Calculation of retention factors in capillary electrochromatography from chromatographic and electrophoretic data

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Calculation of Retention Factors in Capillary Electrochromatography from Chromatographic and Electrophoretic Data

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Key Words:
Capillary electrochromatography (CEC)
Retention factors
Microcolumn liquid chromatography
Capillary zone electrophoresis

Summary
Retention factors in capillary electrochromatography (CEC) were calculated by means of theoretically derived equations and experimentally determined parameters in microcolumn liquid chromatography and capillary zone electrophoresis. It was found that the retention factor of uncharged components in CEC was about 20% higher than was calculated. The derived equations do not take into account alteration of the nature of the stationary phase or distribution constant by the applied electric field. However, the influence of the electric field on the retention in CEC can be estimated. Individual field contributions could not be determined.

1 Introduction
Capillary electrochromatography (CEC) is a relatively new technique that is still in a state of development. CEC is performed in packed microcolumns, i.e., packed fused silica capillaries with an internal diameter (i.d.) of 50–100 μm, and the eluent is driven through the column by electro-osmosis. The electro-osmotic flow is induced by applying an electric field of 20–100 kV m⁻¹ over the column. The i.d. of the column is limited in order to prevent unwanted temperature gradients along the column radius, i.e., Joule heating. Neutral analytes are separated by normal partition between the mobile phase and the stationary phase, similar as in liquid chromatography (LC). In the case of dissociated or ionized analytes also electrophoresis contributes to the separation.

Only a limited number of scientific publications deal with the theoretical aspects of CEC. Knox and Grant [11] studied the effect of the particle diameter on the electro-osmotic velocity, the effect of the particle diameter on the plate height in pressure driven and electrically driven chromatography, and the effect of electrolyte concentration on the mobile phase velocity and the plate height. The theory of band-broadening in CEC was investigated by Tsuda [2,3] and that of extracolumn band-broadening by Rebscher and Pyell [4]. Other studies mostly involved comparative studies between CEC and microcolumn LC [5–7]. This paper discusses the calculation of retention of neutral compounds in CEC by means of retention data obtained by LC and capillary zone electrophoresis (CZE). Retention calculation of charged compounds will not be discussed in this paper.

2 Theory
CEC involves both partition and electrophoretic mobility [2,3]. Therefore, the mean linear velocity of a chromatographically unretained compound \( u_0 \) in EC can be expressed as the sum of the electro-osmotic flow of the mobile phase \( u_{oef} \), the effective electrophoretic mobility the unretained compound \( u_{eff} \) and the linear flow velocity corresponding to pressurized flow \( u_{press} \):

\[
u_0 = u_{oef} + u_{eff} + u_{press}
\]  

(1)

The velocity of a retained compound in chromatography is given by:

\[
u_r = \frac{u_0}{(1 + k)}
\]  

(2)

where \( u_r \) is the linear velocity of the retained compound and \( k \) the retention factor. Substitution of equation (2) in equation (1) gives:

\[
u_r = \frac{u_{oef} + u_{eff} + u_{press}}{(1 + k)}
\]  

(3)

The electro-osmotic flow of the mobile phase \( u_{oef} \) and the effective electrophoretic mobility of a unretained compound \( u_{eff} \) can be expressed as the electro-osmotic mobility \( \mu_{oef} \) and electrophoretic mobilities \( \mu_{eff} \) using the potential drop \( V \) over the total length of the column \( L_{tot} \):

\[u_{oef} + u_{eff} = (\mu_{oef} + \mu_{eff}) \times \frac{V}{L_{tot}}
\]  

(4)

Combination of equations (3) and (4) gives:

\[
u_r = \frac{(\mu_{oef} + \mu_{eff}) \times \frac{V}{L_{tot}} + u_{press}}{1 + k}
\]  

(5)

The retention time \( t_r \) of a compound can be calculated introducing the injector-to-detector length of the column \( L_{id} \) Subsequent substitution of eq. (5) yields:
Calculation of Retention Factors in Capillary Electrochromatography

As the electro-osmotic mobility is related to the time of the electro-osmotic flow marker $t_{cof}$ by:

$$\mu_{cof} = \frac{L_{id}}{V \cdot \eta}$$  \hspace{1cm} (9)

and $u_{press}$ by:

$$u_{press} = \frac{d_{p}^2 \Delta p}{\Phi \cdot \eta \cdot L_{col}}$$  \hspace{1cm} (8)

where $d_{p}$ is the diameter of the stationary phase particles, $\Phi$ the flow resistance parameter, $\eta$ the viscosity of the mobile phase, $\Delta p$ the pressure drop over the column, and $L_{col}$ the length packed with stationary phase particles, an expression for the retention time of a compound can be derived:

$$t_r = \frac{L_{id}(1 + k)}{\mu_{cof} \cdot V + u_{press}} \cdot L_{tot}$$  \hspace{1cm} (6)

In the case that no pressurized flow is used, i.e. $\Delta p$ equals zero. Rearrangement of eq. (9) gives:

$$t_r = \frac{1}{\mu_{cof} \cdot L_{id} \cdot L_{tot}} \cdot \frac{L_{id}(1 + k)}{V}$$  \hspace{1cm} (10)

Retention time can thus be calculated by means of LC experiments, i.e. retention factor, and CZE experiments, i.e. $t_{cof}$ and $\mu_{eff}$, respectively.

3 Experimental

3.1 Instrumentation

Micro-LC separations were carried out with a Phoenix 20 CU syringe pump (Carlo Erba Instruments, Milan, Italy) or a model 100 DM syringe pump (ISCO Inc., Lincoln, NE, USA). Injections were performed manually with a 60 nl C14W injection valve (VICI-AG Valco Europe, Schenkon, Switzerland). Detection was performed with a 785 A Programmable Absorbance detector (Applied Biosystems, San Jose, CA, USA), equipped with a z-shaped detection cell (LC Packings, Amsterdam, The Netherlands), at a wavelength of 260 nm. The column was thermostated with a Ultra-thermostat NB-33369 (Calora Messtechnik GmbH, Lorch, Germany) and a home made water jacket.

The CEC equipment consisted of a Prince Version 1 Programmable Injector (Lauerlabs, Emmen, The Netherlands) for electrokinetic or pressure driven injections of the samples, a HCN 140-35000 DC power supply (FUG Electronic GmbH, Germany) to generate an electric field across the packed capillary columns and an on-column UV absorbance detector (Unicam Analytical Systems, Cambridge, UK). Platinum wires were used to connect the injection unit to the positive electrode and the buffer reservoir. A home-made interface and Caesar software (B*Wise, Geleen, The Netherlands) were employed for data acquisition. All CZE experiments were done on the same equipment as the CEC experiments. A 50-\mu m i.d fused silica capillary (Scientific Glass Engineering, Melbourne, Australia) was used during the CZE analysis.

3.2 Chemicals

Acetonitrile (ACN) used as mobile phase modifier, was, like acetone, purchased from E. Merck (Darmstadt, Germany). The applied buffers were sodium acetate and 3-[morpholinopropanesulfonic acid] (MOPS), both from E. Merck, and 2-[N-morpholine]ethanesulfonic acid (MES) from Sigma Chemical Company (St. Louis, MI, USA). 4-aminoacetoephone, o-nitrophenol, 2,6-dimethylphenol and thiourea were from E. Merck and naphthalene from Fluka AG (Buchs, Switzerland). All chemicals were of analytical-reagent grade. Water was purified with a Milli-Q Water Purification system of Water-Millipore (Milford, MA, USA) prior to use. The individual solvents of the mobile phase were filtered through a 0.45-\mu m filter. The mobile phase was degassed with helium before it was used.

3.3 Column Preparation

The procedure used to pack the fused silica capillaries and the packing equipment were discussed in detail in previous papers [8,9]. Nucleosil 100-5 C18 (Machery-Nagel GmbH & Co KG, Dieren, Germany) was used as the packing material and was suspended in acetone by ultrasonication for a 10 minute period before it was transferred into the slurry vessel by means of a syringe. Packing was carried out at 500 bar and ACN/H2O 70:30 (v/v) was used as the packing liquid. The pressure was maintained for a one hour period.

4 Results and Discussion

4.1 Microcolumn LC Experiments

The retention of a compound in LC is dependent among other things on the distribution of the compound over the mobile and the stationary phase. Therefore, the influence of the pH, ionic strength, and modifier concentration on the retention factor of neutral compounds.

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>pH</th>
<th>Ionic strength (mM)</th>
<th>Fraction modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.00</td>
<td>2.00</td>
<td>0.40</td>
</tr>
<tr>
<td>2</td>
<td>5.00</td>
<td>2.00</td>
<td>0.70</td>
</tr>
<tr>
<td>3</td>
<td>5.00</td>
<td>8.00</td>
<td>0.40</td>
</tr>
<tr>
<td>4</td>
<td>5.00</td>
<td>8.00</td>
<td>0.70</td>
</tr>
<tr>
<td>5</td>
<td>7.00</td>
<td>2.00</td>
<td>0.40</td>
</tr>
<tr>
<td>6</td>
<td>7.00</td>
<td>2.00</td>
<td>0.70</td>
</tr>
<tr>
<td>7</td>
<td>7.00</td>
<td>8.00</td>
<td>0.40</td>
</tr>
<tr>
<td>8</td>
<td>7.00</td>
<td>8.00</td>
<td>0.70</td>
</tr>
<tr>
<td>9</td>
<td>6.00</td>
<td>5.00</td>
<td>0.55</td>
</tr>
<tr>
<td>10</td>
<td>4.78</td>
<td>5.00</td>
<td>0.55</td>
</tr>
<tr>
<td>11</td>
<td>7.22</td>
<td>5.00</td>
<td>0.55</td>
</tr>
<tr>
<td>12</td>
<td>6.00</td>
<td>1.36</td>
<td>0.55</td>
</tr>
<tr>
<td>13</td>
<td>6.00</td>
<td>8.65</td>
<td>0.55</td>
</tr>
<tr>
<td>14</td>
<td>6.00</td>
<td>5.00</td>
<td>0.57</td>
</tr>
<tr>
<td>15</td>
<td>6.00</td>
<td>5.00</td>
<td>0.73</td>
</tr>
</tbody>
</table>
Calculation of Retention Factors in Capillary Electrochromatography

Table 2. Results of the experimental design for neutral compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Intercept</th>
<th>Modifier</th>
<th>Modifier^2</th>
<th>r</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Aminoacetophenone</td>
<td>1.93</td>
<td>-4.3</td>
<td>2.6</td>
<td>0.997</td>
<td>1000</td>
</tr>
<tr>
<td>o-Nitrophenol</td>
<td>10.9</td>
<td>-28</td>
<td>19</td>
<td>0.984</td>
<td>180</td>
</tr>
<tr>
<td>2,6-Dimethylphenol</td>
<td>15.2</td>
<td>-42</td>
<td>29</td>
<td>0.995</td>
<td>630</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>82</td>
<td>-240</td>
<td>180</td>
<td>0.992</td>
<td>330</td>
</tr>
</tbody>
</table>

several neutral compounds, i.e., uncharged components under the applied conditions, was investigated by means of a central composite design. The experimental settings of the design are given in Table 1. All levels of the central composite design were measured twice. The influences of the investigated parameters on the retention factor were calculated for 4-aminoacetophenone, o-nitrophenol, 2,6-dimethylphenol, and naphthalene. A backward elimination procedure was applied to determine the significance of the parameters at the 95% confidence level. Single and quadratic interactions terms were also allowed in the experimental central composite design. The equations describing the dependence of the retention factor of the neutral compounds on the parameters is given in Table 2.

The retention factor is, as expected, not influenced by the pH or the ionic strength of the buffer, since all compounds are uncharged in the applied mobile phases. The pK_a values of 4-aminoacetophenone, o-nitrophenol, and 2,6-methylphenol are 2.29, 7.17, and 10.59 respectively [10,11]. Furthermore, the chance of exceeding the 95% confidence level is very small because all calculated F-numbers are much larger than the theoretical value of 2.56 [12].

Because of Joule heating, the temperature during an CEC experiment will be higher than the ambient temperature, and has therefore to be corrected for. A large number of chromatographic systems show linear relationships between the logarithm of the retention factor and the reciprocal of the column temperature, i.e., van't Hoff plots. The temperature effect can be described by:

\[ \ln k = \frac{\Delta S^0}{R} + \frac{\Delta H^0}{RT} \]  

where \( \Delta H^0 \) is the standard enthalpy, \( \Delta S^0 \) the standard entropy, \( T \) the absolute temperature, and \( R \) the gas constant. As an example, the results of four of the investigated mobile phases are given in Table 3. As can been seen from the results of Table 3, all investigated components show a linear relationship between \( \ln k \) and \( 1/T \).

4.2 Capillary Zone Electrophoretic Experiments

The electrophoretic mobility \( \mu_{eff} \) of the compounds in the different mobile phases was determined with capillary zone electrophoresis and was for almost all the components equal to zero in the investigated mobile phases. Only o-nitrophenol had a \( \mu_{eff} \) of \(-1.01 \times 10^{-4} \text{ cm V}^{-1} \text{ s}^{-1} \). o-Nitrophenol has a relatively low pK_a value of 7.17 and therefore shows some electrophoretic mobility at high pH values or high ionic strengths.

4.3 Capillary Electrochromatographic Experiments

The CEC experiments were carried out with the same column as in the microcolumn LC separations. The total length of the capillary column \( L_{tot} \) was 0.544 m, the length from inlet-to-detector \( L_{id} \) equaled 0.399 m and the packed part \( L_{col} \) had a length of 0.274 m. The specific conductivity \( G \), current \( I \), estimated temperature excess \( \Delta T_{excess} \), and the temperature within the core of the tube \( \Delta T_{core} \) for the previous mentioned example are given in Table 4. The applied voltage was 30 kV. \( \Delta T_{excess} \) and \( \Delta T_{core} \) were calculated from eqs. (12) and (13) respectively [1]. \( \Delta T_{core} \) represents the temperature excess within the core of a capillary column and arises from the heating of the mobile phase due to ohmic loss. \( \Delta T_{core} \) is given by:

\[ \Delta T_{core} = \frac{\Delta T_{excess} \cdot L_{id}}{L_{tot} - L_{id}} \]

where \( \Delta T_{excess} \) is the temperature excess within the core of the investigated mobile phases in CEC. The experiment numbers correspond with Table 1.

Table 3. Temperature dependence of the retention factor with correlation coefficients (n = 5). The experiment numbers correspond with Table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \Delta S^0/R )</th>
<th>( -\Delta H^0/R )</th>
<th>r (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. no. 2.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Aminoacetophenone</td>
<td>-3.3</td>
<td>570</td>
<td>1.000</td>
</tr>
<tr>
<td>o-Nitrophenol</td>
<td>-2.7</td>
<td>720</td>
<td>0.998</td>
</tr>
<tr>
<td>2,6-Dimethylphenol</td>
<td>-2.3</td>
<td>610</td>
<td>0.999</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>-1.8</td>
<td>750</td>
<td>0.998</td>
</tr>
<tr>
<td>Exp. no. 6.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Aminoacetophenone</td>
<td>-3.4</td>
<td>570</td>
<td>0.996</td>
</tr>
<tr>
<td>o-Nitrophenol</td>
<td>-2.4</td>
<td>630</td>
<td>0.999</td>
</tr>
<tr>
<td>2,6-Dimethylphenol</td>
<td>-2.2</td>
<td>590</td>
<td>0.999</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>-1.8</td>
<td>760</td>
<td>0.997</td>
</tr>
<tr>
<td>Exp. no. 8.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Aminoacetophenone</td>
<td>-3.3</td>
<td>530</td>
<td>0.999</td>
</tr>
<tr>
<td>o-Nitrophenol</td>
<td>-2.4</td>
<td>570</td>
<td>1.000</td>
</tr>
<tr>
<td>2,6-Dimethylphenol</td>
<td>-2.4</td>
<td>600</td>
<td>0.997</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>-1.8</td>
<td>710</td>
<td>0.999</td>
</tr>
<tr>
<td>Exp. no. 9.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Aminoacetophenone</td>
<td>-3.3</td>
<td>710</td>
<td>1.000</td>
</tr>
<tr>
<td>o-Nitrophenol</td>
<td>-2.3</td>
<td>850</td>
<td>0.996</td>
</tr>
<tr>
<td>2,6-Dimethylphenol</td>
<td>-2.0</td>
<td>760</td>
<td>0.998</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>-1.6</td>
<td>1000</td>
<td>0.998</td>
</tr>
</tbody>
</table>
\[ \Delta T_{\text{core}} = \frac{E \times l}{4\pi K} \]  

where \( K \) is the thermal conductivity of the mobile phase. With an aqueous eluent \( K = 0.6 \text{ W m}^{-1} \text{ K}^{-1} \).

The temperature excess between the capillary column and the surrounding air \( \Delta T_{\text{excess}} \) was approximated by:

\[ \Delta T_{\text{excess}} = \frac{4 E \times l}{\pi d_0^3} \]  

where \( d_0 \) is the outer diameter of the packed capillary column.

\( \Delta T_{\text{core}} \) is, as can be seen from the data in Table 4, negligible; \( \Delta T_{\text{excess}} \) is about 100 times as large as \( \Delta T_{\text{core}} \) and must be taken into account when retention factors are estimated in CEC. The experimentally obtained retention factors and the temperature corrected factors are given in Table 5. The retention factors were corrected for by using the data in Table 3. The corrected retention factors are somewhat higher than the uncorrected values.

### Table 5. Retention factors and temperature corrected retention factors (in parentheses) in CEC.

The numbers of the experiments correspond to Table 1.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>2</th>
<th>6</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Aminoacetophenone</td>
<td>0.18 (0.18)</td>
<td>0.24 (0.24)</td>
<td>0.20 (0.21)</td>
<td>0.44 (0.46)</td>
</tr>
<tr>
<td>o-Nitrophenol</td>
<td>0.58 (0.60)</td>
<td>– (-)</td>
<td>0.60 (0.63)</td>
<td>1.79 (1.89)</td>
</tr>
<tr>
<td>2,6-Dimethylphenol</td>
<td>0.60 (0.62)</td>
<td>0.76 (0.77)</td>
<td>0.60 (0.63)</td>
<td>1.72 (1.80)</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>1.62 (1.68)</td>
<td>2.07 (2.10)</td>
<td>1.60 (1.70)</td>
<td>5.70 (6.07)</td>
</tr>
</tbody>
</table>

### Table 6. Ratio of the retention factors \( k_{\text{CEC}}/k_{\text{LC}} \), uncorrected and temperature corrected (in parentheses) of neutral compounds.

The experiment numbers correspond with Table 1.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>2</th>
<th>6</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Aminoacetophenone</td>
<td>0.92 (0.92)</td>
<td>1.22 (1.22)</td>
<td>1.17 (1.23)</td>
<td>1.30 (1.35)</td>
</tr>
<tr>
<td>o-Nitrophenol</td>
<td>0.99 (1.03)</td>
<td>– (-)</td>
<td>1.30 (1.37)</td>
<td>1.34 (1.38)</td>
</tr>
<tr>
<td>2,6-Dimethylphenol</td>
<td>0.97 (1.00)</td>
<td>1.19 (1.20)</td>
<td>1.22 (1.29)</td>
<td>1.37 (1.42)</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.96 (0.99)</td>
<td>1.21 (1.22)</td>
<td>1.26 (1.33)</td>
<td>1.43 (1.52)</td>
</tr>
<tr>
<td>Mean</td>
<td>0.96 (0.99)</td>
<td>1.21 (1.22)</td>
<td>1.24 (1.31)</td>
<td>1.36 (1.42)</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>3.1 (4.8)</td>
<td>1.3 (1.3)</td>
<td>4.5 (4.6)</td>
<td>4.0 (4.8)</td>
</tr>
</tbody>
</table>

Since in this study no pressurized flow was applied, \( \Delta p = 0 \), and the electrophoretic mobility \( \mu_{\text{eff}} \) of the uncharged compounds also equals zero, eq. (9) reduces to:

\[ t_e = t_{\text{EOF}} (1 + k) \]  

which is a well known relationship in chromatography. Therefore, retention factors obtained in microcolumn LC and CEC can, in the case of uncharged compounds, directly be compared. The ratio of the uncorrected and temperature corrected retention factors \( k_{\text{CEC}}/k_{\text{LC}} \) are given in Table 6.

As can be seen from the results in Table 6, the retention factors in CEC are about 1 – 1.4 times as high as in microcolumn LC. The temperature corrected retention factors are 5% higher than the uncorrected values. Several factors may increase the retention factor in CEC. Namely, alteration of the nature of the stationary phase due to the application of an electric field, changes in the distribution constants of the compounds of interest over the mobile and stationary phase and electric field inhomogeneities in the packed part of the column. To what extent the different factors contribute to the overall effect is not clear. However, the average overall effect can be determined and equals 1.2 for the investigated mobile phases, i.e., retention in CEC for neutral compounds is = 20% slower than in microcolumn LC using the same stationary phase and mobile phases. Similar observations have been made by Eimer et al. [13]. The same kind of effects are observed for charged compounds but differ in magnitude. This will, however, be discussed elsewhere.

### 5 Conclusions

Electrochromatography was successfully applied in packed fused silica capillary columns of 320-µm i.d to theoretically calculate the retention of neutral compounds in CEC. The derived equation to calculate retention in CEC does not foresee any alterations of the nature of the stationary phase or distribution constants by the applied electric field, or field inhomogeneities, and therefore fails in the exact calculation of the retention times. However, based on the obtained results the overall contribution by the electric
field could be estimated for uncharged compounds. Retention in CEC is ≈ 20% slower than in LC. No distinction could be made between the individual contributions to the effect of the electrical field on the retention in CEC.

References


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