

Chapter 2

Principles of Electromigration Techniques

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Abstract Electromigration techniques provide the separation of analyzed sample components owing to external voltage generating electrokinetic phenomena—electrophoresis and electroosmosis. Taking into account the relatively large number of parameters dealt with during electrophoretic analyses, it is essential to know their influence on the achieved separation of analytes. In this chapter the theoretical and practical aspects of a resolution optimization, as well as the effect of different separation parameters on the migration behavior are described. These, among others, include migration time, efficiency, selectivity, and resolution. The influence of electrodes polarization, applied voltage, temperature, capillary, background electrolyte, and various additives on the separation is also discussed.

Electromigration techniques, due to their huge analytical potential, are widely applied in the determination of various substances. These techniques provide separation of components of an analyzed sample owing to external voltage generating electrokinetic phenomena—electrophoresis (motion of ions in an electrical field) and electroosmosis (volumetric liquid flow in a capillary caused by an electrical field). The separation takes place in a liquid phase in a solution called (acc. to various literature sources) a buffer, electrolyte or background electrolyte, separation buffer or separation electrolyte. It may be either an aqueous solution or based on pure organic solvents or their mixtures.

Owing to their accuracy and precision of determination, they are widely applied in, e.g., the biochemistry of proteins and nucleic acids, molecular biology, pharmacology, forensic medicine, forensic science, medical diagnostics, and analysis of food

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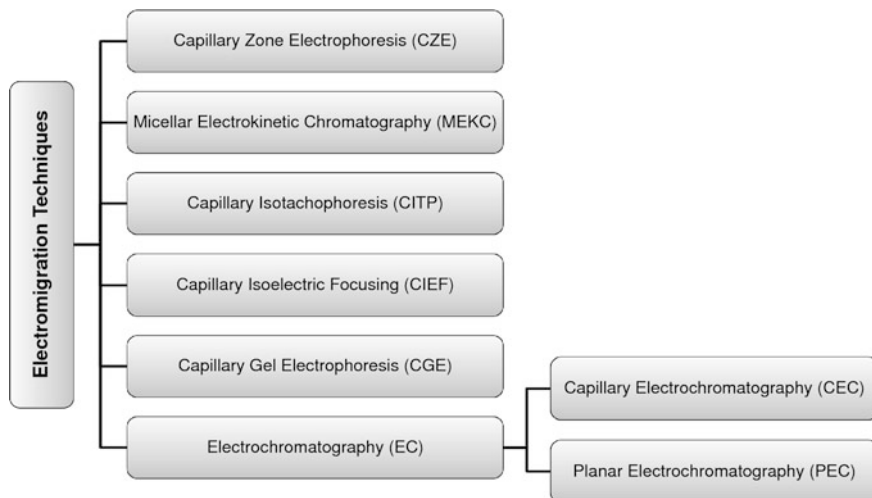


Fig. 2.1 Types of electromigration techniques, according to [11, 20]

[1–10]. All electromigration techniques applied in laboratory practice are derivatives or combinations of three basic types of electrophoreses: Zone Electrophoresis, Isotachopheresis, and Isoelectric Focusing (Fig. 2.1).

2.1 Electrophoretic Mobility

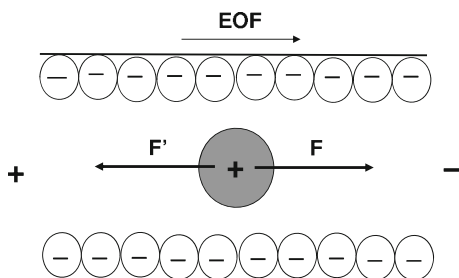
The electrophoretic separation stems from the difference in velocity of charged particles migrating under the influence of the electric field.

In a capillary filled with a separation buffer, the ion is subject to two forces. One is an electrostatic force (F): (Fig. 2.2)

$$F = qE, \quad (2.1)$$

where q is ions charge and E is strength of electric field [V cm^{-1}], which is a function of the imposed voltage and length of the capillary. The second force is

Fig. 2.2 Forces influencing an ion in separation buffer



oppositely directed friction force, F' , which according to Stokes law for spherical particles is expressed by the equation:

$$F' = -6\pi\eta r v_{ep}, \quad (2.2)$$

where η is solution viscosity [$\text{kg m}^{-1} \text{s}^{-1}$], r is ionic radius [m], v_{ep} is electrophoretic velocity [m s^{-1}].

During the electrophoresis (ionic motion), the electrostatic and friction forces are in equilibrium and then the following expression is achieved:

$$qE = -6\pi\eta r v_{ep}. \quad (2.3)$$

Keeping in mind that the electrophoretic mobility (μ_{ep}) is proportional to the charge of particle (q), and inversely proportional to its radius (r) and environment viscosity (η):

$$\mu_{ep} = \frac{q}{6\pi r \eta}, \quad (2.4)$$

obtained is the equation for electrophoretic velocity of individual ions:

$$v_{ep} = \mu_{ep} E. \quad (2.5)$$

It can be deduced from Eq. (2.4) that charged species can be resolved according to the differences in their electrophoretic mobility (charge-to-size ratio) which is the basis of the electromigration techniques. This equation proves that the electrophoretic mobility is greater for small particles with a big charge, and smaller for bigger particles with a small charge. And the electrophoretic mobility of neutral particles equal zero, because $q = 0$. In addition, the ionic mobility is influenced by the changes in temperature which cause changes in the buffer viscosity.

The electrophoretic mobility in a given buffer is a characteristic and constant value for a given ion. A careful selection of the buffer properties makes it possible to control the selectivity of separation by changing the mobility of the determined analytes [11, 12].

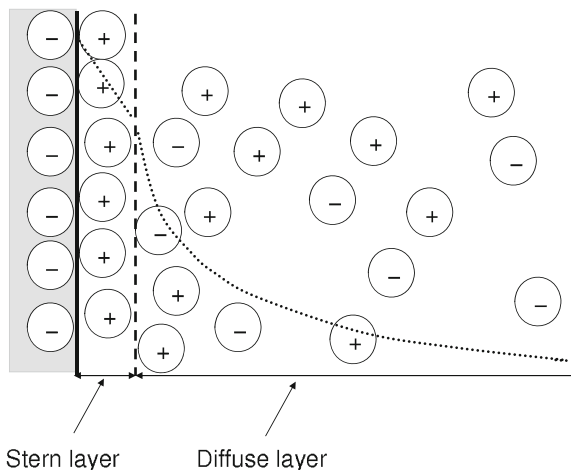
2.2 Electroosmotic Flow

An important role during the electrophoretic separation is played by electroosmotic flow (EOF), i.e., the motion of entire liquid by which the capillary is filled, caused by the difference in potentials. It is connected in the presence of electric charges on the internal surface of the capillary and it depends on:

- ionization of functional groups present on their surface and
- adsorption of ions or other buffer components on the inner capillary surface [12].

Depending on the pH of the electrolyte, silanol groups of the internal wall of the quartz capillary are subject to ionization, according to the following equation of reaction:

Fig. 2.3 Diagram of creation of double electric layer



As a result of dissociation of silanol groups, the internal wall of the capillary receives a negative charge and this leads to the creation of an electric double layer on the border phases of electrolyte/capillary wall (Fig. 2.3) [13].

The negative charge on the capillary wall (SiO^-) attracts cations from the liquid phase which creates a permanent layer, i.e., the Stern layer. However, not all charges on the surface of a capillary are compensated, which leads to the creation of a second layer, more weakly bounded to the surface, i.e., diffusion layer [14, 15]. Only ions from the diffusion layer take part in electrokinetic phenomena because they are subject to a permanent exchange with the buffer ions from the bulk solution. And the part of the double layer which is directly adjacent to the surface does not participate in the migration.

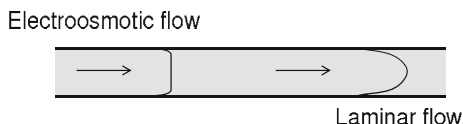
The electrokinetic potential occurring on the border of two layers (mobile and permanent), called a hydrodynamic slip plane, is called a zeta potential (ζ), which is described by the equation of Smoluchowski [16]:

$$\zeta = \frac{\eta \mu_{EOF}}{\varepsilon_0 \varepsilon_r}, \quad (2.6)$$

where η is solutions viscosity [$kg\ m^{-1}\ s^{-1}$], r is ionic radius [m], μ_{ep} is electrophoretic mobility [$cm^2\ V^{-1}\ s^{-1}$], $\varepsilon_0, \varepsilon_r$ are dielectric constant in a solution and electric permeability in vacuum.

Zeta potential is proportional to the density of the charge on the internal wall of the capillary, which depends on the pH. Thus, the value of zeta potential differs, depending on the pH value. Above pH 9 silanol groups are completely ionized and the electroosmotic flow is the quickest. Below pH 4 the level of dissociation of silanol groups is low and then EOF is small [17]. The ionic strength of the buffer is also a significant parameter influencing the zeta potential. The increase in the ionic

Fig. 2.4 Profiles of electroosmotic and laminar flow



strength causes the decrease in the double layers thickness, which causes the decrease in zeta potential and slowing of EOF [18].

According to the following equation, the velocity of electroosmotic flow (v_{eo}) depends on the solution viscosity (η), dielectric constant (ϵ), strength of electric field (E), and the value of zeta potential (ζ) [12]:

$$v_{EOF} = \frac{\epsilon \zeta E}{4\pi\eta}. \quad (2.7)$$

Compared to ions velocity (v), the electroosmotic flow can be so strong that solvated ions of the mobile layer move from anode to cathode, regardless of their charge. A value describing the electroosmotic flow is its electroosmotic mobility (μ_{eo}) expressed by the formula:

$$\mu_{EOF} = \frac{v_{EOF}}{E}. \quad (2.8)$$

After the substitution of Eq. (2.7) into (2.8) we obtain:

$$\mu_{EOF} = \frac{\epsilon \zeta}{4\pi\eta}. \quad (2.9)$$

The electroosmotic flow has two important characteristics. First, compared to chromatographic techniques with laminar flow of a parabolic profile, the profile of electroosmotic flow is flat (Fig. 2.4). Second, according to Eq. (2.7), its velocity is not connected with a decrease in pressure (Δp) and it does not depend on the capillaries diameter through which the liquid flows (if double electric layers of opposite capillary walls are not placed on each other). The flat profile of EOF does not directly cause the dispersion of compounds and widening of peaks [12, 19].

The velocity of electroosmotic flow is considerably influenced by conditions of the electrophoresis. Therefore, it should be controlled by the determination of a neutral (without charge) marker velocity. Owing to the fact that this compound does not have an electric charge, its velocity equals the EOF velocity [20]. Examples of markers are: acetone, thiocarbamide, mesityl oxide, and dimethyl sulfoxide.

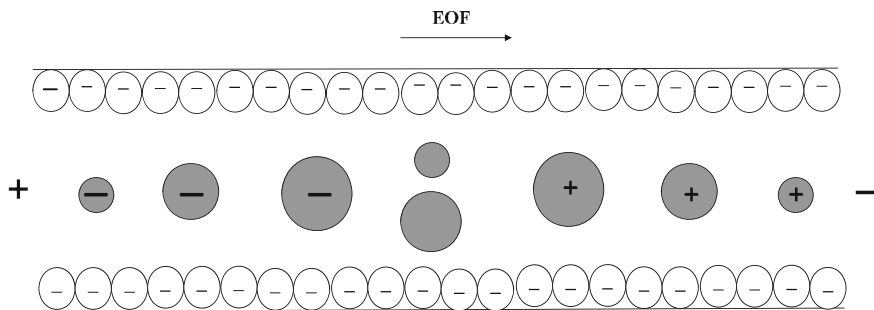


Fig. 2.5 Order of ions migration during the electrophoretic separation

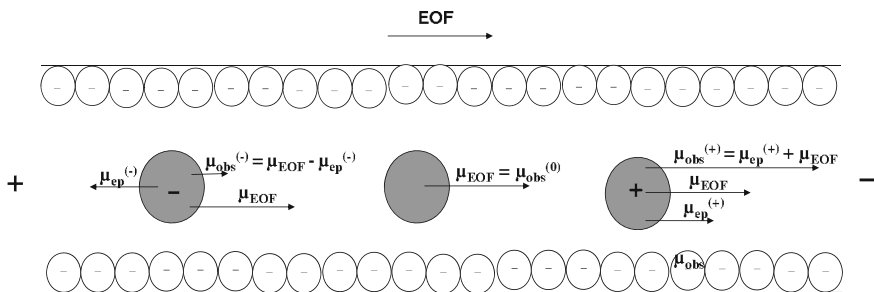


Fig. 2.6 Real versus observed electrophoretic mobility

2.3 Mechanism of Electrophoretic Separation

Under the influence of the electric field strength, particles with electrical charge contained in a sample will move with different velocity, and this is reflected in their separation (Fig. 2.5).

In case of unmodified quartz capillaries, the electroosmotic flow is performed from anode to cathode (presence of negative charge on the internal surface of the capillary) (Fig. 2.6). Owing to the EOF existence it is possible to determine cations and anions at the same time. If the value of electroosmotic flow is higher $\mu_{obs} > \mu_{ep}$ and in the direction opposite to the electrophoretic mobility of anions, then all ions (both anions and cations) will move in the same direction but with different velocity [12, 21].

A detector placed before the cathode is first reached by cations whose mobility is observed by the sum of the electrophoretic mobility and electroosmotic flow:

$$\mu_{obs}^{(+)} = \mu_{ep}^{(+)} + \mu_{EOF} \tag{2.10}$$

Second in order from the detector come non-separated neutral individuals, moving with velocity equal to EOF:

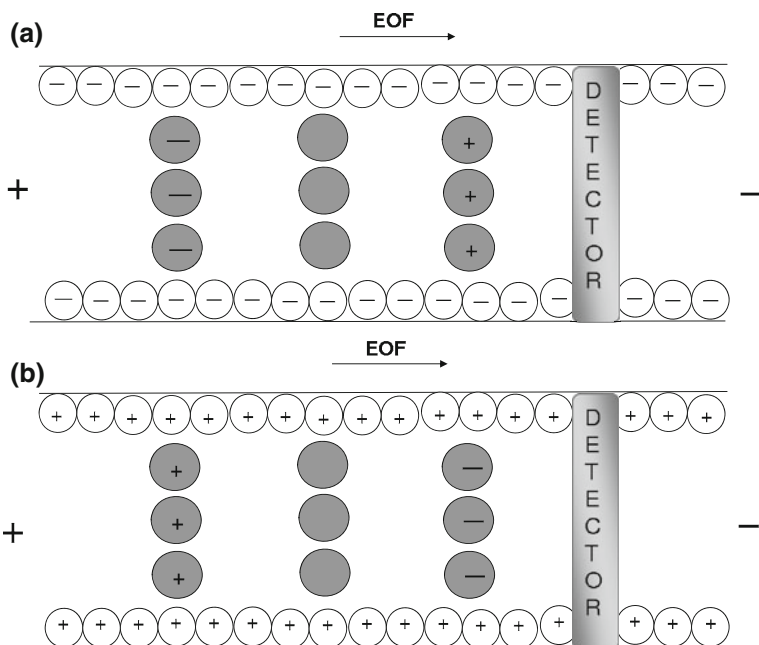


Fig. 2.7 Determination of (a) cations with a normal electroosmotic flow, (b) anions with a reversed electroosmotic flow

$$\mu_{obs}^{(0)} = \mu_{EOF}, \quad (2.11)$$

and in the end anions migrate, and their mobility is observed in the difference between the electrophoretic mobility and electroosmotic flow

$$\mu_{obs}^{(-)} = \mu_{EOF} - \mu_{ep}^{(-)}. \quad (2.12)$$

The above deliberations are simplified. In reality the following kinds of ionic mobility can be distinguished:

- absolute mobility—being a theoretical constant relating to indefinitely diluted solution in a given temperature,
- effective mobility—related to specific conditions of experiments (e.g., a specific composition and pH of a buffer),
- observed (apparent) mobility—the one described above, constituting the sum of the effective and electroosmotic mobility in specific experiment conditions.

Taking the above deliberations into account, in unmodified quartz capillary the order of components migration is as follows:

- small cations with a big charge,
- bigger cations with a smaller charge,
- non-separated neutral individuals,

- big anions with a small charge,
- small anions with a big charge.

If there is a need to determine anions (whose observed migration times can be very long), a reversed electroosmotic flow is applied to shorten the time of analysis. Owing to this a reversed order of ionic migration is obtained (Fig. 2.7b). For analytes to move from an inlet to an outlet of the capillary, the anode should be located behind the detector which is first reached by anions, then neutral particles, and in the end by cations.

The reversal of EOF is achieved by a specific chemical or dynamic modification of the effective charge on walls of the capillary. The change in direction and velocity of the electroosmotic flow depend on the kind and concentration of a modifier [20, 21].

2.4 Parameters of Electrophoretic Separation

2.4.1 Migration Time

The migration time of analytes (t_m) is a time needed for the transport of the determined components of a sample along the capillary to the detector:

$$t_m = \frac{L_{eff} \cdot L_{tot}}{(\mu_{ep} + \mu_{EOF}) \cdot U}, \quad (2.13)$$

with the assumption $L_{eff} \sim L_{tot}$ we obtain:

$$t_m = \frac{L^2}{(\mu_{ep} + \mu_{EOF}) \cdot U}, \quad (2.14)$$

where L_{tot} is the total length of the capillary [cm], L_{eff} is the effective length of the capillary, i.e., a distance from the capillary inlet to a place of detection [cm], μ_{ep}, μ_{EOF} are adequate electrophoretic mobility of the electroosmotic flow [$\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$], and U is voltage [V].

Thus, a shorter capillary, higher voltage, and higher mobility make the migration time of analytes shorter, at the same time shortening the time of the whole analysis [12].

2.4.2 Efficiency

The efficiency in capillary electrophoresis, as in chromatographic techniques, is expressed by the number of theoretical plates determined by the following equation [11]:

$$N = \frac{L^2}{\sigma^2}, \quad (2.15)$$

where L is the length of the capillary [cm], and σ^2 is peak variance.

During the migration of sample components in the capillary, a molecular diffusion can occur, leading to the broadening of band on the electropherogram, defined as the peak variance (σ^2) [12]:

$$\sigma^2 = \frac{2DL^2}{\mu_{obs}U}, \quad (2.16)$$

where D is analyte diffusion coefficient, L is capillary length [cm], and μ_{obs} is observed mobility [$\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$], U is voltage [V].

The total peak variance is the sum of variances of individual factors influencing its broadening [12]:

$$\sigma_{tot}^2 = \sigma_{inj}^2 + \sigma_{det}^2 + \sigma_{dis}^2 + \sigma_{dif}^2 + \sigma_{edis}^2 + \sigma_{EOF}^2 + \sigma_{wall}^2, \quad (2.17)$$

where σ_{inj}^2 is variance resulting from dosing of a sample (e.g., volume, way of dosing, way of preparing of the capillary end) introduced to the capillary, σ_{det}^2 is variance resulting from volume and shape of the measuring cell of the detector, σ_{dis}^2 is variance resulting from a thermal dispersion, σ_{dif}^2 is variance resulting from a longitudinal diffusion, σ_{edis}^2 is variance resulting from electromigration dispersion, σ_{EOF}^2 is variance resulting from EOF, σ_{wall}^2 is variance resulting from interactions of the analyte with internal surface of the capillary.

After the substitution of Eq. (2.16) into (2.15) we obtain dependence:

$$N = \frac{\mu_{obs}U}{2D}, \quad (2.18)$$

where μ_{obs} is observed mobility [$\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$], U is voltage [V], and D is analyte diffusion coefficient.

As the above equation proves, it is possible to improve the circuits efficiency by increasing applied voltage, with the assumption of an effective heat removal. On the other hand, a high diffusion coefficient and a small mobility of analytes decrease the circuits efficiency.

2.4.3 Selectivity

The selectivity of the system (α) in case of the capillary electrophoresis can be expressed as follows [20]:

$$\alpha = \frac{t_{m2} - t_{EOF}}{t_{m1} - t_{EOF}}, \quad (2.19)$$

where t_{m1} , t_{m2} are migration times of adjacent peaks [min], t_{EOF} is migration time of neutral marker [min].

Thus:

$$\alpha = \frac{\mu_1}{\mu_2} \cdot const \quad (2.20)$$

where μ_1 , μ_2 are electrophoretic mobility of two analytes.

Thus, the selectivity depends on the ratio of electrophoretic mobilities, and very often an effective way to change the selectivity is to change the buffer pH [21].

2.4.4 Resolution

A good resolution is achieved when there is a big difference in the electrophoretic mobility between separated analytes. Thus, the resolution (R_s) can be defined as follows [11, 12]:

$$R_s = \frac{1}{4} \frac{\Delta\mu_{obs}}{\mu_{s'r}} \sqrt{N}, \quad (2.21)$$

where $\Delta\mu_{obs} = \mu_2 - \mu_1$.

$\mu_{s'r} = \frac{\mu_2 + \mu_1}{2}$ (average mobility of two ions).

Applying Eq. (2.18) we obtain:

$$R_s = 0.177(\mu_2 - \mu_1) \sqrt{\frac{U}{D(\mu_{s'r} \pm \mu_{EOF})}} \quad (2.22)$$

Equation (2.21) allows to assess at the same time two factors which influence the resolution—selectivity and efficiency. The selectivity is reflected in the electrophoretic mobility of analytes, while the efficiency of the separation process is determined by the number of theoretical plates [11].

The resolution can be improved by lengthening of the capillary, changing buffers parameters, or by adding an organic solvent [20].

...AFFECT →

INCREASING... ↓

	pH	Temperature	Viscosity	Injection volume	EOF	Diffusion	Current	Ionic strenght	Electrophoretic mobility	Capillary surface	Analyte charge
pH					↑				↕	↕	↕
Temperature	↕		↓	↑	↑	↑	↑	↑	↑		
Viscosity				↓	↓	↓			↓		
Ionic strenght		↑		↕	↓					↕	
Current		↑	↓		↓	↑	↑				
Capillary lenght		↑		↑			↑				
Capillary diameter		↑		↓	↑		↑				
Analyte charge				↕					↕		
Analyte size/shape						↕			↕		↕
EOF						↑					
Electrophoretic mobility				↕							

Fig. 2.8 Relation between parameters in the capillary electrophoresis (*up arrow* parameter's growth, *down arrow* parameter's decrease, *up-down arrow* two-direction influence, according to [11])

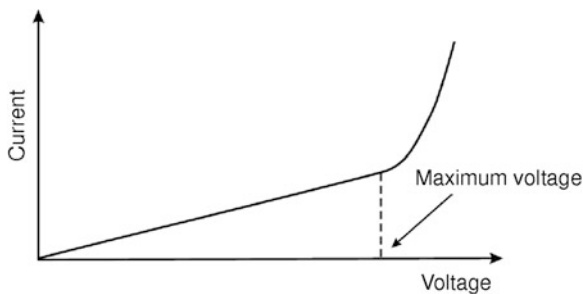
2.5 Optimization of Electrophoretic Separation Conditions

Taking into account a relatively large number of parameters dealt with during electrophoretic analyses, it is essential to know their influence on the achieved separation of analytes (Fig. 2.8).

2.5.1 Polarization of Electrodes

The selection of electrodes polarization should be the first operation before the analysis. As it was mentioned before, during the normal polarization solvated ions of a moving layer move from the anode on inlet to the cathode on outlet, in accordance with the electroosmotic flow. If a reversal of the electroosmotic flow direction is required, the electrodes polarization should also be reversed, so the anode should be located behind the detector which is first reached by analytes with a negative charge [11, 21].

Fig. 2.9 Dependence of current strength on applied voltage



2.5.2 Applied Voltage

The applied voltage largely influences the time and quality of separation. As Eq. (2.22) proves, the resolution is proportional to the square root of voltage, hence a fourfold increase in voltage will lead to a twofold increase in resolution [12, 13]. However, benefits resulting from the voltage increase can be lost after exceeding a maximum voltage which should be utilized for given conditions of the analysis. This is connected with the influence of a generated Joule heat. The maximum voltage can be determined from the dependence of the current strength flowing through the capillary on the applied voltage, according to Ohm's law [13, 20]. According to this law, the strength of flowing current is proportional to the applied voltage.

As one can see in Fig. 2.9, the applicability of Ohm's law depends on the scope of applied voltages. In small voltages the proportionality is maintained, and in the scope of high voltages there is a deviation from linearity which results from a surplus of generated heat, a decrease in resistance, and increase in current strength. In a terminal moment the buffer boils in the capillary, which breaks the electric circuit. Therefore, the point when the proportionality collapses is a value of maximum voltage [11].

Maximum permissible voltage depends on the composition, concentration, and pH of the buffer, and on the length and diameter of the capillary (Fig. 2.10).

2.5.3 Temperature

The repeatability of the results of electrophoretic separations depends on maintaining a fixed temperature. The increase in buffer temperature in order to decrease its viscosity causes acceleration of electroosmotic flow and shortening of migration time [22, 23]. The lack of a thermostatic system of the capillary may cause a noticeable, unfavorable influence of generated Joule heat. In a capillary without a thermostat, depending on its internal diameter, the buffer temperature in the central part of capillary can be higher than at the walls. This effect can cause

a thermal diffusion, as a result of which analytes in the central, warmer zone, migrate quicker than those in the area of the cooler walls of the capillary [21]. The electropherogram then shows broadened peaks. Besides, the unstable temperature of the sample influences the reproducibility of injection and may lead to the dissolution of sample [13, 17]. In case of processes controlled thermodynamically, the lowering of temperature causes the increase of selectivity. If the selectivity is sufficient, the increase in temperature may be utilized to improve the peak shape, to shorten the time of analysis, or it may cause additional, often favorable, effects, such as conformation changes (separation of chiral compounds) [12].

2.5.4 Capillary

The selection of the internal diameter of capillaries may have a huge influence on the applicability of a given method of analysis. For effective heat removal, a high ratio of surface to volume for capillaries of small diameter is responsible. This allows to apply high voltages, thereby increasing resolution and shortening the time of analysis, or high concentrations of buffers, which may also have a favorable influence on resolution [11, 22].

The increase in the capillary length (Fig. 2.10) extends the migration time and assures a better resolution. This is caused by a longer stay of analytes in the

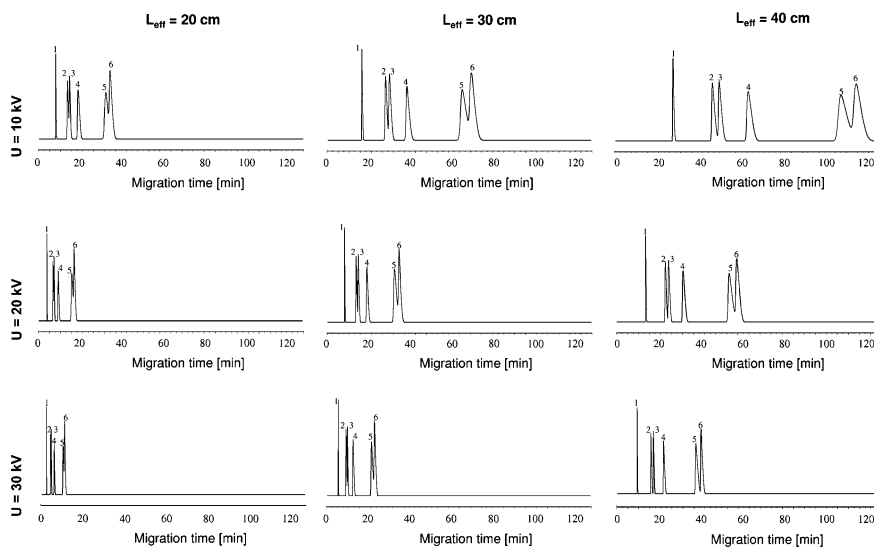


Fig. 2.10 Influence of applied voltage and length of effective capillary tube on the organic acids separation (peaks: 1—malonic acid, 2—tartaric acid, 3—salicylic acid, 4—citric acid, 5—apple acid, 6—formic acid). Conditions of analysis: 5 mmol/L phosphate buffer (pH 2.5)

Table 2.1 Examples of substances added to the buffer to modify EOF

EOF modifier		Ref.
Surfactants	Didodecyltrimethylammonium bromide (DDAB)	[26–29]
	Tetradecyltrimethylammonium bromide (TTAB)	[28, 30]
	Cetyl trimethylammonium bromide (CTAB)	[27, 29, 31]
Metal ions	Ca ²⁺ , Mg ²⁺ , Ba ²⁺ , Na ⁺ , K ⁺	[32–38]
Organic solvents	Methanol, acetonitrile	[26, 27, 39, 40]
Polymers	Pochodne celulozy	[41, 42]
	Polyvinyl alcohol (PVA)	[30, 43–45]
	Poly(ethylene oxide) (PEO)	[46–50]
	Polyethyleneimine (PEI)	[30, 51–53]

Table 2.2 Chemical modification of the internal surface of the capillary

EOF modifier	Influence of modification on EOF	Ref.
Polyacrylamide (PAA)	Reduce/eliminate	[43, 54–60]
Polyvinyl alcohol (PVA)	Reduce/eliminate	[61]
Poly(ethylene oxide) (PEO)	Reduce	[56, 62, 63]
Polyethyleneimine (PEI)	Reverse	[64–66]
Chitozan	Reduce	[67, 68]
Dextran	Reduce	[69–71]
Methylcellulose	Reduce/eliminate	[69, 72]
Cellulose acetate	Reduce	[73]
Hydroxypropylcellulose	Reduce	[56, 74]

capillary. Additionally, the strength of electric field is inversely proportional to the capillary length, hence the increase in the capillary length allows to apply higher voltages [11, 22].

In practice, a chemical character of the internal surface of the capillary is a decisive factor influencing the efficiency and resolution of the system. It depends on a material from which the capillary is made, conditions of conditioning and parameters influencing the direction and size of EOF [12]. The adsorption of analytes on the internal wall of the capillary may cause the broadening of peaks and decrease in resolution. In order to minimize this problem there are a few well-tried ways of running a separation process:

- doing analyses in $\text{pH} < 2$, when silanol groups do not ionize;
- modification of effective analytes charge to prevent their adsorption on the walls;
- adding substances which dynamically modify the internal wall of the capillary (Table 2.1);
- covalent bond of different monomers to the surface of the capillary to block silanol groups (Table 2.2).

Table 2.3 Buffer employed in CE, according to [12]

CE buffers	pKa	pH range
Phosphate	2.12	1.14–3.14
	7.21	6.20–8.20
	12.32	10.80–13.00
Citrate	3.06	3.77–4.77
	4.74	5.40–7.40
	5.40	
Formate	3.75	2.75–4.75
Acetate	4.75	3.76–5.76
MES	6.15	5.15–7.15
PIPES	6.80	5.80–7.80
HEPES	7.55	6.55–8.55
Tris	8.30	7.30–9.30
Borate	9.24	8.14–10.14
CAPS	10.40	9.70–11.10

2.5.5 Buffer

Buffers utilized in the electrophoretic analysis are significant because they assure a correct electrophoretic behavior of individual analytes, a general systems stability, and a satisfactory separation. When choosing a buffer one should take into account an applied detection system, solubility, stability, and the degree of analyte ionization, pH range in which the buffer can be utilized ($pK_a \pm 1$ units). Also, the influence of various buffer modifiers on the Joule heat dissipation should be considered [12, 24]. Table 2.3 shows examples of the most commonly utilized buffer solutions for CE, along with their pK_a values and ranges of pH in which they are used.

2.5.5.1 pH of a Buffer

As it was mentioned before, the degree of silanol groups ionization on the capillary internal wall depends on the buffer pH. Differences in the ionization level may cause differences in electrophoretic mobility and electroosmotic flow [24]. On the other hand, the increase in pH value between 4 and 9 causes the increase of silanol groups ionization, which causes the increase of a negative charge on the capillary walls. Zeta potential is proportional to a charge density on the capillary walls. As a consequence, the increase in potential determines the increase of EOF [17, 24]. One should also remember that the buffer solution pH value may change under the influence of temperature, when buffer ions and organic additions are exhausted [11].

The buffer pH value also influences the ionization of analytes that are weak acids or bases. For weak electrolytes, e.g., acids, a dissociation constant is described by the following equation:

$$K = \frac{C_{H^+} \cdot C_{A^-}}{C_{HA}}, \quad (2.23)$$

where C_{HA} is the undissociated form of a weak acid, and C_{A^-} is the dissociated form of a weak acid.

For a given analyte (weak acid) in a solution of a different pH the dissociation level will be changing (the higher the pH, the greater the ionized form). The electrophoretic mobility for such a substance will be connected with a mobility of a dissociated form and ionization level (α). Terabe et al. [25] found that for two analytes without a big difference in pKa, the optimum pH of separation amounts to:

$$pH_{(optimum)} = pKa - \log 2 \quad (2.24)$$

At low pH values H^+ ionic concentration increases, and the ions neutralize a negative charge of the analyte (anion). Then a decrease of electrophoretic mobility and shortening of the analysis time is observed. In case of determination of cationic analytes, along with buffer pH increase, the level of dissociation decreases, thus, as a result, their electrophoretic mobility is decreased [24]. It is particularly important to maintain a fixed pH value of the buffer during the electrophoretic separation of proteins and peptides which have amphoteric properties. A resultant electric charge of protein is influenced by the ionization of carboxyl and amine groups. Thus, in the environment with $pH > pI$ the protein has a negative charge which will migrate to anode (+). On the other hand, if $pH < pI$ the protein is charged positively and it will move in the direction of the cathode (-); they will not migrate if $pH = pI$ of a given protein/peptide.

2.5.5.2 Concentration/Ionic Strength of a Buffer

Ionic strength or concentration of buffer solutions used are limited by the length and diameter of the capillary, applied strength of electric field, and by the effectiveness of Joule heat removal. In a situation where the temperature is controlled, the increase in concentration or ionic strength of the buffer reduces EOF [26], which leads to the extension of time of analysis and improvement in resolution (Fig. 2.11).

In case the temperature is not controlled, the increase in buffer concentration or ionic strength increases the buffer conductivity, which leads to an unfavorable generation of heat. Then, the viscosity is decreased and EOF is increased [12]. In order to increase the buffer ionic strength or concentration without increase in heat, the effective length of the capillary should be increased, the capillary internal diameter should be decreased or additions of dual ions should be utilized; these actions do not cause a change in the buffer conductivity. In addition, the increase in buffer ionic strength decreased interactions with analyte/analyte or analyte/internal wall of the capillary [11].

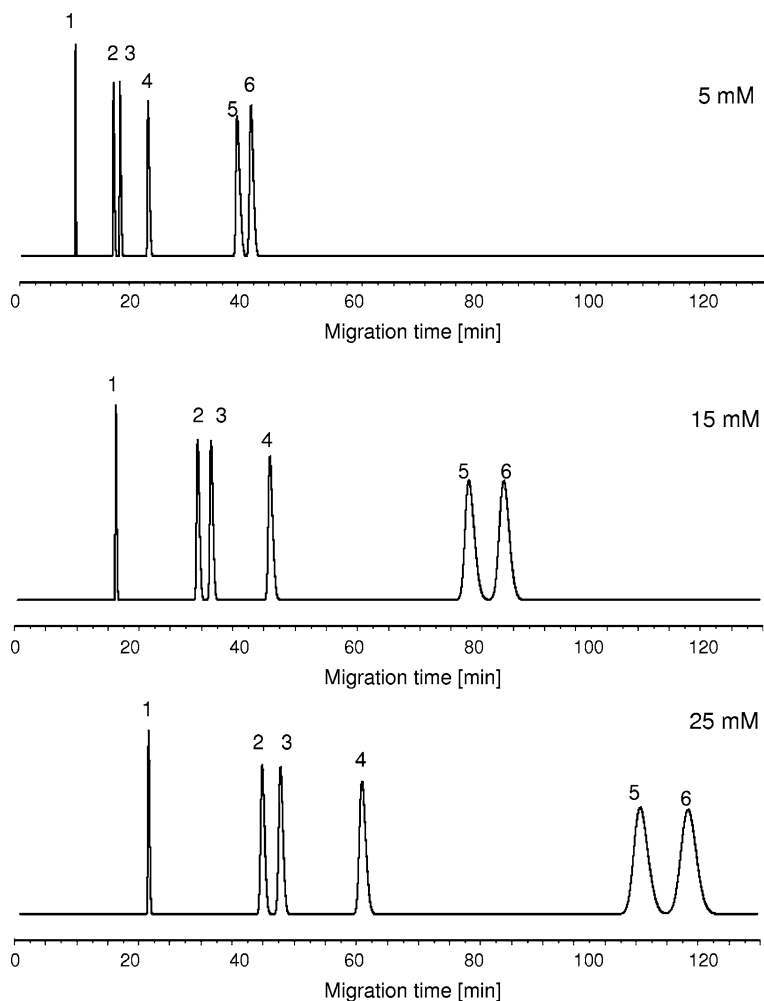


Fig. 2.11 Influence of ionic strength on separation of organic acids (peaks: 1—malonic acid, 2—tartaric acid, 3—salicylic acid, 4—citric acid, 5—apple acid, 6—formic acid). Conditions of analysis: phosphate buffer (pH 2.5); $L_{\text{eff}} = 40$ cm; $U = -30$ kV

2.5.5.3 Substances Modifying EOF Added to the Buffer

In order to improve the circuit resolution, substances modifying EOF are added to buffer solutions (Table 2.1). Their function is to prevent the adsorption of analytes on the capillary walls and to rebuild an electric double layer (change of electrokinetic potential and electroosmotic flow). They directly influence the change in buffer viscosity, its solvative properties, and accessibility of silanol groups on the capillary walls [12, 24]. On the other hand, organic solvents, such as methanol or acetonitrile

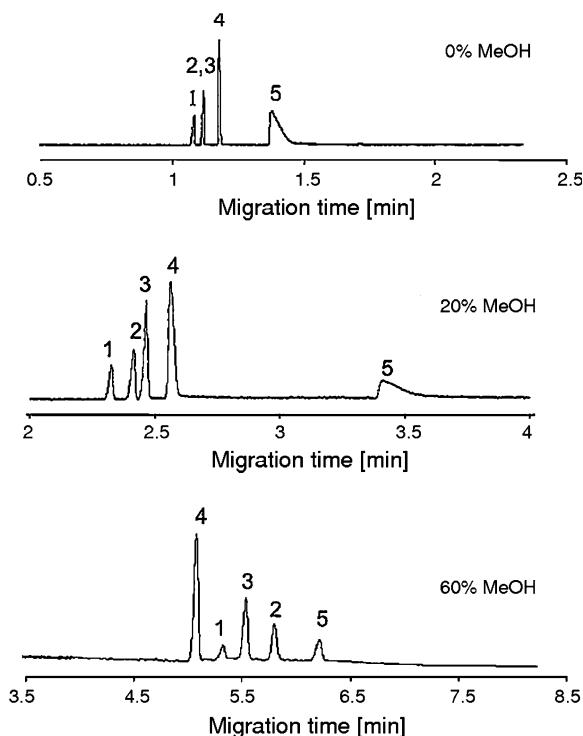


Fig. 2.12 Electropherogram received for a sample containing five non-organic ions. Conditions of analysis: [CTAC] = 0.5 mmol/L, phosphate buffer (pH 8.0), $c = 15$ mmol/L, $L_{eff} = 28.5$ cm, $U = -15$ kV. Peaks on the electropherogram: 1 Br⁻, 2 NO₃⁻, 3 NO₂⁻, 4 I⁻, 5 SCN⁻. Reprinted with permission from Diress and Lucy [27] Electroosmotic flow reversal for determination of inorganic anions by capillary electrophoresis with methanol–water buffers. *J Chromatogr A* 1027:185–191. Copyright 2004 Elsevier

(Fig. 2.12), cause the decrease in buffer conductivity, decrease in thermal diffusion, and a better solubility of analytes. Unfortunately, additions of substances modifying EOF may lead to unwanted modifier/analyte interactions [11, 12].

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