ELSEVIER

Journal of Chromatography A, 924 (2001) 147–154

JOURNAL OF CHROMATOGRAPHY A

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Extension of the application range of UV-absorbing organic solvents in capillary electrophoresis by the use of a contactless conductivity detector

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Abstract

A contactless conductivity detection (CCD) system is used for capillary zone electrophoresis (CZE) with non-aqueous solvents of the buffering background electrolyte, which exhibit strong UV absorbance below 230 nm. It is found that the CCD characteristics with such solvents (propylene carbonate, *N*,*N*-dimethylformamide and *N*,*N*-dimethylacetamide as examples) is the same as with aqueous solutions: the same signal and noise is obtained for a given electric conductance of the background electrolyte, independent of the kind of the solvent. Therefore CCD enables the extension of the application range to solvents with restricted use for common UV detection in CZE due to their unfavourable or even unfitting optical properties. The applicability of CCD is demonstrated by CZE of aliphatic ammonium compounds in these solvents.

Keywords: Contactless conductivity detection; Conductivity detection; Detection, electrophoresis; Background electrolyte composition; Propylene carbonate; Dimethylformamide; Dimethylacetamide

1. Introduction

Organic solvents as constituents of the background electrolyte (BGE) offer some advantages in capillary electrophoresis (CE). Compared to aqueous systems, they enhance solubility for many (especially lipophilic) analytes, and/or they can increase separation selectivity. Therefore their use in capillary zone electrophoresis (CZE) is becoming more and more popular (for reviews cf. e.g., Refs. [1,2]). From the practical point of view, organic solvents should have a number of properties to be favourably used: (i) different solvation should lead to selective changes of the mobilities of the analytes and/or their acido–basic properties compared to water. In this context the solvation ability for all particles involved in the equilibrium reactions of the analytes is of importance, also, e.g., for the proton and the molecular analyte particles. Not to overestimate is the ability of the solvent to dissolve the analytes, and the buffering electrolyte. (ii) The organic solvents should possess physico–chemical properties matching the requirements for practical use, e.g., a not too low boiling point, and a not too high melting point to allow working under feasible temperature conditions. A not too low dielectric constant reduces ion–ion interac-

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)00853-6

tions like ion pair formation (which would be strong in solvents with low dielectric constant). A low viscosity avoids too small mobilities of the analytes (which would lead to long run times, and to an only small separation window), and facilitates the handling of the solutions during filling and rinsing the capillary. It also avoids the presence of gas bubbles, which are often remaining in highly viscous solutions. (iii) Chemical stability is needed to exclude reactions with the sample and buffer. Hygroscopic solvents complicate controlling the content of water. Low toxicity facilitates handling of buffers. (iv) Further favourable aspects are the commercial availability of solvents with high purity, and at a low price. Finally (v) the suitability with the detector is an important parameter. Thus, from the large number of organic solvents known surprisingly only few match these criteria.

A substantial restriction in CE with UV–Vis detection is given by the fact that many solvents have a high absorbance in the wavelength range normally used. The most common solvents used with UV–Vis detection are those also common in high-performance liquid chromatography (HPLC): the lower alcohols (mainly methanol) and acetonitrile. Solvents with higher absorbance – formamide (FA); *N*-methylformamide (NMF); *N*,*N*-dimethylformamide (DMF); *N*,*N*-dimethylacetamide (DMA); dimethyl sulfoxide (DMSO); acetone, propylene carbonate (PC, 4-methyl-1,3-dioxolan-2-one) – were used so far in CZE only in a very limited number of applications, with severe restrictions concerning the choice of the analytes (that absorb at considerably high wavelength) and the separation conditions to meet the demands of UV–Vis detection. This is because these solvents have a optical cut-off around 230 to 260 nm, which make them unsuitable for detection lower than around 240 nm, and leads to high detection noise even at higher wavelengths.

Conductivity detection (CD) has no such restriction as optical detection, and should allow the use of solvents in CZE independent on their optical properties. According to the criteria listed above the following solvents might be generally useful thus: in addition to the restrictedly used amides like DMF and DMA, PC, sulfolane (TMS, tetramethylene sulfone), and DMSO. It should be pointed out that also mixtures of these solvents with other organic solvents, or with water most probably have a great potential. In fact we have used already aqueous DMSO mixtures as solvents for CE in the isotachophoretic mode with conductivity detection [3–5].

One should take into account possible limitations of some of these solvents, e.g., due to a considerably high viscosity (PC, DMSO, especially TMS). However, a potentially advantageous effect accompanied by the low mobilities is the applicability of high voltages, because the currents established are small. However, the low heat conductance of the organic solvents might level this effect.

Many solvents mentioned above have been used in the past for acid–base titrations, and for investigation of ion transport phenomena in physical chemistry. Thus data of pK_a values and ion conductance for an (although small) number of solutes is available from the literature as a starting point for more systematic investigations by CE (see, e.g., the literature cited in Refs. [6,7]). It is the goal of the present contribution to demonstrate the advantage of contactless conductivity detection (CCD) for the use of organic solvents in CE. Detection is carried out with a high-frequency conductivity cell without contact to the solvent.

Three UV-absorbing solvents are selected: DMF, DMA and PC (in addition we did not make any restrictions concerning the UV absorbance of the constituents of the buffer as well). The solvents belong to the class of aprotic dipolar solvents, that means that they have a high dielectric constant (>30; note the dielectric constant of 63 for PC), and are not H-bond donors [8–13]. For this reason they have low ability to stabilise anions compared to water. Although some of them are stronger bases than water (especially DMF and DMSO), they shift the pK_a values of neutral acids (of type HA) significantly to higher values. This is in contrast to cation acids of type HB^+ (the corresponding acids of the bases, B), for which pK_a is influenced much less [7–9,11–13].

Most of the work in the past was dealing rather with the analysis of particular sample constituents than with the investigation of the individual properties responsible for their separation, and it was dealing mainly with solvents with low UV absorbance. The present evaluation will serve as a starting

point to a more detailed and systematic investigation on the properties that determine the electrophoretic behaviour of solutes in solvents with high UV absorbance, namely the pK_a values and the mobilities as those parameters, which reflect the ion–solvent and the ion–ion interactions in solutions.

2. Experimental

2.1. Chemicals

DMF (99.8%) and DMA (99.9%) were obtained from Aldrich (Steinheim, Germany), PC (99%) from Fluka (Buchs, Switzerland). Tetraethylammonium hydroxide (20%), acetic acid (glacial, 99.7%) and 2,6-dihydroxybenzoic acid (98%) were obtained from Aldrich. Tris(hydroxymethyl)aminomethane (Tris) and potassium chloride, both analytical grade, were obtained from E. Merck (Darmstadt, Germany).

Tetramethylammonium (TMA) benzoate (97%), tetrapropylammonium (TPA) perchlorate (98%), dipropylamine (DPA) (99%), *N*-methylcyclohexylamine (MCHA, 98%) and dodecylamine (DA, 98%) were obtained from Fluka. Propylamine (PA) hydrochloride (99%) and *N*,*N*-dimethyldodecylamine (DMDA, 97%) were obtained from Aldrich. Tetrabutylammonium (TBA) bromide, analytical-reagent grade, was obtained from Lachema (Brno, Czech Republic).

2.2. Instrumentation

All experiments were carried out with a ^{3D}CE instrument (Agilent Technologies, Palo Alto, CA, USA) with fused-silica capillaries of 60.0 cm (length to the CCD system 46.0 cm)×50 μm I.D.×363 μm O.D. (Supelco, Bellefonte, PA, USA). The instrument was equipped with a laboratory-made high-frequency contactless conductivity detector [14]. Its construction has some features of the contactless detectors described previously [15–17]. Two metal cylinders (surrounding the separation capillary in series) form two electrodes that are bonded through the capacity of the capillary wall to the electrolyte solution inside the capillary. Both electrodes are supplied by a high-frequency voltage supply with a frequency of 625 kHz. The electronics evaluates the high-frequency current in the circuit, which is dependent on the resistivity of the electrolyte solution present in the gap between the electrodes. A metal disc placed between the electrodes has an auxiliary function: it is connected to ground and prevents from a direct capacitive bond between the electrodes.

The detector cell together with the electronics is built into the cassette of the ^{3D}CE instrument and has a compact construction. Output signal is processed by the HP3900E A/D converter (Agilent Technologies) and analysed using Chemstation software. The capillary column passes through both the conductivity cell and the alignment of the UV diode-array detector. The distance between both detector cells is 6.0 cm. The employed configuration easily enables one to use both detection techniques simultaneously.

2.3. Procedures

The BGEs in organic solvents were prepared by dissolving an appropriate amount of 2,6-dihydroxybenzoic acid in DMF, DMA and PC, respectively, and by adding half of the molar concentration of tetraethylammonium hydroxide (in a 20% aqueous solution). In this way no adjustment of the pH* was needed. The BGE in water was prepared by diluting an appropriate amount of Tris in water, and by adjusting the pH with acetic acid with a glass electrode.

As the result of the use of an aqueous solution of tetraethylammonium hydroxide and of the neutralisation reaction the running electrolytes contains certain amount of water – 1.5% for the 50 mmol l^{-1} buffer in PC, and 0.6% for the 20 mmol l^{-1} buffer in DMF and DMA, respectively.

3. Results and discussion

3.1. Performance of the conductless conductivity detector

In Fig. 1 the CCD response on the conductivity of the BGE solution (the calibration function) is shown for water, DMF, DMA and PC as solvents. The signal is between 220 and 850 mV for a conductivity of the solution ranging from 5 to 1300 mS m^{-1}. It is

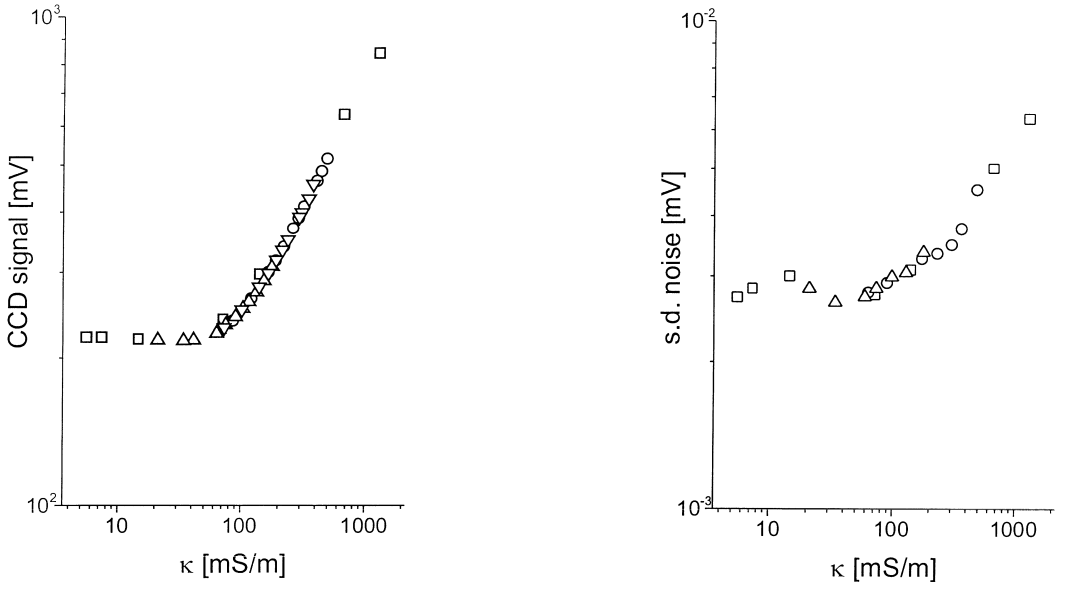

Fig. 1. Conductivity detector signal (left) and standard deviation of the high frequency noise (right) in dependence on the conductivity, κ, of the solution with various solvents. The noise is expressed by the standard deviation from 1200 digital data points collected from the baseline within 2 min at a period of 100 ms in absence of drift or jumps. The background electrolyte in the organic solvents was an equimolar mixture of tetraethylammonium 2,6-dihydroxybenzoate and 2,6-dihydroxybenzoic acid at various concentrations; in water potassium chloride was used. Conductivity in the organic solvents was calculated from the current in the capillary according to Ohm's law. Conductivity in water was taken from tabulated data [25]. Separate capillaries were used for each solvent. Symbols: □ water; ○ DMF; ▽ DMA; △ PC.

seen that, as expected, the detector signal is not depending on the solvent for a particular conductivity of the solution. Therefore it is indeed perfectly suited to the use of organic solvents without any restrictions to their optical properties. From the figure it can be seen that the detector signal is about constant at very low conductivities of the solution. This is due to electronic and mechanical construction details: a crosstalk between two electrodes of the measuring cell causes that some parasitic additional high-frequency signal is added to the useful signal, which passes through the solution in the detector gap. In the conductivity range above 120 mS m^{-1} the calibration function is almost linearly increasing with increasing conductivity, being thus the most favourable working range for quantitative analysis. Note, however, that linearity is not a prerequisite for the usability of a detector in CZE: due to a very small change of the signal in presence of an analyte the peak area can be still linearly dependent on the sample amount even when working in the non-linear part of the calibration function. In Fig. 1 the noise of the detector with frequency higher than about 1 Hz is depicted as a function of the signal, too. The same shape of the curve is obtained as for the response, however, the signal curve has a steeper slope at higher conductivities. The standard deviation of the noise is in the range between 3 and 7 μV.

In Fig. 2 the CCD signal is depicted as a function of the concentration of the BGE in the solution of the different organic solvents. The different slopes of the curves are caused from the different mobilities of the electrolyte ions in the individual solvents. It is known from the literature that the (absolute) mobilities decrease in the sequence DMF>DMA>PC [18], in the same sequence as the solvent viscosity increases (viscosity: DMF 0.7939 cP, DMA 0.927 cP, PC 2.53 cP [19]). Indeed the slopes of the curves give the same ratio as the reciprocal viscosities (in agreement with Walden's rule): for DMF (slope 1 640 mV l mol^{-1}, see Fig. 2) and PC (slope 510 mV l mol^{-1}), e.g., the value of both ratios is 3.2.

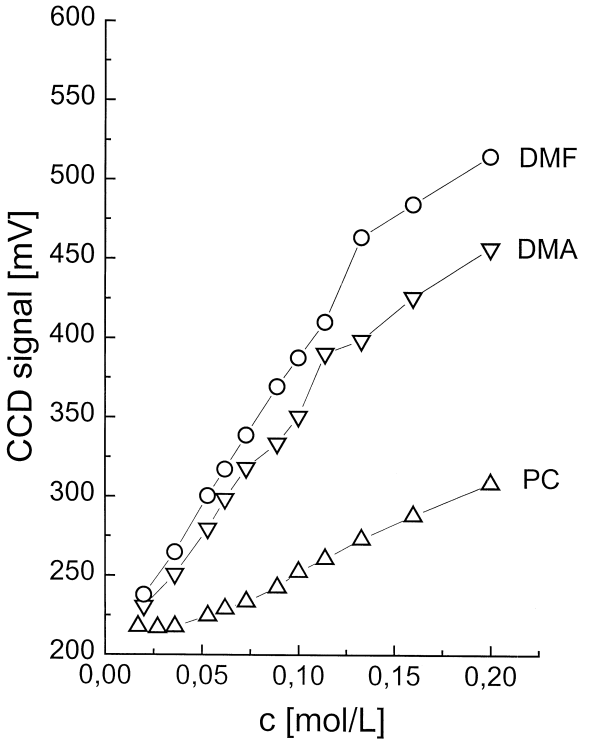

Fig. 2. CCD signal as a function of the electrolyte concentration, *c*, in the organic solvents. In all solvents the electrolyte was an equimolar mixture of tetraethylammonium 2,6-dihydroxybenzoate and 2,6-dihydroxybenzoic acid at various concentrations. Symbols: ○ DMF; ▽ DMA; △ PC.

3.2. CZE with CCD in non-aqueous solvents

In order to evaluate the applicability of CCD for CZE in non-aqueous media, a number of aliphatic amines (and/or their ammonium ions) were run in DMF, DMA and PC, respectively. The pK_a^* values of only two amines (the values are for the respective ammonium as corresponding cation acid) were found in the literature, and only for DMF: they are 9.25 for triethylammonium (water 10.7) and 9.10 for butylammonium (water 10.6) [11–13]. For DMA similar values can be assumed. It can be seen that DMF as solvent slightly decreases the pK_a values of these cation acids, compared to water, which is a well-known phenomenon for many organic solvents (and is in contrast to the effect on the neutral acids of type HA). It should be mentioned that for PC no data for pK_a^* values of cation acids were found. Note that all pK_a^* values are based on a conventional pH* scale [20].

The pH (or pH*) of the BGE was chosen significantly lower than the pK_a^* of the amines to fully protonise the analytes. Thus, 2,6-dihydroxybenzoic acid was taken as buffer constituent, which has a pK_a^* of 3.56 in DMF (water 1.2) [13]. A similarly low value in DMA is expected as well. To avoid systematic measuring errors of the pH* by the use of a glass electrode (calibrated with aqueous buffer solutions, and consisting of a salt bridge with aqueous solutions) an equimolar mixture of free 2,6-dihydroxybenzoic acid and tetraethylammonium 2,6-dihydroxybenzoate was taken as buffer. According to the Henderson–Hasselbalch equation this solution has a pH* equal to the pK_a^*; it does not need further adjustment. In water, acetate–Tris at pH 4.75 was used. The pH* in PC will be discussed below.

For comparison CZE was carried out in aqueous BGE solution as reference. The resulting electropherogram in water as solvent is given in Fig. 3. As usual in conductivity detection, the response of the detector depends on the ratio of the mobility of the analyte and the co-ion of the BGE, respectively. It can be seen that the migration order of the ammonium ions follows roughly their molecular mass (or their mole volume), but not in detail. The deviation is obvious because the mobility is determined rather by the size of the solvated ion than by that of the bare ion in the crystal. Further, even for ions with the same mass the shape of the ion (spherical or elliptical, oblate or prolate) plays a role as well (Ref. [21]). Two analytes are not resolved (TPA and DA), in agreement with the literature values [22] of their absolute mobilities ($24.3 \cdot 10^{-9}$ and $24.7 \cdot 10^{-9}$ $m^2\ V^{-1}\ s^{-1}$).

The electropherogram of the ammonium ions in DMF as solvent is shown in Fig. 4. It can be seen that the detector response is in the same range as for aqueous solutions. The noise is in the same order of magnitude, whereby some baseline drift is seemingly not caused by an instability of the detector cell, but has some chemical reasons concerning the BGE. However, the migration sequence changes drastically compared to water, and all analytes are separated here. The same sequence as in DMF is found with DMA (Fig. 5), whereby a slight decrease of the

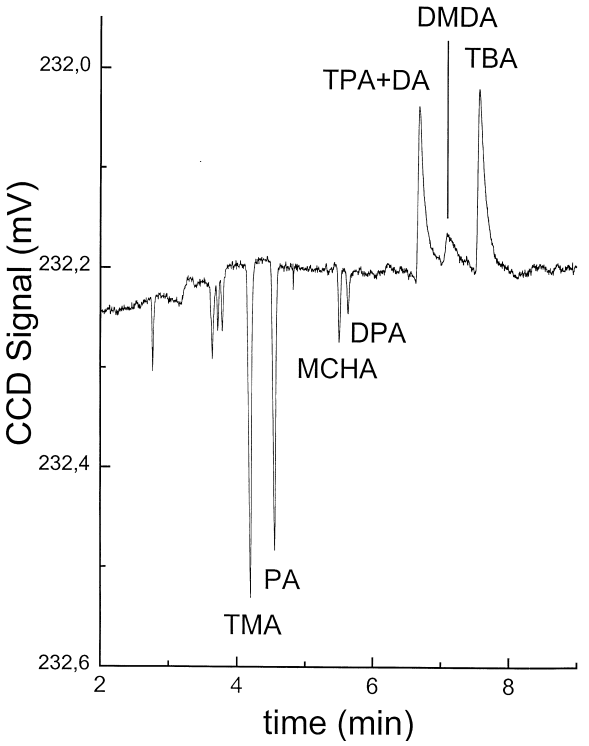

Fig. 3. Electropherogram of the analytes in aqueous BGE solution with conductivity detection. Conditions: capillary: 60.0 cm (length to the detector 46.0 cm)×50 μm I.D.; buffer: acetic acid–Tris acetate (10 mmol l^{-1} each), pH 4.75; voltage: +20 kV; current: ~4.8 μA; sample: analyte concentration 1 mmol l^{-1} each; injection: 50 mbar s. Analytes: TMA, tetramethylammonium; PA, propylammonium; DPA, dipropylammonium; TPA, tetrapropylammonium; MCHA, *N*-methylcyclohexylammonium; TBA, tetrabutylammonium; DA, dodecylammonium; DMDA, *N,N*-dimethyldodecylammonium. The small peaks detected between 2.5 and 4 min stem from Na^+, K^+, Ca^{2+} and Mg^{2+} impurities of water.

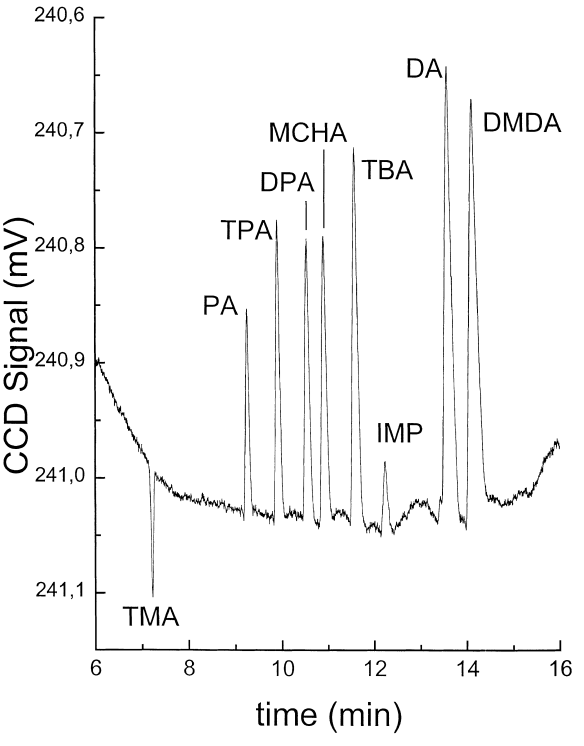

Fig. 4. Electropherogram of the analytes in DMF as solvent. Conditions: buffer: 2,6-dihydroxybenzoic acid–tetraethylammonium 2,6-dihydroxybenzoate (10 mmol l^{-1} each); voltage: +20 kV; current: ~5.8 μA; sample: 1 mmol l^{-1}; injection: 50 mbar s. Capillary and analyte abbreviations as in Fig. 3. IMP is an impurity.

resolution is observed here, especially for DA and DMDA.

PC as solvent has not been used in CE of ionic analytes so far. One application has shown the applicability of PC for the separation of neutral solutes by the aid of ''solvophobic'' interactions with alkylammonium ions by a kind of electrokinetic chromatography [23]. From Fig. 6 it is seen that it is a well-suited solvent for the purpose of CZE of ions, and the detector responses correctly with this solvents as well. Also in this solvent the sharp peaks typical for CZE can be observed, resulting in plate numbers, *N*, of 170 000 for TPA and 200 000 for TMA. These values can be compared with the theoretically reachable limit of around 300 000. The effective voltage, *U*, taken for this calculation is 15 300 V, and the charge number, *z*, is 1. This latter value for the plate number is calculated from the relation $N \approx 20zU$, which should be valid for infinite dilution also in such solvents, under the assumption that only longitudinal diffusion is the cause of peak broadening. This limiting case is not necessarily reached, mainly due to possible electromigration dispersion. Concerning peak broadening due to Joule heating we should take into account that the plate height is proportional to the square of the ratio of electric to thermal conductivity of the BGE [24]. The mobilities of the ions (and thus the electric conductance) are reduced in PC by a factor of around 3, compared to water. By about the same factor the

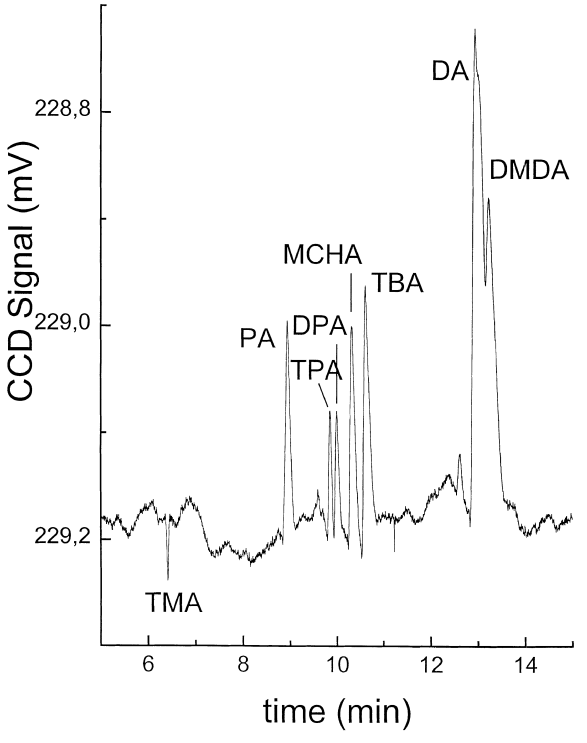

Fig. 5. Electropherogram in DMA as solvent. Conditions: buffer: 2,6-dihydroxybenzoic acid–tetraethylammonium 2,6-dihydroxybenzoate (10 mmol l^{-1} each); voltage: +30 kV; current: ~6.5 μA; sample: 1 mmol l^{-1}; injection: 50 mbar s. Capillary and analyte abbreviations as in Fig. 3.

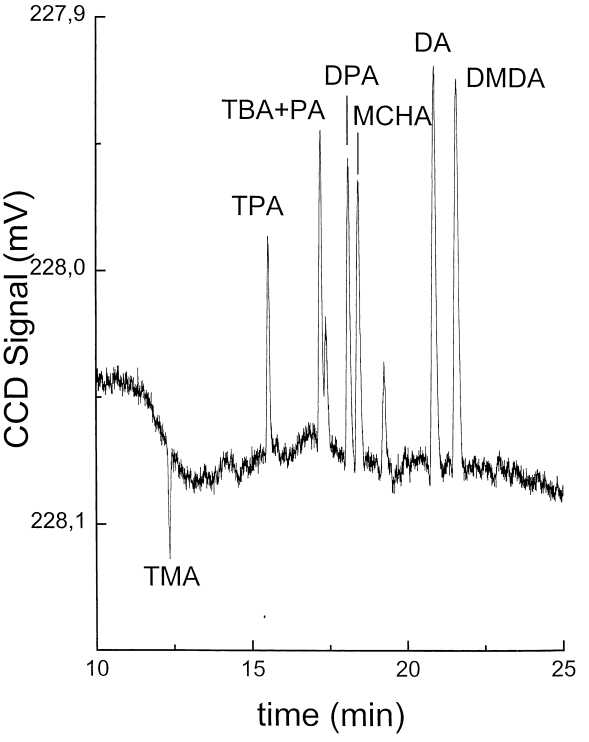

Fig. 6. Electropherogram in PC as solvent. Conditions: buffer 2,6-dihydroxybenzoic acid–tetraethylammonium 2,6-dihydroxybenzoate (25 mmol l^{-1} each); voltage: +20 kV; current: ~3.9 μA; sample: 1 mmol l^{-1}; injection: 50 mbar s. Capillary and analyte abbreviations as in Fig. 3.

thermal conductivity is smaller (the thermal conductivity of most organic liquids at 25°C is in the range of 1.5 to 2 mW cm^{-1} K^{-1}, whereas water has about 6 mW cm^{-1} K^{-1} [25]). This means that the contribution of Joule heating to peak broadening should be in the same order of magnitude for aqueous and organic solvents. It should be pointed out that the given expression for N holds only in the absence of an electroosmotic flow (EOF), which is not strictly fulfilled under the present experimental conditions. However, the EOF is so small that it is neglected here.

More striking in PC than the reasonable efficiency is a totally changing migration sequence: all tetraalkylammonium ions have a higher mobility than the primary, secondary and tertiary ammonium ions. These changes in the total mobilities (the sum of effective mobility and that of the EOF) of the analytes in the different solvents is clearly visualised in Fig. 7. As we do not anticipate that the size of the unsymmetrical ions is selectively increased due to specific solvation by PC, the reason for the smaller relative mobility of these ions lies most probably in

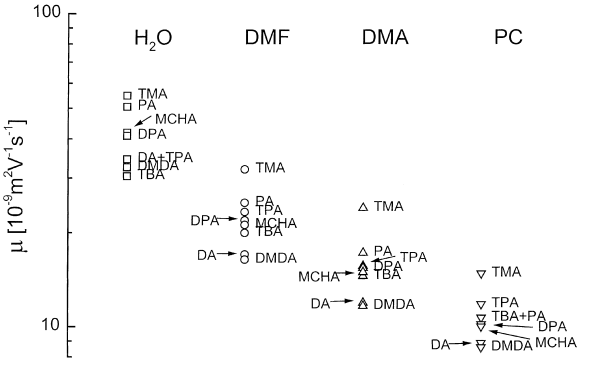

Fig. 7. Total mobility, μ, of the analytes in the different solvents. Abbreviations as in Fig. 3.

their incomplete protonation in this solvent, and thus in a smaller effective charge number.

Unfortunately there are nearly no data available for pK_a^* values in PC, neither for the buffering benzoic acid, nor for the analytes. Only one data is found: salicylic acid (which has some similarity with the 2,6-dihydroxybenzoic acid used as buffer) the pK_a^* increases in PC compared to water by 12 pK units [13]. Given that the magnitude of the change is similar for the buffering 2,6-dihydroxybenzoic acid, the pH* of the BGE would be in the range of 13. Also assumed that PC does not change the acidity of the analytes strongly, we can expect the resulting values of the ammonium cation acids in the range of the pH* of the BGE. As the primary, secondary and tertiary amines will thus not be fully protonised, their charge will be lower than that of the tetraalkylammonium ions with their permanent charge. The mobility is smaller for the former compared to the latter, which explains the changing separation selectivity in PC compared to the other solvents. However, the shift of the pK_a^* values of all the acids needs further investigation. This is the topic of current work.

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