Capillary Electrophoresis

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Key Words

Capillary electrophoresis
Isotachophoresis
Displacement electrophoresis
Capillary zone electrophoresis

Summary

The principles of capillary zone electrophoresis (CZE) and isotachophoresis (ITP) are reviewed. Temperature effects occurring during the passage of an electric current through a capillary are discussed. Special emphasis is directed to migration and dispersion processes in free CZE, including electroosmotic flow. Instrumental aspects are discussed and applications of CZE are presented.

Introduction

Electrophoretic techniques are separation methods of high efficiency which are of extreme importance in the field of chemical and biochemical analysis. They may be classified into two groups with respect to instrumentation: classical electrophoresis and capillary electrophoresis. The classical techniques use stabilizing media; in capillary electrophoresis tubes of small inner diameter are used to decrease the negative influence of temperature gradients on the separation efficiency. The practical impact of this is discussed below.

Temperature Effects in Electrophoresis

During electrophoretic separation Joule's heat is produced, leading to temperature gradients in the system, accompanied by gradients in density, viscosity and mobility, thus increasing dispersion. It is known, for example, that the mobility increases by two percent when the temperature is raised by 1 °C. To decrease the resulting mixing by convection the classical techniques use stabilizing media e.g. paper, cellulose acetate, starch, agarose and polyacrylamide gels. A disadvantage of these classical techniques is that manipulation times are long, detection is tedious, quantification is difficult and automation is, practically, not possible.

The alternative approach, used in capillary electrophoresis to reduce convective dispersion resulting from temperature gradients, is to minimize the diameter of the separation capillary. It is known that the temperature difference ΔT between the center of the capillary and its wall is proportional to the square of the diameter of the capillary. The relationship in the case of an infinately long cylinder within which heat is generated homogeneously and is carried off by conduction through the walls is in accordance with [1]:

$$\Delta T = W \cdot d_c^2/16 \cdot K \quad (1)$$

where W is the rate of heat generation per unit volume within the cylinder, K is the thermal conductivity of the medium and d_c is the inner diameter of the capillary.

For the case where heat is generated from the flow of current through the capillary the heat output W is given by:

$$W = E^2 \cdot \lambda \cdot c \cdot \varphi \quad (2)$$

where c is the molar concentration of the electrolyte, λ is the equivalent conductivity, E is the electric field strength and φ is the total porosity of the medium (φ = 1 for an open tube).

Combining these two equations leads to

$$\Delta T = E^2 \cdot \lambda \cdot c \cdot d_c^2/16 \cdot K \quad (3)$$

for open tubes. It can be concluded from the above equation that the diameter of the capillary has to be reduced to a few hundred µm, or less, in order to be able to work at the desired high potential of several ten thousand Vm^{-1} without a loss in efficiency due to a temperature excess in the center of the capillary.

Although it has been shown [1] that temperature differences inside capillaries are rather small (less than 1 K under realistic conditions), they can be as high as several tens of degrees between the wall of the capillary and the surrounding air. Therefore an effective thermostatting device is important in order to obtain sufficiently reproducible migration times over a long period of use. A schematic representation of the temperature gradients occurring from heat generation during the passage of an electric current in an open tube is shown in Figure 1 [2].

In isotachophoresis (displacement electrophoresis) capillaries – usually made of Teflon – have been used

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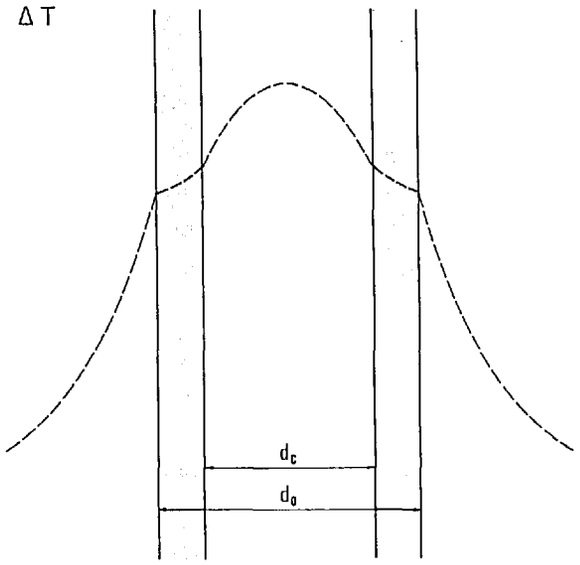

Figure 1
Temperature excess in a capillary filled with electrolyte solution when ohmic heat is released.
d_o and d_c are the outer and the inner diameter, respectively, of the capillary; ΔT is the temperature difference in arbitrary units. According to ref. [2].

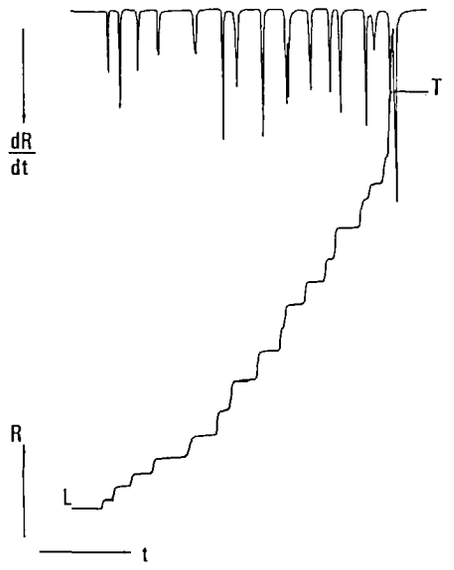

Figure 2
Isotachopherogram of 14 anions as obtained by conductivity detection.
Capillary: polytetrafluoroethane, 30 cm length, 300 µm inner diameter; leading electrolyte: chloride/histidine, 0.01 mol/l, pH 6.0; terminating electrolyte: MES/TRIS, 0.01 mol/l, pH 7; 50 µA constant current.
t = time, R = resistance. L and T are the leading and the terminating ions.

for more than 20 years. In zone electrophoresis the use of capillaries was also proposed in the sixties [3] and the seventies [4, 5], but they have found extended application only since the beginning of the nineteen eighties [6]. It should be mentioned that the instrumentation used for isotachophoresis and zone electrophoresis can also be adapted thus allowing isoelectric focusing to be carried out in the capillaries [7].

Capillary Isotachophoresis and Zone Electrophoresis

The difference between isotachophoresis (ITP) and zone electrophoresis (CZE) lies in the choice and the positioning of the buffer electrolytes. In isotachophoresis two electrolytes are used in the separation system, the leading electrolyte (the leading ion having the highest mobility with respect to all ions of interest) followed by the terminating electrolyte (the terminating ion having the lowest mobility). The sample is injected between these two electrolytes. During separation the electric current is kept constant and the electric field increases according to Ohm's law with decreasing mobility (conductivity) of the consecutive zones. Separation is achieved due to the different velocities v_i of the analyte ions i in the mixed zone formed by the sample:

$$v_i = m_i \cdot E^{(mix)} \tag{4}$$

where $E^{(mix)}$ is the electric field strength in the mixed zone and m_i is the mobility of the species i in this zone. The mobility m_i is thus the physico-chemical property that is responsible for the separation of the analytes. When the sample ions have separated, they all migrate with the same velocity. The resulting concentration profile is rectangular in shape, because the steady state concentration in the sample zones is adapted to the concentration of the leading ion according to the Kohlrausch regulating function.

$$c_A = c_L \frac{m_A \cdot (m_L + m_Q)}{m_L \cdot (m_A + m_Q)} \tag{5}$$

where c_A is the concentration in the zone of the analyte A, c_L is the concentration of the leading electrolyte, m_A, m_L and m_Q are the mobilities of the analyte A, the leading ion L and the counter ion Q in the steady state.

Therefore isotachophoresis is an enrichment method in those cases, where the original concentration of the analyte in the sample is lower than the concentration in the steady state. In isotachophoresis the detection principle mainly used is conductivity, but UV-absorption is also commonly used. A typical isotachopherogram obtained with conductivity detection is shown in Figure 2.

In capillary zone electrophoresis, in contrast to isotachophoresis, the separation system is filled with a single electrolyte of relatively high concentration so that the electric field strength is maintained constant over the entire capillary, a uniform diameter being assumed. Separation takes place due to the different migration of the analyte ions according to Eq. (6), which is given below. Dispersion effects cause band broadening of the analyte zones, acting against separation. Differences of the migration distances are directly proportional to separation time, but peak broadening of the analyte zones (based on space) increases only by the square root of the analysis time: thus separation can be achieved, provided that the

difference in the mobilities is sufficient. The parameters, which are decisive for these two processes in capillary zone electrophoresis will be discussed in the following paragraphs.

Migration: The entire system is uniformly filled with a single electrolyte of relatively high concentration, usually between 0.01 and 0.1 mol/l, which determines the electrical properties of the system as well as the pH. When an electrical potential U is applied to the capillary of length L, and electric field of uniform strength E is established, given by E = U/L.

Ions of the species i of a strong electrolyte migrate electrophoretically in the capillary towards the corresponding electrodes with a velocity v_i, which is, inaccordance with Eq. (4), proportional to the electric field strength E:

$$v_i = m_i \cdot E \tag{6}$$

Different mobilities of the sample components lead to different migration velocities and therefore the individual sample species reach the detector at different times, t_i, given by

$$t_i = L/v_i = L/m_i \cdot E \tag{7}$$

The difference in the migration times, that is the time difference of the peak maxima of compounds i and j, is thus given by

$$\Delta t_{ij} = t_i - t_j = (L/E)\,(m_j - m_i)/(m_j \cdot m_i) \tag{8}$$

In the case of weak electrolytes the parameter which is decisive for separation is not the mobility of the totally ionized particle but the effective mobility m_i^{eff}. This is related to m_i (the mobility of the fully dissociated species i) by the degree of dissociation, α, given by

$$m_i^{eff} = m_i \cdot \alpha \tag{9}$$

Dispersion: Dispersion effects lead to band broadening of the analyte zones. The resulting concentration profile is approximated, as in chromatography, by a Gaussian distribution function, at least in those cases where the conductivity of the sample injected is the same as that of the carrier electrolyte. In contrast to chromatography, where four dispersion processes can be distinguished, i.e. longitudinal diffusion, dispersion due to the flow profile of the mobile phase and dispersion due to the mass transfer in the mobile and in the stationary phase, in pure CZE band broadening occurs only due to a single effect: longitudinal diffusion. Therefore in CZE the dispersion characteristics are more favourable compared to HPLC.

Making the assumption that the sample is introduced into the capillary in a very narrow profile and that no other processes except longitudinal diffusion contribute to the peak dispersion, then the width of the concentration distribution of a sample zone, expressed by the variance based on space σ_z^2, increases linearly with the migration time t

$$\sigma_z^2 = 2 \cdot D \cdot t \tag{10}$$

where D is the diffusion coefficient of the sample component and σ_z is the standard deviation of the Gaussian peak.

In chromatography the dispersion characteristics of a separation system are usually described by the "height equivalent to a theoretical plate", H, which relates the variance based on space to the migration distance z.

$$\sigma_z^2 = H \cdot z \tag{11}$$

From Eqs. (10) and (11) an expression for H in zone electrophoresis can be obtained by substituting z/t by $m \cdot E$:

$$H = 2 \cdot D/m \cdot E \tag{12}$$

The number of theoretical plates N = L/H is thus given by [8, 9]

$$N = m \cdot U/2 \cdot D \tag{13}$$

From this equation it can be concluded that the separation efficiency can only be optimized by varying the applied voltage, as m and D are not properties of the system but of the sample. An excessive increase in voltage is counteracted, however, by the resulting higher temperature gradients. Furthermore, m and D are related according to the Einstein-Nernst equation as follows:

$$D/m = k \cdot T/Q \tag{14}$$

where Q is the electric charge of the particle, k is the Boltzmann constant and T is the absolute temperature. Thus, the plate number, N, given by

$$N = U \cdot Q/2 \cdot k \cdot T \tag{15}$$

is only dependent on the charge and not on the size of the sample ion.

The high separation efficiency, which can easily be achieved in capillary zone electrophoresis can be seen from the electropherogram in Figure 3. The number of theoretical plates calculated from this electropherogram is about 150,000, obtained with a capillary of 75 µm inner diameter and an applied voltage of 20 kV (H ~ 3.5 µm) [10].

Electroosmosis: The sample components, which are shown in the electropherogram in Figure 3, are neutral and anionic species and are introduced into the capillary (made of fused silica) at the side of the anode. They can be detected at the side of the cathode, because of an additional flow of the liquid, the electroosmotic flow, which is directed towards the cathode in the case of capillaries made of fused silica.

Electroosmosis occurs because the acidic silanol groups at the surface of the fused silica capillary dissociate when in contact with an electrolyte solution. The negative charges formed lead to an electric double layer between the surface of the capillary and the electrolyte solution, which is characterized by the zeta potential. When an electrical potential is applied to the capillary, the electrophoretic velocity of the ions is superimposed on the velocity vector resulting from the electroosmotic flow of the bulk liquid, which is equal for all species and is directed, for the material described, towards the cathode. The electroosmotic velocity v_{eo} is given by the following relation

$$v_{eo} = \varepsilon \cdot \zeta \cdot E/4 \cdot \pi \cdot \eta \tag{16}$$

Figure 3

Effect of electroosmosis on the migration of anionic and neutral compounds: zone electropherogram of commercially available riboflavin-5′-phosphate.

All compounds detected are moving towards the cathode. Neutral riboflavin is detected at 9.67 min, the main component is riboflavin-5′-phosphate; compounds with longer migration times are other riboflavin-phosphates.

Capillary: fused silica, 96 cm length, 100 µm i.d., buffer electrolyte: 10^{-2} M KCl/10^{-3} M phosphate, 20 % methanol, pH = 7.5; voltage: 20 kV; detection: fluorescence. For details, see ref. [10].

where ε is the dielectric constant of the liquid in the region of the electric double layer, η is the viscosity coefficient in that region and ζ is the zeta potential.

The total velocity is therefore higher for cations and lower for anions than their electrophoretic velocity. Neutral molecules move with the same velocity as the electroosmotic flow. The migration sequence is thus: cations, neutral molecules and anions. Only those anions with an electrophoretic velocity lower than the electroosmotic velocity reach the detector.

The electroosmotic flow is dependent on the concentration and the pH value of the buffer electrolyte, corresponding to the degree of dissociation of the silanol groups. It can be seen from Figure 4 that the electroosmotic velocity between pH 3 and pH 12 is, under the given conditions, in the order of 0.3 to 2.2 mm/s.

In the above discussion of dispersion effects only electrophoretic migration of the ions was assumed, so that whilst longitudinal diffusion was considered any dispersion due to the flow profile of the buffer electrolyte was not. The electroosmotic flow of the liquid gives rise to an additional contribution to dispersion but, in contrast to hydrodynamic flow electroosmosis, shows a much more favourable flow profile. This is shown in Figure 5 together with the flow profile resulting from laminar flow due to a pressure drop (Poisseulle flow). In contrast to the parabolic profile of the hydrodynamic flow, a plug-like profile is characteristic for electroosmosis (provided that the thickness of the double layer is much smaller than the inner diameter of the capillary), which leads to an essentially smaller contribution to peak dispersion [11, 12]. For this reason electroosmosis is also used in place of a pressure difference in liquid chromatography, to give the method named electrokinetic chromatography.

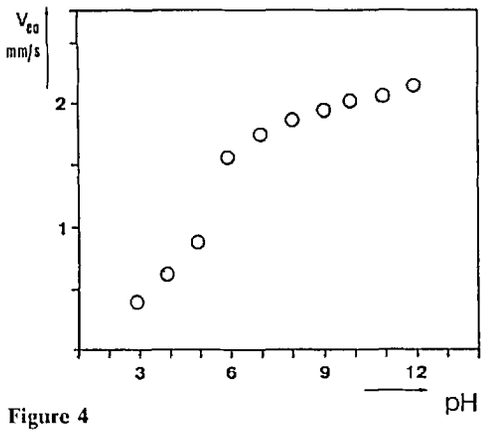

Figure 4

Dependence of the electroosmotic flow, v_{eo}, on the pH of the buffer electrolyte.

Velocities were measured in a fused silica capillary (100 µm i.d., total length 96 cm); the applied voltage was 20 kV. Electrolyte: 10^{-2} M KCl/10^{-3} M phosphate. As marker substances for the electroosmotic flow umbelliferon, riboflavin or 4-nitroaniline were used depending on the pH of the electrolyte.

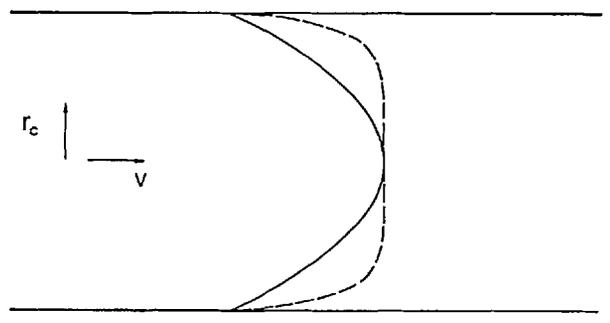

Figure 5

Flow profile of a fluid medium in an open tube with electroosmotic or hydrodynamic (laminar) flow.

v = velocity of the medium, r_c = radius.

dashed line: profile of the electrosomotic flow (as obtained under the conditions of analytical CZE);

full line: profile of the laminar hydrodynamic (Poiseulle) flow resulting from a pressure drop.

Considering the electroosmotic velocity, the expression for the number of theoretical plates can be modified as follows [9]

$$N = (m_i + m_{eo}) \cdot U/2 \cdot D \qquad (17)$$

where m_{eo} can be considered the "electroosmotic mobility" of the bulk liquid analogous to the electrophoretic mobility m_i.

Instrumental and Applications of Capillary Zone Electrophoresis

Before commercial instruments became available within the last year or two capillary zone electrophoresis was carried out in home-made instruments. All instruments, home-made or commercial, have in common the same simple setup as shown in Figure 6. They consist of a separation capillary, a power supply unit, a device for introducing the sample and a detector with a data output. The separation capillary, sample introduction and the detection unit are described in more detail in the following sections.

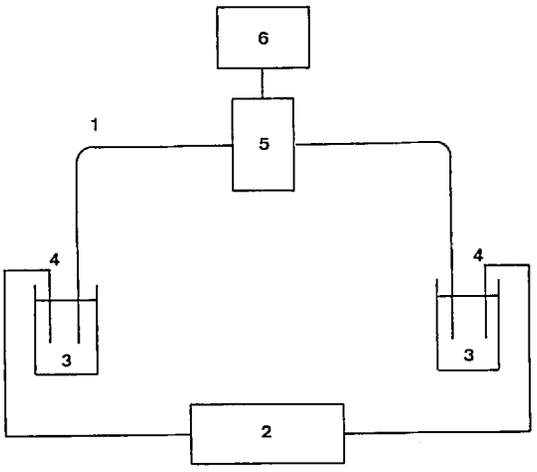

Figure 6
Schematic representation of the instrumentation used for capillary zone electrophoresis.
1 = separation capillary, **2** = high voltage power supply unit, **3** = electrolyte or sample vessels, **4** = electrodes, **5** = detector, **6** = recording device.

Capillaries

Separation capillaries are almost always made from fused silica (in contrast to isotachophoresis, where Teflon capillaries are used). The length of the capillaries varies between about 10 and 100 cm, the inner diameter is between 10 and 150 µm. Open tubes are used for free zone electrophoresis, either without any surface treatment if electroosmotic flow is desired, or with the inner wall modified, so that the zeta potential is eliminated or the chemical activity of the surface is reduced. Gel filled tubes are mainly used for the

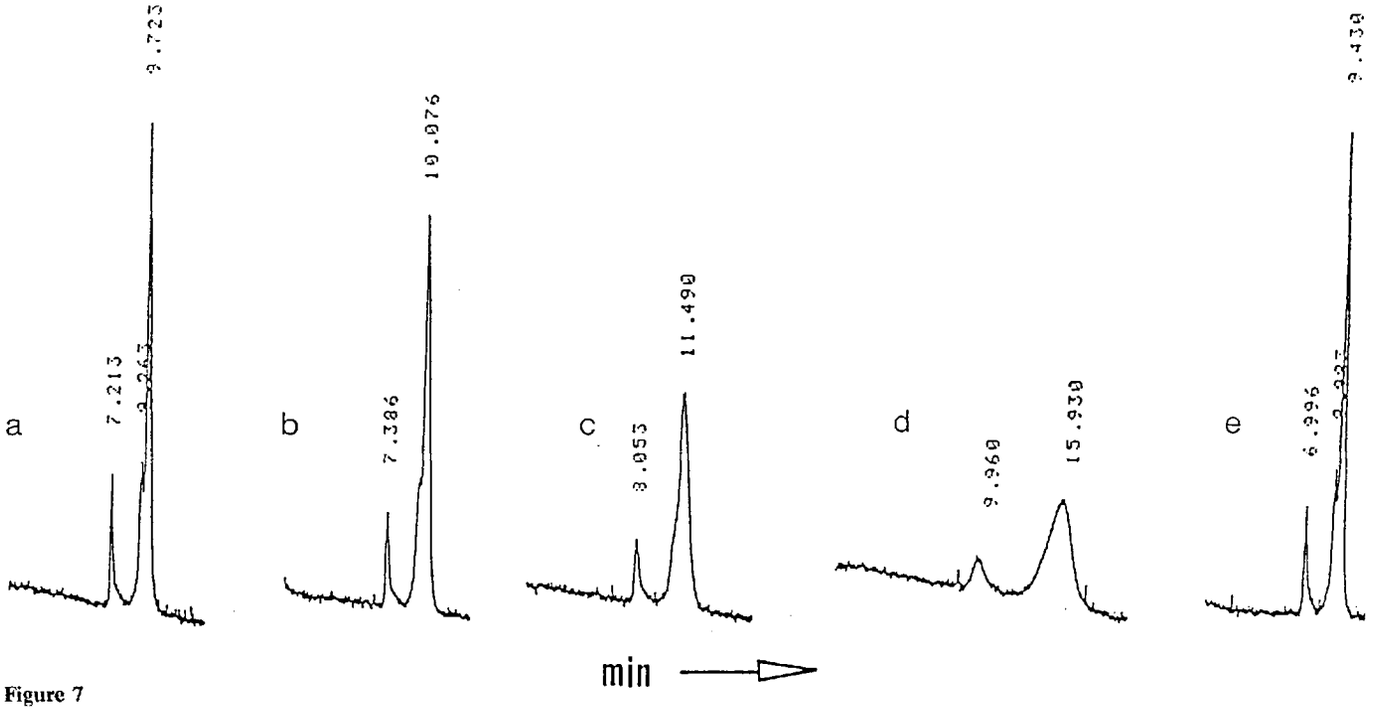

Figure 7
Effect of adsorption of proteins to the wall of a fused silica capillary.
A sample, consisting of two proteins (conalbumin with a migration time of 7.2 min in **a** and ovalbumin having peaks at 9.7 and 9.3 min) were separated in a fused silica capillary by zone electrophoresis. The electropherograms **a–d** were obtained without any rinsing steps between runs. An increase in migration times as well as a deterioration of the separation efficiency due to adsorption of the proteins to the wall can be observed. Rinsing the capillary with NaOH solution (0.1 M) before run **e** restores the initial separation conditions.
capillary: 100 µm i.d., total length 100 cm; electrolyte: 0.01 M borate, pH = 9.1; applied voltage 17.5 kV; detection: fluorescence (280/340 nm). Reprinted with permission from ref. [32].

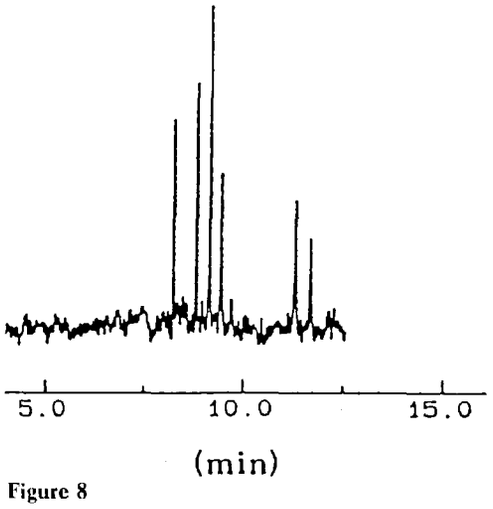

Figure 8

Separation of model proteins.

Elution order: myoglobin (whale skeletal muscle), myoglobin (horse heart), carbonic anhydrase B, carbonic anhydrase A, β-lactoglobulin B, β-lactoglobulin A.

Electrolyte: tricine/KCl, 10/20 mM, pH = 8.22; voltage 20 kV; capillary: overall length 101 cm, length to detector 55 cm, i.d. 52 µm. Reprinted with permission from ref. [12].

separation of oligonucleotides and proteins according to their molecular weight.

With proteins especially, interactions with the untreated wall can lead to poor reproducibility of migration times because of changes in the zeta potential and consequently also in the electroosmotic flow; on the other hand the separation efficiency decreases due to adsorption of the proteins onto the wall. Both effects can be seen in Figure 7: an increase in migration times of the proteins, which are anionic under the given conditions, caused by a decrease in the electroosmotic flow and a drastic broadening of the analyte peaks (a–d). Regeneration of the surface by rinsing the capillary with a solution of NaOH (0.1 mol/l) between runs d and e resulted in regaining the initial conditions, so that the electropherogram e shows the same migration times and efficiency as the first electropherogram a. Such rinsing steps can be done automatically after every run on most commercial instruments. With good optimization of the separation parameters a high separation efficiency can also be obtained for proteins [13]. An electropherogram of model proteins under optimized conditions is shown in Figure 8.

Alternatively, it is possible to prevent unwanted interactions with the wall by deactivating the surface, for instance by coating the capillary with polymer or by chemical modification of the silanol groups (14, 15].

Injection

The problem of introducing the sample in capillary zone electrophoresis arises from the fact that only a very small volume of the sample may be injected, much less as can be handled by the devices, such as syringes or injection loops, used in chromatography. This problem is high-lighted by considering that the total volume of a separation capillary of an inner diameter of 100 µm and of 50 cm length is only about 4 µl. Because of the extremely high separation efficiency of the method the peak volume of a sample component is very small (about 10 nl for the above capillary and a plate number of N = 100,000). It is obviously that, because of the additivity of variances, the sample volume has to be in the low nanoliter range if the high separation efficiency is to be maintained. Therefore direct techniques of sample introduction are used. Thus the sample may be introduced via electromigration (electrophoresis and electroosmosis simultaneously). This very simple method has the disadvantage of being discriminating, which means that the rate of which ions are introduced also corresponds to their charge and mobility. Further the amount of analytes introduced depends on the composition of the sample, and is influenced by the amount of other ionic components present, thus making quantitation difficult.

Another way of introducing the sample is by means of a hydrodynamic flow, which is achieved by a pressure drop. This allows the sample to be sucked or pressed into the capillary without any discrimination. This method is only affected by differences in the viscosity of the sample. Quantitation is better than with electromigration techniques, but the instrumental design is more complicated.

A further means of sample introduction uses a combination of isotachophoresis and capillary zone electrophoresis [16]. The advantage of this technique is that

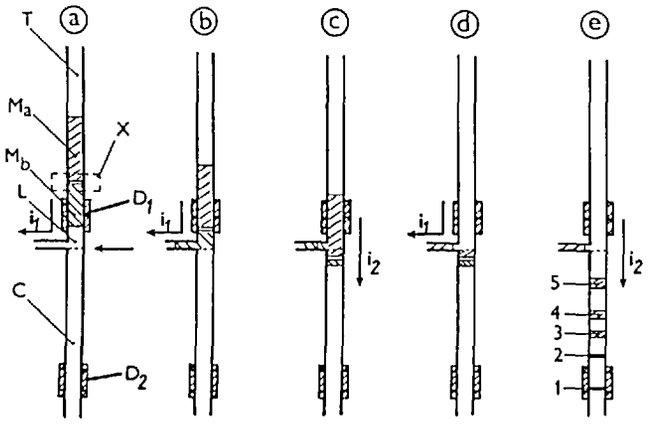

Figure 9

Column coupling of isotachophoresis (ITP) and capillary zone electrophoresis (CZE): schematic illustration of the separation phases.

a) ITP stage: isotachophoretic separation in the first capillary
b) removal of matrix constituents (M_b) from the separation compartment.
c) transfer of the sample fraction containing the analytes (X) into the second capillary
d) removal of matrix components (M_a)
e) CZE stage: zone electrophoretic separation in the second capillary

L = leading electrolyte, T = terminating electrolyte; D_1, D_2 = detectors; i_1, i_2 = directions of the driving current; 1–5 = separated sample components in the CZE stage. Reprinted with permission from ref. [15].

by isotachophoresis a separation of the analytes from most of the matrix compounds as well as a preconcentration can be achieved. The sample volume which is transferred into the second capillary, in which the zone electrophoretic separation is carried out, is of the order of a few nl. A schematic illustration of the separation phases is given in Figure 9.

Detection Units

The requirements on the volume of detector cells are the same as for injectors; these volumes have to be very small, so that no significant contribution to the peak dispersion arises within the detector. The detectors most frequently used are optical detectors using UV-absorption or fluorescence. When comparing the sensitivity of optical detectors, the extremely short absorption path length has to be taken into consideration, because this kind of detection is done on-column with a segment of the capillary being used directly as the detector cell. For UV-absorption, where the intensity of the light beam passing the capillary is measured, the fused silica material which is most frequently used for capillaries, shows optical properties very favourable for this kind of detection. UV-detection can be applied e.g. for the direct determination of underivatized proteins. The detection limit in the case of lysozyme is found to be about 20 fmol (229 nm absorption wave length, inner diameter of the capillary 75 µm) [17].

When fluorescence is used for detection the sensitivity is much higher than with UV-absorption, but the applicability is rather limited, because with only few compounds showing native fluorescence, derivatization becomes necessary. A direct determination is possible for tryptophan-containing proteins. For example with trypsinogen a detection limit of 4 fmol was found using a capillary of 75 µm inner diameter [18]. The sensitivity can be further increased if the intensity of the excitation light source is enhanced by using a laser. With an argon ion laser an extremely low detection limit of 10^{-21} mol for fluorescein isothiocyanate derivatives of amino acids was reached [19]. For proteins and peptides having no intrinsic fluorescence, a post-column reaction laser-induced fluorescence detector is described, giving a calculated detection limit of 4.4×10^{-17} mol for horse heart myoglobin [20]. These devices, however, are not at present commercially available.

Other detectors described in the literature include the electrical conductivity detector [21], which is usually applied for isotachophoresis, but is of only limited use in capillary zone electrophoresis. For the determination of electrochemically active compounds an amperometric detector is described. An electropherogram produced by this method is shown in Figure 10 [22]. The detection limit reached was 0.7 attomol for serotonin in a capillary of 9 µm inner diameter. A semiconductor radioisotope detector has been suggested for the detection of radioactive compounds [23].

Coupling capillary zone electrophoresis to a mass spectrometer has already been demonstrated by

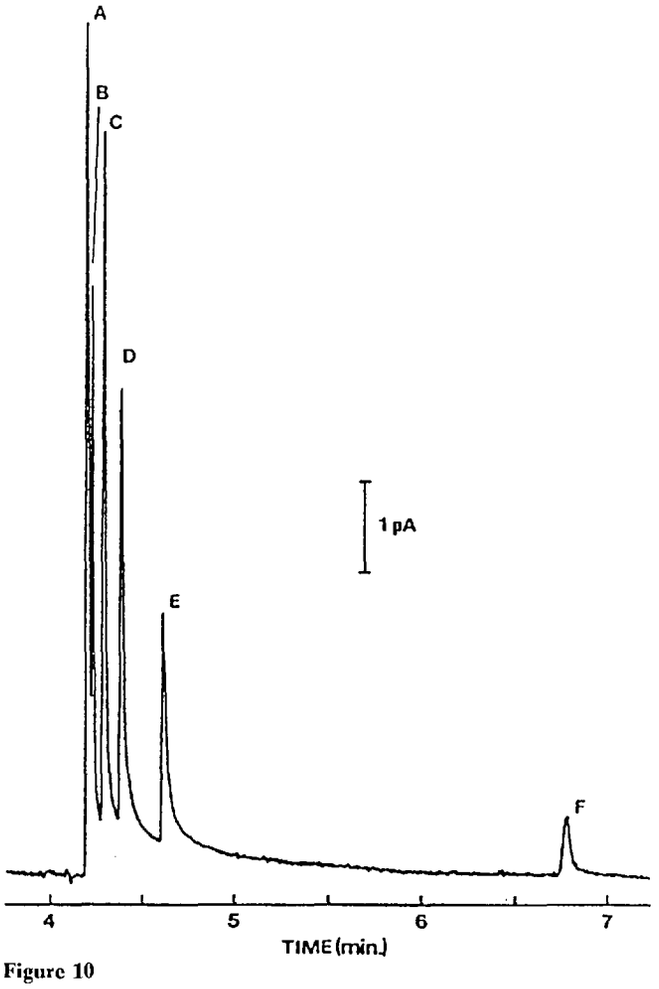

Figure 10

Electropherogram of amines in a capillary of 9 µm i.d. with amperometric detection. Peaks: **A** = dopamine, **B** = serotonin, **C** = norepinephrine, **D** = epinephrine, **E** = isoproterenol, **F** = dihydroxyphenylalanine. Capillary: 9 µm i.d., 68 cm length; electrolyte: 0.025 M MES, pH = 5.5; voltage 30 kV. Reprinted with permission from ref. [21].

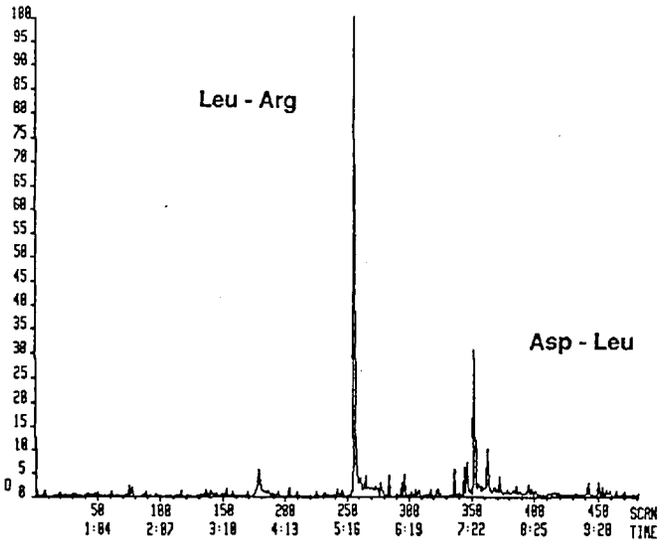

Figure 11

Summed ion electropherogram of the $(M+H)^+$ ions of dipeptides. Electrolyte: ammonium acetate, 0.005 M, pH = 8. Reprinted with permission from ref. [23].

several groups [24, 25]. An example of a direct on-line combination with fast atom bombardment tandem sector mass spectrometry is shown in Figure 11 [24].

Selectivity

In electrophoresis the selectivity of the separation is determined by differences in the effective mobilities of the analytes. The most common way of influencing the selectivity is by changing the degree of dissociation of the sample components by varying the pH of the buffer electrolyte (Eq. (9)). Further possible ways of changing the selectivity, already described in isotachophoresis, are complexation, solvation, interactions with counter ions of different charge and ion-ligand interactions. In addition, in capillary zone electrophoresis new mechanisms which may enhance selectivity, other than varying the degree of dissociation, have been suggested. Of importance is the application of capillaries filled with polyacrylamide gel, in analogy to the classical polyacrylamide gel electrophoresis (PAGE), for the separation of proteins according to their molecular weight and not because of their different mobilities [26]. Such gel-filled capillaries are also utilised for the resolution of oligonucleotides as shown in Figure 12 [27].

The addition of detergents to the buffer electrolyte in concentrations above the critical micelle concentration leads to the formation of micelles, which are negatively charged when sodium dodecylsulphate is used, and which may act as a quasi-stationary phase. If neutral, or in some cases also anionic, analytes are distributed to a different degree between buffer electrolyte and micelles, separation can be achieved. This technique is called "micellar electrokinetic capillary chromatography". It will not be discussed here further, but is is discussed in a review article which deals with this topic in some detail [28].

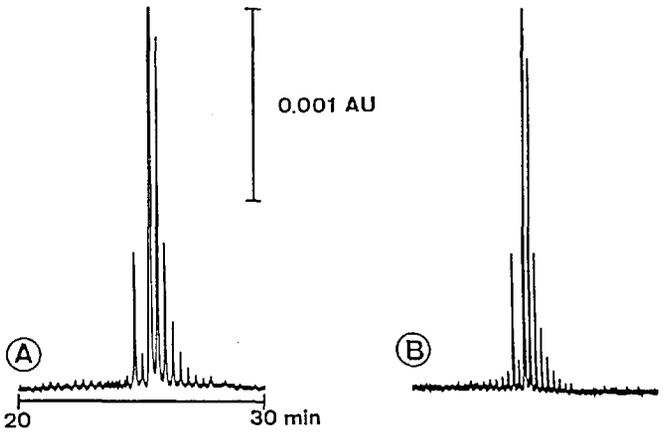

Figure 12

Separation of oligonucleotides by capillary gel electrophoresis [27].
sample: polytimidine ~ 30 mer; fail product
capillary: 40 cm effective length, 100 µm i.d.
A: no surface pretreatment before gel filling,
B: with surface pretreatment;
gel: polyacrylamide, 6 % T, 5 % C;
buffer: 0.1 M TRIS/0.25 M Boric acid/7 M urea; voltage: 250 V/cm;
detection: UV at 260 nm.

Capillary zone electrophoresis is also applied to the separation of chiral compounds. For example the separation of chiral amino acids is achieved by enantioselective interactions with a Cu(II)-Aspartame-complex, which is added to the buffer [29]. Especially high levels of selectivity can be obtained by combining different separation mechanisms. For the separation of oligonucleotides a combination of electrophoresis, complexation and interactions with SDS-micelles has been used [30].

For the characterization of proteins tryptic mapping is often employed. The peptides resulting from tryptic digestion can be analysed by HPLC and CZE. As the retention times in HPLC are almost non-correlated to the migration times in CZE, since the separation principles are completely different for these two techniques, a combination of these two methods significantly increases the information about the sample. An example is the characterization of human growth hormone [31].

Conclusions

Capillary zone electrophoresis is an important analytical technique in the field of bioscience. Compared to classical gel electrophoresis, where stabilizing media for the suppression of convective dispersion are used, electrophoresis in capillaries has a number of advantages.

Foremost amongst these is the high separation efficiency, such that several hundred thousand plates can be reached without any problems. Automation is very simple since detection may be done on-line, enabling the application of a variety of detection principles, including mass spectrometry. The total time of analysis is about some ten minutes or less, and the tedious staining process, often necessary in classical gel electrophoresis, is eliminated. Furthermore, only very small amounts of sample are necessary, because the injection volume is only a few nl.

In spite of the advantages of capillary zone electrophoresis there are still some aspects which need to be improved. One problem is the poor reproducibility of electroosmotic flow. Especially in the free zone electrophoretic analysis of proteins interaction of the samplecomponents with the wall of the fused silica capillary can lead to a decrease of the electroosmotic flow and the separation efficiency. The elimination of the zeta potential, e.g. by a chemical modification of the surface or by coating of the capillary, can reduce this problem; but at the same time the advantage of simultaneously analysing anions and cations is lost.

A further problem is caused by the fact that only very small volumes can be injected, which is considered as an advantage with respect to the amount of sample needed but is a disadvantage with regard to the detectability of the analytes. When using the widely applicable UV-absorption detector a relatively high concentration of the analyte in the sample is required. This applies especially in the determination of feebly UV-active compounds, as for instance a variety of proteins. This high analyte concentration can lead to a

drastic deterioration in separation efficiency, resulting in the dynamic range of the determination being very limited.

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