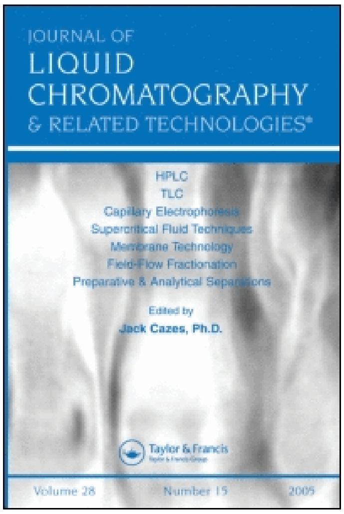

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RETENTION PREDICTION BASED ON THE SOLVATOCHROMIC COMPARISON METHOD IN REVERSED-PHASE CAPILLARY ELECTROCHROMATOGRAPHY

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ABSTRACT

Retention behavior of eleven neutral compounds in reversed phase capillary electrochromatography (RP-CEC) was investigated by using solvatochromic comparison method. The retention behavior in RP-CEC can be predicted quite well based on either multiple solvatochromic parameters of solutes or solvatochromic solvent polarity measurement E_T (30) of mobile phase.

INTRODUCTION

Capillary electrochromatography (CEC), where the column is packed with a HPLC stationary phase and mobile phase is driven by an electric field, provides very high column efficiency and selectivity.[1-6] As a hybrid technique of capillary electrophoresis and micro-HPLC, CEC has many advantages,[1-6] therefore it is developing rapidly. The reports , so far, are mainly concentrated on the demonstration and application of CEC in reversed-phase model. Although a few publications[2-5] in CEC have mentioned the retention behavior of the solutes, a systematic approach to understanding the retention mechanism in CEC is still lacking.

It is generally believed that the retention mechanism for neutral compounds is the same in both CEC and HPLC,[2] because it depends only in the partition equilibrium of solutes between the stationary and mobile phase. However, for charged species, not only partition equilibrium but also electrophoretic migration are involved in the retention behavior in CEC. As we know, the main difference between CEC and HPLC is the driving force of the mobile phase from a pressure field (HPLC) to an electric field (CEC) across an identical separation column; this should not change the chromatographic retention behavior of the solutes as long as the flow rates are the same in both systems. When an electric field is applied across a capillary column in which the solutes are partitioned between the stationary and mobile phase, usually called buffer in CEC, the stationary phase particles as well as solutes will be polarized. The surface of packing particles will form a dielectric layer which is different from that in HPLC.

It is true that the polarizability effects of stationary phase and solutes will influence the chromatographic retention.[7] In order to compare retention mechanisms in RP-CEC with those in RP-HPLC, a simple way is to examine the validity of retention model of RP-HPLC by the experimental data obtained from CEC.

Retention model in reversed-phase liquid chromatography, such as solvophobic theory,[8] statistical-mechanical theory,[9] solubility parameters theory,[10-11] lattice statistical thermodynamic approach,[12] and solvatochromic comparison method,[13-20] which can be used to predict the chromatographic retention has been discussed[8-12] extensively .

Solvatochromic comparison method was generally accepted as a good method to predict chromatographic retention, which is based on linear solvation energy relationship (LSER). It has been applied successfully to study retention both in gas chromatography[13,14] and in RP-HPLC,[13-20] but there is no report on RP-CEC. There are two solvatochromic scales, i.e. the multiparameter scale[15-17] and the single parameter scale,[18-20] E_T (30). Carr et al.[13] first reported the correction of HPLC retention properties using the multiple solvatochromic parameters.

The general correction equation was expressed as below,

$$\log k' = SP_0 + M(\delta^2_m - \delta^2_s)^2 V_i/100 + S(\pi_s - \pi_m)\pi_2 + A(\beta_s - \beta_m)\alpha_2 + B(\alpha_s - \alpha_m)\beta_2 \qquad (1)$$

In term of solvatochromic parameters of the solute, the correction equation becomes,

$$\log k' = SP_0 + mV_i/100 + s\pi_2 + a\alpha_2 + b\beta_2 \qquad (2)$$

where k' is the capacity factor, SP_0 is the intercept of regression equation ,V_i , the cavity term, which is a measure of the intrinsic (Van der Waals) molar volume of solutes, δ^2 , the square of the Hildebrand solubility parameter, and π, α, and β, Kamlet-Taft solvatochromic parameters, of which π measures dipolarity/polarizability, α and β, HBD acidity and HBA basicity, respectively[14] (HBD = hydrogen bond donor, HBA = hydrogen bond acceptor).

Subscripts s and m refer to the stationary phase and mobile phase, and subscript 2, the solute properties. The values of M, m, S, s , A , a , B and b are the coefficients determined by experiments.

According to Eq.1, there are three aspects which will all contribute to the chromatographic retention behavior, namely, stationary phase, mobile phase and solutes. Equation 2 shows that the chromatographic retention can be predicted according to the solvatochromic properties of the solutes in a system with a given stationary and mobile phase. The coefficients m, s, a and b in Eq. 2 are related to the chemical properties of the stationary and mobile phase. Thus, Eq.2 can be used to predict retention with the solute properties and characterize stationary phase based on the coefficients in it.[14]

Dorsey et al.[18-20] developed a method of modeling retention using the single parameter, solvatochromic solvent polarity scale, E_T (30). They found that solvatochromic solvent polarity scale, E_T (30), is truly a good descriptor of mobile phase strength. Plots of log k' vs E_T (30) polarity generally give a good linear relationship.

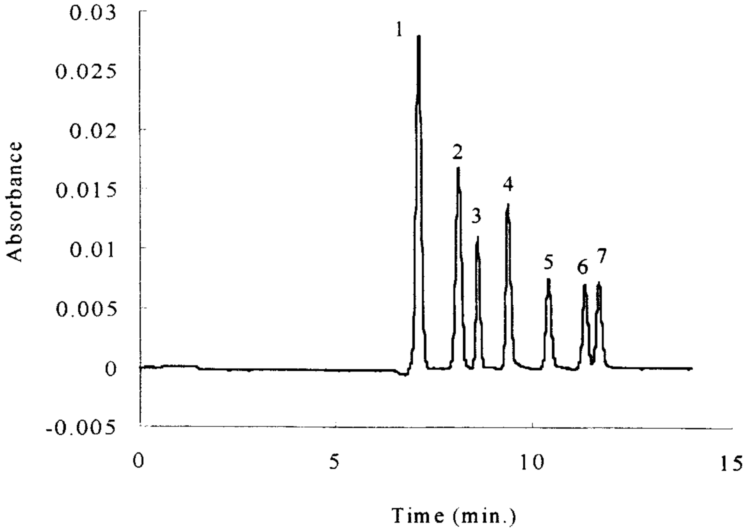

Figure 1. Chromatogram of several substituted benzenes. Experimental conditions: fused-silica capillary, 50 μm (ID) × 37cm/30cm packed with 3μm ODS; Mobile phase: CH_3CN:H_2O (4mM Tris/HCl buffer) =70:30 (v/v); Separation voltage : 30kV; Injection: 10 kv/10s; UV detector: 214 nm, Temperature: 20°C; 1: Thiourea, 2 :Aniline, 3: Acetophenone, 4: o-Nitrotoluene, 5: Toluene, 6: *o*-Xylene, 7: *o*-Chlorotoluene.

Thus, in present work, solvatochromic comparison method is selected as a basic model to test the retention behavior of eleven neutral compounds in RP-CEC. The single parameter scale, E_T (30), is selected to examine the effect of changing mobile phase polarity on chromatographic retention and the multiparameter scale is used to predict retention with the solute properties.

EXPERIMENTAL

Chromatographic System

Experiments were performed with P/ACE 5510 capillary electrophoresis system (Beckman Co., Fullerton, CA, USA). Packed capillary columns used in this work were provided by Unimicro Technologies, Inc. (Pleasanton, CA, USA). One was 27cm× 50μm (i.d.) with 20cm effective length. The other was 37cm× 50μm (i.d.) with 30cm effective length. These columns were packed electrokinetically[6] with 3-µm ODS particles (Micra Scientific, Northbrook, IL, USA). The mobile phase was prepared by mixing different proportions of acetonitrile and Tris/ HCl buffer (pH 8.80). The volume fraction of acetonitrile ranged from 50% to 80%. The mobile phase was filtered through a 0.25 µm membrane and degassed with ultrasonication for 5 minutes before use. Prior to run, the capillary column was conditioned with mobile phase by pressurizing it from the inlet with approximately 500 p.s.i. using a manual syringe pump from Unimicro Technologies, Inc. (Pleasanton, CA, USA). After installation, it was further conditioned by using a low voltage of 5 kV. Capillary temperature was set at 20°C. An electrokinectic injection was performed by applying 5 kv for 5 s or 10 kv for 10s. An on-line UV detector was operated at 214 nm. The applied voltage was 30 kv or 20 kv for different columns. In order to study the effect of salt concentration on retention, the concentration of Tris/HCl ranged from 1 mM to 20 mM. Thiourea, neutral and assumed non-retained, was used as a EOF marker to determine the void time (t_0). The capacity factors of solutes were calculated by $k'=(t_R-t_0)/t_o$, t_R was the retention time of solute, which was the average of three measurements.

Chemicals

HPLC-grade acetonitrile was used as mobile phase modifier. Deionized water was used for all preparation of electrolyte and standard solutions. All other chemicals used were of analytical reagent grade. Eleven test samples were aniline, acetophenone, o-nitrotoluene, toluene, o-xylene, o-chlorotoluene, phenol, chlorobenzene, bromobenzene, benzaldehyde, and anisole. The concentration of samples ranged from 0.1 to 0.8 mg/mL according to their absorbance differences at 214 nm.

RESULTS AND DISCUSSION

Evaluation of Retention with Solvatochromic Parameter E_T (30) Polarity of Mobile Phase

The polarity of mobile phase has a strong influence on chromatographic retention in RP-HPLC. In order to examine the effects of changing mobile phase strength on retention in RP-CEC, we apply the results developed by Dorsey et al.,[18] whose studies indicated that plots of log k' vs. E_T (30) are very often a linear relationship. Figure 1 depicts the chromatogram of several benzene derivatives and Fig. 2 shows the plots of log k' vs. E_T (30) polarity of mobile phase. Table 1 lists the linear regression results for correction between

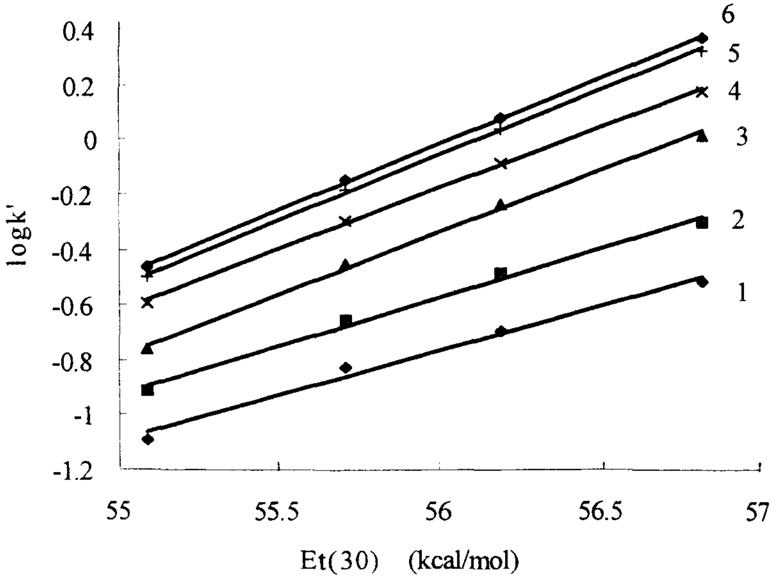

Figure 2. Relationship between log k' and E_T (30) polarity of mobile phase. Experimental conditions as shown in Fig. 1, CH_3CN concentration ranges from 50% to 80% (v/v). 1: Aniline, 2: Acetophenone, 3: o-Nitrotoluene, 4: Toluene, 5: *o*-Xylene, 6: *o*-Chlorotoluene.

Table 1

Relationship Between log k' and $E_T(30)$ Polarity of Mobile Phase*

Solutes	Correction Equations	r
Aniline	logk'= 0.3267 $E_T(30)$ -19.064	0.9930
Acetophenone	logk'= 0.3547 $E_T(30)$ -20.434	0.9961
o-Nitrotoluene	logk'= 0.4488 $E_T(30)$ -25.471	0.9986
Toluene	logk'= 0.4431 $E_T(30)$ -24.993	0.9993
o-Xylene	logk'= 0.4753 $E_T(30)$ -26.674	0.9996
o-Chlorotoluene	logk'= 0.4827 $E_T(30)$ -27.049	0.9997

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* Experimental conditions as in Fig.1.

Table 2

Solvatochromic Parameters and Log k′ of Substituted Benzenes

Solutes	Vi/100	π_2	α_2	β_2	Log k′
Anline	0.562	0.73	0.26	0.50	-0.9078
Phenol	0.536	0.72	0.61	0.33	-0.9560
Acetophenone	0.690	0.9	0.04	0.49	-0.7429
Benzaldehyde	0.606	0.92	0	0.44	-0.7236
Toluene	0.592	0.55	0	0.11	-0.3708
Chlorobenzene	0.581	0.71	0	0.07	-0.3524
Bromobenzene	0624	0.79	0	0.06	-0.3130
o-Nitrotoluene	01.764	0.97	0	0.31	-0.5354
Anisole	0.639	0.73	0	0.32	-0.5098
o-Chlorotoluene	0.679	0.67	0	0.08	-0.2204
o-Xylene	0.668	0.21	0	0.12	-0.2580

Experimental conditions: fused-silica capillary, 50 μm (ID) × 27 cm/20 cm packed with 3 μm ODS(PAH); Mobile phase: $CH_3CN:H_2O$ =7:3 (2 mM Tris/HCl buffer), Separation voltage: 20 kv. Injection: 5 kv/5 s, other conditions as shown in Figure 1. V_i /100, π_2, α_2, β_2 values cited from reference.[21] Log k' data obtained from experiments.

log k' and E_T (30) polarity of mobile phase. A good linear relationship between the log k' and E_T (30) polarity of mobile phase is obtained for the different tested solutes. Therefore, the retention prediction in RP-CEC can be achieved by using the E_T (30) polarity of mobile phase as that in RP-HPLC.

Evaluation of Retention with Solvatochromic Parameters of Solutes

In CEC, when stationary phase is in contact with a buffered mobile phase, a double layer forms on the stationary phase surface. The double layer will be distorted by the strong electric field in CEC and this may have some influence on separation. Solvatochromic parameters comparison method is chosen to evaluate the polarization effect of the solutes and stationary phase. Table 2 lists associated parameters of the solutes obtained from experiment and references.[21]

By using multiple regression analysis, we obtain the relationship between log k' and solvatochromic parameters of solutes. The correction equation is given as below.

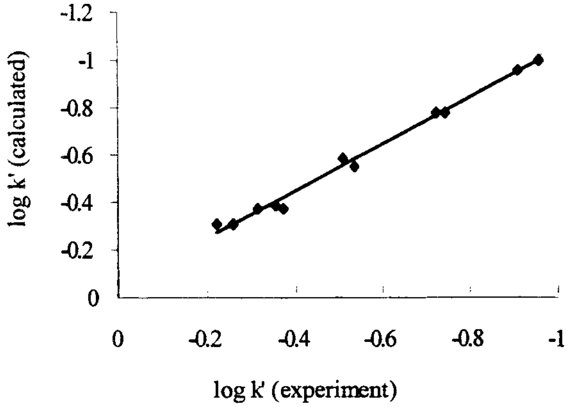

Figure 3. The plot of log k'(experimental values) vs.log k'(calculated values). Experimental conditions as shown in Fig.1.

$$\log k' = -0.5506 + 0.7669V_i/100 - 0.3027\pi_2 - 0.5435\alpha_2 - 0.9452\beta_2 \quad (3)$$

The correlation coefficient (r) of Eq.3 is 0.9953 with standard deviation 0.0064 for eleven solutes. A plot of log k' (experimental values) vs. log k' (calculated values by Eq.3) is shown in Figure 3. It can be seen from Eq. 3 that the correction equation is different from that obtained in RP-HPLC by Carr et al,[18] since the hydrogen bond acidity α_2 is also included. The parameters, V_i /100, π_2 , α_2 and β_2 are of the same importance in the determination of the retention in RP-CEC, as shown by the magnitude of the coefficients. However, in HPLC, Carr et al.[18] found that the most significant factors were solutes size, Vi, and hydrogen bond basicity, β_2. Solutes dipolarity/ polarizability, π_2 was a statistically significant but less important variable.

The results indicate that the retention behaviors in RP-CEC are not the same as those in RP-HPLC. It means that the parameter of hydrogen bond acidity of solutes reflects the effect of the negatively charged surface of stationary phase on retention, while the parameter of polarizability /dipolarity presents the effect of the polarization of both solutes and stationary phase on retention in electric separation system. The values of m, -b in Eq. 3 are in ratio of 1.00/1.23. It is similar with those obtained in RP-HPLC.[18,19] Accordingly, the ratios are 1.00/1.36 at the same concentration 70% (v/v) CH_3CN. It shows that the effects of solute size and hydrogen bond basicity on retention are similar to those in RP-HPLC.

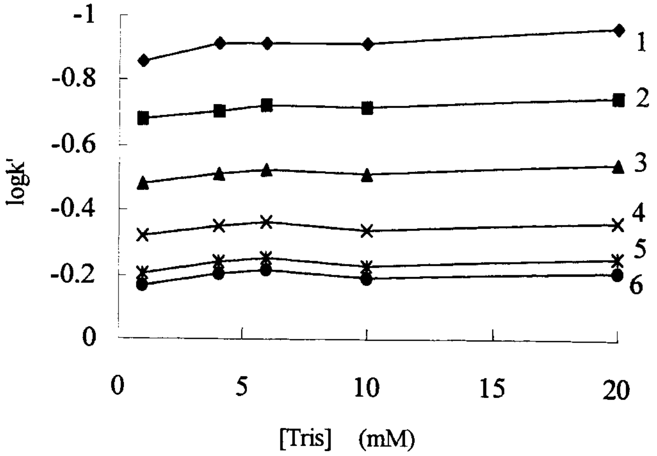

Figure 4. The plot of log k' vs. the concentration of Tris. Experimental conditions: Mobile phase: $CH_3CN:H_2O$ =7:3 (v/v). Other conditions as shown in Fig.1. 1: Aniline, 2: Acetophenone, 3: *o*-Nitrotoluene, 4: Toluene, 5: *o*-Xylene, 6: *o*-Chlorotoluene.

Effect of Salts on Retention

It is necessary to add salts or electrolytes in mobile phase for the performance of CEC. In RP-HPLC, the logarithm of its capacity factor will increase linearly with salt concentration due to the increase of surface tension according to solvophobic theory proposed by Horvath.[8] However, the effect of salts in RP-CEC seems not to be the same as that in RP-HPLC.

Figure 4 is the plots of log k' vs. salt concentration for several substituted benzenes. It shows that log k' decreases with the increase of salt concentration. The reason is that the change of salt concentration will influence the distribution of surface charge of packing particles, as well as double electric layer and separation current. Their effects result in the change of partition of solute between mobile phase and stationary phase.

Meanwhile, the increase of salt concentration in mobile phase usually results in the decrease of linear velocity in CEC,[2,4] while the linear velocity is usually invariant in RP-HPLC. Therefore, the effect of salts in RP-CEC is not a prediction of a simple linear decreasing relationship with the increase of salt concentration in RP-HPLC.[8]

CONCLUSIONS

Based on the solvatochromic comparison method, the retention behavior in RP-CEC was investigated by separating several substituted benzenes. It was found that the retention behavior is not the same in RP-CEC and RP-HPLC. The retention behavior in RP-CEC can be predicted quite well based on either solvatochromic parameters of solutes or solvatochromic solvent polarity measurement E_T (30) of mobile phase. The hydrogen bond acidity and dipolarity/polarization of solutes are the same important parameters to determine the retention in RP-CEC. These results may be due to the polarization effect of solutes and stationary phase induced by electric field in separation system. The effect of salts is complicated due to the effect of applied electric field in CEC.

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