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Short communication

Capillary electrophoretic separation of polynuclear aromatic hydrocarbons using sodium cholate in mixed aqueous–organic buffers

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Abstract

A capillary electrophoretic method for the separation of a series of polynuclear aromatic hydrocarbons is described. Sodium cholate is used with a series of organic modifiers. Analysis times were between 26–80 min depending on sodium cholate concentration and on the type and concentration of organic additives used. Efficiencies in excess of 10^5 theoretical plates were observed.

Keywords: Buffer composition; Polynuclear aromatic hydrocarbons; Sodium cholate; Bile salts

1. Introduction

Polynuclear aromatic hydrocarbons (PAHs) are environmentally and biologically important species due to their suspected mutagenic and carcinogenic properties [1]. Traditional methods for analyzing these pollutants include gas chromatography and high-performance liquid chromatography [2,3].

Capillary electrophoresis (CE) is an attractive method for analyzing polar compounds. The use of surfactants allows the electrophoretic analysis of neutral analytes such as polychlorinated biphenyls or polyaromatic hydrocarbons [4–8]. A number of difficulties are encountered in the analysis of these highly non-polar analytes, including long migration times, poor resolution and low efficiencies. Some of these phenomena can be minimized by incorporation of organic additives [9,10]. Cyclodextrins (CDs) have also been used alone [11] or in the presence of micelles [5,8] to reduce analysis times and improve resolution. A series of eight PAHs were separated using γ-CD and sodium dodecyl sulphate (SDS), with urea as a modifier [5]. Disadvantages associated with the use of CDs include limited water solubility for some CDs and relatively high cost.

More recently, bile salts have been used instead of long-chain surfactants for separating a range of neutral analytes as well as for chiral separations [12–16]. Bile salts, such as sodium cholate, deoxycholate or taurodeoxycholate, are composed of a cholesteroid skeleton and can form small helical aggregates with aggregation numbers from 4 to ~20. The bile salts have a conformation that exposes hydroxyl and charged functional groups to the aqueous solution [13]. The smaller size of the aggregates and different degree of hydrophilicity/hydrophobicity allow bile salts to interact with analyte species to a different extent compared to long-chain surfactant aggregates. In the case of highly hydrophobic analytes, bile salts should allow

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for reduced partitioning and thus faster and more efficient separations. In addition, the mode of analyte interaction with bile salts is different compared to long-chain surfactant aggregates and thus different selectivities may be possible [13]. Although bile salts have been applied to the CE separation of PAHs [13,16], this was done over a limited class of PAHs or a small range of experimental conditions. Cole et al. [13] showed a CE separation of the PAHs anthracene, pyrene and benzo[*a*]pyrene in 50 mmol/l sodium cholate plus 0–20% methanol using laser-induced fluorescence detection. Dabek-Zlotorzynka and Lai [16] show the effect of 0–30% (v/v) acetone in 50 mmol/l sodium taurodeoxycholate on the CE separation of 16 PAHs using UV detection at 214 nm.

This paper describes the analysis of 16 PAHs using CE with sodium cholate (5–60 mmol/l) in the presence of methanol and/or acetonitrile at a total concentration of up to 70% (v/v), ethanol (30–50%), urea (3.2–6.8 mol/l) or methylene chloride (1%). Parameters that were examined as a function of buffer composition included analyte migration times, resolution and efficiency.

2. Experimental

2.1. Instrumentation

A locally constructed CE instrument was used for all experiments [17]. A capillary section was illuminated with a 5 mW average power, 10 mJ pulse energy KrF excimer laser (Potomac Photonics, Model GX-500, Washington, DC, USA) operating at λ=248 nm, 610 Hz pulse repetition rate, and 50 ns pulse width. The excimer laser beam was focused with a 15 mm focal length quartz biconvex lens at right angles to a 50 μm I.D.×190 μm O.D. fused-silica capillary; the polyimide coating of the capillary was burned from the detection region with a gentle flame. A 3 mW, λ=632.8 nm He–Ne laser beam (Melles Griot, Model 05-LHP-151) was focused at right angles to both the capillary and the excimer laser beam with a ×7 microscope objective. The probe beam intensity change was detected 30 cm after the capillary with a 1 mm^2 silicon photodiode. The photodiode output was conditioned with a current-to-voltage converter (1 MΩ feedback resistor in parallel with a 47 pF capacitor) and sent to a two-phase lock-in amplifier (Ithaco, Model 3961, Ithaca, NY, USA), phase referenced to the excimer laser pulse repetition rate. An 80/386 PC collected data from the lock-in amplifier over the IEEE-488 bus. Capillaries purchased from Polymicro Technologies (Phoenix, AZ, USA) were 60 cm long and 55 cm injection-to-detection window.

Samples were injected hydrostatically for 15 s from a height of 4 cm.

2.2. Reagents

Sixteen PAHs were purchased from Aldrich (Milwaukee, WI, USA). Listed in order of increasing molecular mass and hydrophobicity, they were naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz-[*a*]anthracene, chrysene, benzo[*a*]pyrene, dibenz-[*a*,*h*]anthracene, benzo[*ghi*]perylene and ideno-[1,2,3-*cd*]pyrene. The polynuclear hydrocarbons were prepared to 100 ppm in dichloromethane–methanol (1:1, v/v). Mixtures of PAH standards were obtained from Supelco and diluted to 50 ppm with dichloromethane–methanol (1:1, v/v). Sodium cholate, deoxycholate and taurodeoxycholate were purchased from Sigma (St. Louis, MO, USA) and prepared to 0.2 mol/l with Nanopure deionized water. Methanol and acetonitrile were HPLC grade and were obtained from BDH (Toronto, Canada). Running buffer solutions were prepared by mixing different initial volumes of methanol, acetonitrile, stock inorganic buffer solution (10.7 mmol/l NaH_2PO_4–18 mmol/l $Na_2B_4O_7$), sodium cholate and water.

2.3. Calculations

Analyte migration mobilities (μ_{mig}) were calculated from:

$$\mu_{mig} = \frac{Ll}{Vt} \quad (1)$$

where L is capillary length (cm), l is the capillary length to the detector window, V is running voltage (V), and t is analyte migration time (*s*).

Similarly, electroosmotic mobilities (μ_{eo}) were calculated from:

$$\mu_{eo} = \frac{Ll}{Vt_{eo}} \quad (2)$$

where t_{eo} is the methanol (neutral marker) migration time.

Since the PAH separations are performed in the co-electroosmotic mode the net analyte electrophoretic mobility is:

$$\mu_{ep} = \mu_{mig} - \mu_{eo} \quad (3)$$

3. Results and discussion

3.1. Effect of bile salt type and concentration

The effects of sodium cholate concentration in 30% (v/v) methanol on analyte electrophoretic mobilities are shown in Fig. 1. The critical micelle concentration (CMC) of sodium cholate is 12.5 mmol/l [18]. The presence of methanol at this concentration was not expected to influence the cholate CMC, which has been shown to be approximately constant over 0–30% (v/v) methanol [13]. Resolution and efficiency improved at cholate concentrations greater than 40 mmol/l. The working concentration for cholate was chosen to be 60 mmol/l since increased background noise, presumably due to light scattering, was observed at higher cholate concentrations. As Fig. 1 indicates, analyte electrophoretic mobilities decreased, by between 33–45%, over the cholate concentration range 20–60 mmol/l indicating enhanced analyte–cholate interactions. Similarly, electroosmotic flow (EOF) was shown to decrease by about 40% over the range 10–60 mmol/l sodium cholate in media containing 20% methanol and 20% acetonitrile. This EOF trend is similar to the one previously reported for sodium deoxycholate [12] and is probably due to increasing viscosity at the wall–electrolyte interface. Contribution of surfactant wall adsorption to decreasing EOF is minimal as previous studies with SDS have indicated.

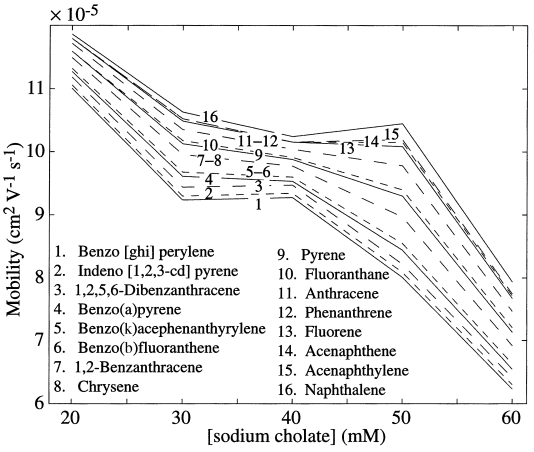

Fig. 1. Plot of analyte electrophoretic mobilities versus sodium cholate concentration (20–60 mmol/l). Buffer composition: 20% (v/v) methanol and 20% (v/v) acetonitrile in 10.7 mmol/l NaH_2PO_4–1.8 mmol/l $Na_2B_4O_7$. The electric field was 250 V/cm.

3.2. Effect of organic modifiers

Organic additives such as methanol, acetonitrile and urea have been used in micellar electrokinetic chromatography to increase the elution window and manipulate selectivity and resolution [9,19–21]. Increasing the concentration of methanol or acetonitrile result in reduced aggregation numbers, thus moderating interactions with hydrophobic analytes.

The effect of methanol concentration on PAH electrophoretic mobilities is shown in Fig. 2. The large increase in analyte electrophoretic mobilities from 20 to 30% methanol indicates a reduction in PAH interaction with cholate aggregates. This decreasing interaction may be due to more effective analyte solvation in the mixed aqueous–organic phase, which reduces partitioning into the cholate micelle. At methanol concentrations from 30–70%, analyte electrophoretic mobilities decreased for all PAHs particularly for the low to moderate hydrophobicity analytes (7–16). At higher organic content, these PAHs tend to interact with the aggregates to a greater extent because of their low to moderate hydrophobicity. In contrast, the higher-molecular-mass PAHs become more solubilized in the mixed aqueous–organic phase and interact less with the pseudostationary phase.

In the case of acetonitrile, decreases in analyte electrophoretic mobilities were observed at between 30–50% (v/v) of added solvent indicating enhanced

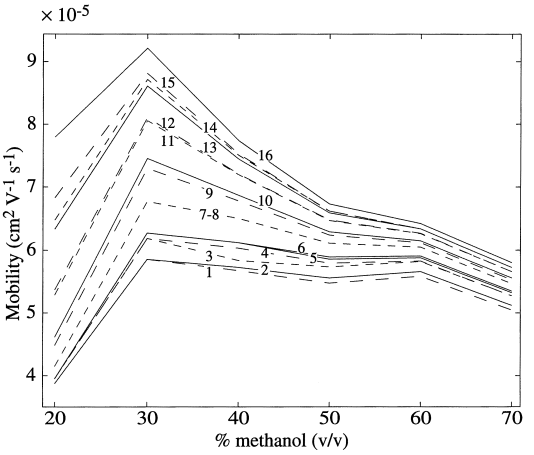

Fig. 2. Plot of analyte electrophoretic mobilities versus percent methanol (v/v) in separation buffer (20–70%). Buffer composition: 40 mmol/l sodium cholate–10.7 mmol/l NaH_2PO_4–1.8 mmol/l $Na_2B_4O_7$. Electric field was 250 V/cm. Compounds are numbered as in Fig. 1.

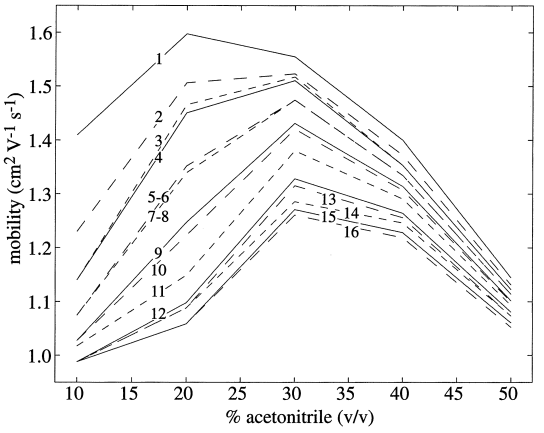

Fig. 3. Plot of analyte electrophoretic mobilities versus percent acetonitrile (v/v) in separation buffer (20–70%). Buffer composition: 50 mmol/l sodium cholate–10.7 mmol/l NaH_2PO_4–1.8 mmol/l $Na_2B_4O_7$. Electric field was 250 V/cm. Compounds are numbered as in Fig. 1.

analyte–cholate interactions. In the concentration range 10–30% (v/v) acetonitrile, increases in electrophoretic mobilities observed for all PAHs parallel the decreasing ε in η (dielectric constant/viscosity) trends observed mixed acetonitrile–water media [22]. A notable difference between Figs. 2 and 3 are the considerably faster electrophoretic mobilities observed with acetonitrile compared to methanol when used at equimolar concentrations. This is due to the slower electroosmotic flow-rates observed in the presence of methanol as opposed to acetonitrile [21].

Two different buffer compositions gave reasonably good separations of the 16 PAHs. A 60 mmol/l sodium cholate–20% acetonitrile buffer additive produced a rather slow separation of the mixture (Fig. 4A), whereas 50 mmol/l sodium cholate–60% methanol produced a similar separation, but in a much shorter period (Fig. 4B). Separation efficiencies under these conditions were found to be 105 000 for ideno[1,2,3-*cd*]pyrene, which indicated rapid non-mass transfer limiting exchange between the mixed aqueous–organic and bile pseudophases. Note that each component is resolved in at least one of the two electropherograms.

A few experiments were carried out to evaluate the effects of ethanol (30–50%, v/v), urea (3.2–6.8 mol/l) and methylene chloride (1%) on the PAH

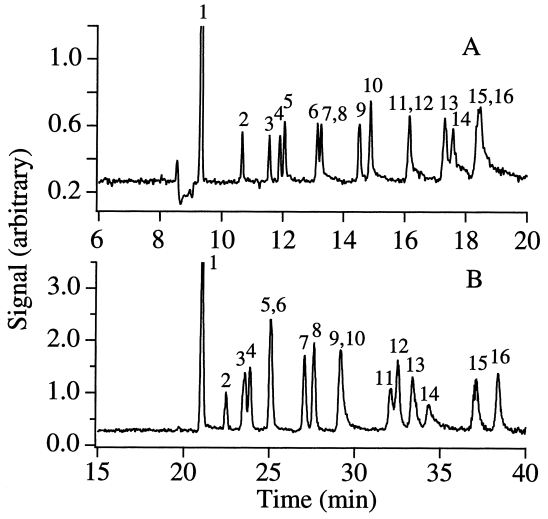

Fig. 4. Electropherograms showing CE separation of 50 ppm PAHs at an electric field of 300 V/cm. Peak identification: 1=naphthalene, 2=acenaphthylene, 3=acenaphthene, 4=fluorene, 5=phenanthrene, 6=anthracene, 7=fluoranthane, 8=pyrene, 9=1,2-benzanthracene, 10=chrysene, 11=benzo[*b*]fluoranthene, 12=benzo[*k*]fluoranthene, 13=benzo[*a*]pyrene, 14=1,2,5,6-dibenzanthracene, 15=indeno[1,2,3-*cd*]pyrene, 16=benzo[*ghi*]perylene. (A) 60 mmol/l sodium cholate, 20% (v/v) acetonitrile, 10.7 mmol/l NaH_2PO_4–1.8 mmol/l $Na_2B_4O_7$. (B) 50 mmol/l sodium cholate, 60% (v/v) methanol, 10.7 mmol/l NaH_2PO_4–1.8 mmol/l $Na_2B_4O_7$.

separation. Ethanol (50%) was effective in resolving as many as 14 PAHs in any given run at the expense, however, of long separation times, >80 min. These excessive analysis times were most likely due to the exceedingly low electroosmotic flow-rates associated with ethanol in comparison to both methanol and acetonitrile at equimolar concentrations [21].

Urea has been used in micellar electrokinetic chromatography to increase the aqueous solubility of sparingly soluble analytes and/or additives [8,10]. In our studies, urea concentrations <6.8 mol/l improved resolution particularly for the higher-molecular-mass PAHs (a total of 13 peaks observed) without compromising analysis times (~12 min). At 6.8 mol/l, poor resolution was observed for most analyte species and broad peaks for the higher-molecular-mass PAHs. Presumably, excessive amounts of urea were effective in solubilizing the PAHs in the aqueous phase thus diminishing their interactions with the bile salt aggregates. When 1% (v/v) methylene chloride was used to aid PAH solubilization in the aqueous phase, buffer precipitation was observed.

3.3. Effect of buffer concentration and pH

Buffer concentrations between 10.7–21.4 mmol/l NaH_2PO_4 and 1.8–3.6 mmol/l $Na_2B_4O_7$ in the presence of 60 mmol/l sodium cholate plus 20% methanol and 20% acetonitrile had no effect on selectivity, although resolution improved at lower buffer concentrations. Such resolution improvements were probably due to a decrease in aggregate size at lower ionic strengths [23] because of less charge shielding and thus greater repulsion between cholate monomers. The smaller aggregate size probably allows for greater analyte differentiation and thus improved resolution.

Changes in pH over the range 6.8–9.2 had little effect on analyte migration times and resolution. Cholic acid has a pK_a (pK_a 6) that lies outside that range; the pH of the buffer is not expected to influence aggregate properties or pseudostationary phase rate and direction of migration [24].

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