Retention Behavior of Neutral Solutes in Pressurized Flow-Driven Capillary Electrochromatography Using an ODS Column

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Abstract

Several alkyl benzenes are separated by pressurized flow-driven capillary electrochromatography using a temperature-controlled capillary column packed with octadecyl siloxane-modified silica gel, and the effect of applied voltage on the retention is investigated. The van't Hoff plot shows good linearity at the column temperature between 305 and 330 K under applications from –6 to +6 kV. The applied voltage causes a relatively large variation in the enthalpy and the entropy of transfer of the solute from the mobile phase to the stationary phase (> 20%). However, the direction of variation in the enthalpy is almost opposite to that in the entropy, both of which might compensate each other. Therefore, the retention factor is not significantly varied (< 4%) by the application of voltage.

Introduction

Chromatographic retention is determined by a wide variety of factors, such as a functional group of the stationary phase, mobile phase composition, and temperature of the column. It is well known that the relationship between the column temperature and the retention factor is described by the van't Hoff's equation as follows:

$$\ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi \qquad \text{Eq. 1}$$

where k is the retention factors of the sample solute, ΔH° is the enthalpy of transfer of the solute from the mobile phase to the stationary phase, ΔS° is the entropy of transfer of the solute from the mobile phase to the stationary phase, R is the gas constant, T is the temperature of the column, and ϕ is the phase ratio of the column. The van't Hoff plot has been often used to study chromatographic behaviors. In 1992, Cole et al. reported that the relationship between $\ln k$ and column temperature depends on the bonding density of a functional group (1,2).

They revealed that the van't Hoff plot had a transition point when the bonding density was over 2.84 µmol/m². Morin et al. observed the dependence of the van't Hoff plot on the pH value of the eluent using a β-cyclodextrin-bonded stationary phase (3). They concluded that the enthalpy–entropy compensation revealed that the retention mechanism of the solute in the β-cyclodextrin-bonded stationary phase was almost independent of the compound's molecular structure, and that it remained the same at pH value of 7 and 7.5, but changes at pH values among 6.5, 8, and 8.5. Sellergen et al. also reported the dependence of the retention and separation factors on temperature using a chiral stationary phase (4).

Capillary electrochromatography (CEC) is a separation technique that combines high-performance liquid chromatography (HPLC) and capillary electrophoresis (5–7). The van't Hoff plot was also used to study the retention upon an applied voltage in CEC. Walhagen et al. separated small linear peptides by CEC and studied the dependence of several electrochromatographic parameters on temperature (8). Carter-Finch et al. studied the decrease in the resolution of D,L-tryptophan in CEC caused by an increase in the column temperature from 288 to 313 K, using a chiral separation column (9).

In our previous study of pressurized flow-driven CEC (p-CEC), it is reported that the application of a voltage affected the retention factors of both ionic and neutral compounds (10–12). The applied electric field may change the properties of the stationary phase or the interaction between the stationary phase and the sample solutes. The application of a voltage on a mixed-mode stationary phase of the octadecyl siloxane (ODS) and anion exchanger varied the retention of neutral compounds (10). Furthermore, the voltage-induced variation in the retention of neutral compounds was also observed in a heptakis-6-bromo-6-deoxy-β-cyclodextrin-modified column but was not significantly in the ODS column (12). In this paper, the effect of an applied voltage on the retention of neutral solutes in p-CEC (using the ODS column in terms of ΔH° and ΔS°, which were calculated from the van't Hoff plot) are studied to reveal the phenomenon of the less variation in the retention factor of the neutral solute under the electric field.

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Experimental

The apparatus consisted of an LC pump (LC-6A, Shimadzu, Kyoto, Japan), UV detector (UV 970, Jasco, Tokyo, Japan), high-voltage power supply (HCZE-30PN, Matsusada Precision, Shiga, Japan), injector (model 7410, Rheodyne, Rohnert Park, CA), laboratory-made capillary column, and laboratory-made splitter (using split injection method). Silica gel particles modified with ODS (5-μm particle size, Develosil ODS-5, Nomura Chemical, Aichi, Japan) were packed into the fused silica capillary (0.075-mm i.d., GL Science, Tokyo, Japan) using a slurry packing method (packed length: 7.9 cm). The capillary column was covered with a small laboratory-made "water jacket" to control the column temperature. A schematic diagram of the water jacket is shown in Figure 1. The water for the temperature control was supplied at the flow rate of 4 mL/min by a micro tube pump (Micro Tube Pump MP-3, Tokyo Rikakikai, Tokyo, Japan), and the water flowed through the water jacket for approximately 30 s. Because the walls of the water jacket are sufficiently thin, the surface temperature of the jacket might be regarded as the water temperature (internal temperature of the column), which was measured using a thermocouple. The open-tubular section of the inlet of a column (shown in Figure 1) is used to control the temperature of the eluent approaching the packed section. The voltage was applied at the outlet of the column. In order to obtain a stable condition, the sample injection was performed 10 min after the change in the applied voltage.

A mixture of 20mM phosphate buffer (pH 6.8) and acetonitrile (40:60, v/v) was used as the eluent. All the sample compounds (uracil, toluene, ethylbenzene, propylbenzene, and butylbenzene) were dissolved in a mixture of distilled water and acetonitrile (40:60, v/v). Uracil was used as the elution time (t_0) marker. All chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Results and Discussion

Evaluation of the water jacket type column temperature control system

The performance of the water jacket was evaluated by the elution time of uracil (t_0). In general, flow velocity (u) and viscosity (η) are inversely related according to the following equation:

$$u = B\,\frac{\Delta p}{\eta} \qquad\qquad \text{Eq. 2}$$

where B and Δp are constant depending on the column and pressure drop, respectively. In this study, because the eluent flowed under constant pressure, Δp is constant. The quantities t_0 and η are correlated by the following equation:

$$t_0 = \frac{l}{u} = \frac{l}{B\Delta p}\,\eta \qquad\qquad \text{Eq. 3}$$

where l is the length from the inlet to the detection window of the column. In general, η and the temperature are related by the Arrhenius equation as follows:

$$\eta = A\exp\left(\frac{E_a}{RT}\right) \qquad\qquad \text{Eq. 4}$$

where A is a constant, and E_a represents the activation energy. Using equation 4, the correlation coefficient (r^2) between η and T of a 60% aqueous acetonitrile solution was over 0.99 (13). Thus, the relation between t_0 and T is described by the following equation:

$$t_0 = \frac{Al}{B\Delta p}\exp\left(\frac{E_a}{RT}\right) \qquad\qquad \text{Eq. 5}$$

In our experiment, the r^2 between $\exp(1/T)$ and t_0 using equation 5 was also more than 0.99 in the entire range of applied voltages. The viscosity of a mixture of water and acetonitrile (40:60, v/v) at 328 K is 60% that at 298 K (13), and the observed t_0 value at 328 K was also approximately 60% that at 298 K. Thus, the column temperature could be adequately controlled using this water jacket system.

A mixture of uracil (t_0 marker), toluene, ethylbenzene, propylbenzene, and butylbenzene was then separated by μ-HPLC without the application of voltage. The repeatability of the retention factors of the sample solutes ($n = 5$) at 298 K and 322 K is listed in Table I. The relative standard deviation of all the retention factors was less than 3%. Therefore, adequate control of the column temperature was achieved during the experiment.
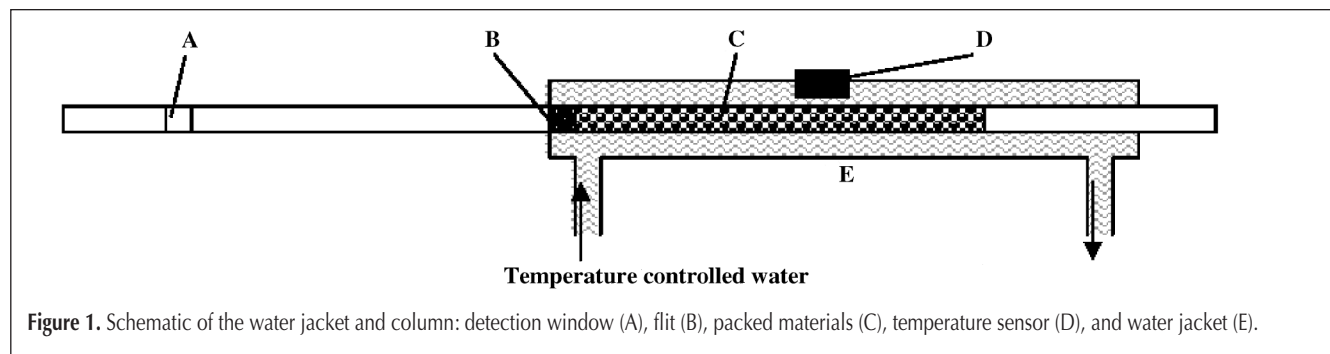

Figure 1. Schematic of the water jacket and column: detection window (A), flit (B), packed materials (C), temperature sensor (D), and water jacket (E).

Temperature controlled water

The effect of applied voltage on retention factor, enthalpy, and entropy

The mixture of uracil, toluene, ethylbenzene, propylbenzene, and butylbenzene was separated by p-CEC. As shown in Figure 2, the increase in the column temperature induced a considerable decrease in the retention time of the sample compounds. This decrease in elution time was provided by both a decrease in retention and acceleration of the pressurized flow velocity, which was caused by the decrease in viscosity of the mobile phase at higher temperature (t_0 value was also decreased). Also, the application of +6 and –6 kV slightly increased and decreased, respectively, the t_0 values (as shown in Figure 2). The deprotonated-residual silanol groups on the packing materials could provide a slightly negative charged surface to generate the electroosmotic flow, by which the t_0 values were varied. Moreover, Figure 3 shows the voltage-induced variation in the relative retention factors (k/k_0, the subscript 0 indicates the absence of an applied voltage) of the neutral solutes under various applied voltages at 298 K. The variation in k/k_0 induced by an applied voltage is only 4% or less.

The variation in retention factors of four neutral solutes because of an applied voltage was also investigated using van't Hoff plots. Figure 4 shows the relationship between ln$k$ and temperature at various applied voltages. The van't Hoff plots show good linearity in the range from 0.0030 to 0.0033 of $1/T$ (from 305 to 330 K of T) but are nonlinear over 0.0033 (305 K) of T. Cole et al. pointed out that the van't Hoff plot had a transition point when the bonding density of the ODS stationary phase was over 2.84 μmol/m^2 (1). In addition, Walhagen et al. reported that the van't Hoff plot using the ODS-modified column was not linear between 303 and 313 K (8). In our study, because the bonding density of the ODS stationary phase was 3.0 μmol/m^2, the van't Hoff plot would have a transition point at approximately 0.0033 of the value of $1/T$. The applied voltage hardly affected the transition point.

The value of ΔH° and $\Delta S^\circ/R + \ln\phi$ between 0.0030 and 0.0033 of $1/T$ (i.e., in the linear range) was calculated using equation 1 and correlated with the applied voltage as shown in Figure 5. The ΔH° value clearly varied depending on the sample solutes, while $\Delta S^\circ/R + \ln\phi$ values showed relatively smaller dependence than ΔH° did. Because lnϕ was constant regardless of the kind of solute because the same column was used, the variation in $\Delta S^\circ/R + \ln\phi$ was mostly controlled by that in ΔS°. Contrary to Figure 5, as was reported the literature (1), the dependency of the ΔS° value on the kind of sample solute (toluene, ethylbenzene, and propylbenzene) was larger than

Table I. Repeatability of k of Alkylbenzenes at 298 and 322 K for Temperature-Controlled μ-HPLC with Water Jacket without Applied Voltage (n = 5)*

Temperature (K)		Toluene	Ethylbenzene	Propylbenzene	Butylbenzene
298	k	0.97	1.47	2.43	3.97
	%RSD[†]	1.4	0.71	1.3	1.5
322	k	0.75	1.15	1.84	2.95
	%RSD	3.0	2.0	2.9	2.8

* The conditions were: column, 0.075-mm i.d.; packed length, 7.9 cm; packed material, Develosil ODS-5; eluent, mixture of 20mM phosphate buffer (pH 6.8) and acetonitrile (40:60, v/v); flow rate, constant pressure mode of 70 kgf/cm^2; and detection, UV 210 nm.
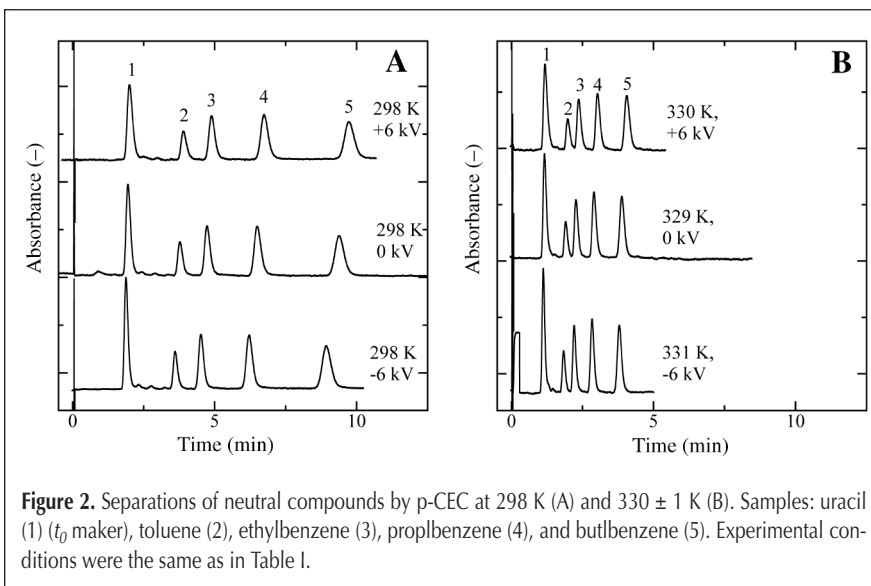[†] RSD = relative standard deviation.

Figure 2. Separations of neutral compounds by p-CEC at 298 K (A) and 330 ± 1 K (B). Samples: uracil (1) (t_0 maker), toluene (2), ethylbenzene (3), proplbenzene (4), and butlbenzene (5). Experimental conditions were the same as in Table I.
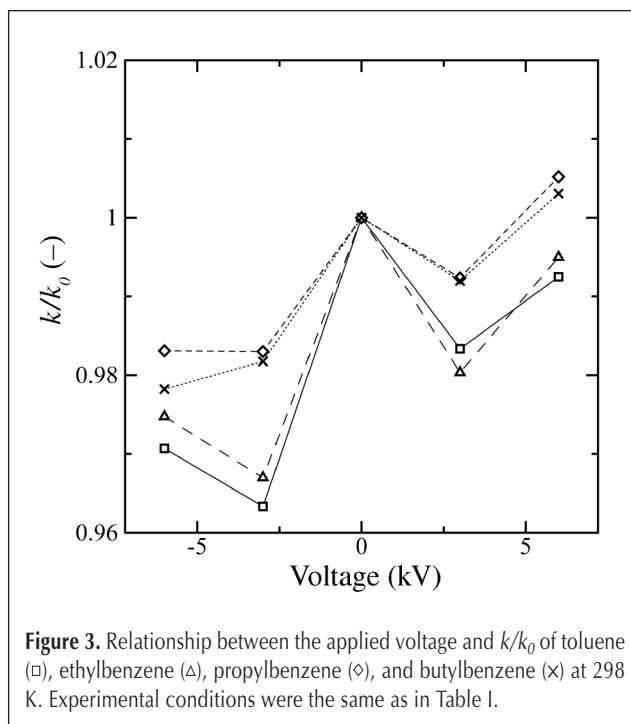

Figure 3. Relationship between the applied voltage and k/k_0 of toluene (□), ethylbenzene (△), propylbenzene (◇), and butylbenzene (✕) at 298 K. Experimental conditions were the same as in Table I.

that of $\Delta H°$. In our experiment, therefore, the contribution of $ln\phi$ to $\Delta S°/R + ln\phi$ might be relatively large. Nevertheless, both the $\Delta H°$ and $\Delta S°/R + ln\phi$ were significantly varied by the application of the voltage. From 0 to 3 kV application, the decrease in $-\Delta H°$ was approximately 22%, and the increase in $\Delta S°/R + ln\phi$ was approximately 23%. The variation in $-\Delta H°$ and $\Delta S°/R + ln\phi$ was larger than that of k shown in Figure 3 (4%). As shown in Figure 5, the voltage-induced variation patterns in $\Delta H°$ were almost opposite to those in $\Delta S°/R + ln\phi$. Therefore,

the variation in $\Delta H°$ was compensated for by the variation in $\Delta S°/R + ln\phi$. This might serve to explain why only the small variation in the retention factor of neutral solutes was induced by even the application of a high voltage in CEC using an ODS column.

Moreover, the application of negative and positive voltage has given the asymmetric variation patterns of both $-\Delta H°$ and $\Delta S°/R + ln\phi$ (i.e., the variation pattern from -6 to -3 kV is almost opposite to of $+6$ to $+ 3kV$). Similar asymmetric voltage-induced variations in retention factor have been obtained in our previous studies (10,12,14,15). The reason for the asymmetric variation has not been clarified sufficiently yet. Further study in this line is currently in progress.

Acknowledgments

Figure 4. van't Hoff plots of toluene (A), ethylbenzene (B), propylbenzene (C), and butylbenzene (D) at the applied voltage of -6 kV (□), -3 kV (△), 0 kV (◇), $+3$ kV(+), and $+6$ kV (×). Experimental conditions were the same as in Table I.

Figure 5. Relationship between the applied voltage and $\Delta H°$ (A) and $\Delta S°/R + ln f$ (B) of methylbenzene (□) ethylbenzene (△), propylbenzene (◇) and butylbenzene (×), and the error bar represents standard deviation. Experimental conditions were the same as in Table I.

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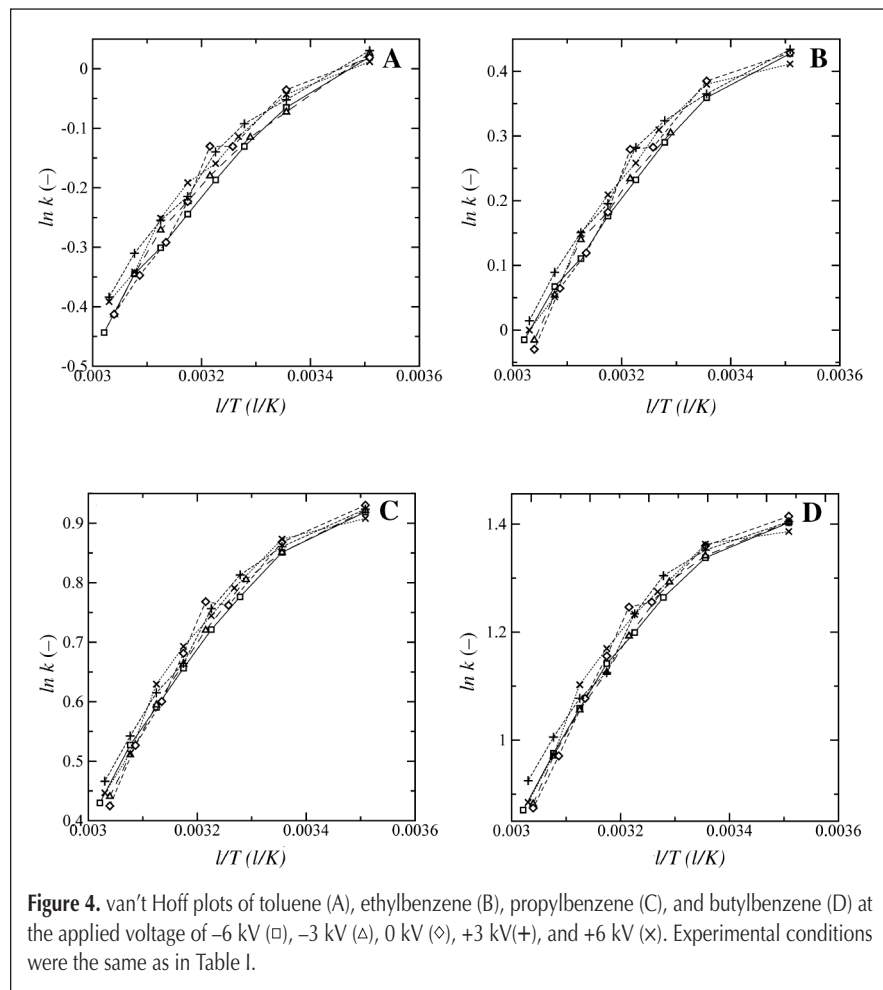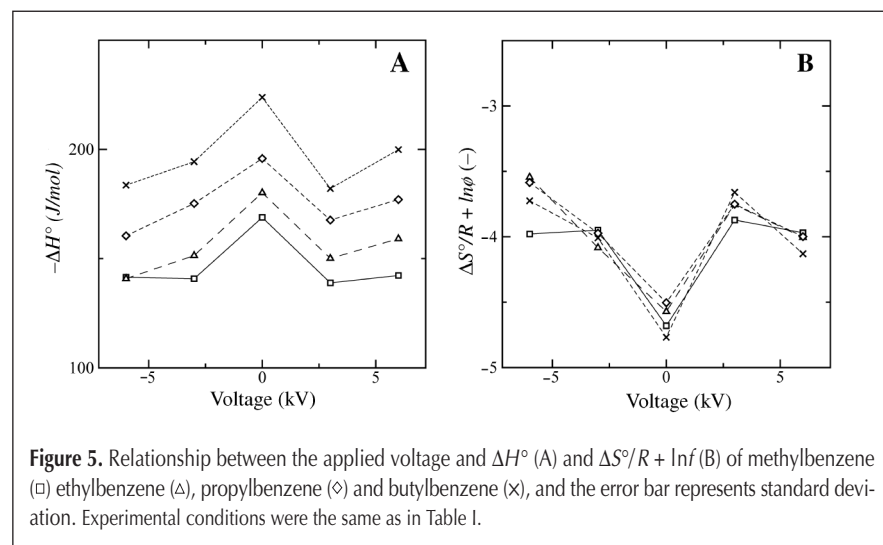
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