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Extremely high electric field strengths in non-aqueous capillary electrophoresis

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Abstract

The influence of high electric field strength on the separation of basic analytes in non-aqueous alcohol background electrolyte (BGE) solutions was investigated. Increasing the separation voltage in capillary electrophoresis (CE) may be advantageous if the conductivity of the BGE solution is low enough to allow fast separations without excessive Joule heating or band broadening. The voltage range tested was 20–60 kV with methanol and ethanol, and 25–60 kV with propanol and butanol as solvent for BGE. The resulting electric field strengths ranged from 660 V cm^{-1} to 2000 V cm^{-1}. Experiments were made with a special laboratory constructed CE instrument. The separation efficiency vs. voltage curve was found to vary with the alcohol BGE solution. The increase in voltage decreased the separation efficiency in the case of methanol BGE solution, but with the other BGEs a clear efficiency maximum was obtained above 30 kV. The highest separation efficiencies were achieved with propanol BGE solution, where the efficiency maximum was reached at 45 kV. However, reasonable efficiency was achieved even at 60 kV. The extent of Joule heating was determined by calculating the temperature inside the capillary and the observed plate heights were interpreted in terms of the Van Deemter equation. The decrease in the separation efficiency with higher voltage was attributed mainly to Joule heating in the case of methanol and ethanol BGE solution and to the analyte adsorption on the capillary wall with propanol and butanol BGE solutions.

Keywords: Non-aqueous capillary electrophoresis; Electric field strength; Joule heating; Adsorption; Efficiency; Background electrolyte composition; Potential

1. Introduction

Capillary electrophoresis (CE) separations are usually performed with electrical potentials no greater than 30 kV. The potential range available in commercial capillary electrophoresis instruments is often limited to −30 kV to +30 kV, mainly because of electrical insulation problems and safety considerations. Additionally, with aqueous background electrolyte (BGE) solutions, the band broadening associated with Joule heating becomes severe above 30 kV because of the high current in the capillary. Hence, for most CE-users, a voltage maximum of 30 kV has been considered acceptable.

If only longitudinal diffusion is contributing to the plate height, the separation efficiency increases linearly with the applied potential:

$$N = \frac{(\mu_{ep} + \mu_{eo})L_{det}U}{2DL_{tot}} \quad (1)$$

where N is the plate number, U is the applied separation potential, and L_{det} and L_{tot} are the capillary length to the detection window and the total

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length of the capillary, respectively. μ_{ep} is the electrophoretic mobility of the analyte, μ_{eo} is the electroosmotic mobility and D is the diffusion coefficient of the analyte. Resolution also increases with the applied potential but only with square root dependence. High electric field strengths also lead to a reduction in the analysis time. For these reasons, high potentials can be considered desirable and potentials as high as 45 kV have indeed been used in several instances, especially in the early years of CE [1–9]. Recently, Hutterer and Jorgenson introduced a unique oil-insulated CE system, which permitted operation with potentials as high as 120 kV [10]. Impressive plate numbers for several peptides were achieved. However, the capillary was almost four meters long, resulting in very long analysis times and low field strengths of about 300 V cm^{-1}. A slightly modified version of the same system has also been applied in micellar electrokinetic capillary chromatography [11].

The use of high potentials is beset by many problems, which must be overcome. First of all, safety considerations must be satisfied to enable safe and reliable operation with the instrument. To overcome the problems associated with Joule heating, which reduces the separation efficiency, the resistance of the capillary needs to be increased. This can be done by increasing the capillary length or by reducing either the inner diameter of the capillary or the conductivity of the separation medium. Increase in the capillary length increases the separation time, while on the other hand, the use of higher potential shortens it. Sample loadability and the sensitivity of the UV detection decrease with decrease in the capillary inner diameter, respectively to the lower cross-sectional area of the capillary and the reduced optical path length. To obtain more advantage from high potentials, it would thus seem better to reduce the conductivity of the BGE solution. Under suitable separation conditions, non-aqueous CE (see, e.g., Ref. [12]) is a good choice where low conductivity is desired. The use of alcohols, especially longer chain alcohols, as solvent for BGE is especially attractive for this purpose. Unfortunately, increase in the alcohol chain length increases the viscosity of the solvent as well and in order to keep the analysis time reasonably short, a long chain alcohol must usually be applied together with a low viscosity solvent, such as acetonitrile [13]. It is clear, however, that pure long chain alcohols may be useful solvents for BGE when extremely high electric fields are applied.

In this work, the effect of high voltages (up to 60 kV) on the separation of some basic analytes was investigated in different non-aqueous alcohol BGE solutions. The alcohols investigated were methanol, ethanol, 1-propanol and 1-butanol. A laboratory constructed CE instrument specially designed for high voltage separations is described.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade and used as received unless otherwise stated. Sodium acetate (E. Merck, Darmstadt, Germany) and glacial acetic acid (Riedel-de Haën, Seelze, Germany) were used to prepare the BGE solutions. HPLC-grade methanol was from J.T. Baker (Deventer, Netherlands), ethanol was from Primalco (Rajamäki, Finland), 1-propanol was from Fisher Scientific (Leicestershire, UK) and HPLC-grade 1-butanol was from Sigma–Aldrich (Gillingham, UK). Amphetamine, dipipanone and levorphanol were obtained from the National Bureau of Investigation Crime Laboratory (Vantaa, Finland), and propranolol was received as a gift from the Department of Pharmacy (University of Helsinki, Finland). Dimethyl sulphoxide (DMSO) was from Lab-Scan (Dublin, Ireland) and sodium hydroxide was from E. Merck. Transducer oil (Neste Trafo 10X) was received as a gift from Fortum (Espoo, Finland) and silicone oils (DC 200) were from Fluka (Buchs, Switzerland).

2.2. Background electrolyte

BGE solutions were prepared by adding equimolar amounts of sodium acetate (NaAc) and acetic acid (HAc) at concentrations of 15 mM to the different alcohols, namely methanol (MeOH), ethanol (EtOH), 1-propanol (PrOH) and 1-butanol (BuOH). BGE solutions were prepared daily by weighing the sodium acetate into a 50 ml volumetric flask and adding about 49 ml alcohol. Acetic acid was then added with a 50 μl syringe (Hamilton Gastight,

Hamilton Company, Reno, NV, USA) and the flask was filled to the mark with alcohol. Special care was taken to seal the flasks properly to avoid moisture uptake from the atmosphere. The BGE solutions were filtered through 0.45 μm Acrodisc filters (Pall Gelman Lab., Ann Arbor, MI, USA) before analysis.

2.3. Sample preparation

The stock solutions of amphetamine, propranolol, dipipanone and levorphanol, were prepared at concentration of 1 mg ml^{-1} each in methanol. A sample solution consisting of all the four analytes (each at concentration of 100 μg ml^{-1}) was diluted from the stock solutions with methanol. The final 10 μg ml^{-1} sample solution was diluted from the 100 μg ml^{-1} solution. DMSO [electroosmotic flow (EOF) marker] was added to final sample solution at concentration of 20 m*M*. Sample and sample stock solutions were stored in a dark coldroom (+7°C).

2.4. Capillary and separation parameters

The total length of the untreated fused-silica capillaries (Composite Metal Services, Hallow, UK) was 30 cm (28.5 cm to the detector window) and the other dimensions were 50 μm I.D. × 375 μm O.D. Capillaries were all from the same stock and a new capillary was introduced daily. Capillary preconditioning was performed by rinsing with 0.1 *M* NaOH in methanol for 30 min. Thereafter the capillary was flushed with MeOH for 20 min and with BGE solution for 30 min. Between the runs the capillary was rinsed with BGE solution for 3 min in the case of methanol and for 5 min with the other alcohols. Capillary thermostating was done by inserting the capillary inside a plastic tube through which air was passed. Before entering the plastic tubing the air was passed through a coiled copper pipe immersed in a water bath (Lauda Dr. R. Wobster, Lauda-Königshofen, Germany) maintained at 23°C.

With the short capillary, it was not possible to lift the inlet vial to perform hydrodynamic injection by siphoning and the sample was thus introduced to the capillary electrokinetically. The injection parameters for methanol, ethanol, propanol and butanol BGE solutions were 5 kV for 1.5 s, 7 kV for 5.0 s, 8 kV for 15 s and 10 kV for 23 s, respectively. To ensure that the same amount of sample was injected to the capillary with each BGE solution, heights and areas of the peaks were calculated and compared. The peak areas were divided by migration times in order to obtain peak areas less dependent on the migration time. The injection parameters were then adjusted so that the peak heights and the corrected areas of the peaks were about the same with each BGE solution. Voltages ranging from 20 kV to 60 kV (25–60 kV for propanol and butanol) were used and all runs were carried out in random order to avoid any systematic error due to the gradual chemical changes on the capillary surface. One voltage series was run each working day (with a single capillary). All runs with specific voltage and BGE solution were repeated five times and the average of the results was used in further calculations.

2.5. CE instrument and control related parameters

The CE instrument was a laboratory constructed model [14], which was modified to allow the use of high voltages. An HPLC detector (Jasco 870-UV, Tokyo, Japan) was used for detection at 208 nm. The original flow-through cell was replaced with a special in-house constructed capillary holder, which allowed replenishment of the outlet BGE solution. A high voltage supply (PS/EH 60R01.5-22, Glassman High Voltage, Whitehouse Station, USA) was used for establishing the electrical field. The maximum voltage of the supply was 60 kV. The CE-currents were calculated from the feedback voltage given by the high voltage supply.

Data acquisition and instrument control were accomplished with an Advantech PCL-812PG laboratory card (Advantech, Irvine, USA) connected to an Intel 486-based personal computer. Software for data acquisition and CE-instrument control was written in the laboratory with C++ programming language [15]. Data collection frequency was 18 Hz for methanol and ethanol BGE solutions and 9 Hz for propanol and butanol BGE solutions. For analysing the data, a peak finding and analysis program called ‘epeaks’ was written for use with Matlab 5.3 software (MathWorks, Natick, USA) [16]. The program can find the peaks from a chromatographic datafile in ASCII format. It can fit different types of peak functions or tangents to peak slopes and

calculate the usual peak parameters from the results. Either Gaussian fitting or tangential method was used to calculate peak characteristics. RedHat Linux 6.1 (Red Hat, Durham, USA) was the operating system for data analysis with Matlab.

2.6. Conductivity measurements

The electrical conductivities were measured with a CDM3 conductivity meter (Radiometer, Copenhagen, Denmark) in a standard measuring cell with 0.94 cm^{-1} cell constant. During the measurements the conductivity measurement flask was kept in a thermostated water bath, which was heated to the desired temperature. The conductivities were measured within the temperature range 20–53°C.

3. Results and discussion

3.1. High voltage insulation

3.1.1. Insulation structure

The use of voltages above 40 kV with air insulation produced sparking, and the high electric fields tended to introduce noise to the electronics. Electrical breakthroughs may also destroy electronic components within the instrument. When air insulation is used around the high voltage region there should be no conductive material near the inlet vial or the high voltage electrode. In most cases, however, metallic parts are located quite close to the vials, and sparking is likely. This problem was solved with oil insulation. The inlet vial was kept in an oil bath inside a special vial holder. When the instrument lifted the vial holder to run position the cylinder around the inlet vial was partly immersed into the oil bath. In this way the cavity where the high voltage parts are exposed was completely insulated electrically from the surroundings. The insulation structure is illustrated in Fig. 1.

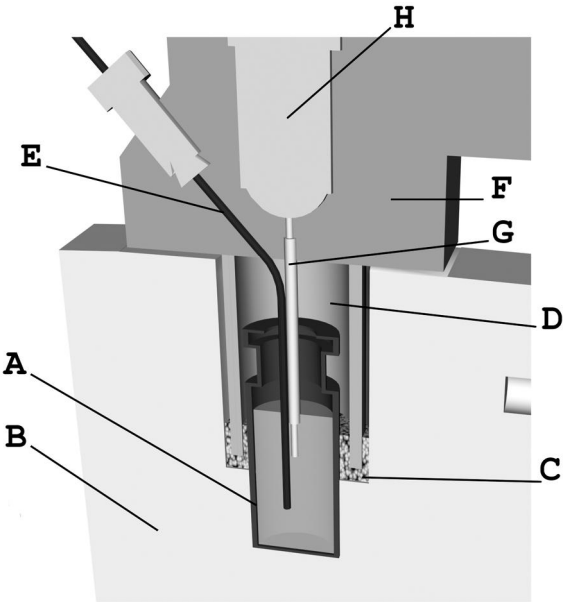

Fig. 1. A cross-section of the electrical insulation structure of the laboratory constructed CE instrument. (A) inlet vial, (B) inlet vial holder, (C) oil bath, (D) insulation cylinder, (E) separation capillary, (F) capillary block, (G) high-voltage electrode, (H) high-voltage cable. The vial holder can move up and down but the capillary block is fixed at a certain level.

3.1.2. Insulation oils

The suitability of different oils for the high voltage insulation was tested by turning on the high voltage supply of the CE instrument only while the other electronic parts were protected by grounding. The breakthrough voltage for olive oil and glycerol was about 55 kV and the mineral and silicone oils of different viscosity provided insulation up to about 55–60 kV. However, the tendency of these oils slowly to climb up from the plastic vial holder when high voltage is applied could constitute a severe contamination risk, as well as voltage breakthroughs if the amount of oil remaining in the vial holder became inadequate for insulation. A high viscosity silicone oil ($\eta \approx 30\,000$ mPas) served well as insulator, but because of operational inconvenience it was not used. Instead transducer oil, which was a very effective insulator and did not rise up from the vial holder, was chosen for the electrical insulation media.

3.1.3. Electric field strength

The electric field strength was in the range of 660 V cm^{-1} to 2000 V cm^{-1} (normally in CE the electric field strength is about 50–600 V cm^{-1}). The high electric field seemed to cause severe stress to the capillary surface and the plate numbers calculated from the initial experiments were not repeatable. To

overcome this problem, we introduced a new capillary each day. Additionally, with methanol BGE solution and 60 kV analyses, the capillary tended to brake. The point of fracture was about 3 cm from the inlet end of the capillary and the fracture was clearly caused by an electric breakthrough from the capillary to the high voltage electrode.

3.2. Migration times and electrophoretic mobilities

Increase in the electric field strength leads to shorter analysis times because the analyte velocity in the capillary increases. The effect of the separation voltage on the migration time with propanol BGE solution is presented in Fig. 2, and similar behaviour was observed with the other BGEs. Clearly, the use of higher potentials is advantageous because analyses can be performed faster.

Electrophoretic mobilities were independent of voltage especially with ethanol, propanol and butanol BGE solutions. In the case of methanol, the presence of Joule heating was indicated by a change in the analyte mobilities as the separation voltage increased.

3.3. Separation efficiencies

The dependencies of the theoretical plate numbers on voltage for different BGE solutions are shown in Fig. 3. The results show the separation efficiencies to be in the same order of magnitude, but the shapes of the theoretical plate number curves differ with the applied voltage. It is worth mentioning that peaks were more symmetrical with propanol and butanol BGE solutions (asymmetry 1–1.5) than with methanol and ethanol BGE solutions except in the case of levorphanol, for which similar peak tailing was observed in all BGE solutions (asymmetry 1.5–2).

With methanol BGE solution, the best separation efficiency was at the low end of the studied voltage range (Fig. 3A). Fig. 4 shows the current in the capillary as a function of the separation voltage with the different BGE solutions. Note that, according to Ohm's law, voltage and current should be directly related at constant temperature, but this is not exactly the case with methanol BGE solution. The slight curvature evident in Fig. 4A is due to the Joule heating, which further leads to band broadening and

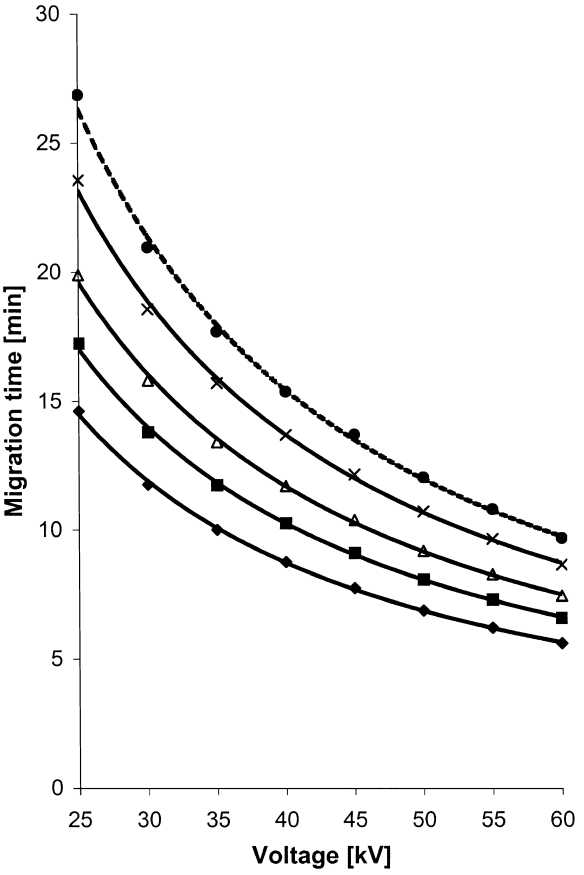

Fig. 2. Dependence of the migration time on the separation voltage with 15 m*M* NaAc+15 m*M* HAc in propanol BGE solution. Capillary, 30 cm (28.5 cm to detector), 50 μm I.D.; detection, UV 208 nm; injection, 8 kV for 15 s; air flow thermostating, 23°C. Symbols: ♦ amphetamine; ■ propranolol; △ dipipanone; × levorphanol; ● DMSO.

decrease in the separation efficiency. The separations with methanol BGE solution were very fast, however, especially with higher electric field strengths, and if some loss of separation efficiency can be tolerated it is advantageous to use high potentials to save analysis time.

Best separation efficiency with ethanol-based BGE solution was obtained when the separation voltage was about 30 kV, as can be seen in Fig. 3B. Because the conductivity of ethanol is relatively low, also the currents were low. At 60 kV potential, the current was less than 10 μA. The current increases more or less linearly with voltage (Fig. 4B) and only the

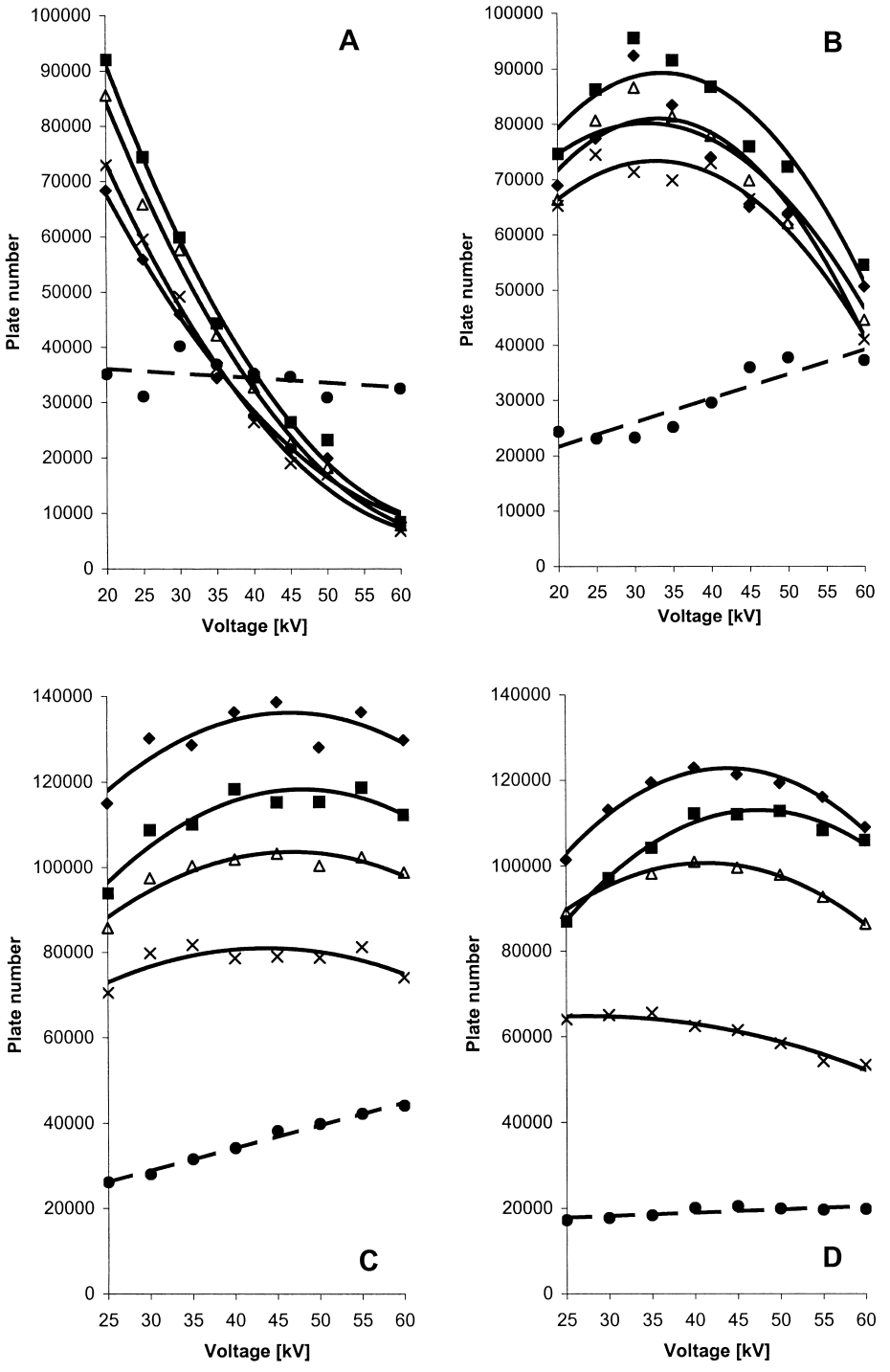

Fig. 3. Dependence of plate number on the separation voltage with 15 m*M* NaAc+15 m*M* HAc BGE in (A) methanol, (B) ethanol, (C) propanol and (D) butanol. Capillary, 30 cm (28.5 cm to detector); 50 μm I.D.; detection, UV 208 nm; injection, (A) 5 kV for 1.5 s, (B) 7 kV for 5 s, (C) 8 kV for 15 s, (D) 10 kV for 23 s; air flow thermostating, 23°C. Symbols: ♦ amphetamine; ■ propranolol; △ dipipanone; × levorphanol; ● DMSO.

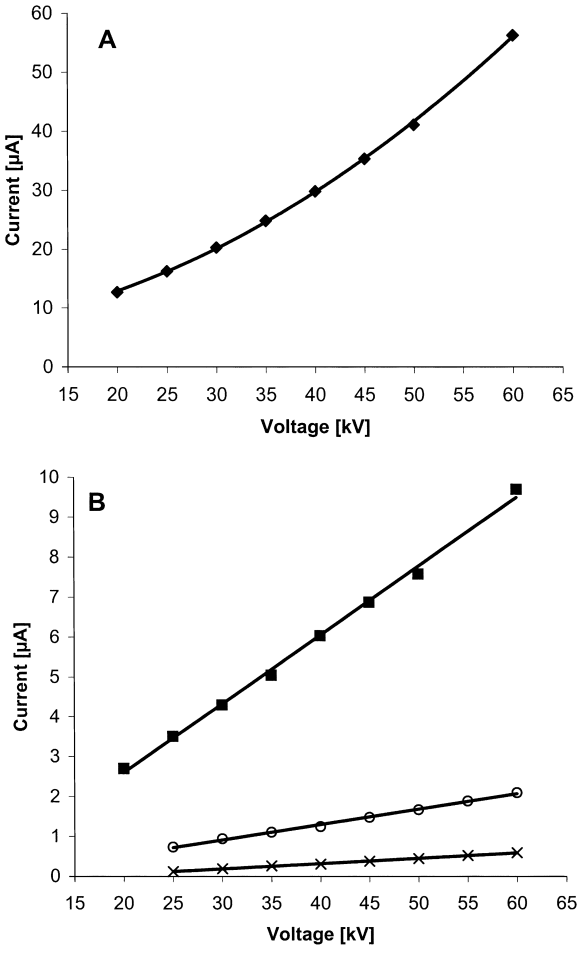

Fig. 4. Electrical currents in the capillary as a function of voltage for BGE solutions with methanol (♦), ethanol (■), propanol (○) and butanol (×). Correlation coefficients were 0.9908, 0.9985, 0.9985 and 0.9990 for methanol, ethanol, propanol and butanol BGEs, respectively.

current value at 60 kV is higher than one would expect from the linearity of the graph.

For propanol BGE solution the separation efficiency remained good over the whole voltage range and increased with applied voltage up to about 45–50 kV (Fig. 3C). The currents increased linearly with voltage and were under 3 μA for all separations (Fig. 4B), which would suggest that Joule heating is not present to any significant extent. The highest separation efficiencies were achieved with propanol BGE solution.

A clear maximum in the separation efficiency with butanol BGE solution occurs at 45 kV (Fig. 3D). Note that the currents for butanol BGE solution (Fig. 4B) were extremely low (<0.6 μA at 60 kV) and linear in regard to voltage.

The voltage dependence of the plate number of neutral DMSO (EOF marker) is fairly linear with all the BGE solutions. With methanol and butanol BGEs, the plate number of DMSO is fairly constant over the whole voltage range, whereas the separation efficiency with ethanol and propanol BGEs increases with the voltage.

3.4. Temperature dependency of conductivity

Fig. 5 illustrates the temperature dependency of conductivity of the BGEs. With methanol BGE solution the conductivity increases linearly with temperature, but with ethanol BGE solution there is slight curvature. The curvature is even stronger in

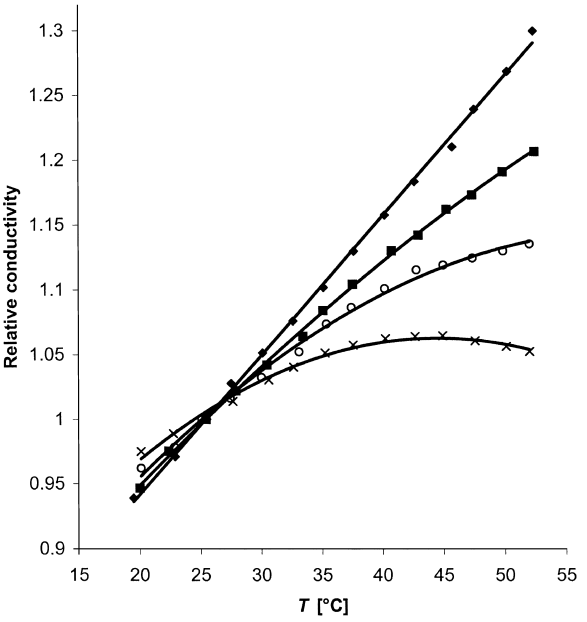

Fig. 5. Temperature dependence of the conductivity (expressed relative to the conductivity at 25°C) on the BGE solutions. BGEs: 15 m*M* NaAc+15 m*M* HAc in methanol (♦), ethanol (■), propanol (○) and butanol (×). The conductivity of the BGE solution at 25°C was taken to represent the value 1 and conductivities at other temperatures were normalised against that value. The absolute conductivity values at 25°C are 928 μS cm^{-1}, 223 μS cm^{-1}, 55 μS cm^{-1} and 18 μS cm^{-1} for methanol, ethanol, propanol and butanol BGE solution, respectively.

the case of propanol BGE solution, and an apparent maximum can be seen between 40°C and 45°C for butanol BGE solution. Note that the absolute values of conductivities of the BGE solutions differ significantly.

3.5. Estimation of the capillary temperature

Heat generation power in the capillary is presented in Table 1. The capillary temperature was first approximated from the temperature dependence of conductivity after calculation of the conductivities of the BGE solution inside the CE capillary from the capillary dimensions and the CE current. However, this rough method did not give satisfactory results and another method had to be used. With this other method, the average surface temperature of the capillary was first estimated by calculating the temperature difference between the outer wall of the capillary and the surroundings as described by Knox and McCormack [17,18]. With forced convection over the capillary, the temperature of the capillary surface T_s can be calculated from:

$$T_s - T_a = \frac{UI}{\pi b R_e^n k_a L_{tot}} \quad (2)$$

where T_a, I, R_e and k_a are the temperature of the cooling medium (air), the current in the capillary, the Reynolds number and the thermal conductivity of the cooling medium, respectively [18]. The constants b and n are dependent on the value of the Reynolds number, which was calculated from:

Table 1
Heat generation power (P) in the capillary with different separation voltages

U(kV)	P(W m^{-1})			
	MeOH	EtOH	PrOH	BuOH
20	0.8	0.18	–	–
25	1.4	0.29	0.06	0.01
30	2.0	0.43	0.09	0.02
35	2.9	0.59	0.13	0.03
40	4.0	0.81	0.17	0.04
45	5.3	1.03	0.22	0.06
50	6.9	1.26	0.28	0.07
55	–	–	0.34	0.10
60	11.3	1.94	0.42	0.12

$$R_e = \frac{u_f d_o}{\eta} \quad (3)$$

where u_f, d_o and η are the fluid velocity over the capillary surface, the capillary outer diameter and the kinematic viscosity of the cooling medium [18]. The dependencies of the thermal conductivity and the kinematic viscosity on temperature were assumed to be negligible.

In view of the relatively low thermal conductivities of organic solvents, it was suspected that there could be severe temperature gradients within the capillary, which would deteriorate the separations. The temperature inside the capillary was thus of special interest. When the surface temperature of the capillary is known, the temperature at the centre of the capillary (T_c) can be calculated from the following heat conduction equation:

$$T_c - T_s = \frac{UI}{2\pi L_{tot}}\left(\frac{1}{2k_b} + \frac{1}{k_s}\ln\frac{r_2}{r_1} + \frac{1}{k_p}\ln\frac{r_3}{r_2}\right) \quad (4)$$

where k_b, k_s and k_p are thermal conductivities of the BGE solution, the quartz wall and the polyimide coating, and r_1, r_2 and r_3 are the internal radius of the quartz tube, the external radius of the quartz tube and the capillary outer diameter (with polyimide coating), respectively [18]. The calculated temperature values are presented in Table 2. As expected, the temperature inside the capillary is very high with the methanol BGE solution. There are also relatively strong temperature differences between the centre and the surface of the capillary with methanol BGE, but the effect is weaker with ethanol BGE. As mentioned above, with methanol BGE solution the current broke down on many occasions when operating with 60 kV, suggesting high temperature in the capillary. The temperature at the capillary centre is close to the boiling point of methanol, which is 64.7°C. Note, moreover, that 17% (5 cm) of the capillary length is in the unthermostated region and the temperature is not uniform in the capillary. With ethanol BGE solution the temperature seems to be moderate and for example, with 60 kV the capillary temperature is about 30°C, which still exceeds the temperature of the cooling system (23°C). During the separation with propanol or butanol BGE solutions, even the temperature at the capillary centre is quite close to the temperature of the cooling system. With

Table 2
Estimates of the temperature in the capillary at different separation voltages[a]

U(kV)	T_s and T_c (°C)[b]			
	MeOH	EtOH	PrOH	BuOH
20	25.0 (25.6)	23.4 (23.6)	– (–)	– (–)
25	26.3 (27.2)	23.7 (23.9)	23.1 (23.2)	23.0 (23.0)
30	27.9 (29.3)	24.0 (24.4)	23.2 (23.3)	23.0 (23.1)
35	30.0 (32.0)	24.4 (24.9)	23.3 (23.4)	23.1 (23.1)
40	32.6 (35.4)	24.9 (25.6)	23.4 (23.5)	23.1 (23.1)
45	35.8 (39.5)	25.5 (26.3)	23.5 (23.7)	23.1 (23.2)
50	39.5 (44.3)	26.0 (27.0)	23.7 (23.9)	23.2 (23.2)
55	– (–)	– (–)	23.8 (24.1)	23.2 (23.3)
60	50.1 (58.0)	27.7 (29.2)	24.0 (24.3)	23.3 (23.4)

[a] Temperatures at the capillary surface (T_s) and centre (T_c, values in parenthesis) were calculated from the heat generation powers.

[b] Flow velocity of the air coolant $u_f = 3.8$ m s^{-1}; $d_o = 375$ μm; $r_2 = 172.5$ μm; $\eta = 0.153$ cm^2 s^{-1}; $R_e = 93$; $b = 0.615$; $n = 0.466$; thermal conductivities, $k_a = 0.02604$ W mk^{-1}, $k_{b\text{-MeOH}} = 0.2028$ W mk^{-1}, $k_{b\text{-EtOH}} = 0.1742$ W mk^{-1}, $k_{b\text{-PrOH}} = 0.1557$ W mk^{-1}, $k_{b\text{-BuOH}} = 0.1535$ W mk^{-1}, $k_{b\text{-silica}} = 1.38$ W mk^{-1}, $k_{b\text{-polyimide}} = 0.1550$ W mk^{-1} [18–20].

propanol the difference is less than 1.5°C (60 kV) and with butanol about 0.5°C (60 kV).

These results would suggest that Joule heating is minimal with propanol and butanol BGEs and should have little effect on the separation efficiency, and the decrease in the theoretical plate number with higher voltages cannot be explained in terms of Joule heating alone.

3.6. Van Deemter graphs

The Van Deemter equation describes the dependency of plate height H ($= L_{det}/N$) on flow (or migration) velocity v:

$$H = A + \frac{B}{\nu} + C_1\nu + C_2\nu \quad (5)$$

where A presents the effect of injection and detection on plate height, and the effect of longitudinal diffusion is presented by the term B/v. The last terms, C_1v and C_2v, respectively present the contribution of wall adsorption and temperature to band dispersion [21,22].

Fig. 6 presents the dependency of plate height on migration velocity for amphetamine and propranolol. The relatively strong curvature of the H vs. v graph

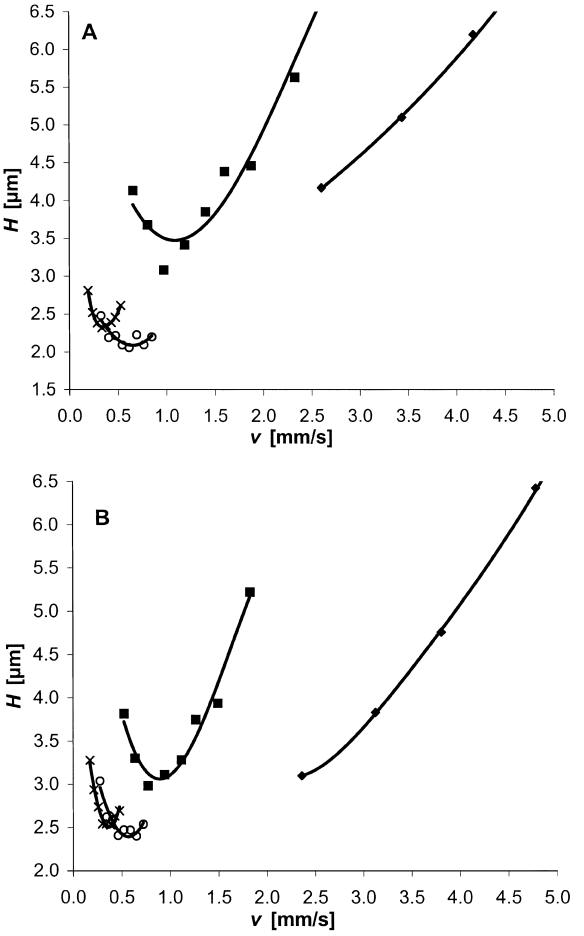

Fig. 6. The plate height dependency on migration velocity for (A) amphetamine and (B) propranolol. BGE solutions: 15 mM NaAc + 15 mM HAc in methanol (♦), ethanol (■), propanol (○), and butanol (×). The H vs. v curve for methanol BGE continues to higher migration velocities (data not shown). Experimental conditions as in Fig. 2.

at higher migration velocities is due either to the Joule heating or to the band broadening caused by wall adsorption. The longer the length of the alcohol chain the lower is the optimum velocity in regard to H. Note the relatively rapid increase of H in butanol BGE solution after the migration velocity exceeds 0.3 mm s^{-1} and the flatter profiles of the H vs. v curves for propanol BGE solution.

From Eq. (5) it can be seen that when the migration velocity is high enough, the initial length of the injection plug begins to influence the sepa-

ration efficiency, as also pointed out by Delinger and Davis [23]. At high voltages and migration velocities the longitudinal diffusion becomes less significant and the separation efficiency begins to be governed by the initial plug length. In this case, Eq. (1) is not obeyed even where the temperature and adsorption effects are negligible. The injection volumes in the present experiments are moderate, 5–8 nl (0.2–0.4% of capillary volume), and probably the contribution of the analyte plug width to the separation efficiency is stronger at high field strengths. According to Eq. (5), however, the influence of the initial plug length (constant term A) should not reduce the separation efficiency with higher migration velocities. As presented in Fig. 6, the plate height begins to increase (and the separation efficiency to decrease) after a certain migration velocity. Thus other terms in Eq. (5) must be affecting the plate height and the separation efficiency.

Probably the positively charged analytes are interacting with the capillary wall. From Fig. 5, it is seen that the plate number of DMSO does not decrease at higher voltages (except for methanol BGE solution). Neutral DMSO should be relatively immune to temperature effects, and also the adsorption on the capillary wall should be minimal. If temperature effects are negligible ($C_2 \approx 0$), which seems to be a reasonable assumption for propanol and butanol BGEs (see Table 2), the only factor increasing the plate height at high migration velocities is the wall adsorption. The term C_1 in Eq. (5) can be expressed as:

$$C_1 = \frac{K^2}{D}\frac{r_1}{r_1 + 2K} + \frac{4K}{(r_1 + 2K)k_d} \quad (6)$$

where K is the distribution coefficient between the bulk solvent and the wall and k_d is the rate constant of desorption [21]. Note the strong dependency of C_1 on K. As the alcohol chain length increases, the solubility of analytes in the separation medium decreases and sample affinity to the capillary wall increases (K increases). Additionally, the diffusion coefficients and the desorption rate constants of the analytes decrease as the alcohol chain length increases, which would again increase C_1. At constant C_1 within, for example, the butanol BGE separation series, the increased sample velocity at higher voltages leads to decreased separation efficiency because of wall adsorption. With ethanol BGE solution, the relatively strong increase in the plate height is probably due to the impact of both temperature effects and wall adsorption on the plate height.

4. Conclusions

The use of high electric field strength is advantageous, because separations can be performed faster with little or no loss of efficiency. With the right choice of solvent for BGE, the heat generation in the capillary can be reduced. In this work, Joule heating impacted negatively on the separations with methanol and ethanol BGE solutions, especially at high field strengths. This deteriorating self-heating effect was strongest with methanol BGE solution. With propanol and butanol BGEs, however, the temperature in the capillary was low even at high field strengths. The nonlinear behaviour of separation efficiency vs. separation voltage with propanol and butanol BGE solutions can be attributed to analyte adsorption on the capillary wall and in part also to injection and detection effects on peak dispersion. The effect of adsorption is similar to that reported by Ward and Khaledi, who concluded that plate numbers of several ANTS-derivatised oligosaccharides, which were separated in formamide BGE solution, decreased at higher voltages because of sample adsorption on the capillary wall [9].

The maximum plate numbers achieved were of the same order of magnitude for all four BGE solutions. However, the highest plate numbers were obtained with propanol BGE solution, evidently due to the not too high viscosity and low conductivity values of propanol. The low viscosity means that analysis times are fairly short which, in turn, keeps the peak broadening due to longitudinal diffusion low. Since the separation efficiency with propanol BGE solution was good at both low and high field strengths, we conclude that propanol is an attractive BGE solution for CE.

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