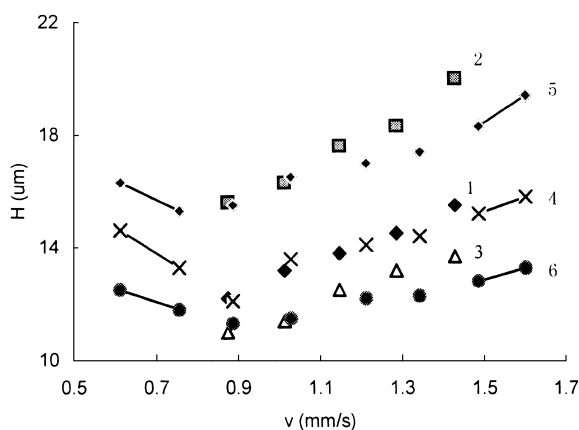


Fig. 5. Relationship between the plate height ( $H$ ) and the flow velocity ( $v$ ) in pCEC and CEC with the change of the separation voltage. Curves 1–3 are results of pCEC with the application of forward pressure of 30 p.s.i., with separation voltages of 17.5, 20.0, 22.5, 25.0, and 27.5 kV. Curves 4–6 are results of CEC, with separation voltages of 12.5, 15.0, 17.5, 20.0, 22.5, 25.0, 27.5, and 30.0 kV. 1, 4 = thiourea, 2, 5 = phenylenediamine, 3, 6 = toluene.

plate height and the flow-rate under these two conditions is almost the same (shown in Fig. 5).

Therefore, it has been concluded that in pressurized capillary electrochromatography with forward pressure, the main driving factor of the separation

is still the EOF. Meanwhile, there is a critical value of the pressure. When the pressure is smaller than the critical value, the EOF decreased with the increase of the pressure; when the pressure is larger than the critical value, the EOF was increased with the increase of the pressure. Anyway, in forward pressurized capillary electrochromatography, the separation is still mainly driven by the EOF.

### 3.2. Influence of the reversed pressure on the separation of pCEC

Fig. 6 shows the separation result of pCEC, in which 25 kV was applied while a pressure of 10–50 p.s.i. was applied to the outlet of the column. From calculation, it was found that with the application of the reversed pressure in pCEC, the repeatability of the migration time obviously increased (shown in Table 2). Moreover, the influence of the voltage on the separation was considered with the constant reversed pressure (shown in Fig. 7). From Fig. 8, it was obvious that the change of the plate height with the flow-rate under the reversed pressurized capillary electrochromatography was almost the same as that of the normal capillary electrochromatography. Ac-

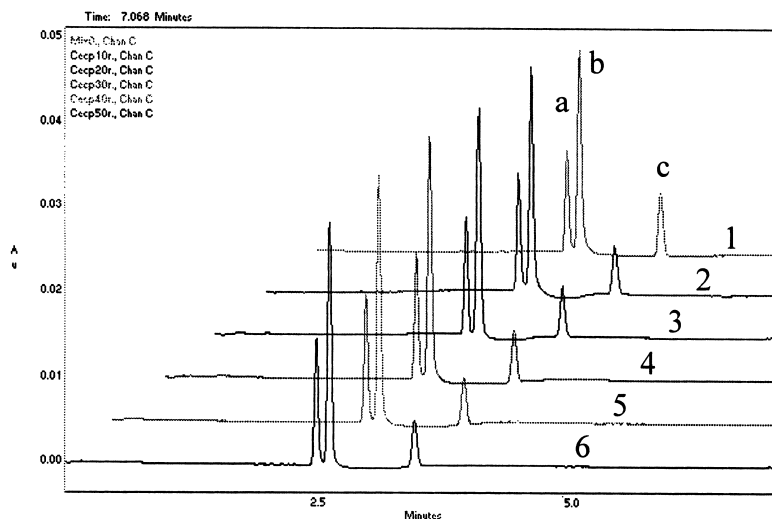


Fig. 6. Influence of the reversed pressure on the separation of pCEC. Traces: 1 = separation of CEC under 25 kV; traces 2–6 = separation of pCEC with the reversed pressures 10, 20, 30, 40, and 50 p.s.i., respectively. Peaks: a = thiourea, b = phenylenediamine, c = toluene. Other conditions as in Fig. 1.

Table 2  
The repeatability of CEC and pCEC with the application of the reversed pressure

$t_R$ (min)	Pressure (p.s.i.)					Average	RSD (%)
	10	20	30	40	50		
Thiourea	2.463	2.467	2.458	2.588	2.629	2.52	3.2
Thiourea in pCEC	2.463	2.463	2.475	2.479	2.492	2.47	0.49
Phenylenediamine	2.583	2.596	2.567	2.592	2.729	2.61	2.5
Phenylenediamine in pCEC	2.538	2.592	2.604	2.608	2.617	2.60	0.52
Toluene	3.388	3.421	3.396	3.463	3.642	3.46	3.0
Toluene in pCEC	3.388	3.417	3.433	3.442	3.458	3.43	0.77

The separation of CEC was under 25 kV with five continuously determination.

According to the above results, it is easy to conclude that in the reversed pressurized capillary electrochromatography, the separation is completely driven by the EOF. The application of the reversed pressure did not change the driving factor or decrease the column efficiency, but increased the repeatability of the separation.

#### 4. Conclusions

In pressurized capillary electrochromatography,

the reversed pressure could increase the repeatability while the larger forward pressure could decrease the separation time. Anyway, in pressurized capillary electrochromatography with the application of the forward or the reversed pressure, the main driving factor of the separation is the EOF all the time.

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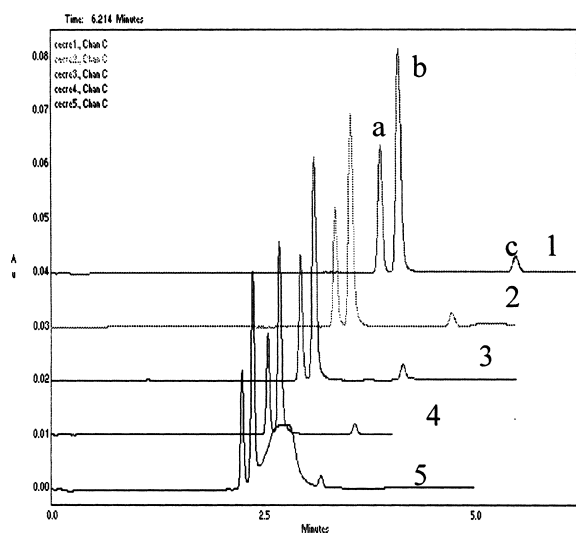


Fig. 7. Influence of voltage on the separation of pCEC with the application of reversed pressure. Traces 1–5: the separation voltages were 17.5, 20.0, 22.5, 25.0, and 27.5 kV. The applied reversed pressure was 30 p.s.i. Peaks: a=thiourea, b=phenylenediamine, c=toluene. Other conditions as in Fig. 1.

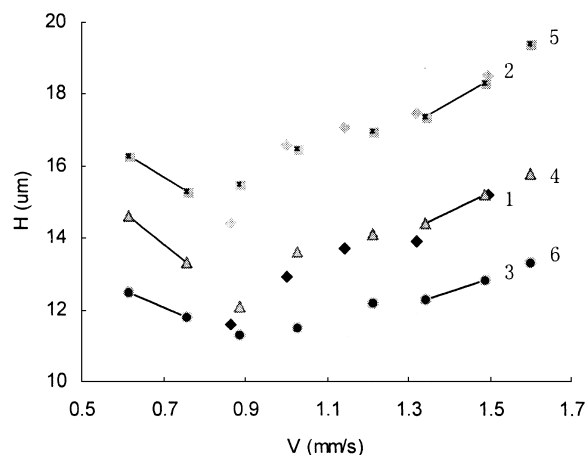


Fig. 8. Relationship between the plate height ( $H$ ) and the flow velocity ( $v$ ) in pCEC and CEC with the change of the separation voltage. Curves 1–3 are the results of pCEC with the application of the reversed pressure 30 p.s.i. and curves 4–6 are the result of CEC. 1, 4=thiourea, 2, 5=phenylenediamine, 3, 6=toluene. Other conditions same as in Fig. 7.

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