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Technical Notes

Nonaqueous Electrophoresis Microchip Separations: Conductivity Detection in UV-Absorbing Solvents

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The use of organic solvents in microfabricated capillary electrophoresis (CE) devices is demonstrated in connection with the separation of aliphatic amines in pure dimethylformamide, dimethylacetamide, dimethyl sulfoxide, or propylene carbonate media. Contactless conductivity detection is employed for monitoring the separated solutes in these UV-absorbing solvents. The effect of the physicochemical properties of the organic solvent upon the migration behavior is investigated. The apparent mobility increases nearly linearly with the reciprocal of the solvent viscosity, while the electroosmotic mobility increases in a linear fashion with the dielectric constant/viscosity ratio. Some deviation from theoretical predictions is observed using propylene carbonate. The nonaqueous CE microchip offers high separation efficiency, reflected in plate numbers ranging from 93 680 to 127 680, using a separation voltage of +3000 V, a dimethylformamide medium, and a contactless conductivity detection. Experimental parameters affecting the analytical performance of the nonaqueous CE/conductivity microchip are examined. Calibration and precision experiments indicate a linear and reproducible response. Such use of organic solvents can benefit microchip separations through extended scope (toward nonpolar solutes) and tunable selectivity.

"Lab-on-a-chip" devices have experienced an enormous growth since their introduction.[1,2] Such microscale devices offer many potential advantages over large analyzers, including speed, efficiency, portability, automation, solvent/reagent economy, integration, and cost. Particular attention has been given to microchip-based capillary electrophoresis (CE) separations.[3,4] Such separations are usually carried out using aqueous run buffers with the exception of a solvent programming separation (using up to 50% acetonitrile).[5] Organic solvents were employed on microchip platforms for eluting analytes during on-chip solid-phase extraction,[6,7] in connection with sample introduction into mass spectrometry,[8] or for performing organic reactions in microreactors.[9]

The use of the organic solvents has been shown to offer several advantages in conventional CE separations, including enhanced solubility of hydrophobic analytes (i.e., extended application range) and tailored selectivity of separations (via changes in solvation or acid−base properties of analytes).[10−12] Yet, despite of these capabilities, little attention has been given to the use of organic solvents to enhance microchip separations.

The goal of this contribution is to demonstrate effective nonaqueous CE microchip separations and to investigate the influence of solvent properties on the performance of microfabricated separation devices. Several organic solvents have been used as alternatives to water in classical capillary electrophoresis.[10−12] Particular attention has been given to the use of methanol and acetonitrile due to their widespread availability and compatibility with UV detection. Yet, the electrophoretic mobility of solutes in methanol and acetonitrile is influenced by ion association or ion-pairing effects, which complicate predictions of the electrophoretic mobilities and rational optimization of the separation process. Other organic solvents, e.g., *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), *N*,*N*-dimethylacetamide (DMA), or propylene carbonate (PC), offer great promise for nonaqueous CE in view of their high dielectric constants (i.e., minimal ion pair formation), high viscosity, and solubility of many potential analytes. However, conventional CE applications of these solvents have been limited due to their optical cutoff around 230−260 nm (that hinders UV detection).[13] In the following sections, we examine the utility of these (UV-absorbing) organic solvents for microchip CE separations in connection with a contactless conductivity detection of model alkylammonium ion

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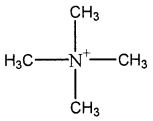
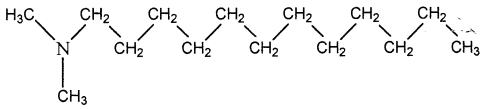
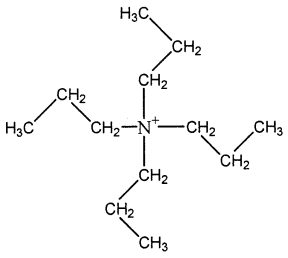
Chart 1. Structural Formulas of the Analytes



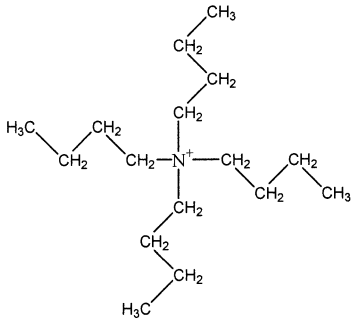
Tetramethylammonium ion
(TMA)



N,N,-dimethyldodecylamine
(DMDA)



Tetrapropylammonium ion
(TPA)



Tetrabutylammonium ion
(TBA)

analytes (Chart 1). Such microchip conductivity detection allows use of diverse solvents, independent of their optical properties, in a manner shown in conventional CE systems.[13]

EXPERIMENTAL SECTION

Reagents. DMF (99.9%), DMA (99.9%), DMSO (99.9%), PC (99.7%), tetramethylammonium bromide (TMA), tetrapropylammonium chloride (TPA), tetrabutylammonium perchlorate (TBA), *N*,*N*-dimethyldodecylamine (DMDA), tetraethylammonium hydroxide (20% solution in water), and 2,6-dihydroxybenzoic acid were received from Aldrich.

The run buffers included DMF, DMA, DMSO, or PC containing 5 mM 2,6-dihydroxybenzoic acid and 5 mM tetraethylammonium 2,6-dihydroxybenzoate.[13] A 0.3% final water content is expected from the aqueous content of the tetraethylammonium hydroxide solution. The pH* of the run buffer was chosen to be significantly lower than the pK_a* of the analytes, hence ensuring their full protonation. This was attained by using an equimolar buffer mixture of 2,6-dihydroxybenzoic acid and tetraethylammonium 2,6-dihydroxybenzoate.[13]

Apparatus. The homemade high-voltage power supply had an adjustable voltage range between 0 and +4000 V. The CE microchip, with the contactless conductivity detection system, was similar to the one described earlier.[14] The simple cross glass microchip was obtained from Micralyne (model MC-BF4-001, Edmonton, Canada); the cover plate of the glass chip was polished to a 150-μm thickness to facilitate the contactless conductivity detection.[14] The microchip had a four-way injection cross connected to three 5-mm-long arms and a 80-mm-long separation channel. The effective length of the separation channel for on-column contactless conductivity detection was 73 mm. Short pipet tips were inserted into the holes of the buffer and sample reservoirs. A Plexiglas holder was fabricated to house the separation chip and the buffer/sample reservoirs.[14] An Agilent 33120A function generator (Agilent, Palo Alto, CA) was used for generating the sinusoidal ac waveform for the conductivity detection. Contactless conductivity detection was accomplished using a frequency of 200 kHz with a peak-to-peak amplitude of 5 V_{p-p}. The electronic circuitry of the conductivity detector was placed on the chip cover.[14] The circuitry was designed in accordance with a previously reported scheme.[14,15]The electronic components were purchased from local suppliers. The rectangular-shaped conductivity electrodes (0.8 mm × 10 mm) were fabricated from two 10-μm-thick aluminum foil strips. The end side of the electrode was widened to 4 mm to facilitate the electrical connection. The electrodes were fixed to the top of the 150-μm-thick cover plate of the glass microchip using a common epoxy, at a 7-mm distance from the end of the microchannel and with a 1.0-mm spacing between them. Thin copper wires (0.1 mm diameter, 15 mm long, Aldrich) were attached to the electrodes using a conducting epoxy (Chemtronics, Kennesaw, GA) and were tin-soldered to the detector electronics. The electrodes were placed in an "antiparallel" orientation to minimize the stray capacitance between them.[14,16]

Electrophoresis Procedure. The channels of the glass chip were treated before use by rinsing with appropriate run buffer for 1 h. The run buffer and unused reservoirs were filled with the organic background electrolyte solution, while the sample reservoir was filled with a mixture of alkylamines. After the initial sample loading in the injection channel, the sample was injected by applying a potential of +1500 V between the sample reservoir and the grounded outlet reservoir. This drove the sample "plug" into the separation channel through the intersection. The separation potential was subsequently applied to the run buffer reservoir with the outlet reservoir grounded and all other reservoirs floating for the separation of the analytes.

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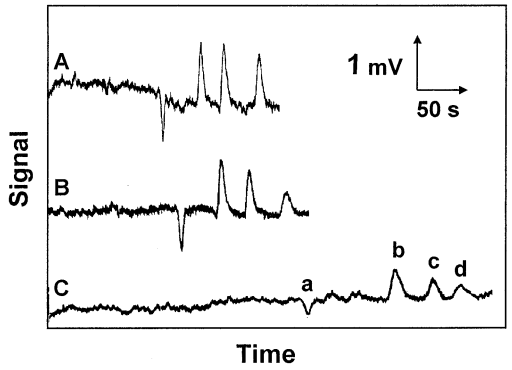

Figure 1. Effect of different organic solvents upon the separation and detection of alkylamines. Solvents: DMF (A), DMA (B), and DMSO (C). Solutes: TMA (a), TPA (b), TBA (c), and DMDA (d), 500 μM each. Separation voltage, 1500 V, injection 1500 V for 1 (A, B) or 2 s (C); frequency, 200 kHz; peak-to-peak amplitude, 5 V_{p-p}. Buffer: 2,6-dihydroxybenzoic acid/tetraethylammonium 2,6-dihydroxybenzoate (5 mM each).

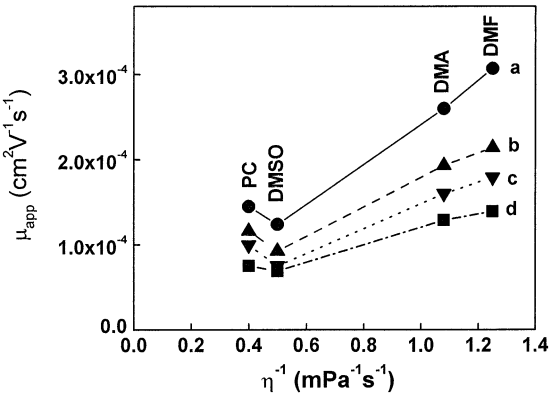

Figure 2. Dependence of the experimentally obtained apparent mobilities upon the reciprocal viscosity of the solvents. Analytes: TMA (a), TPA (b), TBA (c), and DMDA (d). Other conditions, as in Figure 1.

Safety Considerations. *The high-voltage power supply should be handled with extreme care to avoid electrical shock. Tetramethylammonium bromide and N,N-dimethylformamide are highly toxic and other chemicals are irritants. Skin contact and accidental inhalation or ingestion should be avoided.*

RESULTS AND DISCUSSION

The aim of this study has been to demonstrate effective nonaqueous separations on microchip platforms and to establish the factors governing such separations. Figure 1 displays typical electrophoregrams for a mixture of several alkylammonium ions and alkylamine in different organic solvents: dimethylformamide (A), dimethylacetamide (B), and dimethyl sulfoxide (C). Well-defined and resolved peaks are observed in these three nonaqueous media for all four cations. The half-peak widths in the DMF, DMA, and DMSO solvents range from 2.8 to 6.0 s, from 4.1 to 9.3 s and from 6.2 to 13.5 s, respectively. The four analytes are separated within 5−10 min. (Shorter assays, e.g., 2 min, are described below in connection with higher separation voltages). The organic solvent does not affect the migration order (that nearly follows the molecular mass). The defined response, coupled to a favorable signal-to-noise ratio, offers convenient quantification of submillimolar levels of the alkylamine and alkylammonium ions analytes.

The influence of the organic solvent upon the apparent mobilities and electroosmotic mobility has been assessed. It is known that the ion mobility in electrophoresis depends on the solute charge (q), its radius (r), and solvent viscosity (η) according to[17]

$$\mu = q/6\pi\eta r \qquad (1)$$

Therefore, the mobility of the analyte is expected to decrease upon increasing the solvent viscosity. Figure 2 shows the experimentally obtained dependence of the apparent mobilities of the alkylammonium ions and alkylamine analytes upon the reciprocal viscosity of different organic solvents (based on literature values of η[18]). The mobilities of all four analytes are in good agreement with eq 1 and the Walden rule (constancy of the product of μ_{app} and viscosity), i.e., displaying a nearly linear dependence of the apparent mobility of the solutes with the reciprocal viscosity of DMF, DMA, and DMSO. The Walden product ($\mu_{app}\eta$)[19] for a particular ion varies only between 1 and 14% (for DMF, DMA, and DMSO). In contrast, some deviation from the Walden rule is observed for the ion mobilities in propylene carbonate. Such deviation is in agreement with previously published mobility data in that solvent.[19] Note also that the Walden rule is based on a simple model, describing ionic solutes as spherical rigid particles moving in the continuum with a viscosity equal to their macroscopic viscosity. This model thus ignores changes in the solvent structure in the surroundings of the ion or changes in the ionic radii due to different degrees of solvation that may affect the Walden product.[19,20] Such changes may account for the slight changes from constancy in the case of DMF, DMA, or DMSO.

It is also important to examine the influence of the organic solvent upon the electroosmotic mobility. The dependence of the electroosmotic mobility (μ_{eo}) of the bulk liquid on the basic physicochemical properties of the solvent is given by the von Smoluchowski equation:[21,22]

$$\mu_{eo} = -(\epsilon_0\epsilon\zeta/\eta) \qquad (2)$$

where ϵ_0 is the permittivity of vacuum, ϵ is the dielectric constant of the solvent, η is its viscosity, and ζ is the zeta potential. Based on eq 2, the electroosmotic mobility should increase linearly with ϵ/η (considering a constant zeta potential). Figure 3 displays the dependence of the electroosmotic mobility (μ_{eo}) upon the dielectric constant/viscosity ratio of different solvents. (The μ_{eo} was calculated from the time of the electroosmotic flow system peak.[23]) A linear dependence is observed for the DMSO, DMA, and DMF

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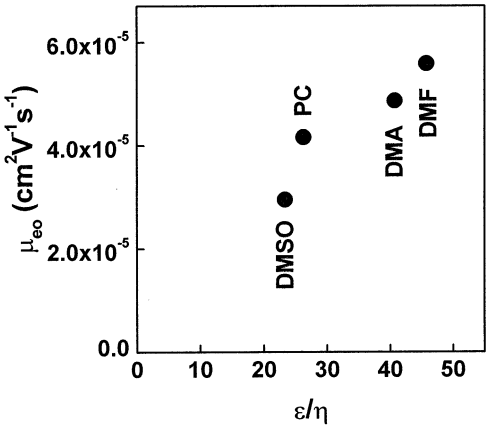

Figure 3. Dependence of the experimentally obtained electroosmotic flow mobilities upon the ratio of the dielectric constant of the solvent and its viscosity (ϵ/η). Other conditions, as in Figure 1.

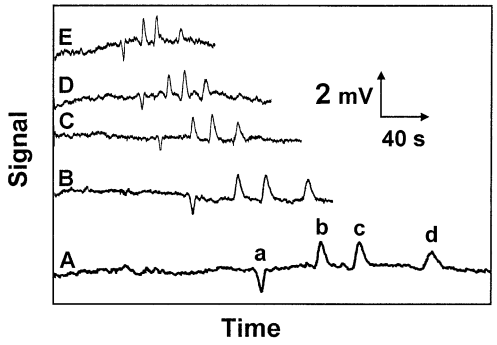

Figure 4. Effect of the separation voltage upon the response of 500 μM TMA (a), TPA (b), TBA (c), and DMDA (d) using DMF as the organic solvent and separation voltages of 1000 (A), 1500 (B), 2000 (C), 2500 (D), and 3000 V (E). Other conditions, as in Figure 1.

solvents; a slight deviation from linearity is observed in the case of PC. Deviations from eq 2 were also reported in conventional CE nonaqueous systems.[22]

Various experimental parameters affecting the analytical performance of the nonaqueous capillary electrophoresis/conductivity microchip were examined and optimized. Figure 4 shows the influence of the separation voltage upon the response of alkylammonium ions in DMF medium using voltages of +1000 (A), +1500 (B), +2000 (C), +2500 (D), and +3000 V (E). As expected, increasing the separation voltage from +1000 to +3000 V (i.e., from a separation field of 125 to 375 V/cm) dramatically decreases the migration times of TMA (a), TPA (b), TBA (c), and DMDA (d) from 168 to 55.5, from 225 to 75.9, from 255 to 86.4, and from 319 to 108 s, respectively. The shorter migration times observed at higher fields are coupled to substantially sharper peaks. The plate number for the four cations thus increases from 84 400 to 127 680 (TMA), from 71 000 to 121 500 (TPA), from 71 400 to 99 500 (TBA), and from 68 360 to 93 680 (DMDA) over the +1000- and +3000-V range. Such values reflect the high separation efficiency of the nonaqueous microchip system and are compared favorably with those reported for aqueous-phase separations of inorganic ions using a similar microchip system.[14] Obviously, the nonaqueous separation media ensure good separation efficiency. The separation voltage has a negligible effect upon the peak-to-peak background noise level for voltages ranging from +1000 to +2000 V. Slightly higher background and noise levels (associated with Joule heating effects)[14] are observed at higher separation voltages. Yet, a nearly flat baseline is observed even at +3000 V (E) reflecting the effective isolation of the detector.

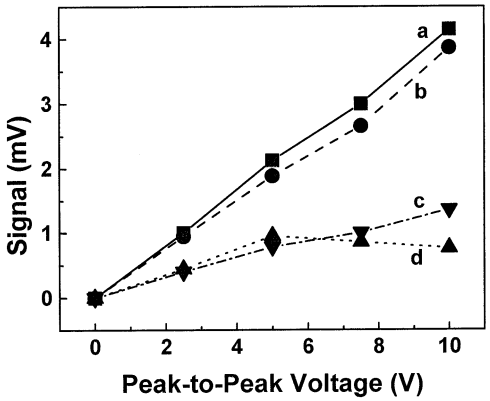

Figure 5. Influence of the peak-to-peak amplitude of the applied ac voltage upon the detector response for 0.6 mM TBA using different organic solvents, DMF (a), DMA (b), PC (c), and DMSO (d). Other conditions, as in Figure 1.

As expected, the response of the contactless conductivity detector is strongly dependent upon the amplitude of the applied voltage. Figure 5 depicts the influence of the peak-to-peak amplitude of ac voltage (V_{p-p}) upon the TBA signal in DMF (a), DMA (b), DMSO (c), and PC (d) solvents. The response increases linearly over the entire range (0−10 V_{p-p}) for DMF, DMA, and PC solvents while in DMSO the response reaches maximum at 5 V_{p-p} and levels off thereafter. This behavior is similar to that observed in aqueous systems where different run buffers resulted in different V_{p-p} effects.[14,24] Such amplitude change results also in a nearly linear increase of the noise level and in a reduced baseline stability. Most favorable signal-to-background characteristics were obtained at a voltage of 5 V_{p-p}.

The nonaqueous microchip capillary electrophoresis/contactless conductivity detector offers a well-defined concentration dependence. Calibration plots for TPA and TBA (in DMF medium) were linear over the 200−1000 μM range with sensitivities of 2.33 and 2.27 mV/mM and intercepts 0.06 and −0.03 mV, respectively (correlation coefficients 0.991 and 0.995). The favorable signal-to-noise characteristics observed for a mixture containing 250 μM TPA and TBA indicated detection limits around 110 μM for both analytes (based on S/N = 3; not shown). This value is higher than those (2.8−6.4 μM) reported for analogous aqueous-phase chip separations of inorganic ions.[14] Further lowering of the detection limit is expected by optimizing the voltage excitation waveform and detector layout.

The good sensitivity and speed of the microchip CE device−contactless conductivity system are coupled to a good reproducibility. A series of eight repetitive injections of a mixture containing 1 mM TMA, TPA, TBA, and DMDA (using DMF as solvent) resulted in reproducible conductivity signals, with relative standard deviations (RSD) of 4.3, 3.4, 3.6, and 4.8%, respectively; the migration times were also reproducible with RSD of 1.42, 1.70, 1.79, and 2.29%, respectively. Reproducible migration times were also found using DMA (RSD of 2.15, 1.84, 1.93, and 2.70% for TMA,

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TPA, TBA, and DMDA, respectively), DMSO (RSD of 2.21, 2.13, 4.27, and 3.49% for TMA, TPA, TBA, and DMDA, respectively), and PC (RSD of 4.07, 4.73, 4.60, and 5.67% for TMA, TPA, TBA, and DMDA, respectively).

In conclusion, the results presented here demonstrate the versatility of nonaqueus CE microchip devices. Our data give useful insights into the factors governing such organic-phase microchip separations and are supported by the theoretical predictions. The use of organic solvents can benefit microchip separations through enhanced solubility of nonpolar solutes (i.e., greater scope) and tailored selectivity. The on-chip conductivity detection poses no restrictions associated with the optical properties of the solvents. Current efforts are aimed at expanding the nonaqueous CE microchip to different organic solvents and their mixtures, to different electrochemical detection modes, and toward the separation and detection of additional hydrophobic compounds (e.g., fatty acids). Such microfabricated nonaqueous CE−conductivity devices are expected to find a wide range of applications.

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