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Solid-phase extraction sorbent consisting of alkyltrimethylammonium surfactants immobilized onto strong cation-exchange polystyrene resin

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Abstract

Presented is a solid-phase extraction sorbent material composed of cationic alkyltrimethylammonium surfactants attached to a strong cation-exchange resin via ion-exchange. The original hydrophilic cation-exchange resin is made hydrophobic by covering the surface with alkyl chains from the hydrophobic portion of the surfactant. The sorbent material now has a better ability to extract hydrophobic molecules from aqueous samples. The entire stationary phase (alkyltrimethylammonium surfactant) is removed along with the analyte during the elution step. The elution step requires a mild elution solvent consisting of 0.25 M Mg^{2+} in a 50% 2-propanol solution. The main advantage of using a removable stationary phase is that traditionally utilized toxic elution solvents such as methylene chloride, which are necessary to efficiently release strongly hydrophobic species from SPE stationary phases, may now be avoided. Also, the final extract is directly compatible with reversed-phase liquid chromatography. The performance of this procedure is presented using pyrene as a test molecule.

Keywords: Solid-phase extraction; Alkyltrimethylammonium compounds; Pyrene; Hydrophobic analytes

1. Introduction

A vast majority of real samples contain analytes that are at a concentration too low for direct instrumental injection. Preparing a sample by concentrating the species of interest prior to instrumental introduction is often necessary. Currently, the most widely used sample preparation method for chromatographic instrumentation is solid-phase extraction (SPE) [1–4]. In SPE, a solid sorbent is most commonly contained within a column, cartridge, or disk, and aqueous solutions containing analytes are passed through the sorbent. Analytes partition into this solid sorbent material and can then be removed by rinsing with a volume of organic solvent. In SPE, hydrophobic forces between the analyte and stationary phase must be broken by using a proper organic solvent. Unfortunately, many organic solvents used to elute strongly sorbed analytes are immiscible with mobile phases used for HPLC and thus incompatible for direct injections. Therefore, a time consuming and environmentally unfavorable procedure of ‘‘blowing-down’’ the organic solvent must first be completed. The residual material must then be reconstituted in the same volume of solvent (methanol or acetonitrile) that is miscible with the mobile

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phase. Methylene chloride, a known carcinogen and possible ozone destroyer, is the most commonly used solvent for strongly hydrophobic analyte elution in SPE. Approximately 10 ml of methylene chloride may be used per determination [5,6]. Collectively, large volumes of this solvent are used as many commercial laboratories determine analytes that strongly sorb [i.e. polyaromatic hydrocarbons (PAHs)].

Surfactant-based extractions are a very active area of chemical research. Cloud point extractions [7,8] and aqueous two-phase extractions [9,10] both utilize surfactants in liquid solution media and are currently studied for their environmental and/or selectivity advantages over the use of conventional organic solvents for the separation of biomolecules. Surfactants immobilized on support materials have been used to aid in the extraction of certain analytes. Extractions of anionic phenols have been accomplished using a C_{18} based sorbent with cationic surfactants which adhere through chain–chain affinities [11]. The exposed cationic functional group of the surfactant now attracts negatively charged species (anionic phenols) electrostatically. Large-scale extraction of organic pollutants using pyridium surfactants bound to a cation-exchange resin has been reported [12,13]. We have demonstrated that cationic surfactants can be used successfully as extractants [14]. These same cationic surfactant molecules can be bound ionically to a strong cation-exchange resin. In the presented approach, alkyltrimethylammonium bound resin material is placed within a column; then organic analytes from an aqueous solution are extracted as they flow through the column. Our overall approach taken is analogous to conventional SPE. However, in the presented approach, the analyte–stationary phase interaction is not broken. Instead, the stationary phase (surfactant) together with the hydrophobic analyte is removed via ion-exchange from counter ions in a water and low molecular mass alcohol rinse solution. The presented sample preparation method does not require the use of harmful organic solvents because strong hydrophobic interactions between the analyte and stationary phase (surfactant) do not have to be broken.

Preparation of traditional SPE media is long and requires several steps to condition the silica particles. Further steps involve mixing silica gel with the appropriate reagent (i.e. C_{18} moiety requires trichlorooctadecyl silane). Large volumes of organic solvents such as benzene and toluene are used as the reaction solvent and for rinsing the prepared media [15,16]. In the proposed protocol, the extraction media is prepared in a very mild, water-based reaction mixture. The mixture is simply water, surfactant, and resin.

Presented are the results of the extraction of a strongly hydrophobic analyte, pyrene, using an alkyltrimethylammonium surfactant–polystyrene strong cation-exchange resin sorbent material. Rinse solution parameters investigated include counter ion type, counter ion concentration, alcohol type, and alcohol concentration. Sorbent parameters studied include crosslinking and surfactant chain length.

2. Experimental

2.1. Chemicals and materials

Polyaromatic hydrocarbons consisting of naphthalene, acenaphthylene, phenanthrene, acenaphthene, anthracene, pyrene, and benzo[*a*]pyrene were purchased from either Aldrich (Milwaukee, WI, USA) or Acros (Fisher Scientific, Pittsburgh, PA, USA). Dowex 50WX2-400, Dowex 50WX8-400 which are sulfonated polystyrene resins, and all alcohols which were HPLC grade were purchased from Fisher Scientific. Salts of all counterions were in the chloride form and purchased from Aldrich.

2.2. Sorbent preparation

These strong cation-exchange resins were obtained in their commercially available H form. A Soxhlet extraction set-up was used to clean this resin for at least 8 h using methanol as the solvent. The resin was dried overnight at room temperature conditions then mixed with the appropriate amount of surfactant (a 1:1 mol ratio) for at least 8 h. The resin–surfactant sorbent was filtered with 1 l of deionized water, then subjected to the Soxhlet extractor for another 8 h. The cleaned sorbent was stored in an all-glass container with a ground glass stopper. All sorbent material was stored in all glass containers. It was discovered that sorbent material placed in polymer based containers became contaminated with plasticizers, therefore all manipulations of sorbents were done with glass containers and glass pipettes.

2.3. Instrumentation

A high-performance liquid chromatographic (HPLC) system was run in the reverse phase mode using a Varian Microsorb C_{18} column (Varian Analytical Instruments, Walnut Creek, CA, USA) connected to a UV–Vis detector (Varian, Model 9050) set at 254 nm. The mobile phase was programmed using methanol–water from 50:50 to 100:0 (v/v) in 30 min with a 1 ml/min flow-rate. Data obtained from the HPLC was used to calculate percent recoveries of pyrene. All injections were at 50 μl using an autosampler (Varian, Model 910). Nitrogen analysis of the sorbent materials was obtained from a Leco Model 100 CHN analyzer (St. Joseph, MI, USA). Data obtained were used to calculate the percentage of surfactant removed from the resin. The surfactant was the only source of N.

2.4. Extraction set-up

All studied sorbents were housed in a 300×10 mm I.D. Adjusta-Chrom (Ace Glass, Vineland, NJ, USA) adjustable chromatography column. In order to accommodate small amounts of sorbent, 15 and 30 cm extenders were used instead of two 15-cm extenders. In our early studies, polymeric based columns with polymeric frits were used. It was not possible to remove PAHs sorbed to this material with mild elution solvents. The Adjusta-Chrom column allows minimal analyte contact with polymeric materials. The column tube is composed of glass, and the frit is silica based. All experiments were performed in trials of three.

3. Results and discussion

3.1. Correlating surfactant removal with pyrene elution

The first set of experiments conducted was aimed at exploring whether there is a direct correlation between the amount of surfactant removed from the resin and the amount of pyrene eluted from the sorbent. In Table 1, the results of the elution of 50 ng of sorbed pyrene from a C_{12} trimethylammonium form Dowex 50WX2 sorbent are presented. Two milliliters of a 25-ppb pyrene standard in 10% methanol were introduced at a dropwise flow onto 2 ml of sorbent, and the eluate was collected. Injection of the eluate revealed that pyrene did not break through the resin. Two milliliters of each rinse solution, which consisted of calcium counter ions with different alcohols at 75%, were passed through the sorbent individually. Percent recovery was based on the first five rinses. These initial results indicate no correlation between the percent pyrene eluted and the percent surfactant removed. Each rinse solution provided a nearly quantitative removal of surfactant, however the percent pyrene eluted correlates to the elution strength of the alcohols. In Table 2, the results of the same experimental procedure were conducted using different percentages of methanol in the rinse solution. A solvent strength–pyrene elution correlation is again observed. The greater the amount of methanol, the greater the percent pyrene eluted. Also noted is percent surfactant removed increases when the rinse solution increases from 50% to 75% methanol. The increased solvent strength is allowing the immobilized alkyl chains to extend or become more soluble in the rinse solution. The chain extension now enables calcium counter ions to more readily exchange surfactant ions off the resin. Although 2-propanol resulted in the best pyrene elution

Table 1
Percent pyrene eluted and percent surfactant removed after rinsing C_{12} immobilized Dowex 50WX2 resin with various alcohol-containing rinse solutions

Rinse solution	Percent pyrene eluted	Percent surfactant removed
75% methanol; 0.25 M Ca^{2+}	60.0±10.1	98.7±0.7
75% ethanol; 0.25 M Ca^{2+}	82.1±7.0	98.3±0.7
75% 2-propanol; 0.25 M Ca^{2+}	98.7±7.5	96.7±1.2

Table 2
Percent pyrene eluted and percent surfactant removed after rinsing C_{12} immobilized Dowex 50WX2 resin with varying percent methanol rinse solutions

Rinse solution	Percent pyrene eluted	Percent surfactant removed
50% methanol; 0.25 M Ca^{2+}	0	65.5±3.3
75% methanol; 0.25 M Ca^{2+}	60.0±10.1	98.7±0.7
100% methanol; 0.25 M Ca^{2+}	72.4±18.9	86.8±4.4

for this study, we initially chose to investigate methanol-based mixtures as rinse solutions. We chose methanol because it is a common mobile phase component in reversed-phase HPLC and thus may be the most compatible alcohol. It was our hope that the experimental parameters studied in Sections 3.3–3.6 may lead to the possibility of using methanol as the alcohol in the rinse solution.

3.2. Nature of pyrene–resin interactions

There exists an attraction between pyrene and the unmodified resin, therefore we chose to explore the nature of the pyrene–resin interaction by conducting experiments on resin that had no immobilized surfactant. Pyrene loading was performed in the same manner as described, and experiments were conducted on Dowex 50WX2 resin in two different forms. Dowex 50WX2 was supplied in the H form. Pyrene was quantitatively removed using a 75% methanol solution. The resin was placed in the Ca form by passing 0.1 M $CaCl_2$ through the H form resin until the pH was neutral according to pH indicator paper. The resin was then rinsed with several bed volumes of deionized water. Pyrene eluted in less than a quantitative yield (77.7±5.3%) with a 75% methanol rinse. Walton [17] observed the increased affinity of this type of resin for pyrene in various metal forms including calcium. Dowex 50WX2 is a polystyrene backbone polymer. It is believed that the electrostatic field generated by resin-bound metal ions such as calcium induces a dipole moment in pyrene and thus strengthens sorption. The strong attraction between pyrene and the resin is due to π–π interactions between phenyl groups on both substances. When the rinse solution contains 0.25 M calcium ions the percent pyrene removal further drops to 54.4±3.1%. This is due to a ‘‘salting-out’’ effect, whereby through the addition of salt, pyrene is becoming less soluble in the rinse solution.

3.3. Effect of counter ion on surfactant removal and pyrene elution

Examined next were rinse solutions containing environmentally friendly cations, namely magnesium and sodium in addition to calcium. Rinse solutions in 75% methanol with counter ions at a 0.5-M charge concentration were prepared from their chloride salts and used for eluting pyrene from Dowex 50WX2 immobilized with C_{12} trimethylammonium surfactant. Results are presented in Table 3. This sorption trend is the same as that reported by Walton [17], for these three ions. Through these preliminary results, it seems sorption is inversely related to the hydrated ion size. The smaller the hydrated ion, the greater the polarization and thus the greater the sorption. Because the rinse solution with magnesium ions provided the best results in terms of percent pyrene elution, it was chosen for further studies in place of calcium ions.

Table 3
Percent pyrene eluted and percent surfactant removed after rinsing C_{12} immobilized Dowex 50WX2 resin with a 75% methanol rinse solution containing different counter ions

Rinse solution	Percent pyrene eluted	Percent surfactant removed
75% methanol; 0.25 M Ca^{2+}	70.0±4.3	98.3±0.6
75% methanol 0.25 M Mg^{2+}	88.4±5.4	98.6±0.2
75% methanol; 0.50 M Na^{+}	53.3±10.3	87.9±0.4

3.4. Optimization of counter ion concentration

A 75% methanol rinse solution with varying concentrations of Mg^{2+} were examined to find optimum conditions for pyrene elution. In Fig. 1, a plot of concentration of Mg^{2+} versus percent pyrene and percent surfactant removed is presented. A trend of increasing surfactant removal is observed as the concentration of the magnesium counter ion is increased. As the counter ion concentration is increased, the equilibrium is expected to shift towards a greater surfactant ion-exchange, thus more surfactant will be removed. The percent pyrene eluted from the sorbent reaches an optimum at a concentration of approximately 0.25–0.30 *M* Mg^{2+}. At greater concentrations, pyrene removal decreases, which is due to the ‘‘salting out’’ effect. Pyrene becomes less soluble in the rinse solution as the concentration of magnesium ion increases. However, at a concentration of 0.10 *M* Mg^{2+}, the percent pyrene removal is less than optimum. There is a balance that is occurring between the concentration of magnesium counter ion and pyrene removal. Though the ‘‘salting out’’ effect will be the weakest at 0.1 *M* magnesium ions, this is not a sufficient concentration of counter ion that will remove enough surfactant to provide optimal pyrene removal. Using a 0.25 *M* Mg^{2+} 75% methanol rinse on the same resin in the magnesium form resulted in a 76.3±12.5% pyrene elution. Optimal rinse conditions using the surfactant bound resin sorbent provided a better pyrene removal. This is possibly due to a shielding effect created by the surfactant that hinders to some extent the interaction of pyrene with the polystyrene-based resin.

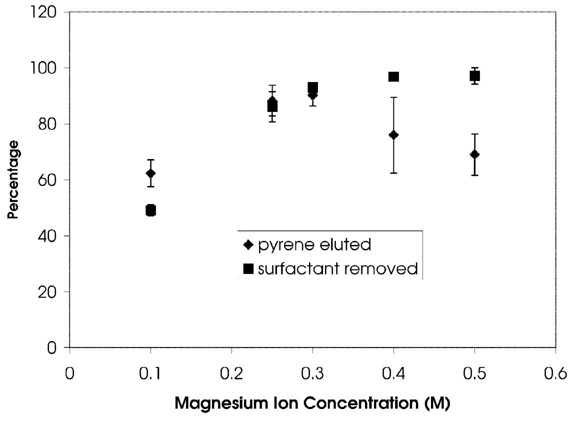

Fig. 1. Pyrene elution and surfactant removal versus rinse solution magnesium ion concentration.

3.5. Effect of resin crosslinking

A more highly crosslinked Dowex 50WX8 resin was investigated for its affinity for pyrene. This resin in the calcium form rinsed with 0.25 *M* Ca^{2+} in 75% methanol resulted in an 80.6±4.1% elution. The X2 resin under the same set of conditions provided a 60.0±10.1% pyrene elution. The weaker affinity of pyrene for the higher crosslinked resin may be due to size exclusion. As the crosslinking becomes greater, the pore size decreases, thus preventing pyrene from entering. When eluting pyrene from C_{12} trimethylammonium immobilized resin, a different trend is observed. With a rinse solution consisting of 0.25 *M* Mg^{2+} in 75% methanol, pyrene elution was 65.4±11.1 and 88.3±5.4% for X8 and X2, respectively. The lower recovery with the X8 resin is most likely due to an inefficient 18.3% surfactant removal. A much greater surfactant removal, 86.1±5.4%, was achieved for the rinsing of X2. The greater amount of surfactant remaining on the X8 resin is retaining pyrene to a greater degree. It becomes more difficult to remove the surfactant ions from the higher crosslinked resin because it takes more time for diffusion to occur in smaller pores.

3.6. Effect of surfactant chain length

C_8, C_{12}, and C_{16} trimethylammonium surfactants were immobilized onto the X2 resin. Using a rinse solution of 0.25 *M* Ca^{2+} in 75% methanol pyrene elution was measured at 53.6±4.5, 70.0±4.3 and 63.0±7.9% for the C_8, C_{12} and C_{16} moieties, respectively. Surfactant removal was 99.9±4.5, 98.3±0.6 and 55.9±3.6%. The higher surfactant removal yet lower pyrene elution for the C_8 versus C_{16} may be the result of a weaker shielding of the polystyrene based resin from the smaller less hydrophobic surfactant chain. However, the C_{16} surfactant being the more hydrophobic ion proves very difficult to remove from the polymeric resin, and thus this sorbent combination strongly retains pyrene as well. Because the C_{12} immobilized surfactant provided the best results for pyrene elution and near quantitative surfactant removal, it was chosen as the optimal sorbent material.

3.7. Choice of 2-propanol as a component in the rinse solution

Methanol as a component in the rinse solution resulted in pyrene present in all five rinse fractions. It is desirable to elute an analyte in as few rinses as possible. For any given analyte, the concentration factor will decrease with increasing rinses. 2-propanol, because it is a stronger solvent than methanol, was reexamined as a possible rinse solution component. From the first set of experiments, it was observed that 2-propanol provided the best results in terms of pyrene elution. A greater fraction of pyrene is removed in early rinses using 2-propanol versus methanol. Quantitative removal is achieved in three rinses for the 2-propanol containing rinse; however, 70.0±4.3% of pyrene was eluted in five methanol containing rinses.

In Fig. 2, the elution of pyrene and removal of surfactant for various magnesium ion and 2-propanol rinse concentrations are presented using the C_{12} trimethylammonium surfactant ion immobilized onto Dowex 50WX2. High percentages of pyrene elution and surfactant removal are achieved for the rinses containing 50 and 75% 2-propanol. The 25% 2-propanol 0.25 *M* Mg^{2+} rinse resulted in less that a quantitative removal of surfactant and no detectable elution of pyrene in five rinses. The solvent strength of this rinse is too weak to remove pyrene from the surfactant and resin. The 90% 2-propanol, 0.25 *M* Mg^{2+} rinse resulted in lower recoveries than the 50 or 75% 2-propanol-containing rinses. A trend is observed at alcohol percentages of 50 or greater: as more surfactant is removed, more pyrene is eluted. This phenomenon where an increase in the alcohol component resulted in less surfactant removed was previously observed in Table 2.

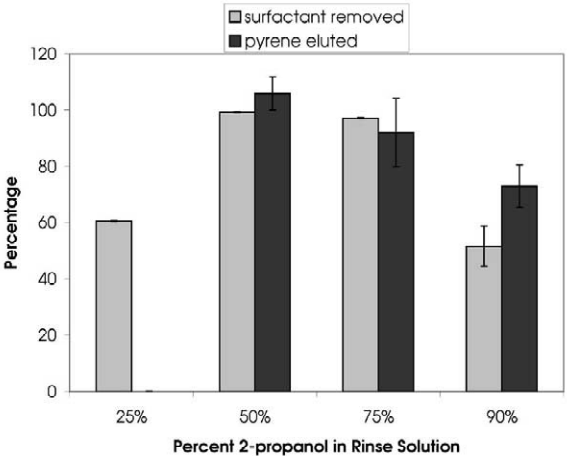

Fig. 2. Pyrene eluted and surfactant removed from C_{12} trimethylammonium surfactant immobilized Dowex 50WX2 resin versus percent 2-propanol in the rinse solution.

3.8. Advantage of removing immobilized surfactant

A control experiment was conducted to examine the significance of removing the immobilized surfactant on pyrene elution. Pyrene was rinsed from C_{12} trimethylammonium Dowex 50WX2 sorbent using a 50% 2-propanol rinse solution with no counter ions. No detectable pyrene peak is observed for five rinses, and no detectable removal of surfactant was measured. In the same experiment using a 50% 2-propanol 0.25 *M* Mg^{2+} solution, it was found that quantitative removal of both pyrene and surfactant was accomplished in three rinses. By removing the surfactant in a quantitative fashion, pyrene was eluted quantitatively.

3.9. Results of higher pyrene loading and hard water study

Investigated next was the efficiency of removing pyrene at a greater loaded amount. Ten times the amount of pyrene from the previous experiments was loaded onto the C_{12} trimethylammonium Dowex 50WX2 sorbent. Two hundred and fifty milliliters of a 2-ppb pyrene solution prepared in deionized water were passed through this sorbent. The amount extracted corresponds to 500 ng of pyrene. Ten 2-ml rinses consisting of 75% 2-propanol 0.50 *M* Mg^{2+} were rinsed through the sorbent sequentially and then individually injected into the HPLC. After five rinses, the percent of pyrene removal was 73.1±7.6%. Only after the ten rinses was a 99+% removal of pyrene observed. A quantitative removal of the surfactant was achieved after five rinses; however, a less than quantitative removal of pyrene was observed after the same number of rinses. Pyrene is not easily removed at this higher amount because of strong hydrophobic interactions with the polystyrene-based resin. A hard water matrix (500 ppm $CaCO_3$), under the same experimental conditions, was investigated for the possibility of surfactant ions being stripped by counterions and thus a possible loss of extraction efficiency. The results were statistically similar to those reported for the deionized water matrix. A 73.2±3.1% elution of pyrene was recorded after the first five rinses, and there was no measurable decrease in the amount of surfactant being removed from the sorbent after the analyte solution had passed through.

3.10. Breakthrough study

Two milliliters of H form Dowex 50WX2 resin and C_{12} trimethylammonium surfactant immobilized onto Dowex 50WX2 resin were placed individually on line using the described extraction column. The extraction column was attached in place of the HPLC column, and a frontal chromatography experiment was performed where a 1.85-ppm naphthalene solution was introduced through the extraction column at 1.00 ml/min. The breakthrough profiles of the resin and sorbent are depicted in Fig. 3. The breakthrough volume (1% of initial analyte absorbance) for the H form Dowex 50WX2 resin was approximately 23.0 ml which corresponds to 42.5 μg of naphthalene sorbed. The breakthrough profile for the C_{12} trimethylammonium surfactant immobilized resin reveals naphthalene breaking through at a much later time than the resin containing no surfactant. The breakthrough volume for the surfactant-immobilized resin is 325 ml, corresponding to 601 μg of naphthalene sorbed. While the H form resin would be expected to have some retention for naphthalene

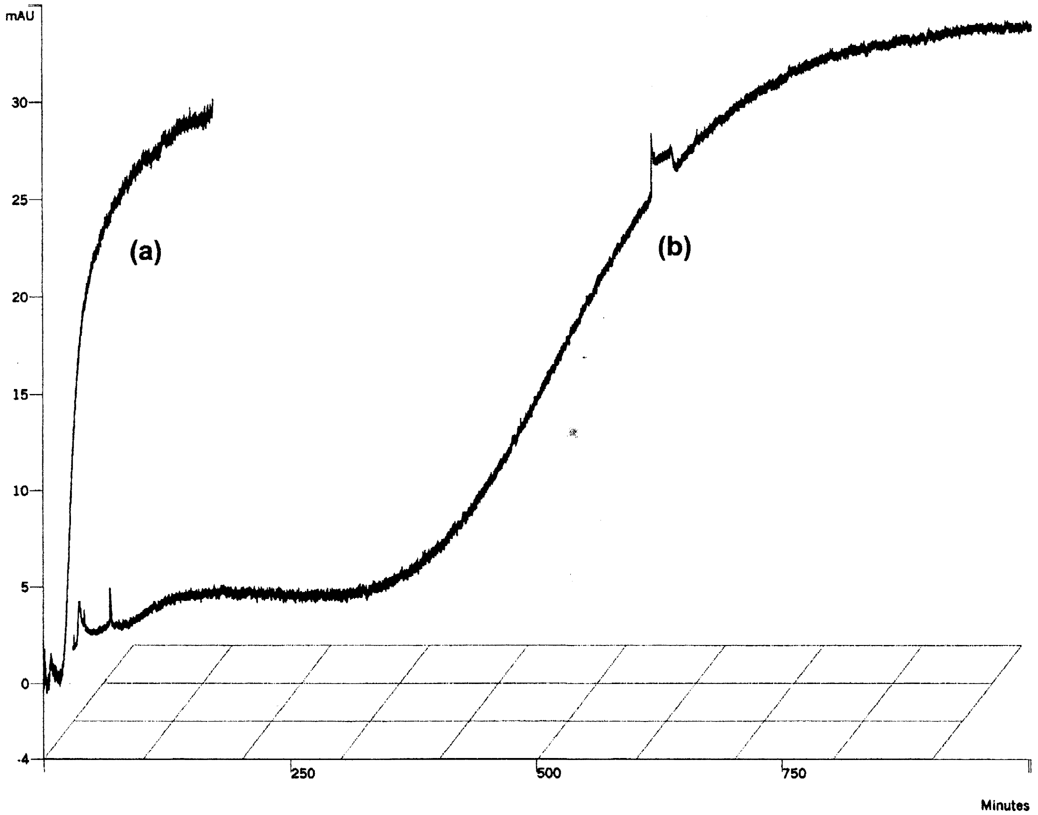

Fig. 3. Breakthrough profiles of (a) unmodified Dowex 50WX2 resin and (b) C_{12} trimethylammonium surfactant modified Dowex 50WX2 resin.

from π–π interactions, a greater than 10-fold increase in trapping efficiency for naphthalene is obtained by having surfactant immobilized onto this resin. Naphthalene was chosen in this experiment because a pyrene breakthrough signal at the same conditions did not appear after 14 h using this surfactant-immobilized sorbent.

3.11. HPLC band broadening study

The large amount of surfactant in the final eluant brings into question the possibility of extra band broadening. To study this possibility a mixture of PAHs at a concentration of 500 ppb was examined in a 50:50 (v/v) methanol–water matrix and in the first rinse matrix. The PAH components were naphthalene, acenaphthylene, phenanthrene, acenaphthene, anthracene, pyrene, and benzo[*a*]pyrene. There was no observable difference in the bandwidths for each of the seven PAHs as observed in Fig. 4. A direct injection of this surfactant-containing matrix is thus feasible. Throughout our studies, no increase in band broadening has been observed.

4. Conclusion

This surfactant-immobilized material shows advantages for the extraction of hydrophobic analytes. Namely, at lower amounts sorbed, removing the surfactant layer allows the sorbed analyte to be removed in fewer rinses. Our future work will focus on ways to improve this technique by investigating resin supports that have a much weaker attraction for phenyl containing substances. In this case, we would expect hydrophobic species to be removed in milder solvent systems and in fewer rinses.

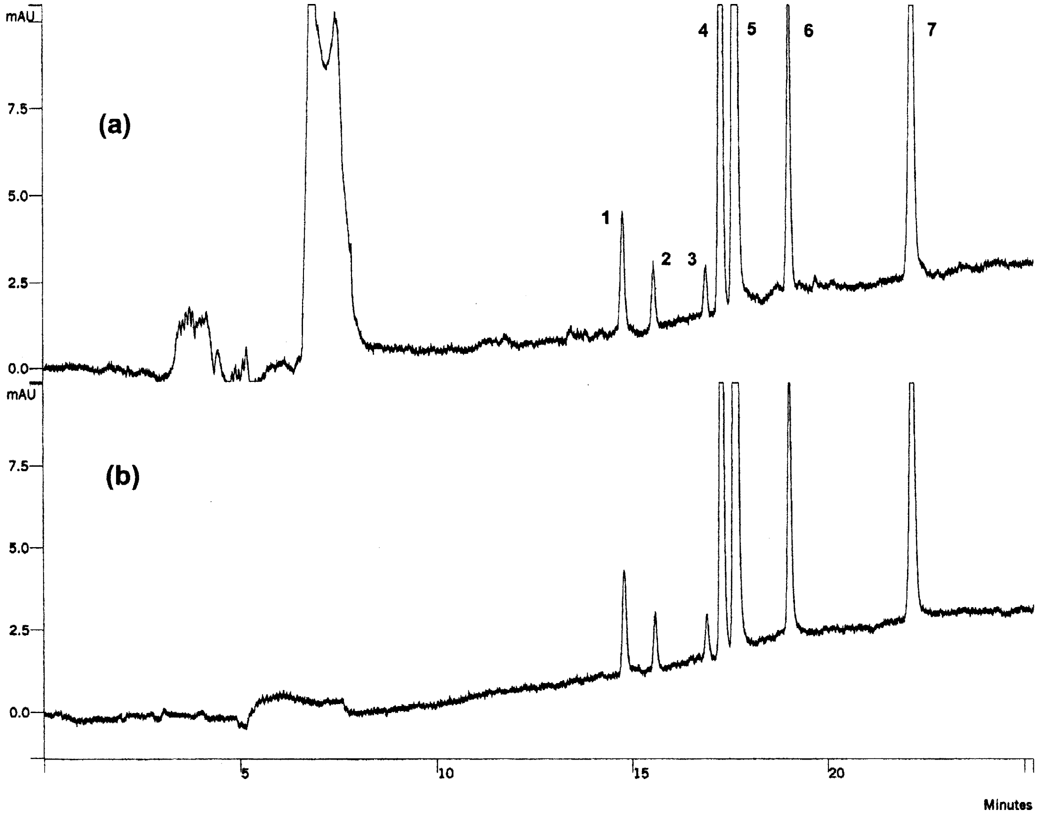

Fig. 4. Separation of seven PAHs in (a) 50:50 (v/v) methanol–water matrix and (b) column eluant containing C_{12} trimethylammonium surfactant. 1-naphthalene, 2-acenaphthylene, 3-acenaphthene, 4-phenanthrene, 5-anthracene, 6-pyrene, and 7-benzo[*a*]pyrene.

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