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Original Research Papers

Electrophoresis versus Electrochromatography

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Electroosmosis

Summary

In separation techniques, such as Liquid Chromatography and Capillary Zone Electrophoresis, separation is performed on the basis of differences in velocity of the various separands, making use of differences in k' and/or effective mobility.

While in chromatography the flow of the eluent is elementary, in electrophoretic techniques the electroosmotic flow is generally suppressed in order to avoid disturbing of the sample zone boundaries, which migrate with a maximal velocity of 10^{-3} m s^{-1}. This holds especially for isotachophoretic separations, where separands migrate in consecutive zones with minimal detectable lengths of about 0.1 mm.

If electroosmotic flow is applied as a transport mechanism, using capillaries as small as about 50 μm, linear velocities of the liquid flow can reach about 2×10^{-3} m s^{-1}. Especially for ionic species with a low effective mobility, this velocity can be a multiple of the electrophoretic migration velocity in the separation compartment. Therefore, anionic, non-ionic, and cationic separands can migrate in the same direction.

Depending on whether repulsive or attractive forces are operative, the electrophoretic separation power can be counteracted or favored. The separation mechanisms making use of (quasi)stationary phases are studied.

Plotting the chromatographic behavior *versus* the electrophoretic shows transition areas to exist between the "purely" electrophoretic techniques and the "purely" chromatographic techniques.

It must be stated that most of the recent publications in CZE, especially those with very narrow bore capillaries, can be allocated to the transition areas, sometimes with a strong chromatographic retention component.

1 Introduction

In his Nobel lecture in 1950, *Tiselius* [1] pointed out that in chromatography basically three different migration modes can be distinguished: zonal, frontal, and displacement. Surprisingly, however, in electrophoresis *Tiselius* preferred to distinguish only between moving boundary and zonal separations [2,3].

Martin and *Everaerts* [4,5], noting the analogy between electrophoresis and chromatography, again distinguished between three main principles:

- zone electrophoresis, which can be compared with the elution principle in chromatography;
- moving boundary electrophoresis, the analogue of chromatographic frontal analysis;
- isotachophoresis, the analogue of the chromatographic displacement principle.

These three main principles suffice to describe every migration mode, but need the addition of isoelectric focusing, and chromatofocusing which technique will not be discussed further.

Combinations of the three main principles are used: *e.g.* disc electrophoresis [6] is a combination of moving boundary electrophoresis, isotachophoresis, and zone electrophoresis. Moreover, additional force fields, more dimensions, additional (chromatographic) separation mechanisms, and methods of detection can be used.

2 Electrophoresis *versus* Chromatography

In electrophoretic experiments carried out in free solutions, chromatographic retention behavior almost always hampers the separation process and is therefore seldom applied. The use of (quasi) stationary phases, *e.g.* cyclodextrins and crown ethers or phases on the solid wall, may sometimes improve separation.

From the other hand, it is well known that due to the mechanical transport in chromatographic processes, streaming potentials and zeta potentials are induced. Here too, these "unwanted" effects can hamper the separation and can cause severe problems, *e.g.* in the packing of HPLC columns.

Electrophoresis, carried out in free solutions, can be considered as an electric field induced transport of electrically charged species in a system that consists of solutions of electrolytes with initially arbitrary local concentrations: anions migrating to the anode and cations migrating to the cathode.

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Equivalent to chromatographic separations, several electrophoretic migration modes can be present in a single electrophoretic experiment. Depending on the separand of interest, one might then call the experiment zone electrophoresis, isotachophoresis, or moving-boundary electrophoresis.

Zone electrophoresis is the most common and most widely applied electrophoretic principle. Numerous zone electrophoretic techniques and procedures have been developed, mainly on an empirical basis, especially for the separation of proteins. Being a zonal separation principle, zone electrophoresis allows a separand to form a zone separated from other zones by the so-called carrier electrolyte. Broadening of the separand zones in zone electrophoresis is caused by diffusion, convection, reversible adsorption, and often electroosmosis.

In zone electrophoresis, inhomogeneity of the electric field in the zone profile leads to non-symmetrical concentration distributions. This leads to so-called boundary anomalies, in which the migration velocity is a function of concentration (problems in retention times may be the result). In the interst of electrophoretic performance, these boundary anomalies have to be avoided.

Although there is a close analogy between elution chromatography and zone electrophoresis, some methodological differences exist. The most profound difference is that Ohm's law must hold in electrophoresis and that the resulting *Kohlrausch* [7] regulating functions govern the electrophoretic process.

Under operational conditions, aqueous solutions, room temperature, and concentrations of the solutes of *ca.* 10^{-1} - 10^{-3} M, ionic mobilities, μ, (in 10^{-9} m^2 V^{-1} s^{-1}) have values of $0<|\mu|<100$, with exception of some mobile ions like OH^- and H^+. E may easily reach a value of 10^4 V m^{-1}. The electrophoretic velocities $v_{eff} = \mu \cdot E$ in ms^{-1} (where E is the electric field in $V.m^{-1}$) in capillaries then amount to $0<|v_{eff}|<10^{-3}$. Of course μ is affected by factors such as pH, solvent, or complexation. Electroosmosis is generally suppressed in electrophoretic experiments by using surface active compounds or stabilizing with gels, because small differences in mobility and thus small differences in velocity demand, in "classic" electrophoretic separation techniques, an abolishment of any mechanical liquid transport during separation, even if (sieving) gels are applied. Sometimes in ITP however, counterflow of electrolyte is used [5, pp. 375 *et seq*].

For small solutes *(e.g.* alkaline earth metals or all sorts of isomers) the use of quasi-stationary phases (crown ethers, cyclodextrins) opens the way to chromatographic interaction in electrophoretic separations.

In chromatography also effective velocities are used taking into account the time a compound is retained due to chromatographic interaction.

Therefore, one way to characterize chromatographic and electrophoretic separation techniques results on comparing their effective velocities, as shown in **Figure 1**.

On the ordinate the "purely" electrophoretic techniques and on the abscissa the purely chromatographic techniques can be found. The two-dimensional field indicates all transition areas. In these transition areas the quasi-stationary phases stand for substances such as crown ethers and cyclodextrins, as used for separations in capillary zone electrophoresis and isotachophoresis. The stationary phases refer to the phases commonly used in liquid chromatography.

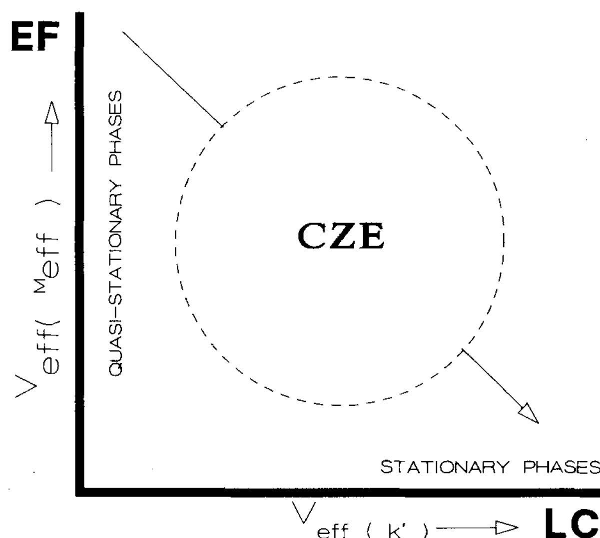

Figure 1

Effective electrophoretic velocities *versus* effective chromatographic velocities. The two dimensional field indicates all transition areas.

If in CZE (as performed in capillaries with internal diameters of *ca.* 50 µm and even smaller) electroosmotic flow is applied to transport simultaneously the various solutes (cationic, anionic, and non-ionic) and the linear velocities of the separands reach a multiple of the electrophoretic velocities, the separations performed in these capillaries have to be considered as chromatography, especially as far as separation of non-ionic solutes are concerned.

If the internal diameter of the capillary increases to values >200 µm, the contribution of electroosmotic flow to transport of the solutes decreases and hence the influence of the "purely" electrophoretic parameter increases.

Figure 2 gives a few possibilities of solute interactions if both electroosmotic flow and electric field strength cause migration of these solutes.

In the separation we can distinguish three solutes fractions *viz.* the anionic, the non-ionic, and the cationic. This contrasts with "pure" zone electrophoretic experiments, where generally cations or anions are separated. Depending on the choice of operating conditions, ampholytes behave as cations or anions. Uncharged solutes, of course, cannot be separated by classical electrophoretic techniques.

Obviously the capillary wall needs to be charged in such analyses because no electroosmotic flow remains if the charge of the wall approaches zero.

The charge at the wall can be chemically bound or result from charge separation of the carrier buffer and the wall. Also, additives present in the carrier buffer, such as SDS, CTAB, polyvinyl alcohol, polyvinylpyrollidone, or derivatives of cellulose may exert a similar effect. The charge at the wall determines the direction of the electroosmotic flow, due to the electric double layer formed. Because this double layer is much thinner than the

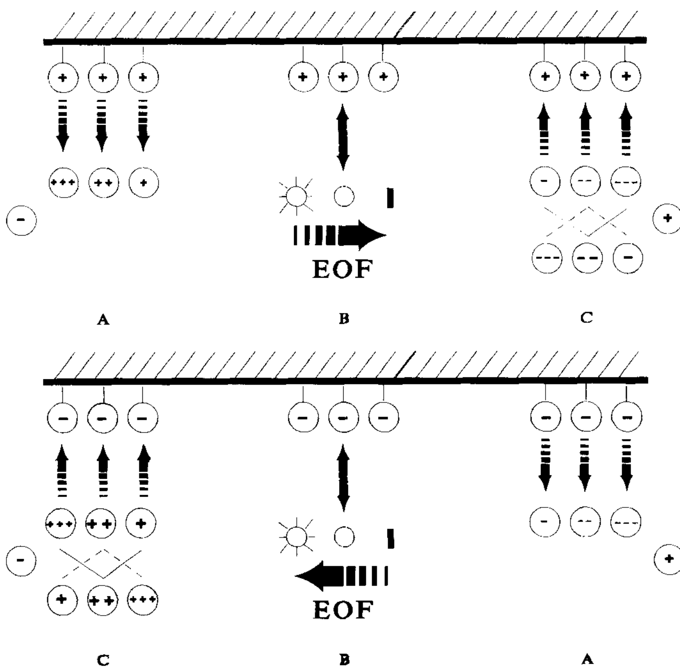

Figure 2

Separation of anionic, non-ionic and cationic solutes in CZE and electrochromatography in capillaries, in which electroosmotic flow is applied (EOF). A: upstream; B: midstream; C: downstream.

inner diameter of the capillary tube, the capillary wall can be considered to be flat, which also leads to a flat velocity profile (non-turbulent piston or plug flow). In capillaries with negative charge at the wall the electroosmotic flow is directed to the cathode, if the wall carries a positive charge the flow is directed to the anode.

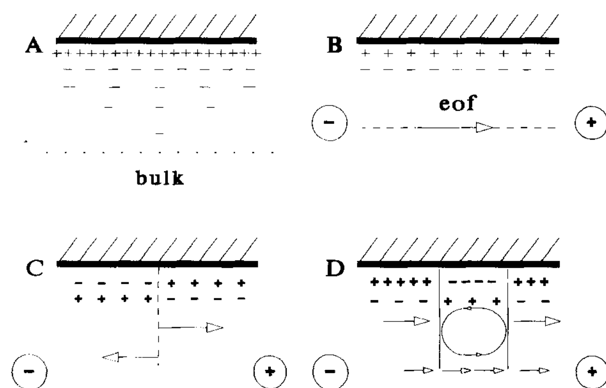

Figure 3

Electroosmotic flow in capillaries.

(a) Charge distribution resulting in an electrical double layer between insulator and solvents.

(b) The average electroosmotic flow is constant because pure solvents are used.

(c) The average electroosmotic flow is not constant due to change of zeta potential of the wall. The flow can even reverse during the experiment. The double layer may also change gradually during an experiment.

(d) The average electroosmotic flow is constant, but due to local changes in pH or the presence of surface active compounds, locally the electroosmotic flow changes.

The electroosmotic velocity is generally expressed by the Helmholz-Smoluchovsky equation, $v_{eo} = \varepsilon\,\xi\,E/4\,\pi\,\eta$, where v_{eo} is the electroosmotic flow velocity, ε is the dielectric constant, ξ is the zeta potential, E is the electric field strength, and η is the value of the viscosity of the liquid. This means, however, that the electroosmotic flow can easily be changed (**Figure 3**) due to the presence of surface active substances in the carrier buffer, and can even reverse direction.

The separands may change the local electroosmotic flow [8], although the average flow often remains constant. It is clear that this will lead to disturbance of the zone boundary in isotachophoresis. In zone electrophoresis it may counteract the disturbance by diffusion. The retention times of the separands may vary and can strongly depend on the concentration of the other separands.

That concentration and pH of carrier buffers influence the electroosmotic flow in capillaries has been described elsewhere [9].

The transport of the non-ionic solutes can be compared with common liquid chromatographic procedures because, except for the electroosmotic flow their transport is unaffected by the electric field strength. These solutes have been used as markers for electroosmotic flow velocity (v_{eo}) determinations [10,11], but a chromatographic retention during this transport will result in inaccurate electroosmotic flow measurements if $k' > 0$ and if this can be measured,. New devices for measurement of the electroosmotic flow [12-14] are under construction.

If non-ionic solutes are ionized through solvent association and/or correctly chosen ionic complexing agents, they can be treated as cations or anions.

In **Figure 4** schematically more detailed information is given from the upper part of Figure 2. If the electroosmotic flow is directed to the anode, the anionic solutes attain a velocity which is the vector sum of the contribution by the electric field, the electroosmotic flow, and chromatographic retention. The more charges such as solute has, the higher the electrophoretic velocity will consequently be. The contribution by the average electroosmotic flow is constant for all solutes: anionic; non-ionic; and cationic. Interaction with the charged wall is favored for those solutes which carry the highest charge of opposite sign. This almost always counteracts migration.

For the cations the opposite holds. The velocity attained by the electroosmotic flow is opposed by the electric field, while the cations are repulsed by the wall: this can favor the separation.

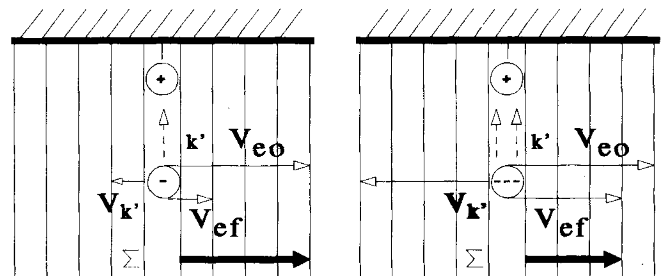

Figure 4

Schematic view of effects that influence the transport of solutes in CZE.

It will be clear that other interactions, *e.g.* complex interaction, can influence the separation via both the electrophoretic and the electroosmotic contribution.

3 Conclusions

CZE with and without non-suppressed electroosmotic flow can be classified as a powerful separation technique, developed in an increasing number of laboratories, *e.g.* [10,11,15-24]. Due to the separation mechanism used and the difference in transport velocity of electrophoretic and electroosmotic mechanisms, it seems appropriate to use, *e.g.*, the name electro (osmotically driven) chromatography [25,26], especially if separations are carried out in capillaries with diameters of 50 μm or less and electroosmotic flow dominates the transport mechanism of the solutes. Electrokinetic chromatography [27] assigns too great a contribution to electrokinetic separation techniques, as compared to chromatographic contributions, although only the electroosmotic flow is applied. The CZE performed in capillaries of > 200 μm and when electroosmotic flow is suppressed [28-34] has to be considered as a "purely" electrophoretic separation technique and classified as CZE.

Abbreviations

CTAB	Cetyltrimethylammonium bromide
CZE	Capillary Zone Electrophoresis
EF	Electrophoresis
EOF	Electroosmotic flow
HPLC	High Performance Liquid Chromatography
ITP	Isotachophoresis
LC	Liquid chromatography
SDS	Sodium dodecyl sulfate
k'	chromatographic retention constant
v_{ef}	electrophoretic velocity
v_{eff}	effective velocity
v_{eo}	average electroosmotic velocity
$v_{k'}$	decrease in velocity due to chromatographic reactions

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