

The Influence of Chemical Modification of Fused Silica Capillary Tubes on the Electroosmotic Flow in Capillary Zone Electrophoresis

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The influence of chemical modification on the electroosmotic mobility was investigated in phosphate buffer. The inner capillary wall was coated with silanes bearing different functional group. Aminopropyl, cyanopropyl, diol-type, dimethyloctadecyl, and HMDS-treated columns were studied in comparison with untreated fused silica capillaries. It was demonstrated that chemical modification results not only in lowering the electroosmotic flow rate but also in changing the hysteresis of pH-electroosmotic mobility. In the case of some hydrophyllic coatings of the capillary wall the electroosmotic flow becomes almost pH independent under investigated conditions.

Badano wpływ chemicznej modyfikacji kapilar na ruchliwość elektroosmotyczną w buforach fosforanowych. Wewnętrzna ściankę kapilary pokrywano silanami z różnymi grupami funkcyjnymi, takimi jak aminopropylowa, cyjanopropylowa, typu dioli, dime-tylooktadecylowa i HMDS. Otrzymane wyniki dla modyfikowanych kapilar porównano z wynikami dla kapilar niemodyfikowanych. Chemiczna modyfikacja prowadziła nie tylko do obniżenia szybkości przepływu elektroosmotycznego, ale również do zmian histerezy na wykresie ruchliwości elektroosmotycznej względem pH. W przypadku niektórych hdrofilowych pokryć przepływ elektroosmotyczny był niezależny od pH.

Movement of a liquid through a capillary tube under the influence of the potential difference applied along the capillary is known as electroosmosis. Although this phenomenon was observed already in 1808 by Reuss, the analytical meaning of electroosmosis started from the pioneer work of Jorgenson and Lukacs where the flow of liquid was exploited as a peculiar mean of transportation along the capillary [1].

The electroosmotic flow (EOF) is a result of charge separation between the capillary wall and the solution. The formation of the so called electric double layer is caused either by the dissociation of active silanol groups present on the capillary wall [2] and/or adsorption on their surface. The value and polarity of ζ potential created in the double layer determines the rate and direction of electroosmotic flow. Because of the negative polarity of fused silica surface being in contact with electrolyte the electroosmotic flow is directed towards the cathode. In Capillary Zone Electrophoresis (CZE) the existence of the EOF gives a possibility of simultaneous analysis of cations and anions. Additionally, the proper ratio between electroosmotic and electrophoretic mobilities is desirable to obtain a higher resolution in comparison with that in the case of pure electrophoresis [3]. However, to obtain good results, stable and reproducible EOF is desired. Among factors influencing the electroosmotic flow under certain operational conditions one can distinguish the material of the capillary column, the physical and chemical properties of the wall, the composition and concentration of the buffer, pH [2,4], the presence of organic modifiers [5], surfactants modifying the column wall [4], and a superimposed radial electric field [6].

Chemical modification of the capillary inner surface is widely used in capillary zone electrophoresis in order to minimize adsorption of analyzed substances and to control the electroosmotic flow rate. A variety of chemical modifications has been applied. Monomeric [7] and polymeric [8–10] coatings from hydrophobic [1] through hydrophilic [11], monomolecular non cross-linked [12] and cross linked polymeric coatings [13], fuzzy and interlocked polymer [14], and polymer gels [15] have been successfully used. Recently a great deal of interest is paid to evaluation of the chemical coating of the capillary column. Except a few more complex attempts [13,16–18], the only information about the quality of the surface modification is either a drop in the electroosmotic flow or peak shape of the analyte. There is still incomplete knowledge on the real surface coverage density and the properties of the capillary used in electromigration techniques. In some works the drop in the EOF flow rate *via* chemical modification of the inner capillary wall was explained by lowering of the ζ potential, whereas in many cases it may not be the only explanation. Hjerten has pointed out that EOF can be reduced either by lowering the ζ potential or increasing viscosity of the solution [12]. It is not necessary to increase the viscosity in the bulk; it is sufficient to do it in the share plane of the double layer. Thus coating of the capillary wall by an archy or fuzzy polymer especially by immobilization of the polymer layer may result not only in elimination of active silanol groups but also in increasing of local viscosity. This is supported by the “arch model” of chemically bonded poly(ethyleneglycol) to the surface of solid support [20]. In the present work we discuss the influence of chemical modification with silanes bearing different functional groups on the electroosmotic flow in capillary zone electrophoresis. In our experiments we have selected a modification type which does not give any fuzzy or archy layer and therefore does not change the viscosity in the region adjacent to the column wall.

EXPERIMENTAL

Apparatus

An Grom Kapilar-Elektrophrese System 100 instrument (Tübingen, Germany) equipped with an air forced cooling system was used for all electrophoretic measurements. Fused silica capillaries (50 mm i.d.) with lengths varying in the range of 50–60 cm and the detection window at 20–30 cm were used. A Radelkis pH meter, Model OP-208/1 (Hungary) was used for pH measurements. Samples of a neutral marker (10% acetone or 0.1% of *N,N*-dimethylformamide) were introduced hydrostatically either by lifting the capillary inlet 10 cm above the outlet for a few seconds or by electromigration at 12 kV for 1 second. On-column ultraviolet (UV) detection was carried out at 200 nm, and at temperature 25°C. Measurements were performed after flushing the capillary with the proper buffer. At least 3 measurements were carried out for each pH value.

Chemicals

Double distilled water (the last step from a quartz apparatus) was used for preparation of the buffers. The stock solutions of KH_2PO_4 and Na_2HPO_4 at concentration of 0.033 mol l^{-1} ($1/30 \text{ mol l}^{-1}$) were prepared and suitable pH value of the desired buffer was obtained by mixing appropriate amounts of the stock solutions under pH control. For the chemical modification, 3-aminopropyldimethylmethoxysilane (NH_2), dichlorocyanoethylmethylsilane (CN) (Aldrich), γ -glycidoxypopyl-dimethoxysilane (DIOL) (Fluka, Chemie AG.), hexamethyldisilazane (HMDS) (POCh, Gliwice, Poland), and octadecyldimethylchlorosilane (ODS) (Wacker, München, Germany) were used.

Preparation of the columns

The capillaries were etched with 0.1 mol l^{-1} sodium hydroxide overnight at room temperature, rinsed with water for 30 min, flushed with 3 mol l^{-1} hydrochloric acid for 6 h (to remove Na^+ from the wall and to produce free silanol groups), again washed with water for a few hours, rinsed with methanol for 0.5 h, and then dried at 110°C by flushing with nitrogen for a few hours. Chemical modification was carried out in a 10% silane solution in toluene at 110°C for 12 h. In the case of HMDS the modification was performed under hexamethyldisilazane-saturated nitrogen at 260°C for 2 h. After completing the silanization step the capillaries were flushed with toluene, methanol, and rinsed with water. The DIOL-type capillary was treated with 0.3 mol l^{-1} hydrochloric acid for the epoxy-ring opening, similarly to the procedure described by Regnier and Noel [21]. The unmodified fused silica capillary was used after etching with sodium hydroxide and rinsing with water.

Calculations

Electroosmotic mobility (μ) was calculated using the formula:

$$\mu_{\text{eo}} = \frac{IL}{V \cdot t_m}$$

where L is the total capillary length (cm), l is the distance from injector to detector (cm), V is the applied voltage (12000 V), and t_m is the migration time (s) of the neutral marker (10% acetone or 0.1% *N,N*-dimethylformamide in water).

Electroosmotic mobility vs. pH were plotted for unmodified, NH_2 , CN, DIOL, HMDS, and ODS columns. For experimental data of the relative standard deviation greater than 3%, the error bars were used to show the scattering of the data.

RESULTS AND DISCUSSION

Although it is well known, that the EOF is dependent on the ionic strength [22], we have carried out our investigations at constant concentration to avoid introduction of other anions to the buffer system and also for practical reasons. Experiments were done for the pH range from 9 down to 4 and again up to 9. In the case of unmodified (uncoated) and cyanopropyl (CN) modified capillaries, additional measurements at pH from 9 down to 4 were performed, because significant differences in the electroosmotic mobilities at pH 9 were observed.

Electroosmotic mobility was plotted against pH value of 0.033 mol/l^{-1} $\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer for uncoated fused silica (Fig. 1), and hydrophilically (Fig. 2a–c) and hydrophobically coated capillary columns (Fig. 2d,e). For a comparison, Figures 2a–c contain the corresponding plot of unmodified fused capillary (dashed lines). In Figure 2e asterisk at pH 4 represents the hypothetical value of the electroosmotic mobility which was calculated basing on a relatively long time after which no peak of the electroosmotic flow marker was observed. Additionally, the representative electroosmotic mobility data for all investigated columns are summarized in Table 1.

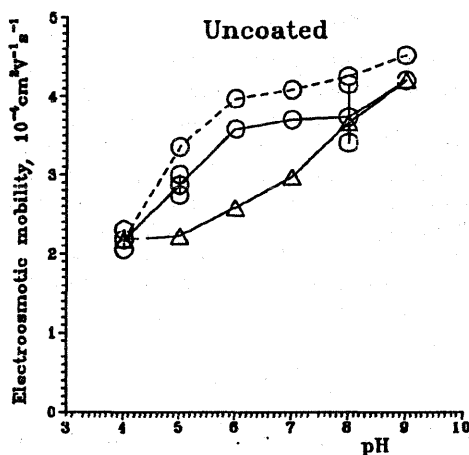


Figure 1. Effect of pH on electroosmotic mobility in fused silica capillary column with phosphate ($0.033 \text{ mol l}^{-1} \text{ KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$) buffer solution. Circles represent the data obtained for the buffer change downward down the pH scale, triangles for the opposite direction. Dashed line represents the first run of experiments down pH range

As one could expect [9,10,14,17,23], there is a significant decrease in the electroosmotic flow for all coated capillaries. However, all diagrams show a hysteresis loop [23] typical for fused silica. The shape of each loop strongly depends on the modification type. According to McCormick [25], phosphate anion can bind to the silica surface at low pH. Due to this chemical modification of the surface the

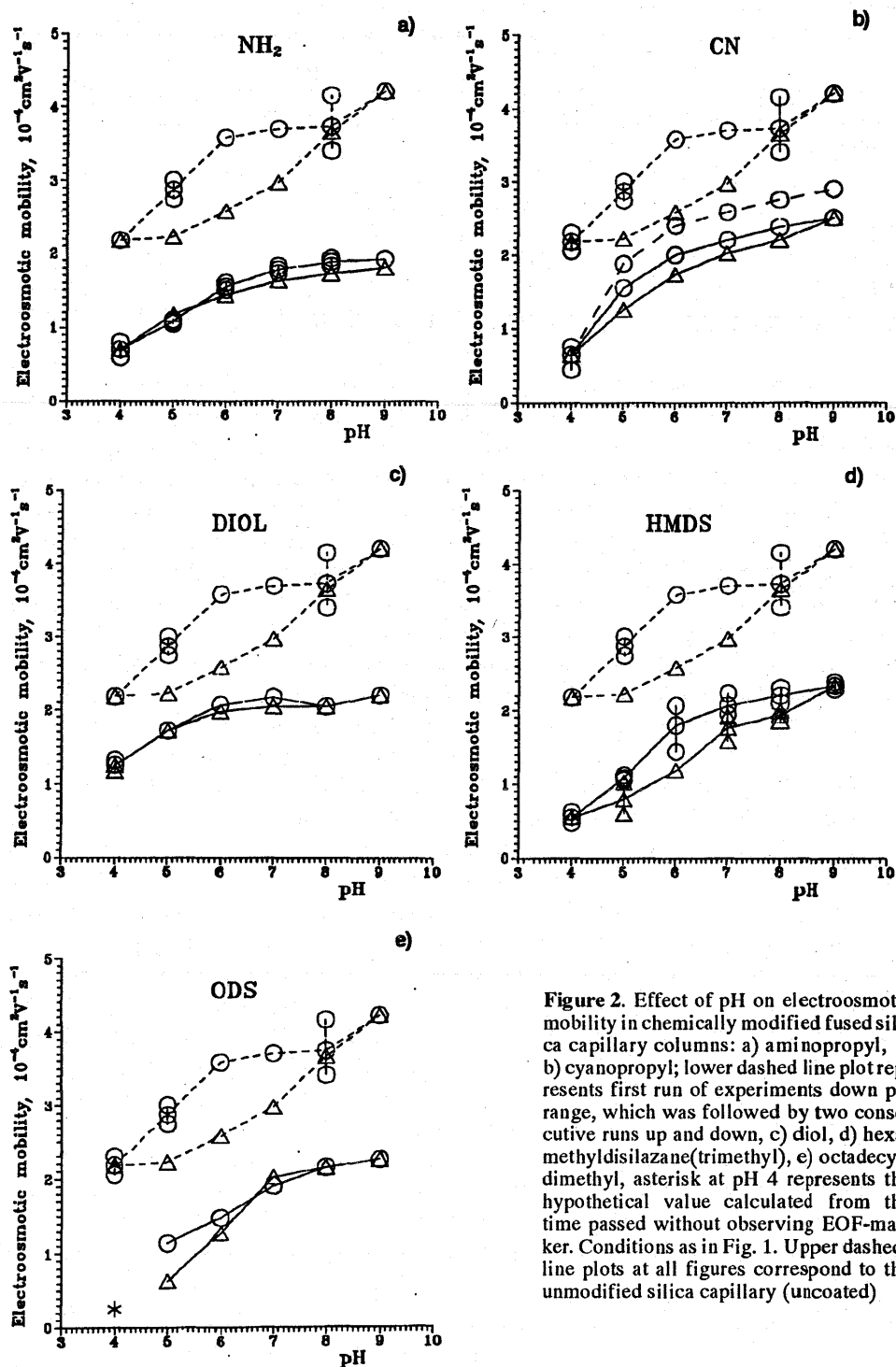


Figure 2. Effect of pH on electroosmotic mobility in chemically modified fused silica capillary columns: a) aminopropyl, b) cyanopropyl; lower dashed line plot represents first run of experiments down pH range, which was followed by two consecutive runs up and down, c) diol, d) hexamethyldisilazane(trimethyl), e) octadecyldimethyl, asterisk at pH 4 represents the hypothetical value calculated from the time passed without observing EOF-marker. Conditions as in Fig. 1. Upper dashed-line plots at all figures correspond to the unmodified silica capillary (uncoated)

Table 1. Electroosmotic mobility data at different pH values for unmodified and different type chemically modified columns, μ_x^{pH4} and μ_x^{pH9} are the electroosmotic mobilities at pH 4 and 9 respectively

Column type	pH 4		pH 9		$\mu_x^{\text{pH4}}/\mu_x^{\text{pH9}}$, %
	μ_x	$\mu_x/\mu_{\text{unc}} \%$	μ_x	$\mu_x/\mu_{\text{unc}} \%$	
Uncoated	2.18		4.2		52
NH ₂	0.7	32	1.86	44	38
CN	0.65	30	2.5	60	26
DIOL	1.25	57	2.2	52	57
HMDS	0.55	25	2.33	55	24
ODS	0.25*	11*	2.25	54	11*

* Calculated for hypothetical value represented by the asterisk at the corresponding plot (Fig. 2e).

electroosmotic flow decreases. Therefore, in the case where silanization is incomplete, two processes can cause the reduction of electroosmotic mobility. One reason for this is the lowering of the zeta potential by elimination of active silanol groups, and the other results from modification by phosphate moiety of the buffer. Although the last mechanism is not clear, we assumed that it contributes to the electroosmotic flow reduction. At pH 9 the electroosmotic mobility of all chemically modified columns is reduced roughly by a factor of two in comparison with the unmodified fused silica capillary. The lowest value is observed for the aminopropyl modified column; therefore one may assume that this type of coverage is more dense in comparison with the others. But this behaviour may also result from the chemical character of the NH₂ group (in the case of a dense coverage one may expect even reversed electroosmotic flow). On the other hand, among chemically modified columns the highest value of electroosmotic mobility at pH 9 has the column with the cyanopropyl chemically bonded layer. This result may be also due to the specific interaction of the CN group with the ions of the buffer solution. At pH 4 the columns with the hydrophobic type of silyl modifier (Fig. 2d,e) exhibit the lowest electroosmotic mobilities. In fact, at pH 4 no peak was observed for the specific time in the case of the ODS type column. This may suggest, that the strongest acidic silanol groups were blocked during the silanization procedure. Relatively high electroosmotic mobility was observed at pH 4 in the case of the DIOL type column. Since we could observe significant reduction of electroosmotic mobility at higher pH, the assumption of low density coverage should be excluded. In this case, exchange of silanol groups (characterized by some degree of heterogeneity) into homogeneous diol groups results in relatively high electroosmotic mobility in comparison with the other chemically modified columns. This effect is clearly shown in the last column of Table 1 where the percentage ratio of the electroosmotic mobilities at pH 4 and pH 9 are expressed. As it has already been mentioned, phosphate anion binds to the capillary wall at low pH. The opposite process is slow in time and requires a higher pH buffer [24]. Therefore a wide hysteresis loop of the pH-electroosmotic flow dependence indicates a large surface of fused silica exposed for interaction with components of the buffer. Conversely, a narrow hysteresis loop may indicate suppression of buffer-silica interaction and thus relatively dense coverage. These are the cases of aminopropyl and especially DIOL-type chemically bonded columns. Addi-

tionally the DIOL column is characterized by relatively stable electroosmotic flow within investigated pH range under applied conditions. This kind of capillary is reported as superior to other coatings [10,13]. Hysteresis effect may cause serious errors in quantitative CZE analysis [23] in the pH range 4–6, and may also offer an explanation why it is often recommended (and built into most commercial instrument software) to rinse the capillary with an alkaline solution between injections.

Conclusions

Chemical modification leads not only to a decrease in the electroosmotic flow but also to a change in the hysteresis of pH–electroosmotic flow rate. The hysteresis loop is the narrowest for the diol type chemical modification which is related to elimination of active acidic silanols on the fused silica capillary wall. An analysis of the shape of the pH–electroosmotic flow may be useful in evaluation of the surface coating density.

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