Journal of Chromatography, 260 (1983) 241–254
Elsevier Science Publishers B.V., Amsterdam — Printed in the Netherlands

CHROM. 15,654

EFFECT OF ELECTROOSMOSIS ON DETECTION IN ISOTACHOPHORESIS

J. C. REIJENGA*, G. V. A. ABEN, Th. P. E. M. VERHEGGEN and F. M. EVERAERTS
Eindhoven University of Technology, Laboratory of Instrumental Analysis, P.O. Box 513, 5600 MB Eindhoven (The Netherlands)

(Received December 28th, 1982)

SUMMARY

The effect of electroosmosis on the relative sharpness of the zone boundaries and hence the detection limit in isotachophoresis is discussed. A high concentration and a low pH of the leading electrolyte were found to be favourable. The magnitude and sign of the ζ-potential of the capillary wall was measured by streaming potential determinations as a function of concentration, pH and additives. Poly(vinyl alcohol), hydroxyethylcellulose and hydroxypropylmethylcellulose decrease the ζ-potential sufficiently, whereas cationic detergents reverse its sign and thus the direction of the electroosmotic flow. Both were found to be favourable in anionic separation. Fused-silica capillaries and the effect of methanol were also investigated.

INTRODUCTION

Electroosmosis is one of the main electrokinetic disturbances in isotachophoresis, whether a high-viscosity, a high-density or a capillary system is used as a stabilizing medium. As the use of capillary systems is most promising from an analytical point of view, consideration of electroosmotic effects in this paper is limited to those in capillary systems. In this case the dimensions of the separation compartment are relatively well defined. Most equations, including those used in ζ-potential measurements, can easily be modified to either a gel system with a well defined pore size distribution or a capillary system with a rectangular cross-section. Electroosmosis is strongly related to the ζ-potential of the capillary material in which it occurs. Electroosmosis often leads to less sharp zone boundaries, which increases the minimal detectable amount considerably. Moreover, electroosmosis generally increases at lower electrolyte concentrations, which are preferred in trace analysis in normal[1,2] or in coupled column systems[3,4]. The effects of (non)-ionic surfactants and polymers, concentration and pH of the electrolyte on the ζ-potential have been measured and related to the relative sharpness of the zone boundaries in the isotachophoretic steady state[5].

Another disturbing factor in capillary systems is the surface conductance. Therefore, both electroosmosis and surface conductance will be estimated in capillaries under operational electrophoretic conditions. Sharp and straight zone boun-

0021-9673/83/$03.00

daries in the isotachophoretic steady state are also disturbed by axial and radial temperature differences[6], especially at higher field strengths and with large capillary diameters. These effects were not dealt with in this investigation.

ELECTROOSMOSIS

The order of magnitude of the thickness of the electrical double layer in capillary systems at commonly used electrolyte concentrations (0.001–0.01 *N*), is much smaller than the inner diameter of the capillary. Therefore, as a first approximation, the capillary wall can be considered to be flat. Assume a volume element of liquid of unit surface area parallel to the liquid–solid interface (see Fig. 1) and thickness dx perpendicular to the wall. In an electric field E parallel to the wall the electric force on the volume element is $\varrho E\mathrm{d}x$, where ϱ is the charge density. In the stationary state this force is equal to the frictional force $-\mathrm{d}(\eta\partial v/\partial x)$, where η is the viscosity and v the linear velocity of the liquid. Thus

$$\varrho E\mathrm{d}x + \mathrm{d}(\eta\partial v/\partial x) = 0 \tag{1}$$

With the assumption that E is independent of x and that for the viscosity of the liquid near the wall an average value of $\bar{\eta}$ is taken, integration between 0 and ∞ gives

$$E\int_0^\infty \varrho x\mathrm{d}x + \bar{\eta}\int_0^\infty x\mathrm{d}(\partial v/\partial x) = 0 \tag{2}$$

with

$$\tau = \int_0^\infty \varrho x\,\mathrm{d}x \tag{3}$$

we have

$$E\tau = [\eta x\partial v/\partial x]_0^\infty - \bar{\eta}\int_0^\infty \partial v/\partial x\mathrm{d}x \tag{4}$$

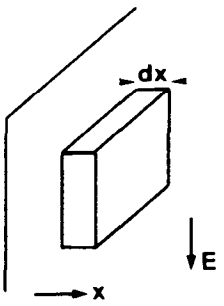

Fig. 1. Choice of coordinates when considering an element of volume of thickness dx near a flat solid–liquid interface parallel to a potential gradient E.

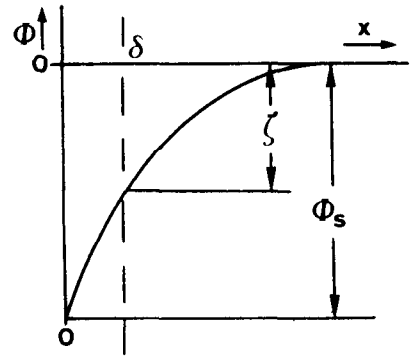

Fig. 2. Potential Φ *versus* distance x from a flat solid–liquid interface with double layer thickness δ.

When $v = 0$ at $x = 0$ and $v = v_{eo}$ (the electroosmotic velocity) and $\partial v/\partial x = 0$ at $x = \infty$, eqn. 5 is obtained:

$$E\tau = \bar{\eta} v_{eo} \tag{5}$$

The relationship between τ and ζ, the ζ-potential at $x = 0$, is obtained from Poisson's equation in a one-dimensional form:

$$\partial^2 \Phi/\partial x^2 = -\rho/\varepsilon \tag{6}$$

where Φ is the potential (see Fig. 2).

Substitution of ϱ in eqn. 3 gives

$$\tau = -\varepsilon \int_0^\infty \partial^2 \Phi/\partial x^2 \cdot x \mathrm{d}x \tag{7}$$

where it is assumed that ε does not depend on x. Integration between δ and ∞ (Fig. 2) gives

$$\tau = -\varepsilon\zeta \tag{8}$$

Substitution of eqn. 8 into eqn. 5 now gives the Helmholtz–Smoluchovsky equation:

$$v_{eo} = -\varepsilon\zeta E/\bar{\eta} \tag{9}$$

Calculating the order of magnitude of the linear electroosmotic velocity (v_{eo}) and comparing it with the isotachophoretic velocity (v_{iso}), a good indication is obtained of the significance of the electroosmotic disturbance during detection. The isotachophoretic steady-state equation (eqn. 10):

$$v_{iso} = mE \tag{10}$$

where m is the effective mobility, combined with eqn. 9, yields

$$v_{eo}/v_{iso} = -\varepsilon\zeta/\bar{\eta}\mathrm{m} \tag{11}$$

The order of magnitude of the ζ-potential in, *e.g.*, glass in 0.001 *M* potassium chloride solution is 0.1 *V*[7,8]. With an anionic mobility (Cl^-) of $m = -76 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}, a dielectric constant of $708 \cdot 10^{-12}$ F m^{-1} and a viscosity $\bar{\eta} = 10^{-3}$ N m^{-2} sec, eqn. 11 gives

$$v_{eo}/v_{iso} \approx -1$$

This means that the electroosmotic velocity cannot be neglected with respect to the isotachophoretic velocity, regardless of the self-correcting properties of the isotachophoretic steady state.

SURFACE CONDUCTANCE

When applying an electric field over a liquid in a capillary, surface conductance has to be considered. Surface conductance of an insulator is caused by the fact that the charge density and concentration near the electric double layer differ from those of the bulk solution. The total conductance of a liquid in a capillary is therefore the sum of both. The total resistance R of a capillary of unit length and radius r_0 is given by

$$1/R = \eta r_0^2 \kappa \, (1 + 2\kappa^\sigma / r_0 \kappa) \tag{12}$$

where κ is the bulk conductance and κ^σ is the surface conductance per unit of capillary circumference. When applying an electric field E across a capillary of unit length, the total current due to surface conductance is

$$i = \kappa^\sigma E = \int_0^\infty \varrho v \, \mathrm{d}x \tag{13}$$

Now, on combining eqns. 1 and 13, with $v = 0$ at $x = 0$ and $\partial v/\partial x = 0$ at $x = \infty$, rearrangement gives

$$\kappa^\sigma = \bar{\eta}/E^2 \int_0^\infty (\partial v/\partial x)^2 \, \mathrm{d}x \tag{14}$$

If we assume that $\partial v/\partial x$ is approximately equal to v_{eo}/δ in the diffuse part of the double layer and integration is limited to the double layer thickness δ, we obtain

$$\kappa^\sigma = \bar{\eta} v_{eo}^2 / E^2 \delta \tag{15}$$

Using eqn. 9 for v_{eo}, this yields

$$\kappa^\sigma = (\varepsilon\zeta)^2 / \bar{\eta}\delta \tag{16}$$

The thickness of the electric double layer δ can be approximated with the Debye–Hückel theory. For relatively dilute solutions of a monovalent electrolyte at 25°C, it may be approximated by:

$$1/\delta \, (\mathrm{m}^{-1}) = 3.29 \cdot 10^9 \sqrt{c} \tag{17}$$

where c is the molarity of the solution. At an electrolyte concentration of 0.001 M, δ = 10 nm. From this it can be concluded that the assumption that the capillary wall is a flat solid–liquid interface was justified. In eqn. 12, $2\kappa^\sigma/r_0\kappa$ is the relative contribution of the surface conductance. Substitution of κ^σ from eqn. 16 gives

$$2\kappa^\sigma / r_0 \kappa = 2\varepsilon^2 \zeta / \bar{\eta} \delta r_0 \kappa \tag{18}$$

For $\zeta = -0.1$ V, $\bar{\mu} = 10^{-3}$ N m^{-2} sec, $\delta = 3.3$ nm at $c = 0.01$ M, $r_0 = 10^{-4}$ m and $\kappa = 0.1$ S m^{-1}, eqn. 18 gives:

$$2\kappa^{\sigma}/r_0\kappa = 3 \cdot 10^{-4}$$

The relative contribution of surface conductance can therefore be neglected under the conditions normally used in isotachophoretic analyses[1]. For very low electrolyte concentrations a small contribution is expected.

ZETA POTENTIAL

As can be seen from the Helmholtz–Smoluchovsky equation (eqn. 9), the ζ-potential of the solid–liquid interface will play an important role in the electroosmotic disturbances during the isotachophoretic analyses. Obviously, electroosmosis can be decreased by increasing the viscosity of the liquid, but according to eqn. 9 this effect is lost at increasing field strength, which is inevitable in the consecutive zones between leading to terminator. Measurement of the ζ-potential of the capillary wall, under operational conditions, therefore gives valuable information for minimizing the effect of electroosmosis. The ζ-potential was measured with either the streaming current or the streaming potential across a capillary, through which an electrolyte solution is streaming under a well defined pressure gradient. If a laminar liquid flow can be considered, due to a pressure gradient P in a capillary with radius r_0 and length l, the Poiseuille velocity profile is given by

$$v(r) = P(r_0^2 - r^2)/4\bar{\eta}l \tag{19}$$

At close range, the capillary wall can be approximated by a flat wall (see eqn. 17). Changing coordinates from r to x with $x = r_0 - r$, the velocity near the wall can be assumed to be equal to

$$v(x) = Pr_0/2\bar{\eta}lx \tag{20}$$

Now, if no external potential is applied, the streaming current per unit wall circumference is equal to the current generated by surface conductance. On substituting eqn. 20 into eqn. 13, we obtain

$$i = Pr_0/2\,\bar{\eta}l \int_0^{\infty} \varrho x \,\mathrm{d}x \tag{21}$$

The integral is now substituted by means of eqns. 3 and 8. The total streaming current in a capillary of wall circumference $2\pi r_0$ is given by $I = 2\pi r_0 i$, so that the equation

$$I = -Pr_0^2\epsilon\pi\zeta/\bar{\eta}l \tag{22}$$

can be derived. This is a direct relationship between the measured streaming current I and the ζ-potential as a function of the radius of the capillary and the pressure drop per unit length P/l. The electrical resistance of the capillary generates a potential drop between the ends of the capillary: the streaming potential, E_{st}. According to Ohms law,

$$E_{st} = -IR \tag{23}$$

The capillary resistance R is given by

$$R = 1/\pi r_0^2 \kappa \tag{24}$$

under the assumption that the surface conductance can be neglected with respect to the total capillary conductance in eqn. 12. If eqns. 22 and 24 are substituted into eqn. 23, eqn. 25 is derived:

$$E_{st} = P\varepsilon\zeta/\bar{\eta}\kappa \tag{25}$$

This equation, from which the capillary dimensions have vanished, gives ζ directly from the streaming potential E_{st} measured as a function of pressure drop P and specific conductance κ of the solution.

EXPERIMENTAL

In selecting the experimental conditions for the determination of the ζ-potential, attention should be paid to the proper choice of dimensions. From eqn. 25 it can be concluded that a high pressure drop will increase the sensitivity. The porosity of the PTFE capillary material limited the pressure to 10^5 N m^{-2}. Care should be taken that the flow remains laminar. A capillary length of *ca.* 150 mm was chosen, with I.D. 0.2 mm. Moreover measurements in a capillary of fused silica (0.22 mm I.D.) were performed, although these capillaries are seldom used in isotachophoresis experiments. The capillary was mounted between two electrolyte-filled vessels (see Fig. 3), in each of which a silver–silver chloride electrode of dimensions 1 × 40 mm was mounted. By means of a series of valves, a 10^5 N m^{-2} nitrogen pressure was applied on each of the electrode vessels alternatively at 15-sec intervals. In streaming potential measurements, the electrodes were directly connected with a high-impedance (10^{12} Ω) mV/pH meter, Type PW 9414 (Philips, Eindhoven, The Netherlands). The output was recorded with a potentiometric recorder, Type BD 41 (Kipp, Delft, The Netherlands). The experiments were continued until a stable signal level was achieved. The viscosity of the electrolyte was measured in the same equipment by flow measurement through the capillary at the same operating pressure of 10^5 N m^{-2}. The conductivity was measured with a digital conductivity meter, Type CDM 83 (Radiometer, Copenhagen, Denmark).

The isotachophoretic experiments were carried out in home-made equipment as described by Everaerts *et al.*[1]. The driving current was 70 μA in a PTFE capillary of 0.45 mm I.D. The sample injected was a thirteen-component standard mixture of anions with a concentration of *ca.* 0.0005 *M* each. The injection volume was 3 μl.

Water was taken from a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.), with a specific resistance of $>10^7$ Ω cm.

The reagents were all of analytical-reagent grade and purchased from either Merck (Darmstadt, G.F.R.) or Sigma (St. Louis, MO, U.S.A.). The operational systems are listed in Table I and the additives to the leading electrolyte investigated are listed in Table II.

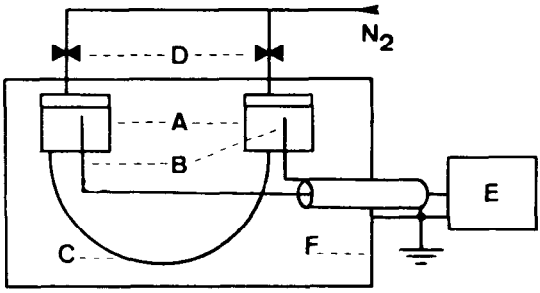

Fig. 3. Equipment for measuring streaming potentials of capillaries. A = Electrolyte vessels; B = Ag–AgCl electrodes; C = capillary; D = magnetic valves for applying nitrogen pressure; E = high-impedance pH/mV meter; F = Faraday cage.

The relative zone-sharpness (σ) was measured by means of the UV signal, defined[5] as the ratio of the theoretical (τ_0) and the actual residence time (τ) of the zone boundary in the detector cell. The former can be calculated from

$$\tau_0 = l/v_{iso} \tag{26}$$

where l is the length of the detector cell along the capillary axis and v_{iso} the isotachophoretic velocity. The actual residence time τ is measured from the UV signal. This signal was sampled at 16 Hz with a Puzzle microprocessor system (E. Steiner, Vienna, Austria) equipped with 12-bit analogue-to-digital (ADC) and digital-to-analogue convertors (DAC), differentiated and plotted together with the original UV signal. Of the differentiated signal, the width of the peaks corresponding to seven "UV–non-UV" zone transitions in the isotachopherogram of the standard mixture was measured, and the τ and σ values were calculated.

After each experiment the capillary was thoroughly rinsed with at least 500 ml of pure water, as a considerable memory effect was observed with some of the additives. Therefore, between the experiments a control run without additives was performed.

TABLE I

OPERATIONAL SYSTEMS USED IN THE ζ-POTENTIAL AND/OR ZONE SHARPNESS EXPERIMENTS

The additives are listed in Table II.

pH	*Leading ion*	*Concentration (M)*	*Counter ion*
3.0	Chloride	0.01	β-Alanine
3.5	Chloride	0.01	β-Alanine
4.8	Chloride	0.01	Creatinine
6.0	Chloride	0.01	Histidine
8.2	Chloride	0.01	Tris
9.0	Chloride	0.01	Ammediol

TABLE II

ADDITIVES TO THE LEADING ELECTROLYTE

Abbreviation	*Name*	*Concentration*	*Manufacturer*
PVP	Polyvinylpyrrolidone	0.1%	Fluka, Buchs, Switzerland
Triton	Triton X-100	0.1%	Rohm & Haas, Philadelphia, PA, U.S.A.
PVA	Poly(vinyl alcohol) (Mowiol)	0.05%	Hoechst, Frankfurt, G.F.R.
HEC	Hydroxyethylcellulose	0.2%	Polysciences, Warrington, PA, U.S.A.
HPMC	Hydroxypropylmethylcellulose	0.2%	Fluka
CTAB	Cetyltrimethylammonium bromide	0.1 m*M*	Merck, Darmstadt, G.F.R.
Priminox	Priminox-32	0.05%	Rohm & Haas

RESULTS AND DISCUSSION

Effect of pH and concentration

Streaming potential measurements were carried out on both PTFE and fused silica capillaries. The effect of the leading electrolyte pH was investigated with a number of 0.01 *M* chloride buffers (Table I) without additives. The results are shown in Fig. 4.

The reproducibility of the streaming potential measurements was generally not within 10%, but it was adequate to conclude that the ζ-potential decreases significantly at lower pH for both PTFE and fused silica capillaries in a comparable manner. The electroosmotic flow will vary correspondingly, as can be seen from eqn. 9. Moreover, the electroosmotic flow will increase from leading to terminator with increasing field strength E in isotachophoretic experiments (eqn. 9). As will be evident from Fig. 2, an increase in the electrical double layer thickness δ at lower electrolyte concentrations (eqn. 17) will result in a higher ζ-potential. This was verified experimentally with three leading electrolyte buffers of different pH (Table I).

In Fig. 5 the measured ζ-potential is shown as a function of leading concentration and pH. These results indicate that increased electroosmotic disturbances are to be expected at high pH and low concentrations. This is in complete agreement with the observation that less sharp zone boundaries are encountered under these conditions. The limit of detection q_{min} in capillary isotachophoresis is defined[5,9] as

$$q_{min} = \pi r_0^2 l c \tag{27}$$

where r_0 is the capillary radius, l the detector length and c the concentration of the sample ion in the zone. The value of q_{min} would decrease significantly if a lower leading electrolyte concentration could be chosen. One important limiting factor in doing so is the electroosmotic disturbance to be expected. Other limitations are the buffering capacity and disturbances due to H^+ or OH^-.

Effect of additives

The effect of additives to the leading electrolyte on the quality of the isotachopherogram has been previously investigated[1]. Special attention was paid to the influence of these additives on the micro-sensing Pt and Pt–Ir (10%) electrodes of the

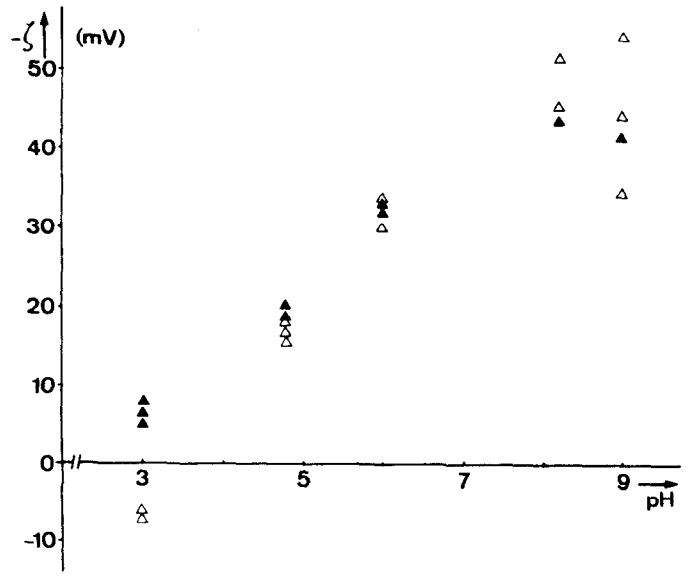

Fig. 4. The ζ-potential of PTFE (△) and fused-silica (▲) capillaries when filled with a 0.01 *M* chloride buffer as a function of pH. For counter ions see Table I.

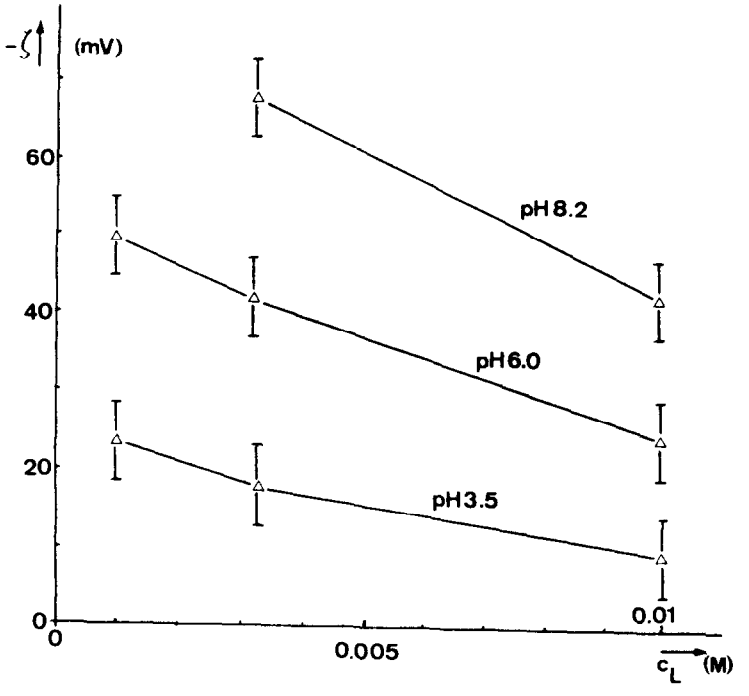

Fig. 5. The ζ-potential of PTFE capillary when filled with chloride buffers of varying concentration and pH. The ζ-intervals indicate estimated 2σ.

conductivity (potential gradient) cell. In order to investigate the mechanism by which these additives decrease electroosmosis, a series of ζ-potential measurements were carried out in PTFE and fused silica capillaries, filled with a pH 6.0 leading electrolyte containing one of the additives listed in Table II. On the basis of the results, summarized in Table III, the additives can be subdivided into two categories:

(a) Those which decrease the ζ-potential to a considerable extent. Of these, Triton X-100 and PVP are least effective, although it may be expected that a higher concentration of any of these additives will increase their effectiveness to a certain extent. An increase in viscosity near the capillary wall is likely, and sometimes the bulk viscosity is also increased, such as with HEC and HPMC.

(b) Those which change the sign of the ζ-potential from negative to positive and thus reverse the direction of the electroosmotic flow. These cationic detergents (CTAB, Priminox) should be added at a sufficiently lower concentration compared with the leading ion concentration, as otherwise a deviation from the ideal isotachophoretic conditions is to be expected. A relatively low concentration, however, is sufficient for the ζ-potential to change sign.

As mentioned earlier, the relative electroosmotic disturbance in isotachophoresis can be described by the ratio of the electroosmotic and the isotachophoretic flow as given by eqn. 11. This is illustrated in Fig. 6, where the above-mentioned ratio is plotted on a logarithmic scale against the effective mobility of the sample ions. It can be seen that the magnitude of the disturbances due to electroosmosis ranges over several decades and that the use of additives is imperative. It should be noted here that, as only the ζ-potential values of the capillary with the leading buffer and the field strengths are considered, variation of the relative electroosmotic disturbance per zone may occur owing to specific properties of the sample ions concerned, resulting from characteristics such as temperature, pH and concentration.

In the experiments mentioned above, and in the derived equations, the possible increase in viscosity near the wall, to be expected with certain additives, is not considered because these data were not available. It will be evident, however, that such an effect will be equally advantageous in reducing electroosmotic disturbances.

TABLE III

ζ-POTENTIAL OF PTFE AND FUSED SILICA CAPILLARIES IN A pH 6.0 BUFFER (TABLE I) WITH DIFFERENT ADDITIVES (TABLE II) AS DETERMINED BY STREAMING POTENTIAL MEASUREMENTS

Additive	κ ($\mu S\ cm^{-1}$)	η (*cp*)	ζ (*mV*)	
			PTFE	*Fused silica*
None	852	1.0	−41	−25
PVP	865	1.0	−3	−4
Triton	860	1.0	−4	−9
PVA	851	1.0	−1	−2
HEC	918	3.1	−1	−1
HPMC	867	4.4	−2	−2
CTAB	870	1.0	+40	+18
Priminox	851	1.0	+21	+14

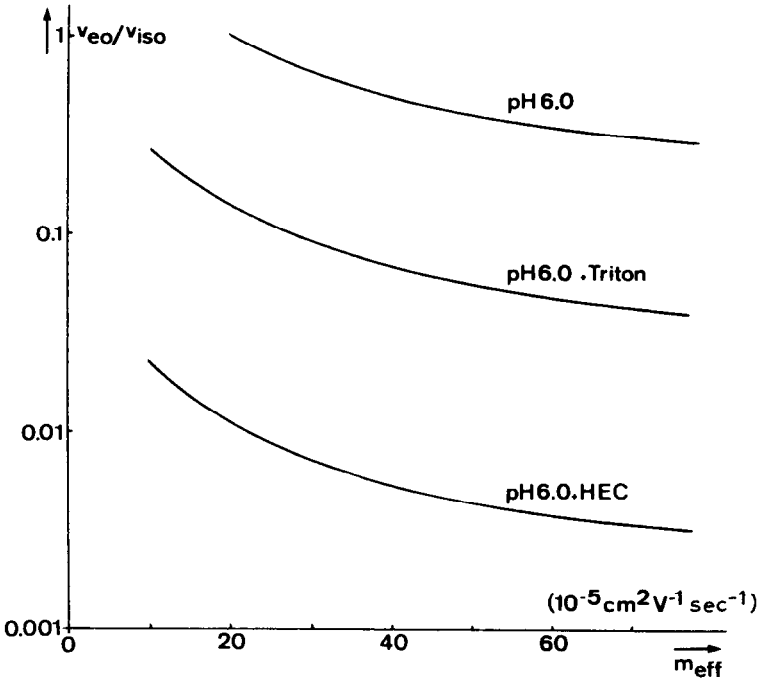

Fig. 6. The relative electroosmotic disturbance v_{eo}/v_{iso} in isotachophoresis, calculated from measured ζ-potential values of the leading electrolyte, obtained in a PTFE capillary as a function of the effective mobility of the sample ion. pH and concentration shift and sample-to-sample differences were not considered.

Zone sharpness

A number of zone sharpness measurements were carried out in order to verify the electroosmotic disturbance semi-quantitatively. Each zone, migrating in the isotachophoretic steady state, is expected to induce its own ζ-potential on to the wall, regardless of the inevitable field strength differences. Therefore, significant zone-to-zone differences of the relative zone sharpness σ are to be expected. For this reason, in the analysis of the pH 6.0 standard mixture of anions, the effect of the additives to the leading electrolyte on seven individual zone transitions was monitored. Fig. 7 shows such a UV trace, where Priminox was added to the leading electrolyte.

The results are summarized in Table IV. Statistical analysis of these data was performed under the assumptions that the minimum detectable relative zone sharpness σ was 0.10 and that the standard deviation of the measurement of each individual zone transition was 0.05σ units. Student's t-test was applied for a 0.95 confidence interval, and yielded the following results for the comparison of the different additives: (a) the use of PVP and Triton X-100 will improve the zone sharpness; (b) PVA, HEC, HPMC, CTAB and Priminox are all more effective than Triton; and (c) CTAB is just as good as PVA, HEC, HPMC and Priminox.

From these results, and those of the ζ-potential measurements (Table III), it can be concluded that the relative zone sharpness is improved by suitably changing the ζ-potential of the capillary wall to a value approximately equal to or above zero.

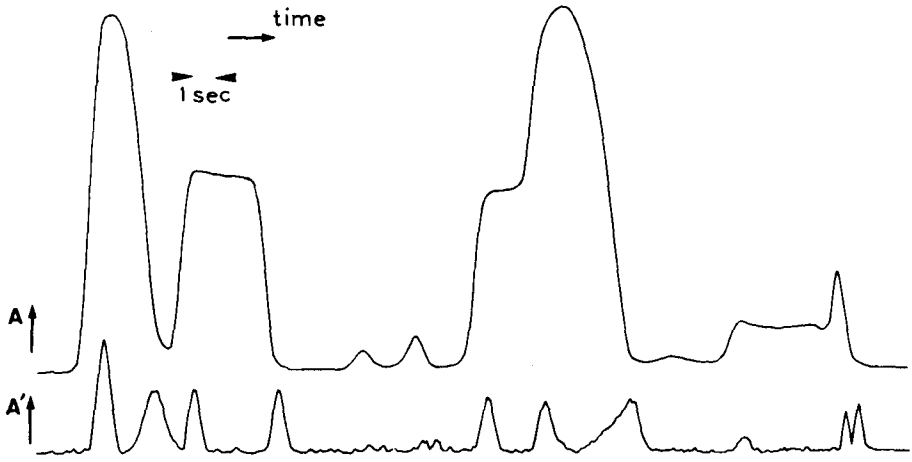

Fig. 7. UV trace (A) and its differential (A') of the analysis of a thirteen-component standard mixture of anions, in a 0.01 *M* chloride buffer at pH 6.0 and with 0.05% Priminox as an additive.

If positive, the magnitude of the ζ-potential is not of importance, at least for anionic separations. For cationic separations, no additives are generally needed, as the electroosmotic flow is in the same direction as the isotachophoretic velocity[5]. If in a cationic separation the ζ-potential of the capillary wall is made more negative, *e.g.*, by the addition of sodium dodecylsulphate, the relative sharpness of the zone boundaries is not significantly increased or decreased.

The choice of additive will depend to a large extent on the sample ions to be separated. The possibility of an interaction between the additive and the sample ions should always be checked, but the same applies to the counter ion. CTAB, for instance, is not recommended for protein determinations, as it binds strongly to the proteins, the effective mobility of which was found to decrease. It may, however, be

TABLE IV

RELATIVE ZONE SHARPNESS σ OF SEVEN ZONE TRANSITIONS IN A pH 6.0 LEADING ELECTROLYTE WITH DIFFERENT ADDITIVES

The isotachophoretic experiments were carried out in a PTFE capillary.

Additive	*E* (V cm^{-1})*							*ζ (mV)*
	116	*121*	*129*	*142*	*188*	*201*	*216*	
None	0.44	0.32	0.42	0.36			0.15	−41
PVP	0.45	0.35	0.54	0.39	0.57	0.59	0.14	−3
Triton	0.48	0.38	0.54	0.51	0.35	0.30	0.17	−4
PVA	0.62	0.51	0.78	0.72	0.66	0.66	0.72	−1
HEC	0.56	0.47	0.66	0.66	0.51	0.38	0.29	−1
HPMC	0.60	0.45	0.69	0.69	0.62	0.60	0.56	−2
CTAB	0.66	0.47	0.72	0.62	0.62	0.62	0.44	+40
Priminox	0.59	0.36	0.69	0.66	0.59	0.53	0.27	+21

* The *E* values refer to the average electrical field strength at the respective zone boundaries.

advantageous when analysing low-molecular-weight substances at a high pH. Another important reason for the use of additives in isotachophoresis is to suppress electrode reactions at the microsensing electrodes of the conductivity or potential gradient detector. Cationic detergents such as CTAB did not entirely inhibit these reactions; a slight drift of some of the isotachophoretic zones, as detected by the conductivity detector, was observed.

Therefore, in practice a combination of additives will often be used. We found a favourable effect in the combination of CTAB to decrease electroosmotic disturbances and HEC to suppress electrode reactions. The concentrations of both additives can be lowered by a factor of up to 10, to decrease the amount of impurities, without seriously changing their effectiveness.

Non-aqueous isotachophoresis

The additional possibility of performing isotachophoresis in mixtures of water and an organic modifier (methanol, acetone) broadens the scope of the technique. Electroosmotic disturbances are to be expected in this instance also (eqns. 9 and 11). Because of generally lower effective mobilities, higher field strengths are expected, whereas on the other hand lower dielectric constants are encountered. The ζ-potentials of PTFE and fused silica were measured with a pH 6.0 buffer containing various percentages of methanol. From Fig. 8 it can be seen that in this instance electroosmosis can be expected to be less, and PTFE is evidently better than fused silica. A higher viscosity and a lower dielectric constant decrease the electroosmotic flow even more than is apparent from Fig. 8, as is shown in Table V. The action of additives to those leading electrolyte buffers containing, *e.g.*, methanol proceeds in a comparable way: PVA decreases the ζ-potential of PTFE to *ca.* -1 mV and CTAB yields a positive ζ-potential.

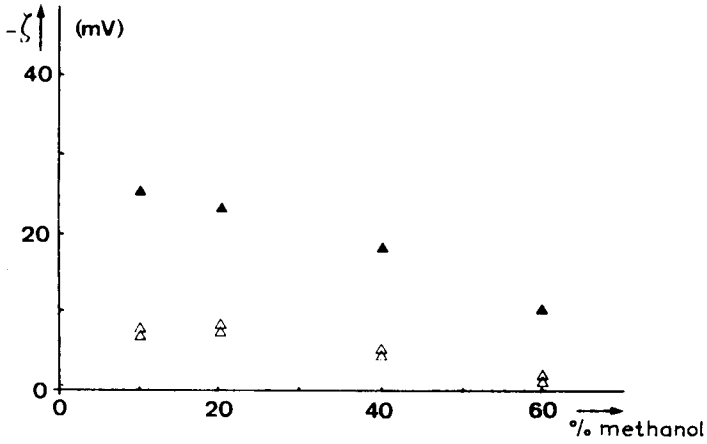

Fig. 8. The ζ-potential of PTFE (△) and fused-silica (▲) capillaries when filled with a pH 6.0 buffer containing varying percentages of methanol.

TABLE V

EFFECT OF METHANOL ON THE ζ-POTENTIAL AND THE RELATIVE ELECTROOSMOTIC FLOW IN A PTFE CAPILLARY FILLED WITH A pH 6.0 BUFFER

Methanol (%, v/v)	ε_r	η	ζ	$\varepsilon_r\zeta/\eta$
0	80	1.0	−41	−3280
10	77	1.3	−7.2	−430
20	73	1.5	−7.8	−380
40	64	1.8	−5.0	−180
60	55	1.7	−1.7	−50

CONCLUSIONS

The relative zone sharpness and the minimum detectable amount in capillary isotachophoresis are adversely affected by electroosmosis when the electroosmotic flow and the isotachophoretic velocity have different directions. This is mostly the case for anionic separations in PTFE or fused-silica capillaries. The relative zone sharpness is improved by decreasing the electroosmosis with PVA, HEC or HPMC (Triton and PVP are less effective) or by reversing the direction of the electroosmotic flow with the use of cationic detergents (CTAB, Priminox). Although the latter are ionic, whenever possible they are to be preferred because of their higher purity (CTAB) and because the tenability of the leading electrolyte buffer is significantly increased. It may be necessary to add a non-ionic detergent also to suppress electrode reactions in the conductivity or potential gradient detector. The use of fused silica instead of PTFE capillaries is generally not recommended when considering electroosmosis only. The additives mentioned act similarly in non-aqueous media, where more electroosmotic disturbances are generally not expected.

REFERENCES

1 F. M. Everaerts, J. L. Beckers, Th. P. E. M. Verheggen, *Isotachophoresis —Theory, Instrumentation, Applications* (*Journal of Chromatography Library*, Vol. 6), Elsevier, Amsterdam, Oxford, New York, 1976.
2 Th. P. E. M. Verheggen and F. M. Everaerts, *J. Chromatogr.*, 249 (1982) 221.
3 F. M. Everaerts, Th. P. E. M. Verheggen and F. E. P. Mikkers, *J. Chromatogr.*, 169 (1979) 21–38.
4 Th. P. E. M. Verheggen, F. E. P. Mikkers, D. M. J. Kroonenberg and F. M. Everaerts, in A. Adam and C. Schots (Editors), *Biochemical and Biological Applications of Isotachophoresis*, Elsevier, Amsterdam, Oxford, New York, 1980, p. 41.
5 J. C. Reijenga, F. M. Everaerts and Th. P. E. M. Verheggen, in C. J. Holloway (Editor), *Proceedings of International Symposium on Isotachophoresis 1982*, Anal. Chem. Symp. Series, Elsevier, Amsterdam, Oxford, New York, in preparation.
6 Th. P. E. M. Verheggen, F. E. P. Mikkers and F. M. Everaerts, *J. Chromatogr.*, 132 (1977) 205–215.
7 A. J. Rutgers, M. de Smet and W. Rigole, in H. van Olphen and K. J. Mysels (Editors), *Physical Chemistry: Enriching Topics from Colloid and Surface Science*, Theorex, La Jolla, CA, U.S.A., 1975, Ch. 21, p. 309.
8 R. J. Hunter, *Zeta Potential in Colloid Science*, Academic Press, London, 1981.
9 J. P. M. Wielders, *Thesis*, Eindhoven University of Technology, Eindhoven, 1978.