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# Interplay of chromatographic and electrophoretic processes in capillary electrochromatography

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**Abstract**

The separation mechanism in capillary electrochromatography (CEC) is a hybrid differential migration process, which entails the features of both high-performance liquid chromatography and capillary zone electrophoresis, i.e., chromatographic retention and electrophoretic migration. The adsorption of the different sample components on the stationary phase can be modified by the presence of the electric field across the column. Here, we use our previously published approach to decouple chromatographic retention from electrophoretic migration that allows us to investigate the ''modification'' of the retention process in CEC. This paper presents a methodology for characterization of changes in the retention of neutral and charged sample components, under identical conditions of stationary and mobile phase.


*Keywords:* Retention; Migration; Separation mechanisms

## 1. Introduction

Capillary electrochromatography (CEC) is an analytical separation technique that is carried out most commonly with packed capillary columns and utilizes electroosmotically driven mobile phase at high electric field strength in an apparatus similar to that used in capillary zone electrophoresis (CZE). Recently, CEC has attracted a lot of interest due to its potential to offer selectivity different to that in high-performance liquid chromatography (HPLC) and CZE and as another high-resolution separation technique [1–16].

Retention factor, $k'$, in HPLC is a dimensionless parameter that quantifies the location of a peak in a chromatogram and provides thermodynamic insights into the interactions between the components and the stationary phase. So far, this definition of the retention factor has also been extended to CEC [17,18]. Recently, the various expressions that have been proposed for the electrochromatographic retention factor were examined [19]. It was concluded that due to the dual separation mechanisms that are in action in CEC, the system is significantly complicated in comparison to that of HPLC and it is not possible to come up with a $k'$ which would have all the attributes that it has in regular chromatography.

The focus of this paper is to examine the modi-

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fication of the adsorption of different sample components on the stationary phase due to the various electrophoretic and electroosmotic interactions that occur in CEC. We use our previously published approach [19] to decouple chromatographic retention from electrophoretic migration for a variety of neutral and charged sample components on different columns. This allows us to quantify the change in the retention properties for the different sample components under identical conditions of mobile and stationary phases.

## 2. Theory

Recently it was shown that the different separation mechanisms of HPLC and CZE preclude the definition of any retention or velocity factor for CEC that would have comparable significance to that of $k'$ and $k_e''$ [19]. However, the CEC system could be defined by a combination of a ''retention factor'' that measures the retention in CEC and a velocity factor that characterizes the electrophoretic migration. The migration velocity, $u_m$, of a charged sample component in CEC is expressed in the literature [1,4,9,12,17–21] by the sum of the velocity of the mobile phase, $u_o$, and the electrophoretic velocity of the migrant of interest, $u_p$, multiplied by the retardation factor, $1/(1+k'')$, as:

$$u_m = \frac{u_p + u_o}{1 + k''} = \frac{u_o(1 + k_e'')}{1 + k''} \quad (1)$$

where, $u_m$, $u_o$, and $u_p$ denote the overall migration flow velocity of the migrant, velocity of the mobile phase, and the electrophoretic velocity of the migrant, respectively. Further, $k''$ is the measure of chromatographic retention under conditions of the CEC experiments, i.e., the retention factor in CEC, and $k_e''$ is the velocity factor. They are given by:

$$k_e'' = \frac{\mu_p}{\mu_o} \quad (2)$$

and:

$$k'' = \frac{t_m(1 + k_e'') - t_o}{t_o} \quad (3)$$

where, $t_m$ and $t_o$ denote the migration time of the analyte and migration time of an inert and neutral tracer, respectively.

It should be emphasized that both $k''$ and $k_e''$ need to be evaluated under conditions used in the CEC experiments [19]. First, the electrophoretic mobility of the sample component, $\mu_p$, is obtained from separate CZE measurements using the mobile phase used in CEC. Then electrosmotic mobility, which is the interstitial electroosmotic flow (EOF) mobility in the packing, is evaluated from the results of measuring the currents and the EOF with CEC columns [12,17]. This allows for calculation of $k_e''$ according to Eq. (2) followed by calculation of $k''$ from Eq. (3) using the migration times of the different sample components in the CEC column. The $k''$ measures the magnitude of retention in CEC due to reversible binding of the analyte to the stationary phase.

## 3. Experimental

### 3.1. Chemicals and reagents

Chemicals, test analytes and reagents were obtained from sources as previously described [16]. The structures, p$K_a$ and log $P$ values of these analytes have been previously published [16].

The Hypersil CEC Basic $C_{18}$ HPLC column (3 μm, 150×4.6 mm) was packed and supplied by ThermoHypersil-Keystone (Runcorn, UK). Media from the same batch of the Hypersil CEC Basic $C_{18}$ was also provided for capillary packing (performed in the laboratory at AstraZeneca) by ThermoHypersil-Keystone.

### 3.2. Instrumentation

For HPLC separations an Agilent 1100 Series liquid chromatograph with Agilent ChemStation v. 8.04 LC software (Agilent Technologies, Cheadle, UK) was used. For CEC and CE separations, an Agilent $^{3D}$CE instrument was used with Agilent ChemStation v. 6.04 CE(C) software. The Agilent $^{3D}$CE instrument has the capability of pressurizing the inlet and outlet vials to 1.2 MPa (provided by an $N_2$ cylinder).

### 3.3. Buffer and mobile phase preparations for HPLC, CE and CEC separations

50 m*M* $NaH_2PO_4$, pH 2.3 was prepared by dissolving the appropriate quantity of buffer salt in ~200 ml of pure water before adjusting the pH of the solution using orthophosphoric acid as required. The volume was then made to 1000 ml before mobile phase compositions of 6:2:2, 5:3:2, 4:4:2 and 3:5:2 (v/v/v) acetonitrile (ACN)–water–50 m*M* buffer were prepared for the separations. Thus, ionic strength was kept constant at each composition.

### 3.4. Capillary electrophoresis conditions

A fused-silica capillary, 48.7 cm (40 cm effective length)×0.1 mm I.D. from Composite Metals (Hallow, UK) was preconditioned by flushing with 1 *M* NaOH for 20 min, then water for 10 min, then 0.1 *M* NaOH for a further 10 min. Conditioning between injections during a run comprised flushing with 0.1 *M* NaOH for 2 min, then with mobile phase for 3 min. Run conditions were 6:2:2, 5:3:2, 4:4:2 and 3:5:2 (v/v/v) ACN–water–50 m*M* $NaH_2PO_4$, pH 2.3, 20 kV, 20 °C, 210/254 nm, 5 kV/3 s injections of basic analyte standards. The EOF was determined by analyzing a 2% aqueous acetone solution ($n=3$), under each eluent.

### 3.5. Capillary electrochromatography packing and evaluation conditions

The capillaries were packed, then equilibrated and tested (acceptability based upon linear velocity and efficiency of designated peaks) as reported previously [16].

## 4. Results and discussion

### 4.1. Separation of basic analytes by reversed-phase (RP) CEC

Dittmann et al. published a study in which separation of basic solutes at low pH by CEC was investigated [1]. Table 1 presents the retention factor in HPLC and CEC and the velocity factor in CZE as reported in the original reference. First, $k''_e$ was calculated as shown in Eq. (2) using the mobility data from Ref. [1] and $t_o$ (time of elution of thiourea for this case) in CZE and in CEC. Next, $k''$ was calculated using Eq. (3) and the elution times of different solutes in CEC. These results are presented in Table 1.

Since the four basic analytes (i.e., procaine, timolol, ambroxol and metoclopramide) are protonated under the chosen conditions, their mobility is faster than the EOF and hence $k''_e$ is always positive. It is seen that the $k''$ values calculated for the various solutes are similar for the Hypersil-$C_8$ and the Spherisorb ODS columns. However, a significant effect is seen on the retention process for the Hypersil-$C_{18}$ column, where it is seen that the solutes are more strongly ''retained'' in CEC mode then in the HPLC mode. It is thought that this is a result of structural changes within the stationary phase resulting in changes in organization of solutes in the stationary phase [22].

It must be emphasized, as seen in Table 1, that measuring retention by using the same expression as in HPLC (Eq. (1)) for this case yields values that are negative and, thus, do not give much insight into the overall process. Calculation of $k''$ using Eqs. (2) and (3) decouples electrophoretic migration from ''retention'' and allows us to compare the retention factors in HPLC and CEC and quantify the modifications to the retention process.

### 4.2. Separation of neutral and charged analytes on sulfonated monoliths

Recently, Bedair and El Rassi published a study on the separation of dansyl amino acids using a novel monolithic stationary phase having long alkyl chain ligands ($C_{17}$) by CEC [23]. The monolithic stationary phase was prepared by the in situ copolymerization of pentaerythritol diacrylate monostearate (PEDAS) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) in a ternary porogenic solvent consisting of cyclodexanol–ethylene glycol–water. They calculated the retention factors $k'$, $k''_e$ and $k''$ using Eqs. (1)–(3) and the results are presented in Table 2.

It is observed that the retention factors in CEC are consistently lower than in HPLC (except for the basic amino acid lysine). The most likely reason for

Table 1
Separation of basic analytes by RP-HPLC and RP-CEC*

| Analyte | Published data [1] | | | Calculations | |
|---|---|---|---|---|---|
| | $k'$ | $k'_{CEC}$ calculated | $k'_{CEC}$ found | $k''_e$ | $k''$ |
| CEC Hypersil-$C_{18}$ | | | | | |
| Procaine | 0.70 | −0.56 | −0.57 | 4.00 | 1.15 |
| Timolol | 0.70 | −0.46 | −0.58 | 2.99 | 1.07 |
| Ambroxol | 0.83 | −0.42 | −0.44 | 2.99 | 1.23 |
| Metoclopramide | 1.51 | −0.31 | −0.32 | 3.80 | 2.26 |
| Naproxen | 0.11 | 0.08 | 0.09 | 0.04 | 0.14 |
| Antipyrine | 0.37 | 0.30 | 0.30 | 0.09 | 0.42 |
| CEC Hypersil-$C_8$ | | | | | |
| Procaine | 0.37 | −0.66 | −0.66 | 3.01 | 0.36 |
| Timolol | 0.32 | −0.60 | −0.61 | 2.24 | 0.26 |
| Ambroxol | 0.32 | −0.60 | −0.61 | 2.24 | 0.26 |
| Metoclopramide | 0.46 | −0.61 | −0.63 | 2.86 | 0.43 |
| Naproxen | 0.22 | 0.18 | 0.17 | 0.03 | 0.21 |
| Antipyrine | 0.32 | 0.24 | – | 0.06 | – |
| Spherisorb ODS-1 | | | | | |
| Procaine | 0.61 | −0.69 | −0.66 | 3.87 | 0.66 |
| Timolol | 0.61 | −0.62 | −0.59 | 2.87 | 0.59 |
| Ambroxol | 0.67 | −0.60 | −0.57 | 2.87 | 0.67 |
| Metoclopramide | 1.24 | −0.54 | −0.54 | 3.63 | 1.13 |
| Naproxen | 0.25 | 0.19 | 0.21 | 0.04 | 0.26 |
| Antipyrine | 1.00 | 0.84 | 0.82 | 0.08 | 0.96 |

* Adapted from Ref. [1]. CEC conditions: columns, 3 μm, 33.5 (25.0)×0.01 cm; mobile phase, ACN–25 m*M* phosphate (8:2 v/v)+0.2% hexylamine, pH 2.5; voltage, 25 kV; temperature, 20 °C. HPLC conditions: columns and mobile phases as for CEC; pressure, 200 bar. CZE conditions: mobile phase as for CEC; capillary, uncoated fused-silica, 335 (250)×0.075 mm.

the reduction in the retention factor in CEC is electrostatic interactions such as repulsion between the negatively charged solutes ($k''_e$ is negative) and the negatively charged stationary phase [23]. Once again, the calculation of $k''_e$ and $k''$ allows us to understand the changes to the retention process in CEC.

### 4.3. Separation of basic analytes by RP-CEC

In order to further understand the modification of the retention process in RP-CEC, experiments were conducted using several basic analytes under varying concentration of the organic solvent. Experiments were performed using identical conditions of the mobile and stationary phase in HPLC, CEC and CZE modes. Next, different migration parameters, $k'$, $k''_e$ and $k''$, were calculated using Eqs. (1)–(3), respectively, and the results are presented in Table 3.

The electrophoretic mobility is positive (codirectional with the EOF) because the analytes are positively charged. For most analytes it is seen that the electrophoretic mobility slightly increases with an increase in percentage ACN, which is predominantly due to changes in eluent viscosity and the solvation radius of the analyte. However, the electromobility of nicotine was found to decrease with increasing proportions of ACN. It is postulated that suppression of the p$K_a$ of the pyridine ring of the nicotine analyte with a concurrent increase in the apparent pH of the eluent was responsible. The net effect is a reduction in the percentage ionized form of the nicotine analyte which will reduce its electromobility. The percentage ionization of the other basic analytes remained unaffected by these changes as their p$K_a$ values are far enough away not to be influenced. Similar observations and extensive reports of the influence of organic solvents in eluents

Table 2
Separation of amino acids on a monolithic column*

| Analyte | Retention factor | | |
|---|---|---|---|
| | $k'$ | $k''_e$ | $k''$ |
| Lys | 0.074 | 0 | 0.074 |
| Gln | 0.68 | −0.257 | 0.25 |
| Ans | 0.72 | −0.263 | 0.27 |
| Thr | 0.79 | −0.269 | 0.31 |
| Ser | 0.81 | −0.281 | 0.3 |
| Glu | 0.83 | −0.317 | 0.25 |
| Asp | 0.95 | −0.323 | 0.32 |
| Ala | 0.99 | −0.281 | 0.43 |
| Gly | 1.02 | −0.3 | 0.41 |
| Met | 1.11 | −0.263 | 0.56 |
| Pro | 1.11 | −0.257 | 0.57 |
| Val | 1.41 | −0.227 | 0.86 |
| Phe | 1.69 | −0.26 | 0.99 |
| Trp | 1.88 | −0.226 | 1.23 |
| Leu | 1.97 | −0.239 | 1.26 |
| Ile | 2.12 | −0.227 | 1.41 |

* Adapted from Ref. [23]. In situ polymerized monolithic column, 33.5 (25)×0.01 cm; mobile phase, ACN–10 m*M* ammonium acetate, pH 4.5 (3.7 v/v); voltage, 25 kV.

on analyte p$K_a$, overall eluent pH and buffer capacity have been reported by others [24].

It is evident from Table 3 that the retention factors in HPLC and CEC ($k'$ and $k''$, respectively) are quite dissimilar for the different analytes, suggesting a strong effect of the electric field on the CEC retention mechanism. Fig. 1 illustrates the retention factors in HPLC and CEC modes for the various analytes. It can be seen that in most cases $k''>k'$ for the positively charged analytes using the Hypersil CEC $C_{18}$ phase. From the observations of Dittmann et al. [1] this is not unexpected. Interestingly, it can be observed that the basic analytes can be divided into two groups in that the CEC retention of the hydrophilic basic analytes is greater than the HPLC retention (i.e., $k''>k'$) over the range studied. The lipophilic basic analytes show a similar trend in that $k''>k'$, but at low percentage ACN (i.e., <30%), a change is seen and LC retention>CEC retention (i.e., $k'>k''$).

If the LC data in Fig. 1 is plotted as log $k'$ vs. percentage ACN, the expected linear relationship is not seen for the lipophilic basic analytes (data not shown), and deviation from linearity (i.e., greater retention than expected is observed) is pronounced at low percentage ACN (i.e., ≤30%). This effect does not appear to be phase dependent as similar observations in LC mode with HyPURITY $C_{18}$ and Discovery $C_8$ stationary phase materials have been made. This suggests that the mechanism of retention is different at low percentage ACN levels, presumably due to a change in the organization of the phase, as the degree of analyte and stationary phase ionization should not be significantly changed. The differences in selectivity observed between CEC and HPLC is likely due to an interplay of the effect of the organic

Table 3
Retention parameters for basic solutes in HPLC and CEC*

| ACN (%) | Thiourea | Benzylamine | Nicotine | Procainamide | Salbutamol | ARC-12495 | Diphenhydramine | Nortriptyline | AR-C68397 |
|---|---|---|---|---|---|---|---|---|---|
| Retention factor in HPLC ($k'$) | | | | | | | | | |
| 30 | | 0.15 | 0.11 | 0.17 | 0.18 | 1.39 | 2.88 | 6.17 | 2.35 |
| 40 | | 0.17 | 0.20 | 0.20 | 0.13 | 0.64 | 0.79 | 1.80 | 0.86 |
| 50 | | 0.15 | 0.11 | 0.17 | 0.10 | 0.39 | 0.63 | 0.84 | 0.44 |
| 60 | | 0.15 | 0.16 | 0.17 | 0.12 | 0.30 | 0.44 | 0.55 | 0.31 |
| Electrophoretic mobility in CZE, $\mu_p$ ($cm^2/V\,s$) | | | | | | | | | |
| 30 | $1.15\cdot10^{-9}$ | $2.60\cdot10^{-8}$ | $3.06\cdot10^{-8}$ | $1.62\cdot10^{-8}$ | $1.37\cdot10^{-8}$ | $1.54\cdot10^{-8}$ | $1.65\cdot10^{-8}$ | $1.57\cdot10^{-8}$ | $1.24\cdot10^{-8}$ |
| 40 | $1.16\cdot10^{-9}$ | $3.09\cdot10^{-8}$ | $3.02\cdot10^{-8}$ | $1.98\cdot10^{-8}$ | $1.73\cdot10^{-8}$ | $2.01\cdot10^{-8}$ | $2.00\cdot10^{-8}$ | $1.93\cdot10^{-8}$ | $1.35\cdot10^{-8}$ |
| 50 | $1.16\cdot10^{-9}$ | $3.16\cdot10^{-8}$ | $2.71\cdot10^{-8}$ | $2.04\cdot10^{-8}$ | $1.82\cdot10^{-8}$ | $2.12\cdot10^{-8}$ | $2.24\cdot10^{-8}$ | $2.12\cdot10^{-8}$ | $1.35\cdot10^{-8}$ |
| 60 | $1.47\cdot10^{-9}$ | $3.29\cdot10^{-8}$ | $2.62\cdot10^{-8}$ | $2.26\cdot10^{-8}$ | $1.98\cdot10^{-8}$ | $2,42\cdot10^{-8}$ | $2.60\cdot10^{-8}$ | $2.46\cdot10^{-8}$ | $1.41\cdot10^{-8}$ |
| Retention factor in CEC ($k''$) | | | | | | | | | |
| 30 | | 0.91 | 1.49 | 0.79 | 0.73 | 1.20 | 1.82 | 2.90 | 2.20 |
| 40 | | 1.14 | 1.46 | 1.06 | 0.90 | 1.36 | 1.38 | 1.83 | 1.41 |
| 50 | | 1.06 | 1.13 | 0.95 | 0.72 | 1.30 | 1.14 | 1.30 | 0.97 |
| 60 | | 1.05 | 0.98 | 0.99 | 0.69 | 1.44 | 1.15 | 1.26 | 0.84 |

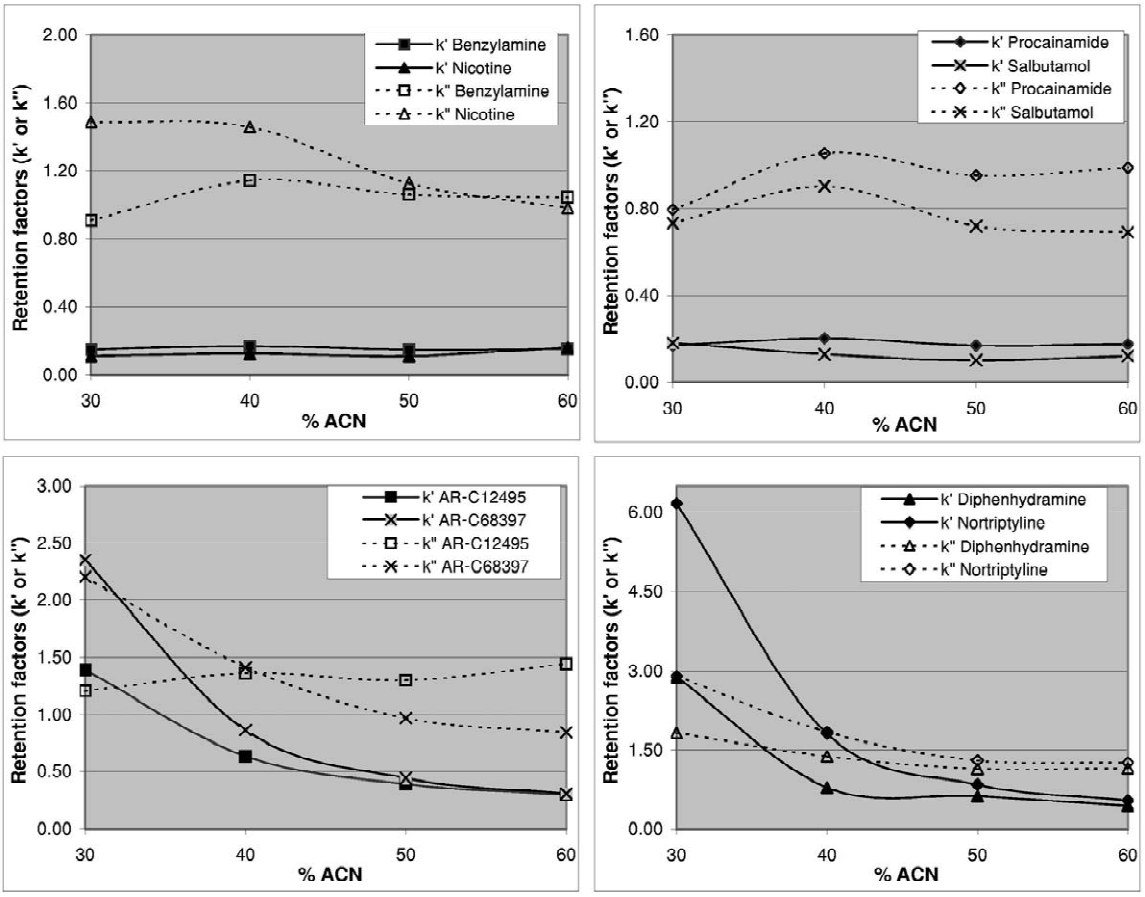


Fig. 1. Retention behaviour of basic solutes in HPLC and CEC.

solvent and the electric field on the analyte (and its physicochemical properties) and the structure, organization and partitioning with the stationary phase.

## 5. Conclusions

Definitions of the key migration parameters in the CEC framework are presented. It is observed that the adsorption of the different sample components on the stationary phase can be modified by the presence of the electric field across the column. In this paper, we use our previously published approach to decouple chromatographic retention from electrophoretic migration for a variety of neutral and charged sample components on different columns. It is shown that the CEC system can be defined by a combination of a ''retention factor'' that measures the retention in CEC and a velocity factor that characterizes the electrophoretic migration. As shown in Tables 1–3, these two parameters together can be very useful in characterization of the overall CEC system. This approach also allows us to investigate the ''modification'' of the retention process in CEC and it is found that indeed, under identical conditions of stationary and mobile phase, there is a big change in the retention of the different sample components, especially when they are charged molecules.

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