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Influence of pH*-value of methanolic electrolytes on electroosmotic flow in hydrophilic coated capillaries

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Abstract

The dependency of EOF on the H^+-concentration and the related so called pH* value of methanolic electrolytes has been examined with poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA) and uncoated capillaries. These results were compared with the pH dependency of EOF of these capillaries using aqueous buffers. In uncoated capillaries the dependency of EOF on the pH(*)-value is very similar for aqueous and methanolic electrolytes. The EOF increases with increasing H^+-concentration and pH-hysteresis is observed. In PVA coated capillaries the EOF is strongly reduced over wide pH* or pH ranges for both methanolic electrolytes and aqueous buffers. The EOF in PEG coated capillaries is surprisingly directed to the anode with methanolic electrolytes whereas a reduced cathodic EOF is observed in aqueous electrolytes. The anodic EOF of PEG-coated capillaries in methanolic electrolytes is independent of the pH*-value. The usefulness of PEG- and PVA-coated capillaries for adjusting the EOF in non-aqueous electrolytes for the analysis of isomeric organic acids was demonstrated.   © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Electroosmosis is an important electrokinetic phenomenon in capillary electrophoresis. The electroosmotic flow is related to the electric double layer at the solid liquid interface and is dependent on the electrolyte composition and on the physico-chemical properties of the surface. The electroosmotic flow characteristics of uncoated fused-silica capillaries using aqueous buffers are well known. The EOF is directed to the cathode and strongly dependent on the pH. The equilibration of fused-silica capillaries after treatment with buffers of different pH is a slow process; this phenomenon has been described as pH hysteresis by Lambert and Middleton [1].

For aqueous electrolytes it is well known that the EOF of fused-silica (FS) capillaries can be manipulated by pH [2,3] and concentration of the buffer, by addition of surface active compounds [4–6], by application of radial electrical fields [7–9] and by chemical modification of the capillary wall [10–12]. Control of electroosmosis is an important task in

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electrophoretic separation techniques as the magnitude and direction of EOF affects the net mobility of analytes in the system. In fused-silica capillaries the EOF changes from one CE run to another, resulting in unsatisfactory high relative standard deviations of migration times in repetitive CE separations. A more reproducible EOF compared to common FS-capillaries was found with synthetic polymer capillaries [13]. The poor stability of EOF in bare fused-silica capillaries, which are nowadays nearly exclusively used in analytical practice, is mainly caused by: (i) adsorption of buffer or sample components to the surface, (ii) drift of buffer pH due to electrolysis, and (iii) incompletely equilibrated capillary surfaces.

For aqueous systems procedures have been developed to improve EOF stability: (A) repetitive rinsing and etching steps between analyses to re-equilibrate capillary surfaces, (B) use of high ionic strength buffers to shield the FS surface by reduction of the ζ-Potential and double layer thickness, (C) operation with electrolytes of high buffer capacity to avoid pH changes by electrolysis during a run, (D) use of appropriate dynamic or permanent capillary coatings.

Particularly the use of capillary coatings is very useful for control of EOF *and* surface adsorptivity. Using differently charged coatings the electroosmotic flow can be increased, reduced or reversed. With non-ionic hydrophilic coated capillaries such as PVA and PEG coatings a reduced electroosmotic flow is observed which is influenced by the aforementioned parameters to a much lower extent compared to uncoated capillaries. Hence, complex rinsing steps and preconditioning steps are usually not required as for bare FS capillaries. Although non-aqueous media have been applied in capillary electrophoresis [14–21], little is known about the characteristics of coated capillaries operated with different non-aqueous electrolytes and how to manipulate and control the EOF in non-aqueous systems.

So far, CE separations in non-aqueous electrolytes have mainly been performed with uncoated capillaries. In such systems the electroosmotic flow has been studied [22–26], but systematic investigations on manipulation of the direction and magnitude of EOF in non-aqueous systems have not been carried out so far. Schützner and Kenndler have however studied the EOF in different organic polymer capillaries with mixed aqueous-organic buffer solutions in detail [13].

It should be of high benefit for analytical practice to optimise non-aqueous CE separations in the same way as it is done for classical aqueous CE by using different permanent or dynamic capillary coatings in order to manipulate EOF and capillary surface adsorptivity. In contrary to standard aqueous CE electrolytes, only unbuffered salt solutions in the respective solvent have been used for non-aqueous CE systems so far. Especially for protic solvents as e.g. methanol this causes changes in the H^+-concentration (pH* value) due to electrolyses during a CE run which may cause alteration of EOF. Therefore it is important to study the dependency of EOF on the pH* value in protic organic solvents too and to develop methods for control of EOF in non-aqueous CE-systems. Recently we were able to show that the EOF in some non-aqueous systems can be reduced using hydrophilic permanent capillary coatings [27]. The coatings showed excellent stability towards methanolic electrolytes.

The dependency of EOF on pH of the electrolyte is a characteristic property of different capillary materials and coatings. The EOF behaviour of PEG and PVA coated capillaries with aqueous electrolytes has already been described in the literature [28,29]. In this work we investigated the pH dependency of the electroosmotic flow in capillaries coated according to procedures developed in our laboratory. The data obtained with aqueous electrolytes were compared with those obtained for methanolic electrolytes of different pH* values.

2. Experimental

2.1. Reagents

All samples, solutions and buffers were prepared using analytical grade chemicals. HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). The water content of methanol was about 0.3% (determined by GC). Ammonium acetate, trifluoro acetic acid (TFA), sodium methanolate, succinic acid, oxalic acid and ammonium hydroxide p.a. (25% (w/w) in water) were purchased from

Fluka (Buchs, Switzerland). Lithium hydrogen succinate was obtained from ICN (Meckenheim, Germany) and ammonium oxalate from Sigma (Deisenhofen, Germany).

2.2. Capillaries

Fused silica capillaries of 50 μm inner diameter have been obtained by Polymicro Technologies (Phoenix, AZ, USA) and Microquartz (Munich, Germany). The permanent PVA coating was generated according to a recently developed method [30]. The PEG coating was applied using the typical static coating method [31,32] as for the preparation of capillaries for gas chromatography by using the PE-1-M-100 coating material from Innophase Corp. (Westbrook, CT, USA) dissolved in methylene chloride.

2.3. Instrumentation

CE experiments were performed with laboratory made equipment with UV-detection which has been described previously [9] and with a BioFocus 2000 Instrument (BioRad GmbH, München, Germany).

2.4. Measurement of pH* and EOF

Calibration of a glass electrode suitable for methanolic solutions was carried out similar to a procedure described by Everaerts et al. [33]. A pH-meter equipped with glass electrode (Metrohm, Switzerland) was placed in stirred methanolic pH* standard solutions. A two point calibration was performed using the following methanolic pH* standard solutions [34]: (A) 0.01 mol l^{-1} oxalic acid with 0.01

mol l^{-1} ammonium hydrogen oxalate for a pH* of 5.79, (B) 0.01 mol L^{-1} succinic acid with 0.01 mol l^{-1} lithium hydrogen succinate for a pH* of 8.75. With the calibrated pH-meter the pH* values of different methanolic solutions of NH_4OAc, TFA and Na-methanolate have been determined. The obtained pH*-values for the respective electrolytes are summarised in Table 1.

The EOF was calculated from the migration time of DMSO. For this purpose about 1 μl of DMSO was added to the inlet buffer (2 ml) and the migration time of the migrating front was determined. In order to examine if pH* hysteresis is observed series of experiments were performed similar to those described by Lambert and Middleton for aqueous systems. Before EOF determination the capillaries were rinsed with about 20 capillary volumes of the respective electrolyte. Series of EOF measurements were performed starting with the most acidic electrolytes proceeding to alkaline conditions and moving back towards acidic conditions.

3. Results and discussion

The performance of the PEG and the PVA coated capillaries was evaluated by separating a test mixture of basic proteins consisting of cytochrome C, trypsinogen, lysozyme and α-chymotrypsinogen before and after the respective EOF measurements, to confirm that the capillaries are uniformly coated and remained stable during the experiments. The efficiencies and peak symmetries achieved were considered as an indication for the uniformity of the coatings and as proof of their stability even after long runs with different electrolytes. Very high separation

Table 1
pH*-values of methanolic electrolytes determined with a pH-meter calibrated with methanol buffers

Electrolyte composition (methanolic solutions)	pH*
2 mol/l^{-1} TFA, 20 mmol l^{-1} NH_4OAc	2.90
1 mol/l^{-1} TFA, 20 mmol l^{-1} NH_4OAc	3.15
10 mmol l^{-1} oxalic acid, 10 mmol l^{-1} ammonium hydrogen oxalate	5.79
10 mmol l^{-1} succinic acid, 10 mmol l^{-1} lithium hydrogen succinate	8.75
20 mmol l^{-1} NH_4OAc	10.51
50 mmol l^{-1} $NaOCH_3$, 20 mmol l^{-1} NH_4OAc	13.50
0.1 mol l^{-1} $NaOCH_3$, 20 mmol l^{-1} NH_4OAc	15.24

efficiencies, e.g. more than 500 000 theoretical plates for cytochrome C using a PEG capillary, were obtained.

3.1. Electroosmosis in aqueous buffers

Plots of electroosmotic flow vs. pH of aqueous buffers obtained with PEG and PVA coated capillaries in comparison to an uncoated capillary are shown in Fig. 1. The EOF of the PVA coated capillary is strongly reduced over a wide pH range, no pH hysteresis, as in the case of bare FS capillaries, is observed.

The magnitude of EOF in the PEG capillary, is considerably reduced compared to the uncoated capillary but slightly higher compared to the PVA-coating, especially at basic pH. Comparing the plots for the PEG and the PVA capillary it can be noted that a weak pH hysteresis is only observed in the case of the PEG coated capillary, indicating a slow equilibration of a PEG capillary when exposed to buffers of different pH. The increase of EOF and the hysteresis in the case of the PEG capillaries might be caused by slow exchange of ions penetrating the coating. Both capillaries exhibited excellent stability

towards the treatment with buffers in the pH range under investigation, as could be concluded from the very high separation efficiencies achieved for the basic protein test mixture even after a large number of experiments with aqueous buffers of basic pH.

3.2. Electroosmosis in non-aqueous electrolytes

A distinctive feature of a capillary surface exposed to an electrolyte is the dependency of EOF on the pH of the electrolyte as discussed above for aqueous buffers. The definition and especially the determination of a pH value in non-aqueous solvents is however problematic. In the case of methanol as an amphiprotic solvent with relatively high dielectric constant it is possible to determine so called pH* values in analogy to aqueous solvents. The pH* value of methanolic electrolytes can be determined with a glass electrode after calibration with methanolic buffers of known pH* values. This has been shown by De Ligny et al. who determined the pH ($-\log c_H \gamma^{\pm}$) for some standard solutions in methanolic electrolytes [33]. Recently the dependency of EOF on estimated pH* values of binary mixtures of methanol and acetonitrile has been determined in
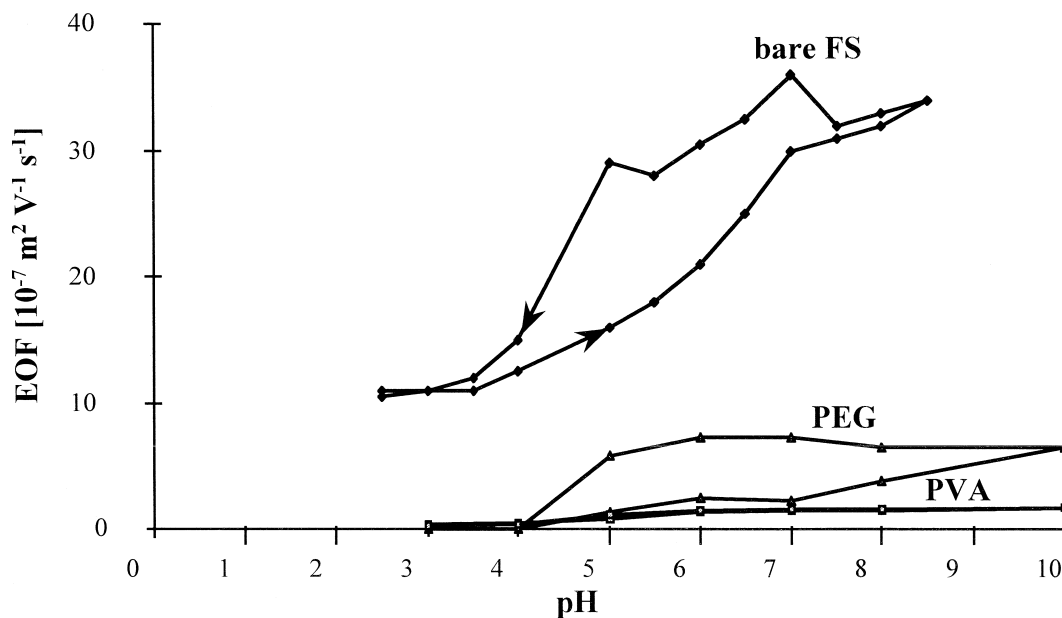

Fig. 1. Dependency of electroosmotic flow on pH of aqueous buffers (40 mmol l^{-1} Na-phosphate) for differently coated columns. Conditions: voltage: 25 kV, capillary: I.D. 50 μm, total length: 50 cm, detection: UV at 200 nm.

uncoated capillaries. In that contribution the pH*
values of unbuffered solutions of a strong acid in the
respective solvents were calculated and used to
calibrate a pH meter [35]. We used the experimental
procedure according to De Ligny et al. for the
determination of pH* values of methanolic elec-
trolytes. In our opinion it should be more reliable to
calibrate a pH meter with *buffered* solutions of
experimentally determined pH* values.

Plots of electroosmotic flow vs. pH* for PVA,
PEG and uncoated capillaries are shown in Fig. 2
and will be discussed separately below.

3.3. Uncoated FS capillary

The plot of EOF vs. pH* is very similar to that
observed in aqueous electrolytes, only the magnitude
of the EOF in methanolic electrolytes is lower
compared to that in aqueous buffers at comparable
pH values. This can be explained by the lower ratio
of dielectric constant to viscosity ε/η for methanol
according to the Smoluchowski equation:

$$\mu_{EOF} = \frac{\varepsilon_0 \varepsilon \zeta}{\eta}$$

where μ_{EOF} is the electroosmotic mobility, ε_0 the
permittivity in vacuum, ε the dielectric constant, ζ
the ζ-potential and η the viscosity. The magnitude of
electroosmotic flow increased with pH* value as for
aqueous electrolytes. EOF hysteresis is observed as
well, indicating a slow equilibration process at the
capillary surface. The data shown in Fig. 2 have been
obtained using a freshly installed FS capillary from
Microquartz. We also determined the EOF in capil-
laries from another manufacturer (Polymicro) and
observed a similar EOF characteristic, but the mag-
nitude of EOF was considerable higher at low pH*
values and the pH* hysteresis was less pronounced,
this is shown in Fig. 3. Because of the inconstancy of
EOF also in non-aqueous systems it is important to
manipulate the EOF over a wide pH range as this is
commonly done for aqueous systems. In order to get
reproducible migration times with high precision in
repetitive analysis the EOF should be independent (i)
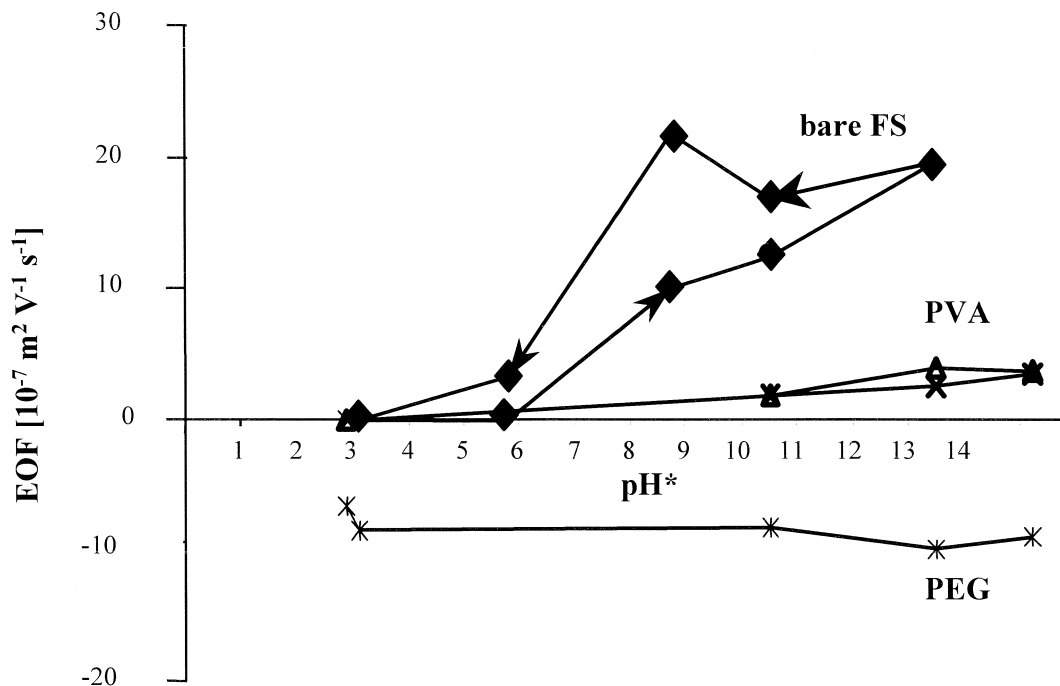from the pH*-value to avoid EOF shifts due to

Fig. 2. Dependency of electroosmotic flow on pH* of methanolic electrolytes for differently coated columns. Other conditions same as for Fig. 1.
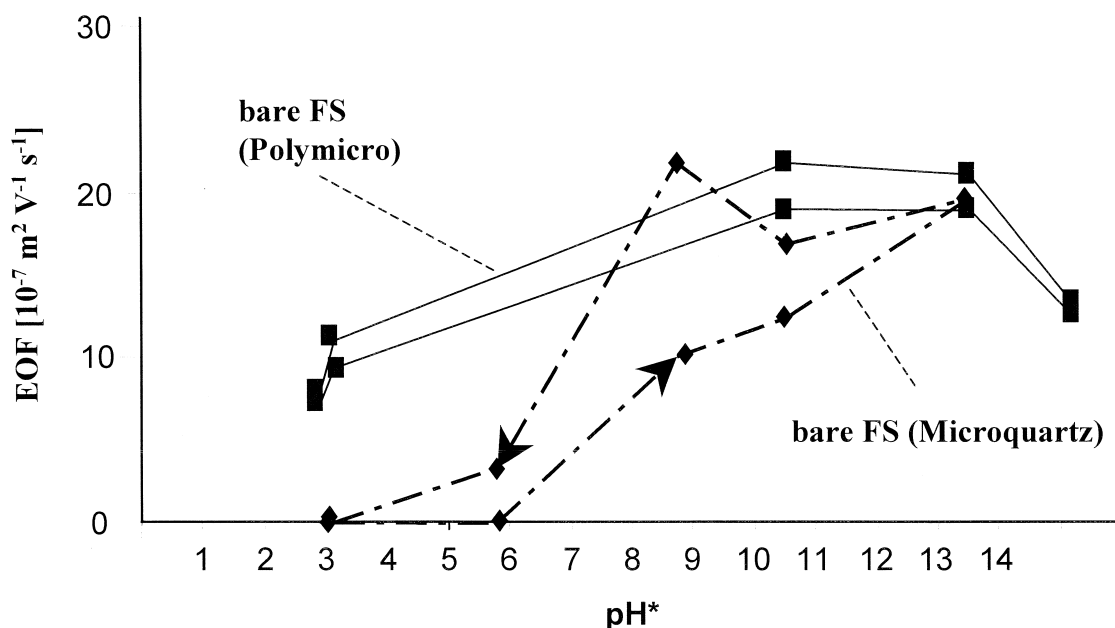
Fig. 3. Dependency of electroosmotic flow on pH* of methanolic electrolytes for bare FS-capillaries from different suppliers. Other conditions same as for Fig. 1.

electrolysis, (ii) from the origin of the bare FS material, (iii) and from the preconditioning history.

3.4. PVA-coated capillary

The EOF of PVA-coated capillaries using methanolic electrolytes is strongly reduced over a wide pH* range as it is with aqueous buffers. A very weak cathodic flow is observed only at extremely basic pH*. Significant hysteresis of EOF was not observed. In analytical application of PVA capillaries for non-aqueous CE the suppressed hysteresis phenomenon should result in an electroosmotic flow which is less sensitive towards the preconditioning history of the capillary. As the EOF is independent from the pH*-value this will result in improved RSDs of migration times in long-time or repetitive CE-runs with a single electrolyte vial since the pH*-value of the electrolyte will change due to electrolysis. PVA-coatings have been generated according to our procedure using FS-capillaries from Polymicro and from Microquartz. The performance and the EOF-characteristics of the coated capillaries were independent from

the origin of the bare FS-capillaries as expected. The PVA coating proved to be stable against treatment with even very basic methanolic electrolytes as it was demonstrated by the aforementioned test for the quality assessment of the coatings. Very high separation efficiencies were obtained in the CE separation of the protein test mixture before and after each series of EOF measurement experiments.

3.5. PEG-coated capillary

While a weak cathodic EOF is observed in aqueous buffers, unexpectedly a considerable anodic electroosmotic flow was observed with methanolic electrolytes which was nearly independent from the pH*-value. A reversed electroosmotic flow directed to the anode indicates, that the surface itself is positively charged. This can be induced when either anionic compounds are migrating out of the coating or cationic substances are adsorbed to the coating. The positively charged surface of PEG capillaries in methanolic-electrolytes can be explained by the complexation of cations by poly(ethylene glycol).

This can formally be described by the following equation:
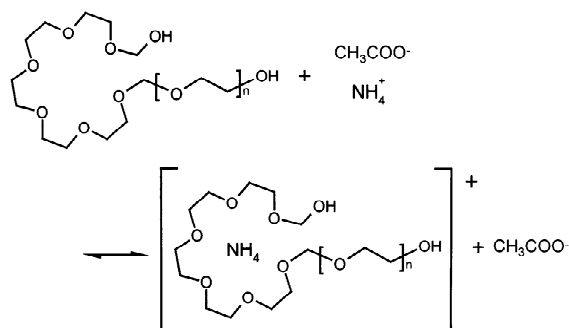
The interaction of polyethylene glycols as polyethers with cationic species is closely related to cation complexation by crown ethers [36–38]. The observation that the EOF is reversed in methanol but not in water can be explained by the better solvation of the cations in water. This leads to higher affinity of the cations to PEG in methanolic electrolytes and induces a positive charge on the surface. As the background electrolyte for these experiments contained 20 mmol L^{-1} ammonium acetate this effect can mainly be attributed to the adsorption of NH_4^+-ions, respectively Na^+-ions at high pH*-values, to PEG. If however strong complexing higher charged cations were present in the electrolyte this could also influence the magnitude of EOF significantly.

PVA as polyhydroxy compound does not interact significantly with cations a reversal of EOF is therefore not observed when the solvent in the electrolyte is changed from water to methanol.

The different electroosmotic flow characteristics using either PEG-, PVA- or uncoated capillaries in non-aqueous CE can be used to adjust the EOF to meet the analytical needs for a given application. The effect of different capillary coatings on the
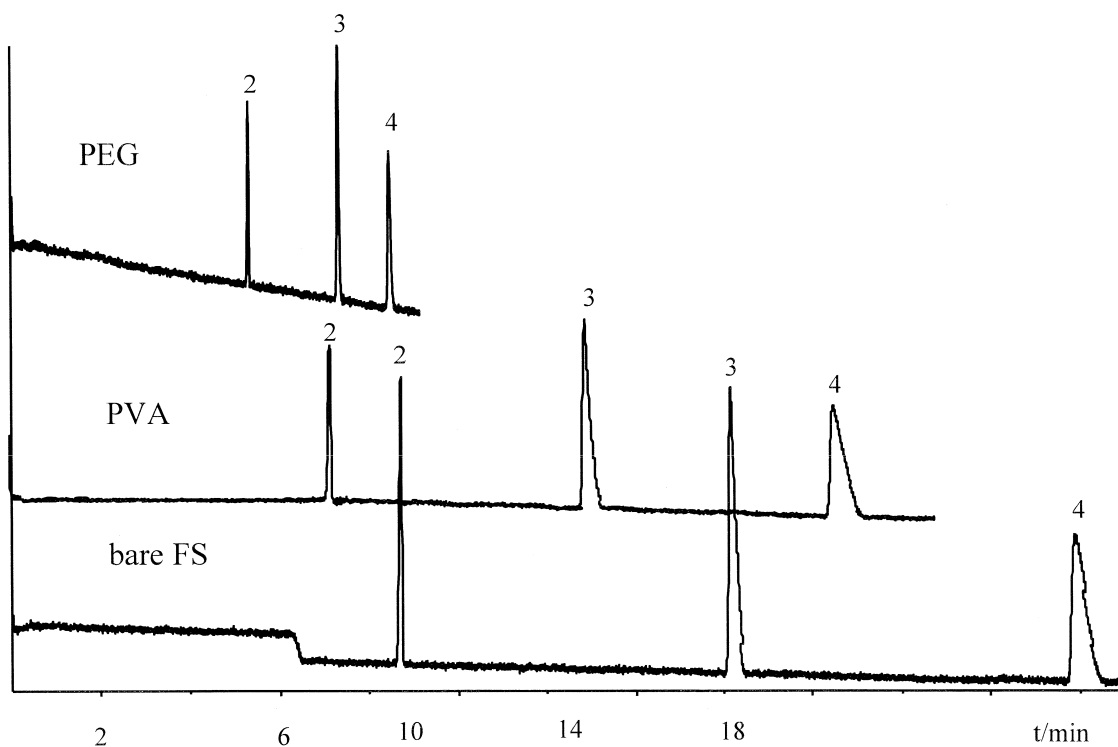

Fig. 4. Influence of capillary coatings on CE separations of acidic compounds using a methanolic electrolyte. Compounds: 2: 2-hydroxybenzoic acid, 3: 3-hydroxybenzoic acid. 4: 4-hydroxybenzoic acid. Conditions: Electrolyte: 20 mmol l^{-1} NH_4OAc in methanol; voltage/current: -20 kV/7 μA; detection UV at 200 nm. Capillary: 55/41 cm, I.D.: 50 μm.

separation of hydroxy benzoic acid isomers in non-aqueous CE is shown in Fig. 4. Using a bare FS capillary the cathodic EOF is in opposite direction to the mobility of the anions causing low effective mobilities of the anions and corresponding long analysis times. With PVA coated capillaries the EOF is decreased leading to shorter migration times of the anionic compounds. When a PEG coated capillary is used the EOF is reversed and now directed to the anode. Hence, the EOF and the electrophoretic mobilities of the analytes are of the same direction and a fast co-electroosmotic separation of the anionic compounds is achieved.

Separation of the hydroxybenzoic acid isomers is also possible using typical aqueous conditions, but the influence of the described capillary coatings is quite different. The separations of the acid isomers using an aqueous phosphate buffer and the differently coated capillaries are shown in Fig. 5. In an uncoated capillary the cathodic EOF is dominant and the anions migrate counterelectroosmotically; they have to be detected at the cathode. Using a PVA or PEG coated capillary the EOF is strongly reduced and the now dominant mobility of the anions leads to an effective migration to the anode with the result of reversed migration order.

4. Conclusion

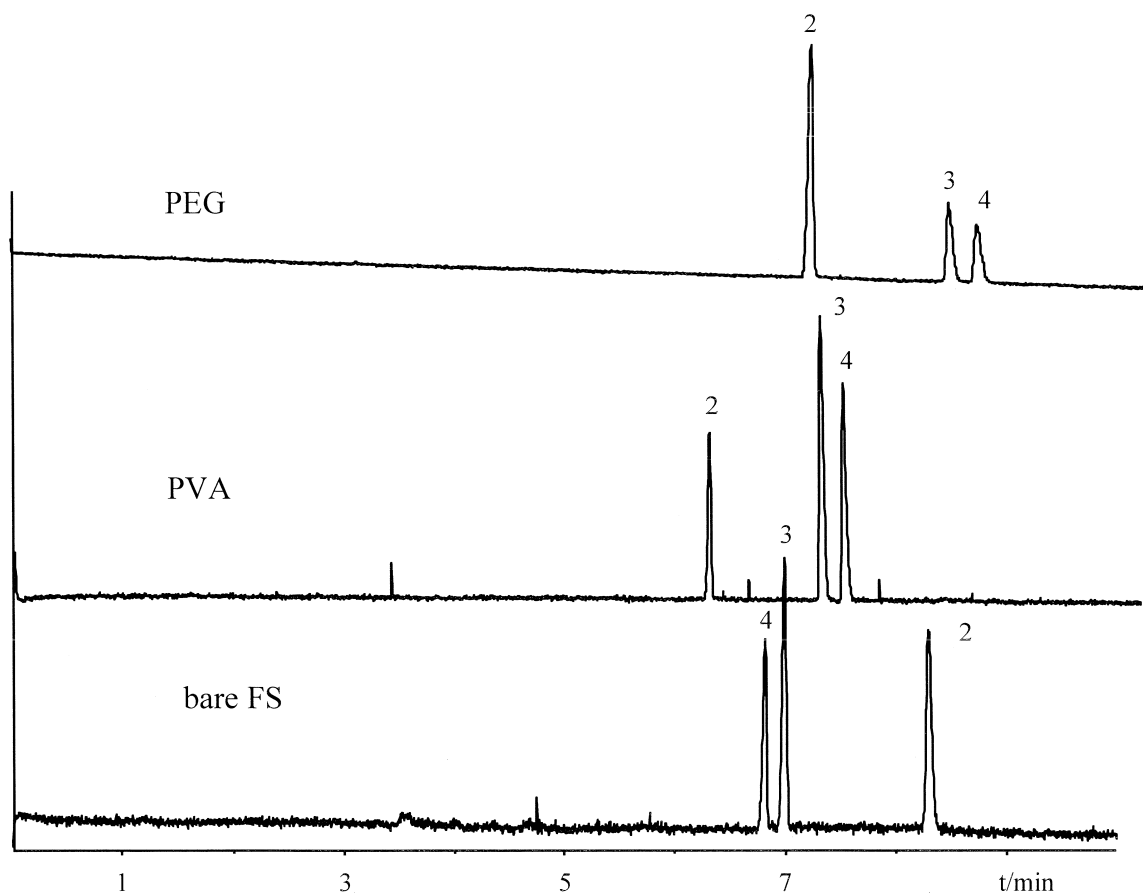The EOF in non-aqueous electrolytes can systematically be manipulated by using differently

Fig. 5. Influence of capillary coatings on CE separations of acidic compounds using an aqueous buffer. Buffer: 20 mmol l^{-1} Na-phosphate pH 7. Voltage: (PEG,PVA) -20 kV, (bare FS) $+20$ kV) Other conditions see Fig. 4.

coated capillaries. The magnitude of EOF in PEG, and PVA coated capillaries operated with methanolic electrolytes is considerably reduced compared to bare FS and exhibits a much lower pH dependency and no pH* hysteresis. Complex capillary rinsing and conditioning procedures, as often applied in non-aqueous CE to obtain a reproducible EOF, could therefore be omitted when PEG and especially when PVA coated capillaries are used. As the direction of EOF in PEG coated capillaries is dependent on the type of solvents used, the alteration of solvent composition can be used to adjust the magnitude and the direction of EOF to meet the analytical requirements. PEG coated capillaries are especially suited for fast coelectroosmotic separation of anionic compounds in non-aqueous CE and therefore especially for CE–MS of anionic compounds. The possible impact of solvent composition on the magnitude and even the direction of EOF should be considered when using non aqueous solvents in CE and CEC.

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