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Capillary electrophoresis with wide-bore capillaries and non-aqueous media

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Abstract

A major drawback of capillary electrophoresis is that its use for semi-preparative purposes is problematic. To overcome the problems associated with wide-bore capillaries, non-aqueous background electrolyte instead of aqueous buffers was used. The effect of the capillary diameter on capillary electrophoretic separation was investigated in ethanol–acetonitrile–acetic acid (50:49:1, v/v) containing 20 m*M* ammonium acetate. This buffer allowed the use of wider capillaries and higher electric field strength than the corresponding buffer containing water in place of ethanol and acetonitrile. Increasing the internal diameter of the capillary from 50 to 200 μm allowed a 16-fold increase in the sample load at the same ratio of injected volume to total volume. In wide-bore capillaries the difference between the inlet and outlet buffer level leads to siphoning, which has a marked effect on the apparent electroosmotic velocity and the efficiency of the separation. The effect of the physical properties of the solvent on siphoning is discussed. Our results indicate that non-aqueous media may have a major role in semi-preparative capillary electrophoresis.

Keywords: Background electrolyte composition; Preparative electrophoresis; Wide-bore capillaries; Non-aqueous media

1. Introduction

The dimensions of the capillary in capillary electrophoresis (CE) have a considerable influence on the separation. While the capillary length typically varies widely, between 25 and 100 cm, most of the CE applications use capillaries with 50 μm internal diameter (I.D.). This internal diameter is a compromise between the need for acceptable sensitivity and low electric currents. For many practical purposes this compromise works well, but sometimes the I.D. of the capillary has to be determined by other requirements and narrower or wider capillaries are used.

The electric current (I) determines the maximum capillary I.D. and maximum electric field strength (E) that can be used with a certain buffer in particular CE equipment. The electric current in a circular cross-section capillary is determined by the applied voltage (U), the specific conductance of the electrolyte (κ), and the capillary dimensions; the capillary radius (r) and the capillary length (L), as follows:

$$I = r^2 \pi \cdot \frac{U\kappa}{L} = AE\kappa \quad (1)$$

where A is the cross-section area. Since the current is proportional to r^2, the capillary radius has a decisive effect on the Joule heat production. The Joule heat production results in a radial gradient of temperature,

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viscosity and, as a result, velocity in the capillary, which can deteriorate the efficiency of the separation. Controlling the temperature of the entire capillary is difficult and, in addition to the radial temperature gradients, several zones at different temperatures may develop along the capillary. Depending on the instrumental design and the capillary length, the unthermostatted part of the capillary may be more than 50% of the total length. Hot spots may be present in the capillary, for example, at fittings or at the detection window. At high electric currents, the heat production may cause the buffer to boil in the unthermostatted parts of the capillary. CE equipment usually has an upper electric current and/or power limit at which the run is automatically terminated, or the voltage is kept below the preset value so that the upper current or power limit cannot be exceeded. For the above reasons, the capillary diameter cannot be increased as much as desired.

One of the drawbacks of CE as compared to high-performance liquid chromatography is that, because of the limited capillary diameter, it is not suitable for preparative purposes. The ratio of injected volume to effective volume determines the limiting efficiency of the separation. Because of the limited possibility of increasing the capillary volume, the sample load in CE is usually very low. To overcome this constraint bundled capillaries [1] or sample enrichment techniques [2] can be used. In both cases, any increase in the capillary radius would further increase the sample load, by a power of four if the injection pressure and time are kept constant and by a power of two if the ratio of injected volume to total volume is kept constant. With pressure injection the injected volume (V_{inj}) can be calculated as:

$$V_{inj} = \frac{ptr^3\pi}{8L\eta} \quad (2)$$

where p is the injection pressure, t is the injection time and η is the dynamic viscosity of the background electrolyte [3].

One step towards semi-preparative CE may be to use low conductivity background electrolytes, which allow increase in the capillary diameter without excessive generation of electric current. Since the electric current is usually weaker in non-aqueous media than in conventional aqueous buffers [4], we studied the utility of organic solvents for separations in wide bore capillaries. Although not a new invention [5,6], the use of non-aqueous background electrolytes has become more and more popular in CE recently [7–14].

In this paper we investigated the usefulness of a non-aqueous background electrolyte in capillaries from 50 μm to 200 μm I.D. Our results and theoretical calculations clearly show that organic solvents may better suit for semi-preparative purposes in CE than water does.

2. Experimental

2.1. Materials

All chemicals were of analytical grade unless otherwise stated. HPLC-grade acetonitrile, glacial acetic acid and ammonium acetate were from Merck (Darmstadt, Germany). Ethanol was from Primalco (Rajamäki, Finland). Benzoic acid was from Fluka (Buchs, Switzerland), *meso*-2,3-diphenylsuccinic acid was from TCI (Tokyo, Japan), probenecid, chlorothiazide and ethacrynic acid were Sigma products (St. Louis, MO, USA), and 1,2-phenylenediacetic acid was synthesised in the Laboratory of Organic Chemistry (University of Helsinki, Finland). HPLC-grade methanol was from J.T. Baker (Deventer, The Netherlands). Distilled water was further purified with a Water I system from Gelman Sciences (Ann Arbor, MI, USA).

2.2. Methods

The experiments were carried out using a HP ^{3D}CE system (Hewlett-Packard, Waldbronn, Germany). Fused-silica capillaries with different internal diameters were used: 50 μm I.D.×375 μm O.D. from Composite Metal Services (Hallow, UK), 100 μm I.D.×375 μm O.D., 150 μm I.D.×375 μm O.D. and 200 μm I.D.×350 μm O.D. from BGB Analytik (Zurich, Switzerland). The detection window was made 8.5 cm from the outlet end of the capillary. The effective capillary length was 25–100 cm. The background electrolyte was ethanol–acetonitrile–acetic acid (50:49:1, v/v) containing 20 m*M* am-

monium acetate. For comparison, an aqueous background electrolyte of water–acetic acid (99:1, v/v) containing 20 m*M* ammonium acetate was used as well. The sample solution was prepared by dissolving benzoic acid, *meso*-2,3-diphenylsuccinic acid, probenecid, chlorothiazide, 1,2-phenylenediacetic acid and ethacrynic acid (100 or 500 ppm each) in methanol. The sample was introduced to the capillary by pressure injection. The injection conditions as well as the applied voltage were changed in accordance with the capillary I.D. and are described in the text. The capillary temperature was kept constant at 20°C. Detection was carried out at 214 nm. The levels of the electrolyte in the inlet and outlet vials were kept the same except when siphoning was studied.

3. Results and discussion

The electric current was measured as a function of the applied voltage in a non-aqueous buffer of ethanol–acetonitrile–acetic acid (50:49:1, v/v) containing 20 m*M* ammonium acetate and in a corresponding aqueous buffer of water–acetic acid (99:1, v/v), also containing 20 m*M* ammonium acetate. The voltage was raised from zero to 30 kV in 5 min. The Ohm plots are shown in Fig. 1.

With the aqueous buffer (Fig. 1A) the currents were high and the Ohm plots seriously deviated from linearity as the I.D. increased. In the 150 μm I.D. capillary the voltage could not be increased to more than about 25 kV because the built-in upper power limit of the CE equipment (6 W) was reached at 240 μA. Increasing the I.D. of the capillary to 200 μm resulted in even higher currents. At about 12 kV the current broke down, most probably because the solvent started to boil and the bubbles disrupted the current.

With the non-aqueous buffer (Fig. 1B) the current was a linear function of the voltage at all capillary diameters. The currents were only one-fourth to one-third as high as in the aqueous buffer. In the 200 μm I.D. capillary the voltage could be increased to about 28 kV, at which point the current was just 100 μA. No further increase was possible because the current broke down, probably as a result of bubble formation in the buffer due to boiling.

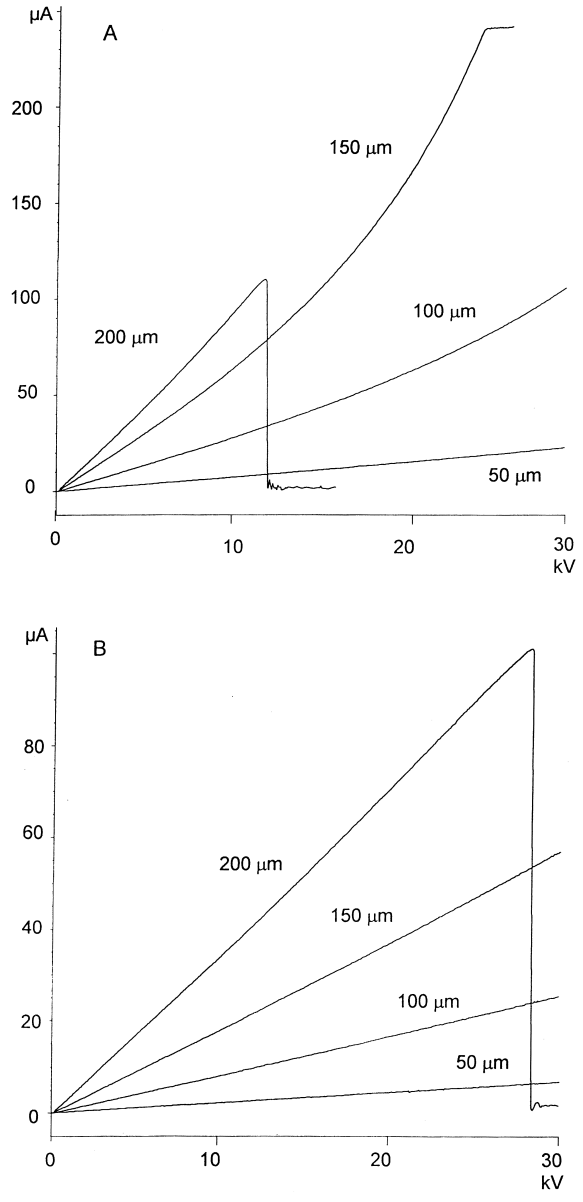

Fig. 1. Ohm plots in 58.5 cm long fused-silica capillaries. The capillaries were filled with (A) water–acetic acid (99:1, v/v) containing 20 m*M* ammonium acetate and (B) ethanol–acetonitrile–acetic acid (50:49:1, v/v) containing 20 m*M* ammonium acetate. The voltage was increased linearly from 0 to 30 kV and the capillary was thermostatted at 20°C.

The results show that non-aqueous buffers allow the use of wider capillaries and higher voltages. While the electric current is generally lower in

organic solvents than in water, the boiling point of many of the organic solvents used in CE is below 100°C. The boiling points of ethanol and acetonitrile, the organic solvents used in this study, are 78 and 82°C, respectively. Apparently, the advantages of lower currents compensate for the disadvantages arising from the lower boiling point of the non-aqueous buffer.

Recently, Valkó et al. [15] and Wright et al. [16] showed that electroosmotic flow develops in many solvents even without the addition of ionic species. Although CE in pure solvents may be impractical because of the lack of buffering, use of organic solvents with low ionic strength would reduce the electric current below the level of conventional CE buffers. The low specific conductance of non-aqueous buffers may reduce buffer depletion due to coulombic titration, which occurs in CE as a result of the ion transport between the inlet and the outlet vials and can be very serious in wide-bore capillaries.

To assess the potential of organic solvents for semi-preparative purposes in CE, we investigated the effect of increased capillary diameter on the separation of six organic acids. Fig. 2 shows electropherograms run under identical experimental conditions where capillaries were of different I.D. To keep the ratio of injected volume to total volume (V_{tot}) constant (0.3%) we varied the injection conditions, as shown in the legend. When the capillary diameter is increased four-fold (from 50 to 200 μm) with V_{inj}/V_{tot} kept constant, the injected mass then increased 16-fold. The efficiency of the separation was slightly lower when the 200 μm I.D. capillary was used, but the loss was well compensated by the increased sample load. The selectivity of the separation is changing when wider capillaries are used as one can clearly notice in panel C. The reason for this may be that the temperature has a strong effect on the apparent pH of the background electrolyte, which in turn, may selectively influence the ionization and therefore the electrophoretic mobility of the analytes.

If the buffer level at the inlet and outlet ends of the capillary is different, a hydrodynamic flow is generated [17]. Since this flow is pressure driven, it has a parabolic profile, which decreases the efficiency of the separation. The siphoning flow velocity at distance x from the centre axis of the capillary (v_x) can be calculated as

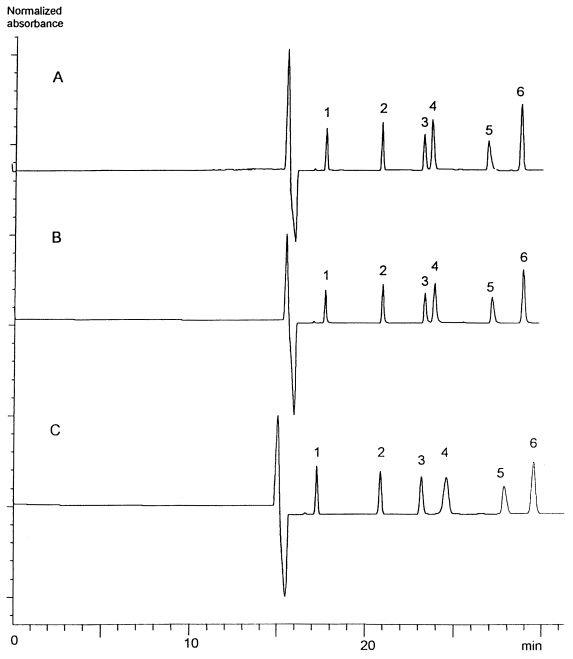

Fig. 2. Effect of the internal capillary diameter on the separation of 100 ppm each of benzoic acid (1), *meso*-2,3-diphenylsuccinic acid (2), probenecid (3), chlorothiazide (4), 1,2-phenylenediacetic acid (5) and ethacrynic acid (6). Effective capillary length 75 cm, internal diameter: 50 μm (A), 100 μm (B) and 200 μm (C). Applied voltage 23 kV, detection wavelength 214 nm, capillary temperature 20°C. The ratio of injected volume to total volume was kept constant at 0.3%. Injection: 41 mbar for 3 s (A), 15 mbar for 2 s (B) and 4 mbar for 2 s (C).

$$v_x = \frac{pr^2}{8L\eta} \cdot (1 - 2r_x^2) \quad (3)$$

where p is the pressure, r is the internal capillary radius, and r_x is the normalized radius variable (x/r) at distance x from the central axis of the capillary [18]. Across the cross-section of the capillary, r_x averages zero, and so the overall linear velocity of the siphoning flow (v) can be calculated by neglecting the term in parenthesis in Eq. (3).

Since the linear flow velocity is proportional to the square of the capillary diameter, the effect of siphoning can be very significant when wide bore capillaries are used. When the inlet level was kept lower than the outlet buffer level, the electropherograms recorded in a 200 μm I.D. capillary became highly distorted, as shown in Fig. 3. The apparent electroosmotic velocity, which can be calculated from the migration time of the negative peak produced by the

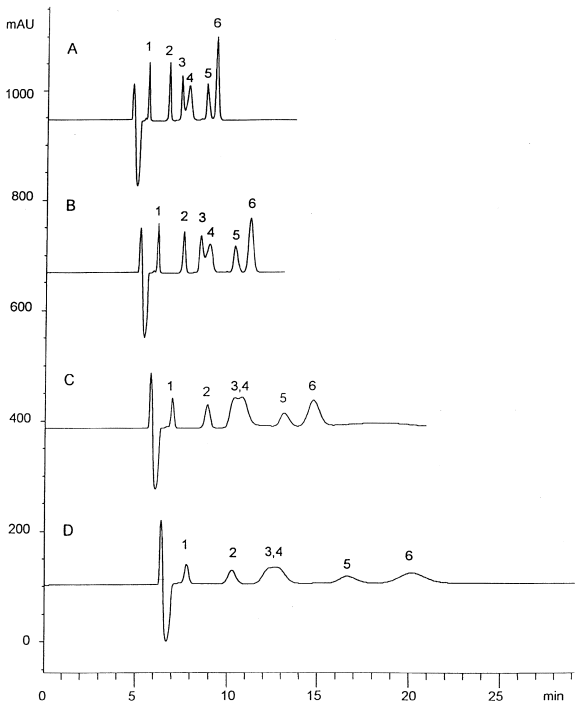

Fig. 3. Siphoning effect on the separation of 500 ppm each of benzoic acid (1), *meso*-2,3-diphenylsuccinic acid (2), probenecid (3), chlorothiazide (4), 1,2-phenylenediacetic acid (5) and ethacrynic acid (6). Capillary: fused-silica, 33.5 cm total length, 200 μm I.D. Background electrolyte: ethanol–acetonitrile–acetic acid (50:49:1, v/v) containing 20 m*M* ammonium acetate. Outlet buffer level: 0 mm (A), 1 mm (B), 2 mm (C) and 3 mm (D) higher than inlet buffer level. Applied voltage 9.3 kV, injection 3 mbar for s, detection wavelength 214 nm, capillary temperature 20°C.

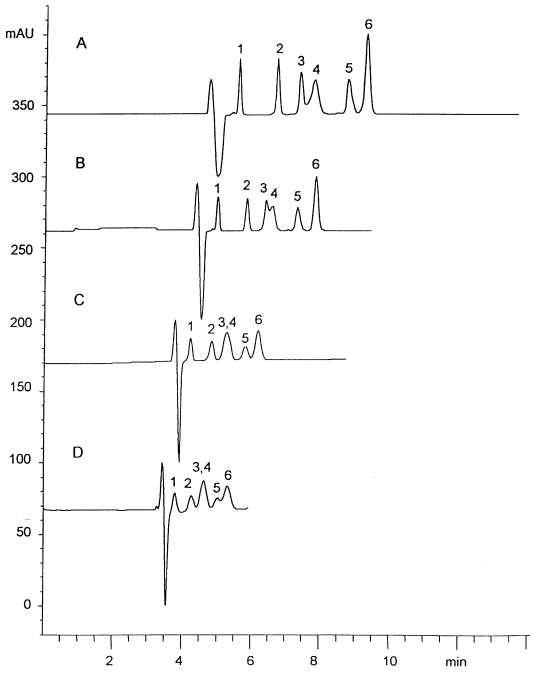

Fig. 4. Siphoning effect on the separation of 500 ppm each of benzoic acid (1), *meso*-2,3-diphenylsuccinic acid (2), probenecid (3), chlorothiazide (4), 1,2-phenylenediacetic acid (5) and ethacrynic acid (6). Capillary: fused-silica, 33.5 cm total length, 200 μm I.D. Background electrolyte: ethanol–acetonitrile–acetic acid (50:49:1, v/v) containing 20 m*M* ammonium acetate. Inlet buffer level 0 mm (A), 2 mm (B), 4 mm (C) and 6 mm (D) higher than outlet buffer level. Applied voltage 9.3 kV, injection 3 mbar for s, detection wavelength 214 nm, capillary temperature 20°C.

sample solvent, is the sum of the flows generated by electroosmosis and by siphoning. In this case the electroosmotic and hydrodynamic flows are in opposite directions, which reduces the efficiency dramatically. Furthermore, the electroosmotic flow carries electrolyte from the inlet to the outlet vial, which further raises the outlet level. In our experiments the apparent electroosmotic flow velocity was 1.49 μl/min, and if we assume a constant flow-rate, then in a 20 min run about 30 μl buffer is transported from the inlet to the outlet vial resulting in a 60 μl difference.

When the inlet level is higher than the outlet (Fig. 4) the hydrodynamic and electroosmotic flows are in the same direction, which results in faster migration and overlapping peaks. The effects of siphoning on the electroosmotic flow and on the slowest migrating analyte, ethacrynic acid, in capillaries of different internal diameter are depicted in Figs. 5 and 6, respectively. In a 50 μm I.D. capillary, the siphoning effect is very weak: it is hardly noticeable if neither the inlet nor the outlet buffer level is lower than 5 mm below the other. At 100 μm I.D. the siphoning effect becomes noticeable and is always more pronounced if the inlet level is lower than the outlet. +4 mm buffer level at the outlet end of a 200 μm I.D. capillary resulted in a four-fold increase in the migration time of ethacrynic acid. The efficiency of the ethacrynic acid peak is plotted as a function of the difference in the buffer levels in Fig. 7. In general, the efficiency decreases with the increasing capillary diameter. Siphoning has a more pronounced effect on the efficiency than on the migration time. The wider the capillary the more sensitive is the efficiency to the difference between the inlet and outlet levels.

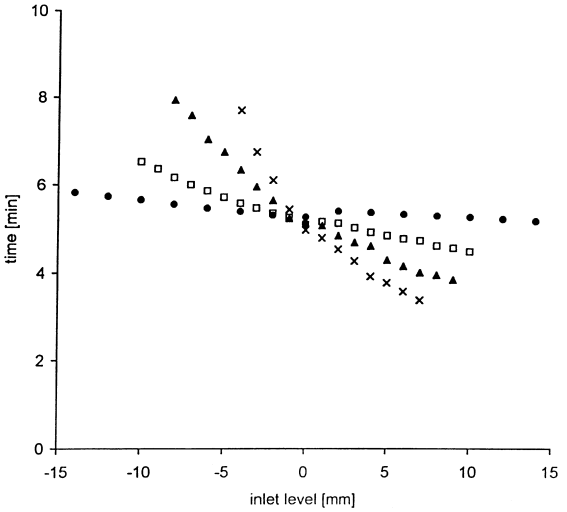

Fig. 5. Effect of siphoning on the electroosmotic flow. Background electrolyte: ethanol–acetonitrile–acetic acid (50:49:1, v/v) containing 20 m*M* ammonium acetate. Capillary length 33.5 cm, internal diameter: (●) 50 μm; (□) 100 μm; (▲) 150 μm; (×) 200 μm.

The solvent properties affect the siphoning through the density (ρ) and the dynamic viscosity of the buffer. The pressure in Eq. (3) is caused by the

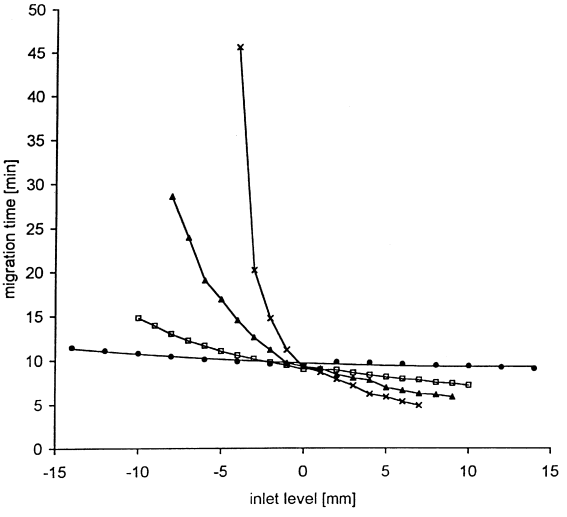

Fig. 6. Effect of siphoning on the migration time of ethacrynic acid. Background electrolyte: ethanol–acetonitrile–acetic acid (50:49:1, v/v) containing 20 m*M* ammonium acetate. Capillary length 33.5 cm, internal diameter: (●) 50 μm; (□) 100 μm; (▲) 150 μm; (×) 200 μm.

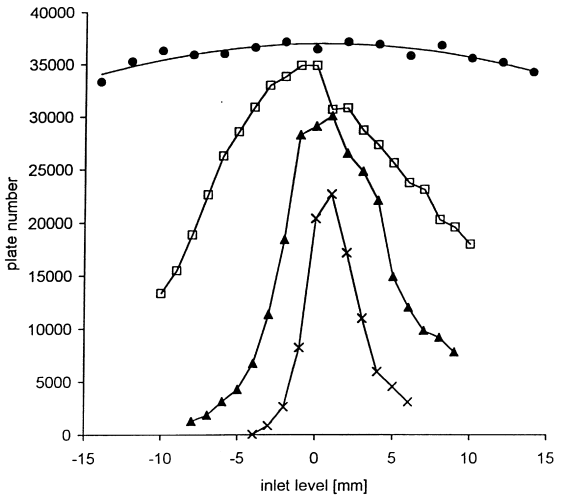

Fig. 7. Effect of siphoning on the plate number of the ethacrynic acid peak. Background electrolyte: ethanol–acetonitrile–acetic acid (50:49:1, v/v) containing 20 m*M* ammonium acetate. Capillary length 33.5 cm, internal diameter: (●) 50 μm; (□) 100 μm; (▲) 150 μm; (×) 200 μm.

weight of the excess solvent, which means that the formula can be rewritten as

$$v = \frac{\rho g d r^2}{8 L \eta} \tag{4}$$

where g is the acceleration due to gravity, and d is the difference of the buffer levels (inlet minus outlet). According to Eq. (4), the unwanted pressure-driven flow can be minimized if the ratio of density to dynamic viscosity (i.e., the reciprocal of the kinematic viscosity) of the buffer is low. Table 1 lists the ρ/η ratios of solvents used in CE as well as the linear velocity of the siphoning flow caused by 1 mm difference in level in a 200 μm I.D. capillary ($v_{1,200}$). The data show that water is clearly not optimal in terms of reducing the siphoning effect. Solvents with lower ρ/η ratios may be advantageous in semi-preparative CE.

When solvent mixtures are used, ρ/η depends on the actual ratio of the solvents in the mixture. Fig. 8 shows that while the density of ethanol–acetonitrile mixtures is almost constant, their dynamic viscosity decreases with increasing acetonitrile concentration, and the ρ/η ratio increases. In semi-preparative capillary electrophoresis the effect of the ρ/η ratio of

Table 1
Density (ρ), dynamic viscosity (η) and ρ/η ratio of selected solvents and the linear velocity of the hydrodynamic flow generated by 1 mm difference in the inlet and outlet buffer levels in 200 μm I.D. capillaries ($v_{1,200}$) at 25°C

	ρ (g/cm^3)	η (mPa s)	ρ/η (s/cm^2)	$v_{1,200}$ (μm/min)
Formamide	1.1292	3.3020	0.3420	751
Dimethyl sulfoxide	1.0958	1.9960	0.5490	1206
N-Methylformamide	0.9988	1.6500	0.6053	1329
Ethanol	0.7850	1.0780	0.7282	1599
Acetic acid	1.0437	1.1550	0.9036	1985
Water	0.9970	0.8903	1.1199	2460
N,*N*-Dimethylformamide	0.9440	0.8020	1.1770	2585
Methanol	0.7866	0.5445	1.4447	3173
Acetonitrile	0.7766	0.3450	2.2510	4944
Acetone	0.7844	0.3040	2.5803	5667

Total capillary length 33.5 cm. Solvents are listed in increasing order of ρ/η ratio. Density and dynamic viscosity data from Ref. [19].

the solvent may be an important consideration. We must point out, however, that optimal separation conditions may lie far from the optimum ρ/η ratio. During method development one should consider both the need for selective separation and minimisation of siphoning. The relative importance of these requirements depends on the particular solvents, capillary diameter and analytes to be separated. Siphoning can be reduced in various ways, such as buffer replenishment and the use of restrictors [17] and wide buffer vials. In even wider capillaries, suppression of the electroosmosis may be necessary because the electroosmotic flow may transport so much buffer in a single run that efficient separation becomes impossible even if the buffer levels are the same at the beginning of the run.

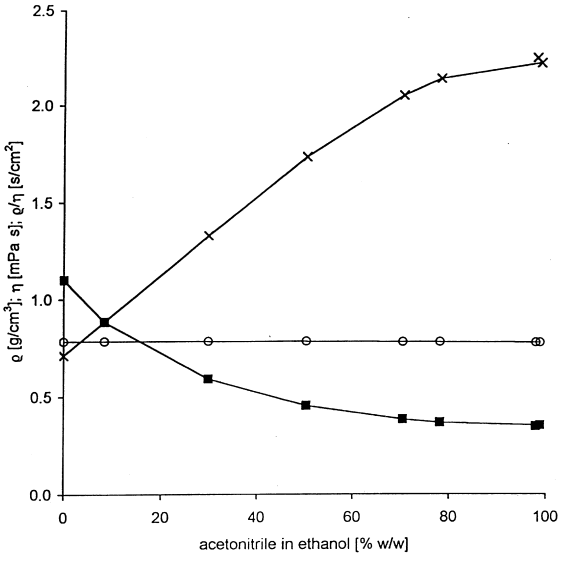

Fig. 8. Properties of ethanol–acetonitrile mixtures at 25°C. Symbols: (○) density (g/cm^3); (■) dynamic viscosity (mPa s); (×) density/dynamic viscosity (s/cm^2). Data from Ref. [20].

4. Conclusions

There are several advantages of using non-aqueous media for semi-preparative purposes in CE. Because the currents are generally lower, higher electric field strength and wider capillaries can be used without reaching the current or power limit of the equipment, and boiling of the buffer may be avoided. The low currents in non-aqueous buffers also reduce the buffer depletion due to coulombic titration. Siphoning has serious undesired effects in wide-bore capillaries, which can be reduced by using organic solvents with low ratio of density to dynamic viscosity.

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