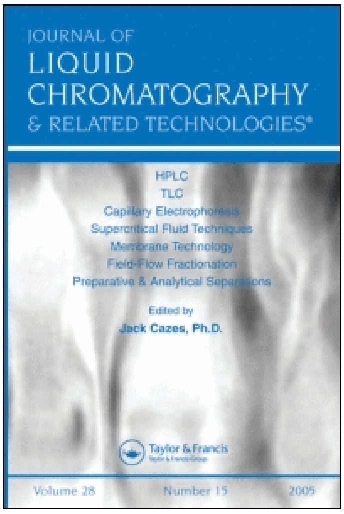

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SEPARATION OF NEUTRAL COMPOUNDS BY HIGH SPEED CAPILLARY ELECTROCHROMATOGRAPHY

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SEPARATION OF NEUTRAL COMPOUNDS BY HIGH SPEED CAPILLARY ELECTROCHROMATOGRAPHY

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ABSTRACT

With using short capillary column packed with porous and non-porous ODS stationary phases, high speed separation of 6 neutral aromatic compounds within 36 s by capillary electrochromatography (CEC) has been performed. Good reproducibility of the migration times for those solutes in high speed CEC was observed with RSD less than 1%. Both the linear velocity of EOF and the current linearly increases with the applied voltage, which means that the thermal effect by Joule heating was small. However, the capacity factor of solutes was found to decrease with the increase of the applied voltage, which was caused by the fact that about several seconds needed for the increase of voltage from 0 to applied value on a commercial CE instrument made larger contributions to the migration times of the early eluted compounds than those of lately eluted ones during high speed CEC, and voltage effect would increase with the higher applied voltage used. The linear relationship between the logarithm of capacity factor and the number of carbon for homologous compounds was observed, and positive value of slope means that the hydrophobicity of solutes is one of the main contribution factors to retention in high speed CEC packed with ODS stationary phases.

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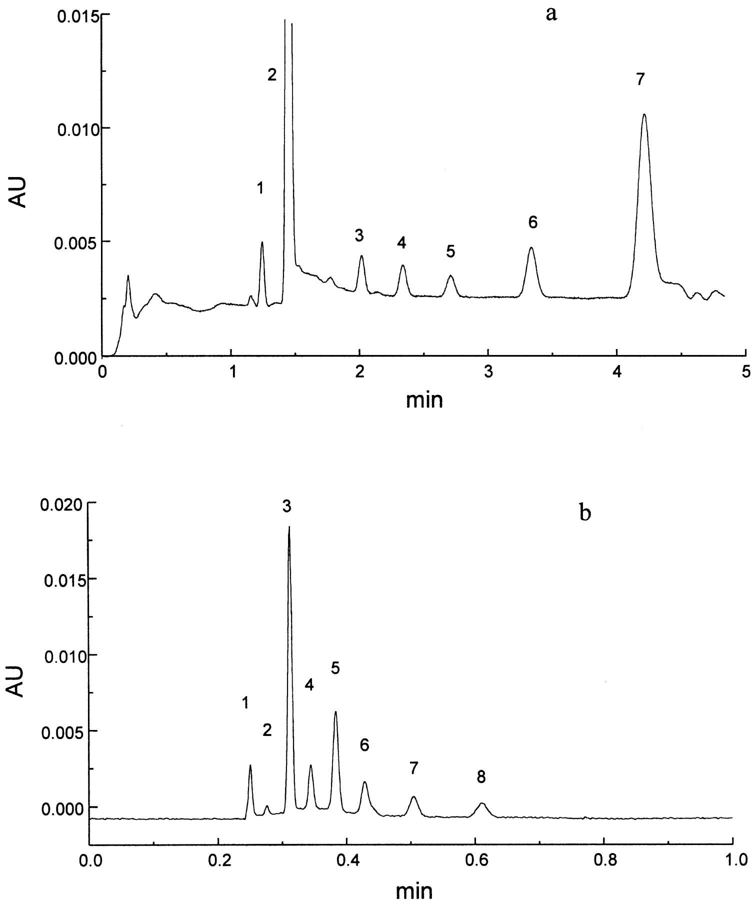


Figure 1. Separation of a neutral test mixture on porous ODS column in high speed CEC. Experimental conditions: Column: 75μm i.d. of capillary column with packed/total length of 6.6/27 cm by 3 μm Spherisorb-ODS2; mobile phase: acetonitrile-buffer (80/20, v/v) containing 4 mM Tris, pH 9.2; detection wavelength 200 nm; electrokinetic injection of sample by voltage 1 kV for 1s. a) Applied voltage: 5kV. Peaks: 1. impurity; 2. thiourea; 3. benzene; 4. toluene; 5. ethylbenzene; 6. propylbenzene; 7. butylbenzene. b) Applied voltage; 30 kV. Peaks: 1. impurity; 2. thiourea; 3. nitrobenzene; 4. benzene; 5. toluene; 6. ethylbenzene; 7. propylbenzene; 8. butylbenzene.

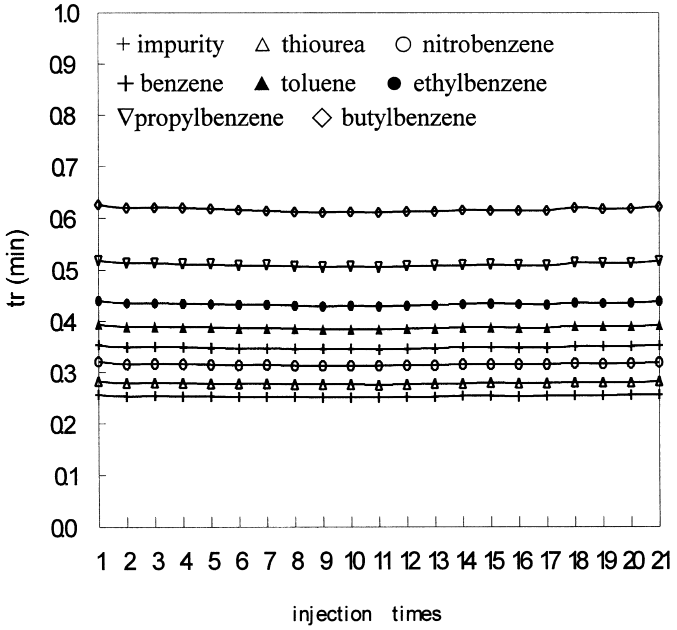


Figure 2. Reproducibility of the migration times by 21 consecutive injections for eight peaks in high speed CEC packed with porous ODS stationary phase.

Table 1

Migration Time (tr) and Theoretical Plate Number per Meter for Solutes (N) at Applied Voltages of 5kV and 30 kV in High Speed CEC

Solute	Tr(min)*	N*	Tr(min)**	N**
Nitrobenzene	---	---	0.321	168000
Benzene	2.02	122000	0.354	157000
Toluene	3.24	127000	0.394	193000
Ethylbenzene	2.71	126000	0.440	107000
Propylbenzene	3.34	127000	0.518	95000
Butylbenzene	4.22	107000	0.626	87000

---

* The data at applied voltage of 5 kV. ** The data at applied voltage of 30 kV.

INTRODUCTION

Since the electric field was introduced into chromatography by Pretorius et al.,[1] capillary electrochromatography (CEC) has attracted more and more attention.[2-11] According to the types of packing material, CEC can be categorized as reversed-phase CEC,[3,4] ion-exchange CEC,[5] chiral CEC,[6,7] etc.

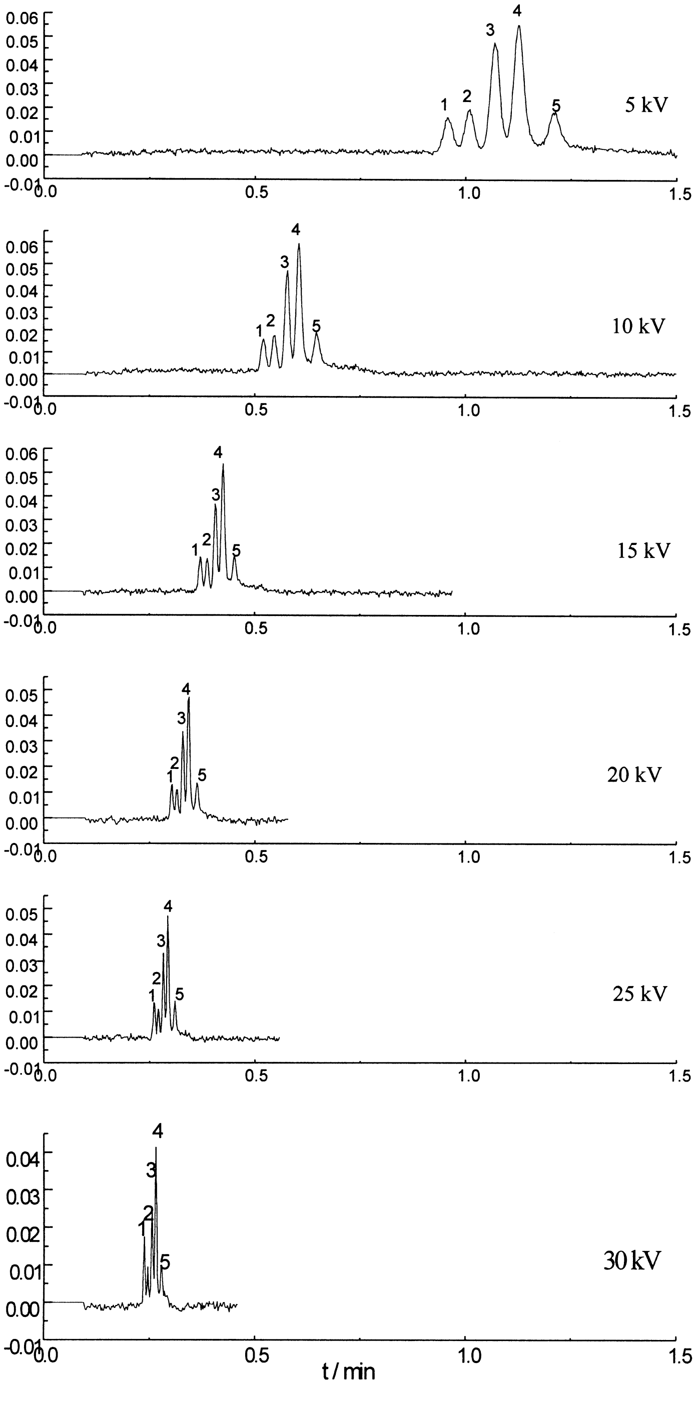
It has been proved theoretically that the theoretical plate number could be doubled by replacing pressurized flow with electroosmotic flow (EOF)[8,9] as the propelling force. In practice, column efficiency as high as 300,000/m has been achieved in RP-CEC.[10,11] While, according to the propelling mode, there is a variant of CEC, pressurized electrochromatography (PEC),[12,13] which can easily eliminate the bubbles that results from Joule heating and frits. PEC can also be utilized to achieve gradient elution.[14,15]

In our previous work,[16] using a unified microcolumn separation apparatus, the retention behavior of neutral solutes in CEC, PEC, and micro-high performance liquid chromatography (μ-HPLC) were investigated and no significant differences were found among these three modes; however, the investigation of column efficiency by Van Deemter's equation showed that the contribution of eddy diffusion and resistance to mass transfer in the stationary phase and mobile phase to plate height in CEC is much lower than those in μ-HPLC.

Another advantage of CEC over μ-HPLC is that no pressure drop is generated along the axis of the column, thus the stationary phases with small diameters (as small as 0.5 μm) and relative long columns can be utilized.

All of the dynamic behaviors mentioned above for CEC are very feasible for high speed analysis. Recently, "short-end injection" rapid analysis CEC has been reported by Bartle et al.[17-19] In this work, separation of 6 aromatic compounds within 36 s in high speed CEC has been achieved with very good reproducibility of migration time. It has been observed that the thermal effect during high speed CEC was small, and several seconds needed for the increase of applied voltage from 0 to fixed value on a commercial CE instrument made the capacity factor of solutes decrease with the increase of the applied voltage. Furthermore, the linear relationship between the logarithm of capacity factor and the number of carbon was obtained in high speed CEC.

Figure 3 (right). Separation of a neutral test mixture at various applied voltages on the non-porous ODS column in high speed CEC. Experimental conditions: Column 75μm i.d. of capillary column with packed/length of 6.3/27 cm by 2 μm non-porous silica ODS; mobile phase; acetonitrile-buffer (60/40, v/v) containing 4 mM Tris, pH 9.2; detection wavelength, 200 nm; electrokinetic injection of sample by applied voltage 1kV for 1s. Peaks: 1. thiourea; 2. benzene; 3. ethylbenzene; 4. propylbenzene; 5. butylbenzene.


5 kV
10 kV
15 kV
20 kV
25 kV
30kV
1
2
3
4
5
0.06
0.05
0.04
0.03
0.02
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0.00
-0.01
0.0
0.5
1.0
1.5
t / min

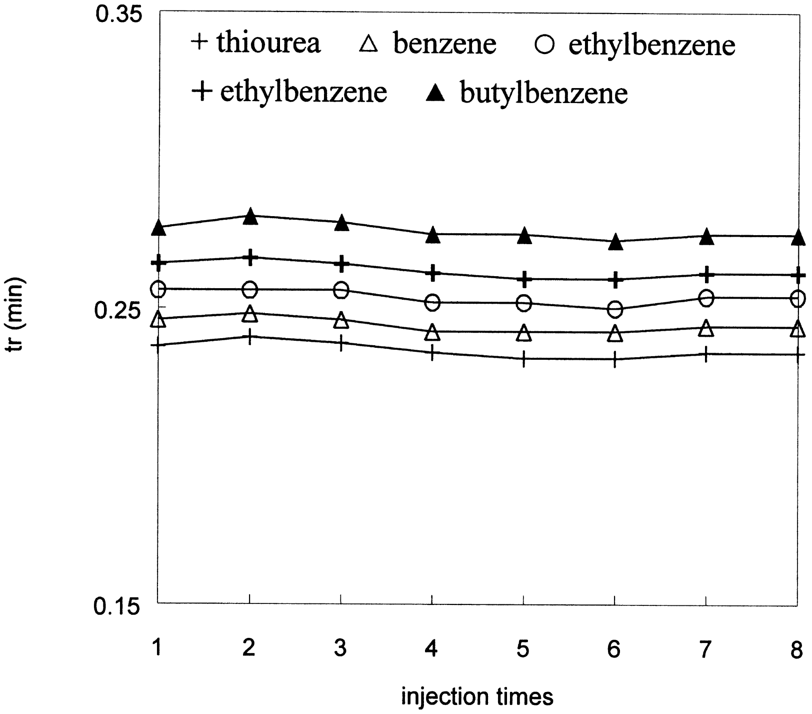


Figure 4. Reproducibility of the migration times by 8 consecutive injections for five peaks in high speed CEC packed with non-porous ODS stationary phase.

Table 2

Theoretical Plate Number of Solutes at Data Rates of 8Hz and 16 Hz in High Speed CEC, with Non-Porous Silica ODS as the Stationary Phase, and Applied Voltage of 30 kV

Solute	Plate Number/m at 8 Hz	Plate Number/m 16 Hz
Thiourea	198000	208000
Benzene	213000	325000
Ethylbenzene	231000	352000
Propylbenzene	242000	247000
Butylbenzene	138000	263000

EXPERIMENTAL

Apparatus

CEC experiments were performed on P/ACE 5510 (Beckman Inc., Fullerton, CA, USA) with a liquid cooling system to control the column temperature at 17 ± 0.1°C, the UV detection wavelength was set at 200 nm and injection of samples was performed by applying voltage of 1 kV for 1 s, unless stated otherwise. A Waters 510 pump (Waters Inc., Milford, MA, USA) was used to pack the capillary columns. Slidewrite Plus 2.0 (Advanced Graphics Software, Inc, Netherlands) was used to process all of the experimental data on a Legend personal computer.

Reagents and Materials

Spherisorb-ODS2 with particle diameter 3 μm was purchased from the Phase Separation (Norwalk, NJ, USA), and the non-porous silica-ODS with particle diameter of 2 μm was obtained from Analytical Department, Dalian Institute of Chemical Physics, CAS. Fused silica capillary was 375 μm o.d. and 75 μm i.d. (Yongnian Optic Fiber Plant, Hebei, China). Acetonitrile (Yuwang Chemical Plant, Shandong, China) was of HPLC grade. All other chemicals used were of analytical grade. Double deionized water was used in all experiments.

Tris was dissolved in water as stored buffer at a concentration of 20 mmol/L. The volume of organic solvent and buffer were measured separately with a different ratio of volume, then mixed together as the mobile phase by keeping Tris at 4 mmol/L and pH at 9.2, unless stated otherwise. The mobile phase was then degassed in an ultrasonic bath for 10 minutes. Samples were dissolved in the mobile phase at a concentration of c.a 60 mmol/L. Dead time of the column was measured by thiourea.

Column Preparation

In order to realize the high speed analysis by CEC, a short packed column should be adopted in order to increase the electric field strength applied over the column, therefore, the capillary columns with total/packed column length of 27/6.6 cm were prepared with satisfying the limitation of column length for the cartridge design of the Beckman CE instrumentation.

A slurry packing method was utilized to pack the column as described in the previous work.[16] The initial frit was sintered with water-wetted 5 μm silica gel particles at the end of the capillary. The slurry, composed of the stationary

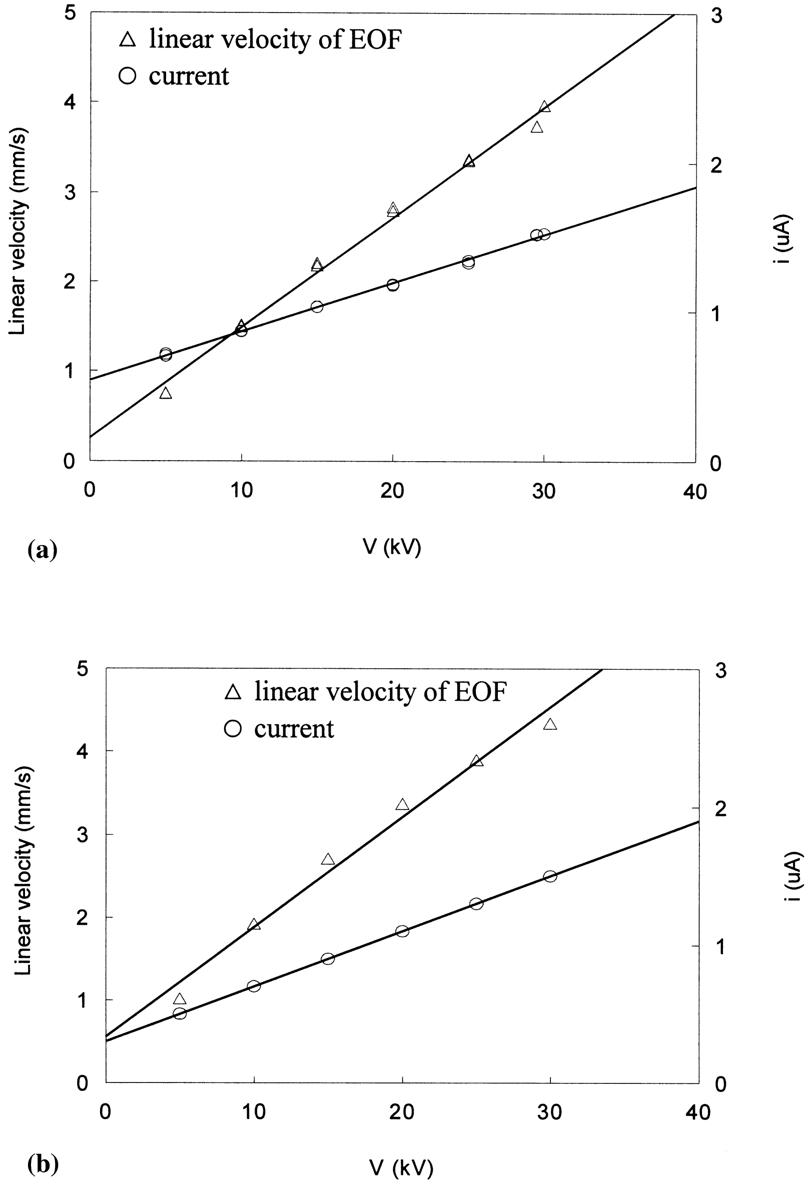


Figure 5. Dependence of the linear EOF velocity (u_{eof}) and current (I) on applied voltage in CEC with packed (a) porous and (b) non-porous stationary phases. Experimental conditions see Figure 1 and Figure 3, respectively.

phase and acetone (c.a. 0.01 g/mL), was prepared in an ultrasonic bath then, was packed into the capillary by HPLC pump under pressure about 350 bar. After the frits at injection and detection ends were made by sintering the stationary phases, the initial frit was cut off, and the residual particles behind the frit at detection end were flushed out by the mobile phase. The detection window was prepared by removing 1 mm polymide coating using a blade, and was about 1 mm from the outlet frit. The voltage of 5 kV was applied for 20 minutes to equilibrate the column by the mobile phase before the run.

RESULTS AND DISCUSSION

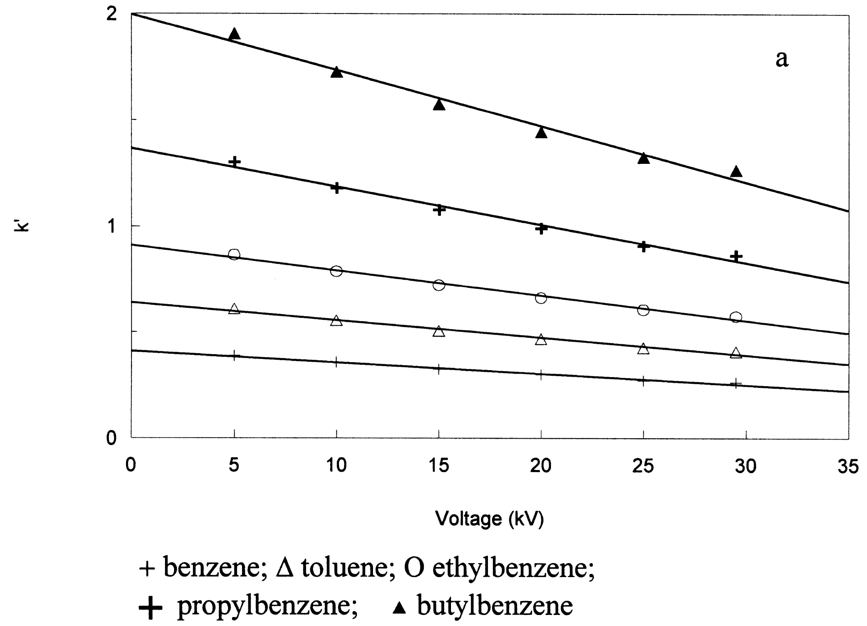
To date, the length of packed capillary has been dictated by the dimension of the cartridge, i.e. for P/ACE 5500 system the total length of capillary column more than 27 cm is necessary. Due to the fact that CEC generates efficiency in the order of three to four fold greater than that in HPLC,[19] it should be feasible to perform rapid analysis on short CEC capillaries and still generate the required resolution.

Figure 1 showed the separation of thiourea, nitrobenzene, toluene, ethylbenzene, propylbenzene, and butylbenzene in CEC by applying voltage at 5 kV and 30 kV, respectively, and the migration time and theoretical plate number calculated for each solute were listed in Table 1.

It can be seen that 6 aromatic compounds can be separated at the baseline within 36 s. The column efficiency at applied voltage of 30 kV for the benzene and toluene seems somewhat lower, but for ethylbezene, propylbenzene and butylbenzene higher than that at 5 kV in CEC. Figure 2 showed the reproducibility of migration times in high speed CEC by 21 consecutive injections; the calculated RSD values (%) for all peaks appearing in Figure 1(b) were below 0.75, which suggested that the reproducibility of migration time in high speed CEC was very good.

Non-porous stationary phase behaves with higher column efficiency than porous stationary phase because of its rapid mass transfer process, and without transport of solute into the pores, and has been used for rapid separation of samples in HPLC recently. As pointed out by Horvath et al.,[20] the permeability of non-porous packings was smaller than that of porous packings, thus the local electric strength on a non-porous column was larger than that on a porous column in CEC.

Figure 3 showed the separation of some neutral solutes on a CEC column packed with the non-porous stationary phase at different applied voltages; and 4 alkylbenzenes can be separated within 15 s. The reproducibility of migration times for thiourea, benzene, ethylbenzene, propylbenzene, and butylbenzene in high speed CEC packed with non-porous stationary phase was illustrated in Figure 4, the calculated RSD values (%) for those solutes were below 1.05.


a
k'
2
1
0
0
5
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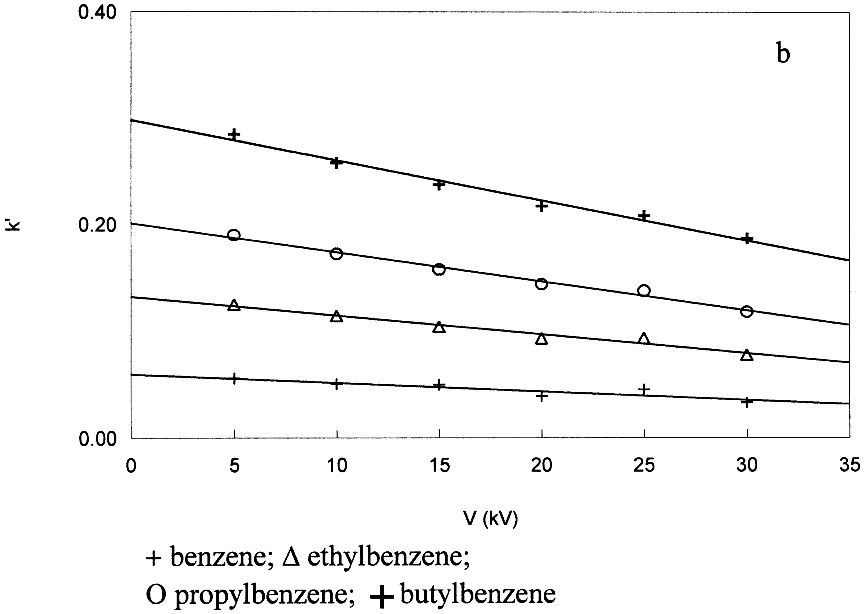
35
Voltage (kV)
+ benzene; Δ toluene; O ethylbenzene;
+ propylbenzene; ▲ butylbenzene


b
k'
0.40
0.20
0.00
0
5
10
15
20
25
30
35
V (kV)
+ benzene; Δ ethylbenzene;
O propylbenzene; + butylbenzene

By comparison of Figure 3 with Figure 1, the migration time of butylbenzene in high speed CEC, with acetonitrile-buffer (60/40) as the mobile phase and non-porous silica ODS as the stationary phase, is much smaller than that with acetonitrile-buffer (80/20) as the mobile phase and porous Spherisorb-ODS as the stationary phase.

This means that the carbon content on non-porous silica ODS is much smaller than that on the porous Spherisorb-ODS. Also, concentration of organic modifier in the mobile phase is one of the important factors to adjust the retention value in high speed CEC.

Due to the extremely rapid analysis resulting from the high speed CEC approach, the effect of data collecting on the resultant peak efficiency was studied, and the obtained result in high speed CEC on non-porous silica ODS is shown in Table 2. It can be seen that the column efficiency for all solutes increases, and especially for benzene, ethylbenzene, and butylbenzene.

There was more than a 50% increase of theoretical plate number by changing the date rate from 8 Hz to 16 Hz, which indicated the importance of appropriate data rate in fast speed analysis by CEC.

Figure 5 shows the dependence of the linear velocity of EOF (u_{eof}) and current on the applied voltage in high speed CEC packed with porous and non-porous stationary phases, respectively. It could be seen that both linear velocity of EOF and current linearly increased with the applied voltage, which means that the thermal effect on the linear velocity in CEC column could be neglected.

This result was the same as one observed in conventional CEC.[16] However, the decrease of capacity factors with the voltage applied for neutral compounds was observed in high speed CEC packed with the porous and non-porous stationary phases as shown in Figure 6. This was quite different from the conventional CEC observation that the retention value of neutral solutes should be independent of the applied voltage.

This result might be caused by the fact that the increase of applied voltage from 0 to fixed value would take about several seconds, which made a larger contribution to the migration time of the early eluted compounds than that of lately eluted solutes during high speed CEC. Such an effect would increase with the higher applied voltage used.

Figure 6 (left). Dependence of the capacity factors on the voltage for the neutral solutes in high speed CED. (a) Porous stationary phase of Spherisorb-ODS2; other experimental conditions see Figure 1. (b) Home made non-porous silica-ODS stationary phase; other experimental conditions see Figure 3.

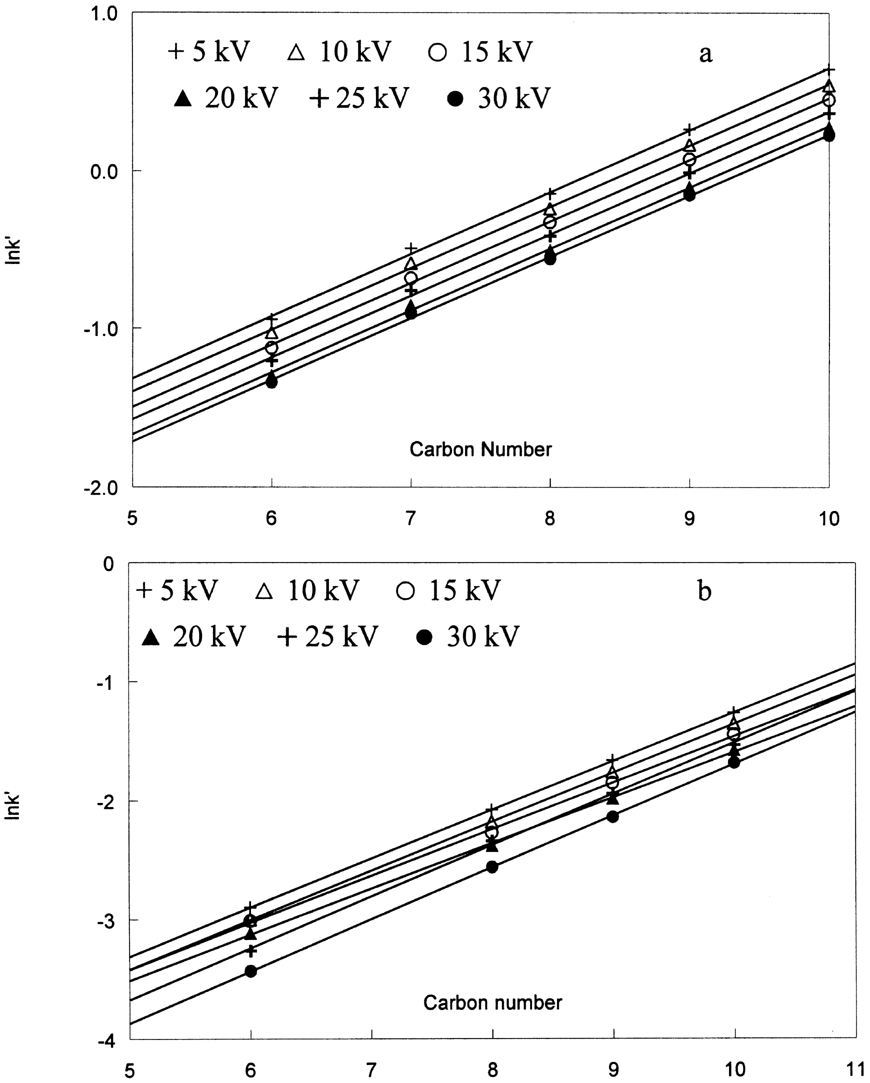


Figure 7. Dependence of logarithm of capacity factor on number of carbon for homologous solutes in high speed CEC packed with a) porous and b) non-porous ODS stationary phase.

Thus, the apparent capacity factor would decrease with the increase of applied voltage, and the apparent capacity factor of later eluted solutes would decrease more seriously than that of early eluted solutes.

The linear relationship between the logarithm of capacity factors versus the number of carbon for homologous compounds is well known in chromatography. Such a linear relationship at various applied voltage in high speed CEC was investigated, and the obtained results were shown in Figure 7. It could been seen that there were very good linear relationships between lnk' and the number of carbon for homologous compounds in CEC at all of the applied voltages. Positive value of a slope means that as in RP-HPLC, the hydrophobicity of solutes is one of the main contribution factors to retention in high speed CEC packed with ODS as stationary phase.

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