PALACKÝ UNIVERSITY IN OLOMOUC

FACULTY OF SCIENCE
DEPARTMENT OF ANALYTICAL CHEMISTRY

POLYPYRROLE COATED CAPILLARIES FOR CAPILLARY ELECTROPHORESIS

DIPLOMA THESIS

Author: Radim Knob
Field of study: Analytical chemistry

Supervisor: RNDr. Vítězslav Maier, Ph.D.

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SHRNUTÍ

Diplomová práce se zabývá přípravou a využitím kovalentně pokrytých kapilár polypyrrolem pro kapilární elektroforézu. Připravené kapiláry byly charakterizovány pomocí závislostí elektroosmotické mobility na pH a využity pro separaci jak achirálních, tak chirálních sloučenin.

V teoretické části je podán přehled využívaných technik pro pokrytí křemenných kapilár pro kapilární elektroforézu, od dynamických pokrytí malými molekulami jako aminy či tenzidy, přes adsorbované polymery nebo iontové kapaliny až po kovalentní pokrytí, která jsou využívána také v kapilární elektrochromatografii.

Praktická část se věnuje přípravě silanizačního činidla pro pokrytí křemenných kapilár. Toto činidlo nesoucí pyrrolovou skupinu umožňuje přípravu polypyrrolového pokrytí na povrchu kapiláry. Toto pokrytí bylo také modifikováno aniontovým polymerem. Charakterizováno bylo jak nemodifikované polypyrrolové pokrytí, tak modifikace dextransulfátem a heparinem. Polypyrrolem pokryté kapiláry modifikované dextransulfátem byly využity pro separaci enantiomerů *R,S*-ibuprofenu pomocí sulfatovaného β-cyklodextrinu. Ve srovnání s nepokrytou křemennou kapilárou bylo dosaženo opačného migračního pořadí enantiomerů za použití stejného základního elektrolytu a chirálního selektoru. Modifikace heparinem byla využita pro studium vlivu pH na separaci modelové směsi vybraných herbicidů micelární elektrokinetickou chromatografií. Polypyrrolem pokryté kapiláry modifikované heparinem a dextransulfátem se vyznačují silným katodickým elektroosmotickým tokem téměř nezávislým na pH a výrazně lepší opakovatelností elektroosmotických mobilit oproti nepokryté křemenné kapiláře.

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CONTENT

1. INTRODUCTION

Capillary electrophoresis (CE) is a fast and highly efficient analytical separation technique based on the relatively simple migration of charged molecules in an electric field. Consumption of chemicals and materials is low, and small sample amounts are needed. One of the main disadvantages is the unsatisfying reproducibility of migration times, strongly depending upon the actual chemical conditions of the silica surface.

Since silica has highly adsorptive surface in its native state, when real samples containing polar matrix components are analyzed, the latter may be irreversibly adsorbed to the surface, altering the net surface charge of weakly acidic silanol groups. Although pH, buffer components, and ionic strength of the running buffer are the main tools for selectivity optimization in CE, their simultaneous influence on the dissociation of ionic analytes on one hand and the dissociation of surface silanol groups on the other may complicate method development. Furthermore, production conditions of fused-silica capillaries seem to be relevant, as significant differences in electroosmotic mobility are observed for capillaries from different manufacturers; even after proper treatment, reproducibility of electroosmotic flow is not always satisfactory.

Over the last decades, different approaches have been suggested how to improve the inertness of the capillary surface and to stabilize the electroosmotic flow (EOF). Surface coatings of various kinds have been suggested for improving the reproducibility and repeatability of EOF by lowering the sorption properties to the silica surface.

A procedure for preparation of polypyrrole-coated capillaries for capillary electrophoresis and their modifications is presented. Unmodified and both dextran sulfate and heparin modified capillaries were characterized by the dependence of electroosmotic mobility on pH. Dextran sulfate modification was utilized in chiral separation of a model mixture or *R,S*-ibuprofen and heparin modification was employed in separation of a model mixture of selected herbicides by micellar electrokinetic chromatography.

2. THEORETICAL PART

2.1 Capillaries for capillary electrophoresis

Fused silica is by far the most common material because of its UV transparency, high thermal conductivity, and commercial availability of coated flexible fused-silica tubing with uniform internal diameter. CE tubes are made of nonporous fused-silica, a material introduced in 1979 for GC columns[1].

Although silica possesses many beneficial characteristics, there are several factors limiting its employment as a universal setting for separations by CE and its modes. Adsorption of basic and hydrophobic compounds is considered as the most serious one, namely in protein separations by CE the loss of peak efficiency and poor recoveries forces researchers to avoid using unshielded silica surface and employ surface modifications or different materials that do not exhibit such affinity toward these compounds. A second often noted difficulty presents a repeatability of silica surface conditions, which usually changes in time and could be significantly different in different part of capillary, negatively affecting its performance[2]. For certain number of applications, a presence of the electroosmotic flow (EOF) could be another major disadvantage as it could extend analysis time or ruin the separation itself. In such cases, the elimination of the EOF could be useful.

In past decades, when it was difficult to obtain very pure silica, some authors turned their attention toward other materials than silica. Utilization of plastic hollow fibers in capillary zone electrophoresis helped to overcome the named difficulties, but it also brought new ones. In the early days of capillary electrophoresis, the pioneers employed hydrophobic polymers such as polyethylene[3,4] and polypropylene[5-7] capillaries, a few papers reported use of poly(vinyl chloride)[3,8] or poly(methylmethacrylate)[9,10]. Properties of these materials were evaluated in detail by Schutzner and Kenndler[4] regarding electroosmotic mobility at different pH and content of organic solvents.

Poly(tetrafluoroethylene) (PTFE)[11,12] or fluorinated poly(ethylene propylene)[13-15] present another material mostly used as an alternative to silica because of its chemical inertness and observation of weaker EOF in contrast to fused silica. These materials are both considered as UV transparent; however, their optical transmittance differs as was compared by Macka et al.[12] in Figure 1.

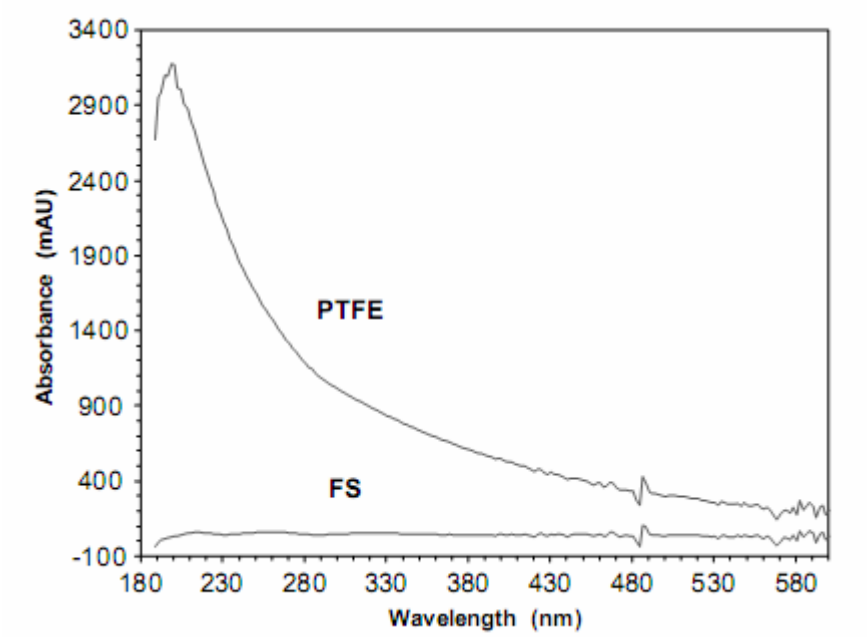

Figure 1. Absorbance spectra of PTFE and fused silica[12].

Another drawback of these fluorinated materials is their mechanical softness, they do not hold the shape properly, so their employment in commercial instruments designed for fused-silica capillaries brings practical difficulties[12].

In several applications, poly(ether ether ketone) (PEEK)[16-19] capillaries were found useful for their tolerance to range of organic and inorganic chemicals and stability even at extreme pH because of the chemical resistance of backbones. However, their lack of optical transparency below 270 nm requires utilization of other than optical detection techniques, e.g. contactless conductivity detection (CCD)[17].

Utilization of other inorganic materials than silica provided another possibility how to eliminate its unsatisfactory properties. Oxides of titanium[20], aluminium[21], or zirconium[22,23] were tested as inner surface of capillaries. They are usually produced by decomposition of appropriate compound on the silica capillary wall.

Modified capillaries exhibited higher hydrolytic stability than silica and a decrease in protein adsorption was observed. These materials exhibit the EOF switchable in direction depending on pH or even by sulfate concentration in case of zirconia[22].

2.1.1 Materials for electrophoresis on microchips

In recent two decades, electrophoresis on microchips underwent very fast development. These devices allowed faster and more efficient analyses in various kinds of application[24,25].

A broad range of polymers have been investigated for the fabrication of microchip devices. These materials are characterized by low cost, high purity, and by an advantage of wide choice of microfabrication techniques, which influences the cost of a chip and possibility to employ them as disposable devices.

Poly(methylmethacrylate) (PMMA)[26] presents one of the most widely used substrate, followed by poly(dimethylsiloxane)[27], polystyrene[28], polycarbonate[29], polyimide[30], poly(ethylene terephtalate)[31], and polycycloolefin[32] (commercial name Zeonor). Glass or fused silica[33] substrate could be also utilized as it presents very attractive material for chips for excellent optical properties and resistance to different solvents; however, preparation of a microchip on a glass surface is not as comfortable as on polymers.

These materials possess uncountable number of surface modification possibilities which are usually useful for prevention of analyte-wall interactions and stabilization of the EOF. The later presents a deal of great importance as injections rely on a reproducible EOF. It is also important to note that approaches of surface modification cannot be transferred from one material to another[34].

2.2 Electroosmotic flow

The electroosmotic flow (EOF) presents a phenomenon occurring in electromigration techniques. In the first experiments, Helmholtz[35] applied an electric field to the end of glass capillary filled with an aqueous electrolyte solution. The inner surface acquires negative charge, which is compensated by the oppositely charged particles from the solution. Helmholtz observed movement of bulk solution caused by migration of solvated cations. The model for description of the EOF was proposed by Smoluchowski[36] according to double layer at the solid-solution interface, which was finally characterized by Stern[37].

In case of commonly used fused-silica capillary, the sources of negatively charged surface are the silanol groups. It is generally accepted that there are 4-5 silanol groups/nm^2 on the fused-silica capillary surface[38]. The ionization of silanol groups is pH-dependent, the pK_a value for surface silanol groups ranges between 2 and 9, as can be seen in the Figure 2. The silica surface is modelled as a polyanion constituted of silanol groups with various pK_a

values. Exact value of pK_a 7.7 was obtained by infrared spectroscopy[39]. Silica capillaries exhibit a pH hysteresis effect – electroosmotic mobility at the same pH differs depending upon whether the pH value was obtained by acidification or alkalization[40].

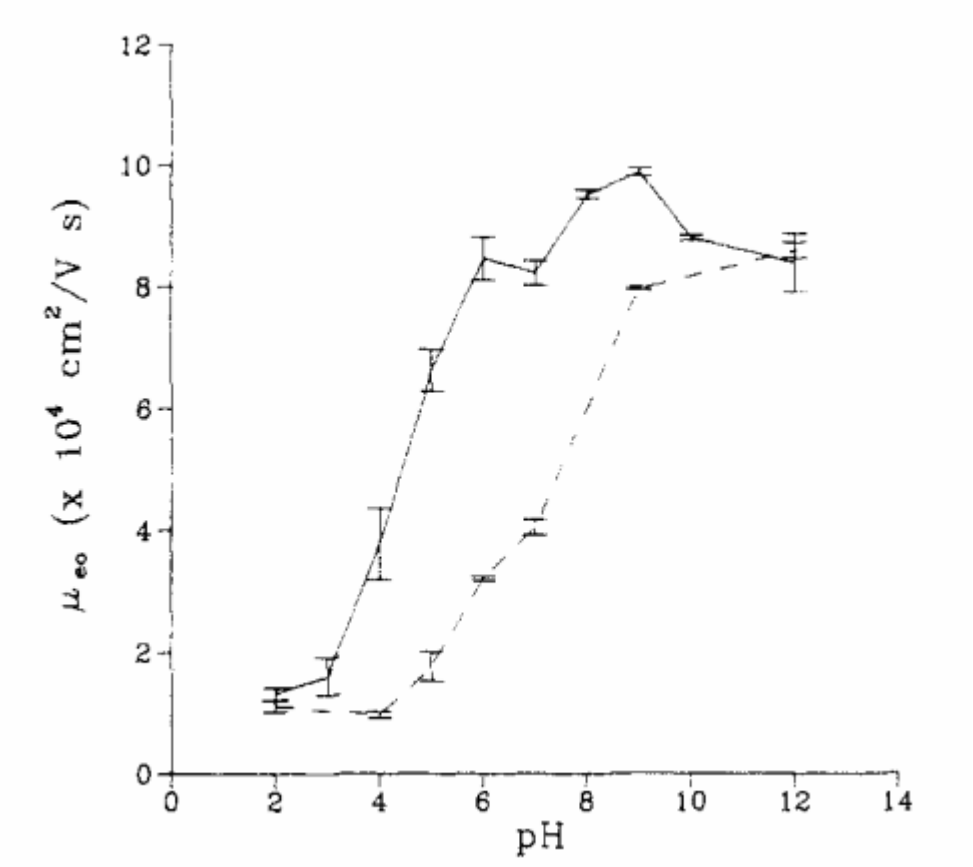

Figure 2. Dependence of the electroosmotic mobility on pH in silica capillaries[40]. Solid line presents data obtained on going from alkaline to acidic conditions, the dashed line represents data with increasing pH.

Other possible mechanism of the EOF origin is the adsorption of species possessing charge. The phenomenon is an explanation for observation of the EOF in teflon capillaries[12]. In other polymeric materials, the EOF can be generated by residual functional groups from substrates.

2.2.1 Manipulation of the electroosmotic flow

Utilization of the EOF flow for the separation may be either useful or undesirable, depending on the purposes and conditions of analysis. The EOF acts as a pump creating a hydrodynamic flow in the capillary and adding the same velocity for all solutes. In contrary to pressure driven systems, the flow profile of the EOF is much narrower than the laminar

flow profile[41] leading to observation of narrower peaks. At certain conditions, the EOF enables separation of both anionic and cationic species in a single run[42].

As the EOF is strongly dependent of pH of background electrolyte, its magnitude at high pH values can be limiting. There are several ways how to control the EOF:

Table 1. Possibilities of control of the EOF[43].

variable	result	comment
electric field	proportional change in the EOF	• affects efficiency, resolution, and Joule heating
buffer pH	decrease of the EOF magnitude at low pH, increase at high pH	• changes the effective charge of analyte, may alter selectivity
ionic strength buffer concentration	increased ionic strength decreases zeta potential and electroosmotic mobility	• high ionic strength generates high current • low ionic strength causes adsorption more problematic • may distort peak shape according to conductivity of zones
temperature	changes viscosity (2-3% per °C)	• temperature control needed
organic modifier	changes zeta potential, viscosity	• changes buffer characteristics, may alter selectivity
surfactant	adsorption at capillary wall	• anionic surfactants can increase the EOF magnitude • cationic surfactant can decrease the EOF magnitude or reverse the EOF
neutral hydrophilic polymer	adsorption at capillary wall	• decrease of the EOF magnitude by shielding surface charge • increase in viscosity
covalent coating	chemical bonding to capillary wall	• modifies surface properties
radial electric field[44]	affects zeta potential	• special instrumentation required

An elimination of the hydrodynamic flow caused by electroosmosis is also practicable by utilization of hydrodynamically closed separation system, where separation occurs in a tube closed with semi-permeable membrane. Such make-up is usually employed in isotachophoretic instruments[45] where the hydrodynamic flow is undesirable. However, the EOF is still present and contributes to dispersion of ionic boundaries[46].

2.2.2 Markers of the electroosmotic flow

As the electrophoretic separation occurs according to different mobility of ions, solutes without an electric charge are not able to migrate. Therefore, when determining electroosmotic mobility, strictly neutral compounds are used, which do not interact with any component of background electrolyte and at the same time provide proper response in the detector. From the other points of view, such marker should be miscible enough with the background electrolyte, available at sufficient purity and price, and should not be very toxic for the environment.

The most often employed markers are dimethylsulfoxide, mesityl oxide, acetone, methanol, thiourea, acrylamide, or benzene. For determination of electroosmotic mobility in the electrokinetic chromatography or chiral separation applications, methanol seems to fulfill enough the requirements of the inert EOF marker[47].

2.2.3 Determination of low electroosmotic mobilities

As the injection and detection of the EOF marker usually takes place at the different ends of capillary, at low electroosmotic mobilities, the time necessary to transport the EOF marker into the detection window reaches becomes very long and ineffective. Therefore, accelerated methods for determination of low mobilities were developed.

At first, it is important to notice a possibility of the EOF marker injection at the short end of capillary (from the outlet) and use the capillary length from the outlet to the detector as the effective length. Electroosmotic mobility is calculated by an equation:

$$\mu_{eof} = \frac{L_{tot} \cdot L_{eff}}{V \cdot t_{migr}} \qquad (1)$$

where L_{tot} presents total length of capillary and L_{eff} is an effective length to the detector, V applied voltage and t_{migr} migration time of the EOF marker.

However, a disadvantage of this method may consist in an inaccuracy as the greater effect of greater variation of injection plug length to the effective length presents an error for mobility determination. Usual lack of thermostating the outlet end of the capillary contributes to the variation of observed mobility. Also, many older electrophoretic instruments are designed for hydrodynamic injection only from the inlet vial. Nevertheless, the method could

be the fastest way to roughly determine low mobilities, if they are measurable in a reasonable time.

In 1996, Williams and Vigh[48] introduced a fast method based on precise pressure rinses. At first, capillary is filled with BGE and then a solution of EOF marker in BGE is injected for time t_{inj}, followed by an application of injection pressure for time t_{tr} on a vial containing BGE. Next, a second band of marker is injected for the same time t_{inj} and transferred a distance for the same time t_{tr} as shown in Figure 3(cit48).

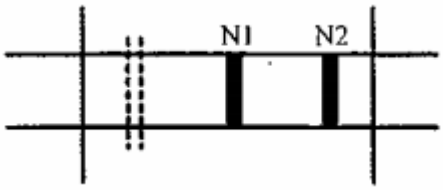

Figure 3. Introduction of two marker bands into thermostated part of the capillary (assigned by two lines). Dashed line presents position of detector[48].

After that, voltage V is applied for the time t_{migr}. During this step, both bands of marker move in the direction of EOF with mobility corresponding to μ_{eof} as shown in Figure 4(cit48).

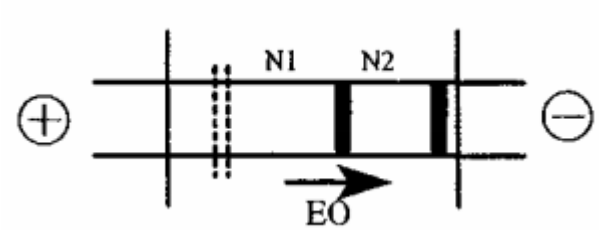

Figure 4. Application of an electric field. EO arrow means the direction of the EOF[48].

Then, a third zone of marker is injected for time t_{inj} as shown in Figure 5(cit48)..

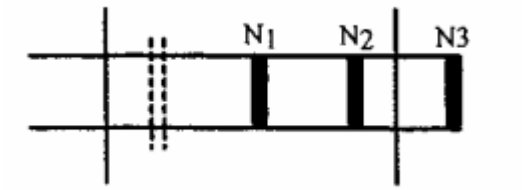

Figure 5. Injection of third band of marker[48].

Finally, injection pressure is applied and capillary is flushed with BGE and times of three peaks ($t_1 < t_2 < t_3$) are registered. Electroosmotic mobility is calculated by an equation:

$$\mu_{eof} = \frac{L_{tot} \cdot L_{eff} \cdot \left(t_3 - 2t_2 + t_1\right)}{V \cdot t_{migr} \cdot \left(t_3 + t_{inj}/2\right)} \qquad (2)$$

Advantage of this methods lies in the ability of use of a thermostated part of capillary, therefore the contribution of generated heat is lowered as much as possible.

Similar procedure was developed by Sandoval and Chen[49] in the same year consists of injection short band of marker for time t_{inj}, application of voltage V for time t_{migr}, then injection of marker for the same time t_{inj} and finally flush the capillary to bring the marker zones into the detector window ($t_1 < t_2$). The electroosmotic mobility is calculated by an equation:

$$\mu_{eof} = \frac{L_{tot} \cdot L_{eff} \cdot \left(t_2 - t_1 - t_{inj}\right)}{V \cdot t_{migr} \cdot \left(t_2 + t_{inj}\right)} \tag{3}$$

The accelerated method seems to be a little bit faster than the William's method; however, it lacks the ability to use just thermostated part of capillary. Authors suggested application power application long enough (at least 5 minutes according to the results) to minimize the temperature effect.

Ermakov et al.[50] proposed a method based on electrokinetic injection of marker solution. The marker band is then flushed to the detector and area of corresponding peak is evaluated according to an equation:

$$\mu_{eof} = \frac{L_{eff} \cdot \delta_{hydr} \cdot A_{EOF}}{V \cdot t_{EOF} \cdot A_{hydr}} \tag{4}$$

where δ_{hydr} presents the length of sample plug injected by a pressure, A_{hydr} presents corresponding area of the marker injected by pressure, t_{EOF} presents electrokinetic injection time and A_{EOF} is corresponding area of the marker injected electrokinetically by the applied voltage V. A possible problem may present the knowledge of value δ_{hydr}, as the calculation requires the viscosity of BGE to be known.

There are also other ways how to determine the electroosmotic mobility: by a measurement of change in level of solution in the receiving vial[51], measuring the time required for the electrolyte in one vial to reach a specific height[52], measuring a fluorescent marker downstream from the detector[53], weighing[54], current monitoring[55], and streaming potential measurement[56]. However, many of them require special equipment and most of them are not suitable for determination of low electroosmotic mobilities.

2.3 Effect of capillary pretreatment

Among manufacturers, the procedures of manufacturing can significantly vary, even batch to batch differences in the electrophoretic behaviour are observed[2].

A presence of trace metallic impurities located at silica surface may behave as additional source of acidic species and increase of acidity of near silanol groups[57]. Capillaries are produced by drawing at temperatures near 2000 °C, when the surface undergoes dehydroxylation. During capillary storage, the surface becomes rehydoxylated depending on the air humidity[1]. Therefore, the capillary pre-treatment is necessary in order to obtain reproducible electrophoretic behavior – a problem hampering larger employment of CE.

The most preferred method presents dynamic etching (in flow) with 1 mol.L^{-1} NaOH for a time ranging from 10 minutes to one hour[2]. The etching has been reported to remove graphite which is covalently bonded by Si-C linkages formed at high temperatures during the drawing of capillary[58].

Acidic conditioning by HF is aggressive towards silica, making the inner surface smoother and more populated with silanol groups[59]. On the other hand, conditioning by HCl does not dissolve the silica, but is essential in order to remove metallic impurities, namely iron, magnesium and calcium[60].

Several papers assessed the effectiveness of combined NaOH and HCl capillary pretreatment[2,59,61]. Rinse with 1 mol.L^{-1} NaOH for at least 1 hour followed by rinse with water and next rinse with 1 M mol.L^{-1} HCl for 1 hour is often the recommended procedure. Gomez et al.[2] also suggested a high flow rinse, as the effectiveness of is strongly dependent on the flow rate of treating solution.

The second step of silica capillary pre-treatment consists of conditioning with background electrolyte. During rinsing, surface undergoes re-equilibration until steady-state surface condition is reached. Such equilibration presents slow phenomenon as it may take weeks until the electroosmotic mobility becomes stable[40].

2.4 Capillary coatings

Capillary coatings present one of the techniques of manipulation of the EOF in order to optimize efficiency, resolution, and speed of analysis. In many cases, the coating is performed in order to reduce the EOF as much as possible; however, often it is useful to provide strong EOF, both in cathodic and anodic directions. The second reason for modification of silica surface is to improve repeatability of consecutive runs and also the reproducibility of the developed methods as the bare-fused silica capillaries may exhibit inconsiderable capillary-to-capillary differences. The third, but often the most important reason, is to reduce analyte-wall interactions, namely in protein separations or when analyzing samples with complex matrices. The properties of used coating may affect the separation selectivity, namely in capillary electrochromatography the separation mechanism is based on analyte's interaction with the stationary phase.

Capillary wall coatings are described as dynamic or static, based on the type of coating attachment to the capillary wall surface. Dynamic coatings involve adsorptive secondary interactions, while static coatings are based on covalent bonding between the capillary wall and the coating material[38]. All attitudes of coating the silica surface possess their own advantages and disadvantages; they will be discussed in following sections separately. In many cases, it is difficult to accurately decide if presented type of coatings belongs into the category of dynamic or adsorbed coating. The authors sometimes did not provide sufficient information about coating agent properties or even coating procedure, electrophoretic performance, or stability.

The characterization of capillaries after the modification is very difficult. Properties of coated capillaries are commonly performed to measure the EOF and investigate its dependence on pH of the BGE[62]. Unfortunately, there is no unified approach for determination the dependence of the EOF on pH. Authors use different BGE with various counter-ions, even at different ionic strength. Therefore it is almost impossible to compare obtained results among the papers. Gomez[2] noted the importance of measure the electroosmotic mobility in buffers with constant ionic strength. Stability of the coating can be expressed by a relative standard deviation (RSD) of migration times among consecutive runs in the same condition. Coating performance could be also studied by a model mixture of proteins[63] regarding the peak efficiency and recovery as a marker of irreversible adsorption on silica surface that might have remained uncoated. Another possibility how to characterize the inner surface presents utilization of scanning electron microscopy (SEM)[64], atomic force

microscopy (AFM)[65,66], or by gas chromatography[62], when a retention of a probe compound on the coating is studied.

Most of works dealing with modifications of capillary surface contain a study of coating lifetime, often in the conditions of the reported application[67] or at extremely pH[68]. These results are usually reported as a percentual decrease of electroosmotic mobility in time[69], or a number of analysis the capillary endured[70]. A report of variation among capillaries or even batches also brings useful insight into coating performance. However, many authors do not provide proper information about the stability. Therefore, the missing data disallows to estimate the robustness of the coating and its suitability for certain applications.

2.4.1 Dynamic coatings

Dynamic coating is realized by rinsing the capillary with a solution containing a coating agent. Small amount of coating agent should be present in the BGE keep the surface properly coated during the analysis. The lifetime of the coating can be extended by usually simple regeneration procedures.

As dynamic coating agents are generally considered compounds interacting with the silica surface via electrostatic interaction or are adsorbed at the surface via hydrophobic interactions. However, other types of interactions are also possible, depending on the structure and properties of the coating agent and the status of the silica surface, namely pH value of surrounding solution affecting its ionization.

2.4.1.1 Amines

Very common small molecule additives are amines based on their use in reversed phase HPLC eliminating tailing of basic compounds due to interactions with uncoated silanol groups[71]. In CE, a number of amines with various structures were reported. For examples see Table 2. Interaction of amines with capillary wall leads to reduce the electroosmotic mobility while reducing its adsorption potential and improving efficiency and repeatability of electrophoretic performance namely in protein separations[72]. Verzola et al.[73] compared different classes of amines in order of reduction of protein adsorption inhibition and found that polyamines as spermine[74] or provided very good results at milimolar concentration while triamines[75] or diamines[76] achieved such results at concentrations ten or hundred times higher.

Amines as weak bases are efficient for the coating only in the protonated form, therefore pH of the BGE presents a critical factor. The efficiency of surface modification is significantly influenced by number of binding centers and distance among them[77].

Righetti et al. introduced a new class of additives named the "Skorpios"[78]. These compounds are trifunctional, possessing a quaternary amine, a tertiary amine and ω-alkyl iodine. Rinsing with such reagent before analysis provided strongly reversed electroosmotic flow even at highly basic pH; however, the protein adsorption remained serious problem.

Table 2. A list of dynamic coatings performed by amines and their applications.

amine	concentration	application	ref.
triethylamine	30 mmol.L^{-1}	separation of anilines	[79]
triethanolamine	27 mmol.L^{-1}	separation of butyric and pentanoic acid	[80]
1,4-diaminobutane (putrescine)	3 mmol.L^{-1}	separation of peptides	[76]
hexamethonium bromide (HMB)	0.75 mmol.L^{-1}	determination of chloride and sulfate in fuel	[81]
spermidine	0 - 1 mmol.L^{-1}	study of effect on electroosmotic mobility	[75]
spermine	0.02 mmol.L^{-1}	determination of transferrin	[74]
tetraethylene pentamine	1.5 mmol.L^{-1}	determination of organic acids in wine	[82]

2.4.1.2 Surfactants

Surfactants present another class of dynamic coating agents. They are amphiphilic compounds possessing both a hydrophobic tail and hydrophilic functional group. At certain concentration considered as critical micellar concentration (CMC), they aggregate into forms of spherical micelles or bilayers[63].

The adsorption of cationic surfactants on the silica surface was described to occur in several stages depending on the concentration of surfactant[83]: (i) at low concentration, the adsorption occurs similarly as in the case of other small molecules, i.e. single molecule interacts with certain silanol group; (ii) increase in concentration leads to lowering the distances among the molecules of surfactant, after exceeding a threshold concentration, an aggregate is formed at the capillary surface. The threshold concentration for silica surfaces is much lower than CMC of corresponding surfactant[84]. Both concentration thresholds are significantly influenced by the BGE[85]. Melanson et al.[86] suggested the concentration above the threshold in order to obtain reproducible migration times. The type of the aggregate depends upon the structure of the surfactant: single-chained molecule as CTAB (cetyltrimethylammonium bromide) forms a micelar layer while double-chained surfactant as

DDAB (didodecyldimethylammonium) forms a bilayer. This phenomenon was studied by AFM and the results[87] confirmed proposed structures shown in Figure 6[(cit86)].

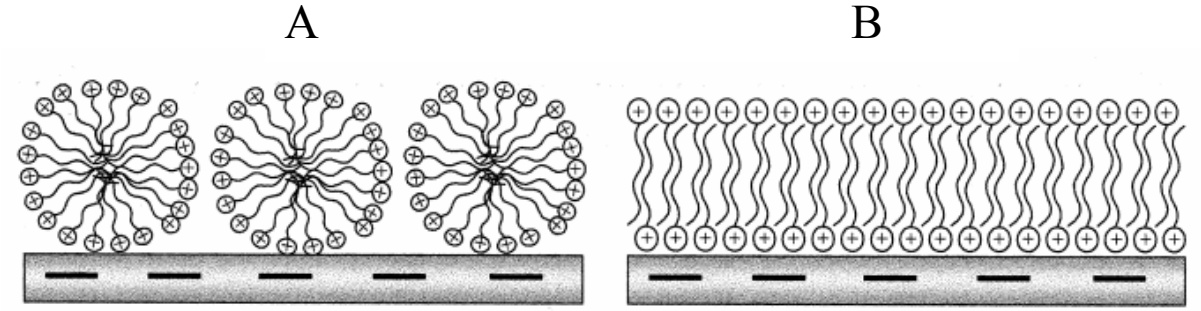

Figure 6. Structures of surfactant aggregates above CMC at the silica capillary wall. CTAB and TTAB form a micellar layer (A) and DDAB form a bilayer (B)[86].

The aggregate structure has an impact on the coating performance: single-chain surfactants must be present in the BGE in order to maintain the coating during the analysis, while double-chained surfactants form semi-permanent coatings, so they do not need to be present in the BGE during the analysis[88].

A major consequence of the aggregate formation is the EOF reversal, which has been widely used in many applications namely in analysis of anions[89,90]. As the magnitude of the EOF in uncoated silica capillaries could be controlled by various cations present in the BGE[86], the alternation of anionic BGE constituents affects the electroosmotic mobility. Examples of frequently employed cationic surfactants are listed in Table 3.

Non-ionic surfactants as Brij-35[99], Triton X-100[92], or Tween 20[91] present polymers composed of hydrophobic head and hydrophilic polyoxyethylene part. These compounds tend to adsorb on the silica surface and prevent undesired adsorption of analytes, e.g. proteins[91]. Triton X-100 was reported to improve stability of baseline[92].

Zwitterionic surfactants as lauryl sulfobetaine[91] present compounds possessing both anionic and cationic groups together with longer hydrophobic tail. Employment of such additives in about milimolar concentration leads to suppression of the EOF and improvement of efficiency and recovery in several protein separations[93].

Table 3. A list of dynamic coatings performed by surfactants.

surfactant	concentration	application	ref.
cetyltrimethylammonium bromide (CTAB)	0.5 mmol.L^{-1}	determination of organic acids in cheese and yogurt	[90]
tetradecyltrimethylammonium bromide (TTAB)	0.3 mmol.L^{-1}	analysis of plant extract	[94]
myristyltrimethylammonium bromide (MTAB)	0.5 mmol.L^{-1}	analysis of wine	[95]
dioctadecyldimethylammonium bromide (DODAB)	0.1 mmol.L^{-1} (rinse only)	separation of proteins	[96]
didodecyldimethylammonium bromide (DDAB)	0.1 mmol.L^{-1} (rinse only)	separation of anions and cations	[97]
1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)	1 mmol.L^{-1} (rinse only)	separation of proteins and steroids	[98]
Brij-35	10 mmol.L^{-1}	determination of olive oil acidity	[99]
Triton X-100	0.0125% (v/v)	determinaton of impurities of foscarnet sodium	[92]
Tween 20	0-3% (v/v)	study of adsorption of proteins	[91]
lauryl sulfobetaine	0-2.8% (v/v)	study of adsorption of proteins	[91]

The employment of surfactants helps to overcome the difficulties of reproducible analyses by CE. The type and concentration of selected surfactant enables to control both magnitude and direction of the EOF easily.

However, a lot factors limit their use. Surfactants can ruin the separation by undesired interaction with analytes, or even precipitation with BGE constituents[100] was reported. Presence of the surfactant counterion presents a significant drawback, bromide anion as the most common counterion for cationic akylammonium salts induces system peak that can interfere with analyte determination[101]. Employment of higher concentration of surfactants is not compatible with MS detection.

2.4.1.3 Adsorbed polymers

Neutral or basic polymers tend to adsorb on the silica surface via weak interactions – by hydrogen bonding or Van der Waals interactions. The capillary pretreatment should produce protonated silanol groups suitable for such interactions.

Neutral polymers are used in order to reduce the EOF by reducing the electrokinetic potential at the capillary wall and also the analyte-wall interactions. Derivatives of cellulose[102-104] present the most frequently used polymers, followed by polyvinyl alcohol

(PVA)[105], polyethylene oxide (PEO)[106] and dimethylacrylamide[108]. The thickness of the coating determines the effectivity of reduction of the EOF and of shielding the surface[114]. The hydrophilicity of the coating polymer is very important for reduction of the interactions of biopolymers, but it also reduces the stability of adsorbed coating[38]. Hydrophilic polymers could be thermally immobilized at the capillary wall[105] or need to be added to the BGE to maintain the coating[103]. Many polymers form sieving matrices used in DNA analysis[115].

Basic polymers as polyethyleneimine (PEI)[110], polyarginine[111], chitosan[112], or poly(diallyldimethylammonium chloride) (PDADMAC)[113] cause the surface to exhibit acquire a positive charged and the direction of the EOF is usually reversed. As in case of neutral polymers, the coating deteriorates during use, therefore reconditioning is necessary after certain number of runs.

A list of frequently used polymers is shown in Table 4.

Table 4. A list of adsorbed polymer coatings and their applications.

polymer	concentration	application	ref.
hydroxypropyl-methylcellulose	0.35% (m/v)	separation of peptides	[102]
hydroxyethylcellulose	0.1% (m/v)	separation of amino acids	[103]
cellulose acetate	4.5% (m/v) (immobilized)	separation of proteins	[104]
poly(vinyl alcohol)	10% (m/v) (immobilized)	determination of organic acids and herbicides	[105]
poly(ethylene oxide)	1.5% (m/v)	determination of amino acids	[106]
poly(vinylpyrrolidone)	2% (m/v)	separation of DNA	[107]
poly(dimethylacrylamide)	3% (m/v)	separation of DNA fragments	[108]
poly(*N*-hydroxyethylacrylamide)	0.1% (m/v)	separation of proteins	[109]
poly(ethylene imine)	0.1% (m/v)	separation of proteins	[110]
polyarginine	0.005% (m/v)	separation of proteins	[111]
chitosan	0.2% (m/v) (immobilized)	analysis of proteins in extract	[112]
poly(diallyldimethylammonium chloride)	0.04% (m/v) (rinse)	separation of peptides	[113]

2.4.1.4 Successive multiple ionic polymer layer

In 1998 Katayama[116] introduced an approach employing two (or more) oppositely charged polymers to create a successive multiple ionic polymer layer (SMIL) on the silica capillary wall. The procedure of preparation of the coating is shown in Figure 7[116]. The established coating enables about 100 runs without significant deterioration and seems to be easily repaired by a simple reconditioning. The coating shows resistance to organic

modifiers, 1 mol.L^{-1} NaOH, and surfactants. Silica surface becomes fully shielded, in case of employment of Polybrene and dextransulfate, capillary-to-capillary reproducibility of less than 1% RSD is reported[116].

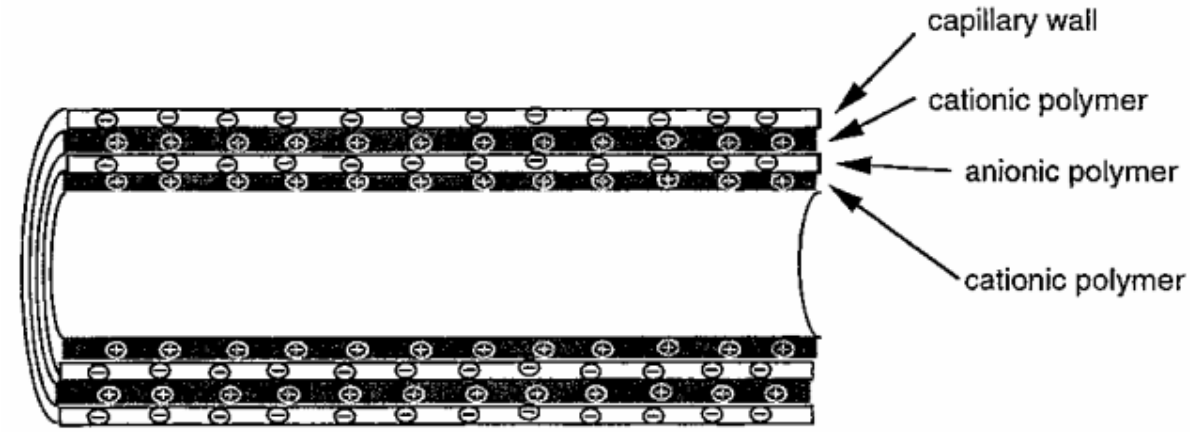

Figure 7. A scheme of SMIL coated capillary[116].

Further studies on this type of coating led to development of numerous modifications, differing for example in the EOF magnitude and direction observed as polyelectrolytes with various characteristics are used. Suppression of the EOF is also possible, MacDonald and Lucy[123] reported use of dimethyldioctadecylammonium bromide (DODAB) and polyoxyethylene 40 stearate. The coating was used for separation of a model protein mixture and excellent repeatability and efficiency was achieved. Research papers utilizing SMIL are summarized in Table 5.

Nehmé and co-workers[125] systematically studied the coating performance regarding preparation condition. Significant effect of ionic strength of coating solutions was reported, followed by proper rinse with BGE and application of voltage after deposition of each layer.

Table 5. A list of SMIL coatings and their applications.

cationic layer	anionic layer	application	ref.
poly(diallyldimethylammonium chloride)	poly(styrene sulfonate)	separation of proteins	[117]
polybrene	dextran sulfate	determination of organic acids	[118]
poly(ethyleneimine), protamine	dextran sulfate	chiral separation of 1′-binaphthyl-2,2′-diyl hydrogen phosphate	[119]
α-chymotrypsinogen A	dextran sulfate	separation of small ions	[120]
poly(diallyldimethylammonium chloride)	dextran sulfate	chiral separation of amino acids	[121]
polybrene	poly(vinyl sulfonate)	sepration of peptides	[122]
dimethyldioctadecylammonium bromide	poly(oxyethylene) 40 stearate	separation of proteins	[123]
poly(diallyldimethylammonium chloride)	SDS	effect of conditions on EOF	[124]

Both adsorption and electrostatic interactions are employed in SMIL coatings, therefore very effective and long-term stable coating is obtained. The use of polymers may make difficult the employment of UV detection for their self-absorption or MS detection. Nevertheless, a successful application of SMIL with MS detection was reported[126]. There are many polyelectrolytes with different charge densities commercially available; together with a number of parameters of these coatings it makes the SMIL coatings a robust and versatile tool.

A drawback of use of polyelectrolytes consists in possible undesirable interactions with analytes, BGE constituents or other additives. For certain proteins or other macromolecules interacting specifically, the SMIL coatings were found unsuitable.

2.4.1.5 Ionic liquids

Ionic liquids are salts with low melting temperature (less than 100 °C), in many cases it is lower than room temperature. They posses low vapor pressure and they have wide range of solubility, polarity, viscosity, or density[127]. The ionic liquid environment differs from the polar or non-polar organic solvents, it presents a new class of solvent. These compounds are formed by a bulky organic cation most frequently composed of *N*-alkyl-imidazolium, *N*-alkyl-pyridinium, *N*-alkyl-N-methylpyrrolidinium, tetraalkyl-ammonium, or tetraalkyl-phosphonium and of a counter-ion like tetrafluoroborate, trifluoroacetate, perchlorate, or nitrate.

They were found useful in GC as stationary phases for their thermal stability and in HPLC as mobile phases or only as additives in the mobile phases.

In CE, ionic liquids could be used as constituents of the BGE in both aqueous and non-aqueous media[128]. However, the utilization as solvent in capillary is limited because of their high conductivity. Several ionic liquids were used to perform chiral[129] and MEKC separations[130].

With regard to the hydrophobic character and possession of the cationic part, they are strongly adsorbed on the capillary wall. Immobilization of these compounds leads to the reduction of the EOF or in many cases in reversal of its direction. The performance is affected mainly by the concentration in the BGE, the nature of cation, and length of alkyl chain. Many applications of ionic liquids as dynamic coating agents have been recently

reported in analysis of polyphenolic compounds[131], halogenated phenols[132], proteins[133], carboxylic acids[134], or flavonoids[135].

Several ionic liquids were reported to be covalently coated to the silica capillary wall in order to analyze sildefanil and its metabolites[136], DNA fragments[137], or alkyl phosphonic acids[138].

2.4.2 Covalent coatings

Permanent surface coating presents another interesting possibility how to eliminate EOF and decrease interaction of analytes with the separation capillary. In contrary to dynamic coating, the procedures for preparing coated capillaries are often more complex and more demanding regarding chemicals and equipment.

Preparation of the coating usually consists of several steps. At first, the capillary pretreatment is necessary. Trace metal ions in silica structure may affect the bonding of stationary phase, its uniformity at the surface leading to a degradation of hydrolytic stability[57]. Then, usually an intermediate layer is introduced on the capillary surface. The layer often consists of a bifunctional silane, namely 3-(aminopropyl)trimethoxysilane[143], 3-methacryloxypropyl-triethoxysilane[153], or 3-isocyanatopropyltriethoxysilane[156]. For improving the yield of silylation, Cifuentes et al.[61] recommended dehydration the capillary surface at 160 °C. After silylation, an attachment of certain coating agent to the silane is carried out. Various compounds can be introduced, whether large molecules or small compounds, e. g. monomers for further *in situ* polymerization. Nevertheless, there is a possibility to perform the preparation in one step – Malik and co-workers[139] filled the capillary with a mixture of surface derivatization agent, free radical initiator, and coating polymer. Brief heat treatment was sufficient to immobilize the polymer film on the surface.

There are many strategies differing in used procedures; almost every research group proposes their developed one, therefore it is impossible to generalize the preparation into a universal process. Development of every type of coatings requires careful optimization of many experimental conditions. Also it is important to notice a need to consider stable storage conditions as usually several capillaries are prepared together.

The most frequent Si – O – Si bonds possess a disadvantage of unsatisfactory hydrolytic stability at alkaline pH[38]. To improve the stability, Cobb[140] prepared coating with Si – C bonds using Grignard chemistry. The coating was reported to be stable even at pH 9.2, while more common siloxane bonds are damaged at less alkaline pH (about 8)[141].

Examples of covalent coatings and their applications are given in Table 6:

Table 6. A list of covalent coatings and their applications.

coating	bridging compound	application	ref.
poly(dimethylacrylamide)	3-(methacryloyl-oxy)-propyltrimethoxysilane	EOF suppression	[142]
poly(acrylamide)	3-aminopropyltriethoxysilane	chiral separation of amines	[143]
hexamethyldisilazane		determination of ephedrine and pseudoephedrine	[144]
tri(ethyleneglycol)	trichlorosilane	separation of proteins	[145]
poly(siloxane)	trimethylsilyltrifluoro-methansulfonate	separation of aromatic cations	[146]
poly(siloxanediol)		separation of biopolymers	[147]
trimethylchlorsilane		DNA fragment analysis	[148]
octadecyltrichlorosilane		separation of proteins	[149]
2-hydroxyethyl methacrylate	trichlorsilyl(chloromethyl-phenyl)ethan	separation of proteins	[150]
poly(acrylate)	vinyltrichlorosilane	separation of fatty acids	[151]
quaternarized piperazine		separation of arylalcanoic acids	[152]
p-allylcalix[4]arene	3-methacryloxypropyl-triethoxysilane	separation of neurotransmitters	[153]
propanediol	3-glycidoxypropyl-trimethoxysilane	separation of proteins	[154]
imidazole epoxy resin	3-methacryloxypropyl-trimethoxysilane	separation of proteins	[155]
poly(ethylene glycol)	3-isocyanatopropyl-triethoxysilane	improvement of biocompatability	[156]

Covalent coatings permit a permanent deactivation of silica surface without any additive present in the BGE. It proves to be useful in order of employment various detection techniques interfering with additives described in the sections above. However, a “bleeding” of the coating should be monitored in order to avoid any undesired surprises. These coatings tend to change less their performance in time in contrary to dynamic coatings and the lifetime of the coating is usually longer.

The main disadvantage of covalent coatings is hidden in their preparation, where numerous material and time demanding steps are performed. At extreme conditions, both pH or in non-aqueous media, their stability is problematic, whereas several types of dynamic coating might prove useful in such situations. The dynamic coatings also have a great ability to be repaired between analyses, providing much more lifetime of the silica capillary itself.

2.4.2.1 Coatings for capillary electrochromatography

Capillary electrochromatography (CEC) presents a technique that potentially combines the separation efficiency of CE with the selectivity and sample capacity of LC[157]. The separation mechanism could be based on both different migration velocity in an electric field and different partitioning between mobile phase and immobilized stationary phase. The transport of mobile phase is in many cases electrodriven instead of use of pressure, offering increase in separation efficiency and better resolution. The high-resolution power of CEC has been reviewed in a number of papers[158-160].

There are several approaches how to introduce stationary phase into capillary. Support particles with chemically bonded stationary phase can be introduced into capillary; however, the all packing methods[161] require lot of skill and experience. Poorly packed capillaries exhibit low efficiency and asymmetric peak shapes. Separation can be performed utilizing classical C18 stationary phases[162], ion-exchangers[163,164], size-exclusion stationary phases[165], or chiral stationary phases[166,167]. The packing material needs to be hold in the capillary in the volume bordered with frits to disallow the stationary phase to reach detector window or be flushed out during conditioning.

The use of open-tubular capillaries is considered as an alternative. Appropriate moiety is bonded to the wall of the capillary allowing an analyte to interact. This technique was applied using mainly ion-exchange[168,169] and chiral[170,171] stationary phases.

Introduction of continuous bed in 1992 by Švec[172] allowed preparing capillaries by much easier *in situ* polymerization of various organic monomers. This approach does not require producing the frits causing lots of difficulties. Developed stationary phases exhibit significantly higher efficiency than small-pore packed materials[173]. Till now, large variety of monolithic media has been described[173,174]. Together with a possibility of use molecularly imprinted polymers[175], continuous bed stationary phases hold great potential for further development of CEC.

2.5 Polypyrrole

2.5.1 Conductive polymers

Chemistry of conducting polymers underwent a great progress in recent decades. These materials are becoming increasingly adaptable to applications which previously belonged to metals. As construction materials, significant weight saving can be obtained. They are used as current carriers or energy-storage materials where they present a less toxic alternative to commonly used materials. Under influence of an electrical current, they can be forced to change color by alternating the intensity and wavelength of their transmission, therefore they are widely used in light emitting diodes and photoelectrical cells[176].

Conducting polymers show almost no conductivity in the neutral state. Their conductivity results from oxidizing or reducing their conjugated backbone or by charge injection from a dopant. Incorporation of ions leads to compensation of charges along the polymer backbone, they show great potential in area of chemical and biological sensors. Synthesis is usually performed by oxidants as $(NH_4)_2S_2O_8$ or $FeCl_3$ leading to solutions of conductive polymers, meanwhile electrochemical deposition on conductive substrates forms films of conductive polymers. The thickness can be controlled by total charge passed during the process[177].

2.5.2 Applications of polypyrrole

Polypyrrole (PP) is one of the most used and intensively studied because of its unique properties. As an electroactive polymer, PP can change its properties when various ions or solvents are used during the polymerization process, and this effect can be exploited in ion selective sensors[178]. The protonation enhances the conductivity of PP, whereas deprotonation causes a lower conductivity[179]. The potentiometric response is linear between pH 2 and 11, the fact makes PP perspective for applications in solid state pH sensors[180]. Together with changes in conductivity, the absorption spectrum is pH-dependent[181] enabling PP to be used as a spectroscopic pH sensor.

Copolymers of pyrrole with various ionophores were reported as selective ion-sensitive materials and suggested for applications in ion-selective electrodes[182]. Introduction of β- and γ-cyclodextrin into PP leads to sensitivity to dopamine, L-3,4-dihydroxyphenylalanine, ascorbic acid and other compounds[183]. Molecularly imprinted

PP was used in recognition of amino acids[184]. Immobilization of receptors like cholesterol esterase[185] or glucose oxidase[186] for amperometric sensors is also possible.

PP has been utilized as an extraction phase for solid-phase micro extraction (SPME)[187] and stir bar sorptive extraction (SBSE)[188]. High extraction efficiencies toward polar compounds, aromatic compounds, and anionic species were reported. In-tube solid-phase microextraction coupled with liquid chromatography (LC) was used for analysis of fluoxetine and norfluoxetine enantiomers in human plasma[187] or β-blockers in urine and serum[189].

2.5.2 Polypyrrole as coating for capillary electrophoresis

Čabala and Frank[190] introduced PP as a coating for CE capillaries. Silylation of silica surface was performed employing a pyrrole derivative of 3-(aminopropyl)triethoxysilane, further oxidization was done using $FeCl_3$ leading into formation of PP inside the capillary. The probable structure of PP is shown on Figure 8[190].

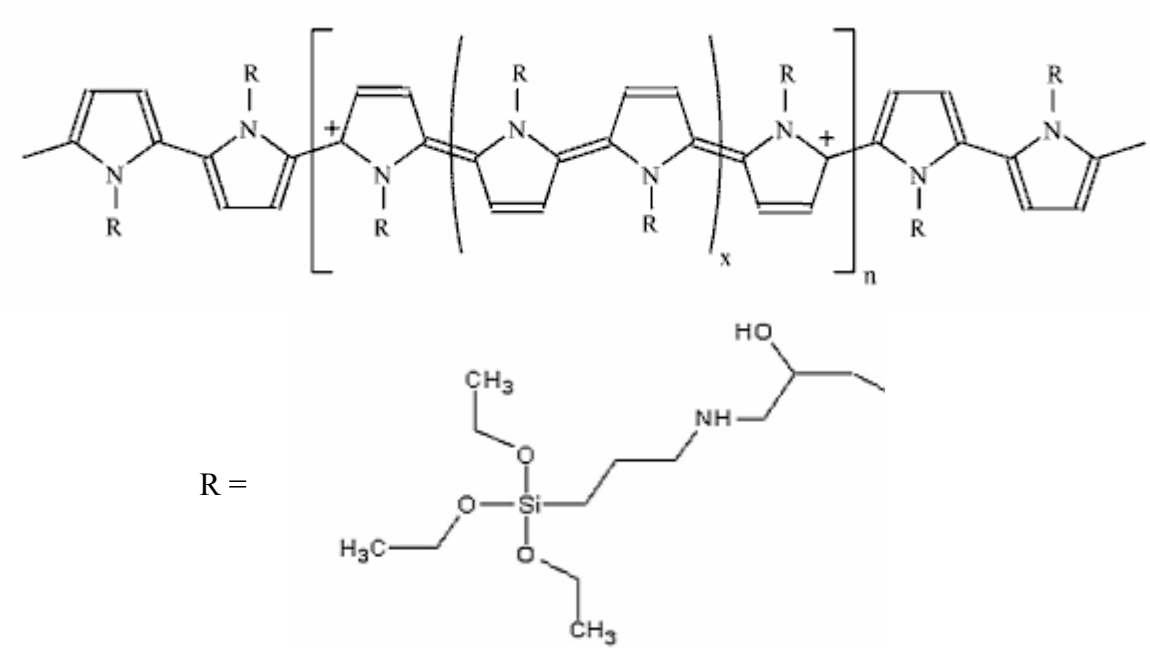

Figure 8. Structure of PP in the oxidized state; counterions are not shown[190].

In another experiment, dextran sulfate modification of PP coated capillary was prepared by polymerization in a mixture of $(NH_4)_2S_2O_8$ and dextran sulfate (sodium salt). Such coating exhibited strong cathodic EOF largely indepent on pH with similar magnitudes as in the case of SMIL coatings with dextran sulfate as the top layer. However, authors did

not keep constant ionic strength of BGEs for determination of electroosmotic mobility. Stability of prepared coating was studied at extreme pH values (2.5 and 9.4) for 80 consecutive runs of EOF magnitude determination and authors did not find any significant deterioration of electrophoretic performance.

The potential of dextran sulfate modified PP coating was demonstrated on the separation of ten selected herbicides in spiked tap water. The capillary zone electrophoresis method showed good selectivity for weak acids at acidic pH and the strong EOF allowed the analysis to be finished in 5 minutes.

3. EXPERIMENTAL PART

3.1 Chemicals and samples

Sodium hydroxide, pyrrole, epichlohydrine, tetrabutylammonium hydrogensulfate, 3-aminopropyltriethoxysilane, ammonium peroxodisulfate, dextran sulfate sodium salt from Leuconostoc spp., heparin sodium salt from porcine intestinal mucosa (HEP), sodium dodecyl sulfate (SDS), 2-(*N*-morpholino)ethanesulfonic acid (MES), *N*-(2-acetamido)-2-aminoethanesulfonic acid (ACES), 3-aminopropyltriethoxysilane, thiourea, acetic acid, acetonitrile, boric acid, nitric acid, phosphoric acid, sodium hydroxide, toluene, methanol, formic acid, *R,S*-ibuprofen, *S*-ibuprofen, and sulfated β-cyclodextrin were obtained from Sigma, Fluka, or Merck.

The herbicide standards mecoprop, dichlorprop, dicamba, 2,4-dichlorophenoxy acetic acid, 4-(2,4-dichlorophenoxy)butanoic acid, 4-chloro-2-methylphenoxy acetic acid, and clopyralid were from Riedel de Haen (Seelze, Germany). All chemicals were analytical grade purity.

3.2 Apparatus and conditions

All separations were performed on a CE instrument equipped with an automated sample injection system and UV-Vis diode array detector (HP^{3D} CE, Agilent Technologies, Waldbronn, Germany).

FT-IR Nicolete 6700 (Thermo Scentific, USA) was used to measure ATR-IR spectra of synthesis products with following parameters: scans: 32, resolution: 4 cm^{-1}, detector: DTGS KBr, beamsplitter: KBr, experiment: ATR (attenuated total reflection), ZnSe crystal.

Fused silica capillaries (Polymicro Technologies Inc., Phoenix, USA, 50 μm I. D. × 365 μm O. D.) with a total length of 48.5 cm and an effective length of 40 cm were used. Capillaries were thermostated at 20 ˚C, the applied voltage was 25 kV unless stated otherwise. All injections were done hydrodynamically by applying a pressure of 30 mbar for 5 seconds.

Background electrolytes were prepared by dissolving the corresponding acid in bidistilled water and by adjusting the pH with 5 $mol.L^{-1}$ NaOH solution to the desired values. SDS or sulfated β-cyclodextrin was added after the pH adjustment.

3.3 Preparation of silanization agent

3.3.1 Synthesis

3-{[3-(*N*-Pyrrole)-2-hydroxypropyl]amino}propyltriethoxysilane was synthetized as described by Faverrole et al.[191].

First step consisted in preparation of an intermediate product *N*-glycidylpyrrole:

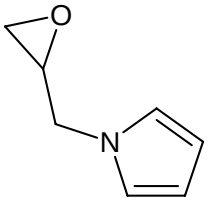

Figure 9. Structure of *N*-glycidylpyrrole.

The mixture of 200 ml of aqueous solution of 50% (m/v) NaOH with 4,8 g tetrabutylammonium hydrogensulfate was prepared and 125 ml of epichlohydrin was introduced into a reactor. The air in the vessel was continuously flushed by nitrogen in order to remove as much oxygen as possible. 40 g of pyrrole were introduced dropwise while stirring the mixture by a teflon stirrer and cooling in an ice bath to keep the temperature between 15 and 20 °C. The mixture was left stirring under a stream of nitrogen for 3 hours. After that, the organic phase was distilled under a low pressure (the pressure was not monitored) and a fraction distilling around 90 °C was separated from parts distilling at lower temperatures. About 30 ml of pure *N*-glycidylpyrrole were obtained. The product was characterized by ATR-IR and NMR.

In next step, 3-{[3-(*N*-Pyrrole)-2-hydroxypropyl]amino}propyltriethoxysilane was prepared by mixing 1.23 g *N*-glycidylpyrrole with 2.21 g 3-aminopropyl(triethoxy)silane (the reaction is shown in Figure 10) in properly dried 50 ml reactor. The air in the vessel was flushed by nitrogen and the mixture was left stirring by a teflon stirrer for about 72 hours at room temperature.

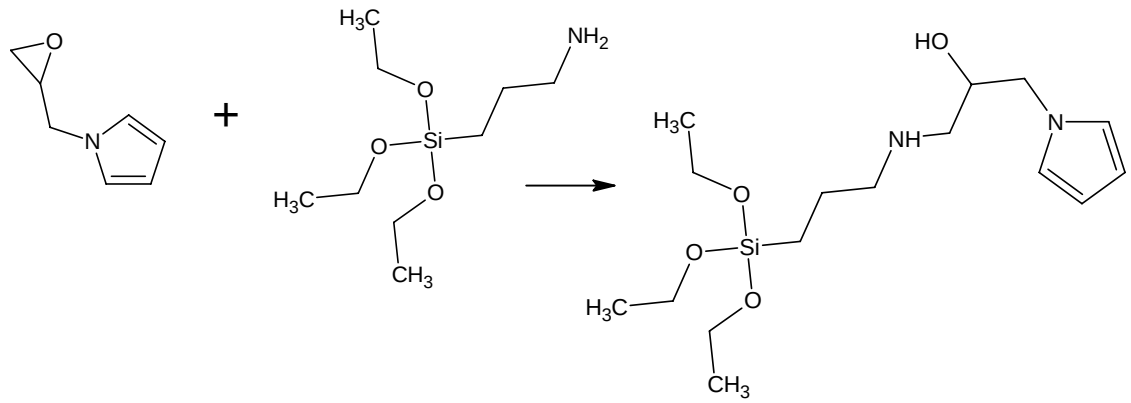

Figure 10. A reaction of *N*-glycidylpyrrole with 3-aminopropyl(triethoxy)silane producing 3-{[3-(*N*-Pyrrole)-2-hydroxypropyl]amino}propyltriethoxysilane.

The intermediate product *N*-glycidylpyrrole and the final product 3-{[3-(*N*-Pyrrole)-2-hydroxypropyl]amino}propyltriethoxysilane were characterized by ATR-IR (see Appendix I).

3.4 Preparation of coated capillaries

Capillaries were preconditioned by rinsing at an external pressure of 0.1 MPa at room temperature for 1 hour each with water, 2.5 mol.L^{-1} NaOH in 50 % (v/v) methanol/water, water, 1 mol.L^{-1} HNO_3 at 70 ˚C, and finally water at room temperature. The capillaries were dehydrated in a GC oven under flowing nitrogen (99,999% purity, Linde, Wiesbaden, Germany) at a head pressure of 0.1 MPa with the following temperature program: 10 min at 25 ˚C, 1 ˚C.min^{-1} to 110 ˚C, hold for 120 min, 1 ˚C.min^{-1} to 180 ˚C, hold for 8 hours. They were cooled to room temperature and flushed for 5 minutes with a solution of 10 % (v/v) 3-{[3-(*N*-pyrrole)-2-hydroxypropyl]amino}propyltriethoxysilane in anhydrous toluene containing 1 % (v/v) acetic acid. After flushing, both ends of the capillaries were sealed by insertion into silicon rubber septa as described by Gomez[2]. The capillaries were heated in a GC oven to 140 ˚C for 24 hours and then opened by cutting at about 1 cm from the septa. They were flushed with dry toluene for 2.5 hours and dried with nitrogen for one hour at room temperature.

For oxidative polymerization of the surface-bonded pyrrole moieties and incorporation of dextran sulfate, or heparin, respectively, into the polymer matrix, the silanized capillaries were rinsed for 24 hours with an aqueous solution of 0.04 mol.L^{-1} ammonium peroxodisulfate containing 3% (m/v) dextran sulfate or 1% (m/v) heparin, respectively. Structures are shown in Figure 11. For the preparation of the plain polypyrrole-coatings, the capillaries were rinsed with an aqueous solution of 0.04 mol.L^{-1} ammonium peroxodisulfate. Finally, the capillaries were flushed with water for 2 hours and stored filled with water until used.

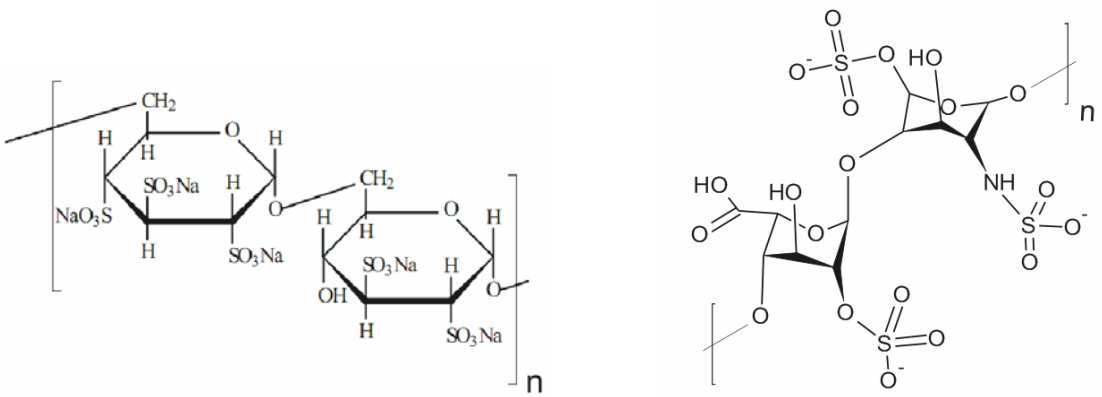

Figure 11. Structural units of dextran sulfate sodium salt (on the left) and heparin anion (on the right).

An optical window was created by scratching the outer polyimide coating away with a sharp single-edged blade over a length of about 2 mm, gently wiping it with a soft tissue wetted with methanol.

3.5 Measurement of electroosmotic mobility

Before use, the coated capillaries were flushed with water (15 min) and BGE (15 min), and the separation voltage was applied for 5 minutes. When changing the BGE, the capillaries were flushed with water (5 min), followed by BGE and application of voltage as above. Between runs, the capillaries were flushed with the actual BGE for 5 minutes. Thiourea (0.5 $g.L^{-1}$) was used as the EOF marker. Electroosmotic mobility was determined 7 times for each experimental point.

For determinations low electroosmotic mobilities, a method proposed by Sandoval[49] was employed (see section 2.2.3); the EOF marker was injected, voltage was applied for a certain time, then the EOF marker was injected again, and finally pressure was applied. The electroosmotic mobility was calculated from the respective peak times. In the case of unmodified polypyrrole-coated capillaries, ± 25 kV was applied for 200 seconds, the polarity depending upon the direction of the EOF at different pH values.

Electroosmotic mobilities were determined between pH 2.5 and pH 9.2 with the following BGEs: pH 2.5: 0.05 $mol.L^{-1}$ sodium phosphate; pH 3.4 and pH 4.3: 0.05 $mol.L^{-1}$ sodium formate; pH 5.0: 0.05 $mol.L^{-1}$ sodium acetate; pH 6.0: 0.025 $mol.L^{-1}$ sodium MES; pH 7.0 and pH 8.0: 0.025 $mol.L^{-1}$ sodium ACES; pH 9.2: 0.05 $mol.L^{-1}$ sodium borate. All BGEs were adjusted with sodium chloride to an ionic strength of 0.05 $mol.L^{-1}$.

Electroosmotic mobilities in the BGEs containing 0.025 $mol.L^{-1}$ SDS were composed of: pH 3.5 and 4.0: 0.05 $mol.L^{-1}$ sodium formate; pH 4.5, 5.0, and 5.5; 0.05 $mol.L^{-1}$ sodium acetate; pH 6.0, 6.5, 7.0, and 7.5: 0.05 $mol.L^{-1}$ sodium phosphate. The ionic strength of the BGEs was not adjusted because of already high conductivities and sufficiently high ionic strength; a further adjustment would have deteriorated the performance.

4. RESULTS AND DISCUSSION

4.1 Characterization of coated capillaries

Figure 12 shows the dependences of electroosmotic mobility upon pH for four different capillary types:

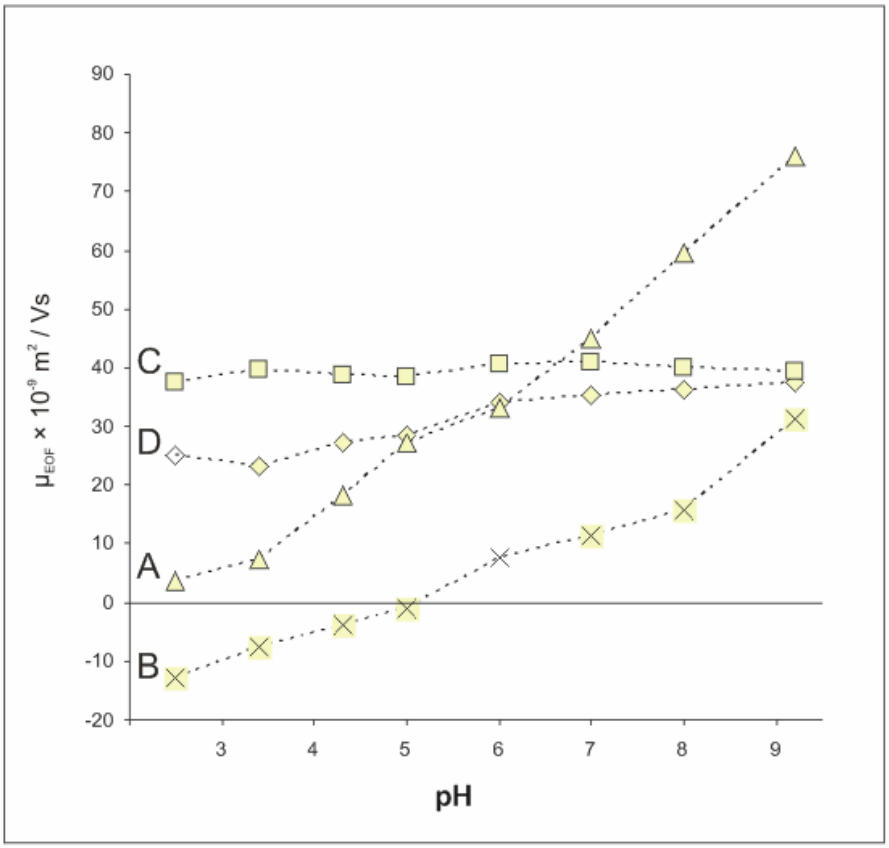

Figure 12. Dependence of electroosmotic mobility on pH; (A) uncoated capillary, (B) unmodified polypyrrole-coated capillary, (C) PP/DS capillary, (D) PP/HEP capillary. For BGEs composition, see section 3.5.

For uncoated capillaries (Figure 12, A), the dependence shows the commonly known increase of electroosmotic mobility with increasing pH. The repeatability of migration time of the EOF marker was 4.11% RSD (average value for all pH values, Table 7) similar to the finding of Gomez[2]. Dextran sulfate-modified capillaries (Figure 12, B; PP/DS) gave the same results (0.35% RSD as an average, Table 7) as reported earlier[190] demonstrating high reproducibility in preparing such coatings. Heparin modified PP-coated capillaries (Figure 12, C, PP/HEP) exhibits slightly lower EOF values as the concentration of heparin during

polymerization is smaller because of its lower solubility. The newly prepared PP/HEP coating showed an average repeatability 0.65% RSD (Table 7). Unmodified polypyrrole-coated capillary prepared with ammonium peroxodisulfate (Figure 12, D) exhibited an EOF-dependence similar to polypyrrole-coated capillaries using iron (III) chloride for polymerization which were prepared by Čabala[190].

Obviously, small anions such as sulfate or chloride are not incorporated into the polymer matrix. It is important to note that the dependence of the electroosmotic mobility on pH as determined by Čabala[190] were obtained with BGEs of variable ionic strength which normally has a strong effect on the formation of the double layer of the capillary surface and the magnitude of the EOF. Therefore, the obtained electroosmotic mobilities are different.

Table 7. Electroosmotic mobilities and their repeatability for uncoated, PP/DS, and PP/HEP capillaries (n = 7).

pH	uncoated capillary		PP/DS coated capillary		PP/HEP coated capillary	
	μ_{EOF} [10^{-9} ^{2}/Vs]	RSD [%]	μ_{EOF} [10^{-9} m^2/Vs]	RSD [%]	μ_{EOF} [10^{-9} m^2/Vs]	RSD [%]
2.5	3.7	5.24	37.5	0.34	25.1	1.35
3.4	7.4	4.38	39.5	0.11	23.2	0.85
4.3	18.1	6.60	38.7	1.03	27.2	0.58
5	27.3	6.18	38.3	0.99	28.6	0.44
6	33.1	4.23	40.5	0.05	33.9	0.16
7	44.9	3.02	41.0	0.03	35.1	0.95
8	59.4	2.20	39.8	0.06	36.1	0.10
9.2	76.0	0.99	39.3	0.17	37.6	0.73

Employment of SDS as an anionic surfactant for micellar electrokinetic chromatography (MEKC) leads into formation of the dynamic coating, providing a strongly negatively charged surface[192,193]. In combination of a positively charged polypyrrole surface with BGEs containing 0.025 mol.L^{-1} SDS, a strong EOF towards the cathode of 40 - 46 × 10^{-9} m^2/Vs were observed over a pH range from 3.5 to 7.5, with average RSD of 0.89 % (Table 8). Pranaityte[124] proposed applying a secondary coating layer of SDS on the capillary wall dynamically coated with PDADMAC and reported similar results with respect to BGEs with different ionic strengths.

Table 8 presents the observed electroosmotic mobility for BGEs containing 0.025 mol.L^{-1} SDS and its repeatability. When comparing the measured electroosmotic mobilities for PP/HEP coated capillary without and in the presence of 0.025 mol.L^{-1} SDS, it is obvious

that additional dynamic coating by SDS occurs. It seems that the capillary surface is shielded by negatively charged heparin but not completely. When comparing the results with those obtained with an unmodified polypyrrole-coated capillary used with BGEs containing 0.025 mol.L^{-1} SDS (Table 8), dynamic coating with SDS provides a stronger EOF than PP/HEP capillary used the same BGEs.

Table 8. Electroosmotic mobilities for separation of herbicides on PP/HEP and unmodified polypyrrole-coated capillary in electrolytes containing 0.025 mol.L^{-1} SDS (n = 7). For BGEs composition, see section 3.5.

pH	PP/HEP coated capillary		unmodified PP-coated capillary	
	μ_{EOF} [10^{-9} m^2/Vs]	RSD [%]	μ_{EOF} [10^{-9} m^2/Vs]	RSD [%]
3.5	40.3	0.47	46.1	0.41
4.0	40.2	1.04	45.9	1.11
4.5	38.9	0.17	45.2	1.20
5.0	39.0	0.98	43.1	0.78
5.5	39.2	0.87	43.2	0.81
6.0	39.4	1.16	42.9	0.66
6.5	40.6	0.69	42.7	1.08
7.0	40.6	1.00	41.9	1.13
7.5	43.6	0.57	40.9	0.94

4.2 Dextran sulfate-modified capillary

The dextran sulfate modified polypyrrole coated capillary showed strong EOF even at low pH. This phenomenon was utilized in chiral separation of *R,S*-ibuprofen and reversal of its migration order in contrary to separation performed in uncoated capillary.

4.2.1 Chiral separations

Separation of enantiomers presents an important field of CE application. Enantiomers do not differ in their mobility in an achiral background electrolyte. In presence of chiral selector which can recognize both enantiomers stereoselectively, a difference in the apparent mobility is achieved due to formation of transient diastereomeric complexes[47].

Various chiral selectors are employed in CE, mainly cyclodextrin, both native and their derivatives. Macrocyclic antibiotics, synthetic or natural chiral polymers, crown ethers, chiral surfactants, or metallic complexes with a chiral component present other classes of selectors[47].

Effectiveness of the chiral separation is usually reported as resolution of the enantiomers and is calculated by an equation:

$$R_s = \frac{2(t_2 - t_1)}{w_1 + w_2} \qquad (5)$$

where t_1 and t_2 are migration times and w_1 and w_2 are baseline peak widths.

4.2.2 Chiral separation of *R,S*-ibuprofen

Ibuprofen is an important member of the nonsteroidal anti-inflammatory drugs, used in the treatment of acute and chronic pain and in a variety of rheumatic and musculoskeletal disorders[194]. Many researchers were involved in its chiral separation, a number of HPLC[195,196] and CE[197-201] methods were developed, mostly utilizing heptakis(2,3,6-tri-O-methyl)-β–cyclodextrin[198,199], avidin[200] and vancomycin[201] as chiral selectors, or derivatization with (naphtalene-1-yl)-ethylamine[196] prior to analysis.

For the demonstration of prepared PP/DS coating, a chiral separation utilizing sulfated β-cyclodextrin in acidic pH was designed. As the BGE, 0.02 mol.L^{-1} sodium formate pH 3.0 containing 4% sulfated β-cyclodextrin was chosen.

At these conditions, ibuprofen (pK_a 4.4) is present mainly in the undissociated form, therefore its electrophoretic mobility is very low at pH 3.0. Sulfated β-cyclodextrin interacts with *R,S*-ibuprofen, producing a negatively charged complex which is attracted towards anode. In PP/DS, strong EOF carries all solutes toward detector. The proposed separation design is shown in Figure 13. Electropherograms are shown in Figure 14. Resolution for *R,S*-ibuprofen was 0.86 (equation 5).

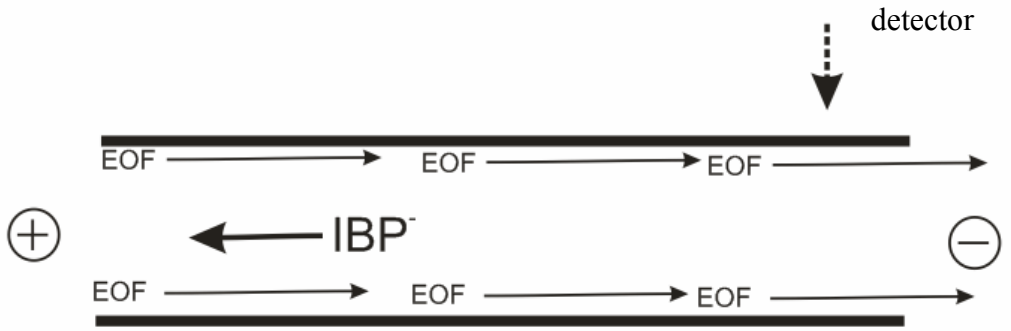

Figure 13. A design for chiral separation of ibuprofen in PP/DS capillary. Direction of migration of IBP$^-$ corresponds migration of both ibuprofen itself and the complex of ibuprofen with sulfated β-cyclodextrin, which is not illustrated for transparency.

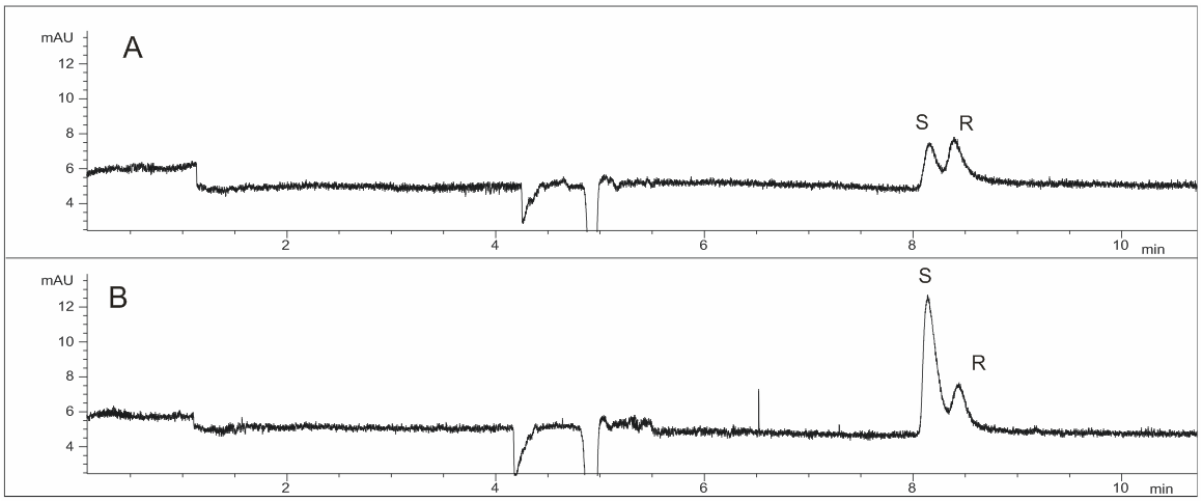

Figure 14. Electropherograms of chiral separation of ibuprofen. Conditions: PP/DS capillary, 0.02 mol.L sodium formate pH 3.0, 4% sulfated β-cyclodextrin, +15 kV, UV detection at 200 nm. (A) concentration of *R*-ibuprofen 1.6×10^{-4} mol.L^{-1}, ratio 1*S* : 1*R*; (B) concentration of *R*-ibuprofen 1.6×10^{-4} mol.L^{-1}, ratio 3*S* : 1*R*.

4.2.3 Comparison with uncoated capillary

The same BGE was used for chiral separation in the uncoated capillary. In contrary to PP/DS capillary, the EOF at pH 3.0 is very weak, therefore it cannot be used for transportation of ibuprofen enantiomers to the detector. Therefore, a reversed polarity must be used. The separation design is shown in Figure 15. The migration of ibuprofen both itself and in the negatively charged complex with sulfated β-cyclodextrin proved to be sufficient to achieve chiral separation within 15 minutes as shown in Figure 16. Obtained resolution for *R,S*-ibuprofen was 2.51.

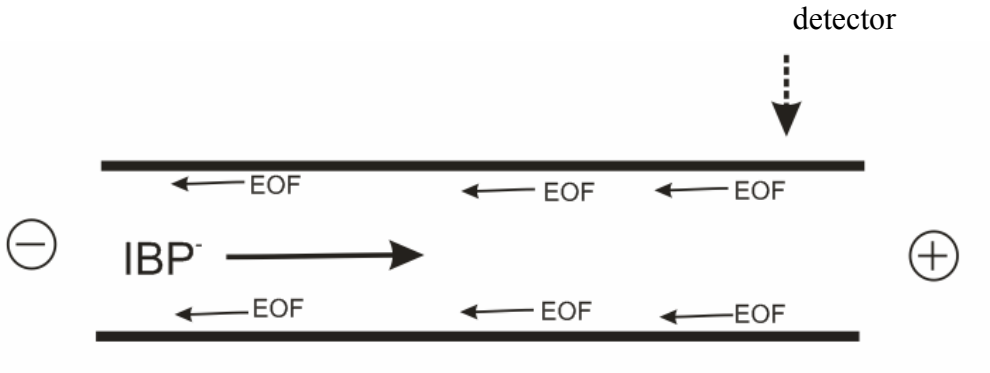

Figure 15. A design for chiral separation of ibuprofen in uncoated capillary. Direction of migration of IBP^- corresponds migration of both ibuprofen itself and the complex of ibuprofen with sulfated β-cyclodextrin, which is not illustrated for transparency.

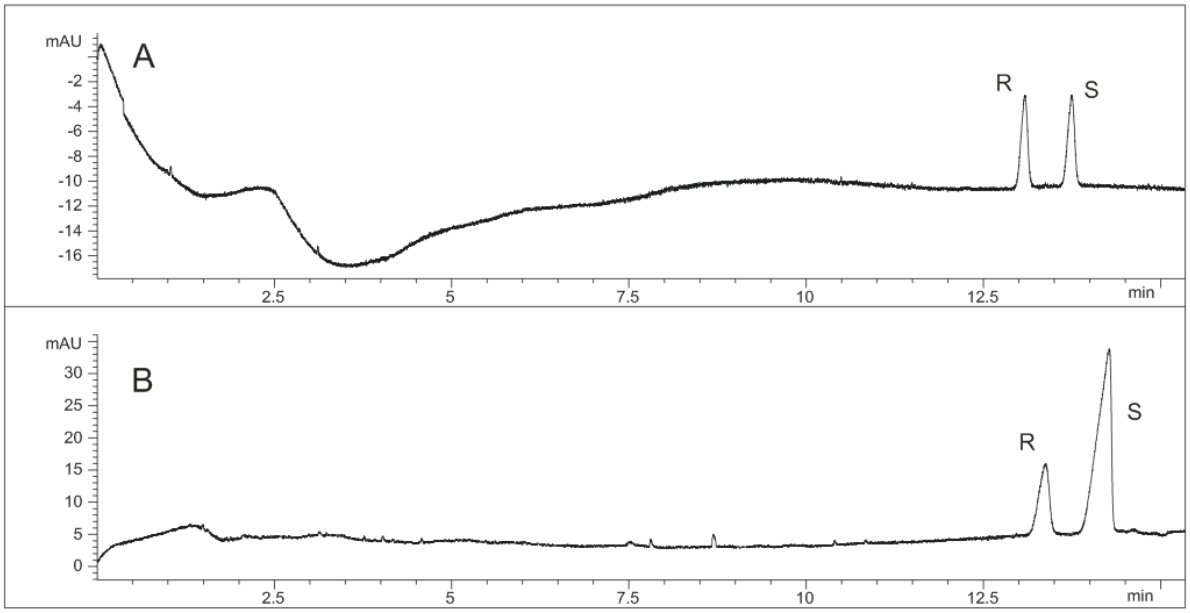

Figure 16. Electropherograms of chiral separation of ibuprofen. Conditions: uncoated capillary, 0.02 mol.L^{-1} sodium formate pH 3.0, 4% sulfated β-cyclodextrin, -15 kV, UV detection at 200 nm. (A) concentration of *R*-ibuprofen 1.6×10^{-4} mol.L^{-1}, ratio 1*S* : 1*R*; (B) concentration of *R*-ibuprofen 1.6×10^{-4} mol.L^{-1}, ratio 3*S* : 1*R*

When comparing the results obtained for PP/DS and uncoated capillary, using the same BGE with the same chiral selector in the same concentration, a reversal of enantiomer migration was achieved by simple change of the capillary and polarity switch. As it is obvious from the results, *R*-ibuprofen makes stronger complex with sulfated β-cyclodextrin than *S*-ibuprofen, therefore, in the case of uncoated capillary, it reaches the detection cell before slower *S* enantiomer.

In PP/DS capillary, the situation is reversed, because *R*-ibuprofen migrates faster against the direction of much stronger EOF, allowing *S*-ibuprofen to be registered before *R* enantiomer. No chiral selectivity of prepared PP/DS coating on *R,S*-ibuprofen was observed during experiments.

Difference in observed resolution for *R,S*-ibuprofen can be attributed to the loss of peak efficiency when counter-EOF (in the case of PP/DS, strong EOF) separation is performed. However, when the reversal of migration order of enantiomers is needed, lower efficiency and resolution do not present a significant drawback.

4.3 Heparin-modified capillary

In order to demonstrate properties of the PP/HEP coated capillary, a micellar electrokinetic chromatography (MEKC) separation of seven selected herbicides (see section 4.3.2) was performed and an influence of BGE pH on the separation selectivity was studied.

4.3.1 Micellar electrokinetic chromatography

MEKC as a mode of CE was introduced in 1984 by Terabe[201]. The separation mechanism is based on both electrophoretic migration and on the different partitioning of the analyte between aqueous and micellar phase, which has different effective mobility[47].

The most important contribution of this method is the ability to separate neutral compounds. Nevertheless, employment of MEKC for analysis of ionic species brings a new dimension of separation selectivity, as the separation is performed according to both electrophoretic mobility and hydrophobicity of the solute.

4.3.2 Selected herbicides

Seven selected herbicides – weak acids were selected as model analytes:

Table 9. List of the selected herbicides with their pK_a values[190] and relative molecular weights.

herbicide	pK_a	M_r
2,4-dichlorophenoxyacetic acid	2.6	221.0
4-(2,4-dichlorophenoxy)butanoic acid	4.8	249.1
4-chloro-2-methylphenoxyacetic acid	3.1	200.6
clopyralid	1.8	192.0
dicamba	1.9	221.0
dichloroprop	3.0	235.1
mecoprop	3.8	214.7

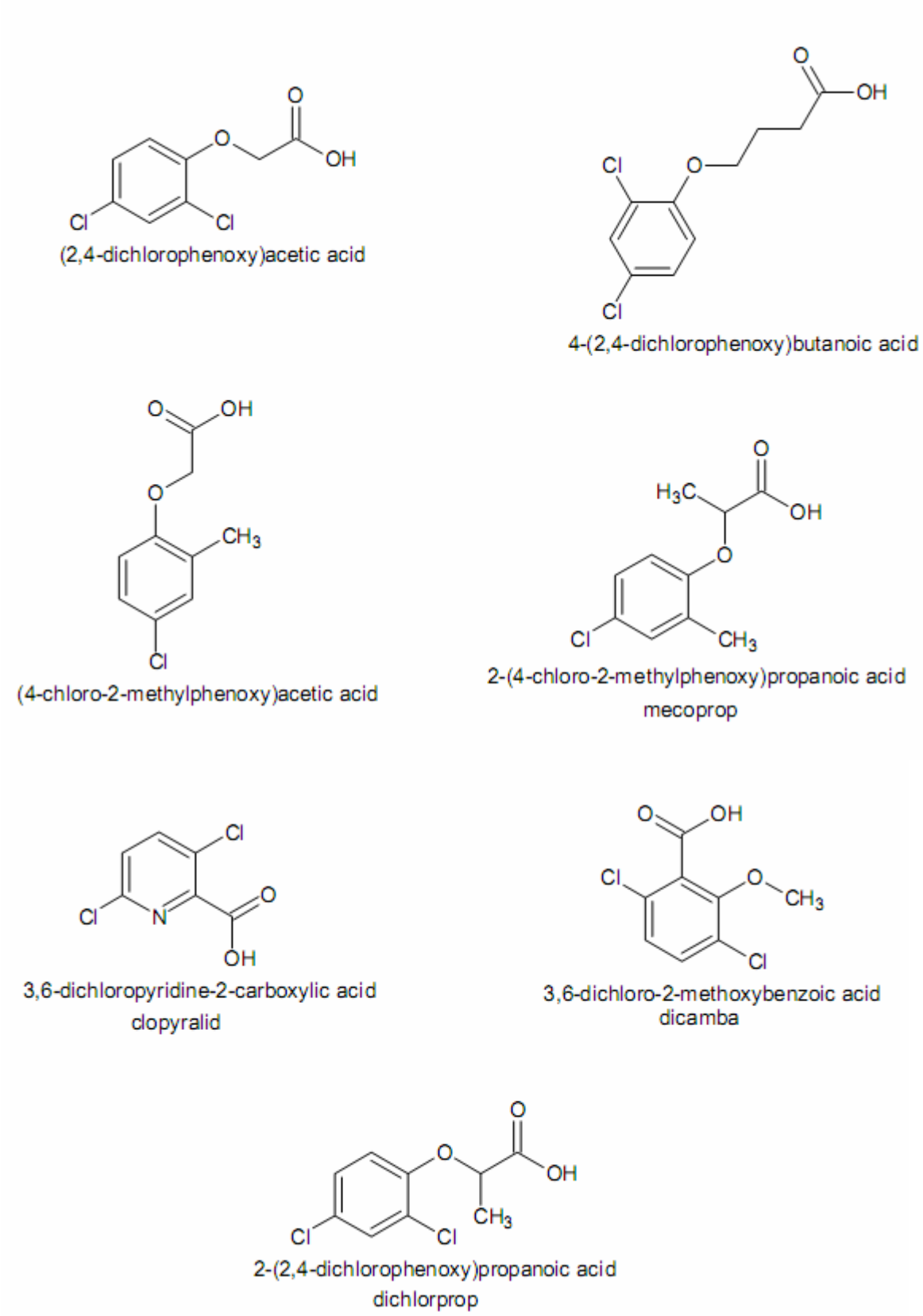

Figure 17. Structures of the selected herbicides with their systematic and trivial names.

4.3.3 Separation of selected herbicides

To demonstrate the properties of the newly prepared heparin-modified polypyrrole-coated capillaries, the separation of selected herbicide standards by MEKC was studied. An advantage of using polypyrrole-coated capillaries with electroosmotic mobility independent of pH is the possibility to perform the separation over a wide range of pH (Figure 18).

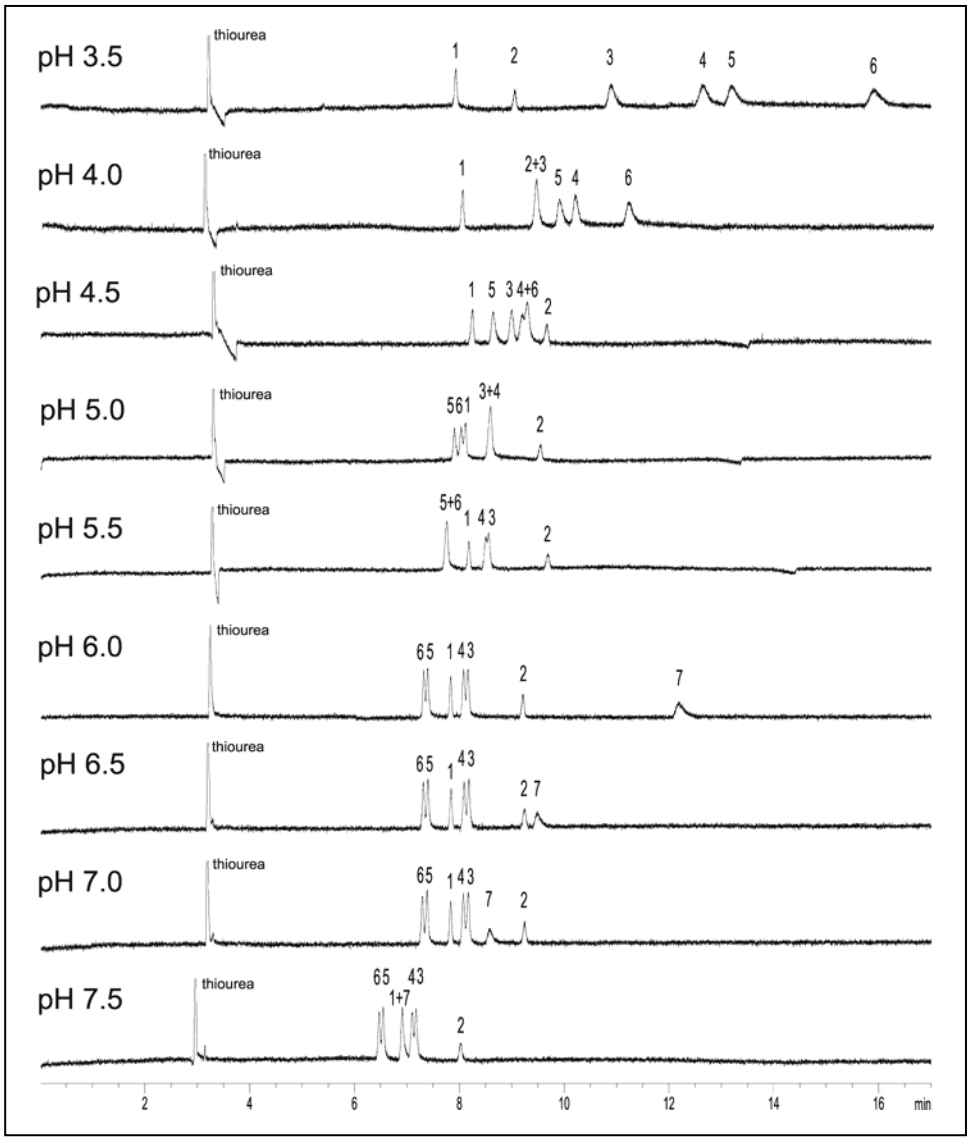

Figure 18. Influence of pH on selectivity. Conditions: PP/HEP modified capillary, 0.05 mol.L^{-1} sodium formate (pH 3.5, 4.0) / sodium acetate (pH 4.5, 5.0, 5.5) / sodium phosphate (pH 6.0, 6.5, 7.0, 7.5), 0.025 mol.L^{-1} SDS; +25 kV; hydrodynamic injection 30 mbar 5 sec, 5×10^{-5} mol.L^{-1} herbicides with 0.05 g.L^{-1} thiourea as the EOF marker. UV detection at 200 nm. Peaks: 1 – dicamba, 2 – clopyralid, 3 - 2,4-dichlorophenoxy acetic acid, 4 - 4-chloro-2-methylphenoxy acetic acid, 5 – dichlorprop, 6 – mecoprop, 7 - 4-(2,4-dichlorophenoxy)butanoic acid.

The strong EOF carries the analytes towards the detection cell, while partition into the SDS micelles carries them away. Since the herbicides are weak acids, they are increasingly dissociated at higher pH and they are repelled from the SDS micelles, and shorter migration times results. The migration order of the herbicides is varying depending mainly on their pK_A values, modified by their hydrodynamic radius and the strength of interaction with the micelles.

Effective mobilities of dicamba or clopyralid as the strongest acids remain approximately the same over the whole pH-range; the effective mobilities of the weaker acids are more strongly influenced by the pH offering a possibility of straight forward adjustment of migration order. 4-(2,4-dichlorophenoxy)butanoic acid as the weakest acid of the selected model analytes was till pH 6.0 mostly carried by the SDS micelles out from the detector, therefore its signal is observed while certain degree of ionization is reached.

5. CONCLUSION

A survey focused on coatings for capillary electrophoresis was made. Utilization of various compounds in order to provide dynamic or covalent coatings is discussed. Improvement of repeatability of analyses and reduction of adsorption of analytes at the capillary surface was achieved by these techniques, possessing their advantages and disadvantages.

In the experimental part, a method for preparation polypyrrole-coated capillaries for capillary electrophoresis is described. Unmodified and both dextran sulfate and heparin modified capillaries were characterized by the dependence of electroosmotic mobility on pH, showing great improvement of repeatability in contrast to uncoated silica capillary. The modified capillaries provided strong cathodic electroosmotic flow.

The dextran sulfate-modified polypyrrole coated capillary was utilized for chiral separation of a model mixture of *R,S*-ibuprofen. The proposed background electrolyte composed of 0.02 $mol.L^{-1}$ sodium formate pH 3.0 and 4% sulfated β-cyclodextrin allowed the separation in 9 minutes with migration order of *S*-ibuprofen first and *R*-ibuprofen as the second. Use of uncoated capillary filled with the same background electrolyte and application of opposite polarity, the reversal of migration order was achieved.

The heparin-modified polypyrrole coated capillary was utilized for separation of a model mixture of selected herbices by micellar electrokinetic chromatography. An influence of pH on separation selectivity was studied; the results showed a possibility to alter migration order of the analytes. The electroosmotic flow largely independent on pH provided a constant transportation force, while different effective mobility and interaction with micelles of sodium dodecylsulfate provided the separation of selected herbicides.

The developed coatings might be found useful in further applications, where excellent repeatability of migration times and resistance against adsorption of hydrophobic analytes is required.

6. LIST OF ABBREVIATIONS

ACES, *N*-(2-acetamido)-2-aminoethanesulfonic acid
AFM, atomic force microscopy
ATR, attenuated total reflection
BGE, background electrolyte
CCD, contactless conductivity detection
CEC, capillary electrochromatography
CMC, critical micelle concentration
CTAB, cetyltrimethylammonium bromide
DDAB, didodecyldimethylammonium
DODAB, dimethyldioctadecylammonium bromide
DS, dextran sulfate
EOF, electroosmotic flow
HEP, heparin
IBP, ibuprofen
MEKC, micellar electrokinetic chromatography
MES, 2-(*N*-morpholino)ethanesulfonic acid
PDADMAC, poly(diallyldimethylammonium chloride)
PEEK, poly(ether ether ketone)
PEI, polyethyleneimine
PEO, poly(ethylene oxide)
PMMA, poly(methylmethacrylate)
PP, polypyrrole
PTFE, poly(tetrafluoroethylene)
PVA, poly(vinyl alcohol)
RSD, relative standard deviation
SEM, scanning electron microscopy
SDS, sodium dodecyl sulfate
SMIL, successive multiple ionic polymer layer

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8. APPENDIX

8.1 Characterization by IR

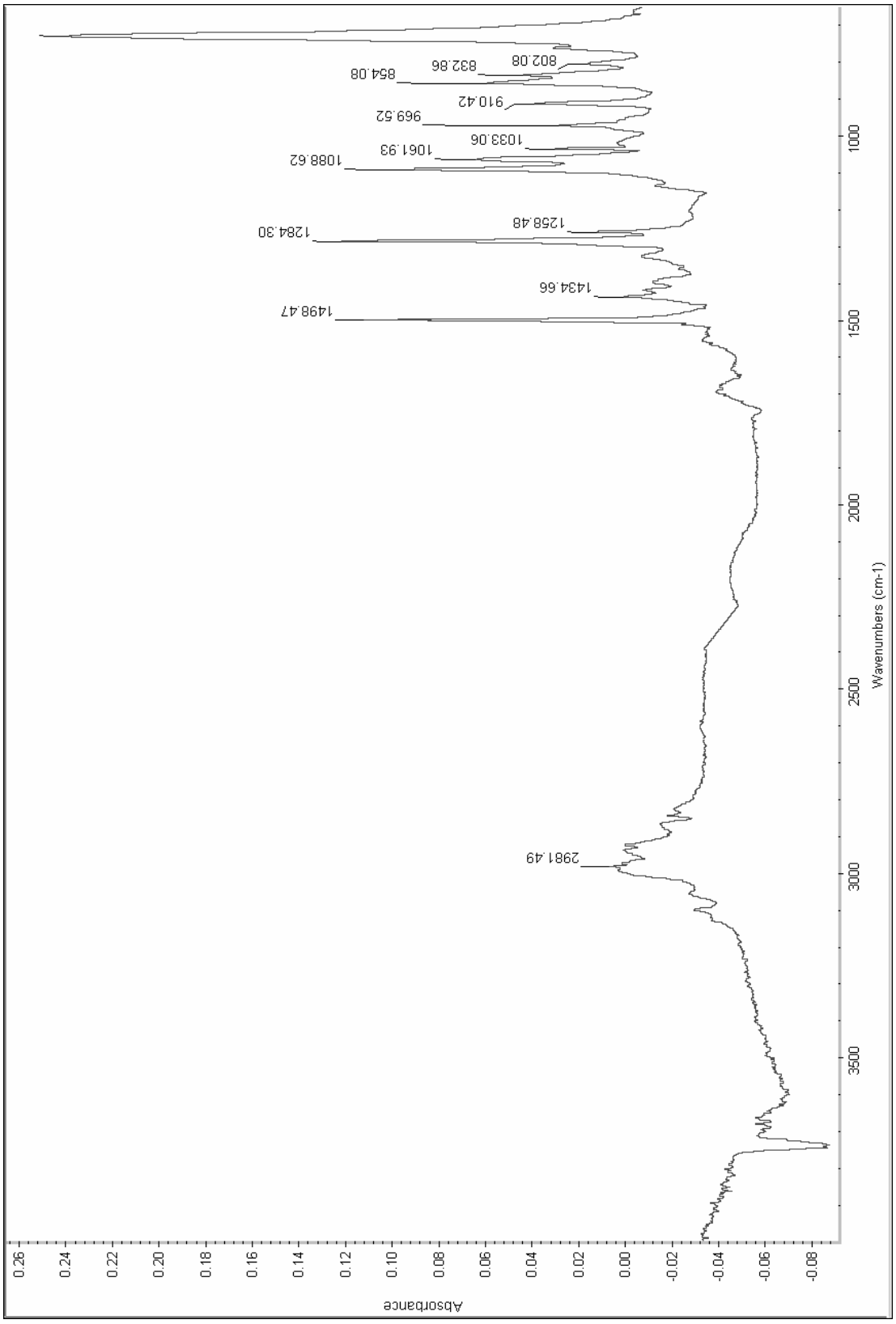

Figure 19. ATR-IR spectra of N-glycidylpyrrole.

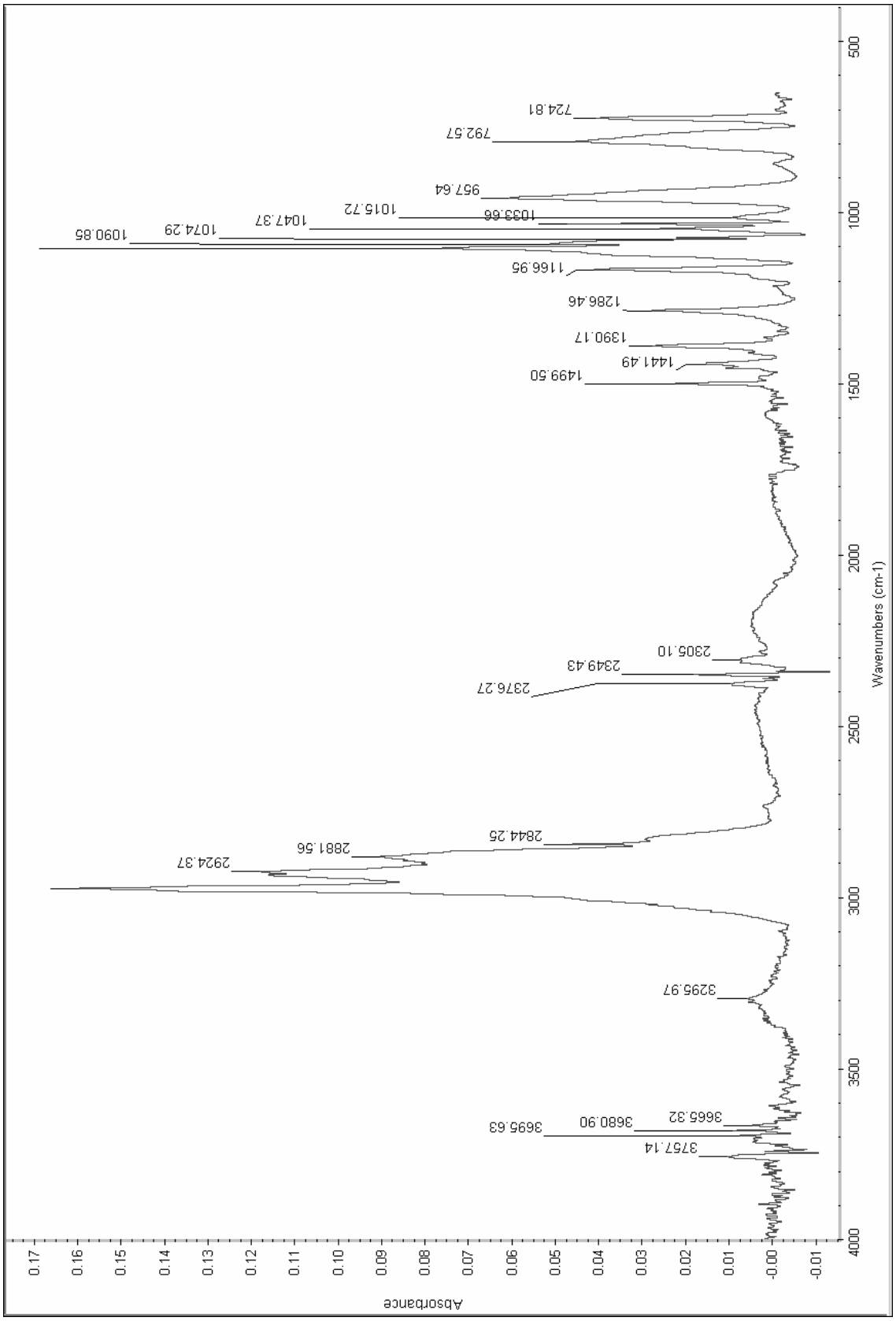

Figure 20. ATR-IR spectra of 3-{[3-(*N*-Pyrrole)-2-hydroxypropyl]amino}propyltriethoxysilane.