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# Behavior of neutral solutes in pressurized flow driven electrochromatography using a mixed stationary phase of ODS and anion-exchange

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## Abstract

The behavior of neutral sample solutes in pressurized flow driven electrochromatography using a mixed stationary phase, which consisted of ODS and anion-exchange (ODS–SAX), was studied. Applications of both positive and negative voltage on a column induced increases in retention factors of sample solutes. The direction of an electroosmotic flow under applications of positive and negative voltage were the same, therefore, the sign of the surface charge density under positive and negative voltage was opposite. We proposed a new equation for the relationship between applied voltage and surface charge density, and the practical electroosmotic flow conformed to this equation. Studying the electroosmotic flow using our proposed equation revealed that the applied negative voltage accelerates the protonation of the quaternary ammonium group and dissociation of the silanol group on packing materials. The retention behavior of a neutral solute was affected by the existence of the charged functional groups. We propose that this phenomenon is applicable to the control of the retention behavior of a sample solute using an electric field.

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## 1. Introduction

Capillary electrochromatography is a separation method with high separability using high voltage [1–4]. The role of high voltage in general capillary electrochromatography is to generate an electroosmotic flow and an electrophoretic migration of a charged solute. A mixture is separated in capillary electrochromatography by two mechanisms, an electrophoretic separation and a chromatographic separation.

Since the applied high voltage induces electrophoretic migration, the electrophoretic separation is dominated by the strength of an applied electric field on a capillary column. Chromatographic separation has been considered as independent from an applied electric field. However, we have found chromatographic separation is also affected by applied high voltage, i.e. an applied electric field induces a variation of the retention factor [5–7]. Jiskra et al. reported that there were differences in thermodynamic behavior between liquid chromatography and capillary electrochromatography using a reversed-phase stationary phase [8]. They concluded that the retention mechanism under capillary electro-

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chromatography differs from that under liquid chromatography.

In our previous papers, the variation of the retention factor has been studied using pressurized flow driven electrochromatography [6,7]. In pressurized flow driven electrochromatography, an electric field is applied to a capillary column in the presence of a pressurized flow generated by a pump. Since the pressurized flow is the main flow to elute a sample solute from the capillary column, both positive and negative high voltage can be applied to the column. In capillary electrochromatography without a pressurized flow, either positive or negative high voltage can be applied to the column to transfer a sample solute to the column outlet. The ability to apply both negative and positive voltage is an advantage in pressurized flow driven electrochromatography to investigate the effects of an applied electric field on the retention factor.

Retention factor variation has been studied using columns packed with anion-exchange [5], fluorinated alkyl chain modified silica gels [6], octadecyl silane (ODS) modified silica gels and hexa-6-bromo-hexa-6-deoxy- $\beta$ -cyclodextrin modified silica gels [7]. The variation has been observed in all packing materials, except an ODS column, and both ionic and neutral solutes have shown variation. Since a neutral sample solute has no electrophoretic behavior under an electric field, the variation of the retention factor calculated from a chromatogram directly reflects the mass balance of a sample solute between stationary and mobile phases [7].

Since certain charges in the stationary phase are favorable to the generation of an electroosmotic flow, the mixed mode stationary phase has often been used in capillary electrochromatography [9–14]. Therefore, the study of capillary electrochromatography with a mixed mode stationary phase is important. In the present paper, pressurized flow driven electrochromatography using a mixed stationary phase, which consisted of ODS and anion-exchange functional groups, was performed, and variation in the behavior of the retention factor of neutral solutes were studied. Since the applied electric field varied electroosmotic flow mobility [15], the behavior of electroosmotic flow under electric field was also studied.

## 2. Experimental

The apparatus for pressurized flow driven electrochromatography used in this experiment was the same arrangement described in our previous reports [6,7]. The apparatus was composed of a pump (LC-10AV, Shimadzu, Kyoto, Japan), an injector (7520, Rheodyne, CA, USA), a laboratory-made splitter (using the split injection method), a laboratory-made capillary column, a UV detector (UV-970, Jasco, Tokyo, Japan), a high voltage power supply (HCZE-30PN, Matsusada Precision, Shiga, Japan) and a laboratory-made reservoir equipped with a platinum electrode. The laboratory-made reservoir was set at the outlet end of the capillary column. In this apparatus, the injector was always grounded for safe operation, and the injector also served as an electrode at the inlet end of the column. Therefore, high voltage was applied at the outlet end reservoir of the capillary column.

The inner diameter, packed length and whole length of the laboratory-made capillary column were 0.1 mm, 120 mm and 204 mm, respectively. The capillary column was packed with silica gel particles modified with ODS–anion-exchange (Kaseisorb LC ODS–SAX Super, Tokyo Kasei Kogyo, Tokyo, Japan). The structure of the ODS–anion-exchange bonded on a silica gel surface is shown in Fig. 1. As

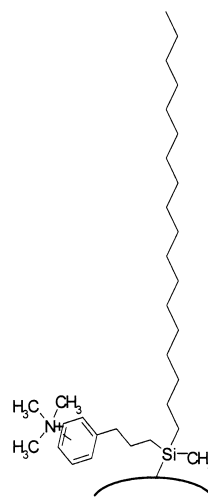


Fig. 1. Structure of the ODS–SAX modified stationary phase on the silica gel surface.

shown in Fig. 1, since a quaternary ammonium functional group is bonded at a silicon atom in octadecyl silane via a spacer, the quaternary ammonium group exists near the surface of a silica gel particle.

The eluent used for this experiment was a mixture of 20 mM  $\text{Na}_2\text{HPO}_4$  aqueous solution and methanol (40:60). As sample solutes, uracil (0.33 mM in sample solution), caffeine (0.53 mM), methyl benzoate (0.80 mM) and diethyl phthalate (0.35 mM) were used. All reagents (Wako, Kyoto, Japan) were guaranteed grade. Uracil was used as a non-retaining sample, i.e.  $t_0$  marker.

### 3. Results and discussion

#### 3.1. Typical voltage-induced variation of the retention factor

Separations of four neutral solutes using the ODS–SAX column with and without application of voltage were performed as shown in Fig. 2. The sample solution was injected after a period of 20 min to obtain a stable column condition, when the value of applied voltage was changed. In Fig. 2, the chromatogram obtained without an application of voltage is just equal to an ordinary capillary liquid chromatogram, and is used as a standard to analyze the variation of the retention factors. Applications of both positive and negative high voltage delayed the elution of samples. Contribution of an electroosmotic flow is the dominant reason why the application of a negative voltage delayed the elution of samples. Since the anion-exchange functional group on the surface of ODS–SAX provides a positively charged surface, an electroosmotic flow streams toward the positive electrode. Therefore, a delay induced by the application of negative voltages at the outlet end of the column is reasonable. Particularly, since the uracil is a non-retained solute, its delay is only caused by a deceleration of the flow velocity in the mobile phase. The elution time of uracil was almost constant with the application of a positive voltage. The constancy of the elution time of uracil means that the flow velocity of the mobile phase did not vary by the application of a positive voltage. There-

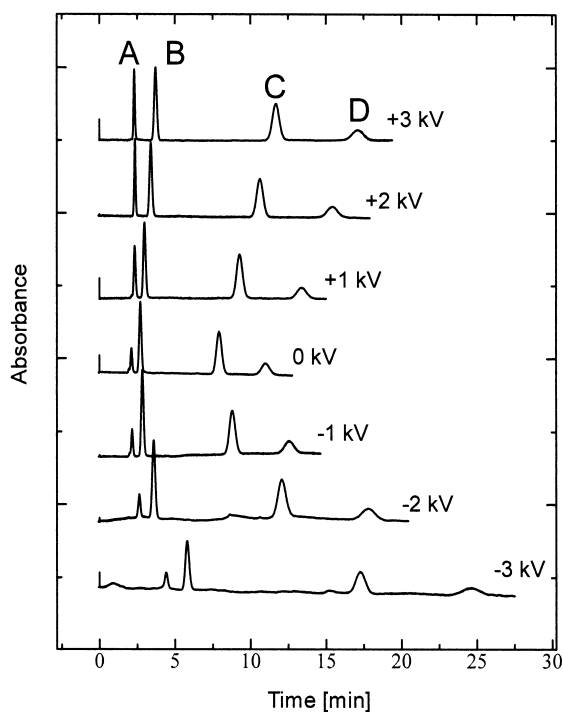


Fig. 2. Variation of elution time of neutral solutes under an electric field using a column packed with ODS–anion-exchange mixed stationary phase. Capillary column: I.D. 0.1 mm, packed length 120 mm, whole capillary length 204 mm; eluent, mixture of 20 mM  $\text{Na}_2\text{HPO}_4$  and methanol (40:60, v/v); flow-rate, constant pressure mode of 60 kgf/cm<sup>2</sup>; peak identification, (A) uracil, (B) caffeine, (C) methyl benzoate, (D) diethyl phthalate.

fore, the delays of elution times of caffeine, methyl benzoate and diethyl phthalate might result from increases of their retention factors.

The relationship between the applied voltage and relative retention factors of caffeine, methyl benzoate and diethyl phthalate are shown in Fig. 3. The ordinate in Fig. 3 shows the relative retention factor, which is calculated by division of  $k/k_{0V}$ , where  $k$  and  $k_{0V}$  are retention factors with and without application of voltage, respectively. This relative retention factor was introduced to compare the magnitude of the voltage-induced retention factor variation of each sample solute [6]. The retention factors of sample solutes under an electric field were calculated from the elution times of a sample solute and uracil. Therefore, the effect of the variation of total flow velocity under the electric field was

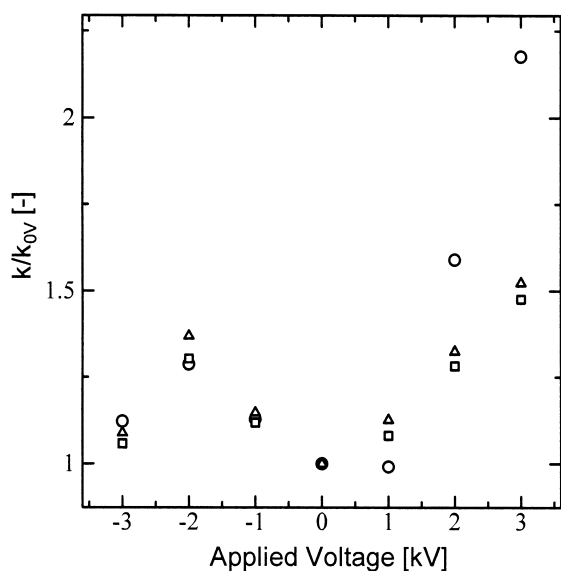


Fig. 3. Relationship between the applied voltage and the relative retention factors of neutral solute of caffeine (circle), methyl benzoate (square) and diethyl phthalate (triangle). Conditions are the same as in Fig. 2.

corrected [7]. The  $k_{OV}$  values of caffeine, methyl benzoate and diethyl phthalate were 0.28, 2.75 and 4.21, respectively. It is clear that the application of a positive voltage increases the relative retention factors of the three neutral sample solutes. Behaviors of the relative retention factors of the neutral solutes under a negative electric field were not linear. The relative retention factor achieved a maximum value at  $-2$  kV. Since all relative retention factors in Fig. 3 were greater than 1.0, both applications of positive and negative voltages induced an increase in retention factors of sample solutes. The retention factor is determined by a balance of the thermodynamic stabilities, i.e. chemical potential, of a sample solute in mobile and stationary phases. Therefore, the application of the voltage might induce an increase of chemical potential in the mobile phase, or decrease in the stationary phase.

### 3.2. Variation in coefficient of electroosmotic velocity

From the elution time of uracil in the electrochromatograms in Fig. 2, it is clear that application of a negative voltage generated an electroosmotic

flow toward the column inlet, however, an electroosmotic flow is almost zero under an application of a positive voltage. This asymmetrical electroosmotic flow velocity suggests that application of a high voltage varies electrophoretic mobility. Calculation of electroosmotic mobility requires values of a potential gradient and electroosmotic flow velocity. The electroosmotic flow velocity could be calculated from a comparison of elution times of uracil with and without the application of a voltage. However, since the conductivity of packed and open tubular parts in a capillary column are not the same values, the potential gradient in a packed part cannot be estimated simply using values of the applied voltage, and packed and open tubular lengths in a column. Therefore, it is difficult to estimate true electroosmotic mobility. To settle this issue, we introduced a new parameter, a coefficient of electroosmotic flow velocity,  $\mu'_{osm}$ , as a substitute for electroosmotic mobility. The coefficient of electroosmotic flow velocity was calculated by dividing electroosmotic flow velocity,  $v_{osm}$ , by the applied electric voltage,  $V$ , i.e.  $v_{osm}/V$ . When the potential gradient in a packed part is proportional to the applied voltage, the coefficient of electroosmotic flow velocity is also proportional to electroosmotic mobility. Since the electroosmotic mobility is reflected in the surface charge density [15], the coefficient of electroosmotic flow velocity is closely related to the surface charge density of the packed materials. The coefficient of electroosmotic flow velocity was used for the assessment of the surface charge density. Since the capillary column was composed of a packed part and an open tubular part, the electroosmotic flow velocity was affected by the properties in both segments. Therefore, the  $\mu'_{osm}$  value includes the influence of the electroosmotic flow generated in the open tubular segment. The following discussion assumes that the effect of the open tubular segment is negligible.

The relationship between an applied voltage and a coefficient of electroosmotic flow velocity is shown in Fig. 4. The positive  $\mu'_{osm}$  value means that the surface charge on a packing material is positive, i.e. an electroosmotic flow streams toward the positive electrode. As shown in Fig. 2, the elution time of uracil under a positive electric field is slightly delayed in comparison to that of 0 kV. Both the applications of positive and negative voltages gener-

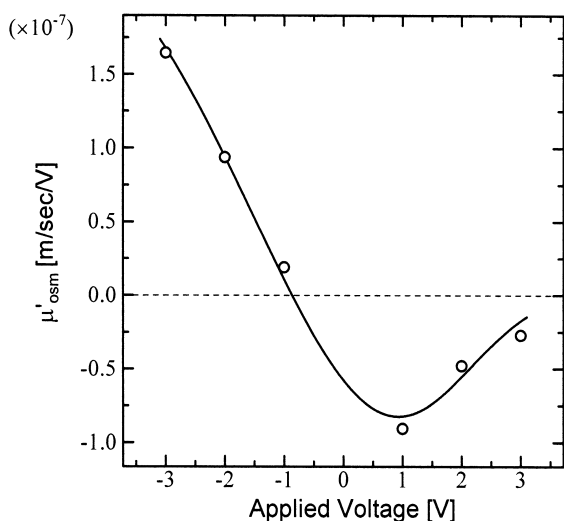


Fig. 4. Relationship between the applied electric field and the coefficient of electroosmotic velocity. Conditions are the same as in Fig. 2.

ated the electroosmotic flow to the column inlet. This phenomenon suggests that the surface of packing materials had negative charges under the application of a positive voltage. Therefore, the sign of  $\mu'_{osm}$  is different under the application of positive and negative voltages. As shown in Fig. 4, an application of a positive voltage reduced  $\mu'_{osm}$  to zero. This phenomenon means that the surface charge density on packed materials reduced to zero. Application of a negative voltage enlarged both  $\mu'_{osm}$  and the positive surface charge density. The behavior of  $\mu'_{osm}$  under a negative voltage is distinctly different from that under a positive voltage, so the origin of the  $\mu'_{osm}$  variation induced by positive and negative voltages might be different.

On a surface of the packing material ODS–SAX, there are two kinds of functional groups that generate surface charge. One is a quaternary ammonium group of anion-exchange, and the other is an unreacted silanol group [16]. These functional groups provide positive and negative surface charges, respectively. The variation of  $\mu'_{osm}$ , i.e. variation of surface charge density, suggests that the applied voltage affects the dissociation of a functional group on a surface. The surface charge density generated by a functional group ranges from zero to its maximum value. A sigmoid shape curve is often

used to describe a relationship between two variables, for example conductance of cell membrane and voltage [17,18], and laser energy and sputtering yield [19]. Therefore, we assumed that the relationship between a surface charge density and the applied voltage forms a sigmoid shape curve (Boltzmann function), i.e. the following equation was assumed:

$$\sigma = \frac{ze\gamma}{1 + \exp(f(V - V_0))} \quad (1)$$

The  $\sigma$ ,  $z$ ,  $e$ ,  $\gamma$ ,  $V$ ,  $V_0$  and  $f$  are the surface charge density, number of charges, elementary charge, number of functional groups in unit area, applied voltage, standard voltage and susceptible parameter to applied voltage, respectively. Since Eq. (1) is a kind of Boltzmann function, the surface charge ranges from zero to  $ze\gamma$ . When  $\mu'_{osm}$  is proportional to the surface charge density, the relationship between applied voltage and  $\mu'_{osm}$  in this experiment can be expressed as the following equation:

$$\mu'_{osm} = \frac{\mu'_{osm,N^+}}{1 + \exp(f_{N^+}(V - V_{0,N^+}))} + \frac{\mu'_{osm,SiO^-}}{1 + \exp(f_{SiO^-}(V - V_{0,SiO^-}))} \quad (2)$$

In Eq. (2), the subscripts of  $N^+$  and  $SiO^-$  mean the quaternary ammonium group of anion-exchange and the unreacted silanol group, respectively. A curve fitting for  $\mu'_{osm}$  to Eq. (2) was performed, and the result is shown as a solid line in Fig. 4. The calculated line was consistent with the measured data. Therefore, our proposed relationship between applied voltage and surface charge density, i.e. Eq. (1), might be appropriate. The calculated parameters are listed in Table 1. Since both  $f$  for  $N^+$  and  $SiO^-$  are positive values, the application of a negative voltage increases the surface charge density derived

Table 1

Values of the coefficient of electroosmotic flow ( $\mu'_{EOF}$ ), susceptible parameter to applied voltage ( $f$ ) and standard voltage ( $V_0$ ) for quaternary ammonium ( $N^+$ ) and silanol ( $SiO^-$ ) groups

	$\mu'_{EOF}$ (m/s/V)	$f$ ( $V^{-1}$ )	$V_0$ (V)
$N^+$	$5.52 \times 10^{-7}$	$0.66 \times 10^{-3}$	-1190
$SiO^-$	$2.56 \times 10^{-7}$	$1.22 \times 10^{-3}$	1850

from both the quaternary ammonium group and the unreacted silanol group. In other words, the negative voltage accelerates both the protonation of the quaternary ammonium group and dissociation of the silanol group. Since the  $f$  value for  $\text{SiO}^-$  is greater than that for  $\text{N}^+$ , the application of a voltage strongly affects the silanol in this experimental condition. Because the  $\mu'_{\text{osm}}$  values of  $\text{N}^+$  and  $\text{SiO}^-$  are  $5.52 \times 10^{-7}$  and  $-2.56 \times 10^{-7}$ , respectively, the  $\mu'_{\text{osm}}$  value under the application of sufficiently high negative voltage is predicted to be  $2.96 \times 10^{-7}$ .

### 3.3. Relationship between surface charge density and retention factor

As shown in Fig. 4, the application of a high voltage can vary the coefficient of electroosmotic flow velocity, i.e. the surface charge density on packing materials. Retention factors of caffeine and diethyl phthalate using the ODS–SAX column without the application of voltage were 0.28 and 4.21, respectively. When the ODS column (I.D. 0.1 mm, length 132 mm, packed with ODS (Capcell C<sub>18</sub> SG-120, kindly donated by Shiseido, Yokohama)) was used without the application of a voltage, the retention factors of caffeine and diethyl phthalate were 0.38 and 7.90, respectively. Therefore, the existence of ionic functional groups on a surface of the packing material might decrease the hydrophobic interaction between a neutral solute and a stationary phase. Fig. 4 indicates that application of a higher positive voltage reduces the surface charge density to zero. Since a neutral solute was retained mainly in hydrophobic interaction with a stationary phase, augmentation of a retention factor under an application of a positive voltage is reasonable.

The application of a negative voltage increased the positive surface charge on a packing material as shown in Fig. 4. However, the retention factors of the neutral solute increased under the application of negative voltage in a range from 0 to  $-2$  kV. The driving force to increase a retention factor of a neutral solute under the application of a negative voltage is still unclear. Although it is only speculation, the interaction between quaternary ammonium and silanol groups might be one such factor. The decrease in retention factor at the  $-3$  kV application might be induced by the higher surface charge density on the packing materials.

As shown in Fig. 4, absolute values of the coefficient of electroosmotic flow at  $-1$  kV and  $+3$  kV are almost the same. However, the relative retention factors are quite different as shown in Fig. 3. The low  $\mu'_{\text{osm}}$  at the application of  $+3$  kV reflects less protonation of the quaternary ammonium groups and dissociation of the silanol groups on the surface, i.e. there are fewer charged functional groups on the surface. The low  $\mu'_{\text{osm}}$  at  $-1$  kV results from a balance between the positive charge of the quaternary ammonium group and the negative charge of the silanol group. These charges on the surface of packing materials are not favorable for the retention of a neutral solute.

## 4. Conclusions

The study of electroosmotic flow behavior has revealed that the applied electric field induced protonation of the quaternary ammonium group and dissociation of the silanol group on the surface of packed silica gel particles modified with ODS–anion-exchange, i.e. the surface charge density is varied by the applied electric field. The retention behavior of a neutral solute was affected as a result of the variation of the charge density in the stationary phase. We suppose that this phenomenon is applicable to the control of the retention behavior of sample solute using an electric field.

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