Apparent baseline irregularities for neutral markers in capillary zone electrophoresis with electroosmotic flow

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Abstract

An explanation is given for experimentally observed baseline irregularities in the trace obtained from the UV absorbance detector in capillary zone electrophoresis. Such disturbances often occur as positive or negative jumps on both sides of the signal of the neutral marker, especially when the marker substance is injected in a solution with an ionic concentration that is different from that of the background electrolyte (BGE). It is demonstrated that a source of these irregularities is the different migration velocity of the concentration boundary between the neutral marker zone and the BGE (which migrates exactly with the velocity of the electroosmotic flow) on the one hand, and the concentration boundary between the electrolyte zones having different salt concentrations on the other hand. Such different migration velocities, leading to an apparent de-mixing of the components of the sample zone, are caused by divergent dependencies of the transference numbers on the concentrations of the ions involved. This de-mixing leads to a kind of system peak resulting from the recorded sum of the absorbance signals of the BGE and the neutral marker. The approach to explain experimentally found irregularities is supported by the results of a computer simulation.

Keywords: Baseline irregularities; Electroosmotic flow; System peaks

1. Introduction

When capillary zone electrophoresis (CZE) is carried out in untreated fused silica (or synthetic polymer) capillaries, electroosmotic flow (EOF) normally occurs. The bulk solution is transported through the separation capillary, and electrically neutral components of the sample (together with its solvent) are moved towards the detector. Provided that the electroosmotic flow has a radially flat velocity profile (this is a good presumption in most cases [1,2]), a neutral component is simply transported along the capillary column and the only phenomenon that it inherently is subject to is axial diffusional dispersion.

Occasionally a UV-active neutral component is added to the injected solution to measure the electroosmotic velocity, by the occurrence of a signal at the UV absorbance detector caused by this component. However, various irregularities of the trace accompanying the marker signal, obtained from the detector, are often observed, but are ignored in most cases. They occur as positive or negative jumps or

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disturbances of the baseline, similar to the so-called system peaks found in HPLC.

In only a few investigations was a rational base for the sources of such phenomena sought. Vinther et al. [3] discuss baseline jumps as a consequence of isotachophoretic effects, leading to concentration adaptation of the subsequent zones according to the Kohlrausch regulation function [4]. Beckers [5–9] relates system peaks and baseline disturbances to moving boundary zones, originating from discontinuities in the co-ion concentration or in the pH. Colyer et al. [10,11] argue that baseline perturbations and emersion peaks are generated at the inlet end of the capillary, due to adsorption of ions of the background electrolyte (BGE) on the liquid–air boundary and by other reasons not fully explained. Nevertheless, from the literature it can be seen that such system peaks have no trivial reasons.

One has to realize that in capillary electrophoresis the composition of the sample solutions differs from that of the BGE in most cases. Consequently, the Kohlrausch regulating function of the sample solution is generally divergent from that of the BGE. After switching the driving voltage on, all ions of the sample are swept out by the electric field after some time and the BGE concentration attains a different value at the original site of the injected sample. This means that, at this site, concentration boundaries of the BGE can originate. They are shifted by the electroosmotic flow, in a similar way as the neutral markers. Nevertheless, their movement is much more complicated than the simple movement and axial diffusion of the neutral marker. Longsworth [12] and Stockmayer [13] have shown that the concentration boundary has its own movement, due to the concentration dependence of the transference numbers of the BGE ions. It has also been shown [14] that the temperature dependence further complicates the movement of these concentration boundaries.

Besides the scientific aspect, it is of relevance to focus attention on such phenomena for practical purposes, as an understanding of the origin of these disturbances is necessary in order to select appropriate experimental conditions e.g. to determine accurately the mobility of the electroosmotic flow. This motivated us to investigate some of the apparent irregularities of the baseline that occur during the transport of neutral compounds by electroosmosis.

We tried to explain one source of baseline jumps, based on the assumption of the movement of the concentration boundaries. A more detailed investigation that concentrates on the theoretical aspect is given elsewere [15].

2. Experimental

2.1. Materials

The substances used for the preparation of the electrolytes were of analytical reagent grade (Merck, Darmstadt, Germany). Water was doubly distilled from a quartz apparatus before use. Mesityloxide (purum, Fluka, Buchs, Switzerland) was used as the neutral marker.

2.2. Apparatus

Electrophoretic measurements were carried out using an instrument (P/ACE System 2000, Beckman, Palo Alto, CA, USA) equipped with a UV absorbance detector, operated at 214 nm. The sample was introduced by pressurized injection. Data collection and acquisition was carried out using Gold software (Beckman).

The uncoated fused-silica capillary (50 or 75 μm I.D.; 56 cm total length, 50 cm to detector; Scientific Engineering, Ringwood, Australia) was thermostated by the liquid thermostating system of the instrument at 25.0°C.

3. Results and discussion

A typical electropherogram depicting irregularities obtained when using a neutral marker is shown in Fig. 1a. Mesityloxide was used as the neutral marker, and HCl–imidazole as the BGE. Both mesityloxide and imidazole strongly absorb at the detector wavelength (214 nm). As is common in practice, the concentration of the electrolyte in the sample and that in the buffer differ in this experiment. A peak of mesityloxide on a straight baseline would be expected; nevertheless, before and after this peak an irregular deviation of the UV absorbance signal is observed.
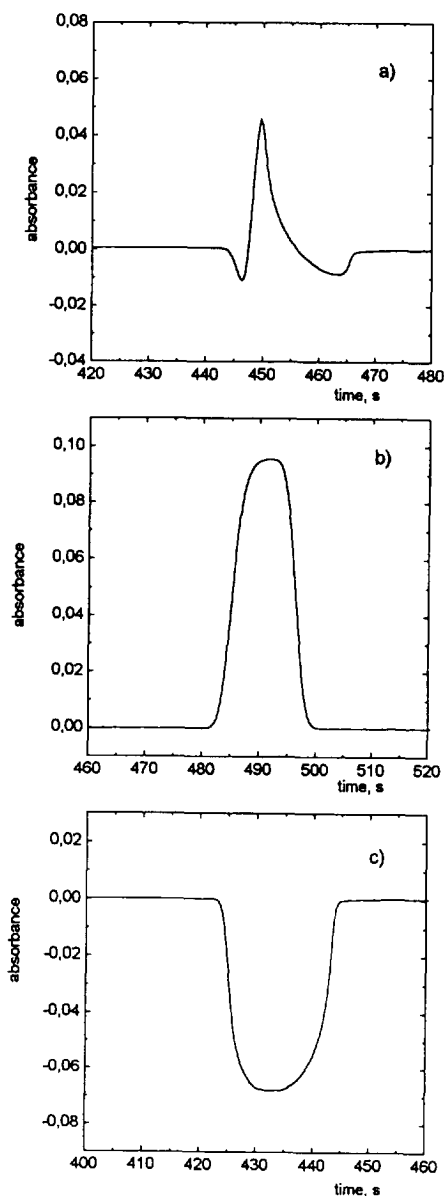
Fig. 1. Electropherograms obtained with imidazole–HCl as the BGE and mesityloxide as a neutral marker. Capillary dimensions: 75 μm I.D., 290 μm O.D., 56 cm (50 cm) length. The BGE was imidazole–HCl, 0.010 mol/l, pH 6.95. The injection time was 10 s (pressurized). UV absorbance was measured at 214 nm (detector placed at the cathode side of the capillary). The voltage used was 10 kV. Sample: a = mesityloxide (0.020 mol/l), dissolved in imidazole–HCl buffer with a concentration of 0.0025 mol/l; b = mesityloxide (0.020 mol/l), dissolved in imidazole–HCl buffer with a concentration of 0.01 mol/l; c = imidazole–HCl buffer with a concentration of 0.0025 mol/l.

Fig. 1b shows an electropherogram obtained with mesityloxide as the neutral marker. In contrast to Fig. 1a, the concentration of HCl–imidazole in the sample solution is the same as that in the BGE. The signal of the marker is apparently without disturbances. Finally, in the third experiment (Fig. 1c), the sample used is the BGE (diluted four-fold). There is a significant asymmetry in the dip on the UV absorbance trace in this case with the left edge being steeper than the right one.

In order to get a clearer insight into the possible source of these phenomena, the model of concentration boundary's movement can be utilized. This approach, described in [12–14], includes as the central aspect, the different dependencies of the mobilities and the transference numbers of the ions involved on the concentration and on the temperature. Unfortunately, these data are not available in the literature for the BGE constituent used here, as no such data exist for imidazole. Therefore, the approach was proved using a system consisting of ions with known data, namely for that of lithium iodide. The mobility of Li^+ and I^- ions have different concentration and temperature dependencies. Moreover, iodide has considerable UV absorption. A solution of this salt as the BGE will obviously have the restriction that it has no buffering capacity, which would limit its use for practical CZE purposes. For our intentions however, it is suitable.

An electropherogram obtained with LiI as the BGE, at a concentration of 0.01 mol/l, with 0.02 mol/l mesityloxide dissolved in 0.0025 mol/l LiI as the sample is shown in Fig. 2a. Again, at the front and the rear side of the mesityloxide signal, two apparent irregularities can be observed. These are seen as a positive and a negative jump on the UV trace. Fig. 2b and Fig. 2c are analogous to Fig. 1b and Fig. 1c. They show the traces obtained for mesityloxide in 0.01 mol/l LiI solution, and that obtained using a BGE that was diluted four-fold, i.e., 0.0025 mol/l LiI, as the sample. It should be pointed out that the trace shown in Fig. 2c exhibits opposite asymmetry compared to Fig. 1c, i.e., in this case the right edge is steeper than the left one.

The concentration and thermal dependencies of the mobilities of Li^+ and I^- ions are available [16]. Using the same approach as described in [14] the following expressions and functions can be given:

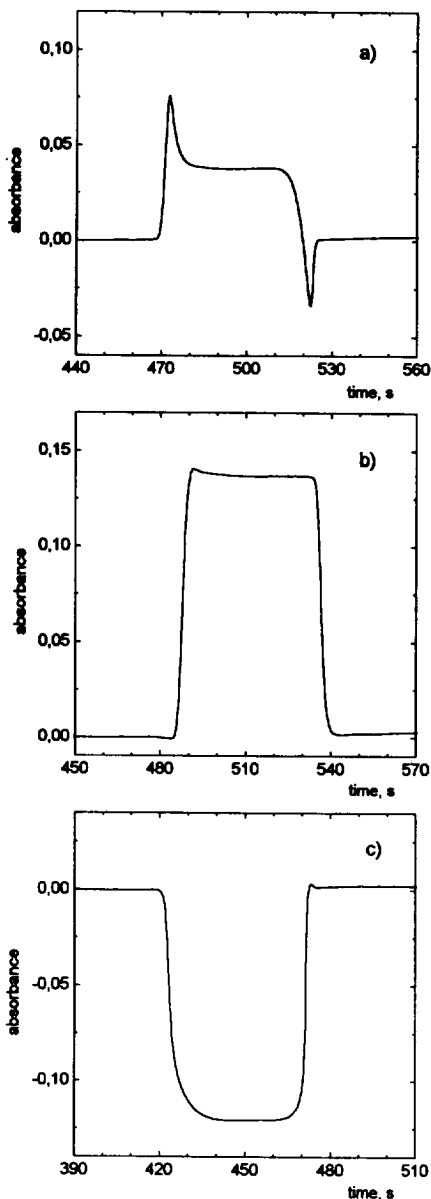Fig. 2. Electropherograms obtained with LiI as the BGE and mesityloxide as a neutral marker. Capillary dimensions: 50 μm I.D., 360 μm O.D., 56 cm (50 cm) length. BGE, LiI (0.010 mol/l); Injection time, 120 s (pressurized); UV absorbance was measured at 214 nm (detector placed at the cathode side of the capillary); Voltage, 10 kV. Sample: a = mesityloxide (0.020 mol/l) dissolved in a 0.0025 mol/l solution of LiI; b = mesityloxide (0.020 mol/l) dissolved in a 0.01 mol/l solution of LiI; c = LiI with a concentration of 0.0025 mol/l.

$$n = \frac{u_{Li}}{u_{Li} + u_I} \tag{1}$$

where

$$u_{Li} = [L_{Li} - (L_{Li}K_1 + K_2)\sqrt{c}]/F$$

$$u_I = [L_I - (L_I K_1 + K_2)\sqrt{c}]/F$$

$$L_{Li} = 18.7322 + T(0.747975 + 2.87634 \times 10^{-3} T)$$

$$L_I = 41.4469 + T(1.37452 + 2.74825 \times 10^{-3} T)$$

$$K_1 = 0.220883$$
$$\quad + T(3.3265 \times 10^{-4} + 1.63986 \times 10^{-3} T)$$

$$K_2 = 31.45 + T(0.63043 + 0.0142788T)$$

Here n is the transference number of the cation, c is the concentration of the BGE in mol/l, T is the temperature in °C, u_{Li} and u_I are the mobilities in cm^2 V^{-1} s^{-1} of Li$^+$ and I$^-$, respectively, and F is the Faraday constant.

The overall velocity of the concentration boundary is given in [13]

$$v = \frac{j}{F} \frac{(n' - n'')}{(c' - c'')} \tag{2}$$

where n' and n'' are the transference numbers of the cation in the two solutions with concentrations c' and c'', respectively.

In the case under consideration, the relatively low electric current of 3.4 μA (corresponding to the current density j = 1732 Am^{-2}), a capillary diameter of 50 μm and liquid thermostating of the outer capillary surface implies that the temperature elevation in the capillary column is small, namely about 0.1°C at the site of the 0.0025 mol/l LiI solution as the sample and 0.03°C at the site of the 0.01 mol/l LiI solution as the BGE [14]. Consequently, the temperature effects will be less serious than the concentration ones. A constant temperature of T = 25°C in all parts of the capillary is therefore assumed.

The transference numbers at 25°C in the solution of LiI with concentration of c' = 0.01 mol/l and c'' = 0.0025 mol/l are 0.327606 and 0.318087, respectively. The corresponding velocity of the concen-

tration boundary according to Eq. 2 is $v = 0.0228$ mm/s. This value is in fact the velocity by which the LiI concentration boundaries are moved, relative to the boundaries of the neutral marker. After about 8 min, which is the approximate time when the sample reaches the detector (see Fig. 2), the relative shift of both kinds of boundaries will reach about 11 mm. As the total velocity of the sample in the capillary column is about 1 mm/s, a shift between both boundaries lasting about 11 s should be seen. It can be deduced from Fig. 2a that this shift is about 6–10 s, which agrees with the predicted value.

The electropherograms can be simulated for the same solutes and conditions using the mathematical model and computer program described in a previous work [14]. The result of the simulation is shown in Fig. 3, where the concentration–time records are given. It is assumed here that the extinction coefficients of iodide and mesityloxide are about equal. Therefore, the absorbance signal is mirrored by the sum of the concentrations of these two compounds.
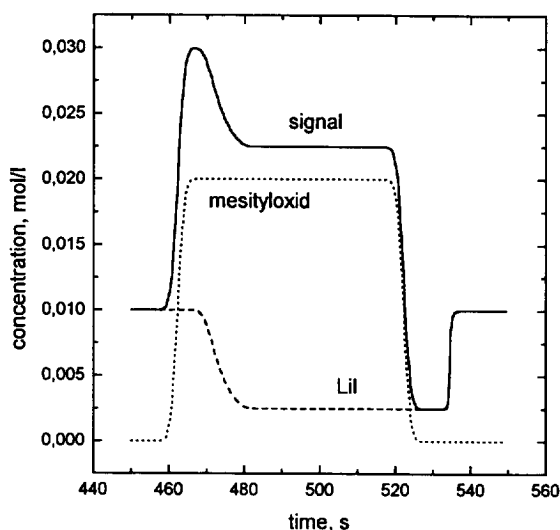

Fig. 3. Simulated electropherograms obtained with LiI as the BGE and mesityloxide as a neutral marker. Time records for mesityloxide and LiI concentration obtained by computer simulation under the following conditions: BGE, LiI (0.010 mol/l); sample, mesityloxide (dissolved at 0.020 mol/l in a 0.0025 mol/l solution of LiI); capillary dimensions, 50 μm I.D., 360 μm O.D.; velocity of the EOF, 1.0 mm/s. The signal is the sum of the concentrations of LiI and mesityloxide. For details see Section 3.

A principal agreement of the simulated electropherogram (depicted as the signal in Fig. 3) with that obtained experimentally (Fig. 2a) is observed. It can be seen that the occurrence of a negative and a positive jump is indeed found at the boundaries of the mesityloxide zone. Even the difference in the steepness of the edges of the LiI concentration–time record (cf. Fig. 2c) due to the non-linear concentration dependence of the transference number resulted from the simulation.

4. Conclusion

The observed apparent irregularities of the UV trace of the neutral marker can be explained as follows: when the sample is injected pneumatically, the injection plug generally has two stepwise gradients between the sample zone and the BGE. It has to be taken into account that both of these gradients strictly consist of two different boundaries; one is the boundary formed by the neutral marker, and the other is formed by the concentration difference of the electrolyte in the sample and the BGE.

It can be concluded from the discussion given above that two different migration processes take place under the influence of the driving electric field: (i) The neutral marker zone migrates exactly with the velocity of the EOF.
(ii) Due to the concentration (and temperature) dependence of the transference number, the electrolyte concentration boundary between the sample zone and the BGE can undergo an additional movement, leading to a slightly different velocity compared to the EOF. For the ions under consideration (Li^+ and I^-), the concentration boundary in fact shifts backwards, in addition to the EOF migration. Additionally, a non-linear character of the dependence of the transference number on concentration and velocity leads to additional distortion of the concentration boundaries, reflected by the different degrees of steepness of the two edges of the recorded sample plug.

The result is an appearent de-mixing of the zones and is one cause of the irregular peaks observed in the electropherogram of UV absorbance.

Acknowledgement

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