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Journal of Chromatography A, 869 (2000) 363–374

JOURNAL OF CHROMATOGRAPHY A

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Some considerations concerning the composition of the mobile phase in capillary electrochromatography

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Abstract

In capillary electrochromatography (CEC) the propulsion of the mobile phase is effected by electroosmosis. The velocity of the electroosmotic flow is dependent on surface properties of the stationary phase and on bulk properties of the mobile phase. Therefore, in CEC the optimization of the mobile phase composition must take more factors into account than in pressure-driven LC. In this paper, the impact of the electrolyte concentration in the mobile phase and of the volume fraction of the organic mobile phase constituent on the velocity of the electroosmotic flow and on the chromatographic efficiency is investigated for CEC with capillaries packed with octadecylsilica gel. Bias of the data by an open section of the capillary has been excluded by employing completely packed capillaries and detection in a packed section. Acetonitrile as organic constituent of the mobile phase is compared to other possible organic modifiers (polar organic solvents) concerning influence on velocity of the electroosmotic flow and retention of solutes.

Keywords: Mobile phase composition; Electrochromatography

1. Introduction

In capillary electrochromatography (CEC) the mobile phase is electroosmotically driven through the chromatographic bed [1–5]. Surface properties of the stationary phase and bulk properties of the mobile phase determine the electroosmotic velocity and (in absence of pressure differences between the capillary inlet and outlet) the velocity of the mobile phase. Hence, in CEC optimization of the composition of the mobile phase must not only consider retention of solutes and selectivity of the chromatographic system (as in pressure-driven LC) but also the obtained velocity of the mobile phase and the chromatographic efficiency. It is evident that strategies for the optimization of the mobile phase in CEC must differ from those that have been developed so far for pressure-driven LC, although the underlying retention mechanism is basically the same.

Parameters determining the electroosmotic velocity in packings of porous particles are not completely understood [6]. Users of CEC must therefore rely on phenomenological recordings of dependencies of chromatographic parameters like velocity of the mobile phase or efficiency on properties of the stationary and mobile phase.

In CEC most of the workers so far have used capillaries packed with octadecylsilica gel and mobile phases consisting of a mixture of acetonitrile with an aqueous buffer. Zimina et al. [7] have measured the electroosmotic mobility μ_{eo} with several octadecylsilica gels as stationary phase ($d_p = 5$ μm, composition of mobile phase kept constant) packed into capillaries of identical geometrical parameters. By the authors no direct correlation of this

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0021-9673/00/$ – see front matter
PII: S0021-9673(99)01052-3

strongly variating magnitude to any known material parameter of the stationary phase that can be suspected to influence μ_{eo} was found.

The influence of the salt concentration in the mobile phase on μ_{eo} and on chromatographic efficiency has been investigated by some workers [8–11]. Few studies are available concerning the substitution of acetonitrile in the mobile phase by an other polar organic solvent [12–14]. These studies have been restricted to methanol and tetrahydrofuran as organic solvents. They show that the velocity of the mobile phase and the chromatographic selectivity are strongly dependent on the organic constituent.

In our study we tried to develop guidelines for the optimization of the composition of the mobile phase in CEC for the separation of non-polar and non-charged solutes. The following parameters have been included into our investigations: the concentration of the salt used to buffer the mobile phase, the volume fraction of the aqueous phase (keeping the ionic strength constant) and the organic solvent employed as organic mobile phase constituent.

2. Experimental

2.1. Preparation of packed capillaries

The packing technique presented in our previous paper [15] was slightly modified. Fused-silica capillaries (100 or 150 μm I.D.; 337 μm O.D.) (CeramOptec, Bonn, Germany) were used as column material. The inlet frit was prepared in the following manner. Native silica gel (Nucleosil 100-3, Macherey–Nagel, Düren, Germany) was wetted with a solution of sodium silicate in water. The end of the capillary was tapped into the wetted silica gel and sintered with a hot resistance wire ($t = 3$ min, $P =$ 2.5 W). The coating is not pyrolyzed during this process.

The columns were packed as described in [15]. The slurry is pumped into the capillary at $p = 600$ bar using a pneumatic pump (DSTV-122, Armaturenbau, Wesel, Germany). During the packing process the capillary and the slurry reservoir are immersed into an ultrasonic bath and the slurry is agitated by an in-house made stirrer bar and a magnetic stirrer.

After packing, the slurry liquid in the capillary is replaced by water and a frit is sintered at the outlet end in the following manner. The packing material is removed over a length of 1 mm and replaced by native silica gel (Nucleosil 100-3) wetted with a solution of sodium silicate in water. This material was heated under the conditions described for the inlet frit. Detection is performed by photometric detection either in a packed section of the capillary or in a non-packed section. In the detection zone the coating is removed by application of hot sulphuric acid.

In the case of detection in an unpacked section of the capillary, an open capillary (length = 100 mm) with identical inner diameter was glued to a completely packed capillary with the help of a two-component resin glue. The junction was mechanically supported by a short fused-silica capillary (length ≈20 mm, I.D. = 500 μm). The distance between the final frit of the packing and the detection window was 50 mm in all cases

The packed capillary was installed in the chromatographic apparatus and equilibrated with the mobile phase by pumping this phase through the column ($p = 50$–100 bar) for at least 30 min.

Nucleosil 100-3-C_{18}, $d_p = 3$ μm, mean pore width = 10 nm, specific surface area = 300 $m^2\ g^{-1}$ (Macherey–Nagel), has been employed as packing material. The capillaries had following dimensions: inner diameter: 100 or 150 μm, total length: 330–440 mm, length to the detector: 280–390 mm.

2.2. Chemicals

All test solutes were used as received without further purification. 2-Amino-4-nitrotoluene, 2,6-dinitrotoluene and *p*-nitrotoluene were obtained from Aldrich, Steinheim, Germany. Pyrene, acenaphthylene and acenaphthene were obtained from Chem Service, West Chester, UK. Benzyl benzoate and *n*-butyl benzoate were obtained from Merck, Darmstadt, Germany. *p*-Nitroaniline was purchased from Riedel, Seelze, Germany. Methyl benzoate, ethyl benzoate, and naphthalene were available at the Department of Chemistry, University of Marburg, Germany.

Solvents employed as mobile phase components

[acetonitrile, acetone, dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and tetrahydrofuran (THF)] were HPLC-grade. Acetonitrile was distilled before use as a component of the mobile phase. Water was doubly distilled. The buffer salts $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$ were of analytical grade. Due to the low solubility of alkyl and aryl benzoates in polar solvents the samples were prepared with pure acetonitrile. The concentration of each solute in the solvent was 1–2 g l^{-1}.

2.3. Calculation of corrected retention factors

With mobile phases containing dimethylformamide or dimethylsulphoxide as organic constituent, photometric detection had to be performed in an open section of the capillary. In order to correct for the time, in which a solute band passes the open section, corrected values for the electroosmotic velocity and the retention factor have been calculated according to [16].

2.4. Chromatographic system

Chromatographic runs were carried out with a laboratory-made apparatus described in [17]. High voltage was generated by a HCN 35-35000 power supply (FUG, Rosenheim, Germany). Detection was performed with a Spectra 100 variable-wavelength UV–Vis CE detector (Thermo Separation Products, San Jose, CA, USA). The samples were injected electrokinetically. The injection parameters have been adjusted to the electroosmotic mobility employing the equations in [15]. The separation voltage was set at 20–25 kV. The temperature of the separation capillary was not controlled. Data were recorded with EZChrom (Scientific Software, San Ramon, USA) software.

Mixtures of one organic solvent with a solution of Na_2HPO_4 and NaH_2PO_4 in water were employed as mobile phases. Except for the studies with varying ionic strength, the concentration of the buffer components in the mobile phase was kept constant at $c(Na_2HPO_4) = 0.5$ mmol l^{-1} and $c(NaH_2PO_4) = 0.5$ mmol l^{-1}. Thiourea was used as marker of the hold-up time.

3. Results and discussion

3.1. Electroosmosis in porous plugs

It was Smoluchowski [18], who showed that Eq. (1) retains its validity, if in the experiment of electroosmosis, an open capillary is replaced by a porous plug [19]:

$$\frac{V}{i} = \frac{\epsilon_D \epsilon_0 \zeta}{\eta \lambda_0} \quad (1)$$

where V = volume of liquid displaced per unit time, i = electric current, ϵ_D = dielectric constant of the bulk liquid, ϵ_0 = electric permittivity of vacuum, ζ = electrokinetic potential (zeta-potential), η = viscosity of the bulk liquid and λ_0 = specific electric conductivity of the liquid.

Smoluchowski argues that in a porous plug, in which the diameter of the pores is much larger than the thickness of the double layer, the lines of force of the applied electric field in the double layer run parallel to the walls of the pores. They cause an electroosmotic movement of the liquid along the wall. Porous plugs may be made of particles of quite irregular geometry, nearly spherical particles, fibres, more or less well orientated, or bundles of capillaries [20]. Smoluchowski has shown that Eq. (1) can be used to describe the electroosmotic flow through a porous plug, provided that the flow is laminar, the local radius of curvature of the particles and the size of the pores are large compared to the thickness of the double layer, and effects of surface conductance are negligible [20]. The material of the plug is considered to be an insulator [19]. It is important to note that the local electric field is distorted by the presence of the particles [21].

When regarding the constraints made by Smoluchowski, it is evident that the third constraint (the size of the pores is large compared to the thickness of the double layer) is not completely fulfilled with porous chromatographic material. It is only fulfilled for the interparticular pores but not for the intraparticular pores. Porous plugs made out of packing of porous material have not yet been extensively investigated by colloid chemists [20,21].

The fourth constraint made by Smoluchowski concerns the surface conductivity. The term surface

conductivity (Oberflächenleitfähigkeit) has been introduced by Smoluchowski [22]. It can be measured as the difference in conductivity between the liquid in the pores of a porous plug to the conductivity of the bulk liquid outside the pores [20]. Surface conductivity of the packing might not be negligible in CEC, if the specific conductivity of the mobile phase is very low.

3.2. Variation of the ionic strength

In an open capillary the electroosmotic mobility μ_{eo} is related to the thickness δ of the double layer adjacent to the wall surface [23]:

$$\mu_{eo} = \frac{\sigma \cdot \delta}{\eta} \quad (2)$$

where σ = surface charge density and η = viscosity; δ is a function of the electric permittivity ϵ of the liquid phase and of the ionic strength [23]:

$$\delta = \sqrt{\frac{\epsilon\, k\, T}{1000 N_A e^2 \sum_i z_i^2 c_i}} \quad (3)$$

where k = Boltzmann constant, T = temperature (in Kelvin), N_A = Avogadro constant, e = charge of electron, z_i = valence number and c_i = molar concentration.

Choudhary and Horváth [8] emphasized that in open capillaries for a given electrolyte (only the concentration of the buffer component is varied) the electroosmotic mobility μ_{eo} is inversely proportional to the ionic strength I ($I = \frac{1}{2}\Sigma_i\ z_i^2 c_i$). The same relationship should be expected for packed capillaries. Indeed, Choudhary and Horváth found a linear relationship between μ_{eo} and the square root of the concentration of added NaCl (c = 20–60 mmol l^{-1}). Dittmann and Rozing [13] found for a packed capillary a decrease of μ_{eo} with increasing buffer concentration (c = 2–20 mmol l^{-1}).

Because of the high volume fraction of the organic component in the mobile phase, however, the concentration of the buffer component in the mobile phase is often restricted to low concentrations ($c \leq$ 1 mmol l^{-1}) because of the limited solubility of the buffer component in the solvent mixtures. Wan [11] determined μ_{eo} dependent on the concentration of sodium phosphate ($c = 10^{-3}$–10^{-5} mol l^{-1}) in the separation medium in an open capillary and in a capillary partly packed with octadecylsilica gel. The expected relationship (μ_{eo} decreases with increasing c(sodium phosphate)) was found with an open capillary. With the partly packed capillary, however, maximum μ_{eo} is at an intermediate concentration of sodium phosphate. It is suggested by Wan that thermal effects or double layer overlap are responsible for the observed dependence of μ_{eo} on the ionic strength in a packed capillary. Wan expected that the double layer overlap effect is operative only in low electrolyte concentrations.

Knox and Grant [9] determined μ_{eo} dependent on the concentration of NaH_2PO_4 [drawn packed capillary, fluorescence detection in the packing, mobile phase acetonitrile–water (70:30, v/v)] and also found maximum μ_{eo} at an intermediate concentration of the buffer component, $c(NaH_2PO_4) = 10^{-3}$ mol l^{-1}.

Choudhary and Horváth [8] showed that in the case of partially packed capillaries, as they are commonly used in CEC, the overall measurable properties are also determined by the open section (''duplex nature of CEC columns''). In order to measure electroosmotic mobilities that are not distorted by the influence of the open section of the separation capillary, we determined μ_{eo} in capillaries completely packed with octadecylsilica gel. Thiourea has been used as an inert tracer of the electroosmotic velocity. The suitability of urea as tracer of the electroosmotic velocity in CEC is corroborated by studies (employing urea as inert tracer) finding an approximately linear relationship between volume fraction of organic modifier vs. logarithm of retention factor [24]. Photometric detection was performed in-column, i.e. in the packing, as described in our previous papers [25,26]. Repetition of the experiment under identical conditions with a second column gives a rough estimate of the column-to-column variability of μ_{eo}. Acetonitrile–water (80:20, v/v) was employed as mobile phase. The mobile phase was buffered (pH 7.2 in the aqueous component of the mobile phase) with NaH_2PO_4/Na_2HPO_4 added in a stoichiometric ratio of 1:1. In Fig. 1 the electroosmotic mobility is plotted against the logarithm of the phosphate concentration [$c(NaH_2PO_4) + c(Na_2HPO_4)$] in the mobile phase.

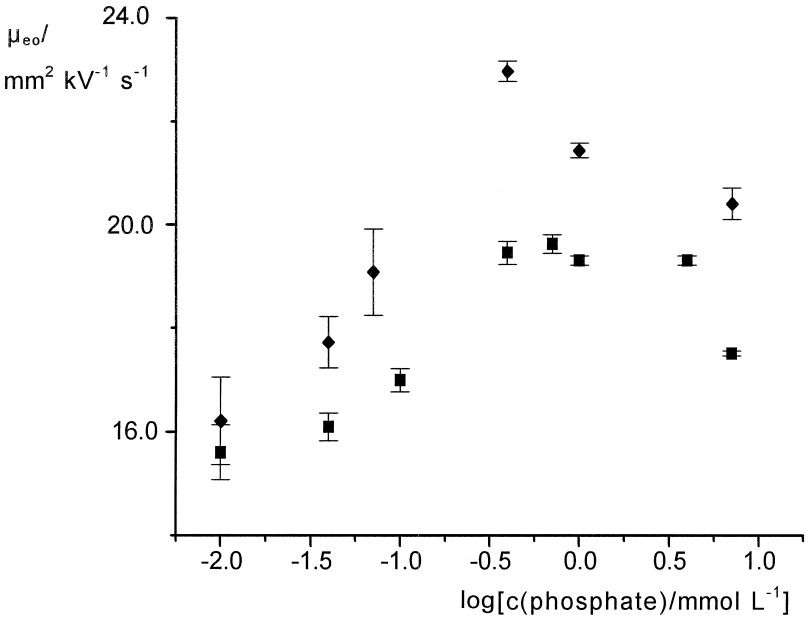

Fig. 1. Electroosmotic mobility vs. buffer salt (phosphate) concentration. ■ = column 1, L = 442 (390) mm × 100 μm I.D., mean of three measurements, ♦ = column 2, L = 422 (376) mm × 100 μm I.D., mean of five measurements, error bars = standard deviation, mobile phase: acetonitrile–phosphate buffer (pH 7.2, c = 0.01–7 mmol l^{-1}) (80:20, v/v), stationary phase: Nucleosil 100-3 ODS, d_p = 3 μm, separation voltage 25 kV, electrokinetic injection 5 s at 5 kV, photometric in-column detection, λ = 230 nm, marker of hold-up time thiourea.

The phosphate concentration was varied in a range from 0.01 to 7 mmol l^{-1}. Results obtained for two capillaries are depicted in the same figure.

For the two capillaries μ_{eo} passes through a maximum. This result deviates strongly from what is expected from Eqs. (2) and (3) and from results obtained with open capillaries [11,23] or with packed capillaries at higher concentration of the buffer component [8,13]. Our results, however, are in accordance with those reported by other workers [9,11]. For the two capillaries the maximum μ_{eo} was obtained at a phosphate concentration of 0.4–4 mmol l^{-1}. We also observed with the two capillaries a lower repeatability (larger standard deviation) of the electroosmotic velocity at electrolyte concentrations lower than 0.4 mmol l^{-1}.

Streaming potential studies on quartz in aqueous solutions of ammonium acetate exhibited a maximum of the measured electrokinetic potential at a salt concentration at around 0.1 mmol l^{-1} [27]. Also with other indifferent electrolytes such as sodium chloride an apparent maximum was obtained at a salt concentration of $6 \cdot 10^{-2}$ mmol l^{-1}. Fuerstenau [27] ascribes this effect to the influence of surface conductance. Possibly, the data presented in this paper in Fig. 1 have to be interpreted keeping the effect of surface conductance in mind. The effect of specific adsorption of mobile phase components can be excluded, as there are neither micelle forming substances nor divalent cations present.

Although the mechanism responsible for the observed maximum in μ_{eo} needs to be elucidated in further studies, from the point of view of minimizing the separation time by enhancing the velocity of the mobile phase, the concentration of the buffer salt(s) is an important variable. The data depicted in Fig. 1 show that too low concentrations of buffer salt(s) have to be avoided, as in this case the velocity of the mobile phase is decreased and a deterioration of the repeatability of retention times affecting the precision of quantitative analysis is observed (possibly due to effects of adsorbed ions). Under the conditions employed, a phosphate concentration of 0.4–4 mmol l^{-1} permits maximum mobile phase velocity without decrease in the repeatability of retention times.

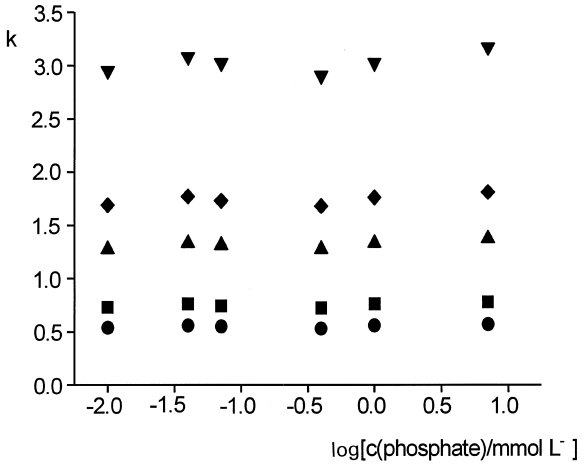

Fig. 2. Retention factors k for several non-charged solutes vs. buffer salt (phosphate) concentration in the mobile phase, column $L=422$ (376) mm×100 μm I.D., other conditions see Fig. 1, solutes: ●=methyl benzoate, ■=ethyl benzoate, ▲=acenaphthylene, ♦=acenaphthene, ▼=pyrene.

In Fig. 2 the retention factors k for several non-charged solutes are plotted against the phosphate concentration in the mobile phase. For five replicate measurements the standard deviation is so small that no error bars can be given. The retention factors of the solutes selected are not significantly dependent on the phosphate concentration in the mobile phase under the conditions employed, hence the buffer concentration does not significantly affect the distribution coefficients of the solutes investigated between the stationary and the mobile phase. This plot also shows that temperature effects can be excluded as being reponsible for the observed dependence of μ_{eo} on the phosphate concentration, as in this case a decrease in k with increasing phosphate concentration would be observed corresponding to the increase in the specific conductivity of the liquid phase in the porous plug and hence an increase in the dissipated electric energy per volume and per unit time. The absence of any dependence of k on the phosphate concentration gives a strong hint, that temperature effects can be neglected in CEC with mobile phases commonly employed. It supports our theory [25] that bubble formation often observed in the beginning of CEC is not due to temperature effects but due to differences in the electroosmotic mobility along a capillary.

In Fig. 3 the plate numbers N determined for selected solutes from the peak width at half height are plotted against the phosphate concentration in the

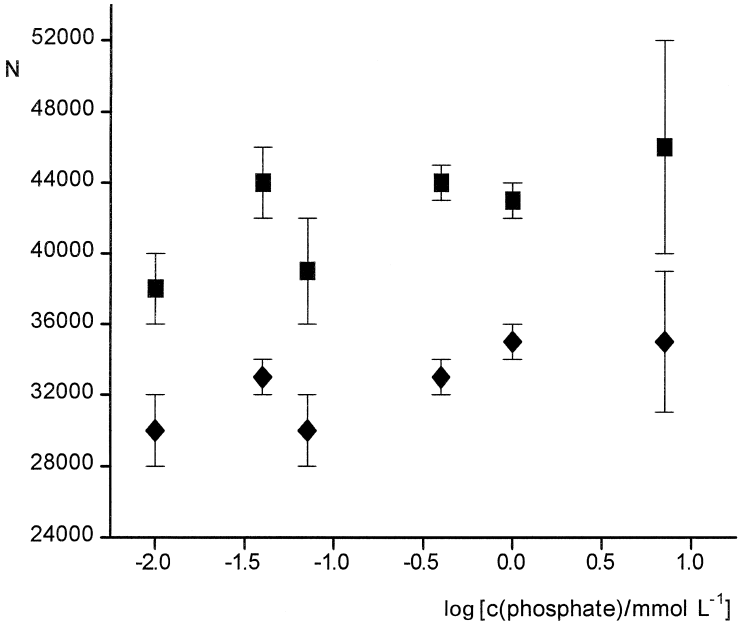

Fig. 3. Plate number N vs. buffer salt (phosphate) concentration in the mobile phase. ♦=acenaphthene, ■=ethyl benzoate, mean of five replicate measurements, error bars: standard deviation. Measurement conditions see Fig. 2.

mobile phase. For the two columns investigated no significant dependence of the efficiency on the concentration of the buffer constituent in the mobile phase was found. The large error bars reflect the fact that the determination of N is inherently imprecise. The injection parameters (electrokinetic injection, 5 kV, 5 s) have been selected so that a significant contribution of the length of the injected sample plug to band broadening can be excluded [15]. In Fig. 4 a typical chromatogram is presented showing that the peak shapes for the solutes are gaussian and symmetrical. The fact that the efficiency is not significantly dependent on the ionic strength of the mobile phase gives a strong hint that the observed dependence of μ_{eo} on the phosphate concentration (at low electrolyte concentrations) must not be due to double layer overlap effects.

3.3. Variation of the volume fraction of the aqueous mobile phase constituent

Schwer and Kenndler [28] measured in open capillaries at constant ionic strength, the influence of selected polar organic solvents (including acetonitrile and acetone) as constituents of the separation electrolyte, on the electroosmotic mobility. They found that at high pH μ_{eo} is generally reduced with increasing volume fraction of the organic constituent. They showed that not only variations of the dielectric constant and the viscosity of the bulk liquid phase are responsible for this reduction, suggesting that alterations of the electrokinetic potential ζ also occur. In contrast to their results Rebscher and Pyell [25] observed a marked increase of μ_{eo} with the volume fraction of acetonitrile in the mobile phase with a capillary packed with octadecylsilica gel. The ionic strength of the mobile phase, however, has not been kept constant. The observation that the dependence of μ_{eo} on the volume fraction of the organic modifier in capillaries packed with octadecyl silica gel differs completely from that found with uncoated open-tubular fused-silica capillaries is corroborated by the results of other workers [8,13,14]. It has to be emphasized, however, that these workers employed partly packed columns (on-column detection in a non-packed segment of the capillary) so that the measured quantities are a result of μ_{eo} in the packed section and μ_{eo} in the non-packed section. Possibly, also properties of the final frits influence significantly the observed velocity [8,25].

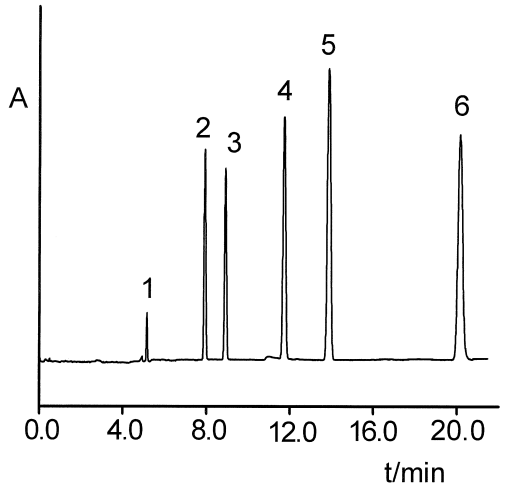

Fig. 4. Separation of alkyl benzoates and polycyclic aromatic hydrocarbons. Mobile phase: acetonitrile–phosphate buffer (c = 0.4 mmol l^{-1}, pH 7.2) (80:20, v/v), solutes: 1 = thiourea (marker), 2 = methyl benzoate, 3 = ethyl benzoate, 4 = acenaphthylene, 5 = acenaphthene, 6 = pyrene, other conditions see Fig. 2.

In order to avoid influence by the open section of the separation capillary, we determined μ_{eo} at constant ionic strength in capillaries completely packed with octadecylsilica gel. Photometric detection was performed in-column. The mobile phase was buffered with NaH_2PO_4/Na_2HPO_4 added in a stoichiometric ratio of 1:1. The phosphate concentration $[c(NaH_2PO_4)+c(Na_2HPO_4)]$ in the mobile phase was kept constant at 0.40 or 0.80 mmol l^{-1}.

In Fig. 5 the electroosmotic mobility is plotted against the volume fraction of the organic mobile phase constituent for acetonitrile and acetone. In both cases there is a continuous increase in μ_{eo} with increasing volume fraction of the organic constituent. This behaviour is differing from that observed in open-tubular fused-silica capillaries [8,28]. Possibly, the difference is due to the octadecylation of the silica surface.

3.4. Selection of the organic constituent in the mobile phase

Most of the work in CEC has been restricted to octadecylsilica gel as stationary phase and aqueous buffer/acetonitrile as mobile phase. In order to rationalize the selection of the organic constituent in

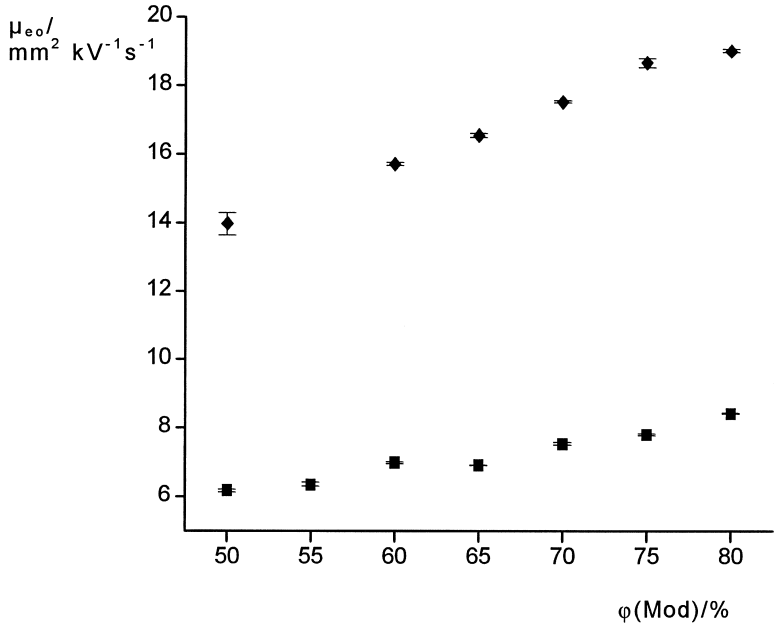

Fig. 5. Electroosmotic mobility μ_{eo} vs. volume fraction of the organic mobile phase constituent. ■ = acetone–phosphate buffer as mobile phase, $c = 0.4$ mmol l^{-1}, pH 7.2, column, $L = 382$ (327) mm × 150 μm I.D., separation voltage 24 kV, electrokinetic injection, 3 s at 2 kV, ♦ = acetonitrile–phosphate buffer as mobile phase, $c = 0.8$ mmol l^{-1}, pH 7.2, column, $L = 442$ (390) mm × 100 I.D., separation voltage 25 kV, electrokinetic injection, 8 s at 2 kV, other conditions see Fig. 1, mean of three measurements, error bars: standard deviation.

the mobile phase, generally Eq. (4) is employed. Eq. (4) (derived for open capillaries) relates the electroosmotic mobility to bulk properties of the liquid phase and to the electrokinetic potential:

$$\mu_{eo} = \frac{\epsilon_D \epsilon_0 \zeta}{\eta} \quad (4)$$

where ϵ_D = dielectric constant of the bulk liquid, ϵ_0 = electric permittivity of vacuum, ζ = electrokinetic potential (zeta potential) and η = viscosity of the bulk liquid.

Assuming that μ_{eo} can be approximated by the linear mobile flow velocity (determined via a non-retarded marker) divided by the electric field strength, Eq. (4) was applied also to porous plugs [13]. Dittmann and Rozing [13] determined the effect of the type of organic constituent (volume fraction = 80%) on μ_{eo} in a capillary packed with Hypersil C_{18}. They observed a decrease in μ_{eo} by a factor of 2.4 when substituting acetonitrile with methanol, and by a factor of three when substituting acetonitrile by tetrahydrofuran. They attributed the

Table 1
Comparison of dielectric constants ϵ_D and viscosities η of various organic solvents (temperature in brackets)

Solvent	ϵ_D	$\eta/(10^{-4}$ kg m^{-1} s$^{-1})$
Acetonitrile (MeCN)	36.64[a] (20°C)	3.69[a] (25°C)
Acetone	21.01[a] (20°C)	3.31[b] (20°C)
N,*N*-Dimethylformamide (DMF)	48.9[a] (20°C)	7.94[c] (25°C)
Dimethylsulfoxide (DMSO)	38.25[c] (20°C)	19.87[c] (25°C)
Tetrahydrofuran (THF)	7.52[a] (22°C)	4.75[a] (25°C)

[a] Data taken from [29].
[b] Data taken from [30].
[c] Data taken from [31].

observed change in μ_{eo} mainly to a decrease in the ratio ϵ_D/η. The changes in selectivity and retention factors, they observed for neutral solutes, occur also in HPLC, as verified by Dittmann and Rozing in a separate experiment.

The results of Schwer and Kenndler [28] for open fused-silica capillaries, however, do not permit to describe the electroosmotic velocity as function of the ratio ϵ_D/η. The results suggest that the electrokinetic potential ζ is generally decreased with increasing volume fraction of the organic constituent in the separation electrolyte. This effect is rather large and mainly contributes to the observed dependence of μ_{eo} on the volume fraction of the organic constituent in the separation electrolyte.

Making the tentative assumption that in CEC the organic constituent in the mobile phase must have UV transparency and a high ratio ϵ_D/η, the organic solvents listed in Table 1 have been selected as candidates [29–31]. As test solutes, amino- and nitrotoluenes and alkyl benzoates have been used. The ionic strength was kept constant. In Fig. 6 a and b the separation of a test mixture employing acetonitrile or acetone as organic constituent is shown. The type of solvent has a large effect on μ_{eo}, retention factors, and selectivity. With THF we were not able to run a chromatogram because of bubble formation. All chromatographic runs have been performed with the same column over a period of 10 days. In all cases symmetrical and gaussian shaped peaks have been obtained. Plate numbers calculated from the peak width at half height are not lowered when acetonitrile is replaced by one of the solvents tested.

In Table 2 μ_{eo} obtained with various organic constituents in the mobile phase is given. The volume fraction φ of the organic constituent has been kept constant at 80%. For acetonitrile and acetone μ_{eo} at $\varphi = 60\%$ was also determined. The data are compared to those obtained by Dittmann and Rozing [13] with a different octadecylsilica gel and $\varphi = 80\%$.

In Fig. 7 the data of the electroosmotic mobility are compared to the ratio ϵ_D/η for a phase containing the organic constituent and water at identical volume fractions. According to Table 2 highest electroosmotic mobilities can be obtained with acetonitrile–water mixtures. At first approximation the unique properties of acetonitrile–aqueous buffer

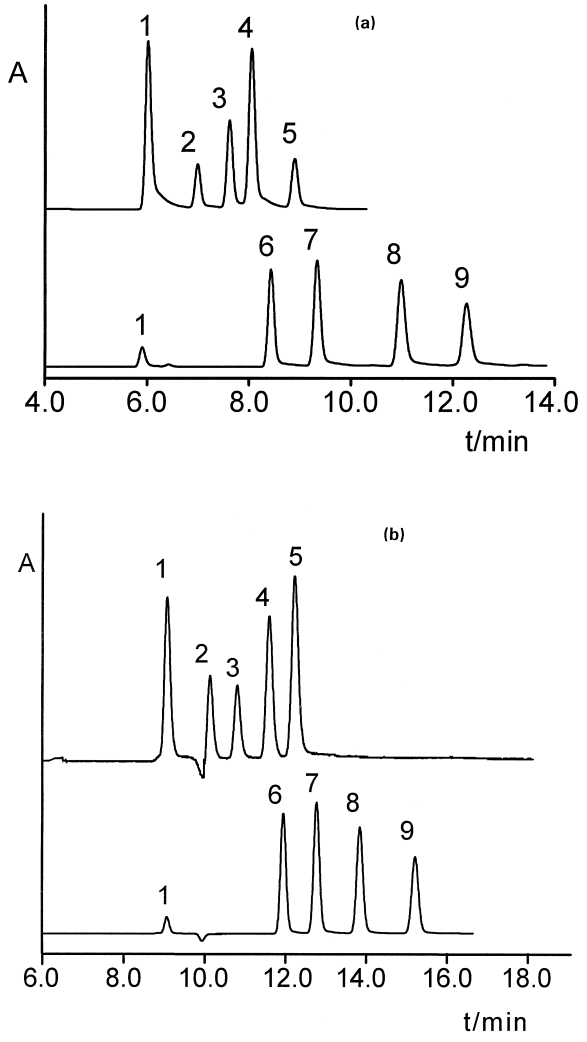

Fig. 6. Separation of benzoates, amino- and nitrotoluenes employing different organic constituents in the mobile phase. Column: $L = 422$ (365) mm × 150 μm I.D., mobile phase: organic constituent–phosphate buffer ($c = 1$ mmol l^{-1}, pH 7.2), (80:20, v/v), organic constituent: (a) acetonitrile, (b) acetone; separation voltage 25 kV, photometric on-column detection, $\lambda = 250$ nm (acetonitrile 230 nm), electrokinetic sample injection (a) 1.9 kV, 3 s, (b) 2.8 kV, 3 s; other conditions see Fig. 1, solutes: 1 = thiourea (marker), 2 = *p*-nitroaniline, 3 = 2-amino-4-nitrotoluene, 4 = 2,6-dinitrotoluene, 5 = *p*-nitrotoluene, 6 = methyl benzoate, 7 = ethyl benzoate, 8 = benzyl benzoate, 9 = butyl benzoate.

mixtures are due to their relatively high dielectric constant and low viscosity. However, although there is a trend, a direct correlation of ϵ_D/η to μ_{eo}, is not possible indicating possibly that ζ cannot be re-

Table 2
Electroosmotic mobility μ_{eo} obtained with various organic constituents in the mobile phase

Mobile phase	μ_{eo} ($mm^2\ s^{-1}\ kV^{-1}$)	ϵ_D (25°C)	η (25°C) (10^{-4} kg $m^{-1}\ s^{-1}$)	ϵ_D/η (10^4 m s kg^{-1})
Acetonitrile–buffer (80:20, v/v)[a]	29.4[c]	44.53[e]	5.03[e]	8.9
Methanol–buffer (80:20, v/v)[a]	13.0[c]	43.1[e]	10.6[e]	4.1
THF–buffer (80:20, v/v)[a]	9.6[c]	18.75[f]	–[h]	–[h]
Acetonitrile–buffer (80:20, v/v)[b]	19.84[d]	44.53[e]	5.03[e]	8.9
Acetonitrile–buffer (60:40, v/v)[b]	13.83[d]	55.2[e]	10.5[e]	5.3
Acetone–buffer (80:20, v/v)[b]	13.01[d]	32.8[e]	11.1[e]	3.0
Acetone–buffer (60:40, v/v)[b]	10.37[d]	43.3[e]	19.1[e]	2.3
DMF–buffer (80:20, v/v)[b]	5.41[d]	52.1[g]	18.0[i]	2.9
DMSO–buffer (80:20, v/v)[b]	2.23[d]	64.7[e]	34.5[e]	1.9

[a] Buffer: TRIS·HCl, $c = 25$ mmol l^{-1}, pH = 8.
[b] Buffer: phosphate–buffer, $c = 1$ mmol l^{-1}, pH = 7.2.
[c] Data taken from [13].
[d] Own measurements, for measurement conditions see Fig. 6, electrokinetic sample injection, 1.9–7.2 kV, 3–5 s.
[e] Data taken from [28].
[f] Data taken from F.E. Crichfield, J.A. Gibson, J.L. Hall, J. Am. Chem. Soc. 75 (1953) 6044.
[g] Data taken from T. Onescu, E. Jurconi, Rev. Roum. Chim. 16 (19711) 1033.
[h] Not determined.
[i] Own measurements.

garded as constant [28] (see Fig. 7). When interpreting this data material, it has also been taken into consideration that the flow in partly packed columns (as used in these experiments) is not only controlled by the electroosmotic mobility and the local electric field strength but also induced by local pressure differences. These local pressure differences are caused by the need to conserve the overall volumetric flow-rate [32]. In this case the viscosity of the mobile phase becomes the dominant physical magnitude, limiting the obtainable linear flow velocity. The unexpectedly high linear flow velocity measured for acetone containing phases might be attributed to this effect.

From comparing Fig. 6a to Fig. 6b, it is evident that acetone is an interesting alternative to acetoni-

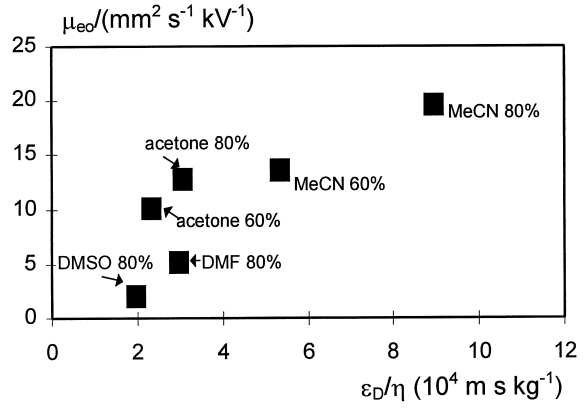

Fig. 7. Electrophoretic mobility (μ_{eo}) plotted vs. ratio dielectric constant (ϵ_D) to viscosity (η) of the corresponding solvent–mixture (experimental conditions see Fig. 6).

Table 3
Retention factors k for selected solutes for different compositions of the mobile phase

Solute	φ(acetonitrile) (%)		φ(acetone) (%)	
	80	60	80	60
p-Nitroaniline	0.23	0.53	0.06	0.07
2-Amino-4-nitrotoluene	0.37	0.96	0.16	0.39
2,6-Dinitrotoluene	0.47	1.52	0.27	0.81
Methyl benzoate	0.54	0.98	0.38	0.41
p-Nitrotoluene	0.65	1.94	0.45	1.13
Ethyl benzoate	0.75	1.58	0.48	0.86
Benzyl benzoate	1.11	3.60	0.63	2.38
Butyl benzoate	1.39	4.23	0.83	2.85

Measurement conditions: mobile phase, organic constituent/phosphate buffer, $c=1$ mmol l^{-1}, pH 7.2 [φ(organic constituent):(100 − φ(organic constituent)] v/v), other conditions see Fig. 6, a and b.

trile as organic component in the mobile phase, reducing μ_{eo} only by a factor of 0.65–0.75 (all other parameters kept constant), offering a significantly different chromatographic selectivity (see Fig. 6 and Table 3). The higher elution strength of acetone is reflected by the reduced retention factors with constant volume fraction of the organic constituent (see Table 3). Because of the small detection volume in case of on-column detection in CEC, the optical properties of acetone as constituent of the mobile phase seem to be tolerable (see Fig. 6b).

With DMF and DMSO containing mobile phases, however, electroosmotic velocities much smaller than those for acetonitrile–aqueous buffer mixtures are obtained (see Table 2). Consequently, DMF and DMSO cannot be regarded as an interesting alternative to acetonitrile, as DMF and DMSO containing mobile phases do not permit short analysis times in CEC.

4. Conclusions

Taking into account that in CEC the composition of the mobile phase does not only determine retention and selectivity of the chromatographic system but also the velocity of the mobile phase, strategies for optimizing the composition of the mobile phase have to be different in CEC from those in pressure-driven HPLC. In order to obtain maximum velocity of the mobile phase, the ionic strength is an important parameter. In order to maintain the high repeatability of separations and hence the precision of quantitation a threshold value should be exceeded.

The presented data suggest that because of its unique properties acetonitrile–aqueous buffer mixtures can be regarded as the mobile phase of choice in reversed-phase CEC with capillaries packed with octadecylsilica gel.

Acknowledgements

Financial support by the German Science Foundation (DFG) is gratefully acknowledged. We thank Mrs. B. Bahmann for having performed some of the measurements. We also thank the Fonds der Chemischen Industrie for financial support.

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