Capillary electrophoresis : an overview

Citation for published version (APA):

Reijenga, J. C. (1992). Capillary electrophoresis : an overview. *Journal of Radioanalytical and Nuclear Chemistry*, 163(1), 155-167. <https://doi.org/10.1007/BF02037490>

DOI:

[10.1007/BF02037490](https://doi.org/10.1007/BF02037490)

Document status and date:

Published: 01/01/1992

Document Version:

Publisher's PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.tue.nl/taverne

Take down policy

If you believe that this document breaches copyright please contact us at:

openaccess@tue.nl

providing details and we will investigate your claim.

CAPILLARY ELECTROPHORESIS AN OVERVIEW

J. C. REIJENGA

*Laboratory of Instrumental Analysis, Eindhoven University of Technology,
P.O. Box 513, 5600 MB Eindhoven (The Netherlands)*

(Received March 20, 1992)

After a short historical introduction, the different modes of separation in capillary electrophoresis are explained and illustrated by practical examples. In addition, the most important parameters that can be used to optimize the selectivity of the separation, are discussed.

Introduction

The theoretical basis of commonly used separation techniques in analytical chemistry is largely understood, although new developments are seen in a number of them recently. In the history of these analytical techniques, stabilization and detection were seen to play key roles in instrumental development.

Successive breakthroughs in these two vital aspects, stabilization and detection, have led to capillary gas chromatography (GC) and high performance liquid chromatography (HPLC) in the 1960's and 1970's. It would be too obvious to state that the same has led to capillary electrophoresis in the 1980's. Some excellent textbooks¹⁻⁴ and review articles⁵⁻¹¹ have appeared in the last decade. In addition, proceedings of several international symposia on isotachopheresis and on capillary electrophoresis¹²⁻²⁰ give a good indication of the development of the technique. A concise historical introduction should therefore suffice.

For decades, electrophoresis has been the method of choice for the isolation and identification of proteins. On-line quantitative determination of these substances did not have a high priority because biological activity was more important than concentration. In addition, also in contrast to chromatography, different modes of electrophoretic separation were applied: zone electrophoresis, immunoelectrophoresis, isoelectric focusing, SDS gel electrophoresis, and combinations of these.

In the 1960's it was demonstrated that stabilization in electrophoresis could in principle be achieved in capillaries of 0.1 to 0.5 mm I. D. This coincided with the introduction of isotachopheresis (ITP). Use of thermocouple detection enabled monitoring of

isotachophoretic zone transitions. Thermocouple detection in capillary electrophoresis is characterized by a slow response and moderate sensitivity to minor temperature differences. For isotachopheresis, a concentrating technique, the latter is less significant. For zone electrophoresis, a diluting technique, sensitivity is of key importance. Improved response was achieved by UV absorption and conductivity, facilitating detection of short zones. Quantitation in isotachopheresis is usually carried out using the time information in the detector signal (measuring zonenlengths), not the signal amplitude (measuring peak areas). More recently, increased sensitivity and miniaturization of UV absorbance and fluorescence detection have enabled the use of zone electrophoresis in capillaries. In open capillaries, electro-osmosis, in combination with micelles, even neutral components can be separated by micellar electrokinetic chromatography (MEKC). These novel techniques ITP, CZE and MEKC can in principle be applied using the same equipment. Recent advances in the development of electrophoretic equipment will no doubt lead to increased use of these techniques in the 1990's.

Selectivity

Key sample component property in capillary electrophoresis is the effective mobility μ , defined as the linear velocity u per unit of field strength E :

$$\mu = \frac{u}{E} \quad (1)$$

Before going into details of the differences between ITP, CZE and MEKC, some general remarks on selectivity are in order. Generally speaking, a difference in effective mobility between the sample components in a mixture is favorable for separation. Some of the more important methods for tuning effective mobility differences and thus selectivity will be discussed.

For weak acids and bases, pH can be used to change the effective mobility by changing the following equilibria respectively:

Effective mobility μ is related to the absolute mobility μ_0 by the relation:

$$\mu = \alpha\mu_0 \quad (4)$$

in which α is the degree of dissociation, given by pH and pK and μ_0 is the mobility of the completely dissociated form. The idea is simply to decrease the average net charge of a sample ion.

For weak acids at a high pH or for strong acids, α is unity so that separation is based only on differences in the absolute mobility μ_0 of the sample components to be separated. These differences do not always result in a good separation. From Eqs (2) and (4) it can be seen that for anions a low pH (around the pK values of the anions) is favorable because different α values for different anions are obtained.

For similar reasons, a high pH is favorable for the selective separation of cations. Some mixtures require even higher selectivity than obtained with pH. For metal ions for instance, pH has little effect. There are several options. The first uses activity, based on the fact that the absolute mobility depends on the ionic strength. For dilute solutions, the Debye-Hückel approximation can be used, giving a linear dependence of the absolute mobility on the square root of the ionic strength. At higher concentrations, the linear relationship does no longer hold. In both cases, the slope of the curve is distinctly different for mono, di and trivalent ions. The ionic strength of the buffer can thus be used for selectivity tuning.

Change of solvent will change solvation of ions and thus lead to different mobilities. The effects are not readily predictable. A third method is using complex equilibria. Consider for instance the change of mobility of Ca by adding citric acid to the electrolyte system. In addition to the three dissociation equilibria of H_3Cit , the following equation will affect the average net charge and thus the effective mobility of Ca:

The equilibrium shown above can also be used to change the effective mobility of citrate by adding calcium to the electrolyte system. Choosing the proper pH, and type and concentration of complex former can result in extremely high selectivities. With micelles, added to the electrolyte buffer, sample components can be distributed between the water phase outside and the organic phase inside the micelle. The fraction of the sample inside the micelle migrates with a velocity equal to that of the micelle, distinctly different from that of the sample molecule in the water phase.

A special kind of interaction is required for the separation of enantiomers. Cyclodextrins are often used for this purpose. Organic molecules fit into the cavity of the cyclodextrin molecule to an extent depending on different functional groups and their orientation. In this case, the interaction does not result in an average change of net charge but rather in an increase of average molecular weight and a lower effective mobility.

Modes of separation

The mode of separation depends on the composition of the electrolyte in different parts of the separation equipment.

Capillary zone electrophoresis

Consider for instance CZE (Fig. 1), where the total equipment is filled with background electrolyte, consisting of ions X^+ and C^- , preferably with a buffering capacity. Somewhere on one side of the capillary a small amount of sample is injected, consisting of ions A^- , B^- and a counter-ion X^+ . A voltage is applied to both ends of the capillary. The effective mobility of the anions A and B is adjusted to the conditions determined by the buffer composition (pH, concentration). With a uniform field strength along the capillary, the resulting differences in velocity lead to a separation of the anions as they move towards the detector. The counter-ions do not interfere because they move away from the detector. Switching polarity will make it possible to separate cations using the same or a different background electrolyte.

Fig. 1. The principle of capillary zone electrophoresis CZE just after sample introduction and after some separation time. The dashed line represents a semipermeable membrane. The location of injection is on the left side. Somewhere on the right side of the capillary, an on-line detector is mounted. In this case, anions are detected as the anode is on the detector side of the capillary. The signal represents a property of the background electrolyte

A detector monitors UV absorbance or another property for quantitation by integrating the Gaussian shaped signals. Migration time is used for identification if the voltage drop across the capillary is held constant. The migration time t_R can be defined as the ratio of the capillary length L and the linear velocity u . With Eq. (1), the mobility μ can be included:

$$t_R = \frac{L^2}{\mu V} \quad (6)$$

in which V is the voltage drop along the capillary. From this equation it is also seen that a high voltage over a short capillary is essential for fast analyses.

An example of a CZE separation is shown in Fig. 2. Peak shape is usually more or less Gaussian so that normal integration procedures, used for chromatography, can be

Fig. 2. CZE of a standard mixture²⁰ of 1 = ATP, 2 = ADP, 3 = UMP, 4 = GMP, and 5 = AMP in a closed 16 cm teflon capillary with 0.2 mm I. D.

employed. Migration times are used for identification. Reproducibility of these migration times in a closed system can easily be better than 1% under isothermal conditions, the temperature coefficient of μ being between 2 and 3% per degree centigrade.

Capillary isotachophoresis

For ITP (Fig. 3a), part of the equipment is filled with leading electrolyte consisting of an anion L^- with a high effective mobility and a counter-ion C^+ with a buffering capacity. Another part of the equipment is filled with terminating electrolyte, consisting of an anion T^- with a low effective mobility and a counter-ion that does not necessarily have to be C^+ . Switching on the high voltage will lead to a different field strength in leading and terminating compartment. The terminating ion moves into the leading electrolyte compartment, adjusting its concentration to the properties of the leading electrolyte according to Kohlrausch's law. The resistance and field strength in zone T remain greater than those of zone L. Consequently, if an anion T enters zone L by diffusion, it meets a lower field strength, resulting in a lower velocity so that it will eventually return

Fig. 3. The principle of a blank analysis in capillary isotachopheresis (ITP) just after starting the driving current and after some separation time, without introduction of sample (see legend to Fig. 1.). The signals represent the resistance or field strength of the zones (a); ITP analysis of a mixture of 2 anions A and B, just after starting the analysis and after reaching the steady state (b). Refer to text for details

Fig. 4. ITP with conductivity detection of heavy metals²¹ in human serum after preconcentration. 1 = Ca, 2 = Fe, 3 = 4 ppm Zn, 4 = 2 ppm Pb, 5 = 1.5 ppm Cu and 6 = 0.4 ppm Al. Selectivity was obtained by addition of HIBA (hydroxyisobutyric acid) as a complex former to the buffer

to its own zone. This self-sharpening effect will last as long as the current is on. The velocity of ions L and T is the same, hence the name isotachopheresis.

Now a sample mixture consisting of anions A^- and B^- (and an arbitrary counter-ion) is introduced at the leading/terminator interface (Fig. 3b). Separation takes place if the mobility of A and B in the mixed zone is different and if they fall within the leading/terminator mobility window. After switching on the voltage, initially the uniform field strength in the mixture will lead to different velocities, resulting in separated zones containing only A and B, respectively. Mixed zones containing A and B will eventually disappear. The sample components are contained in their own zones at a concentration determined by the leading electrolyte. The volume of the zones, or the length in time units when working at constant driving current, are directly proportional to the amount injected. The field strength or resistance of the zones is used for identification.

An isotachopheretic analysis of heavy metals in human serum, after sample pretreatment, is shown in Fig. 4. As mentioned, signal response (step height) is used for identification, and zone lengths for quantitation. For this purpose, the signal is differentiated, where inflexion points between the zones are marked by peaks. Reproducibility of both step height and zone length is 1% or better.

Electrophoresis in open capillaries

The capillaries used in electrophoretic separation are made of insulating material such as polytetrafluoroethylene (PTFE or [®]Teflon) quartz or fused silica. In aqueous solution at pH 2 or higher, the capillary wall has a negative ζ -potential. The volume element of liquid directly outside the electric double layer, therefore, has a positive net charge. In the electric field, this will result in an electro-osmotic plug flow towards the negative electrode.

The electro-osmotic velocity u_{eo} is directly proportional to the ζ -potential of the capillary wall:

$$u_{eo} = \frac{-\zeta \epsilon E}{\eta} \quad (7)$$

in which ϵ is the dielectric constant of the solution, E the field strength and η the viscosity of the solution.

In a capillary that is closed on both ends (Fig. 5a), a zero net flow will result in a flow profile that reverses direction at the capillary axis. This has an adverse effect on the quality of all separation modes. The zeta potential and the electro-osmotic disturbance can be minimized by adding certain surface-active agents.

In open systems (Fig. 5b), all sample components are subject to at least one of two velocity vectors. All are subject to the electro-osmotic velocity. If this flow is directed towards the detector, non-charged sample components (the EOF marker) are also detected. Due to the additional electrophoretic velocity, positive and negative sample components migrate on either side of the EOF marker, except those anions with an electrophoretic velocity that is smaller than the electro-osmotic velocity.

Fig. 5. Electro-osmosis in a closed (a) and an open capillary (b) with a negative ζ -potential in an electric field. The dashed line represents a semipermeable membrane, resulting in a zero net flow in the closed system. In the example, the cathode is on the right side

Fig. 6. CZE of a standard mixture²² in an open capillary where cations and anions migrate on either side of the EOF marker. Detection is by UV absorbance, some peaks show a negative absorbance as compared to the background electrolyte

For identification, the migration time t_R is used. Because of the electro-osmotic flow, t_R is now given by:

$$t_R = \frac{L}{u_{eo} + u} \quad (8)$$

Combination of Eqs (1), (7) and (8) yields:

$$t_R = \frac{L^2}{V[\zeta\epsilon/\eta + \mu]} \quad (9)$$

in which for non-charged sample components the effective mobility $\mu = 0$. Such an EOF marker can be water or methanol, displaying a refractive index effect in UV detection. The sign of μ is different for cations and anions.

An example of CZE in an open system is shown in Fig. 6.

Fig. 7. CZE separation and detection at 206 nm of a racemic mixture of (-)-terbutaline and (+)-terbutaline in a phosphate buffer containing β -cyclodextrin²³

With plate numbers exceeding 100 000, extremely high efficiencies are obtained. If, in addition, a selective buffer composition is chosen, enantiomers can be separated. An example, using an extensively studied additive (cyclodextrin), is shown in Fig. 7. Cyclodextrin migrates with the electro-osmotic velocity as an unchanged molecule. The sample molecule, an aromatic secondary amine, has a positive charge and moves in the same direction as the electro-osmotic flow. The (+) and (–) forms are included in the cavity of the cyclodextrin molecule to different extents, thus decreasing the electrophoretic mobility that is superimposed on the electro-osmotic mobility. This is because the effective mobility of the amine, while in the cavity of the cyclodextrin, is very small as compared to the free amine. A strong interaction therefore leads to a greater migration time. Naturally, the type and concentration and possible functional groups of the cyclodextrin are also of key importance to selectivity.

A good reproducibility of migration times, necessary for identification, requires that the electro-osmotic flow and the ζ -potential is constant and reproducible from run to run (Eq. (9)). This last requirement is not necessary for CZE in closed systems [Eq. (6)], with the restriction that the ζ -potential should be small enough not to effect the plate height.

Micellar electrokinetic chromatography

As is seen in Fig. 6, non ionic sample components are also seen by the detector. Of course, there is no selectivity of these components as there is no migration. To achieve additional separation capability for these components, there are two chromatographic options.

The first uses a stationary phase on the capillary wall. This is actually capillary liquid chromatography with electro-osmosis as a driving force for moving the mobile phase. This technique is beyond the scope of this article. Another option uses micelles, added to the background electrolyte. These micelles always have a net charge such that they move in a direction opposite to the electro-osmotic flow. Micelles with a positive polar group, such as cetyltrimethylammonium bromide (CTAB) migrate in the reversed direction but they also reverse the electro-osmotic flow by inducing a positive ζ -potential.²⁴ In MEKC, sodium dodecylsulfate (SDS) is mostly used.

A non-ionic sample component can be distributed between the background electrolyte and the non-polar interior of the micelle. The fraction inside the micelle moves away from the detector by migration so that retention is obtained. The relation for migration times in MEKC is somewhat more complicated than Eqs (6) and (9) and in addition includes the capacity factor k' , defined as the ratio of the fraction of the solute in the micelle and the fraction in the continuous (aqueous) phase:

$$k' = KV_{mc}/V_{aq} \quad (10)$$

in which K is the distribution coefficient and V_{mc} and V_{aq} the volumes of the micellar and aqueous phase, respectively. Total migration time can now be written as:

$$t_R = \frac{L^2}{V[\zeta\epsilon/\eta + \mu/(k' + 1) + k'\mu_{mc}/(k' + 1)]} \quad (11)$$

in which μ_{mc} is the effective mobility of the micelle and μ the effective mobility of the sample component in free solution. The sign of the second term in the denominator depends on the charge sign of the sample component. For non-charged sample components Eq. (11) naturally simplifies to:

$$t_R = \frac{L^2}{V[\zeta\epsilon/\eta + k'\mu_{mc}/(k' + 1)]} \quad (12)$$

where one has to bear in mind that the two terms in the denominator always have opposite signs.

Fig. 8. MEKC separation of a standard mixture of corticosteroids:²⁵ 1 = hydrocortisone, 2 = hydrocortisone acetate, 3 = betamethasone, 4 = cortisone acetate, 5 = triamcinolone acetonide, 6 = fluocinolone acetonide, 7 = dexamethasone acetate, 8 = fluocinonide

Non-ionic components with different distribution coefficients can thus be separated by Micellar Electrokinetic Chromatography (MEKC). An application of MEKC is shown in Fig. 8, a separation of a standard mixture of 8 corticosteroids²⁵ with a buffer containing SDS and urea. As with CZE in open systems, a reproducible ζ -potential and electro-osmotic flow are essential for identification.

Other developments

As stated in the introduction, the performance of an analytical separation technique is determined by selectivity and sensitivity of both separation and detection.

New stationary phases in MEKC, specific complexation in CZE and the use of gel-filled columns are only a few of the latest developments in separation selectivity.

Selective detection, using fluorescent labeling, will increase the selectivity and sensitivity of detection. Analysis of zeptomole amounts (zepto = 10^{-21}) have been reported using fluorescence detection.²⁶ Extensive sample pretreatment is of course necessary at the low levels. Combinations of different electrophoretic techniques can also be used on-line to perform such a sample pretreatment.²⁷

Conclusions

Some of the more important requirements for large scale routine use of equipment for capillary electrophoresis are: a large linear dynamic range, a greater reproducibility of migration times (used for identification), and a more reproducible injection. If these requirements will be met within the next few years, capillary electrophoresis will no doubt throw capillary gas chromatography in the shade by the year 2000.

References

Textbooks

1. F. M. EVERAERTS, J. L. BECKERS, Th. P. E. M. VERHEGGEN, *Isotachophoresis*, J. Chromatogr. Libr. 6, Elsevier, Amsterdam, 1976.
2. Z. DEYL (Ed.), *Electrophoresis*, J. Chromatogr. Libr. 18A, Elsevier, Amsterdam, 1979.
3. E. HEFTMANN (Ed.), *Chromatography*, J. Chromatogr. Libr. 22A, Elsevier, Amsterdam, 1983.
4. P. BOCEK, M. DEML, P. GEBAUER, V. DOLNIK, *Analytical Isotachophoresis*, VCH, Weinheim (FRG), 1988.

Reviews

5. S.-G. HJALMARSSON, A. BALDESTEN, *CRC Cri. Rev. Anal. Chem.*, (1980) 261.
6. J. W. JORGENSEN, K. D. LUKACS, *Science*, 222 (1983) 266.
7. P. BOCEK, P. GEBAUER, V. DOLNIK, F. FORET, *J. Chromatogr.*, 334 (1985) 157.
8. J. W. JORGENSEN, *ACS Symp. Ser.*, 335 (1987) 182.
9. J. SNOPEK, I. JELLINEK, E. SMOLKOVA-KEULEMANSOVA, *J. Chromatogr.*, 452 (1988) 571.
10. R. WALLINGFORD, A. EWING, *Adv. Chromatogr.*, 29 (1989) 1.
11. W. G. KUHR, *Anal. Chem.*, 62 (1990) 403R.

Symposium proceedings

12. A. ADAM, C. SCHOTS (Eds), *Anal. Chem. Symp. Ser.*, 5 (1980) 1.
13. F. M. EVERAETS (Ed.), *Anal. Chem. Symp. Ser.*, 6 (1981) 1.
14. C. J. HOLLOWAY (Ed.), *Analytical and Preparative Isotachophoresis*, Walter de Gruyter, Berlin, 1984.

15. J. Chromatogr., 320 (1985) 1.
16. J. Chromatogr., 390 (1987) 1.
17. J. Chromatogr., 470 (1989) 1.
18. J. Chromatogr., 545(2) (1991) 1.
19. J. Chromatogr., 559(1 + 2) (1991) 1.

Examples

20. Th. P. E. M. VERHEGGEN et al., J. Chromatogr., 503 (1990) 245.
21. F. M. EVERAERTS et al., J. Chromatogr., 320 (1985) 263.
22. J. L. BECKERS et al., J. Chromatogr., 537 (1991) 407.
23. S. FANALI, J. Chromatogr., 545(2) (1991) 437.
24. J. C. REIJENGA et al., J. Chromatogr., 260 (1983) 241.
25. S. TERABE et al., J. Chromatogr., 545(2) (1991) 359.
26. D. Y. CHEN et al., J. Chromatogr., 559 (1991) 237.
27. D. KANIANSKY, J. MARAK, J. Chromatogr., 498 (1991) 191.