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Journal of Chromatography A, 971 (2002) 199–205

JOURNAL OF
CHROMATOGRAPHY A

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Control of electroosmotic flow in zirconia-coated capillaries

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Received 14 February 2002; received in revised form 19 June 2002; accepted 3 July 2002

Abstract

The inner surface of a silica capillary has been modified using an anhydrous sol–gel method: zirconium propoxide reacted with silanol groups to give, after hydrolysis, a “zirconia-like” surface. The electroosmotic properties of zirconia-modified capillaries have been studied under various conditions: working parameters were nature and concentration of the electrolyte, ionic strength, pH and solvent composition. Using different ions such as sodium, potassium, chloride, nitrate or methanoate, a positive electroosmotic flow (cathodic flow) was observed in the pH range 5–11. But below pH 5, a negative electroosmotic flow (anodic flow) occurred, corresponding to a permanent positive surface charge of the capillary walls. The value of the electroosmotic flow (including the sign) can be easily controlled by adding multivalent ions in the electrolyte. Some of these modifications of surface charge can be made irreversible. The observed electroosmotic flow has been related to surface characteristics using the triple layer model. With the large set of working parameters, the optimisation of separations can be facilitated. An example of flow control is given for the separation of four antihistaminic compounds. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Electroosmotic flow; Coated capillaries; Zirconia; Sol–gel; Antihistaminic compounds

1. Introduction

The application of an electric field to the extremity of a fused-silica capillary may generate an electroosmotic flow inside this capillary. This flow occurs if a charge, positive or negative, has been previously created at the surface of the silica. Then, counterions located close to the surface to compensate the electrical surface charge are able to move under the electrical field producing the electroosmotic flow.

For a silica capillary, the negative charge observed on the surface is the consequence of acid properties

of hydroxide surface groups (silanol groups) $\equiv\text{Si}-\text{OH} \rightleftharpoons \equiv\text{SiO}^- + \text{H}^+$. So, the electroosmotic flow resulting from the positive counter-ions moves from the anode to the cathode. Electroosmotic flow is nil at pH 2 and over the pH range 2–12, the surface charge density of silica increases leading to higher cathodic electroosmotic flow at basic pH.

Some separations could be improved using cathode to anode electroosmotic flow. However, such flow cannot be created using a simple silica capillary: the sign of silica surface cannot be inverted using pH control because silanol groups do not have significant basic behaviour. So, to obtain a positive surface charge using a silica capillary, surface modification must be made or surfactants have to be used [1–3]. The surfactant modification is difficult to

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control and can modify the behaviour of the analytes inside the separation capillary. So a permanent surface modification appears to be a valuable approach for the control of the electroosmotic flow.

Hydroxide groups with an amphoteric behaviour exist on other oxides. Such oxides can be used as negative or positive surface charge promoters. The oxides are characterized by their isoelectric point: at this pH, the apparent surface charge based on electrophoretic measurements is equal to zero. The isoelectric points of silica, titania, zirconia and alumina are, respectively, close to 2, 5, 7 and 9. Zirconia was selected as surface modifier because of its significantly higher isoelectric point and its good pH stability [4–6]. Zirconia [7] and magnesia–zirconia [8] have been coated on a silica capillary as a thick layer and have demonstrated the potential use of zirconia surface. In this work, we modified the inner surface of a silica capillary using an anhydrous sol–gel method: zirconium propoxide reacted with silanol groups producing, after hydrolysis, a “zirconia-like” surface. The electroosmotic properties of zirconia-modified capillaries have been studied under various conditions: working parameters were nature, ionic strength and concentration of the electrolyte, pH, and solvent composition. The observed electroosmotic flow has been related to surface characteristics using the triple layer model to describe surface reactions on oxide material.

2. Experimental

2.1. Reagents and material

Zirconium propoxide was purchased from Sigma–Aldrich (33,397-2). Sodium hydroxide, sodium chloride, sodium sulfate, sodium phosphate, sodium nitrate, potassium chloride, calcium chloride, phosphoric acid, sulphuric acid, methanoic acid, sodium methanoate, were analytical grade. Distilled water was further treated with a Elgastat UHQII purification system. All electrolytic solutions were filtered through 0.45- μm membrane just before use.

Fused silica capillaries (375 μm O.D., 20 or 50 μm I.D.) were obtained from Thermo Separation Products. After modification, these capillaries were

tested using an Agilent capillary electrophoresis system HP^{3D}CE.

2.2. Capillary preparation

Fused silica capillaries were flushed with 1 *M* sodium hydroxide solution for 1 h, washed with water for 1 h and dried overnight under nitrogen at 70 °C. Anhydrous zirconium propoxide solution was diluted in propanol to a concentration of 0.8 *M*. The capillary was flushed with this solution for 1 h at room temperature, then the excess of zirconium propoxide solution was removed using ethanol. The capillary was dried using gas flow at 50 °C. The capillary surface was finally rehydroxylated using 0.1 *M* sodium hydroxide solution. A narrow window (2 mm) was created for detection using a frit burner from Innovatech (UK).

2.3. Capillary characterization

The HP^{3D}CE system had a 0–30 kV high-voltage power supply usable in positive or negative polarity. For all experiments, the temperature was 25 °C, and samples were hydrodynamically introduced into the capillary. Acetone was used as a marker of electroosmotic flow. Vials (inlet and outlet) replenishment was made every two runs. All solutes were dissolved in the mobile phase.

To study the influence of organic solvent on the electroosmotic mobility, electrolytic solutions were prepared from stock solution of 10^{-2} *M* HCl + 5×10^{-2} *M* NaCl. To obtain a MeCN–water (50:50) (or 20:80) solution at pH 3, we mixed 10 ml (or 10 ml) of the stock solution, 40 ml (or 10 ml) of pure water and 50 ml (or 80 ml) of acetonitrile. In all solutions, the amount of sodium ions, chloride ions as well as the amount of hydronium ions was kept constant.

3. Results and discussion

The double layer model has been successfully used to describe the electroosmotic flow observed in fused-silica capillary. This model was originally developed by Gouy [9] and Chapman [10] solving the Poisson–Boltzmann equation and improved by Stern [11] taking into account the size of the ions.

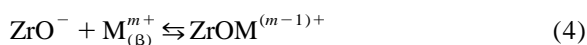
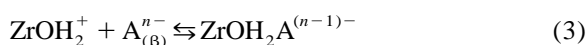
However, this model failed to describe a complex surface system where multiple surface reactions occurred. In order to analyse the large set of experimental conditions used with zirconia-like capillaries, the triple layer model has been more successful. The description of this model can be found in Davis and Leckie's papers [12,13]. It was initially introduced to give a better correlation between data obtained from electrophoretic measurements and those from potentiometric titrations. Fig. 1 illustrates the general scheme of the triple layer model.

For hydroxide surface groups, both acid or basic behaviour takes place according to the reactions:



The subscript (s) indicates that the reaction occurs on the surface of zirconium dioxide.

Furthermore, complexation equilibria can occur between charged sites on zirconia surface and the ions of the electrolytic surrounding solution according to:



The subscript (β) is used to signify that anions and cations are adsorbed on a different plane than protons but below the shear plane which separate the immobilized layer and the mobile layer occurring when an electrical field is applied.

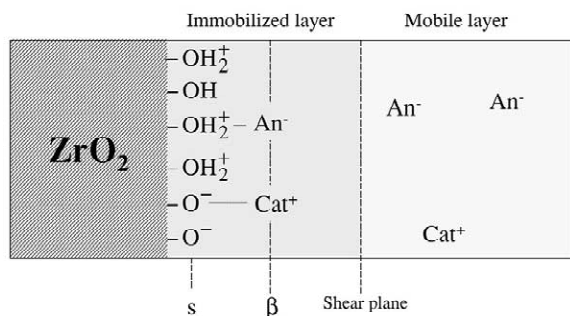


Fig. 1. General scheme of the triple layer model.

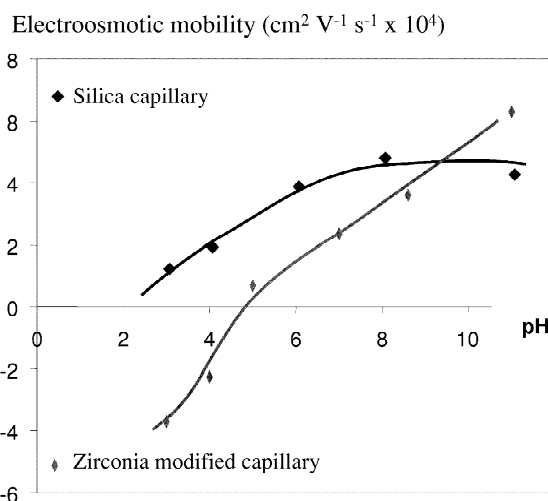


Fig. 2. Electroosmotic mobility as a function of pH using 5 mM sodium chloride solution: ♦, original fused-silica capillary; ◇, zirconia-modified capillary.

3.1. Effect of pH and sodium chloride concentration on the electroosmotic mobility

Fig. 2 shows the electroosmotic flow of the original fused-silica capillary as a function of pH. Sodium chloride solution with a concentration of 5 mM was used to keep the ionic strength constant. The electroosmotic flow was conventionally defined with a positive sign for a displacement directed from the anode (the positive electrode) to the cathode (the negative electrode).

As expected for silica tubing, the electroosmotic flow was positive over the pH range studied: on silica surface, the Si-O⁻ groups, resulting from the deprotonation of silanols, were always present in greater quantities than any positively charged groups. The number of Si-O⁻ groups decreased at low pH values, and the electroosmotic mobility tends to zero at pH 2.

For a zirconium propoxide-modified capillary, the sign of the electroosmotic flow was pH-dependent. Above pH 5, the zirconium propoxide-modified capillary produced a positive electroosmotic flow as for the silica capillary, but below pH 5 the electroosmotic flow (EOF) became negative: the EOF was then directed from the cathode to the anode. The isoelectric point of zirconium propoxide-modified capillary (pH_{iep} = 5) was slightly lower than the

isoelectric point observed for monoclinic zirconia powder ($\text{pH}_{\text{iep}} = 6.5$ [14]). However, as reported in a previous paper [14], the isoelectric point of any oxide (or hydroxide) is known to be dependent on the oxide composition (including impurities) and crystallographic form. Based on the electrophoretic measurements, the surface modification by zirconium propoxide produced zirconia-like capillaries.

Fig. 3 shows the electroosmotic mobility observed for various sodium chloride concentrations for both pH 3 and pH 11: the absolute value of the electroosmotic mobility decreased as the ionic strength increased. Such behaviour was explained using the double layer model as a decrease in the double layer thickness (δ). Using the triple layer model, the increase in sodium chloride concentration in the bulk solution, as for classical equilibrium problem, produced more surface complexation according to reactions (3) and (4): the number of surface charges decreased and so also the number of charges in the mobile layer. The decrease in the electroosmotic mobility was then dependent on the cation and anion complexation constants. These complexation constants were previously reported [15] for sodium and chloride ions on monoclinic zirconia powder $\text{p}K_{\text{Cl}} = -1.5$; $\text{p}K_{\text{Na}} = -0.7$, respectively, for reactions (3) and (4).

3.2. Effect of sodium sulfate electrolyte on the electroosmotic mobility

Fig. 4 shows the electroosmotic mobility of zir-

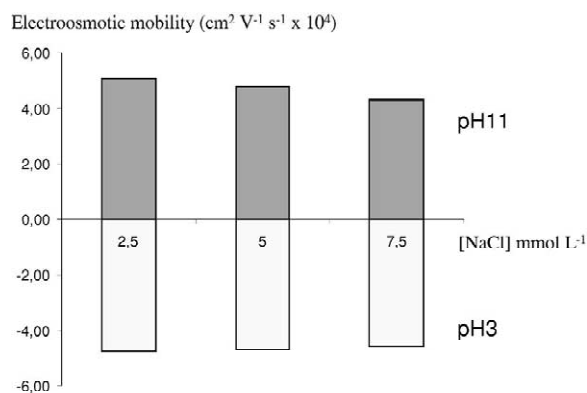


Fig. 3. Electroosmotic mobility of zirconia-modified capillary at pH 3 and pH 11 using various sodium chloride concentrations.

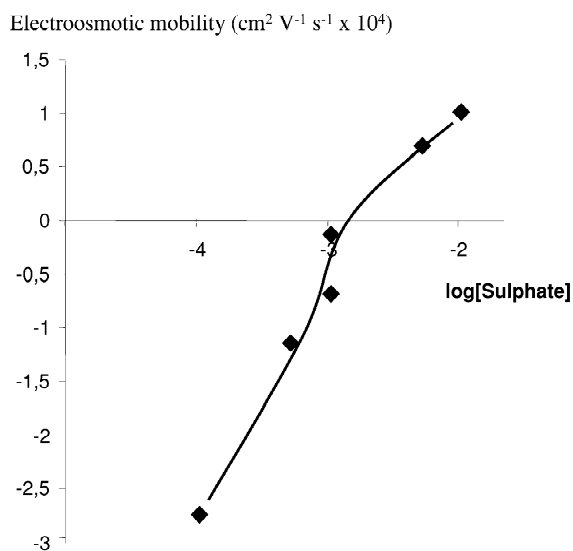


Fig. 4. Electroosmotic mobility of zirconia-modified capillary at pH 3 as a function of sodium sulfate concentration.

conia-modified capillary at pH 3 using electrolytic solutions made of sodium sulfate at various concentrations.

At low sulfate concentrations, the capillary behaved as a normal zirconia capillary using a simple electrolyte (NaCl) at pH 3: the surface charge of the capillary was positive whereas the diffuse layer was negatively charged; the flow was directed from the cathode to the anode (electroosmotic mobility was negative).

When the sulfate concentration increased, the complexation equilibrium corresponding to reaction (3) is shifted to the right. At one time, the number of negative charges below the shear plane coming from sulfate ions was greater than the positive ZrOH_2^+ surface charges: then the charge of the diffuse layer became positive and the electroosmotic flow was directed from the anode to the cathode (electroosmotic mobility was positive).

The complexation constant of sulfate ions on monoclinic zirconia corresponding to reaction 3 was previously reported $\text{p}K_{\text{SO}_4} = -5$ [15]. This reaction behaved as a true equilibrium, a decrease in sulfate concentration in the electrolyte produced a release of sulfate groups from the surface to the bulk solution and the electroosmotic flow came back toward negative values.

This sulfate desorption can be improved by a simple pH shift to basic conditions: in this case, the $ZrOH_2^+$ surface groups were removed as described by reactions (1) and (2) and the sulfate complexation is completely suppressed.

The sign and magnitude of the electroosmotic flow of the zirconium propoxide-modified capillary can be easily controlled by adjusting the sulfate concentration of the electrolyte. Solute interactions with the capillary surface can then be manipulated.

3.3. Effect of other electrolytes on the electroosmotic mobility

Fig. 5 shows the electroosmotic mobility of zirconia-modified capillary at pH 3 with various anions (Na^+ was used as the corresponding cation). Chloride, nitrate and methanoate behave as indifferent electrolyte: they have a very low effect on electroosmotic mobility. Their complexation constants were of the same order of magnitude ($pK \approx -1$).

Conversely, sulfate, citrate and phosphate ions were able to bind the zirconia surface to give negative surface charge and positive electroosmotic flow. Phosphate ions are known to strongly bind the surface of zirconium oxide [16]: a positive electroosmotic flow was maintained even without phosphate ions in the electrolyte. To definitely remove phosphate groups, strongly basic solution (1 M NaOH) was used. The opportunity offered by phosphate binding has been used by Xia et al. [17] to graft hydrophobic chains to the capillary surface in order to perform electrochromatographic separations.

Conversely in the basic pH range, the electroosmotic mobility was controlled by adding calcium

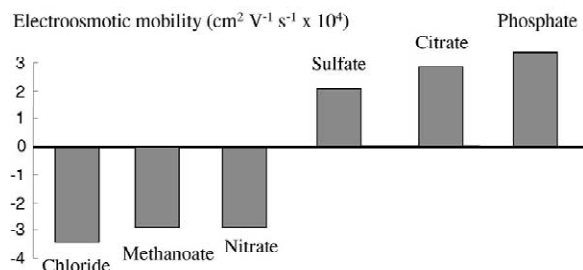


Fig. 5. Electroosmotic mobility of zirconia-modified capillary at pH 3 for various anions.

ions to the electrolyte (data not displayed). Using 5×10^{-3} M calcium nitrate at pH 11, the electroosmotic mobility decreased to $0.5 \times 10^{-4} cm^2 V^{-1} cm^{-1}$.

3.4. Dependence of the electroosmotic mobility on the fraction of organic solvent

The variation of electroosmotic mobility with the content of organic solvent is represented in Fig. 6 for pH 3 and pH 11 solutions for methanol and acetonitrile. The overall trend was the same for the two solvents studied. An initial decrease occurred from 0 to 40% of organic solvent; a reproducible flat portion occurred in the range 40–60% and a final decrease was observed above 60%.

Whatever the layer model used, the electroosmotic mobility is related to the ζ potential (potential at the shear plane), the dielectric constant of the medium and its viscosity by the Smoluchowski equation:

$$u_{eo} = \frac{\epsilon_0 \epsilon}{\eta} \zeta$$

Viscosity and dielectric constant variations could be used to describe the observed tendency in the first

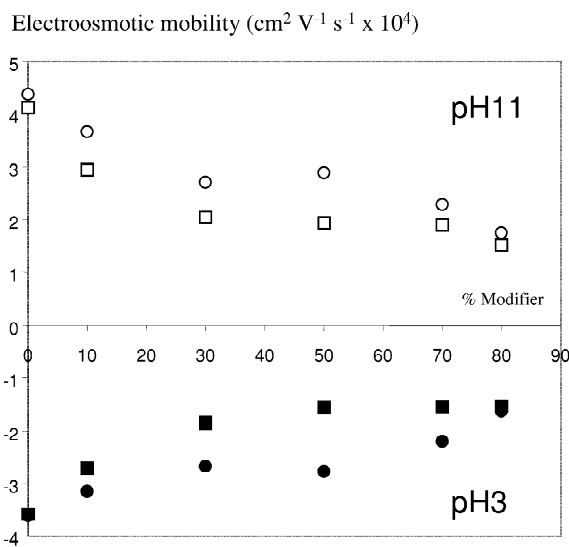


Fig. 6. Electroosmotic mobility of zirconia-modified capillary at pH 3 and pH 11 as a function of organic modifier content. ■, Methanol; ●, acetonitrile; open symbol, pH 11; filled symbol, pH 3.

50% of organic modifier: ε/η decreases in this range and this decrease is smaller for acetonitrile–water mixture than for methanol–water [18]. So, with a constant ζ potential, the electroosmotic mobility should decrease. However, above 60% of organic modifier, the ε/η ratio increases but this trend was not observed on the electroosmotic mobility: the ζ potential cannot be assumed to be constant.

The surface charge, and so the ζ potential, were more affected by a large amount of organic modifier. The constants of the surface reactions were modified by the presence of methanol or acetonitrile: an increase in organic modifier should stabilize non-ionic structures. For acidic solvent, the value of the apparent pK_a should increase: $ZrOH_2^+$ groups were more acidic, the surface charge decreased as well as the ζ potential and so also the electroosmotic mobility. The same trend was observed at basic pH because $ZrOH$ groups were less acidic.

3.5. Application to the separation of four antihistaminic solutes

The separation of four antihistaminic solutes has been carried out using the fused-silica capillary. Such compounds were positively charged below pH 7 whereas the silica surface was negative. So, the electrophoretic mobility of the solutes was in the same direction as electroosmotic flow. Fig. 7A shows the electropherogram obtained using methanoic acid as electrolyte: a poor resolution was obtained for the last three peaks ($m_{eo} = 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$).

If the solute dispersion is predominantly created by solute diffusion, the resolution is related to the electrophoretic mobility and electroosmotic mobility by the equation:

$$Rs = \frac{1}{4\sqrt{2}} \cdot (m_{ep2} - m_{ep1}) \sqrt{\frac{U}{(\bar{m}_{ep} + m_{eo})D}}$$

where D is the diffusion coefficient of the solute and U the applied voltage. In this equation, both electrophoretic and electroosmotic values have to be used in their algebraic form (including the sign). So, if the electrophoretic mobility and electroosmotic mobility have opposite directions, the $\bar{m}_{ep} + m_{eo}$ term would decrease and the resolution could be improved.

The separation has been performed using a zir-

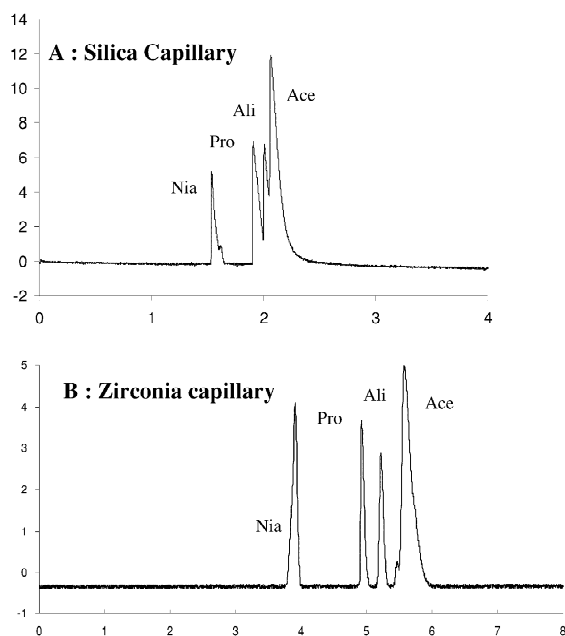


Fig. 7. Separation of four antihistaminic compounds (niaprazine, prometazine, alimemazine, acepromazine). Capillary: 38 cm/effective length 29 cm) \times 50 μm I.D., methanoic acid 60 mM, UV 241 nm. (A) Fused-silica capillary; (B) Zirconia-modified capillary.

conia-coated capillary with the same electrolyte (Fig. 7B). The zirconia surface was positively charged and the electroosmotic flow was negative ($m_{eo} = -1.6 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$). Because the same electrolyte was used, the electrophoretic mobility of the solutes has not changed and so the electrophoretic and electroosmotic flows were in opposite directions. The resolution between the four antihistaminic solutes was then satisfactory and one other compound can even be detected between alimemazine and acepromazine peaks. Furthermore, electrostatic interactions between solute and capillary surface have been minimized and the peak tails observed with silica capillary have been suppressed.

4. Conclusion

The inner surface of a silica capillary has been modified using an anhydrous sol–gel method to give a zirconia-like surface. The resulting column had a cathodic electroosmotic flow (positive) in the pH

range 5–11 and an anodic electroosmotic flow (negative) below pH 5. The effects of several parameters on the electroosmotic flow have been investigated: ionic strength, ion concentration and organic solvent content in the mobile phase. Electroosmotic value (including the sign) can be easily controlled by adjusting all these operating parameters. The optimisation of the separation of basic compounds has been facilitated.

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