

Isotachopheresis with two leading ions and migration behaviour in capillary zone electrophoresis

II. Migration behaviour in capillary zone electrophoresis

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SUMMARY

During a zone electrophoretic analysis, components can migrate in the isotachophoretic mode. If, for example in anionic separation in capillary zone electrophoresis an anion with a high effective mobility is present in the sample at a very high concentration, it migrates forwards into the background electrolyte, separates from the other components and forms an isotachophoretic system with two leading ions together with the anion of the background electrolyte. Some of the sample components will therefore migrate in the isotachophoretic mode for the greater part of the analysis time. Because in the isotachophoretic mode the zone lengths are constant, very small sample zone lengths will give extremely high plate numbers. Of course, migration times will vary strongly depending on the composition of the sample.

INTRODUCTION^a

Electrophoretic techniques can be divided in four main types, *viz.*, isotachopheresis (ITP), zone electrophoresis (ZE), moving-boundary electrophoresis (MB) and isoelectric focusing (IEF). The apparatus needed for all these techniques is identical in essence and consists of modules, *viz.*, separation, injection, detection and electrode modules. The only difference in order to carry out an experiment in a specific mode is the choice of the electrolytes in the different parts of the apparatus. If, for example an ITP analysis has to be performed, the separation module has to be filled with a leading electrolyte the anion (cation) of which has an effective mobility higher than any of the sample anions (cations), whereas the effective mobility of the anion (cation) of the terminating electrolyte must be lower. In ZE the whole apparatus generally will be filled with one background electrolyte.

^a Symbols and abbreviations follow those defined in Part I.

If these requirements are not met, disturbances in the separation can be expected. If in an ITP mode a sample ion is present with an effective mobility higher than that of the leading ion, this ion will migrate into the leading zone and will migrate there in a ZE mode. Analogously, a sample ion with a lower effective mobility than that of the terminator will migrate in a ZE mode in the terminator zone. It will be clear that often one can hardly distinguish between the four main types of electrophoresis as several electrophoretic modes can exist in a single experiment.

Recently, Verheggen *et al.*¹ reported on the phenomenon that extremely high plate numbers could be obtained in the anionic ZE analysis of, *e.g.*, uric and hippuric acid in serum. Schoots *et al.*² recognized the fact that these high plate numbers are due to the presence of the large amount of chloride in the samples and noted the analogy with the solvent effect in chromatography. Plate numbers of over 10^6 could easily be obtained by adding large amounts of chloride to a sample.

Using the concept of plate numbers^{3,4}, generally maximum numbers of 10^6 can be obtained theoretically. Extremely high plate numbers can only be explained if sample ions were to migrate in the ITP mode during a ZE analysis. Because in ITP the zone lengths are constant and the zones are sharp owing to the self-correcting effect of the ITP zones, very small sample zone lengths will give very high plate numbers. The question is, however, why an ITP procedure can exist in a CZE procedure and whether it leads to a steady state. Mikkers and Everaerts⁵ mentioned earlier that ITP with two leading ions can be applied.

In Part I⁶, we gave a mathematical model for isotachophoresis with a leading electrolyte with two leading ions. Calculations showed that at a certain concentration ratio of the two leading ions, also dependent on the effective mobilities of these ions, the terminating zone, consisting of the leading ion with the lowest effective mobility, has a smaller E gradient than that of a specific sample ion, *i.e.*, that this ionic species will migrate in a CZE mode. Isotachophoresis changes into CZE. A close relationship exists in this way between ITP and CZE, as often the first stage in the CZE separation is a moving-boundary procedure followed by a non-steady-state ITP separation. Later in the separation procedure a CZE procedure is the result.

The idea was now that in the first instance the large amount of chloride separates from the other components, migrates forwards into the background electrolyte and hence will create an ITP separation mode with a leading electrolyte with two leading ions, *viz.*, the chloride ions and the anions of the background (MES). By this means, some other sample ions will migrate in an ITP mode behind the two leading ions (see Part I), showing extremely high plate numbers.

Because it is not possible with our apparatus to follow the migration procedure with detectors at the injection point, simulations are used to show these migration phenomena in the first stage of CZE separations.

THEORY

A typical difference between electrophoretic and chromatographic techniques is that in electrophoresis at any point the situation is determined by the initial conditions. Kohlrausch formulated this in 1897 by his "regulation function". This means that in electrophoresis the concentration of the injected sample adapts to the initial concentration of the background electrolyte migrating in the separation capillary tube.

Very dilute samples will be concentrated. Using electromigration injection, this concentration effect occurs directly during the injection by a moving-boundary migration. In that event, no representative aliquot will be introduced. The quality of the CZE separation depends for the greater part on this concentration effect. Parameters such as mobilities and concentrations of all ions present in the system play an important role.

In order to illustrate the effect of these parameters, simulations of the CZE process are carried out by means of a numerical solution of the basic transport equation:

$$\frac{\delta c}{\delta t} = - \frac{\delta(mEc)}{\delta x} + D \cdot \frac{\delta^2 c}{\delta x^2} \quad (1)$$

where c is the concentration of the ionic species and x is the position in the separation tube. The electric field strength E was calculated from the electric current density i by

$$i = E \sum_j c_j m_j F z \quad (2)$$

whereby F is the Faraday constant and j represents the summation over all ionic particles.

The diffusion constant D was calculated using the equation

$$D = mkT/ze \quad (3)$$

where k is the Boltzmann constant, T is the absolute temperature and ze is the charge of the ionic species.

Concentration effect of the sample

During the first stage of CZE, the sample ionic species in diluted samples will be concentrated. In Fig. 1 this effect is shown by a simulation for the introduction of a dilute sample consisting of two anions. The conditions for the simulation of the zone electrophoretic separation are given in Table I. The sample is introduced into the separation chamber between the positions 1 and 2 mm. After 0.01 s the sample anion 1 is partially concentrated at the original boundary between leading ion and sample ions (see Fig. 1A 2 mm) to about 0.0016 M and shows a diffuse front. At the same time sample anion 2 shows a similar behaviour but shows also an increase in concentration at the rear side (see Fig. 1B). There it is partially separated from sample anion 1 and its concentration is adapted to the original total concentration of both sample anions. If sample anion 1 were to be present at a higher concentration, this effect would also be much stronger. The background anions do not pass the sample ions (not shown in the figures) because their mobility is lower than those of the sample anions. In fact, at the rear side the anions migrate in the ITP mode. Further, it can be noted that at the injection point the E gradient is very high because of the low concentration of the sample ions. Owing to this very high E gradient between positions 1 and 2 mm, the concentration effect occurs very quickly.

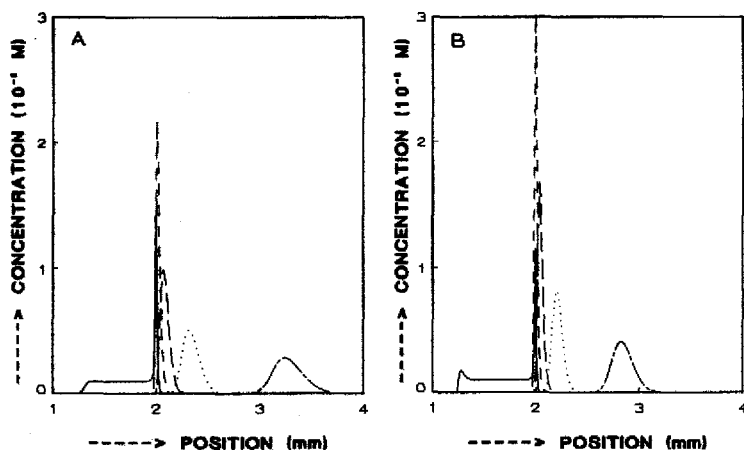


Fig. 1. Concentration profiles for the simulation of a zone electrophoretic separation of two anions. (A) Concentration profiles of anion 1 and (B) those of anion 2 after a separation time of (—) 0.01 s, (---) 0.04 s, (· · · · ·) 0.1 s, (- · - · -) 0.3 s and (- - - -) 1 s. On the horizontal axis the position in the separation tube is given in mm. The sample introduction was between 1 and 2 mm.

After 0.04 s the concentration effect is completed and the concentration of sample anion 1 is about 0.0022 *M* and that of sample anion 2 is 0.0033 *M*. The peaks are very sharp. The simulation at 0.1, 0.3 and 1 s show that in the first stage of the ZE separation the effect of the zone broadening is very strong.

Effect of two leading ions in ITP

In Fig. 2 the simulation is given for an anionic ITP separation using two leading ions after 15 s. In Table II the conditions for this simulation are given. In the simulation it is clearly shown that sample anion 1 migrates in the ITP mode and follows the leading zone L1/L2 whereas sample anions 2 and 3 migrate in the ZE mode with peaks broadening in time.

The original injection point was between 1 and 2 mm and at $t=15$ s the terminating L2 zone is still adapted to the original concentrations of the sample anions.

TABLE I

CONDITIONS FOR THE SIMULATION OF AN ANIONIC ZONE ELECTROPHORETIC SEPARATION

Electric current, 20 μ A; capillary I.D., 0.2 mm.

Species	Concentration (<i>M</i>)	Ionic mobility $\times 10^5$ ($\text{cm}^2/\text{V} \cdot \text{s}$)
Background anion	0.01	-20
Counter ion	0.01	+15
Sample anion 1	0.0001	-75
Sample anion 2	0.0001	-50

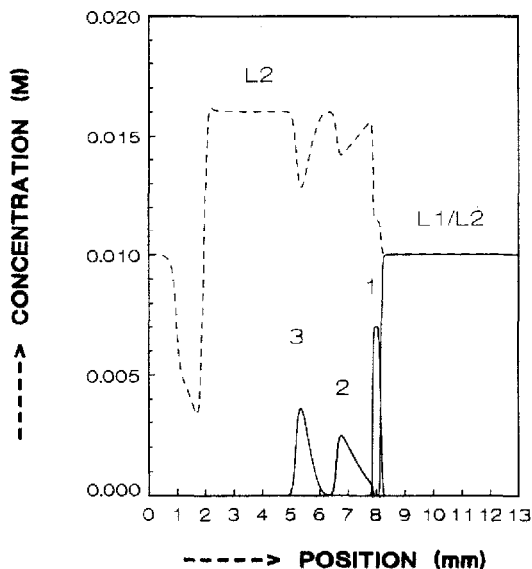


Fig. 2. Concentration profiles for the simulation of a separation of three anions in ITP with two leading ions. Sample anion 1 migrates in the ITP mode behind the leading ions L1 and L2. The sample anion 2 and 3 migrate in the ZE mode.

Effect of an excess of one of the sample ions in ZE

In Figs. 3 and 4 the simulations are given for an anionic ZE separation of a sample containing one of the components in a large excess. The conditions for the simulation are given in Table III. The original injection point was between 1 and 2 mm.

In Fig. 3 the concentration profiles of the four components after 4, 7, 10, 13, 16

TABLE II

CONDITIONS FOR THE SIMULATION OF AN ANIONIC ITP SEPARATION USING TWO LEADING IONS

Electric current, 20 μ A; capillary I.D., 0.2 mm.

Species	Concentration (M)	Ionic mobility $\times 10^5$ ($\text{cm}^2/\text{V} \cdot \text{s}$)
<i>Leading electrolyte:</i>		
Leading L1	0.01	-75
Leading L2	0.01	-15
Counter	0.02	+15
<i>Terminating electrolyte:</i>		
Terminating L2	0.01	-15
Counter	0.01	+15
<i>Sample anions:</i>		
Sample anion 1	0.002	-50
Sample anion 2	0.002	-30
Sample anion 3	0.002	-20

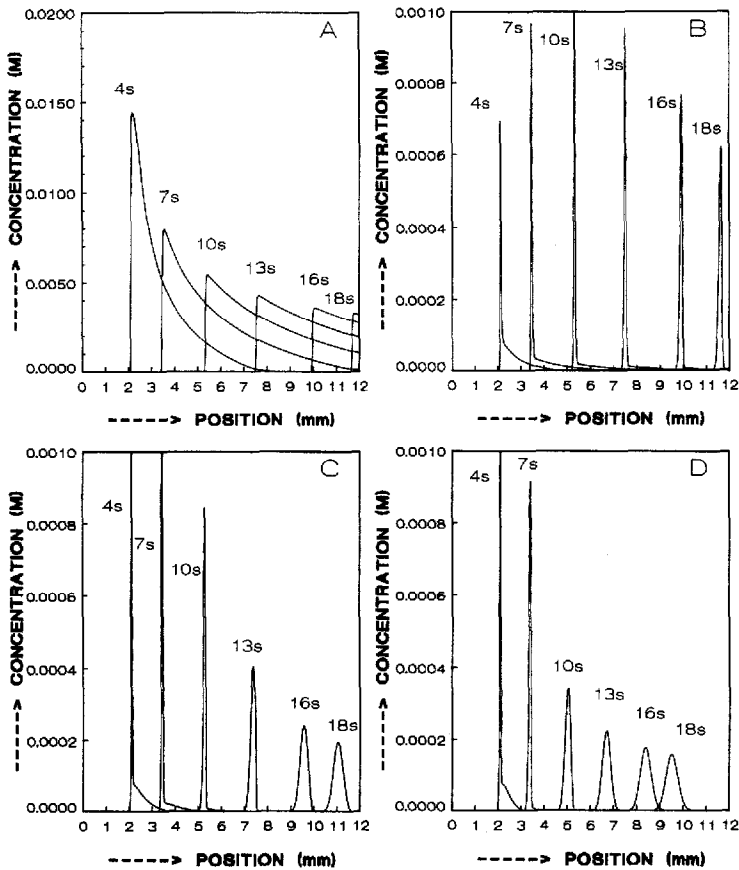


Fig. 3. Concentration profiles for the simulation of a ZE separation of four anions. The anion 1 with the highest mobility is present in excess in the sample. In A, B, C and D the concentration profiles are given for the sample anions 1, 2, 3 and 4, respectively, at separation times of 4, 7, 10, 13, 16 and 18 s.

and 18 s are shown. In Fig. 3A it can clearly be seen that the rearside of the component present in a large excess in the sample is sharp and that the component diffuses into the background electrolyte. This component forms together with the anion of the background an ITP leading electrolyte with two leading ions. The concentration of this component in this leading electrolyte decreases with time, as a result of which only the most mobile of other components will ultimately migrate in the ITP mode. This can be seen in Fig. 3B, where the most mobile of the sample ions migrates ultimately in the ITP mode and shows nearly no peak broadening with time. From Fig. 3C and D it can be concluded that these anions migrate from 13 and 10 s, respectively, in the CZE mode (zone broadening).

In Fig. 4 the concentration profiles of all sample components are given after 18 s. In Fig. 4 the concentration of the component in excess is ten times higher as indicated. The similarity with the ITP separation with two leading ions in Fig. 2 can clearly be seen. In Fig. 2, sample anion 1 migrates in the ITP mode behind the leading zone with

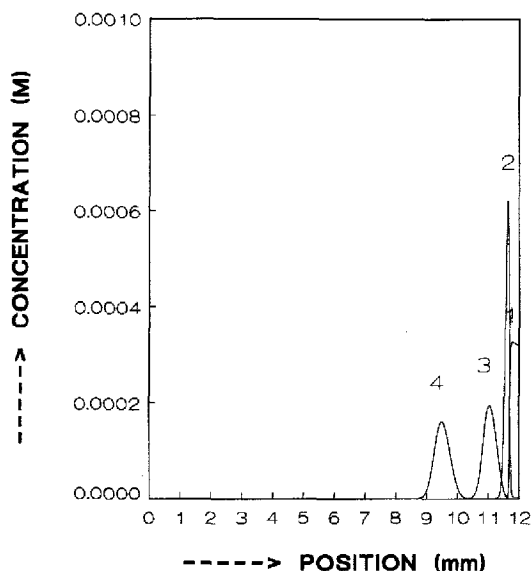


Fig. 4. Concentration profiles for the simulation of a ZE separation of four anions after 18 s. The anion 1 with the highest mobility is present in excess in the sample.

two leading ions (L1 and L2) where as in Fig. 4 the anion 2 migrates in the ITP mode behind the leading zone consisting of the anion of the background electrolyte and anion 1, the sample anion present in the sample in excess. The other two sample anions migrate in the ZE mode and show lower concentrations and zone broadening.

TABLE III

CONDITIONS FOR THE SIMULATION OF AN ANIONIC ZONE ELECTROPHORETICAL SEPARATION OF A SAMPLE CONTAINING A LARGE EXCESS OF ONE SAMPLE COMPONENT

Electric current, 20 μ A, capillary I.D., 0.2 mm.

<i>Species</i>	<i>Concentration (M)</i>	<i>Ionic mobility $\times 10^5$ ($\text{cm}^2/\text{V} \cdot \text{s}$)</i>
<i>Background electrolyte:</i>		
Anion	0.01	-20
Counter ion	0.01	+15
<i>Sample anions:</i>		
Sample anion 1	0.02	-75
Sample anion 2	0.0001	-50
Sample anion 3	0.0001	-40
Sample anion 4	0.0001	-30

CONCLUSIONS

If in ITP the leading electrolyte contains two leading ions, some sample components will migrate in the ITP mode and others in the CZE mode (see Part I). If in CZE a sample is analysed with a large amount of, e.g., chloride, in first instance the ions separate and if the separation is (partially) completed the chloride ions migrate in front of the other sample ions. The chloride ions migrate from the injection position into the background electrolyte and will form in first instance an ITP system with two leading ions. Depending on the concentrations and the effective mobilities, some sample ions will migrate in the ITP and others in the CZE mode. The composition of the two leading ion system changes continuously because chloride migrates through the background electrolyte. This means that the concentration ratio $L1/L2$ of the 2L system decreases with time and more components will finally migrate in the CZE mode. If the capillary tube is long enough, all components will ultimately migrate in the CZE mode.

The number of plates strongly depends on this procedure. A component migrating all the time in the CZE mode will show plate numbers determined by the CZE migration behaviour. A component migrating (nearly) all the time in the ITP mode will show extremely high plate numbers, because the zone lengths are constant. This means that the use of the traditional concept of plate numbers often will be not useful. The separation capacity is often determined by the ITP behaviour. The use of migration times can also be troublesome, especially when working in the electroosmotic flow (EOF) mode. Further investigations are needed to decide what the effects are of sampling, sample composition and the use of EOF on separation capacity, plate numbers and migration times. It is extremely important to report sufficient experimental conditions in papers concerning CZE experiments.

REFERENCES

- 1 Th. P. E. M. Verheggen, A. C. Schoots and F. M. Everaerts, *J. Chromatogr.*, 503 (1990) 245.
- 2 A. C. Schoots, Th. P. E. M. Verheggen, P. M. J. M. de Vries and F. M. Everaerts, *Clin. Chem. (Winston-Salem, N.C.)*, 36 (1990) 435.
- 3 J. W. Jorgenson and K. DeArman Lukacs, *Anal. Chem.*, 53 (1981) 1298.
- 4 J. H. Knox, *Chromatographia*, 26 (1988) 329.
- 5 F. E. P. Mikkers and F. M. Everaerts, in F. M. Everaerts, F. E. P. Mikkers and Th. P. E. M. Verheggen (Editors), *Analytical Isotachopheresis*, Elsevier, Amsterdam, 1981, pp. 1-17.
- 6 J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, 508 (1990) 3.