

Original

Voltage-induced Variation of Capacity Factors for Neutral Solutes by Using Hexa-6-bromo-hexa-6-deoxy- β -cyclodextrin Modified Column in Electrochromatography

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Abstract

The voltage-induced variation of capacity factors of neutral compounds in pressurized flow driven capillary electrochromatography were investigated. The capillary column packed with hexa-6-bromo-hexa-6-deoxy- β -cyclodextrin modified silica gel and neutral solutes were used. The capacity factors of four neutral solutes were able to vary with the application of voltage. All solutes took maximum capacity factors with the application of -3 kV. The voltage-induced variation of relative capacity factor did not depend on the kind of solutes. The variation of the column conductance was also observed. The comparison between hexa-6-bromo-hexa-6-deoxy- β -cyclodextrin and ODS modified columns suggested that the surface charge of the packing material is one of the key factor of the voltage-induced phenomena. The direct control of the retention behavior might be possible by using application of the voltage in same extent.

Keywords: electrochromatography, capacity factor, conductance, electric double layer, β -cyclodextrin, ODS

Introduction

The capillary electrochromatography (CEC) takes an important position in the separation sciences [1-8], and its further development will contribute to the analytical chemistry field. Therefore the study of the electrochromatographic behavior is very important. The key factor of CEC is the electric field applied along a capillary column. In general, the role of the applied electric field in CEC is to provide two kinds of the flow, *i.e.* electroosmotic flow and electrophoresis of the charged solute. The mechanisms and roles of these two flows are well known [1, 10]. In addition, the applied electric field induces some interesting phenomena [2-9].

One of the phenomena is voltage-induced variation of the capacity factor [5-8]. The voltage-induced variation of the capacity factors in the electrochromatography using the column packed with ion-exchange supports were described in our former reports [6, 7]. The unique point of this phenomenon is that the variation of the capacity factor recognized the direction of the electric field. When the application of a negative voltage decreases the capacity factor of the solute, the positive-voltage application increases its capacity

factor. Tsuda reported that the elution time was depended on the period from the beginning of the application of the voltage [2], and Unger and Eimer also reported that the period to obtain the stable retention time after the release of the voltage was more than 4 hours [3]. These phenomena might also reflect the voltage-induced retention variation.

Other phenomenon is the dependence of the electric resistance of the capillary column on the applied electric field [5, 9]. The electric resistance of the capillary column varies with the application of the voltage. This phenomenon also recognizes the direction of electric field, and the variation pattern of the column resistance was governed by the surface charges of the packing materials. The variation of the column electric resistance might suggest that the variation of the ion concentrations around the surface of the packing materials. Namely applied electric field might change the condition around the packing materials.

These phenomena might suggest that application of the electric field along the column changes the condition of the stationary phase. The investigation of this phenomenon is necessary to the

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further development of CEC. In this report, the voltage-induced phenomena in CEC using the column packed with hexa-6-bromo-hexa-6-deoxy- β -cyclodextrin (β -cyclodextrin-Br) modified silica gel are described. The neutral solutes were used to observe the variation of the capacity factors without electrophoretic movement.

Theory

In general, the elution time of the solute in the chromatography is able to describe by using following equation.

$$t_e = \frac{L(k+1)}{v_{press}} \quad (1)$$

where t_e , L , k and v_{press} are elution time of the solute, column packed length, capacity factor and pressurized flow velocity, respectively. In case of electrochromatography, the velocity of the charged solutes in the mobile phase, v_{app} , is given by

$$\begin{aligned} v_{app} &= v_{press} + v_{osm} + v_{eph} \\ &= v_{press} + (\mu_{osm} + \mu_{eph}) \Delta E \end{aligned} \quad (2)$$

where v , μ and ΔE are velocity, mobility and electric potential gradient, respectively. The subscripts of *osm* and *eph* mean the electroosmosis and electrophoresis. The combination of equations 1 and 2 gives

$$t_e = \frac{L(k+1)}{v_{press} + (\mu_{osm} + \mu_{eph}) \Delta E} \quad (3)$$

In general chromatography, the capacity factor is defined by the following equation.

$$k = \frac{c_m}{c_s} \cdot \frac{V_m}{V_s} \quad (4)$$

where c and V mean concentration and volume of the phase, respectively. Subscripts of *m* and *s* mean mobile and stationary phase. On the other hand, the capacity factor is able to estimate from the chromatogram by using following equation.

$$k = \frac{t_e}{t_0} - 1 \quad (5)$$

In an equation 5, t_0 is the elution time of the non-retained compound, *i.e.* $k = 0$. In electrochromatography, the k values in equations 4 and 5 are different, when the solute has electrophoretic mobility.

The k in equations 4 and 5 are re-defined as k_{plate} and k_{chrom} , respectively. The relationship between k_{plate} and k_{chrom} is given by

$$k_{chrom} = \frac{\mu_{osm} \Delta E + v_{press}}{(\mu_{osm} + \mu_{eph}) \Delta E + v_{press}} (k_{plate} + 1) - 1 \quad (6)$$

When the μ_{eph} is zero, the k_{chrom} and k_{plate} are same. Therefore the variation of k_{chrom} of the neutral compounds suggests the variation of k_{plate} .

Experimental Section

The apparatus for the pressurized flow driven electrochroma-

tography used in this experiment was the same arrangement described in the former reports [4, 5, 8]. The apparatus was composed of a pump (LC-6 A, Shimadzu, Kyoto), an injector (7410, Rheodyne, CA), a splitter (the split injection method was used), a laboratory-made capillary column, a UV detector (UV-970, Jasco, Tokyo) and high voltage power supply (HCZE-30 PN, Matsusada Precision, Shiga). In this apparatus, the injector was always grounded for the safety of an operator. Therefore the description of "applied +X kV" means that the +X kV was applied the reservoir at column outlet. For the measurement of an electric current to estimate a conductance of the column, the injector was grounded via a constant resistance of 1 k Ω . The β -cyclodextrin-Br modified silica gel (CHIRAL β -CD BR, dp. 5 μ m, kindly donated by YMC, Kyoto) and ODS modified silica gel (CAPCELL C-18 UG, dp. 5 μ m, kindly donated by SHISEIDO, Yokohama) were packed by using slurry-packing method [5]. The inner diameters of all capillary columns were 150 μ m. The β -cyclodextrin-Br was bonded to the surface of the silica gel via amino functional group, namely, γ -amino propylsilane was bonded on the surface of the silica gel.

The solution of 10% methanol aqueous solution containing 5 mM phosphate buffer (pH 5.0) was used as the eluent for β -CD BR packed column. The packed and whole lengths of the capillary column were described in each figure caption. As sample solutes, o-cresol, m-cresol, o-nitrophenol, m-nitrophenol, phenol, toluene and benzene were used. All the reagents (Wako Pure Chemical Industries, Ltd., Kyoto) were guaranteed grade. All sample solutes, of which pKa values are over 8.0, exist as neutral compounds in the mobile phase. Two types of eluent, 10% and 40% methanol aqueous solution containing 5 mM phosphate buffer (pH 5.0), were used in the measurement of the conductance of the capillary column packed with β -CD BR (packed length : 18.2 cm, whole length : 26.2 cm). The ODS packed column (packed length : 22.0 cm) was also used to observe the electrochromatographic behavior of the neutral solute with the eluent of 50% methanol aqueous solution containing 5 mM phosphate buffer (pH 5.0).

Results and Discussion

Variation of the capacity factors of neutral solutes

The voltage-induced variation of the capacity factors of the neutral compounds has been investigated in the pressurized flow driven electrochromatography using a β -CD BR packed column (packed length : 27.5 cm). The typical electrochromatographic behavior of neutral solutes is shown in Figure 1. Peaks of A and B in Figure 1 are o-cresol (0.5 mM) and phenol (2.9 mM), respectively. The t_0 in Figure 1 were measured by using the methanol. The capacity factors of o-cresol and phenol without applied voltage were 1.01 and 1.36, respectively. The application of +5 kV at column outlet accelerated the elution of all solutes, because of the elec-

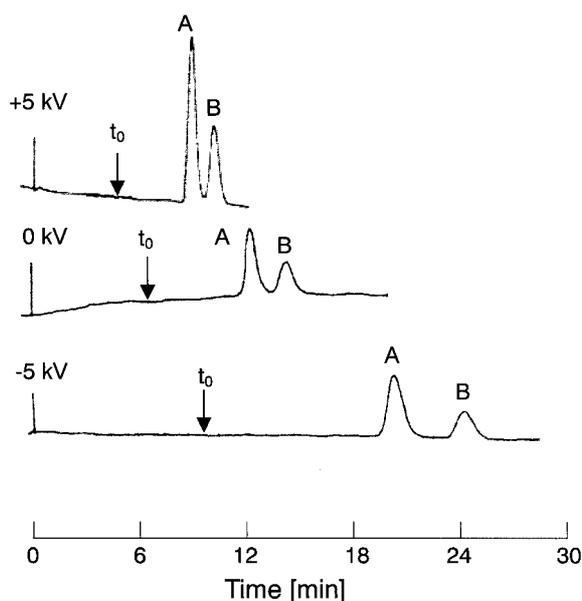


Figure 1. Typical electrochromatographic behavior of neutral solutes using a column packed with β -CD BR. Peaks of A and B are o-cresol and phenol, respectively. Column : i. d. 150 μ m, packed length 27.5 cm, whole length 34.7 cm, packed with β -CD BR; Eluent : 10% methanol aqueous solution containing 5 mM phosphate buffer (pH 5.0); Flow : constant pressure of 150 kgf/cm²; Detection : UV 210 nm.

trosmotic flow that directed to the positive electrode. The application of -5 kV decelerated the elution of all solutes. The capacity factors of o-cresol with application of +5 kV and -5 kV were 0.92 and 1.10, respectively, and those of phenol were 1.20 and 1.51. Namely the application of +5 kV decreased both capacity factors of o-cresol and phenol, and the application of -5 kV increased them.

The variations of the capacity factors of neutral solutes were further investigated by using a β -CD BR packed column (packed length : 20.3 cm). The capacity factors of four sample solutes of m-cresol (0.2 mM), m-nitrophenol (0.2 mM), phenol (0.5 mM) and toluene (0.5 mM) were 1.40, 3.39, 1.35 and 2.78, respectively, without application of voltage. These capacity factors, calculated from the chromatogram by using equation 5, were defined as k_0 . With the application of -3 kV, the capacity factors of 1.56 (m-cresol), 3.68 (m-nitrophenol), 1.43 (phenol) and 2.98 (toluene) were obtained. All capacity factors were measured after the conditioning for over 1 hour, when the applied voltage was changed. The capacity factors of all neutral solutes were varied with every application of the voltage. Namely the application of the electric field induced the variation of the capacity factor of the neutral solute in electrochromatography using the β -cyclodextrin-Br modified column.

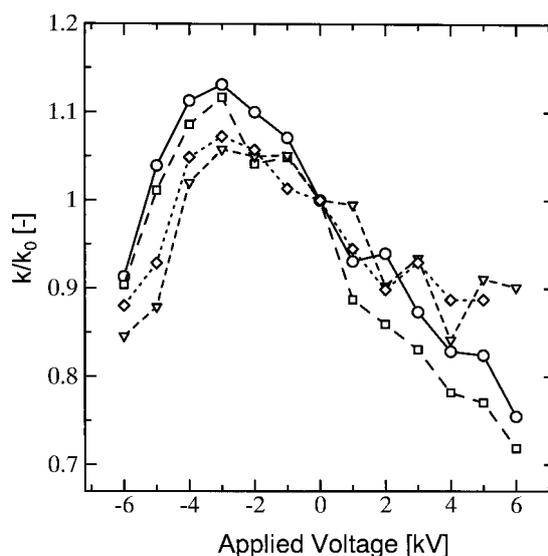


Figure 2. Relationship between applied voltage and relative capacity factors of neutral solutes of m-cresol (○), m-nitrophenol (□), phenol (△) and toluene (◇). Column : i. d. 150 μ m, packed length 20.3 cm, whole length 28.9 cm, packed with β -CD BR. Other conditions are same in Figure 1.

To compare the magnitude of the voltage-induced capacity factor variation of each compound, the relative capacity factor, defined as the k/k_0 , is introduced [8]. The Figure 2 shows the relationship between applied voltage and relative capacity factors of neutral compounds. The relationship in Figure 2 indicates that the voltage-induced variation pattern of the relative capacity factor of the neutral solute is almost same. Namely, the voltage-induced variations of the relative capacity factor dose not depend on kinds of neutral solutes under this experimental condition. The retention order of the sample solutes was constant in every electrochromatograms, and drastic changing of the peak shape was not observed. The relative standard deviation (RSD) of the capacity factor was less than 3% ($n=2$ to 4) at each application of voltage in one series. The slimier variation pattern was observed in all capillary columns packed with β -CD BR. The capacity factor calculated from the chromatogram (k_{chrom}) is equal to that in plate theory, k_{plate} , when the solute is neutral. Therefore the application of the voltage along the column affects a magnitude of the interaction between neutral solute and the β -cyclodextrin-Br in the stationary phase. All neutral compounds show the maximum of relative capacity factor at the application of -3 kV. In addition, the slope at the left side of maximum is steeper than the right side slope.

Variation of the conductance of the column

The conductance of the capillary column was estimated by us-

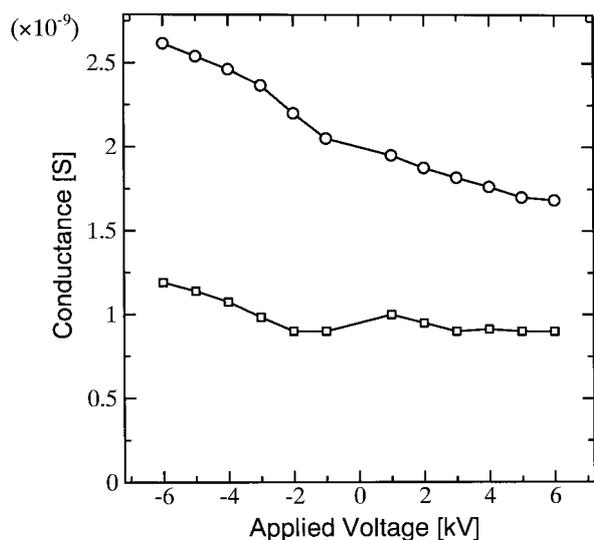


Figure 3. Relationship between applied voltage and conductance of the column. Column : i.d. 150 μm , packed length 18.2 cm, whole length 26.2 cm, packed with β -CD BR ; Eluent ; 10% (O) and 40% () methanol aqueous solution containing 5 mM phosphate buffer (pH 5.0).

ing Ohm's law from the applied voltage and the electric current. The relationship between applied voltage and electric conductance of the column is shown in Figure 3. The conductances of the column are in increase and decrease with the application of the negative and positive voltage, respectively, and the application of higher voltage results the larger variation of the column conductance. The slope of the variation of the conductance shown in Figure 3 is negative. The magnitude and sign of the slope of the voltage-induced conductance variation is governed by the surface charge [9]. The negative slope suggests the surface charge of the packing material is positive. The surface of the β -CD BR has positive charges, because of the existence of amino functional group between β -cyclodextrin-BR and silica gel surface. Therefore the negative slopes in Figure 3 are reasonable.

The charge on the surface attracts counter ions around surface and generates the electric double layer (EDL) [10]. For example, when the surface of the stationary phase has positive charge, the concentration of negative ions around the surface becomes higher than those of bulk solution. Therefore the conductivity at EDL becomes higher compared to the bulk solution [11]. Because the conductivity around the surface is different from that of bulk solution, the electric current through the capillary column might be able to divide two roots. One is the current through the bulk solution, i_{bulk} , and the other is the current through the EDL, i_{EDL} . In general, the conductivity of the bulk solution can be regarded as constant. Therefore the variation of the conductance of the capillary column might reflect the variation of the conductivity of the EDL. The

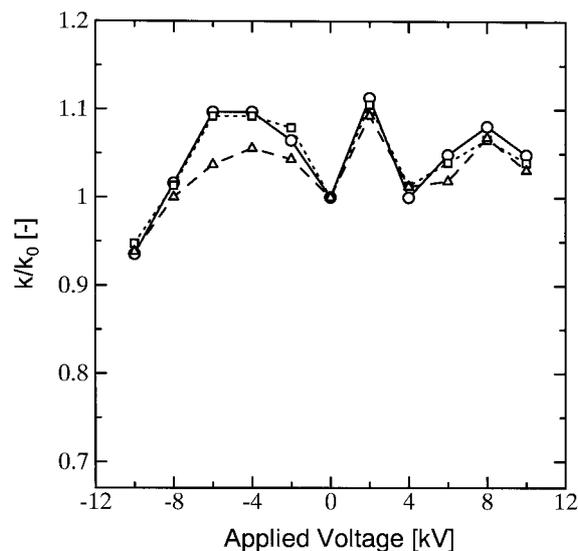


Figure 4. Relationship between applied voltage and relative capacity factors of neutral solutes of o-cresol (O), o-nitrophenol () and benzene (). Column : i.d. 150 μm , packed length 22.0 cm, packed with ODS ; Eluent ; 50% methanol aqueous solution containing 5 mM phosphate buffer (pH 5.0) ; Flow : constant pressure of 150 kgf/cm^2 ; Detection : UV 254 nm.

variation of column conductance suggests that the applications of positive and negative voltage induce decreasing and increasing of the conductance in EDL, respectively.

Comparison of β -cyclodextrin-BR and ODS packed column

The electrochromatographic behavior of three neutral solutes of o-cresol (0.2 mM), o-nitrophenol (0.2 mM) and benzene (1.8 mM) were also studied by using the column packed with ODS modified silica gels for further investigation of the voltage-induced capacity factor variation. The relationship between applied voltage and relative capacity factor, k/k_0 , is shown in Figure 4. The Figure 4 does not show the significant voltage-induced variation of the relative capacity factor compared to the β -CD BR packed column (Figure 2). In addition, the conductance of the column packed with ODS was almost constant in our former report [9]. Therefore magnitudes of above two phenomena, voltage-induced variations of the capacity factor and the conductance, might not so large in the case of using the ODS column. The surface charge density of ODS silica gel is quite smaller than that of the β -CD BR, of which comes from the amino functional group. The surface charge density and its sign have significantly governed the voltage-induced conductance variation [9].

The increase of the column conductance suggests the increase of the conductivity of the EDL, and the high conductivity of the EDL might be resulted from the increment of the concentration of

the ion in the EDL. In general, the ion concentration of the solution varies the surface tension and the dielectric constant of the solution [12]. In case of the reversed phase liquid chromatography, the retention is governed by the surface tension and the dielectric constant [13]. Therefore the voltage-induced variation of the ion concentration in EDL might be one of the factors in the variation of the capacity factor shown in Figure 2.

Conclusions

The application of the electric field along the column, packed with β -CD BR, induced the both variation of capacity factors of neutral solutes and conductance of the column. These voltage-induced phenomena were not much observed when the column packed with ODS silica gels was used. These voltage-induced variations are relevant to the surface charge of the packing material. The precise detail of the mechanism of the variation of the capacity factor is still unclear. However our result suggests the possibility of the direct control of the chromatographic behavior by using the electric field application in same extent. We may be able to develop the newly field of the electrochromatography by using this phenomenon.

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