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Hydrophobic interaction electrokinetic chromatography for the separation of polycyclic aromatic hydrocarbons using non-aqueous matrices

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Abstract

Capillary electrophoresis methodology is developed to provide a rapid, inexpensive and robust technique for screening polycyclic aromatic hydrocarbons (PAHs) in water using additive complexation. A series of conventional RPLC ion-pairing agents are investigated in three different totally non-aqueous separation solvents, and the relative role of hydrophobic interaction versus electrostatic association is evaluated. Methanol is found to provide optimal selectivity when coupled with the tetrahexylammonium cation providing total analysis times of approximately 15 min for the analysis of thirteen 2–7-ring PAH pollutants. Solid-phase microextraction is demonstrated to be an effective sample preparation technique for extraction/preconcentration of PAHs from water into methanol run buffer prior to injection.

Keywords: Electrokinetic chromatography; Hydrophobic interaction electrokinetic chromatography; Non-aqueous capillary electrophoresis; Polynuclear aromatic hydrocarbons

1. Introduction

Since its inception, capillary electrophoresis (CE) has predominantly been associated with the separation of ionic species in aqueous solution. In such a conventional format CE excels in providing rapid, high efficiency separations, requiring minimal sample volumes and using exceptionally convenient and affordable separation columns. Walbroehl and Jorgenson published an early demonstration of the use of totally nonaqueous solvents for zone electrophonesis in 1984 [1], and the same authors subsequently investigated the separation of neutral organics through hydrophobic association in acetonitrile–water mixtures in 1986 [2]. However, with the development of micellar electrokinetic chromatography (MEKC) and subsequently electrochromatography, little serious attention has been given to the zone electrophoresis of neutrals or the use of predominantly non-aqueous matrices until the last few years [3–9].

Polycyclic aromatic hydrocarbons (PAHs) are US Environmental Protection Agency (EPA) and European Union priority pollutants produced via natural and anthropogenic combustion processes, and their measurement in water and sediments constitute a large segment of modern environmental analysis as a result of their documented carcinogenicities [10]. Although chromatographic methods are well established for PAH quantitation, there remains opportunity for improvement in their measurement, particularly in instances where rapid screening would be

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useful. In particular, GC methods are not reliable for larger isomers (e.g., coronene) due to volatility constraints, and RPLC methods require specialty columns and may exhibit lengthy analysis times. In recent years a number of interesting approaches incorporating CE have been advocated for PAH separation including MEKC using charged surfactants with or without cyclodextrin [11–20], the use of charged cyclodextrins in the absence of micelles [21–24] and the use of bile salts [25–27]. While each method has successfully demonstrated at least moderate resolution of PAH standard mixtures, in most cases limitations still remain for their practical implementation. For example, highly hydrophobic solutes such as PAHs typically associate so significantly with micelles that minimal resolution is achieved via MEKC without the addition of organic solvents or other modifiers, often resulting in lengthy analysis times. Charged cyclodextrins (e.g., sulfonated cyclodextrins) capable of forming inclusion complexes with PAHs in the absence of a micellar environment certainly appear more promising, but commercially available products are often expensive, poorly soluble in predominantly non-aqueous media and exhibit a batch-to-batch variation due to differences in degree of anionic derivatization [28]. Ren et al. recently demonstrated another promising approach using sodium cholate bile salts in aqueous mixtures of methanol, ethanol and acetonitrile (although maximal resolution required analysis times of 26–80 min) [27].

In each of the aforementioned strategies for PAH analysis, organic solvents have comprised only a fraction of the overall composition of the electrophoresing buffer, in most cases because the complexing agents are ineffective in totally non-aqueous phases due to formation or solubility constraints (e.g., micelles, sulfonated cyclodextrins). A potential advantage of using a totally non-aqueous matrix for PAH or other hydrophobic analyte separations lies in the inherently greater solubility of these substances in a predominantly (or wholly) organic matrix. In fact, due to their inherently low concentrations resulting from limited aqueous solubility, PAH analyses of natural water samples typically require some means of extraction and preconcentration using techniques including static headspace or purge-and-trap sampling for GC, and solid-phase extraction (SPE) or solid-phase microextraction (SPME) for HPLC [29,30]. Recently, several groups have demonstrated the capacity to couple SPME in particular with CE analysis [24,31,32]. Here, solutes extracted onto a silica fiber impregnated with polydimethylsiloxane or other phases are eluted in a tiny volume of solvent and introduced directly into the CE capillary for analysis.

In the present work we investigate the use of totally non-aqueous matrices including methanol, acetonitrile and *N*-methylformamide for PAH analysis via so-called hydrophobic interaction electrokinetic chromatography (HI-EKC) as coined by Ahuja and Foley [33]. As in our prior work, conventional HPLC ion-pairing agents are evaluated as matrix additives for in-situ association with each solute [34,35]. In this case, this interaction is sufficient to effectively create a net charge on the neutral PAHs (hence, an induced electrophoretic mobility) that can be correlated with their overall hydrophobicity. Electrostatic interactions have also been suggested as an alternative mechanism for the observed migration behavior with tetraalkylammonium salts in particular [9,36,37]. Since anionic as well as cationic additives of varying alkyl chain lengths are considered in our work, this fundamental question regarding the relative importance of hydrophobic interaction is evaluated. In addition to developing relatively rapid and robust PAH separations, we demonstrate the practicality of the totally non-aqueous approach by coupling our methodology with SPME for extracting and preconcentrating aqueous samples prior to injection.

2. Experimental

2.1. Instrumentation

All electropherograms were collected using a Beckman (Fullerton, CA, USA) P/ACE 5000 CE system equipped with UV–Vis filter and photodiode array detectors and System Gold software. Temperature was maintained at 25°C and injections were performed hydrodynamically for 5 s (unless otherwise indicated) at a relative pressure of 0.035 bar. Uncoated fused-silica capillary (363 μm O.D.×50 μm I.D.) was obtained from Polymicro Technologies

(Phoenix, AZ, USA) and cut to appropriate lengths using a ceramic file. Effective lengths (L_{eff}) varied from 40 to 70 cm and field strengths (E) from 390 to 640 V/cm as indicated. An SPME fiber holder and associated 24 guage, 100 μm polydimethylsiloxane fibers were obtained from Supelco (Bellefonte, PA, USA).

2.2. Reagents

HPLC-grade sodium acetate, ammonium acetate, methanol and acetonitrile were obtained from Fisher Scientific and used as received (Atlanta, GA, USA). Complexing agents including tetramethylammonium (TMA^+) chloride, tetraethylammonium (TEA^+) bromide, tetrabutylammonium (TBA^+) bromide, tetrahexylammonium (THA^+) bromide, tetraoctylammonium (TOA^+) bromide, butanesulfonic (BSA^-) acid, heptanesulfonic (HSA^-) acid and decanesulfonic (DSA^-) acid were obtained from Aldrich (Milwaukee, WI, USA), as was *N*-methyl formamide. All PAHs (naphthalene, fluorene, phenanthrene, anthracene, 2,3-benzofluorene, pyrene, chrysene, benzo[*k*]fluorene, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, benzo[*ghi*]perylene and coronene, see Fig. 1) were used as received from Aldrich. Stock solutions of PAH standards were prepared weekly in the analysis buffer, stored refrigerated and diluted to make sample solutions. All aqueous samples and buffers were prepared in deionized water with a resistivity >18.0 MΩ cm.

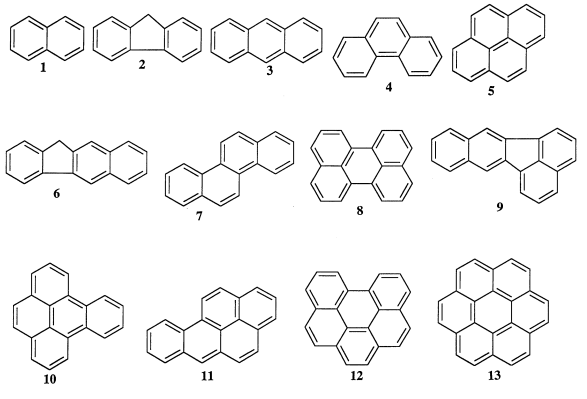

Fig. 1. Structures of PAH solutes investigated. (1) naphthalene; (2) fluorene; (3) anthracene; (4) phenanthrene; (5) pyrene; (6) 2,3-benzofluorene; (7) chrysene; (8) perylene; (9) benzo [*k*] fluoranthrene; (10) benzo[*e*]pyrene; (11) benzo[*a*]pyrene; (12) benzo[*ghi*]perylene; (13) coronene.

2.3. Procedures

New capillaries were initially conditioned with 1 *M* NaOH for 15 min prior to first use. For greatest reproducibility between successive injections the capillary was rinsed only with deionized water for 5 min prior to re-introducing the non-aqueous buffer. Electrophoretic mobilities (μ_e) were calculated based on the use of water as an electroosmotic flow marker and using the relationship $v_T = E(\mu_{eo} + \mu_e)$ where v_T = total velocity (cm/s), E = electric field strength (V/cm), μ_{eo} = coefficient of electroosmotic flow and μ_e = electrophoretic mobility in units of cm^2 s/V.

For SPME experiments, the SPME probe was initially conditioned per manufacturer's instructions (Supleco). PAH solutions prepared in deionized water were extracted from a 3 ml total volume for 30 min using magnetic stirring. Adsorbed PAHs were subsequently eluted from the fiber into methanol CE electrophoresis buffer for 2 min, and immediately injected into the CE system.

3. Results and discussion

3.1. Mechanism of separation

As indicated, Walbroehl and Jorgenson first proposed the mechanism of hydrophobic (termed ''solvophobic'') interaction for CE for the separation of five PAHs using tetrahexylammonium perchlorate (THA^+) as a complexing additive in acetonitrile–water matrices, i.e. ''hydrophobic'' model [2]. Later, Ahuja and Foley attempted the separation of three PAH solutes in non-micellar aqueous media using 3 m*M* sodium dodecyl sulfate (SDS) as the complexing additive but without success [33]. In part due to the apparent discrepancy between the separation observed using the THA^+ versus SDS (albeit in different solvents at significantly different concentrations), it has been proposed that hydrophobic interaction does not account for separations observed with THA^+ [36,37]. Bowser et al. have recently provided an excellent review of analyte-additive

interactions in non-aqueous CE, and the important types of dynamic complexation are discussed as a function of the identity of both the additive and solvent [8]. In low dielectric media such as non-aqueous solvents, the significant π-delocalization present in PAHs is suggested to facilitate polarization in the presence of THA^+, leading to electrostatic association — i.e., an ''electrostatic'' model — that results in differential electrophoretic mobilities [8,36,37].

In an effort to shed additional light on the issue of electrostatic versus hydrophobic interaction for PAHs in particular, we began our studies using linear alkylsulfonates in totally non-aqueous media. Non-aqueous solvents were selected based on a requirement of high dielectric constant (>30) to support conductivity, UV transparency at $\lambda>250$ nm, and appreciable solvency for complexing additives and PAHs. The three solvents selected (acetonitrile, methanol and *N*-methylformamide) also differ quite considerably in their dielectric values ($\epsilon=38$, 33 and 182, respectively), which should have considerable impact on their relative utility as non-aqueous solvents via HI-EKC [8]. Fig. 2 shows the separation of five PAHs in 100% methanol containing 50 m*M* decanesulfonate (DSA^-) and 25 m*M* ammonium acetate. While only four major peaks are observed in this electropherogram, the correlation between electrophoretic mobility and solute size is evident with the larger analytes (those most highly associated with DSA^-) migrating most slowly. In comparison with BSA^- and HSA^-, DSA^- provided improved selectivity even at significantly lower concentrations, consistent with its ability to more fully hydrophobically associate with each solute. Clearly then, sufficient hydrophobic interaction occurs with PAHs even in non-aqueous matrices to invoke appropriate use of the terminology HI-EKC as proposed by Ahuja and Foley in their separation of alkyl aryl ketone homologues (C_{18}, C_{20}, C_{22}, etc.) [33].

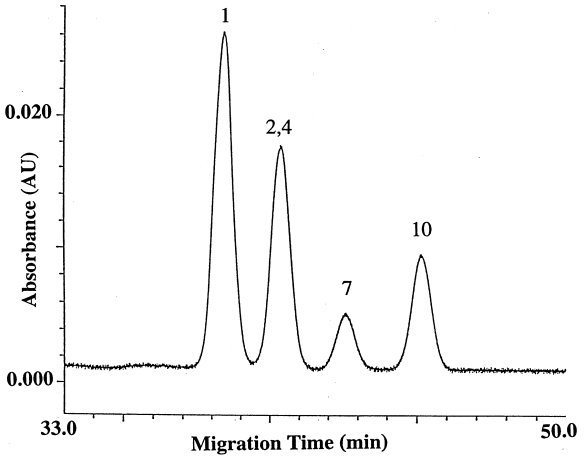

Fig. 2. Electropherogram of 50 μ*M* each (1) naphthalene, (2) anthracene, (4) phenanthrene, (7) chrysene and (10) benzo[*e*]pyrene using 25 m*M* ammonium acetate–50 m*M* DSA^- in 100% methanol. Capillary: $L_{eff}=40$ cm, $E=640$ V/cm, $\lambda=254$ nm.

In a second experimental set, sodium acetate was employed as a buffer additive to support conductivity, replacing ammonium acetate in equimolar concentration. Overall migration times were dramatically decreased due to enhanced Eo flow, necessitating the use of longer capillaries. Surprisingly, selectivity (hence, resolution) was reduced in the presence of the sodium salt. Based on a mechanism that considers *exclusively* hydrophobic interaction as the basis for separation in this buffer system, these differences in mobility as a function of the presence of the ammonium cation are difficult to reconcile, and suggest additional factors (e.g., polarizability) may contribute to separation.

A third series of experiments investigated the use of tetraalkylammonium halides ranging from one carbon (TMC^+) to eight (TOC^+) in the alkyl chain. Here again, the larger the alkyl group on the complexing additive, the greater was the observed resolution for PAH analytes (e.g., $TMA^+<<THA^+$, TOA^+) even at reduced concentrations. (Since the reduced solubility of TOA^+ versus THA^+ in the solvents considered makes this additive more difficult to use on a practical basis, THA^+ was generally favored for PAH analysis as shown in Fig. 3). The first notable difference in the THA^+ separation versus DSA^- separations is the reversal in the order of migration observed, consistent with a hydrophobic interaction model that assumes the larger, more hydrophobic analytes interact more strongly with the additive than do smaller PAHs. The hydrophobic model is likewise consistent with the significant selectivity differences observed as a function of alkyl chain length. However, another striking feature relative to the sulfonate separations is the vastly

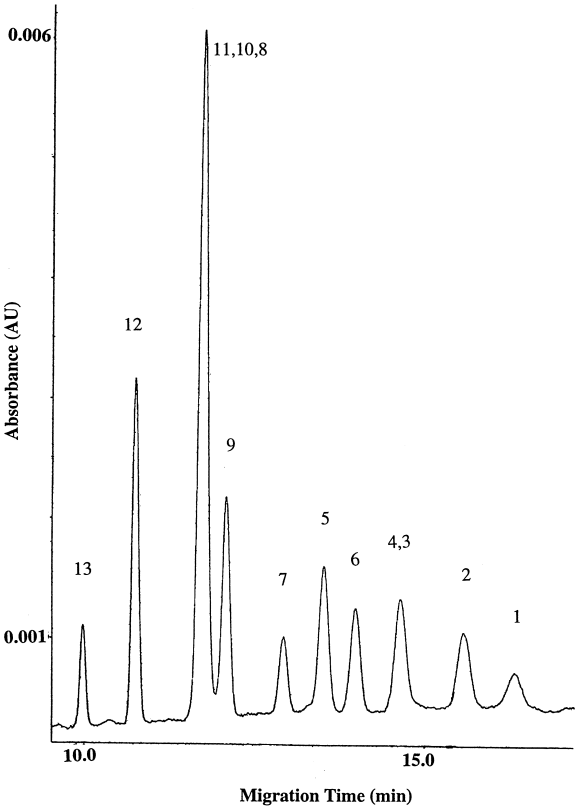

Fig. 3. Electropherogram of (13) coronene, (12) benzo[*ghi*]perylene, (11) benzo[*a*]pyrene, (10) benzo[*e*]pyrene, (8) perylene, (9) benzo[*k*]fluoranthrene, (7) chrysene, (5) pyrene, (6) 2,3-benzofluorene, (4) phenanthrene (3) anthracene, (2) fluorene, and (1) naphthalene using 50 m*M* ammonium acetate–100 m*M* THA^+ in 100% methanol. All solutes approximately 40 μ*M* concentration. Capillary: $L_{eff}=40$ cm, $E=532$ V/cm, $\lambda=254$ nm.

superior efficiencies and overall improved resolution observed, especially considering that these solutes effectively migrate *with* rather than *against* Eo flow and thus have reduced opportunity for additive interaction (note migration times). This indicates that there are indeed special features of the tetraalkylammonium cations (and also the ammonium cation) that provide enhanced separations beyond simple hydrophobic interaction.

In the work of Miller et al., PAH polarizability was correlated with electrophoretic mobility data to demonstrate the importance of electrostatic association for separations that utilized planar organic cations as a charge-transfer separation medium [37]. We have provided a similar correlation between electrophoretic mobilities measured with THA^+ and polarizability [38] for ten PAHs as shown in Fig. 4A. It is useful to note that polarizability essentially correlates with molecular volume (note units), hence, we might also expect a high correlation if only hydrophobic interactions were invoked. In fact,

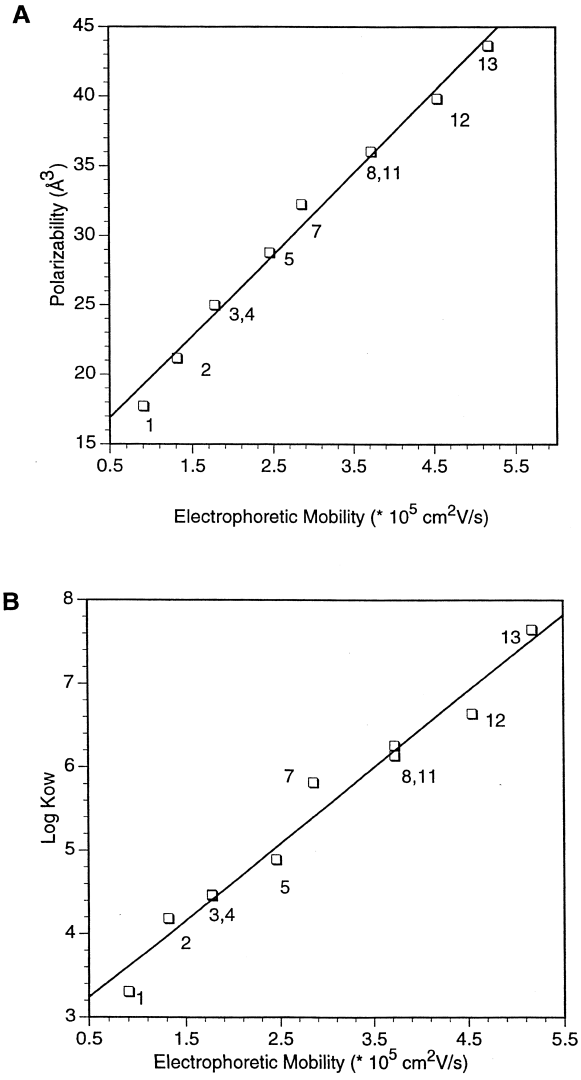

Fig. 4. (A) Electrophoretic mobility vs. PAH polarizability, (1) naphthalene, (2) fluorene, (3) anthracene, (4) phenanthrene, (5) pyrene, (7) chrysene, (11) benzo[*a*]pyrene, (8) perylene, (12) benzo[*ghi*]perylene, (13) coronene. All conditions as in Fig. 3; $y=5.9x+13.97$, $r=0.992$. (B) Electrophoretic mobility vs. log K_{ow} for PAHs; All solute identities as in Fig. 1, all CE conditions as in Fig. 3; $y=0.916x+2.78$, $r=0.985$.

plotting electrophoretic mobility vs. log K_{ow} (octanol–water partition coefficient) only slightly reduces the value of the correlation yielding $r > 0.98$ as shown in Fig. 4B. Further, in some cases we were able to achieve at least partial resolution between structural isomers (e.g., perylene vs. benzo[*a*]pyrene, see below) even though these solutes exhibit identical values for polarizability.

Taken together these data suggest that even in methanol, a solvent expected to provide a relatively poor environment for hydrophobic interaction based on the model of Bowser et al. [8], significant hydrophobic interaction between hydrophobic additives and PAHs is an essential feature of the separation mechanism. Nonetheless, the substantial improvement in resolution observed in the presence of tetraalkylammonium cations in particular does reflect that solute polarization complements this interaction. This is further supported by the improvements observed using anionic complexation in the presence of the ammonium cation, which presumably also electrostatically associates with the PAHs via polarizability phenonoma, hence creating an effective ion-pair with the hydrophobic complexing anion.

3.2. Separation optimization and system performance

In the work of Walbroehl and Jorgenson using THA^+ in acetonitrile–water matrices, evaluation was limited to five PAH test solutes differing by one aromatic ring with a total separation time approaching 25 min [2]. Subsequent separation of four PAHs by Nie et al., using high-sensitivity CE–laser-induced fluorescence (LIF) detection with 25 m*M* THA^+ in acetonitrile–water likewise demonstrated even less separation with 12 min migration times [39]. Recently, several publications have demonstrated that extremely rapid ZE separations can be achieved in pure acetonitrile phases, which is at least partially attributable to the moderately high dielectric value [8] and very low viscosity (0.34) of this solvent [5–7]. Fig. 5 shows the separation achieved for five PAHs and pyridine in pure acetonitrile containing 100 m*M* THA^+. Resolution is enhanced relative to the earlier published work [2,39] and separation times are reduced to approximately 6 min in this phase using a 77 cm capillary with a field

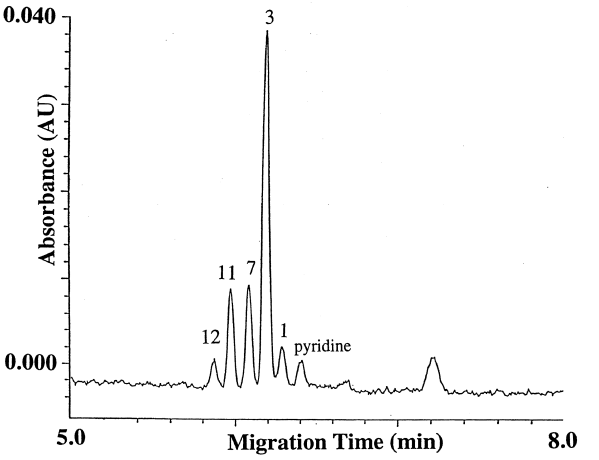

Fig. 5. Electropherogram of 50 μ*M* each (12) benzo[*ghi*]perylene, (11) benzo[*a*]pyrene, (7) chrysene, (3) anthracene, (1) naphthalene and pyridine using 100 m*M* THA^+ in 100% CH_3CN; capillary: $L_{eff} = 70$ cm, $E = 390$ V/cm, $\lambda = 254$ nm.

strength of 390 V/cm. Mixtures of methanol–acetonitrile were also evaluated as separation matrices, with a 1:1 ratio yielding at least partial resolution of a mixture of 7 (of 10) PAHs in approximately 12 min (77 cm capillary, 100 m*M* THA^+, 390 V/cm, data not shown). It is apparent, however, that resolution was significantly reduced in acetonitrile relative to methanol even though these solvents exhibit nearly identical dielectric values [8], emphasizing the importance of solvent evaluation to ascertain optimal analyte selectivity.

N-Methylformamide is significantly more viscous than acetonitrile, methanol or water, but its exceptionally high dielectric value [8] creates a solvent that rivals acetonitrile in producing rapid rates of electrophoretic migration. Analysis times were <12 min for our PAH test solutes, however separation was virtually nonexistent even with 100 m*M* THA^+ present as a complexing additive. The extreme differences in PAH *selectivity* between *N*-methylformamide and acetonitrile or methanol underscores the importance of electrostatic interactions to the overall separation process (which are expected to be significantly greater in low dielectric media [8]).

Two sets of structural isomers were included among the thirteen test solutes listed in Fig. 1, namely (1) anthracene and phenanthrene; and (2) perylene, benzo[*e*]pyrene and benzo[*a*]pyrene. As

noted in Fig. 3, these isomers were not fully resolved using the THA^+ system. However, when only perylene and benzo[*a*]pyrene were simultaneously present in our system, partial resolution was observed, with perylene eluting slightly faster as confirmed using photodiode array detection. This indicates that some shape selectivity does exist that may be manifested in increased resolution using longer capillaries (at the expense of longer analysis times) or smaller I.D. capillaries (at the expense of sensitivity). The smaller isomeric pair, phenanthrene and anthracene, did not demonstrate any resolution in the optimized system. Nonetheless, these solutes can be successfully quantitated by simultaneously monitoring the wavelengths 254 and 276 nm due to their significant differences in molar absorptivity (i.e, at 276 nm, $\epsilon_{\text{anthracene}} < 300$ vs. $\epsilon_{\text{phenanthrene}} > 5000$). Linear calibration data were obtained for resolved PAH solutes over a concentration range of 2–20 μM as shown in Table 1. In general, shorter injection times (3 s) provided superior calibration data as indicated.

3.3. Solid-phase microextraction for PAH analysis

As indicated, one reason for selecting a totally non-aqueous solvent system for HI-EKC is the potential for efficiently coupling this straightforward separation methodology with SPME. Several groups have recently made significant progress in developing direct capillary interfaces between the SPME fiber and the CE separation capillary that minimize the required volume of elution solvent following preconcentration, and one application using charged cyclodextrins has been successfully demonstrated for PAH analysis [24,31,32]. To date, however, commercially available SPME fibers do not exhibit appropriate dimensions to be readily integrated with this approach. Many data have been published in the last few years regarding the application of SPME to PAH analysis in particular [40,41]. As a simple demonstration of the applicability of this technology to our separation system, we investigated the analysis of water samples spiked with varying concentrations of PAHs and subsequently extracted using commercial polydimethylsiloxane fibers. Fig. 6 provides a sample electropherogram obtained following our SPME procedure. Overall resolution for these PAH analytes is commensurate with Fig. 3, and concentration sensitivity is encouraging (albeit inadequate for routine screening) considering the relatively large volume of elution solvent utilized following extraction. Table 1 summarizes additional data for response linearity versus PAH concentration in water for SPME analysis. It is noteworthy that the lowest quality calibration data were obtained at the two extremes with respect to solute hydrophobicity, i.e, coronene (7-ring PAH) and fluorene (3-ring PAH).

Table 1
Correlation coefficients (*r* values) for selected PAHs based on CE calibration data[a]

PAH	*r*		
	3 s injection[b]	5 s injection[b]	SPME extraction 3 second injection[b,c]
Coronene	0.944	0.998	0.807
Benzo[*ghi*]perylene	0.998	0.989	0.936
Perylene	0.996	0.985	0.982
Benzo[*k*]fluorene	0.998	0.982	0.916
Chrysene	0.998	0.976	0.857
Pyrene	0.996	0.989	0.980
2,3-Benzofluorene	0.997	0.982	0.984
Anthracene	0.980	0.975	0.992
Fluorene	–	0.979	0.880
Naphthalene	–	0.984	–

[b] All CE run conditions as provided in Fig. 3.

[c] SPME conditions as provided in Fig. 6.

[a] Linear regression data for each PAH were based on plotting peak areas for single injections vs. 2.0, 5.0, 10.0, 15.0 and 20.0 μM concentration in methanol (or water for SPME analysis).

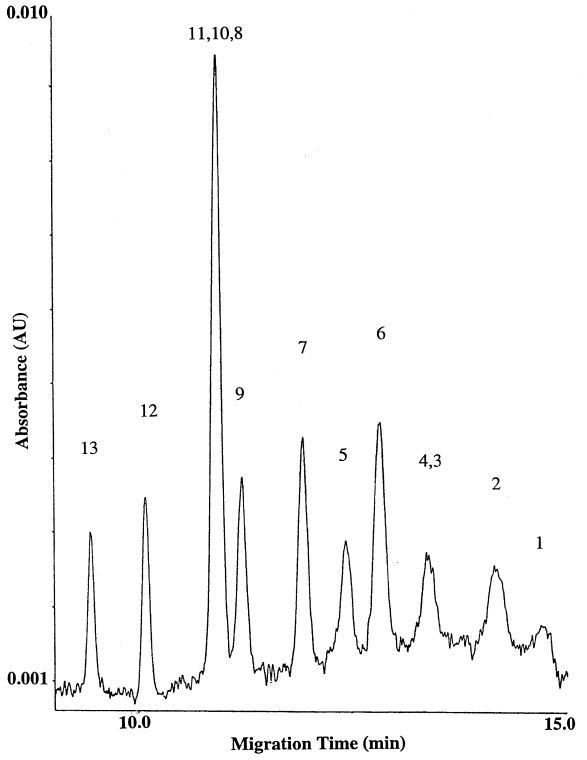

Fig. 6. Electropherogram of PAHs extracted via SPME procedure. Extraction of 3.0 ml volume of all solutes in deionized water (5 μ*M* each) for 30 min followed by elution with 200 μl of 25 m*M* ammonium acetate in methanol. All CE conditions and peak identities as in Fig. 3.

Without question the most significant drawback observed in our studies was the limited day-to-day extraction reproducibility, which ranged from <4% for higher-molecular-mass PAHs to as high as 27% (based on triplicate measurements) for smaller PAHs. This general lack of precision has been observed by others performing PAH extractions via SPME [40,41], and may be associated with the poor reproducibility of mechanical agitation provided by stirring. Ultrasonication has been suggested as a superior technique for enhancing reproducibility [41], and we are currently evaluating this approach as well as the reduction of methanol volume used for PAH desorption and LIF detection for enhanced sensitivity/selectivity to be reported in a subsequent submission.

4. Conclusion

Efficient and robust separations of a series of PAH analytes have been explored using a variety of traditional HPLC cationic and anionic ion-pair agents in several totally non-aqueous solvent systems. Hydrophobic interaction is shown to be an essential factor involved in separation, yet electrostatic interaction due to polarization effects is clearly demonstrated for cationic additives based on observed differences in solvent selectivities. Methanol provides the best overall combination of solvent transparency, PAH resolution, and analysis time of the solvents investigated, and the tetrahexylammonium cation is demonstrated to be an exceptionally convenient and effective complexing agent. Solid-phase microextraction shows significant promise as a sample pretreatment option that will permit rapid CE analysis for screening PAH samples extracted from water at minimal cost and optimal convenience.

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