

Electric Resistance Variation of Packing Materials in Capillary Electrochromatography by Using Fluorinated-Bonded Silica and Octadecylsilane Phases

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The linear relationship between electric resistance of the packed column for capillary electrochromatography and applied voltage was observed in several different effluents and packing materials (fluorinated-bonded silica and octadecylsilane). These linear relationships had different slope values which depended on the charges on the surface of the stationary phase. If there are the negative charges on the stationary phase during the application of the electric field, the slope was negative value as observed in the fluorinated-bonded silica packed column. On the other hand, the slope was positive value in the fluorinated-bonded silica packed column using 0.1% tetrabutylammonium chloride as an effluent. The inclination of the linear relationship between the electric resistance and applied voltage also depended on the kind of the effluent, the concentration of effluent and the ratio of the organic modifier used. In the case of octadecylsilane packed column, the electric resistance of the column did not depend on the applied voltage. The relationship between the electric resistance and the electroosmotic mobility was also studied.

(Received October 14, 1999; Accepted February 7, 2000)

The alternation of the nature of the stationary phase due to the application of the electric field was observed in electrochromatography.¹⁻⁷ In electrochromatography, the retention time and peak height of a solute depended on the period from the beginning of the voltage application.^{2,3} The capacity factor in electrochromatography was found to depend to some degree on the value of the applied voltage.⁴⁻⁶ The application of the electric field on the ion exchange packing supports in electrochromatography induced the release of the sample solutes from the stationary phase⁵ and the variation of distribution coefficient.⁶ During the application of electric voltage, the surface charges of the fluorinated-bonded silica phase altered.⁷ These phenomena indicated the alternation of the nature of the stationary phase due to the application of electro voltage which was also evidenced in the dependence of the electric resistance of the column on applied voltage as was discovered in the cation exchange resin support.⁴

In this article, the relationship between the electric resistance variation and the surface charges of some packing materials in electrochromatography was studied. This provides additional evidence of the alternation of the nature of the stationary phase caused by the electro voltage application.

Experimental

Apparatus, column and materials

The same instrument for pressurized flow driven electrochromatography as described in the former reports^{3,4,7,8} was used with different types of columns. A high-voltage power

supply (dc \pm 30 kV, type HCZE-30P, Matsusada Precision, Kusano, Shiga) was used. The expression "applied + X kV" means that +X kV is applied to the column outlet and the split injector is grounded. Fluorinated-bonded silica (type Fluofix 120E, 80421, particle diameter 5 μ m, pore size 120 Å, surface area 300 m²/g, Neos Co., Ltd., Kobe) in the capillary column (150 μ m i.d., 18.6 cm packed part, 28.6 cm total capillary length) was used as the main column. The other column used was octadecylsilane phase (Chemcobond 5-ODS-H, particle diameter 5 μ m, Chemco Ltd., Osaka) in the capillary column (150 μ m i.d., 19.9 cm packed part, 33.6 cm total capillary length). All reagents (Wako Pure Chemical Industries, Ltd. and Tokyo Chemical Industry Co., Ltd.) used were guaranteed grade. All solvent and buffer solutions were degassed by ultrasonic bath prior to use. Each chromatographic condition is listed in the text.

Electric resistance of column

The body of the split injector of the instrument was grounded via a constant resistance of 1 k Ω . An electric field was applied along the column in pressurized flow driven capillary electrochromatography. Electric voltages were applied in the range between +6 kV and -6 kV with 500 V steps. The electric resistance of the column was estimated by measuring the electric voltage at both ends of the 1 k Ω resistance. The electric resistances for the total packed column (R_{total}) under chromatographic conditions were estimated.

The electric resistance of the open-tubular fused-silica capillary (same dimensions as the packed column) (R_{cap}), and the open-tubular capillary with a column inlet and end frit (same component as used in packed column) ($R_{cap,frit}$) were also estimated under the same chromatographic conditions as those of the packed column. The electric resistance of the frit (R_{frit}) was estimated by subtracting the R_{cap} from the $R_{cap,frit}$. The

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electric resistance of the packed part of the packed column (R_{packed}) was obtained by subtracting the sum of R_{frit} and R_{cap} (with the real value depending on the length of the open-tubular part of the packed column) from R_{total} .

In fluorinated-bonded silica column, the R_{total} depended on the applied voltage as a linear relationship. For example, the R_{total} ($\text{M}\Omega$) for the 10 mM disodium hydrogenphosphate (Na_2HPO_4) effluent was expressed as

$$R_{\text{total}} = 133 - 4.16E \quad (1)$$

where E was the applied voltage (kV); the correlation factor was 0.998.

The estimated R_{cap} and $R_{\text{cap, frit}}$ were not constant over the range of applied voltage. This was due to the Joule heating of the capillary column. Meanwhile the linear relationship between the estimated R_{cap} or $R_{\text{cap, frit}}$ and absolute value of the measured current ($|I|$) (μA) was observed. This linear relationship (the value of slope is little) was used for the estimation of the R_{cap} and $R_{\text{cap, frit}}$. The R_{cap} and $R_{\text{cap, frit}}$ for the same chromatographic effluent were expressed as $R_{\text{cap}} = 97 - 0.35 |I|$, $R_{\text{cap, frit}} = 108 - 0.40 |I|$ and $R_{\text{frit}} = 11 - 0.05 |I|$. The R_{packed} became

$$R_{\text{packed}} = 88 - 4.16E + 0.17 |I| \quad (2)$$

For example, when E is 2 kV and the measured current (I) is 16.3 μA , the R_{packed} became 82.4 $\text{M}\Omega$ as observed in Fig. 1. The estimated R_{total} and R_{packed} were used to calculate the potential gradient.

Estimation of potential gradient

To determine the electroosmotic mobility (μ_{osm}), the estimation of the potential gradient (ΔE) (V/cm) value was very necessary. The experimental applied voltage could not be used to determine the potential gradient directly because the potential gradient depended on the electric resistance of the total column, the packed part and the open-tubular part. The dependence of the electric resistance of the column and applied electric field were used to estimate the potential gradient of the packed part of the column by using the former equation concerning the electric resistance:⁴ $\Delta E = [R_{\text{packed}}/R_{\text{total}}][E/L]$. The term L is the packed column length. For instance, ΔE of the fluorinated-bonded silica column, L is 18.6 cm, with 10 mM disodium hydrogenphosphate (Na_2HPO_4) effluent could be estimated from the following equation:

$$\Delta E = [88 - 4.16E + 0.17 |I|][133 - 4.16E]^{-1}[E/18.6] \quad (3)$$

When the applied voltage is 2 kV, the measured current is 16.3 μA , and the ΔE is 71.1 V/cm.

Estimation of mean linear flow velocity and pseudo-electroosmotic mobility

In pressurized flow driven electrochromatography, the electroosmotic velocity (V_{osm}) are given by^{1,8} $V_{\text{osm}} = L/(Rt_n) - V_{\text{pres}}$. L and R are the column length and the retardation factor $1/[1 + k]$; k is the capacity factor, t_n is the elution time of a neutral marker which was methanol. V_{pres} is the pressurized flow velocity, which can be determined by using 1,3,6-naphthalene trisulfonate sodium salt in the experiment using fluorinated-bonded silica column.⁷

The pseudo-electroosmotic mobility (μ_{pseudo}) value was introduced for the study of the relationship between the electric resistance and the surface charges of the stationary phase. This value is equal to the electroosmotic mobility (μ_{osm}) in the region of positive applied voltage, but gives the negative value of the

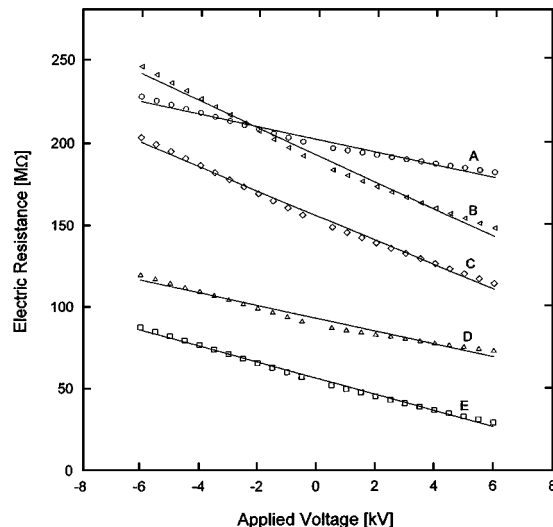


Fig. 1 Relationship between electric resistance and applied voltage for different effluent chromatographic conditions. Column, fluorinated-bonded silica column; effective length (from column inlet to detector), 18.6 cm; total capillary length, 28.6 cm; inner diameter, 150 μm . Electric resistance was obtained under the pressurized flow at the constant pressure mode of 50 kgf/cm^2 . Effluent: [A] methanol:20 mM ammonium acetate (35:65); [B] 10 mM potassium dihydrogenphosphate (KH_2PO_4); [C] methanol:20 mM phosphate buffer pH 6.2 (35:65); [D] 10 mM disodium hydrogenphosphate (Na_2HPO_4); [E] 20 mM disodium hydrogenphosphate (Na_2HPO_4).

μ_{osm} in the region of negative voltage application. The μ_{pseudo} of each voltage application could be calculated from the relationship of the V_{osm} and the potential gradient under chromatographic condition supposed as

$$V_{\text{osm}, i} = \mu_{\text{pseudo}, i} |\Delta E_i| \quad (4)$$

$V_{\text{osm}, i}$ is the electroosmotic velocity of each i applied voltage. $|\Delta E_i|$ is the absolute value of the potential gradient of each i applied voltage. $\mu_{\text{pseudo}, i}$ is the pseudo-electroosmotic mobility calculated from the relationship of the $V_{\text{osm}, i}$ and $|\Delta E_i|$ at each i voltage application.

Results and Discussion

Relationship between electric resistance and the surface charges of the fluorinated-bonded silica phase

In pressurized flow driven electrochromatography, the electric resistance of the packed part of the column under several chromatographic conditions and packing materials depended on applied voltage as a linear relationship, as shown in Figs. 1 and 2. Figure 1 shows the linear relationship in fluorinated-bonded silica column. The high electronegativity of fluorinated structure induced the negative charges on the silica surface of the fluorinated-bonded silica phase during application the electric field,⁷ and the negative charge might play an important role on the negative slope shown in Fig. 1.

The electric resistance of the packed part was different, as observed in Fig. 1. These differences were effected by the electrolyte composition in the effluent used. High electrolyte concentration in the mobile phase gives low electric resistance value. However, it does not contribute to the variation of the electric resistance as observed from the almost constant value of

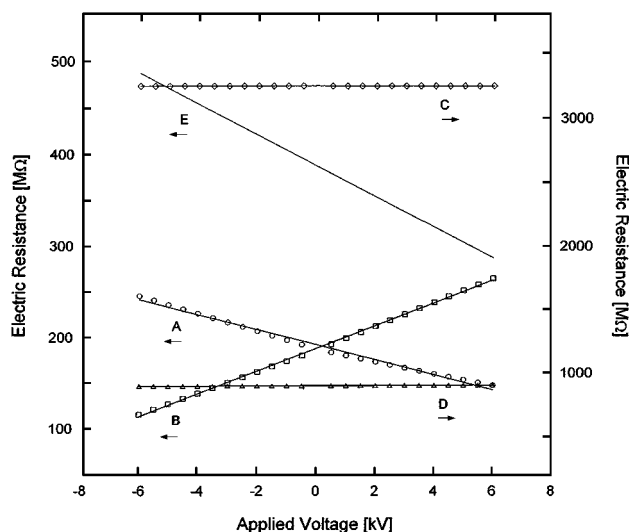


Fig. 2 Relationship between electric resistance and applied voltage for three different columns. Column [A]: packed fluorinated-bonded silica column under 10 mM KH_2PO_4 effluent; Column [B]: packed fluorinated-bonded silica column under 0.1% tetrabutylammonium chloride (TBAC) effluent; Column [C]: packed octadecylsilane (ODS) column under methanol:20 mM phosphate buffer pH 6.2 (80:20). Effective column length 19.9 cm; total capillary length 33.6 cm, inner diameter 150 μm . Electric resistance was obtained under the pressurized flow at the constant pressure mode of 50 kgf/cm^2 . Column [D]: packed ODS column under methanol:20 mM phosphate buffer pH 6.2 (35:65); Column [E]: packed cation exchange supports, IC-Cation-SW column.⁴ Effective column length 25.4 cm, total capillary length 34.3 cm, inner diameter 75 μm . Electric resistance was obtained under the pressurized flow at the constant pressure mode of 70 kgf/cm^2 .

electric resistance in octadecylsilane (ODS) packed column (Lines C and D in Fig. 2).

The applied voltage had an influence on the variation of the electric resistance. The variation of the slope value between the electric resistance and applied voltage could be observed in fluorinated-bonded silica phase. For example, the slope values of the 10 and 20 mM disodium hydrogenphosphate, Na_2HPO_4 , effluents used were -3.97 and -4.95 $\text{M}\Omega/\text{kV}$. The surface charges of the fluorinated-bonded silica phase were assumed to have a lower density of negative charges when positive voltage was applied compared to the application of the negative voltage.⁷ This alternation of the density of surface charges on the stationary phase might play a role in the variation of the slope between electric resistance and applied voltage.

From Fig. 1, the slope values of the 35/65 methanol/20 mM ammonium acetate effluent used (Line A) and 35/65 methanol/20 mM phosphate buffer at the same pH as an effluent (Line C) were -3.86 and -7.53 $\text{M}\Omega/\text{kV}$ respectively. When the ammonium acetate reagent was used as an effluent, it adsorbed on the fluorocarbon stationary phase.⁷ It might suppress the negative charges that would be on the surface of the phase.⁷ The fact that the electroosmotic velocity was less in the ammonium acetate chromatographic condition than in the phosphate buffer effluent⁷ might support this conjecture. These phenomena are part of the explanation of the difference on the slope value between Lines A and C in Fig. 1.

Comparison of electric resistance variation between different packing materials

In the experiment, the packed column of fluorinated-bonded

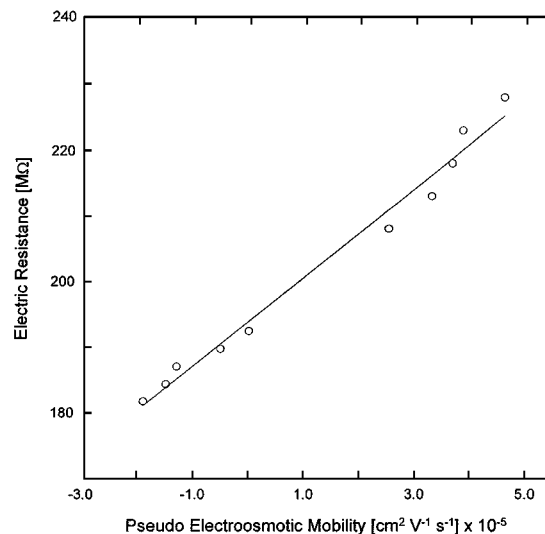


Fig. 3 Relationship between electric resistance and pseudo-electroosmotic mobility (μ_{pseudo}) for methanol:20 mM ammonium acetate (35:65). Other conditions were the same as in Fig. 1.

silica phase with the medium of 0.1% tetrabutylammonium chloride (TBAC) shows the linear relationship between the applied voltage and the electric resistance value of the column, and it has the positive slope value with the correlation factor of 0.997 as shown as Line B in Fig. 2. TBAC which is a positive charge reagent might adsorb strongly on the surface of the stationary phase,^{9,10} and the surface charge on stationary phase might turn to positive. Thus the positive charge on the stationary phase might effect the slope value and yield a positive slope value.

In the former report⁴ using a cation exchange resin capillary column under chromatographic condition, the negative slope value of the electric resistance and applied voltage might be due to the negative charges on the stationary phase, as shown by Line E in Fig. 2.

In ODS packed column, the nearly constant value of the electric resistance, shown in Fig. 2, is due to the neutrality on the surface of the stationary phase during the voltage application.

Relationship between electric resistance and the pseudo-electroosmotic mobility (μ_{pseudo}) in fluorinated-bonded silica column

In the experiment using fluorinated-bonded silica column, the pseudo electroosmotic mobility (μ_{pseudo}) values under pressurized flow electrochromatography were estimated. The linear relationship between the electric resistance and the μ_{pseudo} was observed under several chromatographic effluents. Figure 3 showed the linear relationship between the electric resistance and the μ_{pseudo} using the effluent of the mixture of methanol and 20 mM ammonium acetate (35:65), and the correlation factor was 0.994. As the μ_{pseudo} is related to the charge density on the stationary phase, the electric resistance might also be related to it in some extent.

Acknowledgements

C. Chaiyasut gratefully acknowledges the Ministry of Education, Science, Sport and Culture of Japan for the grant support as part of a study program in Japan.

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