

Wei Wei^{a)},
Luo Guoan^{a)},
Yan Chao^{b)}

Calculation of retention factors for charged solutes in capillary electrochromatography

^{a)} Department of Chemistry,
Tsinghua University, Beijing,
100084, PR China

^{b)} Unimicro Technologies, Inc.,
First St., Pleasanton, CA
94566, USA

An equation describing the capillary electrochromatographic (CEC) retention factor is derived using the fundamental chromatographic concept of retention factor. This retention equation couples both the chromatographic retention factor and the electrophoretic velocity factor, which can explain well the charged solute retention in CEC. The known CEC retention expressions are compared. A simple experimental method for obtaining the CEC retention factors for charged solutes is demonstrated.

Key Words: Capillary electrochromatography (CEC); Retention factor; Charged solute

Ms received: August 14, 1998; accepted: June 1, 1999

1 Introduction

Capillary electrochromatography (CEC) is a promising separation technique due to its relatively high separation efficiency compared with HPLC and better selectivity compared with capillary zone electrophoresis (CZE). In CEC, an electric field, rather than pressure, drives the mobile phase through a packed capillary column. The electroosmotic flow (EOF) of the mobile phase in CEC results in a laminar (plug-like) flow profile and thus in higher column efficiency. These advantages of CEC have been demonstrated experimentally [1–9] and proved theoretically [4, 8]. However, the retention mechanism of CEC has not been thoroughly investigated [6, 9, 10, 13]; hence the conclusions drawn regarding retention in CEC are inconsistent. Tsuda [6] observed different solute retention behaviors between pressure-driven CEC and liquid chromatography. He attributed the results to the stationary phase, which became polar when charged solutes adsorbed onto the surface [7]. Vissers et al. [9] reported that retentions of neutral solutes in CEC and HPLC were different because the electric field significantly influenced retention [11]. They stated that “retention in CEC for neutral compounds is $\approx 20\%$ slower than in micro-column LC using the same stationary and mobile phases”. Eimer et al. [12] reported similar observations for charged compounds but of a different magnitude. However, Zhang et al. [13] found no significant differences between CEC, pressurized CEC, and micro-column liquid chromatography after a systematic investigation of the retention behaviors of 27 neutral solutes under the same experimental conditions. They at-

tributed the incongruity between their results and the previous reports to the properties of the test solutes. Whether or not there is a fundamental difference between solute retention in CEC and in micro-LC remains unclear. Physically, chromatographic retention depends on solute distribution between stationary phase and mobile phase. Regardless of the effect of electric field on stationary phase, for the neutral solute, the CEC retention should be equal to that found in liquid chromatography. However, this assumption fails in practice. For a charged solute, retention in CEC depends on both chromatographic retention and electrophoresis. Electrophoresis drives the charged solute through the capillary at a rate which depends on its mass-to-charge ratio while chromatographic interaction based on its chemical constitution retards its passage. Thus, retention in CEC should combine both chromatography and electrophoresis. The charged solute retention in CEC should be more complicated compared with that for traditional liquid chromatography or electrophoresis.

Several authors [4, 7, 10] have proposed different CEC retention models. Tsuda [7] introduced the following relationship [Eq. (1)].

$$t_R = \frac{L}{u_{pre}/(1 + k'_{LC}) + u_{ep}} \quad (1)$$

In Eq. (1), u_{pre} and u_{ep} are the corresponding migration velocities under pressure and in an electric field. The variables, t_R and L are solute retention time and capillary length, while k'_{LC} is the chromatographic capacity factor. Here CEC retention is separated into one chromatographic factor and one electrophoretic factor. According to Eq. (1) when u_{pre} and u_{ep} have the same mathematical sign, the CEC retention will decrease; otherwise, it will increase. However, the CEC retention factor (k') is not included in Eq. (1) above and therefore cannot be evaluated.

Correspondence: Dr. Luo Guoan, Department of Chemistry, Tsinghua University, Beijing, 100084, PR China.

E-mail: luoga@chem.tsinghua.edu.cn

Fax: +86 10 6784764

Knox and Grant proposed that the overall migration velocity (u_x) of any charged solutes in CEC could be expressed by Eq. (2) [4], where, k'_{LC} is the chromatographic retention factor of a solute and u_{eo} is the electroosmotic flow velocity.

$$u_x = \frac{u_{eo} + u_{ep}}{1 + k'_{LC}} \quad (2)$$

Similar to the model proposed by Tsuda [Eq. (1)], the Knox formalism [Eq. (2)] also describes the CEC retention in terms of migration velocity. The latter worker attributed the CEC retention again to two independent factors, one chromatographic and the other electrophoretic. In neither case does the CEC retention factor (k') enter into the retention expressions above. This omission hinders the physical understanding of the CEC retention factor.

Recently, Rathore and Horvath [10] introduced the concept of virtual migration distances into CEC. Based on this theory, the migration process in CEC can be divided into separative and non-separative components. The separative component describes selective interactions with the stationary phase and differences in the electrophoretic migration velocity while the non-separative component describes migration by convection that does not contribute directly to separation. Rathore and Horvath defined CEC retention factor, k' , as [10]:

$$k' = k'_{LC} + k'_e + k'_e \quad (3)$$

$$k'_e = u_{ep}/u_{eo} \quad (4)$$

where k'_e is defined as the electrophoretic velocity factor.

All features of CZE and liquid chromatography (LC) are coupled in Eq. (3). In principle, this model should apply to any chromatographic system and evaluate the CEC retention well. However, the CEC retention factor of a charged solute cannot be calculated directly using the available CEC retention models (see above) because these retention expressions include the electrophoretic velocity item, u_{ep} , which cannot be obtained from CEC experimental data. Even if one could obtain the mobility value for a solute in CZE, it is not directly transferable to CEC because the electrophoretic environments differ [4, 8].

The purpose of this work is to obtain the CEC retention factor from experimental parameters. For the purpose of this manuscript, it is assumed that the interactions of the charged solute due to the applied electric field are incorporated and do not decouple as such from chromatography. The CEC retention factor is re-evaluated using the traditional LC definition. A simple experimental method for obtaining the CEC retention factor is presented below.

2 Theory

The CEC retention factor, k' , can be expressed by its definition

$$k' = \frac{t_R - t_0}{t_0} = \frac{u_0 - u_x}{u_x} \quad (5)$$

where t_0 and u_0 are the dead time and the migration velocity of an unretained solute, respectively.

By substituting Eq. (2) into Eq. (5), we obtain the following expression of k' (Eq. 6),

$$k' = \frac{k'_{LC} - k'_e}{1 + k'_e} \quad (6)$$

which can be re-arranged to yield Eq. (7).

$$k'_{LC} = k' + k'k'_e + k'_e \quad (7)$$

Combining Eq. (7) and Eq. (2), we obtain the following equation,

$$u_x = \frac{(1 + k'_e) \cdot u_{eo}}{1 + k' + k'k'_e + k'_e} = \frac{1}{1 + k'} u_{eo} \quad (8)$$

For neutral solutes, k' equals k_{LC} , as required. In this case, the solution is driven through the capillary by electroosmotic flow. We also assume here that the electric field has no influence on the retention of neutral solutes. For charged solutes, a plot of u_{eo} vs. u_x [Eq. (8)], follows a linear relationship. Then, k' is calculated from the slope of the line obtained by changing the applied voltage. Since the electrophoretic effect is taken into consideration in this model, Eq. (8) provides a reliable method for directly calculating the CEC retention of a charged solute from CEC experimental data.

3 Experimental

3.1 Chromatographic system

CEC experiments were performed on a P/ACE 2200 capillary electrophoresis system (Beckman, Fullerton, CA, USA). The chromatographic columns used in this work were obtained from Unimicro Technologies, Inc. (Pleasanton, CA, USA). The capillary columns were 20 cm in effective length with an inner diameter of 100 μm (27 cm total length), with 20 cm from the inlet frit to the detection window. The chromatographic column was packed using the method of electrokinetic packing [14]. The packing material used was a strong cation exchanger resin with 300 \AA pore size on 3–6 μm diameter silica to which a

polymeric layer containing sulfuric acid functionalities was bonded (Micra Scientific, Inc. Northbrook, IL). Prior to each run, the capillary was pre-conditioned with 10 mM HCl by pressurizing the column inlet to approximately 500 p.s.i. with a manual syringe pump (Umimicro Technologies, Inc. Pleasanton, CA) or pressurizing with a handheld vise. After the column was installed in the P/ACE system, it was further conditioned with the mobile phase using a low applied voltage of 3 kV. The thermostat was set to 25°C. Electrokinetic injections were performed. An on-line UV detector was operated at 214 nm with a detection range of 0.05 a.u. The mobile phase was prepared by mixing acetonitrile with phosphoric acid buffer. The mobile phase was filtered through a 0.25 μm filter and degassed in an ultrasonic bath for three minutes prior to use.

3.2 Reagents

HPLC-grade acetonitrile was used as a mobile phase modifier. Phosphoric acid and sodium dihydrogen phosphate were analytical grade reagents. The water was deionized. Berberine hydrochloride (I), palmatine hydrochloride (II), jatrorrhizine hydrochloride (III), and codeine phosphate (IV) were reference compounds identified by thin-layer chromatography and obtained from the Chinese National Institute for the Control of Pharmaceutical and Biological Products. Toluene, phenol, and *o*-toluidine dihydrochloride (V) were analytical grade. Phenol and toluene were used as EOF markers. The structures of these alkaloids are shown in **Figure 1**. The concentration of the charged solutes was ca. 5–10 ppm while the concentration of the neutral solutes, phenol, and toluene were ca. 100 ppm.

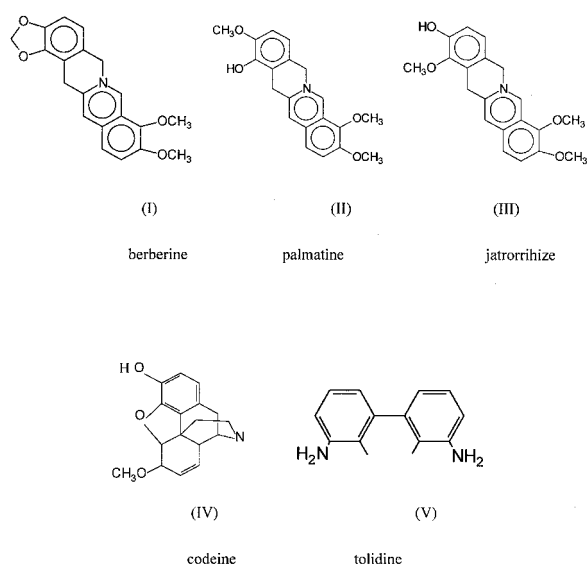


Figure 1. The structures of model compounds.

4 Results and discussion

The CEC retention factor [Eq. (6)], k' , may have a negative or positive value depending on the experimental conditions as addressed by Tsuda [7]. We performed two sets of CEC experiments using capillary columns packed with strong cation exchange resins. In one experiment, the charged solutes elute before the neutral mark, and in the others, the elution order of solutes is similar to that found in traditional LC, i.e., the elution order of the peak is determined by chromatographic retention. Several basic compounds were selected because they are ionic under the experimental conditions used and all possess UV absorbance.

The patterns observed in two different electrochromatograms (**Figure 2** and **Figure 3**) under two different se-

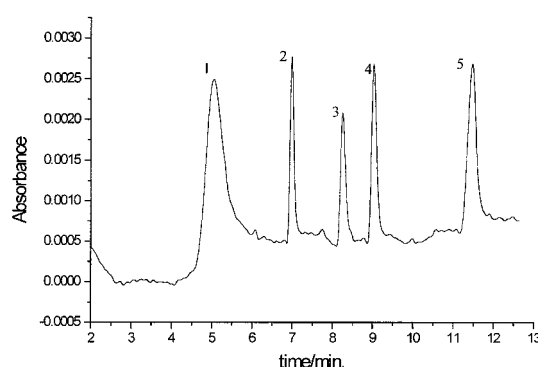


Figure 2. Electrochromatogram for test mixture obtained in CEC. Experimental conditions: column: 20 cm packed, 100 μm ID \times 27 cm total; packing: 3–6 μm SCX; mobile phase: 70% $\text{CH}_3\text{CN}/30\%$ 5 mM H_3PO_4 (pH 2.67); applied voltage: 12 kV, injections: 5 kV for 5 s; UV detection: $\lambda = 214$ nm, 0.05 a.u.; rise time: 0.3 s; temperature: 25°C; solutes: 1) toluene, 2) berberine hydrochloride, 3) palmatine hydrochloride, 4) codeine phosphate, 5) *o*-toluidine dihydrochloride.

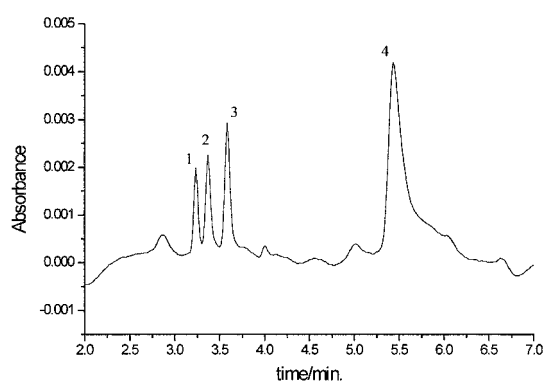


Figure 3. Electrochromatogram for test mixture obtained in CEC. Experimental conditions: mobile phase: 60% $\text{CH}_3\text{CN}/40\%$ 10 mM $\text{H}_3\text{PO}_4\text{-NaH}_2\text{PO}_4$ (pH 3.05); applied voltage: 12 kV, injections: 3 kV for 3 s; other conditions as in Figure 1; solutes: 1) berberine hydrochloride, 2) palmatine hydrochloride, 3) jatrorrhizine hydrochloride, 4) phenol.

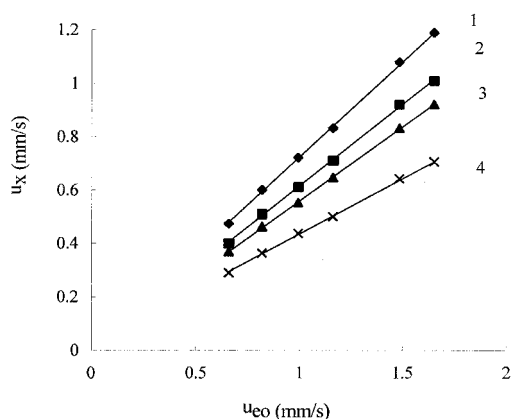


Figure 4. Relationship between u_{eo} and u_x . Applied voltage: 6, 8, 10, 12, 15, 18 kV, other conditions as stated in Figure 2. 1) berberine hydrochloride, 2) palmatine hydrochloride, 3) codeine phosphate, 4) *o*-toluidine dihydrochloride.

paration conditions are described well by Eq. (6). In the first experiment, (Figure 2), a mobile phase with a low ionic strength (5 mM H_3PO_4) was chosen. Owing to the weak elution capacity of the mobile phase, the interactions between the solutes and the stationary phase dominate retention. In other words, the chromatographic retention (k'_{LC}) is larger than the electrophoretic retention (k'_e), and a position value for CEC retention factor ($k' > 0$) is obtained. Therefore neutral solutes elute first with the EOF and cationic compounds elute later. Berberine is least retained and elutes first, while *o*-toluidine, with two positive charges, is strongly retained and elutes last.

In the second experiment (Figure 3), a mobile phase with a high ionic strength (10 mM Na^+) was used. Here, the chromatographic retention was weak (a small k'_{LC}). The CEC retention was dominated by the electrophoretic velocity factor (k'_e). The sequential elution order is: berberine; palmatine; jatrorrhizine; and phenol. The migration velocity profile of charged solutes follows the CEC retention in the case where the mobile phase is driven by both EOF and electrophoretic migration. Here, the migration velocity of neutral solutes is equal to EOF. For charged compounds [Eq. (6)], the electrophoretic velocity factors (k'_e) are larger than the chromatographic retention factors (k'_{LC}), and a negative CEC retention factor ($k' < 0$) is observed. A negative k' means that the charged solutes should elute before the unretained phenol.

It is convenient to obtain different values of u_{eo} and u_x by changing the applied voltage. The relationship between u_{eo} and u_x obtained in two sets of experiments (Figure 4 and Figure 5) are linear [Eq. (8)] as expected. The CEC retention factor of the solute is then calculated using the slope of the line. The applied voltages were 6, 8, 10, 12, 15, 18 kV and 12, 15, 18, 21, 27, 30 kV for Figure 4 and Figure 5, respectively. Table 1 lists the regression equa-

Table 1. Regression equations describing the relationship between u_x and u_{eo} .

Solutes	Regression equations	k'	r^2
Berberine ^{a)}	$u_x = 0.7232u_{eo}$	0.3828	0.9997
Palmatine ^{a)}	$u_x = 0.6155u_{eo}$	0.6247	0.9999
Codeine ^{a)}	$u_x = 0.5599u_{eo}$	0.7860	0.9999
<i>o</i> -Toluidine ^{a)}	$u_x = 0.4316u_{eo}$	1.3170	0.9983
Berberine ^{b)}	$u_x = 1.6382u_{eo}$	-0.3896	0.9918
Palmatine ^{b)}	$u_x = 1.5729u_{eo}$	-0.3642	0.9924
Jatrorrhizine ^{b)}	$u_x = 1.4818u_{eo}$	-0.3252	0.9933

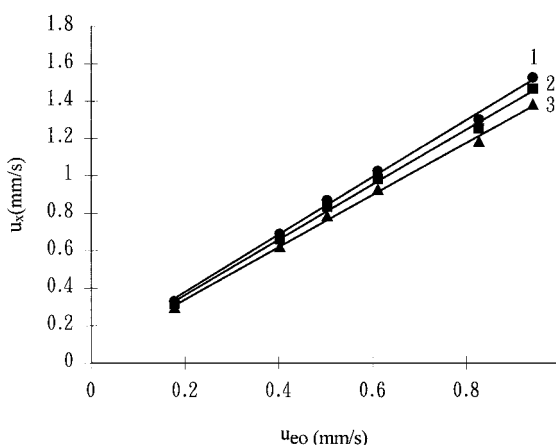


Figure 5. Relationship between u_{eo} and u_x . Applied voltage: 12, 15, 18, 21, 27, 30 kV, other conditions as in Figure 3. 1) berberine hydrochloride, 2) palmatine hydrochloride, 3) jatrorrhizine hydrochloride.

tions, regression coefficients, and the CEC retention factors (k') for the test solutes. The positive and negative values (Table 1) for CEC retention factors obtained were predicted by this theory [Eq. (6)].

Although both the Rathore model and the model presented here consider the coupling of chromatographic and electrophoretic components [compare Eq. (2) with Eq. (6)], they differ in form. From the retention equation presented in this work, k' may be larger or smaller than the corresponding liquid chromatography retention factor, k'_{LC} , depending on the magnitudes of k'_{LC} and k'_e and the sign of k'_e . If k'_e is larger than k'_{LC} with a positive value, the overall k' may be negative. However, according to the Rathore equation, when k'_e is a positive value (where the electrophoretic and EOF velocities are in the same direction), the CEC retention factor, k' , is always larger than the LC chromatographic factor, k'_{LC} , and is also a positive value, which contradicts Eimer's findings [12]. Our results are also at odds with the Rathore and Horváth model. Let us re-evaluate Rathore and Horváth's equation by combining Eqs. (2), (3), and (4), to yield the following expression for CEC retention factor:

$$k' = \frac{t_R(1 + k'_e)^2 - t_0}{t_0} \quad (9)$$

Here [Eq. (9)], the retention factor is always a positive value as a retention factor in traditional LC should be. The alternative definition of CEC retention used by Rathore and Horváth results in a different expression and physical meaning for the CEC retention factor in both cases. The retention model presented here is based on the fundamental chromatographic theory, i. e., Eq. (5), the basic definition of retention factor in LC, while Rathore and Horváth's model was derived from Eq. (9).

5 Conclusions

The CEC retention factor of charged solutes is re-evaluated based on the definition of retention factor. The CEC retention model here combines both chromatography and electrophoresis. A convenient approach for calculating the CEC retention factor of charged solutes from CEC experimental data is also presented. This model explains the case where solutes elute before the t_0 mark in a typical CEC experiment. This work also provides a basis for an improved understanding of the physical meanings of the different retention factors determined by different techniques.

Acknowledgments

Financial support obtained from the National Science Foundation of China. Authors wish to gratefully acknowl-

edge Unimicro Technologies, Inc. for providing the capillary packed column for this work and Dr. Michael Shortreed's helpful discussion in the preparation of this manuscript.

References

- [1] V. Pretorius, B.J. Hopkins, J.D. Schieke, *J. Chromatogr.* **1974**, *99*, 23–30.
- [2] C. Yan, D. Schaufelberger, F. Emi, *J. Chromatogr.* **1994**, *670*, 15–23.
- [3] H. Resbscher, U. Pyell, *Chromatographia* **1994**, *38*, 737–743.
- [4] J.H. Knox, I.H. Grant, *Chromatographia* **1991**, *32*, 317–328.
- [5] N.W. Smith, M.B. Evan, *Chromatographia* **1994**, *38*, 649–657.
- [6] T. Tsuda, *Anal. Chem.* **1987**, *59*, 521–523.
- [7] T. Tsuda, *Anal. Chem.* **1988**, *60*, 1677–1680.
- [8] J. H. Knox, I.H. Grant, *Chromatographia* **1988**, *26*, 329–337.
- [9] J.P.C. Vissers, H.A. Claessens, P. Coufal, *J. High Resol. Chromatogr.* **1995**, *18*, 540–544.
- [10] A.S. Rathore, Cs. Horváth, *J. Chromatogr. A* **1996**, *743*, 231–246.
- [11] W. Wei, Y. Wang, G.A. Luo, C. Yan, *J. Liq. Chromatogr.* **1998**, *21*, 1433–1443.
- [12] T. Eimer, T. Adam, K.K. Unger, poster 233, 19th International Symposium on Column Liquid Chromatography and Related Techniques, Innsbruck, 1995.
- [13] Y. Zhang, W. Shi, L. Zhang, H. Zhou, *J. Chromatogr. A* **1998**, *802*, 59–71.
- [14] C. Yan, *Electrokinetic Packing of Capillary Columns*, US Patent 5453163, 1995.