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Deviation from Ohm's law in electric field assisted capillary liquid chromatography

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Abstract

Earlier studies of electric field assisted LC (EF-LC) have shown that the effect on charged analytes of the application of an electric field over a capillary LC column is relatively small. Charged analytes can only be affected by the electric field while present in the mobile phase, which makes the effective time for influence of the electric field t_0 independent of retention time. Because the charged analytes only can be affected for a short time the electric field strength ought to be high in order to increase the impact of the electric field on the separation. We have, however, found that only a relatively low electric field strength can be used in EF-LC when pressure is used as main driving force. The useful field strength was limited by a dramatic increase in the current. This increase in current was found to origin from an increased concentration of buffer ions that have an electrophoretic mobility towards the pumped flow.

Keywords: Ohm's law; Conductivity; Electric field assisted capillary liquid chromatography; Pressurized electrochromatography

1. Introduction

Packed capillary columns are today utilized in two major techniques, capillary liquid chromatography (LC) and capillary electrochromatography (CEC). The main difference between the two techniques is that in capillary LC, pressure and in CEC, an electric field is used to transport the mobile phase through the column. In addition to these techniques there are methods combining the two approaches, i.e., both pressure and electric field is applied to the system. The combination of pressure and electric field was introduced by Tsuda and Muramatsu [1]. Two different types of instrumentation can be used. In CE instruments, which frequently are used for CEC, a relatively low pressure can be applied at the column inlet in order to create a pressurized flow [2–6]. This technique is often called pressurized electrochromatography (PEC) or pressurized capillary electrochromatography (pCEC). The second approach is to use modified capillary LC instruments equipped with a high voltage supply allowing higher pressure to be used for isocratic [7–18] as well as for gradient elution [19–26]. In addition to the names above,

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pseudo electrochromatography (PEC), electro high-performance liquid chromatography (electro-HPLC) and electric field assisted capillary liquid chromatography (electric field LC) have been used for this technique. In this work we have used the latter approach and consider electric field assisted capillary liquid chromatography (EF-LC) to be the appropriate name. This is in order to emphasize that all ordinary benefits of HPLC still are present and only the option of an electrical field has been added.

The purpose of combining pressure and electric field is often to eliminate bubble formation, achieve a stable flow and add the option of gradient elution to existing CEC methods. In this work we have, however, focused on the possibilities of using an electric field as additional selectivity factor for charged analytes in a capillary LC method. The electric field offers selectivity between charged and uncharged analytes, but selectivity can also be provided for charged analytes having different electrophoretic mobilities. Apffel et al. [25] and Andersson and Blomberg [19] have isolated the effect of electric field on charged analytes by using low pH in the mobile phase to suppress the electroosmotic flow. It was found that the effect of the electric field on the analytes was relatively small. We have in this work performed studies to identify the limits of the EF-LC method.

2. Experimental

2.1. Column and coated capillary preparation

Both the packed columns and the coated capillaries were made in the laboratory. The columns were packed with supercritical CO_2 according to Malik et al. [28]. An ultrasonic bath and a Series 600 SFC pump from Lee Scientific were used for this purpose. The coating of the capillaries was performed according to Wan et al. [29].

2.2. Instrumentation

The micro (μ)-HPLC instrument was from Micro-Tech Scientific (Vista, CA, USA). Injection was performed by an electronically actuated valve and the detection was on a 50 μm I.D. piece of fused-silica by a Linear UV–Vis 200 detector (Linear Instruments, Freemont, CA, USA). The high-voltage supply was from Matsusada Precision (Kusatsu, Japan). A Pt wire inside a piece of polyether ether ketone (PEEK) tubing that was mounted on a 0.15 mm I.D. Valco PEEK coupling tee was used to apply high voltage at the end of the column, Fig. 1. A 1/16 in. screening filter was applied at both ends of the column to fixate the packing material. When open capillaries were used, the high voltage was applied at a 0.15 mm I.D. stainless Valco steel union at the end of the column [8] (1 in.=2.54 cm). The injector valve and the waste outlet were grounded.

3. Results and discussion

3.1. Critical parameters

As shown earlier the effect of applying an electric field over a capillary LC column, on charged analytes, is small [19] especially when gradient elution

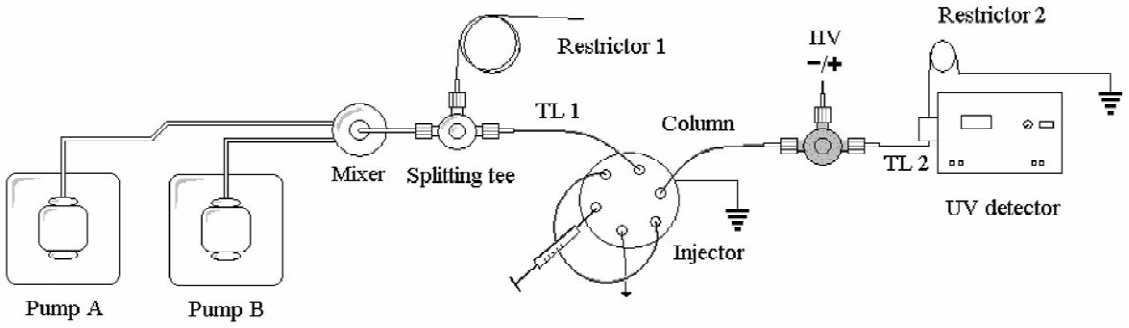

Fig. 1. Schematic setup of the EF-LC system.

is used [25]. The change in retention time of an ionic analyte between a chromatographic run with and without electric field t_{change} is proportional to the electrophoretic mobility μ, the electric field strength E and the time t that the ion is affected by the electric field. Apffel et al. investigated the condition that ions can be affected by the electric field only while they are in the mobile phase [25]. Thereby the time the ionic analytes are affected by the electric field becomes equal to the dead time t_0 regardless of retention time.

The electrophoretic mobility μ depends on the charge of the ion and the viscosity of the mobile phase but little can be done to increase the t_{change} by increasing μ. If the goal is, as in our case, to have a capillary LC system working at its best and use the electric field as an extra separation parameter, then it is not desirable to increase t_0 in order to increase t_{change}. The only parameter that significantly can increase t_{change} is therefore the electric field strength E. The limitation of the magnitude of the electric field that can be applied over a capillary LC column is mainly set by the current through the column and the Joule heating that the current produces.

3.2. Deviation from Ohm's law

Ohm's first law, $U=RI$, describes the linear relationship between current and voltage. In normal cases the capillary column in an electro-assisted chromatographic system is assumed to act as a resistor according to Ohm's law. Deviations from Ohm's law can however be observed if the Joule heat, produced by the electric current, can not be effectively dissipated [30]. Apart from temperature dependence Kitagawa et al. reported a deviation from Ohm's law when using ion-exchange columns [12].

We have in this study observed a significant deviation from Ohm's law when applying high voltage over a capillary column with a pump driven flow. The characteristics of this deviation can be seen when studying an Ohm's law plot from a 150 μm I.D. capillary with 75 m*M* acetate buffer at pH 4 as mobile phase, Fig. 2. Generally linearity can be observed at low voltage but at a higher voltage a dramatic increase in current is observed. Further we found that the magnitude of the current at a given voltage is depending on the direction of the current.

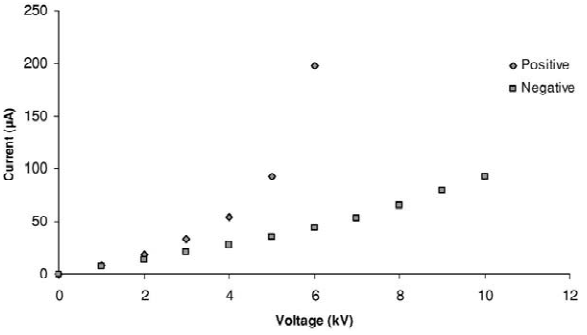

Fig. 2. The difference in current at given voltage depending on the direction of the current through a packed capillary column. Column: 15 cm×150 μm I.D. capillary packed with 3.5 μm YMC Basic. Mobile phase: 75 m*M* acetate, pH 4 with 1% ACN.

In this case, higher current is observed when the positive pole is positioned at the end of the column. The dramatic increase in current creates a voltage window, i.e., an electric field window useable for EF-LC because the high current results in too high Joule heating.

We have found that the reason for the deviation from Ohm's law is that the buffer ions, due to their electrophoretic mobility, are moving towards the pumped flow, causing an increased ionic strength in the column. When these ions enter the electric field their velocity will decrease and as more ions enter with the same velocity the concentration of ions will, at equilibrium, be higher than if no voltage was applied. A similar effect was used by Tsuda and Muramatsu [1]. By applying an electric field during injection a large sample volume could be injected because the analytes stopped moving in the electric field and they were thereby up-concentrated in the beginning of the column.

If an electric field is applied over a capillary, with polarity as in Fig. 3, the negative ions will accelerate and the positive ions will decelerate as they enter the column. The concentration of the ions will thereby depend on their electrophoretic velocity. For a solution with one ion pair, polarity as Fig. 3 and assuming constant flow velocity in the cross section the ionic strength C_{ion} is:

$$C_{ion} = C^{+}\left(\frac{v}{v - v_e^{+}}\right) + C^{-}\left(\frac{v}{v + v_e^{-}}\right) \quad (1)$$

where C^{+} and C^{-} are the concentration of each ion

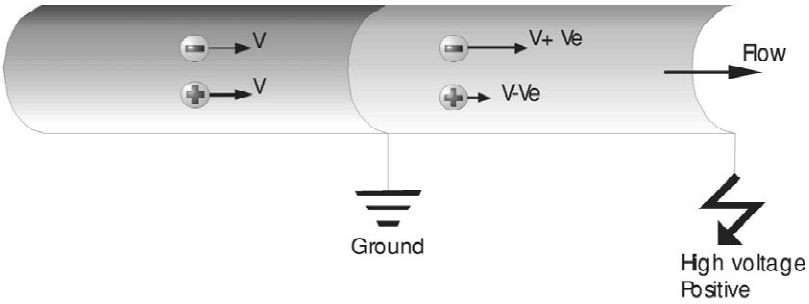

Fig. 3. The change in ion velocity in a capillary as ions with opposite charge enters an electric field. v is the flow velocity and v_e is the electrophoretic velocity of the ions.

in the original solution and v and v_e are the flow velocity and the electrophoretic velocity, respectively. A derivation of Eq. (1) is presented in Appendix A. The ionic strength can also be expressed as a function of the electric field strength, E, or voltage, U:

$$C_{\text{ion}} = C^+\left(\frac{v}{v-\mu^+E}\right) + C^-\left(\frac{v}{v+\mu^-E}\right) = C^+\left(\frac{v}{v-\mu^+\frac{U}{L}}\right) + C^-\left(\frac{v}{v+\mu^-\frac{U}{L}}\right) \quad (2)$$

where μ^+ and μ^- are the electrophoretic mobilities of each ion and L is the length of the column.

Fig. 4 illustrates the theoretical change in ion concentrations as the electrophoretic velocities increase. The decrease in concentration of the accelerated ions is quite moderate compared to the large increase in concentration of the decelerated ions. As a consequence the ionic strength will increase with increasing electrophoretic velocity.

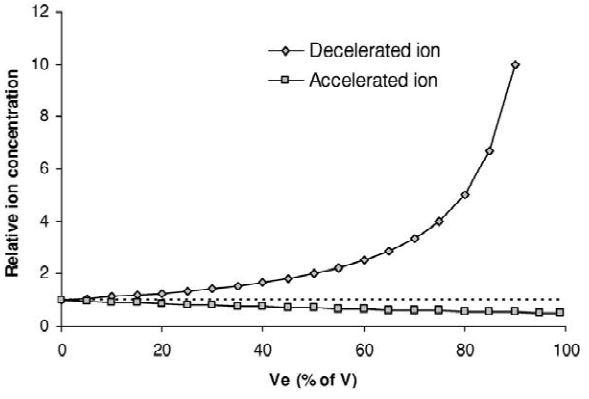

Fig. 4. From theory calculated relative concentration of the decelerated and accelerated ions as a function of the electrophoretic velocity v_e according to Eq. (1).

The conductivity, κ, in a single ion pair solution is depending on concentration according to [31]:

$$\kappa = z^+F\mu^+C^+ + |z^-|F\mu^-C^- \quad (3)$$

where z is the charge of the ion and F is Faradays constant. Combining Eqs. (2) and (3) gives an expression for κ which compensates for the change in ion concentration due to the electric field:

$$\kappa = z^+F\mu^+C^+\left(\frac{v}{v-\mu^+E}\right) + |z^-|F\mu^-C^-\left(\frac{v}{v+\mu^-E}\right) \quad (4)$$

The difference in magnitude of the current due to different direction of the current shown in Fig. 2 can be explained by Eq. (4). As was shown in Fig. 4 it is the decelerated ion that has the largest impact on the overall ionic strength. When reversing the direction of the current, the oppositely charged ions will be decelerated. If the two ions have different electrophoretic mobilities, as in the case shown in Fig. 2, the conductivity at a given field strength will be different. Thus if both ions would have the same electrophoretic mobility, no difference in magnitude due to different direction of the current should be observed. To test this, a KNO_3 solution was pumped through a coated capillary. A coated capillary was used to assure that the flow velocity was constant

regardless of the applied electric field. Potassium and nitrate ions have approximately the same electrophoretic mobility, $76.2 \cdot 10^{-5}$ and $74.0 \cdot 10^{-5}$ $cm^2/V\,s$, respectively [32], and therefore the magnitude should be independent of the direction of the current. This was also found, Fig. 5.

According to Eq. (1) the ionic strength should be dependent of the velocity of the flow through the column, v. If the electrophoretic velocity, v_e, is varied in capillaries at different flow velocity Eq. (1) predicts that the dramatic increase in number of ions that was seen in Fig. 4 occurs with lower v_e, i.e., electric field, when the flow velocity is lower, Fig. 6a.

The currents dependency of the flow velocity through a 50 μm I.D. coated capillary was investigated. A coated capillary was used in order to assure that the flow was independent of the electric field. Measurements of current with increasing negative voltage at the end of the capillary were performed at different flow velocities. To compare the result with the theory, the conductivity was calculated from:

$$\kappa = \frac{IL}{UA} \tag{5}$$

where A is the area of the capillary and I is the current running through it. The conductivity was plotted as a function of the theoretical electrophoretic velocity of NO_3^-, which was calculated from reference data [32] and the voltage, Fig. 6b. Eq. (4) predicts that the conductivity is almost entirely depending on the electrophoretic velocity of the decelerated ion. This is the reason why only the electrophoretic velocity of the NO_3^- ions and not the K^+ ions is considered.

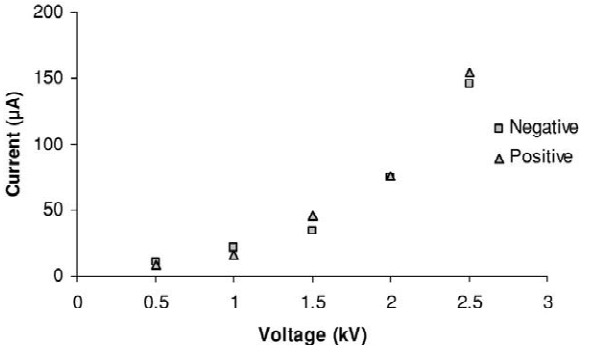

Fig. 5. Current as a function of voltage through a 10 cm×50 μm I.D. coated capillary when pumping 20 mM KNO_3. The inlet of the capillary was grounded and positive or negative voltage was applied at the outlet.

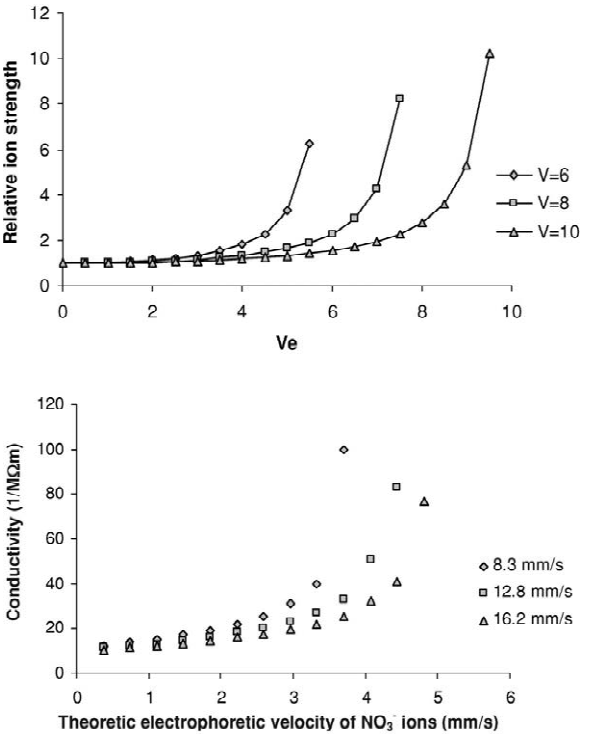

Fig. 6. (a) From theory calculated relative ionic strength in a capillary as a function of increasing electrophoretic velocities, v_e, at different flow velocities v according to Eq. (1). (b) Conductivity as function of theoretic mobility of NO_3^- at different flow-rates. Capillary and KNO_3 solution as in Fig. 5.

According to Eq. (3) the conductivity can be assumed to be directly proportional to the ion strength if the ions have similar mobilities. There is a clear correlation between Fig. 6a, which was predicted from Eq. (1), and Fig. 6b which was based on experimental data and Eq. (5). In the theoretic model, however, the number of ions dramatically increases when v_e approaches v. In the experimental data this was not the case, however. A dramatic increase in conductivity occurs in the experimental data at an electrophoretic velocity that is lower than the flow velocity. The reason for this is that the flow velocity not is constant in the cross section of the capillary as assumed. The flow profile of the pumped

flow is parabolic while the electrophoretic velocity leads to a plug formed flow.

As a consequence of the result presented in Fig. 6b, the flow velocity can be used to increase the electric field window that can be used for EF-LC. Flow velocities as high as in Fig. 6b are however seldom used in LC. Apart from increasing the flow velocity, switching to buffers with lower electrophoretic mobility is a method that can increase the electric field window. By switching buffer from sodium acetate buffer to Tris–acetate at pH 4 a small increase in electric field window was achieved because Tris has lower electrophoretic mobility than sodium, Fig. 7.

We have in this work used conditions to suppress the electroosmotic flow. This was, as mentioned before, done to isolate the parameters affecting the current. The electroosmotic flow can in the same way as changing the pumped flow, as in Fig. 6b, both increase and decrease the electric field window depending on the direction. Note that in the case of conventional non-pressurized CEC the flow will obviously increase as the voltage and the electrophoretic velocity of the negative buffer ions increase. In this case there will be no deviation from Ohm's law. Another important feature, of a capillary LC instrument equipped with a high voltage supply that has great importance for the deviation from Ohm's law, is that apart from a few exceptions [5,24] the voltage is applied directly into the flow line without any kind of reservoir at the ends of the column. In

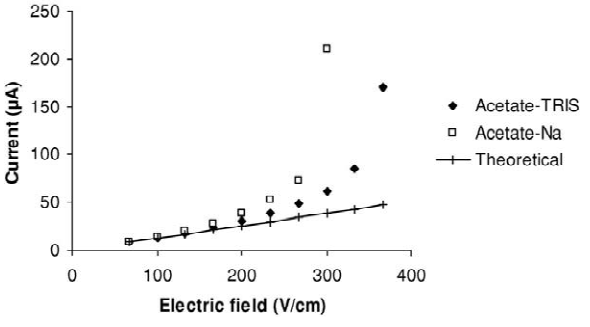

Fig. 7. Current as a function of the electric field using 75 m*M* acetate buffer at pH 4 with Na or Tris as cation. Positive poles positioned at the end of the column. Column as in Fig. 2. The theoretical line shows the current as it would have been if the conductivity had been constant.

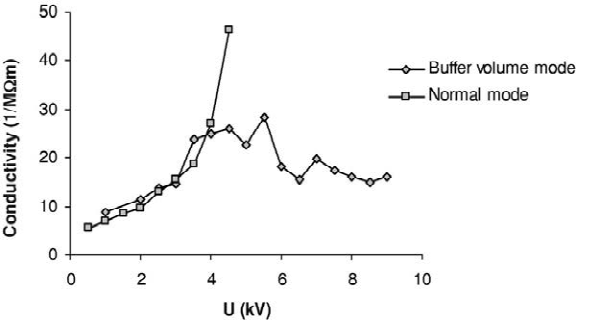

Fig. 8. Conductivity as a function of voltage in a 10 cm×50 μm I.D. coated capillary when pumping 10 m*M* KNO_3. In the buffer volume mode a grounded, pressurized reservoir is applied at the inlet of the capillary. In the normal mode the voltage is applied inline.

CE is it not uncommon that the mobility of the ions that move in the opposite direction of the flow due to their electrophoretic mobility is higher than the flow. This is the case when coated capillaries are used in capillary electrophoresis (CE). This does not result in an increased current in CE systems with reasonable large buffer reservoirs at each end of the capillary. However, in a system with the voltage applied directly in the flow line there is no volume to buffer the buffer ions that due to their electrophoretic mobilities are moving towards the pumped flow. These ions will instead, as has been described above, create a high ionic strength in the column. A grounded and pressurized reservoir was applied at the inlet of a coated capillary in order to investigate if a larger electric field could be used with this setup. It was found that the dramatic increase in conductivity that was observed when the voltage was applied inline was limited to a four time increase of the conductivity which was gradually decreased as the voltage was increased further, Fig. 8. At voltage above the maximum conductivity the current was quite unstable. This makes the use of this setup for EF-LC limited.

4. Conclusions

We have found that the useful field strength in EF-LC is limited by a dramatic increase in current, which deviates from Ohm's law. The increase in

current has been found to origin from an increased concentration of buffer ions that have an electrophoretic mobility towards the pumped flow. To minimize the effect it is especially important to use mobile phases with having ionic strength and buffers with low electrophoretic mobility. The useable electric field strength can also be increased by increasing the flow velocity but only to a limited extent.

Acknowledgements

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Appendix A. Derivation of Eq. (1)

The number of a specific type of ions in a column n can be calculated according to:

$$n_{\text{column}} = \frac{aL}{v_{\text{column}}} \tag{A.1}$$

where a is the number of ions per time unit entering the column, L is the length of the column and v_{column} is the velocity of the ion in the column.

The number of ions entering the column per time unit can be expressed as:

$$a = C_{\text{in}} v_{\text{in}} A \tag{A.2}$$

where C_{in} is the concentration of the ion in the solution entering the column, v_{in} is the velocity of the ions entering the column and A is the cross section area of the column.

Combining Eqs. (A.1) and (A.2) gives:

$$n = \frac{C_{\text{in}} v_{\text{in}} AL}{v_{\text{column}}} \tag{A.3}$$

Because the concentration of the ion in the column C_{column} is:

$$C_{\text{column}} = \frac{n}{AL} \tag{A.4}$$

the concentration in the column can be expressed as:

$$C_{\text{column}} = \frac{C_{\text{in}} v_{\text{in}}}{v_{\text{column}}} \tag{A.5}$$

If v_{in} is equal to v_{column}, which is true in most cases, C_{in} is equal to C_{column}.

If an electric field is applied over the column v_{column} will depend both on v_{in} and the electrophoretic velocity v_{e} of the ion in the column. This gives the expression:

$$C_{\text{column}} = \frac{C_{\text{in}} v_{\text{in}}}{v_{\text{in}} \pm v_{\text{e}}} \tag{A.6}$$

In order to get the total ionic strength C_{ion} the concentrations of each type of ion in the solution are added. For an ion pair this gives Eq. (1):

$$C_{\text{ion}} = C^{+}\left(\frac{v}{v - v_{\text{e}}^{+}}\right) + C^{-}\left(\frac{v}{v + v_{\text{e}}^{-}}\right) \tag{1}$$

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