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Separation of hydrophobic compounds by electrokinetic chromatography with tetraalkylammonium ions

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Abstract

Tetraalkylammonium ions were evaluated as potential pseudo-stationary phases for the separation of highly hydrophobic compounds with electrokinetic chromatography (EKC) in aqueous–organic media. The direction of the electroosmotic flow and, as a consequence, the migration behaviour of the hydrophobic compounds, is shown to be strongly dependent on the tetraalkylammonium concentration and the organic modifier content of the applied electrolyte system. The potential of tetraalkylammonium pseudo-stationary phases in EKC is illustrated by the separation of several geometric isomers of polycyclic aromatic hydrocarbons.

Keywords: Buffer composition; Tetraalkylammonium ion; Polynuclear aromatic hydrocarbon

1. Introduction

Micellar electrokinetic chromatography (MEKC) is a highly efficient separation technique, especially suitable for the determination of neutral species [1,2]. Because of the lipophilic interior of micelles, usually applied in aqueous electrolyte systems, uncharged compounds can be separated, even if they are only partly soluble in water. A unique advantage of MEKC in method development is the possibility of changing rapidly the chemical nature of the pseudo-stationary phase in the electrolyte system by rinsing the capillary. Since the introduction of MEKC by Terabe et al. [1,2], different micellar systems have been applied in order to control migration behaviour and to optimize selectivity for a great variety of substances from various fields of chemistry [3–5].

However, for the determination of highly hydrophobic compounds with MEKC, the presence of a limited elution range forms a major limitation [6]. Due to high partition coefficients between the aqueous mobile phase and the micellar pseudo-stationary phase, these compounds possess retention factors far too high to obtain optimum resolution [7]. They migrate close to or at the migration time of the micelles.

Different strategies have been described to overcome these problems. Terabe et al. [8] described the use of cyclodextrins in MEKC for the separation of polycyclic aromatic hydrocarbons (PAHs). Also, several organic modifiers such as methanol, acetonitrile and 2-propanol have been applied, in order to reduce the affinity of the sample compounds for the micellar phase [9,10]. However, the amount of organic solvents that can be used is limited as at

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modifier concentrations above ca. 20–30% (v/v) sodium dodecyl sulphate (SDS) micelles are generally not stable. Separations at higher organic modifier concentrations have been reported [11,12], but the mechanism for these analyses is based on interactions with surfactant monomers rather than on micellar solubilization. Recently, several authors reported the application of highly branched macromolecular structures, such as unimolecular polymerized micelles [13,14], ionic polymers [15,16] and dendrimers [17–19]. These macromolecules form stable pseudo-stationary phases, allowing relatively high amounts of organic modifiers to be used.

Walbroehl and Jorgenson [20] applied electrolyte systems of tetrahexylamonium ions (THA^+) in mixed water–acetonitrile media for the separation of several PAHs. Due to solvophobic interactions of these hydrophobic compounds with the THA^+ ions, positively charged species are formed which possess an effective mobility and migrate in an electric field. This separation principle has much in common with MEKC [1,2] and, therefore, this technique can be regarded as a form of electrokinetic chromatography (EKC) in aqueous/organic media with tetraalkylammonium ions as pseudo-stationary phase. Baseline separation was obtained for five PAHs with a different number of aromatic rings in a water–acetonitrile (50:50, v/v) electrolyte system containing 25 m*M* THA^+. However, lower resolutions were obtained for these PAHs with mixed water–acetonitrile electrolyte systems containing tetrabutylammonium ions [21], illustrating that the alkyl chain length plays an important role in the solvophobic interaction mechanism. Therefore, we investigated the applicability of two long-chain tetraalkylammonium surfactants, viz. tetraoctylammonium (TOA^+) and tetradecylammonium (TDA^+) ions, as pseudo-stationary phases for the separation of highly hydrophobic compounds with EKC in aqueous–organic media.

2. Experimental

2.1. Chemicals

Tetraoctylammonium bromide (TOAB), pentylbenzene and acetonitrile were obtained from Merck (Darmstadt, Germany), tetradecylammonium bromide (TDAB) from Sigma (St. Louis, MO, USA), propylbenzene and butylbenzene from Aldrich (Milwaukee, WI, USA) and hexylbenzene, octylbenzene, nonylbenzene, dodecylbenzene and all PAHs from Fluka (Frankfurt, Germany). Water was filtered by a Milli-Q purification system (Waters Millipore, Milford, MA, USA).

2.2. Instrumentation and separation conditions

All experiments were carried out on a BioFocus 3000 Capillary Electrophoresis System (BioRad, Hercules, CA, USA) at a constant voltage of 20 kV. In the anionic mode the cathode was placed at the inlet side and the anode at the outlet side of the capillary, respectively, and vice versa in the cationic mode. A 75 μm I.D. fused-silica capillary (Chrompack, Middelburg, The Netherlands) was used, total length 70.0 cm, distance between injection and detection 65.4 cm. From both ends of the capillary the polyimide coating was removed in order to prevent dissolving in the aqueous/organic electrolyte systems. The temperature was kept constant at 25°C and the wavelength of the detector was set at 220 nm. Samples were introduced by pressure injection with an injection constant of 2 p.s.i. s (1 p.s.i.= 6894.76 Pa). Between each run the capillary was flushed subsequently for 2 min with acetonitrile, 2 min with 0.1 *M* NaOH, 2 min with deionized water and 2 min with the electrolyte solution. All samples were dissolved in the electrolyte system at a final concentration of at least 25 times smaller than the concentration of tetraalkylammonium ions.

3. Results and discussion

3.1. Electroosmotic flow and migration mode

In Fig. 1A an electrokinetic chromatogram is shown for the separation of a hydrophobic sample mixture, including several PAHs, in the cationic mode, applying a water–acetonitrile (50:50, v/v) electrolyte system containing 10 m*M* TOAB. In this experiment all compounds migrate in the downstream mode [22], i.e. they are detected before the electroosmotic flow (EOF). The migration mechanism for this kind of analysis was proposed by

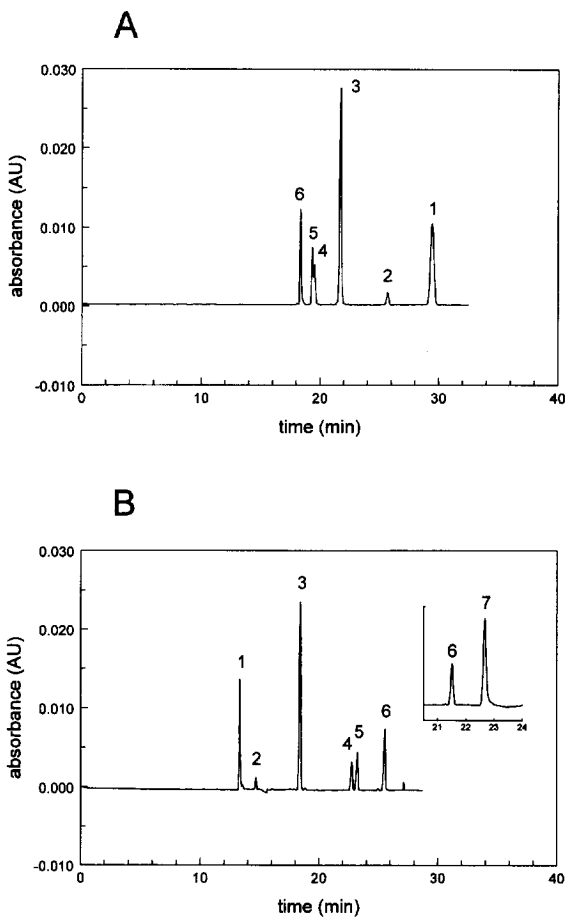

Fig. 1. Electrokinetic chromatogram of the separation of (1) formamide (neutral EOF marker), (2) propylbenzene, (3) naphthalene, (4) anthracene, (5) phenantrene, (6) pyrene and (7) chrysene in electrolyte systems of: (A) water–acetonitrile (50:50, v/v), and (B) water–acetonitrile (60:40, v/v), each containing 10 m*M* TOAB. The inset in part (B) is from a different run.

Walbroehl and Jorgenson [20], involving an association of a positively charged surfactant monomer and a noncharged sample compound. This association will be stronger for larger, more hydrophobic compounds, resulting in a higher migration velocity (see Fig. 1A). They suggested that the association of a sample compound with more than one THA^+ ion is unlikely, due to electrostatic repulsion. However, TOAB and TDAB are known to show micellar aggregation in organic media [23]. Therefore, a micellar pseudo-stationary phase can be formed by these surfactant systems in mixed water–acetonitrile electrolyte systems. Moreover, the formation of a dynamic coating of positively charged hemimicelles on the capillary wall may cause a change in the sign of the ζ-potential, resulting in a reversal of the direction of the EOF [24]. This is illustrated in Fig. 1B where the same sample mixture is separated in the anionic mode, applying a water–acetonitrile (60:40, v/v) electrolyte system containing 10 m*M* TOAB. Notice that the migration order is reversed compared to Fig. 1A. In this situation all compounds migrate in the upstream mode, i.e. they are detected after the EOF. Fig. 1B also illustrates that separation is obtained for several geometric PAH isomers, e.g. anthracene and phenanthrene or pyrene and chrysene (see inset), using TOAB as the pseudo-stationary phase. These results demonstrate that a difference of 10% (v/v) acetonitrile in the electrolyte system strongly influences the condition on the capillary wall and the migration behaviour.

In order to study the influence of the composition of the electrolyte system on the EOF and the migration mode of the sample compounds in more detail, experiments were carried out in water–acetonitrile electrolyte systems at various volume ratios, containing different concentrations of TOAB and TDAB, respectively. The results, shown in Fig. 2, illustrate that for electrolyte systems containing more acetonitrile an EOF reversal is obtained at higher surfactant concentrations. Due to a lower critical micelle concentration, TDAB causes an EOF reversal at lower concentrations than TOAB at intermediate water–acetonitrile ratios.

Here it should be noted that with several of the applied electrolyte systems the repeatability of the EOF and, consequently, of the migration times of the sample compounds was rather poor. This problem may be attributed to adsorption of tetraalkylammonium ions on the capillary wall and/or the formation of an unstable dynamic coating.

3.2. Influence of electrolyte system on migration behaviour

To investigate the influence of the concentration TAA^+ ions on the migration behaviour of hydrophobic species, experiments were carried out with the sample mixture in water–acetonitrile (60:40, v/v) electrolyte systems containing different concen-

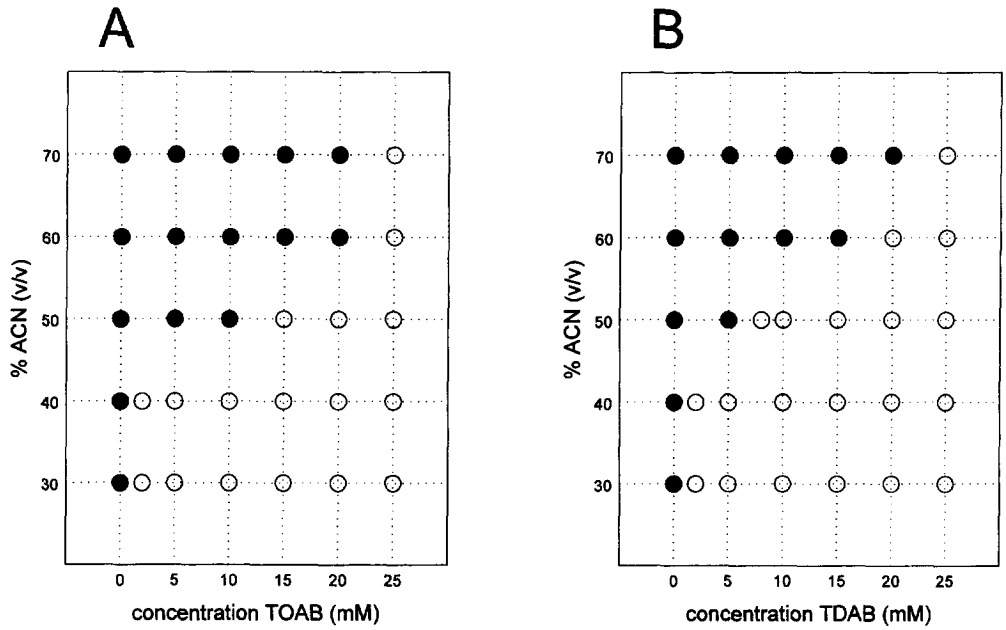

Fig. 2. Influence of surfactant concentration and amount of acetonitrile on EOF direction and migration mode for: (A) TOAB, and (B) TDAB. (●) EOF directed to cathode, downstream mode, (○) EOF directed to anode, upstream mode.

trations of TOAB. In all these experiments the sample compounds migrate in the upstream mode. Since the migration time of the tetraalkylammonium ions is unknown, pseudo-effective mobilities, m_{eff}^{ps}, were calculated for peak identification according to [25]:

$$m_{eff}^{ps} = \frac{l_c l_d}{t_S V} - \frac{l_c l_d}{t_{EOF} V} \quad (1)$$

where l_c is the length of the capillary, l_d is the length from injection to detection, t_S is the migration time of the sample compound, t_{EOF} is the migration time of the EOF and V is the applied voltage. In Fig. 3 all pseudo-effective mobilities are shown as a function of the TOAB concentration, illustrating that m_{eff}^{ps} increases with increasing TOAB concentration. This effect is more pronounced for larger, more hydrophobic compounds which possess stronger interactions with the pseudo-stationary phase. At higher TOAB concentrations the curves level off, as the limiting value of m_{eff}^{ps} is the effective mobility of the pseudo-stationary phase.

Besides the TAA^+ concentration the water–acetonitrile volume ratio will also influence the migration behaviour of the sample compounds [20]. This is illustrated in Fig. 4 for the separation of four hydrophobic compounds in electrolyte systems containing 5 m*M* TOAB and 10 m*M* TDAB, respectively. All sample compounds migrate in the upstream mode in the electrolyte systems with 30% or 40%

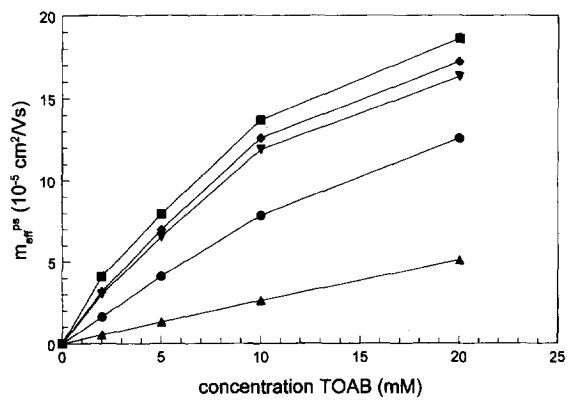

Fig. 3. Pseudo-effective mobility, m_{eff}^{ps}, versus concentration of TOAB in a water/acetonitrile (60/40, v/v) electrolyte system for (▲) propylbenzene, (●) naphtalene, (▼) anthracene, (♦) phenantrene and (■) pyrene.

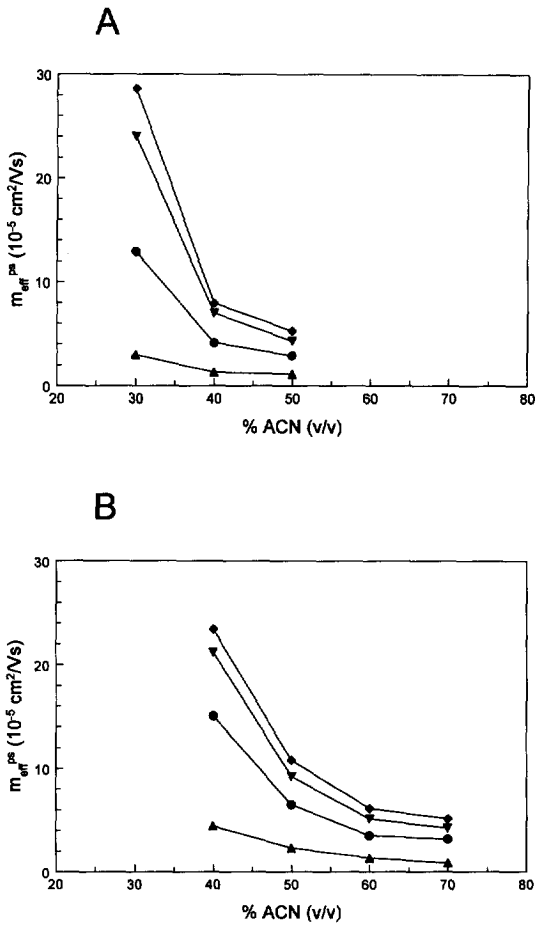

Fig. 4. Pseudo-effective mobility, m_{eff}^{ps}, versus concentration of acetonitrile in electrolyte systems containing: (A) 5 m*M* TOAB, and (B) 10 m*M* TDOB for (▲) propylbenzene, (●) naphtalene, (♦) phenanthrene and (■) pyrene. For migration mode, see text.

(v/v) acetonitrile containing 5 m*M* TOAB and with 40% or 50% (v/v) acetonitrile containing 10 m*M* TDAB, respectively, whereas in the other electrolyte systems all sample compounds migrate in the downstream mode. On increasing the amount of acetonitrile, solvophobic interactions will decrease, resulting in lower pseudo- effective mobilities. As would be expected, a stronger decrease is obtained for larger, more hydrophobic species. These results demonstrate that the resolution can be controlled by the composition of the electrolyte system, i.e. the concentration of the TAA^+ and water–acetonitrile volume ratio.

3.3. Mobility of the pseudo-stationary phase

The conformation of the pseudo-stationary phase in this form of EKC will depend strongly on the composition of the aqueous/organic electrolyte system. If the sample compounds are migrating in the downstream mode, the pseudo-stationary phase consists mainly of single TAA^+ ions. However, if the sample compounds are migrating in the upstream mode, the pseudo-stationary phase consists of both single TAA^+ ions and micellar aggregates, formed by more TAA^+ ions. The aggregation number of TAA^+ ions and, consequently, micellar volume and shape, strongly depends on the amount of water in the aqueous/organic medium [23].

In order to study the migration behaviour of the tetraalkylammonium pseudo-stationary phase, experiments were carried out with a homologous series of alkylbenzenes. For a homologous series with an increasing number of methylene groups, $\log k$ will increase linearly with carbon number, according to [26]:

$$\log k = az + b \tag{2}$$

where k is the retention factor, representing the distribution equilibrium between the aqueous–organic phase and the pseudo-stationary phase, z is the carbon number of the homologues and a and b are constants. In Fig. 5 the electrokinetic chromatogram is shown for the separation of seven alkylbenzenes in the downstream mode, applying a water/acetonitrile (40/60, v/v) electrolyte system containing 20 m*M* TOAB. Providing that the solvophobic interaction mechanism of the hydrophobic solutes is fast enough and that the migration behaviour of the TAA^+ ions is not markedly influenced by this interaction, the pseudo-effective mobility of the species, m_{eff}^{ps}, can be expressed by:

$$m_{eff}^{ps} = \frac{k}{k+1} m_{eff,TAA} \tag{3}$$

where $m_{eff,TAA}$ is the effective mobility of the tetraalkylammonium pseudo-stationary phase. A combination of Eqs. (2,3) leads to:

$$m_{eff}^{ps} = \frac{10^{az+b}}{1+10^{az+b}} m_{eff,TAA} \tag{4}$$

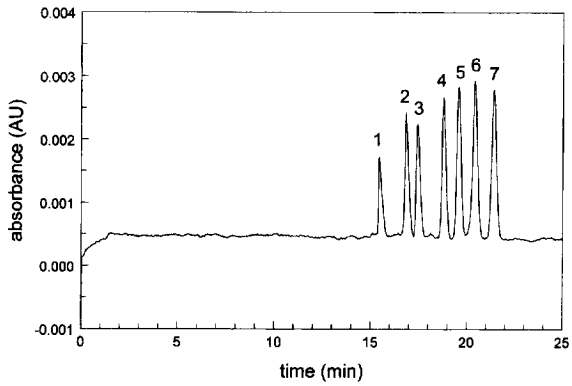

Fig. 5. Electrokinetic chromatogram of the separation of (1) dodecylbenzene, (2) nonylbenzene, (3) octylbenzene, (4) hexylbenzene, (5) pentylbenzene, (6) butylbenzene and (7) propylbenzene in a water–acetonitrile (40:60, v/v) electrolyte system containing 20 m*M* TOAB.

For the same homologous series of alkylbenzenes as in Fig. 5 m_{eff}^{ps} was determined in the upstream mode, applying electrolyte systems of water–acetonitrile (60:40, v/v), containing 20 m*M* TOAB and water–acetonitrile (50:50, v/v), containing 10 m*M* TDAB, respectively. From these migration data, $m_{eff,TAA}$ could be calculated by curve fitting Eq. (4). This is illustrated in Fig. 6. For these calculations experimental data in the upstream mode were applied because in this mode the migration of the pseudo-stationary phase is probably less affected by the interaction with the homologues and better results for

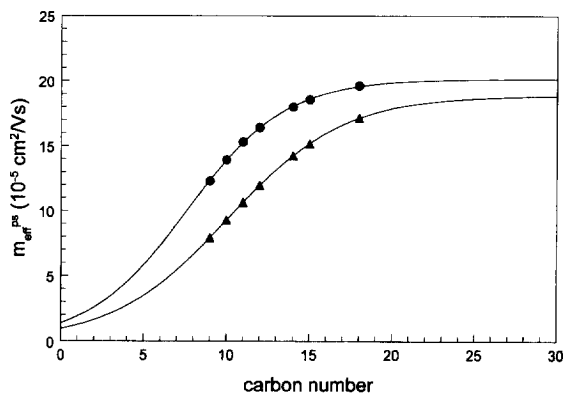

Fig. 6. Pseudo-effective mobility, m_{eff}^{ps}, as a function of carbon number for a homologous series of alkylbenzenes in water–acetonitrile electrolyte systems of (●) (60:40, v/v) containing 20 m*M* TOAB and (▲) (50:50, v/v) containing 10 m*M* TDAB. Drawn lines represent the results of the curve-fitting procedure.

Table 1
Effective mobility of two tetraalkylammonium pseudo-stationary phases, $m_{eff,TAA}$ (10^{-5} cm²/V s), with standard deviations (in parentheses), calculated by curve fitting and an iteration procedure

Electrolyte system	$m_{eff,TAA}^{a}$	$m_{eff,TAA}^{b}$
Water–acetonitrile (60:40, v/v) 20 m*M* TOAB	20.15 (0.11)	20.40
Water–acetonitrile (50:50, v/v) 10 m*M* TDAB	18.90 (0.05)	18.98

[a]Curve fitting of Eq. (4).
[b]Iteration procedure according to [4,10].

the curve fitting procedure were obtained. In Table 1 $m_{eff,TAA}$ is listed for both pseudo-stationary phases. In this table also the effective mobilities, calculated by an iteration procedure as described previously [4,10], are included, giving identical results.

4. Conclusions

Tetraalkylammonium ions were shown to be suitable pseudo-stationary phases for the separation of highly hydrophobic compounds by electrokinetic chromatography in aqueous–organic media. The direction of the EOF and the migration behaviour and, consequently, the resolution of the sample compounds strongly depend on the surfactant concentration and the organic modifier content of the electrolyte system.

A hydrophobic sample mixture, including several geometric PAH isomers could be separated in the upstream mode, applying a water–acetonitrile (60:40, v/v) electrolyte system containing 20 m*M* TOAB. The effective mobility of TOAB and TDAB pseudo-stationary phases was determined from the migration data of a homologous series of alkylbenzenes by curve fitting, giving identical results as an iteration procedure.

Acknowledgments

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