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Coelectroosmotic separations of anions in non-aqueous capillary electrophoresis

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Abstract

The suitability of hexadimethrine bromide (HDB) as electroosmotic flow (EOF) modifier in non-aqueous capillary electrophoresis was investigated. Using HDB as EOF modifier a reversed EOF resulted, which allows very fast separations of anionic compounds in the coelectroosmotic mode. This is demonstrated for the separation of hydroxy- and dihydroxybenzoic acids in various acetonitrile–methanol mixtures. According to the decreasing ratio of dielectric constant and viscosity the EOF mobility showed a linear decrease with increasing methanol concentration in the electrophoresis medium, whereas the analytes showed non-uniform behaviour on addition of methanol. The EOF reproducibility of EOF migration time was excellent, if the concentration of HDB in the running electrolyte was 0.001% (w/v). With this concentration a reproducibility of <0.5% R.S.D. was achieved for nine consecutive runs. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

The use of non-aqueous capillary electrophoresis (CE) was first demonstrated by Wahlbroehl and Jorgenson in 1984 [1]. In recent years a number of successful reports on separations of anionic as well as cationic species using non-aqueous CE have appeared [2–12] and it has been demonstrated that high selectivity in the separation of small molecules may be obtained by using various organic solvents in

place of water in the electrophoresis medium [4,7,8]. The separations of anions by non-aqueous CE have all been performed in counterelectroosmotic mode and until now no electroosmotic flow (EOF) modifier enabling co-electroosmotic separations of anions have been described.

In untreated fused-silica capillaries, which are employed for the most CE applications, anionic compounds migrate counterelectroosmotically, i.e., in the direction opposite to the EOF, leading to often unnecessarily long analysis times. Moreover, the simultaneous determination of slow anions and anions with mobilities higher than the EOF is not possible this way. Thus, in aqueous CE, various

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cationic electrolyte additives have been employed to modify or even revert the EOF by dynamically coating of the capillary wall. Most often, cationic hemimicelles formed by long-chain alkyltrimethylammonium salts like decyl- [13], dodecyl- [13] tetradecyl- [13–15] or cetyl [15–17] trimethylammonium bromide below the critical micelle concentration (cmc) were used for this purpose. Alternatively, polycations like hexadimethrine bromide (HDB, Polybrene) [18–20], or poly(1,1-dimethylenepyrrolidinium chromate) [21] can adsorb strongly to silica capillaries [18,22] and reverse the EOF.

Using these approaches anionic compounds migrate coelectroosmotically, and thus rapid analysis of anionic compounds is provided [23].

In aqueous coelectroosmotic CE the addition of organic modifiers to the electrolyte was found to be advantageous to reduce hydrophobic and electrostatic interactions of anionic compounds with the EOF modifier and thus adsorption at the modified capillary wall [23].

Nevertheless, no attempt was made to use EOF modifiers in non-aqueous CE buffers, where hydrophobic adsorption phenomena should be minimised to the greatest extent.

Alkyltrimethylammonium salts which are commonly employed as EOF modifiers in aqueous CE are of limited use in organic solvents, as both the critical hemimicelle concentration and the ionisation degree are strongly affected even at rather small amounts of organic modifiers in aqueous buffers [24]. However, cationic polyelectrolytes like HDB interact very strongly with the negatively charged silica capillary wall [18,22,25] due to the presence of many positive charges. Furthermore, the shorter alkyl sections of HDB provide less hydrophobic interactions with organic solvents compared to the long chain alkyltrimethylammonium ions and thus higher stability of the dynamic coating on addition of organic solvents is generally observed [23].

In this paper HDB is investigated as potential EOF modifier for non-aqueous CE. Non-aqueous coelectroosmotic separations of hydroxy- and dihydroxybenzoic acids in various acetonitrile–methanol mixtures with direct UV are demonstrated using HDB as EOF modifier.

2. Experimental

2.1. Apparatus

The experiments were carried out on a laboratory-built CE system, equipped with a Lambda 1000 UV detector (Bischoff, Leonberg, Germany) and a high-voltage power supply type HCN 6 M-30000 from FUG (Rosenheim, Germany). Data acquisition was performed using a ERC A/D converter (Altenglofheim, Germany) with Apex Chromatography software (Autochrom, Milford, MA, USA).

Untreated fused-silica capillaries (CS-Chromatographie Service, Langerwehe, Germany) of 60 cm (43 cm to the detector) \times 75 μ m I.D. were employed. Hydrostatic injection was used (5 cm for 10 s) with this system. The separation was performed at ambient temperature. For the experiments investigating effect of various electrolyte compositions an HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany) with on-column diode array detection was used. A detection wavelength of 214 nm, bandwidth 16 nm, was used for all samples in this experiment. The separation was performed in fused-silica capillary [48.5 cm (40 cm to the detector) \times 75 μ m I.D.] at 25°C. The potential drop over the capillary was set to -0.5 V/cm. Samples were injected using a pressure of 5 kPa (50 mbar) for 1 s.

2.2. Chemicals

Gradient grade acetonitrile (MeCN) and methanol (MeOH) as well as acetic acid, sodium acetate and ammonium acetate were purchased from Merck (Darmstadt, Germany) All standard reagents were of analytical grade. Hydroxybenzoic acids and dihydroxybenzoic acids, pyromellitic acid as well as HDB were obtained from Fluka (Buchs, Switzerland).

2.3. Procedures

Standard solutions were prepared by dissolving the reagents in MeOH. A stock solution of HDB was prepared in MeOH at a concentration of 0.1% (w/v) which was further diluted to the appropriate concentrations in the running electrolytes. All electrolyte

solutions were degassed prior to use in CE experiments. The apparent pH values (pH^*) of the non-aqueous electrolytes was measured in the electrophoresis media using a pH meter (Radiometer, Copenhagen, Denmark) equipped with a glass-calomel electrode. The electrode was calibrated using standard aqueous buffers.

Before use, new capillaries were rinsed with 1 M sodium hydroxide for 60 min, 0.1 M sodium hydroxide for 20 min, distilled water for 20 min and MeOH for 20 min.

For conditioning after storage the capillary was rinsed 5 min with water, 10 min with 0.1 M sodium hydroxide, 5 min with water and MeOH and 30 min with running buffer in order to achieve a reproducible dynamic coating. After each run, the capillary was rinsed with running buffer for 3 min. For storage overnight the capillary was rinsed with water and dried with air.

3. Results and discussion

3.1. Separation of hydroxy- and dihydroxybenzoic acids

The suitability of HDB as EOF modifier was examined in various MeCN–MeOH mixtures with ammonium acetate as buffer compound. MeOH and MeCN were chosen as these solvents previously have shown to provide high selectivity towards compounds with equal or almost equal mass-over-charge ratios [12]. However, only mixtures of the

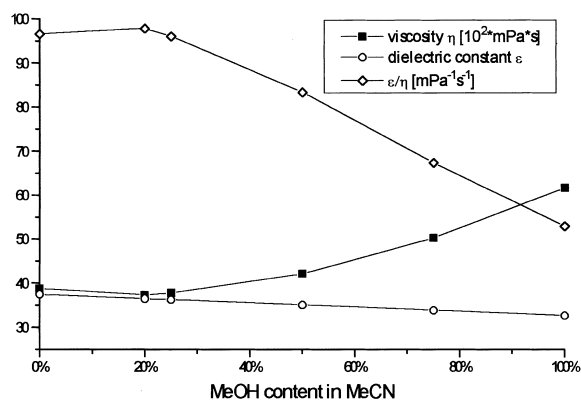


Fig. 1. Physical properties of MeCN–MeOH mixtures; ϵ from Ref. [26], η from viscosity measurements [28].

two solvents and neat MeOH have been investigated as the solubility of some of the electrolytes in MeCN may be a problem. A mixture of hydroxy- and dihydroxybenzoic acids was used as test solutes. The different electrolyte compositions are listed in Table 1 together with the determined apparent pH values (pH^*). Apart from the very similar pH^* values other physical properties, which affect the analyte and EOF mobilities vary in different mixtures of MeCN and MeOH. Fig. 1 shows the dependencies of dielectric constant ϵ [26], viscosity η [28] and the ratio ϵ/η on the composition of the organic solvents. The viscosity of the binary solvent mixture increased with increasing MeOH content, whereas the dielectric constant decreases slightly. Consequently, the ratio ϵ/η was found to be high at low MeOH concentration with a maximum value at 20% MeOH.

Table 1
Electrophoresis media employed with apparent pH (pH^*)

Solvent composition	Electrolyte composition	pH^*
MeOH–MeCN (25:75)	25 mM Ammonium acetate, 0.001% HDB	8.11
MeOH–MeCN (40:60)	25 mM Ammonium acetate, 0.001% HDB	8.00
MeOH–MeCN (50:50)	25 mM Ammonium acetate, 0.001% HDB	8.02
MeOH–MeCN (60:40)	25 mM Ammonium acetate, 0.001% HDB	7.88
MeOH–MeCN (75:25)	25 mM Ammonium acetate, 0.001% HDB	7.87
MeOH 100%	25 mM Ammonium acetate, 0.001% HDB	7.78
MeOH–MeCN (50:50)	25 mM Ammonium acetate, 1 M acetic acid, 0.001% HDB	5.31
MeOH–MeCN (50:50)	25 mM Ammonium acetate, 250 mM acetic acid, 0.001% HDB	6.28
MeOH–MeCN (50:50)	25 mM Ammonium acetate, 100 mM acetic acid, 0.001% HDB	6.77
MeOH–MeCN (50:50)	25 mM Ammonium acetate, 50 mM sodium acetate, 0.001% HDB	8.28
MeOH–MeCN (50:50)	25 mM Ammonium acetate, 100 mM sodium acetate, 0.001% HDB	8.48

ε/η largely determines the mobilities of both the EOF (according to Smoluchowski's equation) and the analytes and thus a high ε/η ratio indicates a potentially high mobility [27]. As ε/η for water is about $78.4 \text{ mPa}^{-1} \text{ s}^{-1}$ it is significantly higher in a wide range of MeOH–MeCN composition than in water.

Fig. 2 shows the separations of eight hydroxy- and dihydroxybenzoic acids and pyromellitic acid (as very fast migrating compound) using electrolytes with different compositions of MeCN and MeOH. The dihydroxybenzoic acids with a hydroxy group in the *ortho* position migrates faster than the other dihydroxybenzoic acid isomers corresponding to the lower $\text{p}K_{\text{a}}$ values of the *ortho* isomers in aqueous media (see legend to Fig. 2). Slight changes in selectivity and resolution were observed with the different solvent mixtures. The changes in selectivity may be caused by differences in intramolecular hydrogen bonds as observed by Fujiwara and Honda [29] but different solubilization of the analytes may also play a role. Complete resolution of all compounds within 2 min was achieved using MeOH–MeCN (50:50). Other ratios of MeOH and MeCN lead to worse resolution or even comigrating compounds.

In Fig. 3 an overview of the mobilities of all compounds including the EOF in the different solvent compositions is given. The EOF mobility shows a linear decrease with increasing MeOH concentration according to the linear decrease in the ε/η ratio in the investigated range of solvent composition (see Fig. 1). The linear behaviour indicates that the HDB coating is not largely influenced by the change of solvent composition, which would affect the ζ potential of the modified capillary wall and thus the EOF mobility in a rather different way.

The analyte mobilities, however, show no uniform dependence on the MeOH–MeCN composition. The mobility of some compounds decreased with increasing MeOH content according to the EOF but other analytes show the reversed effect, presumably due to changes in solubilization. For 3,5-dihydroxy- and 4-hydroxybenzoic acid even a reverted migration order was observed above a concentration of 40% MeOH.

The electrolyte composition (see Table 1) was varied in a solvent mixture of MeCN–MeOH

(50:50) in order to investigate the separation at various pH^* values. Changes in selectivity was also observed when changing the electrolyte composition (Fig. 3b). The observed changes are not only caused by the changes in pH^* . The variations in ionic strength of the electrophoresis medium as well as the nature of the electrolyte also play a role e.g., pyromellitic acid was not detected when the separation was performed in electrophoresis media containing sodium acetate, probably due to a low solubility of the sodium salt of pyromellitic acid in MeCN–MeOH (50:50).

3.2. Reproducibility of the dynamic coating with HDB

In aqueous CE very small concentrations (0.001%, w/v) of HDB are sufficient to reverse the EOF with sufficient reproducibility of the EOF migration time.

In order to examine the reproducibility of the dynamic coating with HDB in non-aqueous CE, an electrophoresis medium consisting of 25 mM ammonium acetate in MeCN–MeOH (50:50) was used with three different HDB concentrations ranging from $6.25 \cdot 10^{-5}$ to 0.001% (w/v). Fig. 4 shows a plot of the EOF migration times for nine consecutive runs using these electrolytes. Excellent reproducibility (0.45% R.S.D.) was observed when, like in aqueous systems [23], a concentration of 0.001% (w/v) was employed in the running electrolyte. However, with lower HDB concentrations a marked deterioration of reproducibility occurred. Thus a concentration of 0.001% (w/v) HDB seems to be suitable also for MeOH–MeCN mixtures. Higher concentrations of HDB should be avoided to prevent any potential adsorption of analytes with HDB.

4. Conclusions

HDB was shown to be an adequate EOF modifier for non-aqueous CE using MeCN and MeOH as solvents. Very fast separations of anionic compounds were achieved in the coelectroosmotic migration mode. The dynamic coating of HDB was found to be stable and reproducible when HDB was used in a concentration of 0.001% (w/v) in a mixture of MeCN and MeOH. Thus HDB is found to be useful

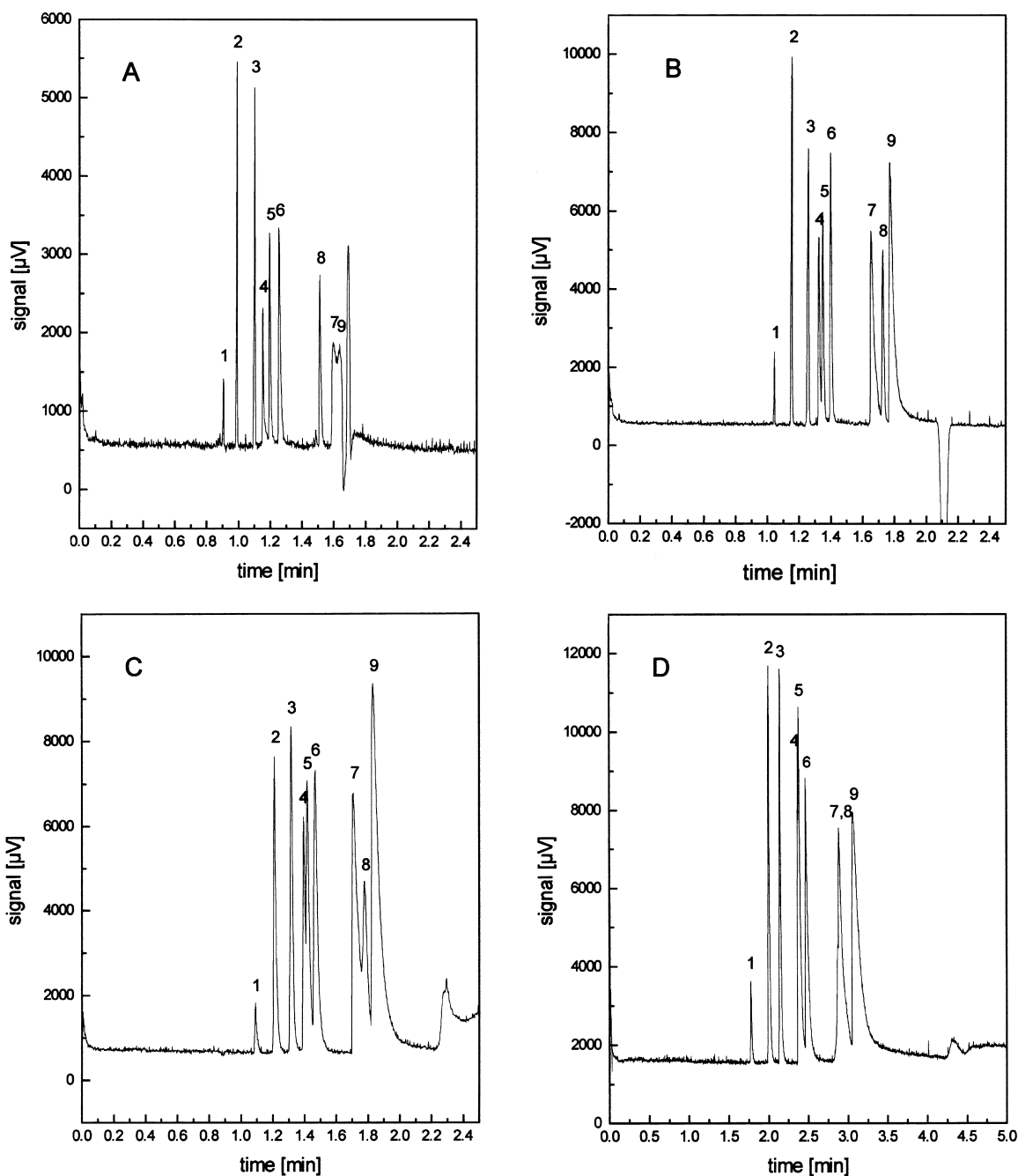


Fig. 2. Non-aqueous coelectroosmotic separations of hydroxybenzoic acids and dihydroxybenzoic acids using different MeOH–MeCN mixtures and HDB as EOF modifier. Peak identification with pK_a values in aqueous solution: (1) pyromellitic acid, (2) 2,6-dihydroxybenzoic acid ($pK_a=1.3$), (3) salicylic acid ($pK_a=2.9$), (4) 2,3-dihydroxybenzoic acid (pK_a not found), (5) 2,5-dihydroxybenzoic acid ($pK_a=3.0$), (6) 2,4-dihydroxybenzoic acid ($pK_a=3.4$), (7) 3,5-dihydroxybenzoic acid ($pK_a=4.1$), (8) 4-hydroxybenzoic acid ($pK_a=4.5$), (9) 3,4-dihydroxybenzoic acid ($pK_a=4.5$). Electrophoresis media: 25 mM ammonium acetate, 0.001% (w/v) HDB in (A) MeOH–MeCN (25:75); (B) MeOH–MeCN (50:50); (C) MeOH–MeCN (60:40) and (D) MeOH. Capillary: 60 cm (43 cm to detector) \times 75 μ m I.D.; injection: hydrostatically, 5 cm, 10 s. Other conditions: voltage -30 kV, UV detection at 214 nm.

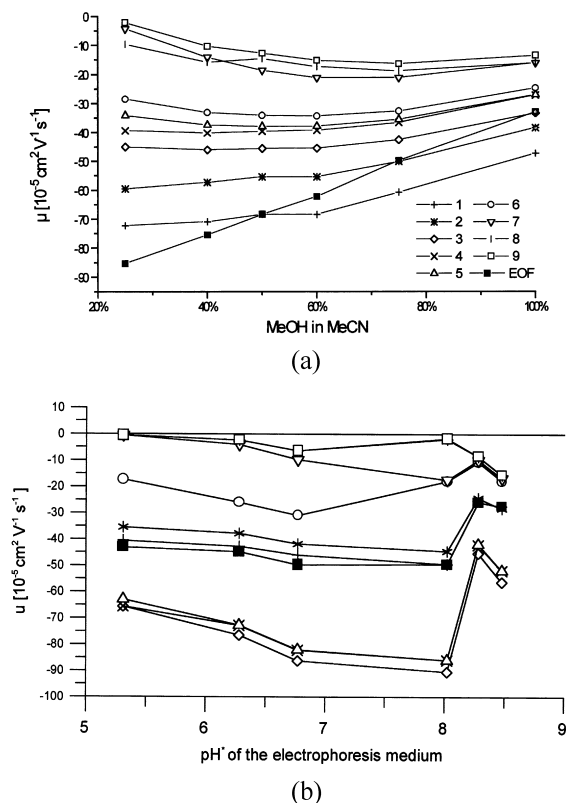


Fig. 3. (a) The effect of changing the solvent composition on the mobility of analytes and EOF; peak numbers and conditions as in Fig. 2. (b) The effect of changing the pH* by using various electrolytes in MeOH–MeCN (50:50) as indicated in Table 1; identification of compounds as in Fig. 3a. Conditions: capillary 48.5 cm (40 cm to the detector) \times 75 μ m I.D., injection: 1 s at 5 kPa. Voltage –22.5 kV, UV detection at 214 nm.

as an EOF modifying additive for separations in non-aqueous CE.

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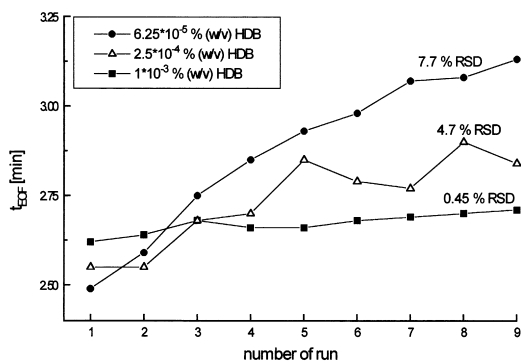


Fig. 4. Comparison of the reproducibility of EOF migration times for three different HDB concentrations.

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