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Modeling of retention behavior in capillary electrochromatography from chromatographic and electrophoretic data

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Abstract

A phenomenological approach was presented to describe the retention behaviors of solutes in capillary electrochromatography (CEC). Equations for calculation of the retention time and actual chromatographic retention factor for ionic solutes, weak monoprotic acid and weak monoprotic base were derived, which can be described by two general expressions regardless the charge status of the solute. The general expressions enable calculation of the retention time and retention factor in CEC from chromatographic and electrophoretic data, which were experimentally verified with a variety of compounds and a variety of CEC modes. Based on this approach, the chromatographic retention and the electrophoretic migration in the CEC systems studied were found to be two independent processes. The validity of this approach was also discussed.

Keywords: Retention behavior; Acid–base equilibrium; Electrochromatography

1. Introduction

Capillary electrochromatography (CEC) [1–3] has been drawing increasing attention recently. As a hybrid technique, CEC combines the advantages of both high-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE). The mobile phase is propelled by an electroosmotic flow (EOF) of flat plug-like profile, rather than a pressure driven flow of parabolic profile like in HPLC. Thus CEC behaves with greatly enhanced separation efficiency relative to HPLC. Besides the difference in the electrophoretic mobility, the migrations of the solutes are also subject to the difference in the chromatographic interactions with the stationary phase. Thus CEC offers wider selectivity and enables separation of neutral compounds as well as charged compounds. Since a CEC column serves not only as the separation channel but also as the pumping device to transport the mobile phase through the system, one may ask, ‘‘do the two different processes, i.e., the chromatographic retention and the electrophoretic migration, influence each other or act independently?’’ In addition, it is necessary to know how to calculate the retention factor in CEC, particularly for charged solutes.

So far, three definitions of retention factor different from conventional definition have been used in the literature. One is the electrochromatographic

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retention factor, k_{cec}, proposed by Rathore and Horváth [4], which is given as:

$$k_{cec} = k_c + k_c k_e + k_e \quad (1)$$

in which k_c is the actual retention factor caused by chromatography alone in CEC, k_e is called electrophoretic velocity factor expressed by the electrophoretic mobility normalized to the electroosmotic mobility (i.e. μ_{ep}/μ_{eo}). This definition was obtained from a unified theory using virtual migration distances, which applies to any separation process. It reflects the concurrence of chromatographic and electrophoretic processes, expressed in the product of the chromatographic retention factor and the electrophoretic velocity factor. It also takes into account the coupling in velocity and time domains. It is noteworthy that k_{cec} has no direct relation with the chromatogram. The second definition is the apparent overall retention factor, k^*, first employed by Wu et al. [5], which is expressed as:

$$k^* = \frac{t_r - t_0}{t_0} \quad (2)$$

where t_r and t_0 are the retention time of the solute and the void time, respectively. Since the value of k^* can be easily obtained from the chromatogram, it is widely used in literature. Obviously, this parameter takes into account contributions of both chromatographic and electrophoretic processes. Based on this expression, Wu et al. [5] discussed theoretically and verified experimentally tuning of the elution of peptides by adjusting the applied field and supplementary pressure in pressurized CEC. Ye et al. [6] derived different expressions for simple ion, weak acid and weak base, and discussed theoretically the influence of the pH value. Recently, Ye et al. [7] employed the parameter k^* as a criterion for study of competitive binding of enantiomers to protein by using affinity CEC. The third definition is the expanded capacity factor, k', proposed by Bower et al. [8,9]:

$$k_i' = \frac{\text{the amount of analyte as species } i}{\text{the amount of free analyte}} \quad (3)$$

Evidently, this definition is far different from the conventional definition by which the retention factor is expressed as the ratio of the amount of an analyte in the stationary phase to that in the mobile phase. According to this definition, each species of an analyte has its own capacity factor. The free analyte has a capacity factor of 1. Although this definition is useful for deriving generally applicable equations of capacity factor, separation factor and resolution for all separation techniques including capillary electrophoresis without or with dynamic complexation, chromatography and ultracentrifugation, the relationship between the apparent retention factor and the expanded capacity factors of all analyte species in a chromatographic system with secondary equilibria is not clear. Based on this definition, Bowser et al. [8,9] define a new separation factor, γ, which is the ratio of the average migration rates of two solutes. This is also different from conventional definition of separation factor, which is expressed as the ratio of the retention factors of two solutes. Unlike the above definitions, here we proposed a new concept, actual chromatographic retention factor (k_c), which is the actual retention factor due to a chromatographic process alone. Note that we use the term retention factor instead of capacity factor because the chromatographic retention is emphasized. Clearly, this is an extension of the conventional concept of retention factor. In traditional chromatographic systems such as HPLC, since there is only a chromatographic process, the actual chromatographic retention factor can be calculated by the conventional equation as the following:

$$k_c = \frac{t_r - t_0}{t_0} \quad (4)$$

where t_r and t_0 have the same meaning as in Eq. (2). In CEC, since it involves chromatographic and electrophoretic processes, we refer to the corresponding term as actual chromatographic retention factor under an electric field, $k_{c,e}$. This parameter is of importance in theory since it reflects the contribution of the chromatographic process. On the contrary, the use of the former definitions may cause misunderstanding in the mechanism. For example, in an enantioselective CEC system with a chiral stationary phase, the selectivity should be determined by the chromatographic process alone if no interplay exists between the chromatographic retention and the elec-

trophoretic migration because the effective electrophoretic mobility is the same for a pair of charged enantiomers. Assuming $k_{c,e,1}=0.1$, $k_{c,e,2}=0.2$, $\mu_{ep,1}=\mu_{ep,2}=\mu_{eo}=0.02$ (dimensionless unit), the separation factor is calculated to be 1.17, 1.09 and 2 from the values of k_{cec}, k^* and $k_{c,e}$, respectively (see later Eq. (44) for calculation of k^*), while the γ value is 1.04 (see Ref. [9] for the calculation of γ). The different values for the separation factor result in a false impression that the selectivity changes in this system due to the use of electric field. Therefore, it is necessary to know how to calculate the actual chromatographic retention factor in CEC.

If an expression of the actual chromatographic retention factor in CEC is available for any solute regardless of its charge status, the initial question may be simplified as ''does the retention factor under an electric field equal to that under a pressure?'' However, up to now, there is no widely acceptable agreement on this issue even only for neutral compounds. While Vissers et al. [10] observed that the retention in CEC for neutral compounds of 4-aminoacetophenone, *o*-nitrophenol, 2,6-dimethylphenol and naphthalene was about 20% higher than that in HPLC, the work of Yan et al. showed that the retention in CEC and HPLC for benzyl alcohol, benzaldehyde, benzene and naphthalene was approximately the same (see Fig. 9 in Ref. [11]). The latter was supported by the work of Zhang et al. [12], in which the retention factors of 27 neutral compounds in pressurized CEC, CEC and HPLC on identical column were systematically investigated but no significant differences were observed.

In this work, two general equations were derived to predict the retention time and actual chromatographic retention factor in CEC based on the data from liquid chromatography (LC) and CZE, regardless the charge status of the solute. These equations were experimentally confirmed with a variety of compounds including neutral compounds, bases and acids and a variety of column formats including reversed-phase (RP) packed column, RP-open tubular column, enantioselective open tubular column, and monolithic silica column. The results also verified that the chromatographic and electrophoretic processes are independent in the systems investigated. The factors influencing the validity of the equations were also discussed.

1.1. Theory

A phenomenological approach, which has been applied to HPLC [13], micellar electrokinetic chromatography (MEKC) [14,15], and CEC [6], is used in this work. Our consideration is limited to CEC systems that are operated under constant applied voltage, constant temperature and isocratic elution mode. We assume that (1) the electric field is constant throughout the column; (2) there is no irreversible interaction between the solute and the stationary phase; and (3) the migration velocities of the solute species in the stationary phase are negligible. It should be emphasized that no limitation is needed on the separation mechanism because the total strength of the interactions between the solute and the stationary phase can be characterized by the retention factor whatever the interactions are. Based on these assumptions, the velocity of electroosmotic flow (EOF) (v_{eo}) within the column and that of effective electrophoretic migration (v_{eo}) of an ionic species can be considered as constant and expressed as:

$$v_{eo} = \mu_{eo}E \tag{5}$$

$$v_{ep} = \mu_{ep}E \tag{6}$$

where

$$E = \frac{V}{L_t} \tag{7}$$

μ_{eo} and μ_{ep} are the electroosmotic mobility and electrophoretic mobility, respectively, E is the electric field across the column, V is the applied voltage, and L_t is the total length of the capillary. The void time (t_0) and retention time (t_r) are given by:

$$t_0 = \frac{L_d}{v_{eo}} = \frac{L_d}{\mu_{eo}E} \tag{8}$$

$$t_r = \frac{L_d}{v} \tag{9}$$

where L_d is the capillary length from inlet end to detector, and v is the migration velocity of the solute.

1.1.1. Fully ionized solutes

We consider the case of an anion as an example.

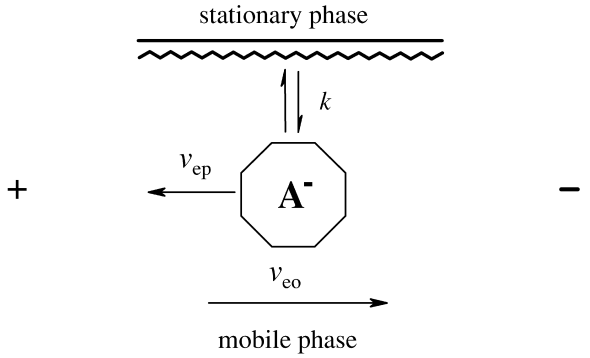

Fig. 1. Schematic illustration of migration process of anion in CEC.

Fig. 1 illustrates the migration process of an anion in CEC. Because of the absence of secondary chemical equilibria, the migration process is relatively simple. The stoichiometric fractions (F) of the anion in the stationary phase and the mobile phase are given as:

$$F_s = k_{c,e}/(1 + k_{c,e}) \tag{10}$$

$$F_m = 1/(1 + k_{c,e}) \tag{11}$$

where the subscripts s and m refer to the stationary and mobile phase, and $k_{c,e}$ is the retention factor of the anion. Under the influence of the applied electric field, the anion has an apparent electrophoretic velocity in the mobile phase (v_m), which is the vector sum of its effective electrophoretic velocity and the velocity of EOF:

$$v_m = v_{eo} + v_{ep} \tag{12}$$

Thus the average-weighted migration velocity of the anion (v_{aw}) is given by:

$$v_{aw} = F_m v_m + F_s v_s \tag{13}$$

As the migration velocity of the anion in the stationary phase (v_s) is assumed to be zero, Eq. (8) becomes:

$$v_{aw} = F_m v_m = \frac{1}{1 + k_{c,e}} v_m \tag{14}$$

A combination of Eq. (9) with Eqs. (5), (6), (8), (12) and (14) yields:

$$t_r = \frac{1 + k_{c,e}}{1 + \mu_{ep}/\mu_{eo}} t_0 \tag{15}$$

which can be re-arranged as:

$$k_{c,e} = \frac{(1 + \mu_{ep}/\mu_{eo})t_r - t_0}{t_0} \tag{16}$$

1.1.2. Weak acids

The migration process and the secondary equilibrium of a weak monoprotic acid, HA, are illustrated in Fig. 2. The dissociation of the acid in the mobile phase is governed by the following equilibrium:

$$HA \rightleftharpoons A^- + H^+$$

where A^- is the conjugate base and H^+ is the solvated proton. The acid dissociation constant in the mobile phase, K_a, is given by:

$$K_a = \frac{[H^+]_m[A^-]_m}{[HA]_m} \tag{17}$$

which can be re-written as:

$$\frac{[A^-]_m}{[HA]_m} = \frac{K_a}{[H^+]_m} \tag{18}$$

where $[H^+]_m$, $[A^-]_m$, and $[HA]_m$ are the concentrations of the solvated proton, the dissociated, and undissociated acid in the mobile phase, respectively. $[A^-]_m$ and $[HA]_m$ can be calculated according to the following equations:

$$[A^-]_m = C_{A^-}/(1 + k_{A^-}) \tag{19}$$

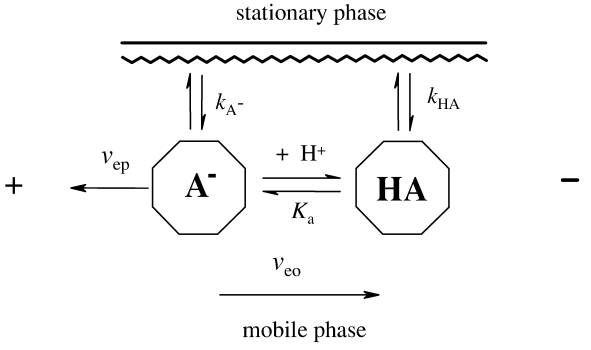

Fig. 2. Schematic illustration of migration process of weak acid in CEC.

$$[HA]_m = C_{HA}/(1 + k_{HA}) \quad (20)$$

where C_{A^-} and C_{HB} are the total concentrations of the dissociated and undissociated acid, respectively; k_{A^-} and k_{HA} are the retention factors of the dissociated and undissociated acid, respectively. The stoichiometric fractions of A^- and HA are given by:

$$F_{A^-} = C_{A^-}/(C_{HA} + C_{A^-}) \quad (21)$$

$$F_{HA} = C_{HA}/(C_{HA} + C_{A^-}) \quad (22)$$

The velocity of the HA in the mobile phase, $v_{HA,m}$ is equal to the velocity of EOF,

$$v_{HA,m} = v_{eo} \quad (23)$$

As the velocities of A^- and HA in the stationary phase are assumed to be zero, the average-weighted velocities of A^- and HA, $v_{A^-,aw}$ and $v_{HA,aw}$ are given by:

$$v_{A^-,aw} = (v_{eo} + v_{ep,A^-})/(1 + k_{A^-}) \quad (24)$$

$$v_{HA,aw} = v_{eo}/(1 + k_{HA}) \quad (25)$$

Thus the average-weighted velocity of the weak acid is given by:

$$v_{aw} = F_{A^-} v_{A^-,aw} + F_{HA} v_{HA,aw} \quad (26)$$

A combination of Eq. (9) with Eqs. (5), (6), (8), (18)–(22) and (24)–(26) yields:

$$t_r = \frac{(1 + k_{HA})[H^+]_m/K_a + k_{A^-} + 1}{1 + [H^+]_m/K_a + \mu_{ep,A^-}/\mu_{eo}} t_0 \quad (27)$$

where μ_{ep,A^-} is the effective electrophoretic mobility of the dissociated acid. The apparent retention factor is given by:

$$k_{c,e} = F_{A^-} k_{A^-} + F_{HA} k_{HA} \quad (28)$$

Substituting Eqs. (18), (21) and (22) into Eq. (28) gives:

$$k_{c,e} = \frac{k_{HA}[H^+]_m/K_a + k_{A^-}}{1 + [H^+]_m/K_a} \quad (29)$$

Combining Eqs. (28) and (29) gives:

$$t_r = \frac{(1 + [H^+]_m/K_a)(1 + k_{c,e})}{1 + [H^+]_m/K_a + \mu_{ep,A^-}/\mu_{eo}} t_0 \quad (30)$$

which can be re-arranged as:

$$k_{c,e} = \frac{\left(1 + \frac{\mu_{ep,A^-}/\mu_{eo}}{1 + [H^+]_m/K_a}\right) t_r - t_0}{t_0} \quad (31)$$

On the other hand, the apparent electrophoretic mobility of the weak acid (μ_{ep}) is given by:

$$\mu_{ep} = F_{A^-} \mu_{ep,A^-} + F_{HA} \mu_{ep,HA} \quad (32)$$

Because the electrophoretic mobility of HA is equal to zero, combining Eqs. (21) and (32) gives:

$$\mu_{ep,A^-} = (1 + [H^+]_m/K_a)\mu_{ep} \quad (33)$$

By substituting Eq. (33) into Eqs. (30) and (31), Eqs. (30) and (31) change into Eqs. (34) and (35), respectively.

$$t_r = \frac{1 + k_{c,e}}{1 + \mu_{ep}/\mu_{eo}} t_0 \quad (34)$$

$$k_{c,e} = \frac{(1 + \mu_{ep}/\mu_{eo})t_r - t_0}{t_0} \quad (35)$$

1.1.3. Weak bases

The ionization of a weak monoprotic base, B, in the mobile phase takes place according to the following equilibrium:

$$BH^+ \rightleftharpoons B + H^+$$

where BH^+ is the conjugate acid. The equilibrium is generally characterized by the acid dissociation constant of the conjugate acid in the mobile phase, K_a, which is given by:

$$K_a = \frac{[H^+]_m[B]_m}{[BH^+]_m} \quad (36)$$

By using the same process as for weak acids, the expressions for retention time and retention factor for weak base can be obtained, which are described as:

$$t_r = \frac{(1 + k_B)K_a/[H^+]_m + k_{BH^+} + 1}{1 + K_a/[H^+]_m + \mu_{ep,BH^+}/\mu_{eo}} t_0 \quad (37)$$

or

$$t_r = \frac{(1 + k_{c,e})(1 + K_a/[H^+]_m)}{1 + K_a/[H^+]_m + \mu_{ep,\,BH^+}/\mu_{eo}} t_0 \quad (38)$$

and

$$k_{c,e} = \frac{\left(1 + \dfrac{\mu_{ep,\,BH^+}/\mu_{eo}}{1 + K_a/[H^+]_m}\right) t_r - t_0}{t_0} \quad (39)$$

where μ_{ep,BH^+} is the effective electrophoretic mobility of the conjugate acid. If the apparent electrophoretic mobility of the weak base is used, then Eqs. (38) and (39) become:

$$t_r = \frac{1 + k_{c,e}}{1 + \mu_{ep}/\mu_{eo}} t_0 \quad (40)$$

$$k_{c,e} = \frac{(1 + \mu_{ep}/\mu_{eo})t_r - t_0}{t_0} \quad (41)$$

Eqs. (27), (29) and (37) involve the limiting retention factors of the neutral and fully ionized forms of a weak acid and weak base, i.e. k_{HA}, k_{A^-}, k_B, and k_{BH^+}. Horváth et al. [13] proposed a least squares analysis method to measure these parameters and the values of k_{HA} and k_{A^-} for some organic weak acids in RP-HPLC were measured. Foley et al. [16] also measured these parameters for a few organic acids in HPLC. The general trend is that the retention factor for the neutral form is greater than that for the fully ionized form. However, the difference changes dramatically with compound. For some compounds, k_{HA} is greater than k_{A^-} by 1 order of magnitude, whereas for some compounds, k_{HA} is just slightly greater than k_{A^-}. It seems that the retention times for a weak acid or weak base in CEC can be predicted according to Eqs. (27) and (37) if the parameters involved are known. However, this is nearly impossible in practice. First, the effective electrophoretic mobility of the fully ionized form is difficult to measure under a pH that can not let the weak acid or weak base of interest to be fully ionized. In addition, the dissociation constants of acid and base are usually measured with aqueous buffer, but the mobile phases usually used in CEC contain a certain concentration of organic modifier. The addition of organic modifier may change not only the pH of the buffer [17–19] but also the pK_a values of the solute [20]. Since the apparent effective electrophoretic mobility of a weak acid or weak base can be obtained by using CZE experiments, Eqs. (34), (35), (40) and (41) is more useful in practice.

By using the same approach, Eqs. (15) and (16) are found to be also valid for polyprotic acids and polyprotic bases. Thus, Eqs. (15) and (16) are the general expressions for retention time and retention factor in CEC, respectively, no matter what the charge status of the solute is and no matter whether a secondary acid–base equilibrium exists or not in the system. When the general equations are applied to ionic compounds, the parameter μ_{ep} represents the effective electrophoretic mobility. But when applied to a weak acid or weak base, it means the apparent electrophoretic mobility of the acid or base, which can be obtained with CZE under otherwise identical conditions. Table 1 summarizes the expressions for retention time and retention factor in CEC. For

Table 1
Expressions for retention time and retention factor in CEC

	Retention time	Retention factor
General equations	$t_r = \dfrac{1 + k_{c,e}}{1 + \mu_{ep}/\mu_{eo}} t_0$	$k_{c,e} = \dfrac{(1 + \mu_{ep}/\mu_{eo})t_r - t_0}{t_0}$
Full equations for monoprotic weak acid	$t_r = \dfrac{(1 + k_{HA})[H^+]_m/K_a + k_{A^-} + 1}{1 + [H^+]_m/K_a + \mu_{ep,A^-}/\mu_{eo}} t_0$	$k_{c,e} = \dfrac{\left(1 + \dfrac{\mu_{ep,\,A^-}/\mu_{eo}}{1 + [H^+]_m/K_a}\right) t_r - t_0}{t_0}$
Full equations for monoprotic weak base	$t_r = \dfrac{(1 + k_B)K_a/[H^+]_m + k_{BH^+} + 1}{1 + K_a/[H^+]_m + \mu_{ep,\,BH^+}/\mu_{eo}} t_0$	$k_{c,e} = \dfrac{\left(1 + \dfrac{\mu_{ep,\,BH^+}/\mu_{eo}}{1 + K_a/[H^+]_m}\right) t_r - t_0}{t_0}$

neutral solute, $\mu_{ep}=0$, thus Eqs. (15) and (16) become:

$$t_r = (1 + k_{c,e})t_0 \quad (42)$$

$$k_{c,e} = \frac{t_r - t_0}{t_0} \quad (43)$$

These two equations are the same as the expressions in HPLC. By substituting Eq. (15) into Eq. (2), the relationship between the actual chromatographic retention factor and the apparent overall retention factor can be expressed as:

$$k^* = \frac{1 + k_{c,e}}{1 + k_e} \quad (44)$$

where k_e has the same meaning as in Eq. (1).

During the above derivation approach, the chromatographic retention and the electrophoretic migration are considered as two independent processes; or in other words, there is no interplay between the two processes. This is the reason why the apparent retention factor of a weak monoprotic acid in CEC has the same relationship with the limiting retention factors as that in HPLC [13]. Eqs. (15) and (16) offer two ways to verify experimentally whether the chromatographic and electrophoretic processes in a CEC system influence each other or not. If the retention time can be predicted from chromatographic and electrophoretic data under otherwise identical conditions according to Eq. (15), or alternatively if the retention factor calculated from Eq. (16) is equal to the retention factor observed in a pure chromatographic process under otherwise identical conditions, the two processes work independently; otherwise, the two processes influence each other or the expressions presented are invalid.

2. Experimental

2.1. Instrumentation

All experiments including CEC, CZE and LC separations were performed on a Beckman P/ACE 5000 instrument (Fullerton, CA, USA). Data collection and instrument control were realized with a Beckman P/ACE Station on a personal computer. UV detection was carried out at 200 or 214 nm. The temperature was kept constant at 20 or 25 °C, unless otherwise stated. Several types of column were used. For reversed-phase open tubular CEC (RP-OTCEC), the column was 57 cm (50 cm to detector)×50 μm I.D. etched C_{18} modified open tubular column purchased from Silicon Valley Separation Media (San José, CA, USA). For RP-CEC, the column was 27 cm (6.5 cm to detector)×50 μm I.D. capillary packed with 5 μm Chemcosorb ODS-H packing obtained from Chemco (Osaka, Japan). For enatioselective OTCEC, the column was 47 cm (40 cm to detector)×50 μm I.D. fused-silica capillary with physically adsorbed avidin stationary phase. For CEC, another column of monolithic silica capillary of 27 cm (20 cm to detector)×50 μm I.D. was also used, which was supplied from Professor Nobuo Tanaka at Kyoto Institute of Technology. For LC separations, the same columns were used under otherwise identical conditions. The applied pressure was 3.45 kPa (0.5 p.s.i.) for the open tubular columns and 138 kPa (20 p.s.i.) for the packed column and monolithic silica column. For CZE separations, open tubular capillaries of the same dimensions were used and the electrode polarity was changed if necessary, while the other conditions were the same as in CEC mode. Except for the case of packed column where the sample was injected from the short-end with electrokintic mode (−1 or −2 kV for 1 or 2 s), the samples were all injected by a pressure of 3.45 kPa for 1–2 s for open tubular columns and 30–60 s for monolithic silica column. In the case of separations of basic compounds, a pre-rinse with 1.5–2 column volumes of mobile phase was added before each run. CEC, LC and CZE measurements were carried out three times and average values were taken.

2.2. Column preparation

The enantioselective OTCEC column was prepared according to the method proposed previously [21,22]. The packed column was prepared according to the slurry method reported in literature [12].

2.3. Chemicals and solutions

All the chemicals used were of analytical or chromatographic grade. Avidin and donepezil were

gifts from Eisai (Tokyo, Japan). Mexiletine HCl, chlorpheniramine maleate and ephedrine were gifts from Nippon Boehringer Ingelheim (Hyogo, Japan). Phenol, toluene, anthracene, propanolol HCl were purchased from Nacalai Tesque (Kyoto, Japan). Naphthalene was purchased from Kanto Chemical (Tokyo, Japan). Naproxen was purchased from Tokyo Chemical Industry (Tokyo, Japan). Haloperidol was purchased from Aldrich (Milwaukee, WI, USA). R,S-Ketoprofen, R,S-fluribiprofen, R,S-ibuprofen, benzylamine and morpholine were purchased from Wako Pure Chemical (Osaka, Japan). Except that the samples of phenol, toluene, naphthalene and anthracene were prepared with methanol, the other samples were all prepared with water. The concentrations of the samples were about 0.05–0.2 mg/ml. Water was purified with a Milli-Q Labo system (Nihon Millipore, Yonezawa, Japan). Buffers of 100 m*M* phosphate (pH 6.00, 7.00 and 8.60) and a buffer of 50 m*M* tris(hydroxymethyl)–aminomethane (Tris)–HCl (pH 8.00) were used as stock buffers. The mobile phases or running buffers were prepared by adding appropriate volume of stock buffer and appropriate volume of organic modifier to a calibrated flask, and then mixing with enough water to the calibration mark, without adjusting the pH value.

3. Results and discussion

3.1. Experimental verification

Joule heating is a characteristic of an electro-driven system, which gives rise to increase in the column temperature and thus influences the retention factor and the migration velocity in the system. The temperature excess within the core of a capillary (ΔT_{core}) and the temperature excess (ΔT_{air}) between the capillary wall and the surrounding air in an unstirred system can be estimated by the following equations [23]:

$$\Delta T_{core} = EI/(4\pi K) \quad (45)$$

$$\Delta T_{air} = 1.3EI/d_o^{0.3} \quad (46)$$

where E is the field strength, I is the current, K is the thermal conductivity of the mobile phase, and d_o is the outer diameter of the capillary. $K = 0.6\ \mathrm{Wm^{-1}\,K^{-1}}$ for an aqueous eluent. The values of ΔT_{core} and ΔT_{air} for the CEC systems used were estimated in accordance with above equations. The values of ΔT_{core} were found to be negligible, only 0.04 K at maximum. The values of ΔT_{air} were 100 times as high as ΔT_{core}, from 0.2 to 4.5 K. If the capillary is cooled by forced air at 10 m/s, the temperature excess can be reduced to about 1/5 of its unstirred values [23]. In the work, except for the short packed column of ODS for which the ΔT_{air} was only 0.2 K, the columns were all controlled by coolant through a thermostat. Therefore, the thermal effect can be ignored in this work.

The CEC systems used in the work and corresponding experimental conditions such as pH values are listed in Table 2. Four kinds of columns were used to observe the retention behaviors of solutes of different natures. The C_{18}-modified open tubular capillary was used for investigations of neutral and basic compounds. The main interaction responsible for the retention is hydrophobic interaction. But adsorption also takes part in the retention mechanism for basic compounds. The enantioselective open tubular capillary is used for investigations of acidic compounds. And chiral affinity interactions are responsible for retention on this column. The packed ODS capillary was employed for investigations of neutral compounds, on which hydrophobic interaction makes main contribution to the retention. The monolithic silica capillary was employed for investigations of basic compounds, the mechanism on which is the most complicated. The work of Wei et al. [24] showed that CEC separation of basic compounds on a bare silica column involves multiple mechanisms including reversed-phase, cation-exchange and normal-phase mechanisms. The test compounds and pK_a values are also given in Table 2. The degree of dissociation (α) of an acid or a base can be estimated from its pK_a value and the pH of the mobile phase according the following equation:

$$\alpha = 1/\left(1 + 10^{\pm(\mathrm{p}K_a - \mathrm{pH})}\right) \quad (47)$$

where the sign is plus for an acid and minus for a base. An acid at a pH of pK_a +1.5 or a base at a pH of pK_a −1.5 can be considered as fully ionized

Table 2
Comparisons of experimental and calculated retention times and of experimental retention factors in LC and calculated retention factors in CEC

Mode	pH	Organic modifier	Compound	Solute type (pK_a)	$t_{r\ exp.}$	$t_{r\ cal.}$ [a]	Diff.%	$k_{c\ exp.}$	$k_{c,e\ cal.}$ [b]	Diff.	Diff.%
RP-OTCEC[c]	7.00	10% (v/v) methanol	Naphthalene	Neutral	9.719	9.638	−0.84	0.088	0.097	0.009	9.28
			Anthracene	Neutral	10.796	10.796	0.00	0.217	0.217	0.000	0.00
		30% (v/v) methanol	Donepezil	Base	8.851	8.673	−2.02	0.091	0.114	0.023	25.68
		20% (v/v) methanol	Chlorpheniramine	Base (9.13)	6.898	7.060	2.35	0.113	0.089	−0.023	−20.75
			Haloperidol	Base (8.66)	8.602	8.788	2.16	0.264	0.237	−0.027	−10.24
			Propanolol	Base (9.53)	5.675	5.620	−0.96	0.100	0.111	0.011	10.94
	8.60	10% (v/v) methanol	Chlorpheniramine	Base (9.13)	9.905	9.699	−2.08	0.736	0.774	0.038	5.20
			Donepezil	Base	9.170	9.230	0.65	0.538	0.528	−0.010	−1.93
RP-CEC[d]	8.00	80% (v/v) acetonitrile	Phenol	Neutral (9.99)	2.370	2.339	−1.30	0.224	0.236	0.012	5.08
			Toluene	Neutral	4.159	4.192	0.8	1.194	1.174	−0.020	−1.70
			Naphthalene	Neutral	4.694	4.739	0.96	1.480	1.464	−0.016	−1.09
			Anthracene	Neutral	7.472	7.540	0.91	2.945	2.942	−0.003	−0.10
Enantioselective OTCEC[e]	6.00	without	R,S-Ketoprofen	Acid (4.0)	5.561	5.473	−1.58	0.093	0.111	0.018	19.35
					6.401	6.374	−0.42	0.273	0.279	0.006	2.20
			R,S-Fluribiprofen	Acid (4.14)	5.392	5.444	0.96	0.083	0.073	−0.010	−12.05
					5.692	5.761	1.21	0.146	0.133	−0.013	−8.90
			R,S-Ibuprofen	Acid (4.4)	5.417	5.476	1.09	0.069	0.058	−0.011	−15.94
					5.655	5.744	1.57	0.121	0.104	−0.017	−14.05
CEC[f]	7.00	50% (v/v) methanol	Mexiletine	Base (9.2)	3.626	3.620	−0.16	0.533	0.539	0.006	1.18
			Propanolol	Base (9.53)	4.395	4.312	−1.88	0.693	0.727	0.034	4.91
			Morpholine	Base (8.49)	3.325	3.294	−0.94	0.580	0.591	0.011	1.93
			Ephedrine	Base (10.3)	3.737	3.798	1.62	0.705	0.685	−0.020	−2.86
			Benzylamine	Base (9.50)	3.090	3.052	−1.24	0.578	0.615	0.037	6.38
	8.60	50% (v/v) Methanol	Propanolol	Base (9.53)	5.641	5.574	−1.18	0.760	0.762	0.002	0.25

[a] Calculated according to Eq. (15) using k_c instead of $k_{c,e}$.
[b] Calculated according to Eq. (16) using experimental t_r.
[c] Column, 57 cm (50 cm to detector)×50 μm I.D. C_{18} OTCEC column; mobile phase, 5 m*M* phosphate buffer containing different percentage of methanol; applied voltage, 25 kV; temperature, 25 °C.
[d] Column, 27 cm (6.5 cm to detector)×50 μm I.D. with 5 μm Chemcosorb ODS-H; mobile phase, 5 m*M* Tris buffer containing 80% (v/v) acetonitrile; applied voltage, −5 kV; temperature, ambient temperature.
[e] Column, 47 cm (40 cm to detector)×50 μm I.D. capillary with physically adsorbed avidin; mobile phase, 10 m*M* phosphate buffer; applied voltage, −20 kV; temperature, 20 °C.
[f] Column, 27 cm (20 cm to detector)×50 μm I.D. monolithic silica capillary; mobile phase, 5 m*M* phosphate buffer containing 50% (v/v) methanol; applied voltage, 15 kV; temperature, 25 °C.

($\alpha = 97\%$). Thus it can be seen that the weak bases used of known pK_a value (8.5–9.5) were fully protonated at pH 7.00, but partially protonated at pH 8.60, while the weak acids ($pK_a = 4.0$–4.4) were fully ionized at pH 6.00, without considering the influence of organic modifiers in the mobile phase.

The precision of the measurements of CEC, CZE and LC was investigated. The maximum of RSD was 1.2, 0.9 and 2.1% for retention time in CEC, electrophoretic mobility in CZE and retention factor in LC, respectively ($n = 6$). The experimental and calculated retention times, the experimental retention factors of LC and the calculated retention factors for CEC for the systems studied are summarized in Table 2. The calculated retention times from Eq. (15) using k_c instead of $k_{c,e}$ are in agreement with the experimental values, with the maximum relative difference of 2.4%. The calculated retention factors for CEC from Eq. (16) using experimental t_r are very close the experimental values for LC, with the maximum absolute difference of 0.04. The relative differences between the calculated retention factor and the experimental LC retention factor are significant in some cases, the reason for which is the absolute retention factors are too small. If the calculated retention time is plotted against the experimental retention time, a linear equation is obtained as the following:

$$t_{r\,cal.} = 0.026 + 0.996 t_{r\,exp.}$$
$$(r^2 = 0.9984, \mathrm{SD} = 0.095, n = 24) \quad (48)$$

Similarly, the relationship between the calculated retention factor for CEC and the experimental retention factor of LC can be described by the following equation:

$$k_{c,e} = 0.002 + 0.998 k_c$$
$$(r^2 = 0.9991, \mathrm{SD} = 0.020, n = 24) \quad (49)$$

The intercepts of about 0 and the slopes of about 1 indicate that the calculated retention time and retention factor for CEC nearly equal to the experimental retention time and retention factor of LC, respectively. Therefore, we can conclude that Eqs. (15) and (16) can be used to calculate the retention time and the actual chromatographic retention factor in CEC, respectively, and that the chromatographic and electrophoretic processes in CEC are independent.

This work is consist with the findings of Yan et al. [11] and Zhang et al. [12], but in dispute with the conclusion of Vissers et al. [10] The discord is perhaps related to the column configure used in the latter work. Although the column configure for LC mode was not clearly described, for CEC mode the length from inlet to detector was 39.9 cm whereas the length of packed part was only 27.4 cm. That is different from the common configure in which the detection window is very close to the packed part. The long void tube before the detector may have paid some unclear role in the retention.

3.2. Factors influencing the validity

In the mathematic treatment above, we assumed that the electric field is constant throughout the column. This is true in the open tubular column and the monolithic silica column. However, this assumption fails in the packed column. In a partially packed column, due to the different electric resistances of the packed and unpacked parts, the local electric filed across the two parts are different. On the other hand, the EOF within the column is generated not only by the packed material but also by the unpacked part of the column. As a result, the velocity of EOF within the packed column and the effective electrophoretic velocity of a charged solute cannot be calculated directly from Eqs. (5) and (6), and Eqs. (15) and (16) are thence invalid. For a neutral compound, which has no electrophoretic mobility, however, Eqs. (15) and (16) are still valid in such a case because the inhomogeneity of electric field and EOF only influences the void time. This is the reason why Eqs. (15) and (16) successfully predicted the retention time and retention factor for neutral compounds in the case of PR-CEC with the partially packed column. When the packed column was applied to basic compounds, it was found that calculated retention times from Eq. (15) are longer than the experimental values by 36%. That is due to fact that a noticeable inhomogeneity of electric field existed. By simply monitoring the apparent current through an open tubular column of identical dimension, the apparent current through the packed column and the current due to leakage (through surrounding parts instead of the column itself) under the same voltage, the local field strength across the packed part and unpacked part in the packed column can be estimated to be 478 and 94 V/cm[1], respectively. As

[1]Method for estimating the local field strength across the packed part and un-packed part in the packed column: Since the same voltage was applied to the open tubular column and the packed column,

$$I_o R_o = I_p R_{pp} + I_p R_{upp}$$

where I_o and I_p are the effective currents through the open tubular column and packed column, which equals to the corresponding apparent current minus the current due to leakage; R is the resistance, the subscript o, pp and upp represent the open tubular column, the packed part and the un-packed part of the packed column. The R_o and R_{upp} can be given by

$$R_o = \frac{V}{I_o} \quad \text{and} \quad R_{upp} = \frac{L_{upp}}{L_o} R_o$$

where V is the applied voltage, L_{upp} the length of the un-packed part of the packed column, and L_o the length of the open tubular column. Thus, the R_{upp} can be given by

$$R_{pp} = \left(\frac{I_o}{I_p} - \frac{L_{upp}}{L_o} \right) R_o$$

Therefore, the local electric field strength across the packed part (E_{pp}) and that across the un-packed part (E_{upp}) can be calculated by the following equations:

$$E_{pp} = \frac{I_p R_{pp}}{L_{pp}} = \left(\frac{I_o}{I_p} - \frac{L_{upp}}{L_o} \right) \frac{I_p V}{I_o L_{pp}}$$

$$E_{upp} = \frac{I_p R_{upp}}{L_{upp}} = \frac{I_p V}{L_o I_o}$$

where L_{pp} is the length of the packed part of the packed column.

a contrast, the apparent field strength across the packed column was 187 V/cm.

We also assumed that there is no irreversible interaction between the solute and the stationary phase. On the C_{18} open tubular column and monolithic silica column, adsorption played an important role in the retention of basic compounds. If the adsorption–desorption kinetic is too slow, the adsorption may influence the validity of Eqs. (15) and (16). Irreversible adsorption of basic compounds can be evaluated through monitoring the current within the column. Reversible adsorption gives a nearly straight current whereas irreversible adsorption results in a serious descending current. As shown in Fig. 3, the current when propanolol was injected was quite stable, which gave good prediction accuracy for the retention time. As a contrast, when ephedrine or benzylamine was injected, the current descended dramatically from 5.7 μA at the beginning to 4.4 μA after the solute migrated out of the column, giving poor prediction accuracy. Pre-rinse of the column before each separation of basic compounds seemed necessary, which can reduce possible adsorption in a previous run. For example, when no pre-rinse was employed, the differences between the calculated and experimental retention times and between the calculated retention factor for CEC and the experimental retention factor for LC were −13.4% and 42.5%, respectively. In comparison, when a pre-rinse with 1.5 column volume of mobile phase was employed, the corresponding differences were only −0.2% and 1.2%.

3.3. Donnan exclusion effect

When the approach is applied to packed column and monolithic silica column, Donnan exclusion effect of the stationary phase must be taken into account. Most of the packing materials used in HPLC and CEC are silica-based. Under common pH condition used in CEC, the silica matrix of the packing material is negatively charged. Therefore, anionic solutes cannot penetrate into the occluded liquid phase within the mesopores of the packing material because it is repelled by the anionic silanol groups. Thus anionic solutes will be eluted faster than a neutral t_0 marker if there is no other interaction with the stationary phase to offset the Donnan exclusion effect. Because the mobile phases used in CEC are usually of low ionic strength, the Donnan exclusion effect may be noticeable. As shown in Fig. 4, significant Donnan exclusion effect was observed in LC separations on both the monolithic silica column and packed column of ODS packing. In CEC

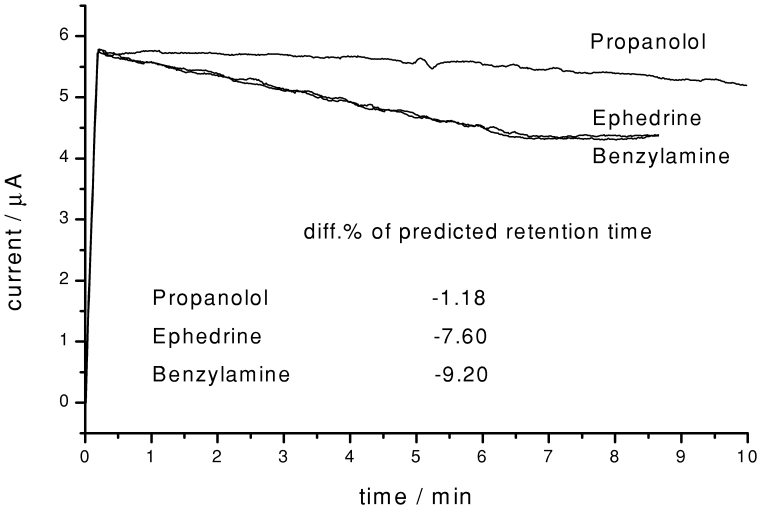

Fig. 3. Relationship between the current stability on the prediction accuracy of Eq. (15). Conditions: column, 27 cm (20 cm to detector)×50 μm I.D. monolithic silica capillary; mobile phase, 5 m*M* phosphate buffer containing 20% (v/v) methanol, pH 8.60; applied voltage, 15 kV; temperature, 25 °C.

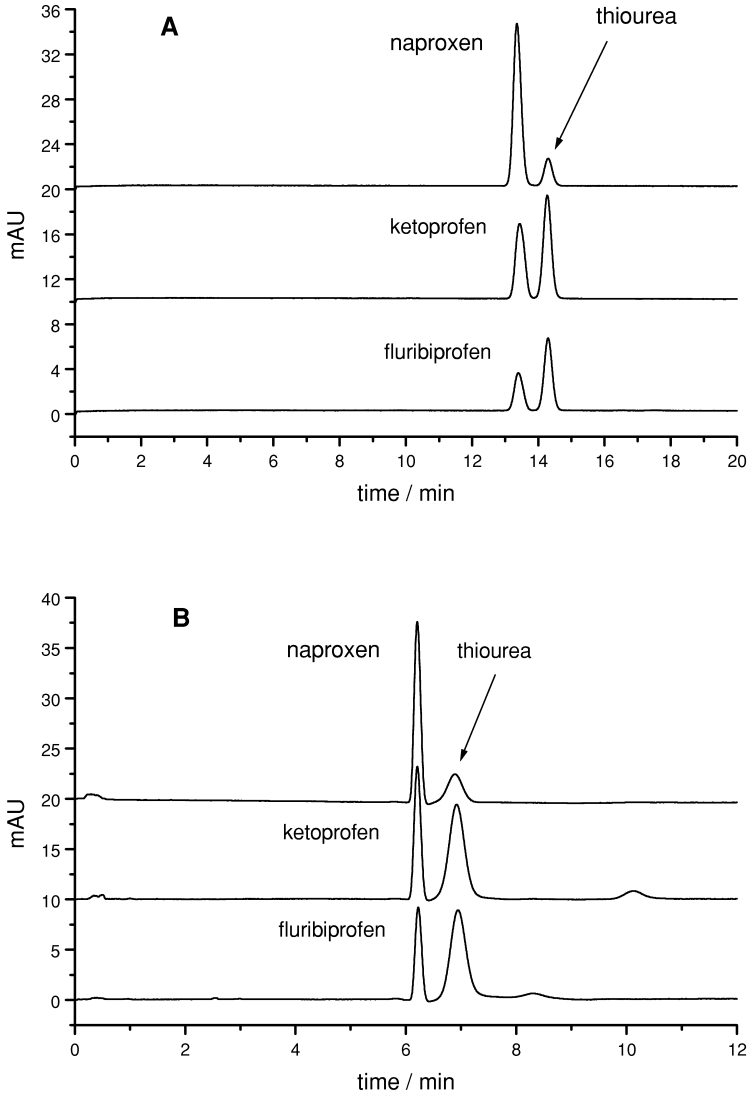

Fig. 4. Donnan exclusion effect in LC separations of acidic compounds on monolithic silica column (A) and packed reversed-phase column (B). Conditions: column, (A) 27 cm (20 cm to detector)×50 μm I.D. monolithic silica capillary, (B) 27 cm (6.5 cm to detector)×50 μm I.D. with 5 μm Chemcosorb ODS-H; mobile phase, (A) 5 m*M* phosphate buffer containing 20% (v/v) methanol, pH 7.00, (B) 5 m*M* Tris buffer containing 80% (v/v) acetonitrile, pH 8.00; applied pressure, 138 kPa; temperature, (A) 25 °C, (B) ambient temperature.

separation, the Donnan exclusion effect offers a positive contribution to the migration of an anionic solute. For example, the values of apparent electrophoretic mobility without considering chromatographic process for ketoprofen and naproxen in CEC were -6.6×10^{-3} and -8.4×10^{-3} $cm^2\ V^{-1}\ min^{-1}$, respectively, whereas those in CZE were -9.4×10^{-3} and -9.8×10^{-3} $cm^2\ V^{-1}\ min^{-1}$, respectively. Note that the presence of Donnan exclusion effect does not invalidate the general equations proposed, but invalidates the measurement of void time if the t_0 marker employed is not subject to Donnan exclusion effect. In a case where Donnan exclusion effect exists, in theory, an un-retarded charged compound having identical repulsion interaction with the anionic silanols as the solute has should be employed

as a marker for the void time for the solute under study. However, it is rather hard to find such a marker in practice.

4. Conclusions

The general equations obtained from the phenomenological treatment have been experimentally verified. The test analytes involved neutral, basic and acidic compounds. The CEC systems studied included RP-CEC (on both open tubular column and packed column) where hydrophobic interaction played a main role, enantioselective OTCEC where hydrophobic, electrostatic, steric and hydrogen bonding interactions could take part in the retention process, and monolithic silica CEC where multiple mechanisms including reversed-phase, cation-exchange and normal-phase mechanisms could occur. These general equations allow calculation of the retention time and retention factor in CEC from related chromatographic and electrophoretic data. The chromatographic process and the electrophoretic process in the CEC systems studied were found independent. Although these equations seem to be valid for other CEC systems because no assumption is made for the separation mechanism, discreetly to say, universal validity of these equations needs further experimental confirmation with more diverse CEC systems and analytes. When using these equations, three factors must be taken into account: (1) inhomogeneity of the electric field and EOF in partially packed column; (2) irreversible interaction between the solute and the stationary phase; and (3) the Donnan exclusion effect of the stationary phase.

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