Title: Hemimicelles of Alkyl Carboxylates Chemisorbed onto Magnetic Nanoparticles. Study and Application to the Extraction of Carcinogenic Polycyclic Aromatic Hydrocarbons in Environmental Water Samples

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ABSTRACT

In this work, magnetic nanoparticles (MNPs) coated with hemimicelles of alkyl (C_{10}–C_{18}) carboxylates, which have traditionally been used to prepare ferrofluids for technological applications, were used for the first time to extract organic contaminants from environmental water samples. The nanoparticles were coated by following a simple, rapid procedure (30 min at 85 ºC) that uses commercially available magnetite of 20–30 nm mean particle diameter. The strong chemical bonding between the surfactant and magnetite relative to the electrostatic forces involved in conventional hemimicelle-based sorbents precludes leaching of the surfactant, and facilitates its reuse and the obtainment of surfactant-free extracts. Tetradecanoate hemimicelles were used here to extract carcinogenic polycyclic aromatic hydrocarbons (CPAHs) prior to analysis by liquid chromatography (using a C_{18} Supelcosil LC-PAH column and a gradient elution program with water and acetonitrile) and fluorescence detection. The procedure involved stirring filtered aqueous samples (350 mL) with 200 mg of tetradecanoate-coated MNPs for 15 min, isolating the sorbent with a Nd-Fe-B magnet and eluting the CPAHs with a mixture of acetonitrile and tetrahydrofuran. The extraction efficiency was not influenced by salt concentrations up to 1 M and pH values over the range 2.5–9. The preconcentration factor was 116. No clean-up of the extracts was needed and the method proved matrix-independent. The limits of quantitation thus obtained, 0.2–0.5 ng L^{-1}, meet the stringent water quality requirements established by the recently amended European Water Framework Directive 2000/60/EC and also the US EPA for the determination of CPAHs in surface and ground waters. The proposed method was successfully applied to the determination of these pollutants in surface and ground environmental water samples collected from various places in southern Spain. Recoveries from samples spiked with CPAHs at concentrations of 1–10 ng L^{-1} ranged from 85 to 94%, and relative standard deviations from 1 to 7%. CPAHs were only present in the sample from Navallana reservoir, at concentrations over the range 0.42–0.96 ng L^{-1}.

INTRODUCTION

Solid-phase extraction (SPE) continues to be the leading technique for the extraction of pollutants in aquatic systems.[1] The use of SPE in this field has been fostered by the development of new sorbent materials (*e.g.* molecularly imprinted polymers, carbon nanotubes, hemimicelles, admicelles) and formats [*e.g.* solid phase microextraction (SPME), stir bar sorptive extraction (SBSE)], which have greatly expanded the scope and experimental convenience of this extraction technique.

Hemimicelles and admicelles are flexible materials with unique features for SPE.[2–4] Hemimicelles are tepee-like monolayers of ionic surfactants adsorbed onto the surface of oppositely-charged mineral oxides. Admicelles are roughly spherical aggregates formed by adsorption of an additional surfactant on hemimicelles via hydrocarbon chain interactions. By virtue of the amphiphilic character of surfactants, hemimicelles and admicelles possess regions of disparate polarity allowing the solubilisation of solutes of highly different nature. Thus, surfactant hydrocarbon chains solubilise hydrophobic pollutants; while surfactant polar groups can retain solutes through hydrogen bonding, π-cation and/or electrostatic interactions; and amphiphilic compounds are strongly retained by mixed aggregate formation. A number of pollutants including surfactants,[2,4-6] chlorophenols,[6,7] phthalates,[6] pesticides,[3,8] endocrine disrupters,[9] drugs[10] and perfluorinated compounds[11] have been successfully extracted from surface, ground and waste water by exploiting the previous interactions. The multiple retention mechanisms that may come into play make hemimicelles and admicelles excellent sorbents for multiclass pollutant extraction (*e.g.* pesticide multiresidue analysis[12]). Most of their uses in this field involve alumina or silica as the mineral oxide and an alkyl sulphate or quaternary ammonium salt as the surfactant.

The outstanding properties of hemimicelles and admicelles warrant research into ways of overcoming their intrinsic and format-related drawbacks, which have so far restricted their applicability or experimental convenience. The greatest disadvantages of these sorbents arise from disruption of surfactant aggregates during analyte elution; this produces extracts containing high surfactant concentrations that may suppress ionization in MS or interfere with MS, UV or fluorescence detection. Also, the extracts are incompatible with separation techniques such as gas chromatography and capillary electrophoresis. On the other hand, hemimicelles/admicelles are preferentially used in cartridge format, which results in slow extraction and low breakthrough volumes despite their widespread use in SPE.

In this work, we assessed the ability of new surfactant–mineral oxide sorbents (*viz.* alkyl carboxylate–magnetite mixtures) to circumvent the above-described shortcomings. MNPs coated with single or double layers of alkyl carboxylic acids have been widely used to prepare ferrofluids, which are stable colloid suspensions of magnetic nanoparticles in a carrier liquid. Ferrofluids are widely used in dynamic loudspeakers and also in sealing, computer hardware, aerospace and mechanical engineering applications;[13–14] lately, they have also attracted much attention among biomedical and biological researchers.[15–17] The coating of magnetite with single layers of alkyl carboxylates occurs via the formation of bidentate mononuclear complexes between carboxylate groups and iron atoms;[18,19] this facilitates the dispersion of magnetite in nonpolar solvents.[20-22] Unlike conventional hemimicellar sorbents generated by electrostatic interaction, alkyl carboxylate-coated MNPs possess excellent chemical stability and hence the potential for reusing and the obtainment of surfactant-free extracts. In addition, magnetic SPE affords straightforward extraction even with large sample volumes.

Magnetic SPE initially used MNPs coated with silica or charcoal[23] and, more recently, polymers[24] to avoid alteration of the magnetic properties of magnetite or its oxidation below pH 4. These coatings were subsequently functionalized with organosilanes and/or affinity ligands in order to enable the selective extraction of organic contaminants[25] and metals.[26,27] Also, alkyl quaternary ammonium salts were physisorbed onto MNPs, both uncoated[28] and coated with silica,[29] and sodium dodecyl sulfate was physisorbed on alumina-coated MNPs,[30] in order to obtain hemimicelles and admicelles; this approach, however, failed to produce surfactant-free extracts.

In this work, we assessed the ability of MNPs coated with alkyl (C_{10}–C_{18}) carboxylates to extract and concentrate carcinogenic polycyclic aromatic hydrocarbons (CPAHs) from surface waters. Their structures, octanol–water distribution constants and estimated carcinogenic potency are shown in Table 1. The US EPA has set highly restrictive limits for CPAHs in surface and ground waters (3.8 ng L^{-1} each)[31] as a criterion for water quality; on the other hand, the European Union has established maximum allowable concentration limits of 50 ng L^{-1} for benzo(*a)*pyrene, 30 ng L^{-1} for the combination of benzo(*b)*fluoranthene and benzo(*k*)fluoranthene, and 2 ng L^{-1} for benzo(*ghi)*perylene and indene(*1,2,3-cd)*pyrene.[32,33] Few currently available SPE and liquid–liquid extraction (LLE) methods provide the limits of quantitation (LOQs) required to determine such low levels.[34,35] Also, they involve labour-intensive, time-consuming procedures; use large sample volumes (1–50 L) and substantial amounts of hazardous organic solvents (in LLE); and require evaporation and clean-up prior to LC-FL. Several microextraction techniques have been proposed to simplify the sample treatment involved. SPME–GC–MS/MS[36] and SBSE–LC-FL[37] afford CPAH LOQs of 0.20–0.98 and 0.7–4.9 ng L^{-1}, respectively. The extraction times involved, however, are too long (60–120 min) and recoveries are influenced by CPAH concentration[37] and sample matrix.[36] Alkyl carboxylate-coated MNPs might provide a simple, expeditious and inexpensive choice for the routine quantitation of CPAHs in surface and ground waters at the ultra-low levels required by existing legislation. In this work, the variables governing the coating process and extraction step were optimised, and compatibility between the sorbent and the additives used to prevent adsorption of CPAHs on container surfaces was assessed. To the best of our knowledge, this is the first time hemimicellar sorbents providing surfactant-free extracts are used for the extraction of contaminants.

EXPERIMENTAL SECTION

Chemicals

All chemicals were analytical reagent-grade and used as supplied. Fe (II,III) oxide magnetic nanoparticles of mean diameter 20–30 nm and sodium alkyl (C_{10}–C_{18}) carboxylates were obtained from Sigma (Steinheim, Germany). The target compounds chrysene (Chry), benzo(*ghi*)perylene (BghiP), benzo(*b*)fluoranthene (BfF), benzo(*a*)pyrene (BaP), dibenzo(*a,h*)anthracene (DahAn) and indeno(*1,2,3-cd*)pyrene (IP) were purchased from Sigma–Aldrich, while benzo(*a*)anthracene (BaA) and benzo(*k*)fluoranthene (BkF) were obtained from Fluka (Steinheim, Germany). Tetrabutylammonium bromide (TBABr) was purchased from Sigma. Tetrahydrofuran (THF), methanol (MeOH) and LC-grade acetonitrile (ACN) were supplied by Panreac (Sevilla, Spain) and ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Madrid, Spain). Stock standard solutions containing each individual CPAH at a concentration of 200 mg L^{-1} were prepared in acetonitrile and stored at 4 ºC in the dark. Working-strength solutions containing a mixture of CPAHs were made by appropriate dilution of the stock solutions with a 50:50 v/v mixture of ACN and THF.

Instrumentation

The liquid chromatographic system used (Breeze HPLC, Waters, Milford MA) consisted of a 1525 binary pump, a 717plus automatic injector, a 1500 series column heater, a 2475 multiwavelength fluorescence detector and a 2898 photodiode array detector. The stationary-phase column employed for the analysis of CPAHs was a Supelcosil LC-PAH model (particle size 3 μm, internal diameter 3 mm, length 10cm). RW-14-Basic mechanical stirrers from Ika (Staufen, Germany) were used to facilitate extraction. An Nd-Fe-B magnet (*viz.* a disc 2 cm in diameter and 1 cm thick from Farplas, S.L., Barcelona, Spain) was used to separate MNPs from the samples after extraction.

Optimisation of the Coating of MNPs with Alkyl Carboxylates

The amount of alkyl carboxylate chemisorbed onto the magnetic nanoparticles (200 mg) was determined under variable experimental conditions as regards the length of the alkyl chain (C_{10}–C_{18}), amount of surfactant (0.01–0.5 mmol) and reaction time used (1–120 min). Nanoparticles were coated in a 50 mL glass beaker by heating a mixture of MNP, surfactant and 25 mL of distilled water at 85 ºC. Then, coated MNPs were separated from the reaction medium by means of an Nd-Fe-B magnet and washed five times with methanol or ethanol in order to remove physisorbed surfactant. The amounts of surfactant present at equilibrium and in the successive washing solutions were determined by LC-UV, using a Supelcosil ABZ+PLUS column (particle size 5 μm, inner diameter 4.6 mm, length 15 cm) and detection at 205 nm. The mobile phase consisted of water (A) containing 60%, 85%, 90% and 95% acetonitrile (B) for the analysis of C_{10}, C_{14}, C_{16} and C_{18} alkyl-carboxylate sodium salts, respectively. The flow rate was set at 1 mL min^{-1} and the column temperature at 40 ºC. Calibration curves were obtained over the concentration range 0.05–20 mg mL^{-1}, using an injected volume of 2.5 μL.

Synthesis of Tetradecanoate-Coated MNPs

The following coating procedure was used to prepare the SPE sorbent for extraction of CPAHs: an amount of 200 mg of Fe_3O_4 was mixed with 50 mg of tetradecanoic acid sodium salt in 25 mL of distilled water. The mixture was homogenized by hand and allowed to stand in a thermostatic bath at 85 ºC for 30 min. Following chemisorption, C_{14}-coated MNPs were separated from the equilibrium solution by means of an Nd-Fe-B magnet and washed three times with 4 mL of methanol or ethanol to remove excess surfactant. The SPE material thus obtained was stored in a closed glass container at 4 ºC until use. The amount of material obtained can be adjusted at will by choosing an appropriate, constant Fe_3O_4/sodium tetradecanoate/water proportion.

Determination of CPAHs in Surface and Ground Waters

Sample Collection and Preservation

Water samples were collected in dark glass containers from a well (Almodóvar), two rivers (Guadalquivir and Rabanales), a reservoir (Navallana) and the Mediterranean Sea (Los Álamos and La Malagueta beaches) in January 2009; all sampling sites were located in either Córdoba or Málaga (southern Spain). Immediately upon collection, the samples were spiked with a 50 mg L^{-1} concentration of tetrabutylammonium bromide (TBABr) in order to prevent adsorption of CPAHs onto container surfaces. This was followed by passage through filters of 0.45 μm pore size from Watman GF/F Osmonics (France) in order to remove suspended solids, acidification to pH ~4.5 with 12 M hydrochloric acid and storage at 4 ºC until analysis.

Magnetic Solid Phase Extraction

A volume of sample of 350 mL at pH ~4.5 containing 50 mg L^{-1} TBABr and 200 mg of C_{14}-coated MNPs were mixed in a 500 mL glass cone (Pobel, S.A., ref. no 912003) prior to extraction with a mechanical stirrer at 500 rpm for 15 min. Then, MNPs were isolated by placing an Nd-Fe-B magnet in the bottom of the cone and pouring the solution away. Excess water retained on MNPs (~100 µL) was removed by passing a nitrogen stream at ~1 bar for 2 min. The sorbent was then washed twice with 1 mL first and 2 mL then of a 50:50 v/v THF:ACN mixture to elute CPAHs. The eluate was filtered through nylon mesh of 0.22 μm pore size and a 6 μL aliquot directly injected into the LC-FL system for analysis.

Liquid Chromatography/Fluorescence Detection

The separation and quantitation of CPAHs was accomplished by using liquid chromatography with fluorescence detection in combination with water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.65 mL min^{-1} as eluents. The column temperature was set at 32 ºC. The gradient elution program used was 75% B during the first 10 min, ramp from 75% to 80% B over the next 7 and 80% B for another 4. Reconditioning the column took about 5 min. Using a column with a small inner diameter (3 mm) allowed a low flow rate to be employed and solvents saved without losing resolution. The fluorescence detection program was performed at the following excitation and emission wavelengths: Chry and BaA (λ_{ex} 275 nm, λ_{em} 400 nm); BbF and BkF (λ_{ex} 284 nm, λ_{em} 450 nm); BaP (λ_{ex} 282 nm, λ_{em} 400 nm); DahAn and BghiP (λ_{ex} 290 nm, λ_{em} 420 nm); and IP (λ_{ex} 300 nm, λ_{em} 500 nm). Individual CPAHs were quantified by measuring the areas under the chromatographic peaks provided by 6 μL aliquots of standard solutions of ACN:THF (50:50 v/v) containing analyte concentrations over the following ranges: 0.020–10 μg L^{-1} for BkF and BaP; 0.025–10 μg L^{-1} for BaA; 0.040–10 μg L^{-1} for Chry, BbF, DahA and BghiP; and 0.05–10 μg L^{-1} for IP.

RESULTS AND DISCUSSION

Coating of MNPs with Alkyl Carboxylates

Sodium alkyl carboxylates react readily with magnetite at high temperatures to give surfactant monolayers chemisorbed onto the oxide surface.[21,38] The resulting tight hydrophobic sheath makes MNPs stable in nonpolar solvents, thereby facilitating the obtainment of ferrofluids for a variety of applications.[13,20-22] Most such applications rely on the preparation of 10–15 nm MNPs that are subsequently[38-41] or simultaneously[42] coated with either saturated straight-chain alkyl (C_{10}–C_{18}) carboxylates or oleate at high temperatures. Once coated, the MNPs are washed with polar organic solvents to remove physisorbed surfactant. MNPs in particle sizes above 20 nm are rarely used since they are more difficult to disperse and hence less suitable for producing ferrofluids.

In this work, we examined the potential of magnetite coated with saturated straight-chain alkyl (C_{10}–C_{18}) carboxylates as SPE materials. We chose to use commercially available magnetite in particle sizes of 20–30 nm for this purpose in order to facilitate preparation of the sorbent, and saturated straight-chain alkyl (C_{10}–C_{18}) carboxylates on the grounds of their high ability to coat magnetite surfaces. In fact, the surface area occupied by these molecules on MNP particles about 10 nm in size is similar to that occupied by fatty acids as monomolecular films on water (20–22 Å^2 per molecule) irrespective of chain length.[40] An area of 20-22 Å^2 represents the compressive limit for a condensed film, which is reached when molecules become tightly packed together.[43] Oleate coatings are more readily expanded (36–38 Å^2 per molecule)[40,41,44] and hence less useful than octadecanoate coatings for the intended purpose, which led us to exclude the former from the study.

Because the packing density of alkyl carboxylates depends on several variables including the size of magnetite particles,[20] we examined their variation with the length of the alkyl chain, amount of surfactant and reaction time on magnetite particles 20–30 nm in size (magnetite surface area ~ 60 m^{2}g^{-1}).

Figure 1A shows the chemisorption isotherm for a monolayer of tetradecanoate on magnetite. The amount of surfactant added to the mineral oxide ranged from 0.05 to 2.5 mmol g^{-1}. The other alkyl carboxylates studied (*viz.* decanoate, hexadecanoate and octadecanoate) behaved similarly in this respect. Chemisorption of the alkyl carboxylate onto magnetite increased in a virtually linear manner with surfactant coating concentrations below 1 mmol g^{-1} and approached saturation at higher levels. The maximum surfactant coverage was *ca.* 0.35 mmol g^{-1} irrespective of chain length (Y-axis in Figure 1A). An amount of alkyl carboxylate of 1 mmol g^{-1} was thus selected as optimal to prepare the sorbents. The concentration of surfactant in the equilibrium solution increased up to around 17 mM in the interval tested (X-axis in Figure 1A). The surface area occupied by each surfactant molecule on the isotherm plateau was ~ 28 Å^2; therefore, the packing density was *ca.* 25% lower than that for smaller NMP sizes (*e.g.* 20–22 Å^2 for 7 nm particles).[39,40] These results agree with those recently obtained in the chermisorption of oleate on magnetic particles of variable size.[20] The surfactant coverage of saturated straight-chain alkyl carboxylates on 20–30 nm magnetite particles (28 Å^2 per molecule) was higher than that of oleate on 7–10 nm particles (36–38 Å^2 per molecule); this confirms that saturated straight-chain alkyl carboxylates coat MNP surfaces more tightly than does oleate. The time needed for surfactant coverage to peak was around 30 min at 85 ºC irrespective of the particular alkyl carboxylate. By way of example, Fig. 1B shows the variation of the chemisorption rate of tetradecanoate on magnetite.

The above-described optimum conditions lead to magnetite being coated with both chemisorbed (~ 75%) and physisorbed (~ 25%) surfactant. Removal of the latter was attempted with various solvents including methanol, ethanol and distilled water. Washing with distilled water (5 mL × 3) sufficed to remove more than 99% of physisorbed decanoate. However, water failed to efficiently remove physisorbed surfactants of longer alkyl chains, even when used at 50 ºC or alkalized to pH 9. Complete removal (> 99%) of physisorbed tetradecanoate required washing the MNPs with three 4 mL aliquots of ethanol or methanol, the volume needed increasing slightly with increasing chain length (*e.g.* around 15 mL of ethanol was needed for hexadecanoic acid sodium salt).

Optimization of the Extraction of CPAHs with Alkylcarboxylate-Coated MNPs

This was accomplished by conducting triplicate tests that involved extracting spiked distilled water with coated MNPs under a variety of experimental conditions (C_{10}–C_{18} alkylcarboxylates, 30–400 mg of sorbent, 0.15–175 ng of each CPAH, 100–500 mL of sample, pH 2–9, 0.1–1 M NaCl and 1–120 min stirring). The optimum conditions were established in terms of recoveries of the target compounds.

Selection of the Sorbent

Four different sorbents (*viz.* MNPs coated with decanoate, tetradecanoate, hexadecanoate and octadecanoate) were tested for the extraction of CPAHs. Parallel tests with bare magnetite were also performed in order to determine the actual extraction efficiency of the surfactant layer. As can be seen from Table 2, recoveries exceeding the threshold value recommended by various international bodies for the extraction of contaminants,[45,46] 70%, were only obtained with alkyl chains of more than 14 carbon atoms; also, no significant improvement was obtained from C_{14} to C_{18} —not even with amounts of sorbent of up to 100 mg (results not shown).

The significant increase in recoveries from C_{10} to C_{14} relative to that from C_{14} to C_{18} could be a result of the suggested structure for the chemisorbed surfactant monolayer, which makes MNPs stable in ferrofluids. The formation of the monolayer has been interpreted in the light of the electric double layer model and carboxylate anions are assumed to be chemisorbed on the inner Helmholtz plane.[47] It has been suggested that the four methylene groups adjacent to the carboxylate ion are submerged within the double layer and not exposed to the aqueous medium (*i.e.* C_{10} must behave as a C_6 chain in terms of water solubility and steric stabilization of MNPs[46]). Surely, the length of this chain length is too small to accommodate CPAHs and/or provide enough Van der Waals interactions, which would account for the poorer recoveries obtained. Based on the foregoing, we selected tetradecanoate-coated MNPs as sorbents to extract the target compounds.

Effect of Experimental Variables on Recoveries

Recoveries were found not to be affected by the sample volume when an amount of 100–500 mL of distilled water containing 7 ng of each CPAH was subjected to C_{14}-MNP extraction with 200 mg of sorbent. A volume of 350 mL was thus adopted for further testing as it afforded sufficient concentration factors (~90–100) to meet LOQ requirements (~0.5 ng L^{-1}). The influence of the amount of sorbent (30–400 mg) is illustrated in Table 3. As can be seen, recoveries were quantitative at 200 mg, which was thus chosen as optimal.

Recoveries exhibited no change with the amount of CPAH or NaCl over the ranges 0.15–175 ng and 0–1 M, respectively. Bare magnetite nanoparticles are known to be oxidized and easily lose their magnetism below pH 4. We examined the influence of this variable over the range 2–9 and found CPAH recoveries to remain constant from pH 2.5 to 9; this suggests that the carboxylate shield effectively protects nanoparticles over this range. The protective action, however, was inefficient at lower pH values (*e.g.* CPAH recoveries were only around 55–65% at pH 2).

No leaching of tetradecanoate from the MNPs was observed under the recommended extraction conditions (see Experimental), as checked by chromatographic analysis of the extracts. Obtaining clean extracts obviously facilitates the accurate quantitation of the target analytes. This supports the use of hemimicelles in liquid chromatography, especially in combination with mass spectrometric detection. Extracts containing high surfactant concentrations have precluded the development of a number of MS applications by effect of their adverse effects on analyte ionization and/or detection.

The reusability of the sorbent after elution of CPAHs was assessed in various water matrices. The recoveries of the target compounds from river and reservoir water samples were found to remain constant after reusing the sorbent 5 times without further treatment. After that, recoveries progressively decreased (e.g. 20-25% after 7 extractions) probably due to the progressive accumulation of matrix components. Treatments for cleaning the sorbents after five uses (e.g. two washing steps with 5 mL each of acetonitrile, methanol or water) were not successful in removing strongly adsorbed matrix components. On the other hand, seawater extraction produced non-reusable sorbents, -not even if the sorbent was washed with distilled water to remove excess salt. Sorbents behaved similarly in distilled water solutions containing salt (i.e. NaCl) at seawater concentrations, but although salt was identified as the agent causing non-reusability, no explanation for this behaviour was found.

Selection of the Desorption Solution

The desorption of CPAHs from MNPs was studied with MeOH, ACN and THF, using various elution programs that involved solvent volumes from 0.5 to 5 mL and 1–5 runs; both variables were found to directly influence recoveries. Using a 500 mL cone instead of a 500 mL beaker permitted the use of low solvent volumes and facilitated retention of MNPs on a small magnet. Complete removal of water in the vessel and adsorbed onto the MNPs was required to avoid a decrease in solvent desorption efficiency and changes in the final volume. Water was displaced from MNPs by passing a nitrogen stream at ~1 bar for ~2 min while they were retained on the magnet.

Recoveries above 90% were obtained for all CPAHs by using 3 mL of THF in two steps (1+2 mL). Similar results were obtained with ACN (1+2 mL) for the less hydrophobic CPAHs (*viz.* BaA, Chrys, BbF, BkF and BaP); this solvent, however, provided poorer recoveries for the most hydrophobic compounds, DahAn (82±3%), BghiP (69±3%) and IP (74±3%).On the other hand, methanol (1+2 mL) was rather inefficient, with CPAH recoveries from 24 to 50% only.

Injection of a 6 µL aliquot of the THF extract into the chromatographic system and use of a mobile phase consisting of 75:25 (v/v) ACN:Water reduced analyte peak resolution owing to the lower polarity of the former. We found a 50:50 (v/v) mixture of ACN and THF to have the same elution capacity as THF and to result in no degradation in peak resolution, which led us to adopt this solvent mixture for elution of the CPAHs. Manual agitation for about 30 s, followed by standing for 1 min, sufficed to ensure complete desorption. Under these conditions, the magnetite coating retained its integrity and the sorbent was reusable.

We assessed the possibility to concentrate the 3 mL of eluate to 1 mL by evaporation under a gentle stream of nitrogen. No losses were found to result from this drying operation thanks to the low volatility of PAHs with 4–6 rings. This allowed the sensitivity to be increased threefold. However, the LOQs obtained without drying, 0.2–0.5 ng L^{-1}, are low enough to meet the quality criteria of current legislation, so we propose dispensing with the evaporation step in order to make the procedure simpler and faster for routine analyses.

Influence of the CPAH Preservation Conditions on Recovery

Polycyclic aromatic hydrocarbons are strongly adsorbed onto container surfaces, which causes substantial losses during storage of samples[48] (*e.g.* losses as high as 15%[49] have been reported for BaP after storage in glass containers for 24 h). Two commonly used strategies for preserving PAH-containing water are the addition of an organic solvent (*e.g.* acetonitrile, isopropanol or methanol in proportions between 10 and 20% v/v)[50-52] or a surfactant [*e.g.* sodium dodecyl sulfate (SDS),[48] Brij 35[53]] in amounts exceeding its critical micelle concentration (cmc). The former usually results in poor extraction recoveries and the latter can interfere with chromatographic or detection systems.

We initially assessed the ability of methanol (5–30%) and tetrabutylammonium bromide (1–500 mg L^{-1}), a well-known hydrotrope, to keep CPAHs in solution. Tests were carried out on spiked river samples that were previously filtered and adjusted to pH 4.5 with concentrated nitric acid. The samples were simultaneously supplied with stabilizing agents and CPAHs prior to storage at 4 ºC in the dark until direct analysis by LC/FL on a daily basis for a week. All tests were performed in triplicate. The addition of 20% of methanol or a 50 mg L^{-1} concentration of TBA prevented the loss of CPAHs (maximum analyte concentration tested 600 ng L^{-1}) for at least 5 days. This concentration of TBA (0.15×10^{-3} M) is well below its cmc ($\sim75\times10^{-3}$ M); therefore, the enhanced water solubility of the CPAHs must have arisen mainly from the establishment of hydrophobic and π-cation interactions with the monomer rather than with aggregates. The use of TBA above the cmc did not prevent the adsorption of CPAHs for a longer period.

The extraction efficiency for C_{14}-MNPs in the presence of these additives at the previous concentrations was assessed. Recoveries were found not to be affected by the presence of the hydrotrope TBA; on the other hand, those for most of the studied CPAHs were significantly decreased by the presence of 20% of methanol (*e.g.* to 50±4, 52±4, 73±3, 79±2 and 77±4 for BaA, Chrys, BbF, BkF and BaP, respectively). The decrease was exclusively due to enhanced partitioning of CPAHs to the aqueous sample since the carboxylate shield was not affected; as a result, recoveries remained constant in successive reuses. This led us to preserve CPAHs by adding a 50 mg L^{-1} concentration of TBA to the samples.

Analytical Performance

Table 4 lists the analytical figures of merit of the proposed method. The instrumental limits of detection (LODs) and quantitation (LOQs) were calculated from blank determinations at a signal-to-noise ratio of 3 and 10, respectively. Such limits were used to estimate the LODs and LOQs for the proposed method with provision for the actual concentration factors obtained (~100). The method LOQs for the individual CPAHs ranged from 0.2 to 0.5 ng L^{-1} (see Table 4). Consequently, the proposed method affords their quantification at concentrations below the ultra-trace levels established by EPA (3.8 ng L^{-1}) and those set by EU for surface waters (2–50 ng L^{-1}). Under the proposed experimental conditions, CPAHs recoveries ranged from 88% to 93% throughout the studied concentrations range (~1–500 ng L^{-1}), and standard deviations from 1 to 8%.

The potential interference of matrix components co-eluting with CPAHs was assessed by comparing the slopes of the calibration curves (n = 7) obtained from standards with those provided by blank eluates of sea water (n = 2) and fresh water (n = 2) spiked with known amounts of CPAHs. The difference between such slopes and those obtained for environmental samples were found to be statistically insignificant in terms of Student's t.[54] In fact, the calculated t-values fell in the range 0.2–0.7 and were below the critical value (3.17), the significance being set at the 0.01 levels. Therefore, matrix components are expected not to interfere with the determination of the target compounds.

The precision of the method was evaluated by extracting 11 different spiked samples of fresh (n = 7) and sea water (n = 4). The results for the eight CPAHs, expressed as relative standard deviation (RSD), ranged from 3% to 6%.

Analysis of Environmental Water Samples

The suitability of the proposed method for use with real-world samples was established by applying it to the determination of CPAHs in samples from two rivers (n = 3), a reservoir, two beaches and a well. Table 5 shows the results, expressed as the mean value of three independent determinations, in addition to their corresponding standard deviations before and after spiking with variable concentrations of CPAHs over the range 1–10 ng L^{-1}. As can be seen, recoveries ranged from 85% to 94% and relative standard deviations from 1 to 6%.

The target compounds were only present in one of the studied samples, namely: water from Navallana reservoir, which contained BkF, BaP, DahA, BghiP and IP, all at very low concentrations (0.42–0.96 ng L^{-1}), however. These values are below the EPA and EU limits. Figure 2 shows the chromatograms for a standard solution in 50:50 (v/v) ACN:THF (A), water from Los Alamos beach before and after spiking (B) and water Navallana reservoir (C). As can be seen, no interference from matrix components was detected in any sample.

CONCLUSIONS

Alkylcarboxylate hemimicelles chemisorbed onto magnetic nanoparticles constitute a useful alternative to conventional ionic surfactant hemimicelles physisorbed onto oppositely charged mineral oxides for the extraction of hydrophobic compounds. Major general advantages are the obtainment of surfactant free extracts, pH-independent surfactant loads, extended working pH range and reusability to a certain extent.

Based on the results obtained in this work, these sorbents operate in an efficient manner under real-world conditions. Their use for the extraction of CPAHs (4-6 ring PAHs) has permitted the development of a simple, rapid, matrix-independent method for the routine determination of these carcinogenic compounds at the levels required by EU and US EPA in a variety of environmental water samples. The extraction procedure is robust; also, no cleanup of the extracts or solvent evaporation is needed, extractions take only 15 min (recoveries 85-94% with RSDs (1-6%), and the sorbent is compatible with the presence of stabilizing agents for preservation of the analytes during storage of samples. Compared to SBSE, which is the best suited microextraction technique for CPAHs,[55] the proposed method surpasses it in: a) extraction time (i.e. around 24 h are required to reach equilibrium conditions in SBSE[55] and selected extraction times range from 60 min[55] to 14 h.[56] Additionally, a slow desorption in at least two steps is required[35]); b) recoveries, which are low (e.g. 29-87%,[55] 60-85%,[35] 50-90%[56]) and matrix-dependent; and c) relative standard deviations (e.g. 14.2-21.6%[55]*)*.

This work opens up new avenues for developing admicelle-based sorbents from alkylcarboxylates containing polymerizable bonds (*i.e.* bonds similar to those used in the formation of molecular micelles) and tunable sorbents with a variety of functionalities (*e.g.* from bolaamphiphiles such as dicarboxylic acids, alkylbetaine surfactants, polymers with carboxylic functional groups) useful for multiresidue determinations. Both hemimicelles and admicelles synthesized in this way provide surfactant-free extracts, which opens up interesting prospects for use in LC–MS, GC and CE in combination with various detector types. Work currently under way in our laboratory may lead to further advances in this area in the near future.

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FIGURE CAPTIONS

Figure 1. (A) Isotherm for the chemisorption of tetradecanoate (0.05–2.5 mmol) onto 1 g of magnetite. (B) Amount of tetradecanoate chemisorbed as a function of time (tetradecanoate added = 1 mmol g^{-1}). All measurements were made in triplicate.

Figure 2. LC/fluorescence chromatograms for (A) a standard solution in 50:50 (v/v) acetonitrile:tetrahydrofuran (0.5 μg L^{-1}); (B) Los Alamos beach sample before (1) and after spiking at 2 ng L^{-1} (2); and (C) Navallana reservoir sample.

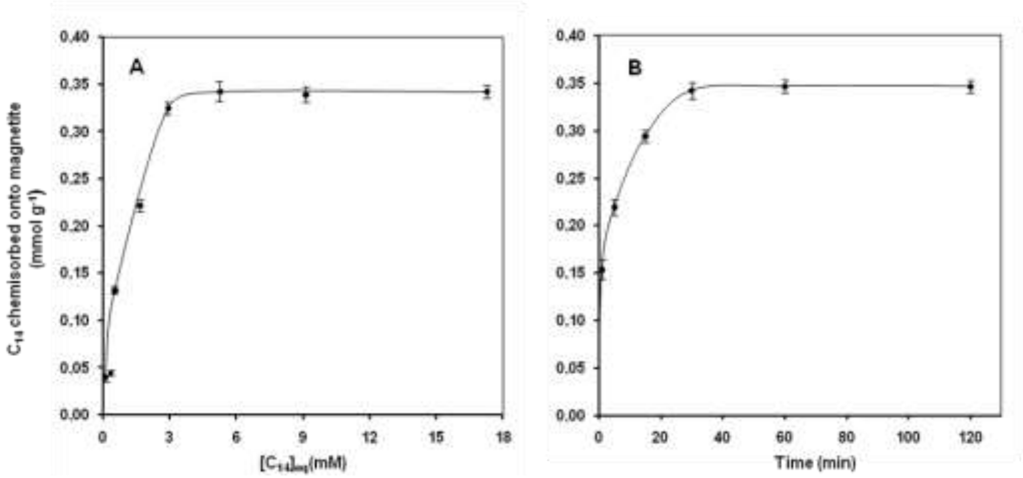

A
B
C14 chemisorbed onto magnetite (mmol g-1)
0,00
0,05
0,10
0,15
0,20
0,25
0,30
0,35
0,40
0
3
6
9
12
15
18
[C14]eq (mM)
0
20
40
60
80
100
120
Time (min)

Figure 1

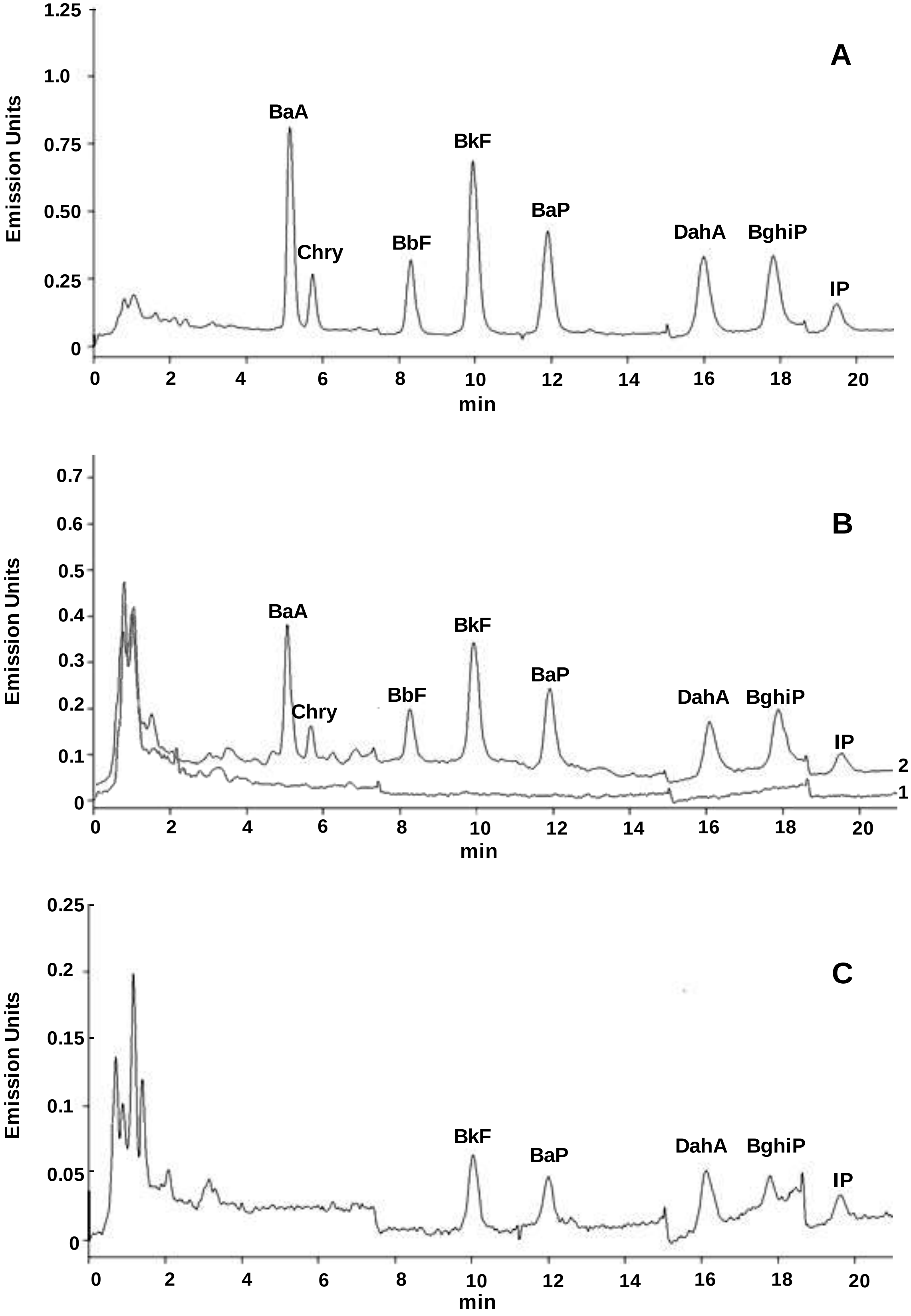
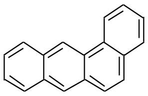
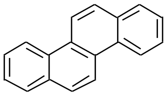
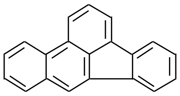
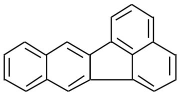
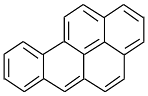
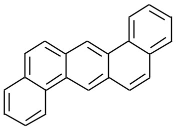
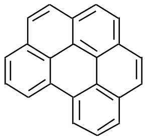
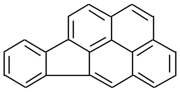

A
Emission Units
1.25
1.0
0.75
0.50
0.25
0
BaA
Chry
BbF
BkF
BaP
DahA
BghiP
IP
0
2
4
6
8
10
12
14
16
18
20
min
B
Emission Units
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BaA
Chry
BbF
BkF
BaP
DahA
BghiP
IP
2
1
0
2
4
6
8
10
12
14
16
18
20
min
C
Emission Units
0.25
0.2
0.15
0.1
0.05
0
BkF
BaP
DahA
BghiP
IP
0
2
4
6
8
10
12
14
16
18
20
min

Figure 2

Table 1
Structure, octanol:water partition coefficient and estimated carcinogenic potency of priority carcinogenic polycyclic aromatic hydrocarbons

PAH	Structure	Log K_{ow}[a]	Carcinogenic potency[b]
Benzo(*a*)anthracene, BaA		5.91	2B
Chrysene, Chry		5.91	2B
Benzo(*b*)fluoranthene, BbF		6.40	2B
Benzo(*k*)fluoranthene, BkF		6.40	2B
Benzo(*a*)pyrene, BaP		6.40	1
Dibenzo(*a,h*) anthracene, DahAn		7.14	2A
Benzo (*ghi*) perylene[c], BghiP		6.89	3
Indeno(*1,2,3-cd*) pyrene, IP		6.89	2B

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[a] Calculated with the software Advanced Chemistry Development (ACD/Labs) V9.04 for Solaris

[b] IARC classification: carcinogenic to humans (1), probably carcinogenic to humans (2A), possibly carcinogenic to humans (2B), (3) not classifiable as to carcinogenicity to humans

[c] BghiP was included in the study despite its being deemed noncarcinogenic owing to its low legal limits

Table 2
Recoveries (%) ± standard deviations[a] obtained in the extraction of CPAHs with bare and alkyl carboxylate-coated magnetite

	Bare magnetite	Decanoate-coated	Tetradecanoate-coated	Hexadecanoate-coated	Octadecanoate-coated
BaA	8±2	28±1	85±2	88±5	86±2
Chrys	13±5	30±3	85±5	89±1	85±4
BbF	19±3	54±1	90±4	93±2	90±1
BkF	19±4	51±1	90±3	93±2	93±3
BaP	22±4	43±1	89±2	93±4	92±2
DahAn	32±4	55±4	85±1	92±3	90±2
BghiP	36±5	60±5	84±2	92±2	90±3
IP	33±6	54±1	82±3	89±5	88±3

[a] n = 3; Extraction conditions: 250 mg of sorbent, 300 mL of distilled water spiked with 6 ng of CPAHs

Table 3

Recoveries (%) ± standard deviations[a] obtained in the extraction of CPAHs with variable amounts of C_{14}-coated MNPs (mg)

	30	60	100	200	300	400
BaA	26±3	61±1	75±2	90±2	96±6	96±4
Chrys	24±2	54±3	79±1	89±5	94±5	95±2
BbF	35±6	65±1	80±4	92±4	95±2	96±4
BkF	39±5	67±5	82±2	92±3	98±1	100±3
BaP	37±2	63±4	81±2	91±2	101±3	102±1
DahAn	41±5	65±4	83±3	89±1	98±2	97±1
BghiP	40±4	64±5	82±4	87±2	95±3	100±1
IP	39±4	66±3	77±2	88±3	95±3	98±1

[a] *n* = 3; Extraction conditions: 350 mL of distilled water spiked with 7 ng of CPAHs

Table 5

Table 4
Analytical performance of the proposed method

Target compound	[a] Capacity factor	External calibration			LOQ[c] (ng L^{-1})	LOD[d] (ng L^{-1})
		Linear range (µg L^{-1})	Slope ±SD [(×10^{3}) mV L µg^{-1}]	[b]r		
BaA	5.4	0.025–10	183.1±5.3	0.998	0.25	0.15
Chry	6.1	0.04–10	50.2±2.3	0.998	0.4	0.25
BbF	9.4	0.04–10	84.5±4.0	0.998	0.4	0.25
BkF	11.4	0.020–10	228±8.2	0.998	0.2	0.1
BaP	13.9	0.020–10	143±7.2	0.998	0.2	0.1
DahA	18.9	0.04–10	132±3.9	0.998	0.4	0.25
BghiP	21.2	0.04–10	126±3.4	0.997	0.4	0.25
IP	23.5	0.05–10	48.3±1.8	0.997	0.5	0.25

[a] column void marker: acetone ; [b] correlation coefficient; n = 7; [c] estimated limits of quantitation of the method; [d] estimated limits of detection of the method

Mean concentration (ng L^{-1}) ± standard deviation (n = 3) of the CPAHs found in surface water samples, and percent recoveries ± standard deviations (n = 3) obtained upon spiking with the target analytes

Sample origin	**BaA**	**Chry**	**BbF**	**BkF**	**BaP**	**DahA**	**BghiP**	**IP**
Well[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	92±6	91±4	91±5	94±4	89±2	87±3	87±3	87±4
River Guadalquivir (site 1)[c]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	87±1	89±1	88±6	91±3	91±4	87±3	87±2	88±3
River Guadalquivir (site 2)[d]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	88±6	87±5	90±1	90±3	90±6	86±2	85±3	85±5
River Rabanales[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	88±5	87±4	90±6	90±4	92±5	88±5	87±2	86±4
Navallana reservoir[a]	n.d.	n.d.	n.d.	0.51±0.03	0.42±0.01	0.75±0.03	<LOQ	0.96±0.05
	92±1	93±2	91±1	93±3	88±1	90±1	88±5	89±5
La Malagueta beach[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	88±3	89±3	90±5	94±5	91±6	89±5	86±2	85±4
Los Alamos beach[c]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	92±2	90±6	91±2	90±2	89±4	90±1	86±5	86±5

Spiking level: [a] 1 ng L^{-1} ; [b] 5 ng L^{-1}; [c] 2 ng L^{-1}; [d] 10 ng L^{-1} ; n.d. = not detected; < LOQ = detected, but below the limit of quantitation