



ELSEVIER

Journal of Chromatography A, 716 (1995) 97–105

JOURNAL OF  
CHROMATOGRAPHY A

## Control of the electroosmotic flow by metal-salt-containing buffers

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### Abstract

New possibilities to suppress electroosmotic flow (EOF) were investigated. Buffers prepared from salts of zinc (up to 50 mmol/l), aluminium (up to 0.5 mmol/l) and barium (up to 250 mmol/l) lead to an effective reduction of EOF in Tris buffer, pH 7.4, 50 mmol/l. The dependence of EOF on different metal-salt concentrations is documented. The usefulness of barium-salt-containing buffers is demonstrated for pH values between 5 and 9.2. If the suppression is too strong, the EOF can be adjusted by mixtures of sodium and barium salts.

### 1. Introduction

The walls of fused-silica capillaries are negatively charged above pH 2.5, because the silanol groups are partly deprotonated. However, the net charge of a capillary is zero as there is an excess of cations in the buffer solution to compensate for the fixed negative charge at the wall. These cations are partly mobile. Normally, they are concentrated close to the capillary wall. If a voltage is applied, they migrate towards the cathode, along with the buffer solution due to hydrate binding and friction. The result is a flow of the bulk solution towards the cathode. This is called electroosmotic flow (EOF).

The electrokinetic behaviour of a charged surface depends on its  $\zeta$  (zeta) potential. The corresponding theoretical principles have been understood for a long time [1]. Two effects compensate for each other: electrostatic attraction and diffusion. The solution of the corre-

sponding differential equations leads to a Boltzmann distribution. The excess of cations decreases exponentially with distance from the capillary wall.

However, the details of this theory are still under discussion (reviewed in Ref. [2]). The contributions of specific adsorption, the consequences of competition of different ions for binding sites, and effects like secondary adsorption are not yet fully understood. Therefore, it is not simple to control the EOF via the zeta potential.

The EOF affects the resolution in capillary electrophoresis (CE). Usually a low EOF is desirable. There are many possibilities to achieve this: high buffer concentration, additional modifiers, low buffer pH and coated capillaries (reviewed in Refs. [3,4]). However, these methods often influence the selectivity as well. Especially when working with buffers of high pH, it is not easy to suppress the EOF effectively.

There are CE methods that use zinc [5–12] and barium salts [13] to improve the resolution.

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In these cases the selectivity was not influenced, but the EOF decreased. This effect is studied systematically in this paper. According to earlier studies, salts of different metals should influence the zeta potential similarly [14]. Thus, the study of a few different salts should be sufficient to investigate the entire phenomenon.

## 2. Experimental

### 2.1. Effect of salt concentrations on EOF

The dependence of the EOF on different salt concentrations was investigated with a laboratory-built instrument [3], similar to the one described in Ref. [15]. The power supply HCN 35-35000 (FUG, Rosenheim, Germany) and UV detector Spectra 100 (ThermoSeparations, Darmstadt, Germany) were used. The detection wavelength was 214 nm. A recorder Linear 2030 000 (Gamma Analysen Technik, Bremerhaven, Germany) was employed at a chart speed of 1 cm/min.

Fused-silica capillaries from the same batch (Polymicro), 50  $\mu\text{m}$  internal diameter, 25 cm effective and 40 cm total length, were used. Fresh capillaries were pretreated by rinsing with 0.1 M NaOH for 5 min, applying 10 kV for 15 min, rinsing with buffer, and equilibrating for 30 min under the subsequent running conditions. Between experiments the capillary was rinsed with buffer for 5 min. If the species or the concentration of the additive was changed, the capillary was first rinsed with 0.1 M NaOH, followed by the new buffer, 5 min each. Then 15 kV was applied for 30 min ( $\text{Al}^{3+}$ : 60 min) to equilibrate the capillary.

If not stated otherwise, chemicals were of analytical grade, supplied by Merck (Darmstadt, Germany). All measurements were performed in Tris [tris(hydroxymethyl)aminomethane] buffer, pH 7.4, 50 mmol/l. This was prepared by dissolving 606 mg Tris (laboratory reagent grade; Boehringer-Mannheim, Germany) in 42.0 ml of 0.1 mmol/l HCl (volumetric solution), adding an appropriate amount of metal-salt stock solution and filling up to 100.0 ml with HPLC-grade

water (Millipore, Eschborn, Germany). Barium-containing buffers were prepared by dissolving the appropriate amount of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  directly in Tris buffer. As stock solutions 2726 mg/200 ml (100 mmol/l)  $\text{ZnCl}_2$  and 120.7 mg/500 ml (1 mmol/l)  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  (laboratory reagent grade; Baker, Deventer, Netherlands) were used. All solutions were prepared fresh every day.

If possible, a separation voltage of 15 kV was used. The current was ca. 30  $\mu\text{A}$  at rather low concentrations of metal-salt buffer additives. If the additive concentration exceeded 10 mmol/l, a voltage lower than 15 kV was used. For most experiments the cathode was at the capillary outlet; for  $c_{\text{Zn}^{2+}} \geq 25$  mmol/l and  $c_{\text{Al}^{3+}} \geq 0.5$  mmol/l the anode was at the outlet. Normally, the EOF mobility was estimated by using acetanilide (330 mg/l, dissolved in Tris buffer) as neutral marker. If the EOF was very low ( $c_{\text{Zn}^{2+}} = 2\text{--}10$  mmol/l,  $c_{\text{Ba}^{2+}} \geq 100$  mmol/l), neostigmine bromide (516 mg/l, dissolved in Tris buffer, pharmacopoeia quality; Hoffmann-La Roche, Basle, Switzerland) was used as the cationic marker. Here the EOF was estimated indirectly from the known electrophoretic mobility of neostigmine.

The samples were injected electrokinetically (5 kV, 15 s). Mobility measurements were typically performed in triplicate.

### 2.2. Effect of addition of barium to buffers of various pH on EOF

The EOF obtained by addition of barium to buffers of various pH was investigated on a P/ACE 2100 (Beckman Instruments). Unless stated otherwise, the experimental conditions were as described above for barium salts. The thermostat was set to 30°C. Separation voltage was 20 kV. Capillary length was 30 cm effective, 37 cm total. A pressure difference of 34 hPa was applied for 4 s to inject the samples. Prior to each run, the capillary was rinsed for 2 min with separation buffer. Before the buffer system was changed, the capillary was rinsed for 30 min with 0.1 M NaOH, followed by a rinse of 5 min with

separation buffer and equilibration at 20 kV for another 30 min.

### 2.3. Preparation of borate buffers

The borate buffers, pH 9.2, 60 mmol/l (32.07 mmol/l  $\text{Me}^+$ ) were prepared by dissolving 371 mg of boric acid,  $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$  and 0.1 M NaOH with HPLC-grade water to 100.0 ml solution. The buffer with %Ba = 0 contained 0 mg of  $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$  (= Ba) and 32.07 ml 0.1 M NaOH (= Na); the others were prepared analogously: %Ba = 6.25: 31.5 mg Ba, 30.07 ml Na; %Ba = 12.5: 63 mg Ba, 28.06 ml Na; %Ba = 25: 126.5 mg Ba, 24.05 ml Na; %Ba = 50: 253 mg Ba, 16.03 ml Na; %Ba = 62.5: 316 mg Ba, 12.03 ml Na; %Ba = 100: 506 mg Ba; 0 ml Na.

The barium-free buffer was prepared by dissolving 233 mg of boric acid and 213 mg of sodium tetraborate decahydrate with HPLC-grade water to 100.0 ml solution. The barium-containing borate buffers, pH 8.5, 60 mmol/l (10.48 mmol  $\text{Me}^+$ ), were prepared by dissolving 371 mg of boric acid,  $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$  (= Ba) and 0.1 M NaOH (= Na) with HPLC-grade water to 100.0 ml solution. %Ba = 6.25: 11 mg Ba, 10.48 ml Na; %Ba = 12.5: 22 mg Ba, 9.78 ml Na; %Ba = 25: 44 mg Ba, 8.39 ml Na; %Ba = 50: 88 mg Ba, 5.59 ml Na; %Ba = 62.5: 110 mg Ba, 4.19 ml Na; %Ba = 100: 176 mg Ba.

The same recipes as for the 60 mmol/l buffers were used, but all weights and  $V_{\text{NaOH}}$  were multiplied by 2.862 ( $60 \times 2.862 = 171.7$ ).

### 2.4. Preparation of imidazole buffers

The buffers were prepared by dissolving 272 mg of imidazole, 20.0 ml of 0.1 M HCl,  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  and NaCl with HPLC-grade water to 100.0 ml solution.

The buffer with %Ba = 0 contained 0 mg of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  (= Ba) and 187.5 mg of NaCl (= Na), corresponding to 32.07 mmol  $\text{Me}^+$ ; the others were analogously prepared: %Ba = 6.25: 24.5 mg Ba, 176 mg Na; %Ba = 12.5: 49 mg Ba, 164 mg Na; %Ba = 25: 98 mg Ba, 140 mg Na; %Ba = 50: 196 mg Ba, 94 mg Na; %Ba = 62.5: 249 mg Ba, 70 mg Na; %Ba = 100: 392 mg Ba.

### 2.5. Preparation of acetate buffers

The buffers were prepared by dissolving 1.60 ml of 1 M acetic acid (volumetric solution), sodium acetate trihydrate (= Na) and barium acetate (= Ba) with HPLC-grade water to 100.0 ml solution.

The buffer with %Ba = 0 contained 0 mg of Ba and 436 mg of Na, corresponding to 32 mmol/l  $\text{Me}^+$ ; the others were analogously prepared: %Ba = 6.25: 25.5 mg Ba, 409 mg Na; %Ba = 12.5: 51 mg Ba, 382 mg Na; %Ba = 25: 102 mg Ba, 327 mg Na; %Ba = 50: 205 mg Ba, 218 mg Na; %Ba = 62.5: 256 mg Ba, 163 mg Na; %Ba = 100: 410 mg Ba.

## 3. Results and discussion

### 3.1. Estimation of the mobility

The mobility,  $\mu$ , is defined as the ratio of velocity  $v$  and electrical field strength  $E$  (Eq. 1). The velocity is calculated by measuring the time  $t_M$  needed by the sample to pass the column length  $l$  to the detection window (Eq. 2);  $E$  is defined as the ratio of voltage  $U$  and overall column length  $L$  (Eq. 3).

If the EOF is high, its velocity  $v_{\text{EOF}}$  can simply be determined by Eq. 2, using the migration time of a neutral marker. However, if the EOF is low, it takes a very long time to measure it. In this case the EOF can be estimated indirectly. In preceding experiments the electrophoretic velocity  $v_e$  of a positively charged marker compound is determined from its total velocity  $v$  and the velocity of a neutral marker  $v_{\text{EOF}}$  (Eq. 4). Now  $v_{\text{EOF}}$  can be calculated from the total velocity of the cationic marker (Eq. 5), if this can be considered independent of the buffer composition.

$$\mu = \frac{v}{E} \quad (1)$$

$$v = \frac{l}{t_M} \quad (2)$$

$$E = \frac{U}{L} \quad (3)$$

$$v_e = v - v_{\text{EOF}} \quad (4)$$

$$v_{\text{EOF}} = v - v_e \quad (5)$$

However, in principle the electrophoretic mobility is dependent on ionic strength. Thus, the changes of  $v_e$  with buffer composition had to be investigated. The mean of  $v_e$  was  $(2.77 \pm 0.09) \cdot 10^{-8} \text{ V}^{-1} \text{ s}^{-1} \text{ m}^2$  for barium-salt concentrations between 0 and 10 mmol/l ( $n = 12$ ). The average of three measurements at 0 and 10 mmol/l barium was  $2.67 \cdot 10^{-8}$  and  $2.85 \cdot 10^{-8} \text{ V}^{-1} \text{ s}^{-1} \text{ m}^2$ , respectively. The mean of  $v_e$  was  $(2.56 \pm 0.16) \cdot 10^{-8} \text{ V}^{-1} \text{ s}^{-1} \text{ m}^2$  for zinc-salt concentrations between 0 and 1 mmol/l ( $n = 9$ ). The average of three measurements at 0 and 1 mmol/l zinc was  $2.63 \cdot 10^{-8}$  and  $2.60 \cdot 10^{-8} \text{ V}^{-1} \text{ s}^{-1} \text{ m}^2$ , respectively. No significant dependence was observed between electrophoretic mobility and ionic strength under these conditions.

### 3.2. Relationship between the concentration of different metal salts and EOF mobility

The dependence of the electrophoretic mobility on the salt concentration is demonstrated in Figs. 1-3. These results can be understood easily

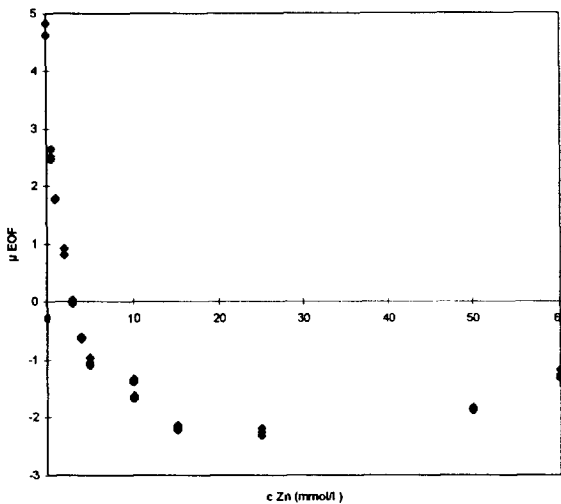


Fig. 1. Dependence of the EOF ( $\mu$  in  $10^{-8} \text{ V}^{-1} \text{ s}^{-1} \text{ m}^2$ ) on the zinc-salt concentration, measured in Tris buffer, pH 7.4, 50 mmol/l. Mobility measurements were performed in triplicate.

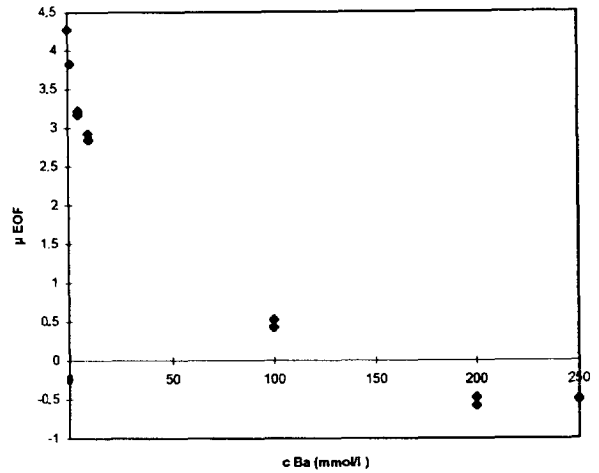


Fig. 2. Dependence of the EOF ( $\mu$  in  $10^{-8} \text{ V}^{-1} \text{ s}^{-1} \text{ m}^2$ ) on the barium-salt concentration, measured in Tris buffer, pH 7.4, 50 mmol/l. Mobility measurements were typically performed in triplicate (except  $c = 250$  mmol/l, which was measured twice).

(compare Fig. 4). The EOF towards the cathode is caused by the negatively charged silica glass surface (i). If cations are adsorbed to this surface, its charge and thus the EOF decrease (ii). At high cation concentrations the surface charge can even be inverted. The resulting excess of solvated anions leads to an EOF towards the

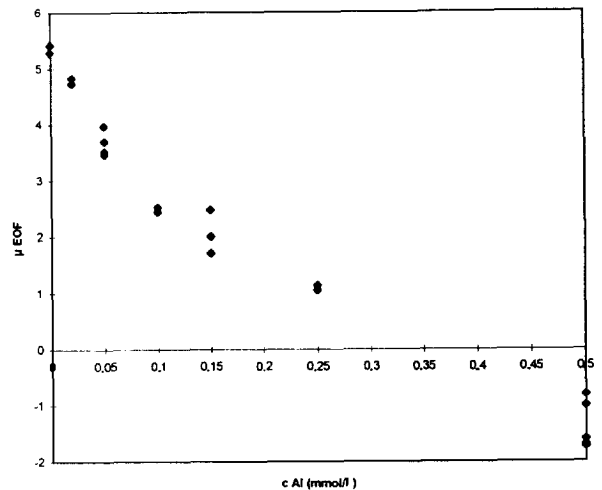


Fig. 3. Dependence of the EOF ( $\mu$  in  $10^{-8} \text{ V}^{-1} \text{ s}^{-1} \text{ m}^2$ ) on the aluminium-salt concentration, measured in Tris buffer, pH 7.4, 50 mmol/l. Mobility measurements were performed at least in triplicate.

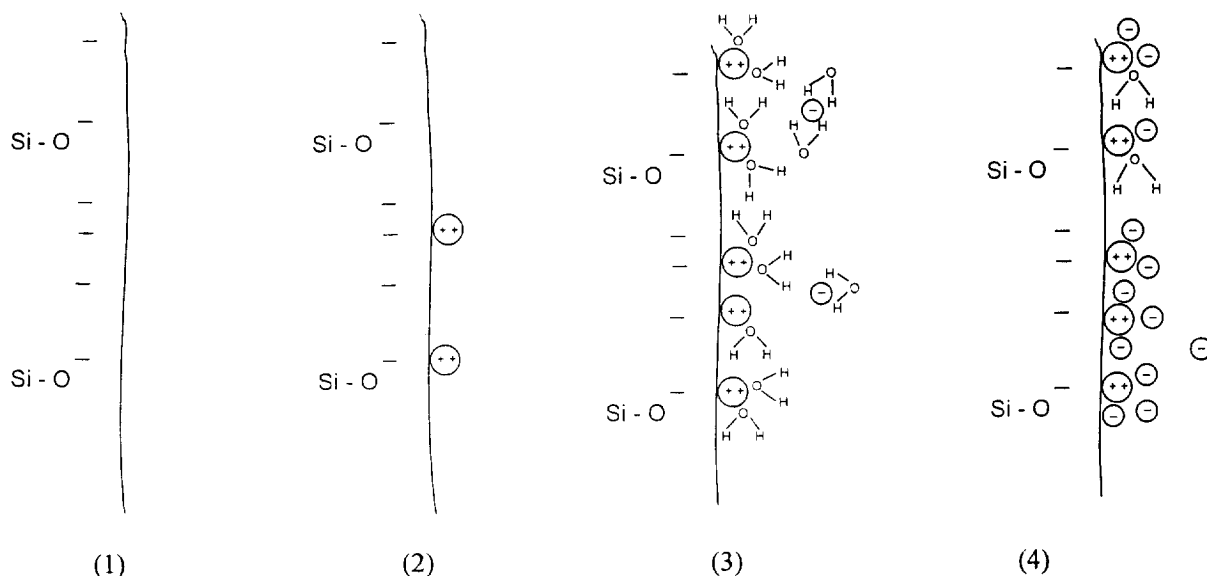


Fig. 4. Interpretation of results shown in Figs. 1–3. For details see text.

anode (iii). If the salt concentration is increased further, no additional adsorption of cations takes place, because all the binding sites are already saturated. The EOF towards the anode is not increased further. On the contrary, at high concentrations the anions displace the solvating water molecules on the surface of the cations [2,16]. A salt layer begins to form on the capillary surface. The surface charge and the EOF decrease again (iv).

The shape of the curves obtained with different metal salts depends on their affinity to the silica surface and the solubility of the cations.

### 3.3. Comparison with earlier investigations

Many investigations have analysed the dependence of the zeta potential on the concentration of various metal salts. The results obtained here are in agreement with earlier measurements, concerning the influence of aluminium and barium concentrations on the zeta potential of different glass surfaces ([17,18]; Table 1). Thus, the behaviour of other metal salts in CE can be predicted from older studies as well. Data about lithium ([14], p. 405), sodium [14,17], potassium, silver, magnesium, calcium, strontium, copper,

lead, mercurium, uranium, lanthanum, cerium and thorium salts are available ([14,17–19]; Table 1). However, significant effects of alkaline ions were only observed at high concentrations, and only the influence of potassium ions was investigated at these relevant concentrations.

The precision can be estimated by comparing data from different authors about the same ion. There are significant deviations, probably caused by different pH values, impurities of the reagents, different instrumentation to measure streaming potentials, heterogeneous glass materials, different capillary histories and equilibration statuses. Nevertheless, the concentration range in which different metal ions may be useful to control the EOF can be estimated.

The expected EOF mobility,  $\mu_{\text{EOF}}$ , can be simply calculated from the  $\zeta$  potential by:

$$\mu_{\text{EOF}} = -\frac{\epsilon\zeta}{\eta} \quad (6)$$

Assuming a permittivity  $\epsilon$  of  $7.08 \cdot 10^{-10} \text{ A s V}^{-1} \text{ m}^{-1}$  and a viscosity  $\eta$  of  $1 \cdot 10^{-3} \text{ N m}^{-2} \text{ s}$  for the electrolyte,  $7.08 \cdot 10^{-8} \text{ V}^{-1} \text{ s}^{-1} \text{ m}^2$  corresponds to a  $\zeta$  potential of 100 mV [20]. The data of Figs. 1–3 were incorporated into Table 1 using this relationship.

Table 1

The influence of different metal ions on the  $\zeta$  potential depending on the ion concentration  $c$ . Data of earlier publications are compared with the data shown in Figs. 1–3

Ion	Investigated $c$ range (mmol/l)	$\zeta$ potential range (mV) observed	$c$ (mmol/l) where $\zeta$ becomes 0 mV	$c$ (mmol/l) causing half of the maximal effect	$c$ (mmol/l) causing the maximal effect	Ref.
K <sup>+</sup>	0.01–1	–140–110		0.16	1	[14], p. 405 <sup>a</sup>
K <sup>+</sup>	0.05–10	–5.3––1.8 <sup>b</sup>		0.95	10	[14], p. 538 <sup>b</sup>
K <sup>+</sup>	0–1	–350––4			1	[18] <sup>a</sup>
K <sup>+</sup>	0–100	<sup>c</sup>	100	0.27	100	[17] <sup>c,d</sup>
K <sup>+</sup>	0–0.39	<sup>c</sup>		0.14	0.39	[17] <sup>c,e</sup>
Ag <sup>+</sup>	0–40	<sup>c</sup>	40	0.047	40	[17] <sup>c,d</sup>
Mg <sup>2+</sup>	0–18	–53––31		9	18	[14], p. 405 <sup>a</sup>
Ca <sup>2+</sup>	0–18	–53––25		7	18	[14], p. 405 <sup>a</sup>
Sr <sup>2+</sup>	0–18	–53––22		7	18	[14], p. 405 <sup>a</sup>
Ba <sup>2+</sup>	0–18	–53––20		6	18	[14], p. 405 <sup>a,f</sup>
Ba <sup>2+</sup>	10 <sup>–3</sup> –10	–115–0	10	0.33	10	[14], p. 405 <sup>a,f</sup>
Ba <sup>2+</sup>	0.02–10	–4.7––1.0 <sup>b</sup>		0.78	10	[14], p. 538 <sup>b</sup>
Ba <sup>2+</sup>	0.01–1	–139––1.1			1	[18] <sup>a</sup>
Ba <sup>2+</sup>	0–250	–60–+7 <sup>g</sup>	>100	ca. 50	250	Fig. 2
Hg <sup>2+</sup>	0–0.03	<sup>c</sup>		3.8 · 10 <sup>–3</sup>	0.01	[17] <sup>c,d,h</sup>
Zn <sup>2+</sup>	0–60	–66–+31 <sup>g</sup>	3	1.5	25	Fig. 1
UO <sub>2</sub> <sup>2+</sup>	0–1	<sup>c</sup>	1	0.05	1	[17] <sup>c,d</sup>
Pb <sup>2+</sup>	5 · 10 <sup>–3</sup> –5					[19] <sup>i</sup>
Cu <sup>2+</sup>	0.13–10					[19] <sup>i</sup>
Al <sup>3+</sup>	0–0.5	–350–+1.4	8 · 10 <sup>–4</sup>		3 · 10 <sup>–3</sup>	[18] <sup>a</sup>
Al <sup>3+</sup>	0–0.5	–75–+24 <sup>g</sup>	0.35	0.2	0.5	Fig. 3
Al <sup>3+</sup>	0–0.1	<sup>c</sup>	0.1	4.5 · 10 <sup>–3</sup>	0.1	[17] <sup>c,d</sup>
Al <sup>3+</sup>	0–1.6 × 10 <sup>–3</sup>	<sup>c</sup>	1.6 · 10 <sup>–3</sup>	2.4 · 10 <sup>–4</sup>	1.6 · 10 <sup>–3</sup>	[17] <sup>c,e</sup>
La <sup>3+</sup>	2 × 10 <sup>–3</sup> –10	–5.2–+0.2 <sup>b</sup>	8.7	0.18	10	[14], p. 538 <sup>b</sup>
La <sup>3+</sup>	0.01–1	–30–+20	0.25	0.1	1	[14], p. 405 <sup>a</sup>
Ce <sup>3+</sup>	0–0.44	<sup>c</sup>		8.6 · 10 <sup>–3</sup>	0.44	[17] <sup>c,d</sup>
Th <sup>4+</sup>	10 <sup>–3</sup> –1	+45–+160		2 · 10 <sup>–3</sup>	0.1	[14], p. 405 <sup>a</sup>
Th <sup>4+</sup>	0–0.01	<sup>c</sup>	0.01	2.7 · 10 <sup>–3</sup>	0.01	[17] <sup>c,d</sup>
Th <sup>4+</sup>	0–3.8 · 10 <sup>–3</sup>	<sup>c</sup>	1.9 · 10 <sup>–3</sup>	4.4 · 10 <sup>–4</sup>	3.8 · 10 <sup>–3</sup>	[17] <sup>c,e</sup>

<sup>a</sup> Chlorides were used;  $\zeta$  potential obtained by measuring streaming potentials. Type of the glass capillary not specified, nor pH of solutions.

<sup>b</sup>  $\zeta$  potential of SiO<sub>2</sub> gel; nitrates were used. pH of solutions not specified.

<sup>c</sup> The absolute values of the  $\zeta$  potential were not determined. However, the given data can be considered as proportional to the  $\zeta$  potential.

<sup>d</sup> Chlorides, nitrates and sulphates were used; type of the glass capillary not specified, nor pH of solutions.

<sup>e</sup> Chlorides, nitrates and sulfates were used; capillary from fused-silica, pH of solutions not specified.

<sup>f</sup> Different measurement series.

<sup>g</sup> 7.08 · 10<sup>–8</sup> V<sup>–1</sup> s<sup>–1</sup> m<sup>2</sup> corresponds to a  $\zeta$  potential of 100 mV [20]; compare text and Eq. 6.

<sup>h</sup> The author states that the measurements with mercurium “were not performed with the same care like the others”. Nevertheless “it seems to be clear that there are strong effects caused by mercurium ions.”

<sup>i</sup> Cited in [14], p. 405.

Positively charged surfactants provide another possibility to control the EOF and to reverse the charge of silica surfaces [3,4]. The properties of alkylamines [21] and alkyltrimethylammonium salts [22,23] were studied systematically. These

cations are monovalent. Charge reversal becomes possible by the formation of hemimicelles at the silica surface.

In most cases these cationic surfactants also influence the electrophoretic mobility considera-

bly [22]. The hemimicelles offer additional binding sites for the adsorption of analytes. Moreover, the formation of hemimicelles depends strongly on the pH. Therefore, control of the EOF using cationic surfactants is less attractive. The use of metal ions may provide a good alternative for a number of applications.

### 3.4. Reproducibility of the EOF

The experiments were reproducible when zinc or barium salt was added to the buffer. The %C.V. of the migration time was always below 2% and typically below 1%. The investigations of zinc salt were repeated on two capillaries of the same batch. The results were nearly identical in both series. Therefore, these metal ions are useful to control the EOF for method development. The characteristics of barium-salt-containing buffers were studied more thoroughly at different pH values.

The deviations were much higher (>10% C.V.) when aluminium salt was added. The EOF decreased from run to run at all concentrations under investigation, independent of the EOF direction. It is possible that aluminium, which is a Lewis acid, catalyses the hydrolysis of the silica glass. This would lead to a gel-like structure on the surface, and thus the EOF would slow down (compare [24]).

### 3.5. Control of the EOF by addition of barium salts to buffers of various pH

Next, control of the EOF was investigated regarding reproducibility and influences of pH and of other ions. Four buffer pH values were selected between 5 and 9.2. Different amounts of barium salts were added. The EOF depends on the total buffer ion concentration. Thus, the buffers were adjusted using sodium salts to achieve comparable experimental conditions. The molarity of the anions was kept constant within a series, as well as the total amount of positive charge from metal ions: to vary the experimental conditions, each mmol of  $\text{Ba}^{2+}$  was replaced by 2 mmol of  $\text{Na}^+$ . In this way the

conductivity remained very similar within a series. However, the ionic strength could not be kept constant at the same time.

Different total ion concentrations at the same pH were also tested, and the influence of the capillary was investigated on two capillaries from different batches (Table 2). If barium ions were the only metal ionic compound of the buffer (%Ba = 100%), no EOF was detectable within 1 h ( $t_{\text{EOF}} > 60$  min, thus  $\mu_{\text{EOF}} < 2 \cdot 10^{-9} \text{ V}^{-1} \text{ s}^{-1} \text{ m}^2$ ), except at pH 5.05. Here a signal of the EOF marker occurred after 8 min, corresponding to a mobility of  $1.17 \cdot 10^{-8} \text{ V}^{-1} \text{ s}^{-1} \text{ m}^2$ .

The mobilities given in Table 2 are the averages of three measurements. The maximal deviations from this average were typically less than 2%. Exceptions are given as footnotes.

Good reproducibility was achieved in most cases. However, in some cases it was poor, especially when borate buffers were investigated. Secondary effects may result when borate ions bind to the surface. Generally, an equilibration time of 30 min was sufficient for measurements of the EOF in Tris buffer. This equilibration time had to be increased with aluminium-containing buffers. The reproducibility of some of the experiments could be improved by longer equilibration times. In general, more information is required about equilibration processes and equilibration times to obtain sufficient reproducibility.

However, a number of general conclusions are possible. No charge reversal was observed at barium concentrations up to 32 mmol/l. After this concentration, though, the EOF mobility dropped to almost zero, independently of the buffer pH. The relationship between barium concentration and EOF mobility depends decisively on the buffer pH. The mobilities obtained in Tris buffer (Fig. 2) and imidazole buffer (Table 2, pH 6.95) are very similar.

The effect of the barium ions is more pronounced at higher pH. Here the EOF is faster in the absence of barium salts, but it also decreases more sharply if barium salts are added. At rather low barium concentrations (2 mmol/l), the EOF is slower in alkaline than in neutral or acidic buffers.

The EOF can be controlled by mixtures of

Table 2

The dependence of the EOF on the buffer pH and the barium ion concentration, given as percentage of positive charge from metal ions contributed by barium [ $\%Ba = 100\% \cdot 2c_{Ba^{2+}} / (2c_{Ba^{2+}} + c_{Na^+})$ ]

%Ba	$\mu_{EOF}$ ( $10^{-8}$ V <sup>-1</sup> s <sup>-1</sup> m <sup>2</sup> ) at pH					
	5.05 <sup>a</sup>	6.95 <sup>b</sup>	8.5 <sup>d</sup>	8.5 <sup>e</sup>	9.2 <sup>g,h</sup>	9.2 <sup>g,i</sup>
0	3.45	4.55	6.20	5.50	6.20	6.38
6.25	4.03	3.82	4.70	3.72	2.12	4.13
12.5	3.58	3.70	3.70	2.82	2.95	1.70 <sup>j</sup>
25	3.12	3.15	2.92	2.20	1.80	1.23 <sup>j</sup>
50	2.53	2.78	2.07	0.93 <sup>f</sup>	0.57	≈0.6
62.5	2.41	3.45 <sup>c</sup>	1.17	1.00	0.75	0.63 <sup>j</sup>

EOF marker: acetanilide, effective column length: 30 cm, overall length: 37 cm; separation voltage: 20 kV. Typical total metal ion concentration corresponding to Me<sup>+</sup>: 32 mmol/l (compare Experimental).

<sup>a</sup> Acetate buffer, 48 mmol/l; average of 2nd and 3rd injection.

<sup>b</sup> Imidazole buffer, 40 mmol/l; average of 2nd and 3rd injection.

<sup>c</sup> One injection only.

<sup>d</sup> Borate buffer, 60 mmol/l ( $c_{Me^+} = 10.48$  mmol/l); average of 3 injections.

<sup>e</sup> Borate buffer, 171.7 mmol/l ( $c_{Me^+} = 30$  mmol/l); average of 3 injections.

<sup>f</sup> Maximal deviations of 4% from measurement to measurement.

<sup>g</sup> Borate buffer, 60 mmol/l; two different capillary batches.

<sup>h</sup> Average of 3 injections, maximal deviation: 8%.

<sup>i</sup> Average of 2nd and 3rd injection.

<sup>j</sup> Average of 2nd and 3rd injection, maximal deviation: 6.5%.

barium and sodium salts. The competition of the ions at the surface decreases the effect of the barium ions on the surface charge. A similar effect, the competition of cationic surfactants and sodium ions, has been observed by other authors [21]. The EOF depends more on the absolute barium concentration than on the ratio of sodium and barium ions.

#### 4. Conclusions

The dependence of the zeta potential on metal-salt concentrations has become clear. The effects are in principle similar for different salts, but the concentration ranges in which these effects are observed differ. The affinity to the silica surface depends strongly on the type of metal ion.

The results presented here are in good agreement with earlier findings. Thus, information about other useful metal salts for CE can be easily adapted from earlier publications.

The possibility of using barium salts as buffer

additives was demonstrated. The EOF was measured at different pH values.

Barium salts are effective reagents to suppress the EOF even at high pH values.

#### Acknowledgements

We thank Mr. G. Walter for the construction of the laboratory-built instrument. We are grateful to Beckman Instruments, especially to Dr. C. Nutzhorn, for supporting these measurements.

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