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Mixed aggregate-based acid-induced cloud-point extraction and ion-trap liquid chromatography–mass spectrometry for the determination of cationic surfactants in sewage sludge

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Abstract

Alkyl ammonium surfactants were extracted and concentrated from sludge samples using the acid-induced cloud-point extraction (ACPE) technique. Reversed-phase liquid chromatography–electrospray ionization in positive ion mode ion-trap mass spectrometry was then used for the separation, quantitation and identification of single homologues of the cationic surfactants. Variables affecting the ACPE efficiency were optimized using a spiked activated sludge. Total method recoveries ranged from 91 to nearly 100%. Detection limits for cationics in the sludge were between 40 and 75 ng/g. The analysis of cationic surfactants in activated and dehydrated sludge collected from two different sewage treatment plants revealed the presence of dialkyldimethyl, alkylbenzyldimethyl and alkyltrimethyl ammonium in the concentration range from 0.1 to 34 mg/kg. Alkylpyridinium surfactants were not detected. The persistence of alkyl ammonium surfactants in sewage sludge after treatments applied at municipal sewage plants was confirmed.
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1. Introduction

Contamination of the environment by surfactants arising from their widespread domestic and industrial use is of public concern. Much effort has been expended in the last few years to understand the fate of surfactants when they reach soil and natural waters via the application of sewage sludge on land, effluents from wastewater treatment plants (WWTPs) and industrial discharges into freshwater and marine

sites. Cationic surfactants, mainly based on quaternary ammonium compounds, only account for about 4–5% of total surfactant production [1]. However, their fate in the environment is of concern since there is a lack of data on their degradation [2] and they are known to be toxic even at low concentrations [3,4]. They are primarily used in domestic fabric softeners, as the positively charged hydrophilic head tends to interact strongly with negatively charged fabric fibres.

Cationic surfactants have a strong affinity for the surface of particles in sewage sludge, which are mostly negatively charged. Therefore, a significant proportion of the surfactant load of raw sewage will

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be associated with the particulate fraction. Adsorption percentages of about 95% have been found for quaternary ammonium surfactants in activated sludge [5,6]. Since the predominant treatment of sludge from primary settling tanks in WWTPs is anaerobic digestion, and cationic surfactants, except for ester-quats [7], are known to be resistant to degradation under anaerobic conditions [8], these surfactants can pass through a WWTP relatively untreated. Application of sludge to agricultural land may have a detrimental effect on the soil biota and, therefore, there is a need of further research to understand the fate of cationics recalcitrant to anaerobic degradation in the environment. For this purpose, there is a requirement for the development of reliable and rapid methods for their determination.

Analytical methods to determine cationic surfactants in sewage sludge are confronted by several major problems. Thus, since they are strongly adsorbed on the sludge by hydrophobic and electrostatic interactions, their extraction is difficult. Also, there are many types of cationic surfactants, which are produced as a mixture of homologues, and this makes their prior separation (generally chromatographic) mandatory. An important aspect is that most cationic surfactants lack a chromophoric group, so direct determination with UV detection is not possible. Because of the low concentrations expected in the environment, preconcentration methods are generally required

Extraction of cationics from sewage sludge has traditionally been performed using a conventional liquid extraction method with acidic methanol, followed by a sequence of liquid–liquid partitioning analysis [9–12]. Although recognized as efficient, this method is tedious and it takes about 1 week for the treatment of four samples [13]. Supercritical fluid extraction, which overcomes some of the above drawbacks, has been proposed as an alternative [13,14]. On the other hand, liquid chromatography with normal or reversed phase [11,13,15–19] is a more reliable technique for the specific analysis of cationic surfactants. Conductivity detection [11,15] or post-column ion-pair formation with phase separation and UV detection [13,20] have been used to solve the problem of the lack of chromophores. However, because of the complexity of sludge samples and the incomplete chromatographic separation of all homologues, more specific detection systems should be used to monitor the environmental fate of cationic surfactants. Recently, electrospray mass spectrometry has been demonstrated to be a suitable technique for the identification and quantification of some groups of cationic surfactants in water samples [16,18].

The objective of this study was to develop a rapid, simple and reliable method for the determination of cationic surfactants recalcitrant to anaerobic degradation in sewage sludge. The approach used combines the ability of acid-induced anionic surfactant phase separation [21] (acid-induced cloud-point extraction, ACPE) to extract cationic surfactants on the basis of the formation of extractant–analyte mixed aggregates [22], with the performance of liquid chromatography–electrospray ionization ion-trap mass spectrometry (LC–ESI-IT-MS) for the separation, quantitation and identification of the target analytes. The feasibility of the method is illustrated by the analysis of cationic surfactants in activated and dehydrated sludge from two WWTPs. To our knowledge this work is the first reported cloud-point extraction based on the formation of mixed aggregates between analytes and extractant, demonstrating that this approach is an interesting alternative for the extraction of amphiphilic compounds in complex matrices. Also, as far as we are aware this is the first reported identification and quantification of some groups of cationic surfactants (e.g. benzyl-alkyldimethyl ammonium and alkyltrimethyl ammonium) in sewage sludge.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical reagent grade and used as supplied. Sodium dodecanesulphonate (SDoS) was obtained from Fluka (Madrid, Spain), hydrochloric acid and HPLC-grade methanol from Panreac (Sevilla, Spain) and ammonium formate and formic acid from Merck (Darmstadt, Germany). Cationic surfactants were supplied by Aldrich (Milwaukee, WI, USA) (dodecyltrimethyl ammonium bromide, DTAS; tetradecyltrimethyl ammonium bromide, TTAS; dihexadecyldimethyl ammonium bro-

mide, DHDAS; benzyldodecyldimethyl ammonium bromide, BDDAS; benzyltetradecyldimethyl ammonium chloride, BTDAS), Fluka (ditetradecyldimethyl ammonium bromide, DTDAS; didodecyldimethyl ammonium bromide, DDDAS), Sigma (St. Louis, MO, USA) (*N*-hexadecylpyridinium bromide, HPS; hexadecyltrimethyl ammonium bromide, HTAS) and Merck (*N*-dodecylpyridinium chloride, DPS). Stock solutions of the analytes were prepared in methanol.

2.2. Samples

Activated and dehydrated sludges were collected from two WWTPs (Pozoblanco and Linares) in the south of Spain in April 2002. Pozoblanco WWTP mainly receives domestic effluents and Linares WWTP receives about 30% industrial effluents (mainly from automobile and textile manufacturing) mixed with about 70% domestic waste waters. Samples were completely dried in air, finely ground (<0.5 mm), and stored in amber bottles at 4 °C until analysis.

Spiked "aged" samples were prepared from activated sludge collected from the Córdoba WWTP (October 1999). Samples were dried and ground according to the procedure described above. Then, distilled water to give a slurry and 12 mL of a methanolic solution of cationics (0.5 mg/mL each) were added to 20 g (dry weight) of sludge [13]. Samples were allowed to dry for 4 days under nitrogen to prevent aerobic degradation and under stirring to permit the target analytes to interact with the natural organic matter. Then activated sludges were completely dried, ground (<0.5 mm), and stored in amber bottles at 4 °C. Extractions were carried out 1 year after spiking in order to check whether time affects the strength of the interactions between cationics and sludge.

2.3. Acid-induced cloud-point extraction

Eight sludge samples were treated simultaneously. Each (0.1 g of the dry and homogenized sludge) was mixed with 10 mL of 0.1 *M* HCl in a closed centrifuge tube and stirred at 700 rpm for 5 min. The acid solution containing alkaline and alkaline-earth metals was separated by centrifugation and discarded. Then, 10 mL of 2% SDoS in 3 *M* HCl was

added to the solid residue and the samples stirred at 700 rpm for 1 h in a water circulating thermostated (40 °C) beaker. It was then centrifuged at 5000 rpm for 10 min. Three phases were observed in the centrifuge tube: the nondissolved solid matrix at the bottom, a small volume of anionic surfactant-rich phase containing the cationic surfactants at the top and an aqueous phase in the middle, containing the anionic surfactant at a concentration near the critical micellar concentration. In order to facilitate the separation of the surfactant-rich phase, the temperature was reduced to 0 °C, at which this phase turns gelatinous, dense enough to be completely separated from the liquid phase using a simple tool (e.g. a spatula). At room temperature, the gelatinous phase is liquid (between 1.4 and 1.6 mL) and was diluted to the mark with methanol in a 2 mL vial. Before injecting an aliquot into the chromatographic system, the sample was filtered through a 0.45 μm nylon membrane filter.

2.4. Liquid chromatography–mass spectrometry

The analytes were separated, identified and quantified using a liquid chromatography–electrospray ion-trap mass spectrometry system (1100 Series LC–MSD, Agilent Technologies, Waldbronn, Germany). In all experiments, a Rheodyne 7125NS injection valve with a 20 μL sample loop was used. The stationary phase column was a 15 cm Nova Pack C_8 column, 3.9 mm I.D. and 5 μm particle diameter from Waters (Milford, MA, USA). Methanol and 50 m*M* ammonium formate buffer (pH 3.5) were used as eluent solvents at a flow-rate of 0.8 mL/min. The gradient elution program was: 0–30 min (linear gradient from 70 to 90% methanol); 30–45 min (isocratic conditions with methanol–ammonium formate, 90:10).

Surfactant analysis was carried out in the "ESI(+)" mode. To optimize the ESI-MS parameters, a mixture of cationic surfactants (10 μg/mL of each standard compound) in methanol–ammonium formate (80:20, v/v) was analyzed directly using a KD Scientific, Model 100, syringe pump (New Hope, MN, USA) at 600 μL/h. Selection of the best value for each parameter was based on the criteria of sensitivity and precision for the molecular ion. The set of parameters used is shown in Table 1.

Table 1
ESP parameter set for the analysis of cationic surfactants

Parameter	Value
Capillary voltage	5.0 kV
Capillary exit voltage	30 V
Skimmer	30 V
Trap drive	30
Source temperature	350 °C
Drying gas	10 L/min
Nebulizer gas	80 p.s.i.
Scan	200–500 m/z
Max. accumulation time	50 ms

Quantitation was carried out under full-scan conditions by using the extracted molecular ion chromatograms for each homologue and the corresponding peak areas were used to quantify them. Smooth chromatograms were obtained using the Gauss function (width, 5 points; cycles, 1). Correlations between peak areas and homologue concentrations (0.2–20 ng absolute amount) were determined by linear regression and were typically $r^2 = 0.997$. Structural identification was performed by MS–MS experiments after the parent ion was isolated and fragmented using the ion-trap mass spectrometer. The isolation width was set to 4 m/z units. Different dissociation energies were used and are specified with the associated results. Excitation of the ions was accomplished through collision with helium.

3. Results and discussion

3.1. Chromatographic separation and mass detection

Gradient elution programs with various combinations of acetonitrile or methanol and ammonium formate buffer at several concentrations were tested for the separation of the target compounds. The chromatographic resolution obtained was similar with the two organic solvents, however the time required for separation of dialkyldimethyl ammonium surfactants was considerably reduced in the presence of methanol. The use of the acidic buffer as
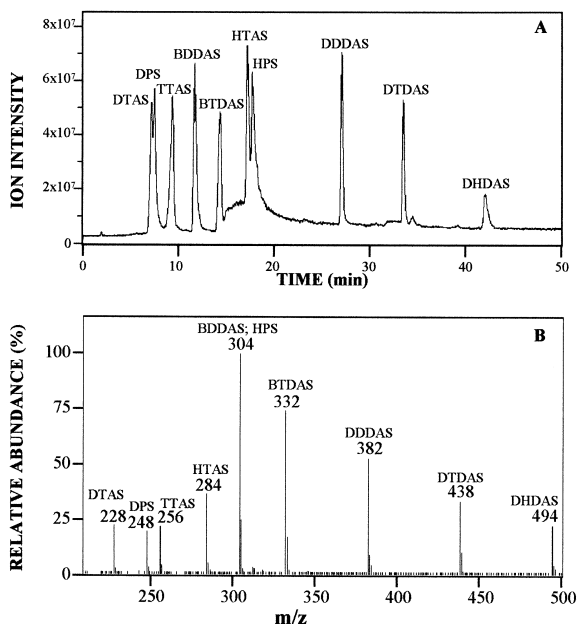

Fig. 1. (A) LC–MS total ion chromatogram obtained by injecting a standard solution containing 20 ng of each target compound. (B) Mass spectrum for cationic surfactants obtained by direct injection of a mixed solution (10 µg/mL each) using a syringe pump.

a component of the mobile phase was found to be essential to minimize the strong interaction of cationics with the silanol groups of the column packing. Fig. 1 shows the total ion chromatogram and the corresponding mass spectrum, collected by direct injection with a syringe pump, for a standard mixture of the target compounds. The mass spectrum for each homologue consisted only of the molecular ion signal. A compromise between chromatographic resolution and analysis time was adopted. Higher resolution can be achieved by using other gradient elution programs, but about 80 min are required for chromatographic development. It was checked that coelution of DTAS/DPS and HTAS/HPS did not affect quantitation due to space-charge effects in the ion trap. Thus, the calibration curves obtained were identical for standard solutions containing individual or mixtures of coeluted analytes, and quantitation of DTAS/DPS and HTAS/HPS mixtures when one surfactant concentration was much greater than the other (e.g. 50-fold) did not produce interference.

3.2. Mixed aggregate-based ACPE of cationic surfactants

The organic matter in domestic wastewater mainly consists of carbohydrates, fats and proteins [23]. It is mostly negatively charged, therefore it exerts a strong attraction for amphiphilic substances of opposite charge (e.g. cationic surfactants) via hydrophobic and electrostatic interactions. The extraction of alkyl ammonium surfactants from sludge samples has traditionally been based on their solubilization in organic solvents, in which their hydrophobic character is mainly exploited. It seems logical to believe that an extractant exploiting both the hydrophobic and electrostatic nature of cationic surfactants can greatly enhance the extraction efficiency.

The inherent properties of the acid-induced cloud-point extraction technique [21], which uses an anionic surfactant as extractant, make this technique especially suitable for the extraction of cationic surfactants from sewage sludge samples on the basis of mixed aggregate formation by oppositely charged surfactants. Thus, aqueous solutions of surfactant mixtures are known to undergo cooperative self-association to form mixed aggregates [22]. In this process, both ideal and nonideal mixing contributions may occur. Since the hydrophobic effect [24], which drives the overall process, is not specific to the surfactant head group, the formation of randomly mixed surfactant aggregates will tend to be favored. This can be viewed as leading to the ideal component of mixing in the aggregate. On the other hand, electrostatic interactions between the head groups of different surfactant types can provide the basis for the nonideal component of mixing in the aggregate. The synergism observed in the effects on the properties of systems (e.g. critical micelle concentration, interfacial tension, etc.) of mixtures of different surfactant types with respect to unmixed surfactants can be attributed to nonideal mixing effects in the aggregates, and this synergism has led to both theoretical [25–28] and practical [22] interest in developing a quantitative understanding of the behavior of mixed-surfactant systems.

A widely used model for treating mixed aggregation is the pseudophase separation approach [25–28], which assumes that the mixed aggregate can be treated much like a separate phase. In this equilibrium thermodynamic-based approach, the net difference in interaction energy between the mixed and unmixed system (β) for a binary mixture can be expressed as:

$$\beta = N(W_{11} + W_{22} - 2W_{12})/RT$$

where W_{11} and W_{22} are the energies of interaction between molecules in the pure aggregate and W_{12} is the interaction between two species in the mixed aggregate; N is Avogadro's number. Measurements on a large number of different binary surfactant mixtures have shown that nonideal interactions become progressively stronger (i.e. the dimensionless interaction parameter β becomes more negative) in going from mixtures of the same surfactant type to those of opposite charge. That is, nonionic–nonionic or cationic–cationic interactions are almost ideal ($\beta \approx 0$), cationic–nonionic interactions are significantly stronger ($-5 \leq \beta \leq -1$), but somewhat weaker on average than anionic–nonionic interactions, and anionic–cationic interaction are much stronger ($\beta \leq -10$).

Therefore, the formation of mixed aggregates between the anionic surfactant used as extractant and the cationic surfactants, by strong hydrophobic and electrostatic interactions, greatly enhances the extracting ability of ACPE. On the other hand, the acid medium used to induce the phase separation of the anionic surfactant favors the desorption of cations from the sludge, probably due to an ion-exchange mechanism. Furthermore, the cloud-point technique permits the simultaneous extraction and preconcentration of analytes in an aqueous medium.

3.3. Optimization of the extraction process

As no standard reference materials were available, optimization of the parameters potentially influencing the extraction efficiency of cationic surfactants (surfactant amount, temperature, HCl concentration and time of extraction) was carried out through the recoveries obtained for each homologue of the target compounds using spiked, aged activated sludge. Three replicates were analyzed to obtain a mean value.

Before extraction, the removal of alkaline-earth metals from the matrix was necessary since they

Table 2
Mean percent recoveries[a] obtained for the extraction of cationic surfactants from sewage sludge as a function of SDoS concentration

SDoS (%)	DDDAS	DDTAS	DHDAS	DTAS	TTAS	HTAS	BDDAS	BTDAS	DPS	HPS	Recovery[b] (%)
1	79	76	76	63	61	57	61	59	56	65	65
1.5	92	86	90	73	71	74	73	70	68	76	77
1.75	93	91	92	93	96	80	90	80	84	95	90
2	99	95	96	94	99	96	91	95	91	93	95
2.5	98	96	97	94	98	96	91	95	92	95	95

[a] Based on three replicates; range of RSD values 2–6%.
[b] Average recoveries of the cationic surfactants. Experimental conditions: 40 °C, 3 M HCl and 1 h.

yield very insoluble salts with anionic surfactants bearing sulfonate groups [29]. Stirring of the sample with 0.1 M HCl for 5 min was enough to remove the alkaline-earth metals present in sludge as carbonates without loss of cationics in the acid phase discarded. Partitioning of the analytes to the surfactant-rich phase from the water phase was found to be quantitative, presumably due to the strong cationic–extractant interaction and the low critical micellar concentration of SDoS in the acid medium required for extraction. Therefore, the target compounds were assumed to partition between the sludge and the surfactant-rich phase.

Table 2 shows the recoveries obtained for the target compounds as a function of SDoS concentration. The recoveries calculated for total cationic surfactants are also included. The extraction efficiency of ACPE was found to be practically independent of the hydrophobicity of the analytes. Increments of recoveries between 20 and 35% were observed when the percentage of anionic surfactant ranged from 1 to 2%. Since the phase volume ratio (volume of surfactant-rich phase/volume of aqueous solution, after the extraction step) increases linearly with surfactant concentration (e.g. 0.05 to 0.15 for

SDoS percentages ranging from 1 to 3%), a compromise between recovery and preconcentration has to be adopted when using the cloud-point technique. Percentages of 2% SDoS giving rise to about 1.4–1.6 mL of surfactant-rich phase is recommended for the application proposed.

The influence of temperature on the efficiency of ACPE to extract cationic surfactants from sludge was studied in the range 25–80 °C (results not shown). The percentage extraction increased between 5 and 10% when the temperature ranged from 25 to 40 °C. No significant increments were observed for higher temperatures. Therefore, it is recommended that extractions are carried out at 40 °C.

Acid conditions (2.5–5 M HCl) are necessary to obtain the separation of SDoS aqueous solutions into two isotropic phases [21]. The influence of this parameter on cationic surfactant recoveries was examined in the range 0.01–5 M. For extractions using hydrochloric acid concentrations lower than 2.5 M (no cloud conditions), a volume of acid necessary to achieve a final concentration in the interval 2.5–5 M had to be added after extraction in order to obtain phase separation before measurement. Table 3 shows some representative results. The

Table 3
Mean percent recoveries[a] obtained for the extraction of cationic surfactants from sewage sludge as a function of HCl concentration

HCl (M)	DDDAS	DDTAS	DHDAS	DTAS	TTAS	HTAS	BDDAS	BTDAS	DPS	HPS	Recovery[b] (%)
0.01	94	91	90	90	91	85	88	80	86	89	88
0.3	102	97	85	99	97	95	92	94	81	85	93
1.2	94	97	91	90	98	95	90	94	91	96	94
3	99	95	96	94	99	96	91	95	91	93	95

[a] Based on three replicates; range of RSD values 3–10%.
[b] Average recoveries of the cationic surfactants. Experimental conditions: 40 °C, 2% SDoS and 1 h.

recoveries of cationics were hardly affected by the hydrochloric acid concentration in the range examined, however they showed high variability when extractions were carried out under no cloud point conditions. The lower acid concentrations examined (e.g. 0.01 M) were enough to favor ion exchange between protons and cationic surfactants in the matrix, probably due to the low concentration of analytes in the sludge. The influence of acid concentrations below 0.01 M could not be investigated since 2% SDoS was not soluble at the temperature recommended for extraction.

On the other hand, SDoS did not separate into two isotropic phases for acid concentrations above 4 M. In order to examine this behavior, the influence of matrix components on the separation of the surfactant-rich phase was examined by adding different acid concentrations to 0.1 g of sludge. SDoS phase separation was found to occur in the HCl concentration interval 2.2–3.3 M. Volumes of the surfactant-rich phase for this interval were between 2.7 and 1.5 mL, respectively. Phase separation also occurred between 3.3 and 3.9 M HCl, however it took about 4 days. Similar results were obtained from parallel investigations carried out using 0.2 g of sludge (SDoS phase separation useful for practical purposes was not obtained for HCl concentrations above 3.1 M). It is evident from this study that matrix sludge components, probably of organic nature, influence the HCl concentration versus SDoS concentration phase diagram and, therefore, when no phase separation occurs or surfactant-rich phase volumes greater than about 1.4–1.6 mL are obtained in the extraction of a sludge sample, the appropriate hydrochloric acid concentration to be used should be established. A HCl concentration of 3 M is proposed for the extraction of the spiked aged activated sludge used for optimization purposes.

The influence of the extraction time on the ability of SDoS to extract cationic surfactants was examined in the range between 5 and 90 min. Extraction times of less than 10 min produced low precision recovery measurements (standard deviations ranged between 4 and 10%). The percentage recovery value increased from about 55 to 95% in the 5–60 min interval. Because of their greater hydrophobicity, extraction of the maximum amount of dialkyldimethyl ammonium surfactants required an additional 15 min

with respect to the other target compounds. An extraction time of about 1 h is recommended.

The influence of sample size on the extraction efficiency for cationic surfactants was also investigated. These efficiencies were identical for the two different sludge amounts analyzed (0.1 and 0.2 g). Therefore, 0.1 g of sludge is recommended for analysis.

Total method recoveries for cationics ranged from 91 to nearly 100% and, therefore, we assumed that sample preparation (e.g. sludge dried in air) did not cause a loss of cationics. Also, the recoveries obtained demonstrated that no degradation of cationics occurred under the recommended storage conditions (e.g. dried sludge at 4 °C in amber bottles). Recoveries were similar for aged and recently spiked sludges, which probably indicates the strong affinity of cationics for negatively charged surfaces

3.4. Calibration data

Quantification was carried out by extracting the molecular ion chromatogram (EIC) for each homologue from full-scan spectra, in the range 200–500 m/z, in order to improve S/N ratios and selectivity. We checked in the initial steps of our research that working in SIM mode (e.g. reducing the acquisition range to cover a single mass as in quadrupole mass filter instruments) only resulted in a S/N enhancement of 1.7 compared with the recommended working mode. Common S/N enhancements achieved in LC–IT-MS in going from full-scan to SIM mode are in the range 2–3 [30] since SIM mode in this technique only shortens one part of the scan program (e.g. the time spent to produce the spectrum) and does not permit the transmission of a single mass to the ion trap. While this modest gain in sensitivity may be beneficial in some cases, the additional information provided by full scan is typically more significant. Because of the complexity of sludge samples we decided to work in full-scan mode.

Table 4 shows the figures of merit for the calibration curves obtained for each target compound in the range of injected amounts examined, from 0.2 to 20 ng. The correlation coefficients indicate good fits. The instrumental detection limits were calculated using a signal-to-noise ratio of 3 (the ratio between

Table 4
Analytical characteristics of the method

Compound	m/z	Slope±SD ($\cdot 10^5$)	Intercept±SD ($\cdot 10^5$)	r[a]	LOD[b]
DDDAS	382	51.8±0.2	2±3	0.998	0.06
DDTAS	438	37.1±0.1	5±6	0.998	0.06
DHDAS	494	19.4±0.9	2±8	0.995	0.07
DTAS	228	42.2±0.3	0.3±6	0.997	0.06
TTAS	256	72.8±0.3	1±7	0.997	0.05
HTAS	284	46.8±0.4	2±9	0.995	0.06
BDDAS	304	89.4±0.3	1±8	0.995	0.04
BTDAS	332	91.2±0.4	3±9	0.997	0.04
DPS	248	64.9±0.4	2±5	0.995	0.05
HPS	304	81.7±0.3	3±8	0.996	0.05

[a] Correlation coefficient ($n=9$).
[b] Limit of detection calculated as three times the noise (ng injected); dynamic range from 0.2 to 20 ng of each cationic surfactant injected.

the peak intensity for each compound in the standard solution under the extracted ion chromatogram conditions and noise intensity). Detection limits were in the range from 0.04 ng for BDDAS and BTDAS to 0.07 ng for DHDAS. From these values and taking into account the amount of sample extracted, the volume of extract injected and the recovery obtained from spiked samples, the detection limits of the cationic surfactants in sludge samples were calculated. They ranged from 40 to 75 ng/g.

The possible interference of matrix components that could coelute with the target compounds causing ion suppression and/or space-charge effects on the ion trap was assessed by comparison of calibration curves obtained from standards and sludge fortified with known amounts of cationics. The analytical characteristics of both types of calibration curves were similar and therefore the matrix components did not interfere in the developed method under the conditions described for operation. Accordingly, we recommend external calibration for the determination of cationic surfactants in sludge samples. However, it is advisable to check the influence of matrix components when the sludge to be analyzed is expected to have a composition very different to those investigated here (e.g. sludges from industrial sites)

The precision of the method was evaluated by extracting 11 independent sludge samples. The relative standard deviation ranged from 2 to 4%, which

indicated a good repeatability of the approach developed in this work.

3.5. Structural studies

Cationic surfactant identification was carried out with MS–MS spectrometry using the ion trap instrument. In these studies, the molecular ion corresponding to each target compound was isolated and fragmented. Figs. 2 and 3 show the typical fragment ions obtained from different cationic surfactant groups and the variation of the intensity of molecular and fragment ions as a function of the resonance excitation applied.

The most abundant fragment ions produced from alkyl ammonium surfactants resulted from cleavage of a carbon–nitrogen bond and the subsequent loss of (a) alkane (e.g. fragment ions at m/z 212 from the surfactants BDDAS and DDAS, see Fig. 2) or (b) alkene (e.g. fragment ions at m/z 136 from BDDAS, m/z 214 from DDAS, see Fig. 2, and m/z 80 from alkylpyridinium surfactants, see Fig. 3A). Fragmentation of benzylalkyl compounds also produced a typical stable tropylium ion at m/z 91 (Fig. 2A).

Alkyltrimethyl ammonium surfactants were found to be very stable ions under the different experimental conditions of fragmentation examined. The fragmentation pathway of these compounds favored the formation of $[C_nH_{2n+1}]^+$ ions from long chain alkyl breakdown (e.g. ions at m/z 71 and 85 corresponding to C_5 and C_6 alkyl chain lengths, respectively).

The abundance of molecular and fragment ions was dependent on the resonance excitation applied (Figs. 2 and 3). Substantial fragmentation occurred using resonance excitation between about 0.8 and 1.2 V, which provided good structural information and a sufficient ion intensity signal for the target compounds. The decrease in signal intensity for the fragment ions compared with the parent ions (between 10 and 1000 times) advises against the use of MS–MS for quantification.

An advantage of using an ion-trap mass spectrometer is that the instrument can switch between full-scan MS and a collision-induced dissociation (CID) product scan in the presence of helium collision gas, with no loss in signal or CID efficiency. Therefore, from the same chromatographic peak, you can quantify cationics with the maximum
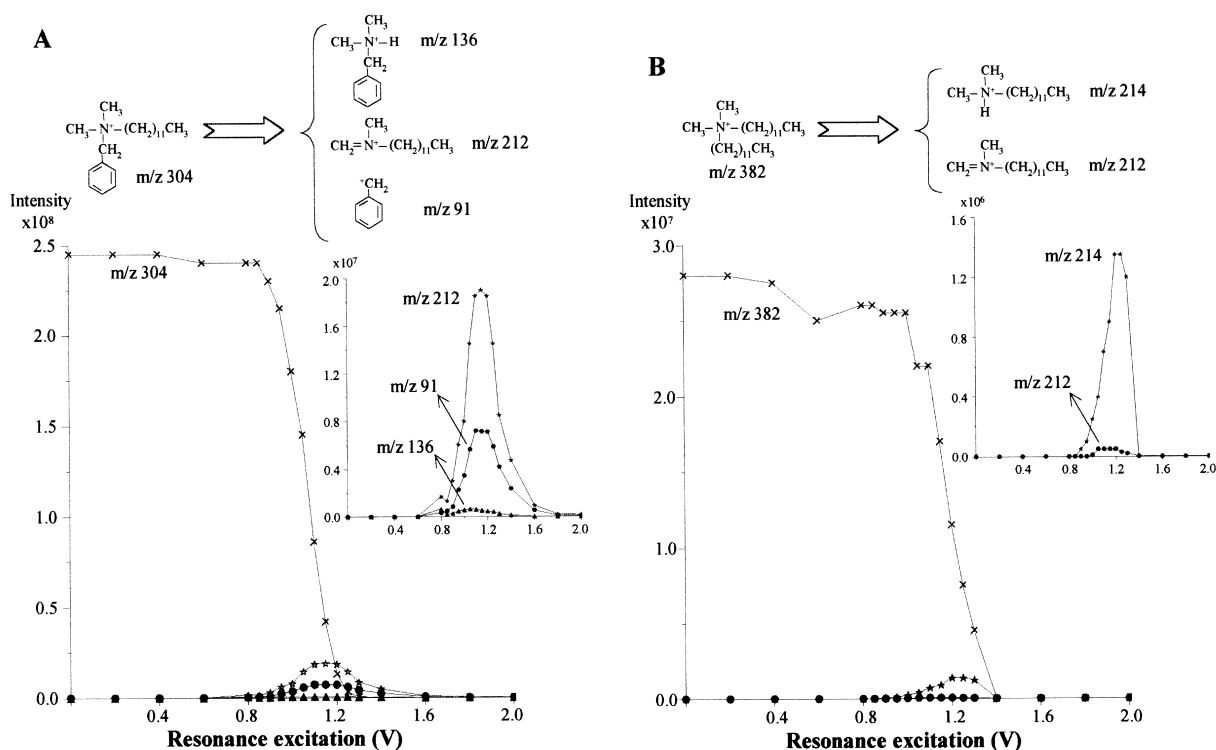
Fig. 2. Proposed fragmentation ions and fragmentation pattern obtained with ESI-MS–MS in positive ionization mode by direct injection of (A) MeOH–50 mM HCOONH$_4$ (80:20)–BDDAS (10 μg/mL) solution and (B) MeOH–50 mM HCOONH$_4$ (80:20)–DDDAS (10 μg/mL) solution. Cut-off mass 27% of parent ion; CID time 40 ms.

sensitivity from single-stage mass spectrometry and identify them from MS–MS analysis.

3.6. Analysis of sewage sludge samples

The method developed was applied to the analysis of activated and dehydrated sewage sludge from two WWTPs. Both of them apply biological sewage treatment. Table 5 lists the concentrations found for the target compounds, expressed as the mean value ($n=3$) and the corresponding standard deviation. Confirmation of the identity of compounds was obtained by MS–MS experiments, considering the main fragmentation ions (see Figs. 2 and 3). Extracted ion chromatograms obtained for the different cationic surfactants found in dehydrated sludge samples are depicted in Fig. 4. Total method recoveries were assessed by spiking the sludge samples with 1 mg/kg of each target compound. The re-

covery values obtained were similar to those found for the spiked aged activated sludge used for optimization purposes (i.e. >91%).

Volumes of surfactant-rich phase between 1.4 and 1.6 mL were obtained for all samples analyzed, except for the activated sludge from Linares, using the concentration of HCl recommended (3 M) for the extraction of the spiked aged activated sludge. The acidity conditions used to extract cationics from the Linares activated sludge sample were found by applying the ACPE procedure to 0.1 g of sample using HCl concentrations between 2.5 and 5 M. Adequate volumes of surfactant-rich phase (1.4–1.6 mL) were obtained from 3.8 M hydrochloric acid. The acid concentration selected for analysis of this sample was 4.2 M. The different influence that the matrix of this sample exerts on the phase separation behavior of anionic surfactants with respect to the other analyzed samples can probably be attributed to
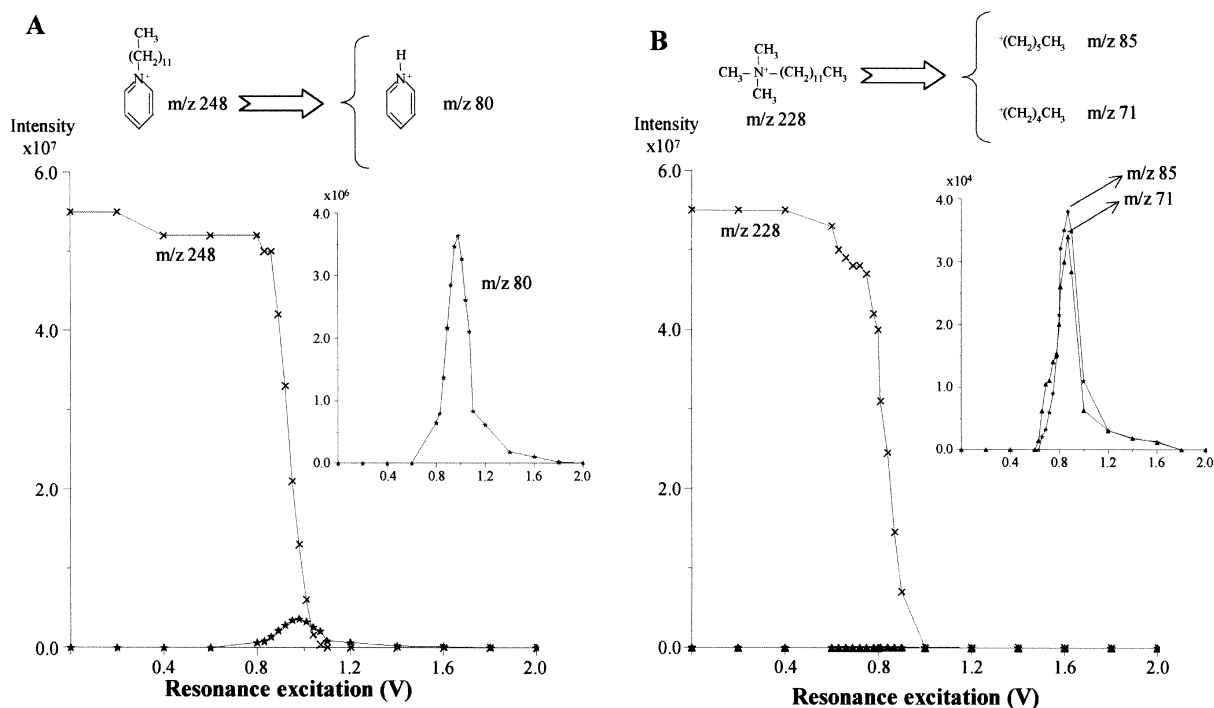
Fig. 3. Proposed fragmentation ions and fragmentation pattern obtained with ESI-MS–MS in positive ionization mode by direct injection of (A) MeOH–50 m*M* HCOONH$_4$ (80:20)–DPS (10 µg/mL) solution and (B) MeOH–50 m*M* HCOONH$_4$ (80:20)–DTAS (10 µg/mL) solution. Cut-off mass 27% of parent ion; CID time 40 ms.

the different composition or proportion of organic matter that it contains, taking into account that the Linares WWTP receives both industrial and domestic wastewater.

Although the aim of analyzing different sludge

samples was to demonstrate the applicability of the ACPE–LC–ESI-IT-MS technique in the determination of cationic surfactants, some conclusions can be derived from the results obtained. Thus, the low concentrations found for dialkyldimethyl ammonium

Table 5
Mean concentration (mg/kg)±standard deviation of cationic surfactants[a] found in sludges collected from two WWTPs, analysed by the ACPE approach

Surfactant	Sludge from Pozoblanco		Sludge from Linares	
	Activated	Dehydrated	Activated	Dehydrated
DDDAS	1.4±0.3	1.5±0.3	0.8±0.1	8.6±0.9
DDTAS	0.24±0.05	–	0.21±0.03	0.92±0.10
DHDAS	4.5±0.2	2.5±0.2	3.7±0.3	4.1±0.4
DTAS	0.14±0.04	0.10±0.02	0.08±0.02	–
TTAS	–	–	–	–
HTAS	1.3±0.2	1.1±0.1	0.39±0.07	34±2
BDDAS	1.1±0.1	0.84±0.06	0.65±0.05	28±2
BTDAS	0.80±0.1	0.53±0.08	0.40±0.04	18±1
DPS	–	–	–	–
HPS	–	–	–	–

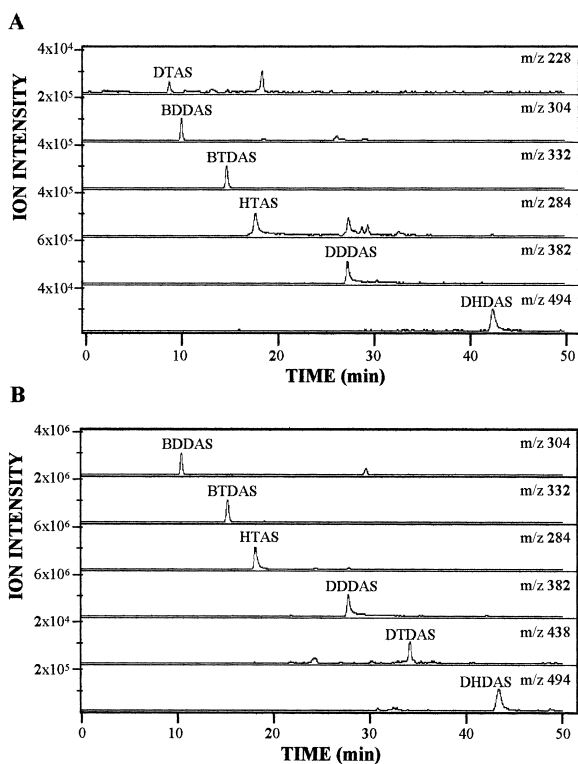[a] Based on three replicates; "–" below the detection limit of the method.

Fig. 4. LC–MS extracted ion chromatograms of cationic surfactants obtained from dehydrated sludge samples of WWTPs in (A) Pozoblanco and (B) Linares.

surfactants (Table 5) are probably due to their increasing substitution by more biodegradable cationic surfactants, such as esterquats. On the other hand, the nonbiodegradability of quaternary ammonium salt-based surfactants with the common anaerobic treatments applied in WWTPs was confirmed. As can be seen from Table 5, dehydrated sludges contain cationic surfactants. No conclusions, however, can be derived from some of the results, such as the difference found for the surfactants HTAS, BDDAS and BTDAS in the activated and dehydrated sludges from Linares. Since samples were collected on the same day and therefore represent different loads for the WWTP, a possible explanation for this dramatic difference may be the inter-day variability of the surfactant load.

The presence of cationic surfactants belonging to three of the four groups investigated was confirmed in the sludge samples analyzed (Table 5). To our knowledge, this is the first confirmation of the presence of benzylalkyldimethyl and alkyltrimethyl ammonium surfactants in sewage sludge. Taking into account the demonstrated toxicity of alkylammonium surfactants, further studies should be undertaken to establish their fate in sludge-amended soils.

4. Conclusions

A new approach based on ACPE–LC–ESI-IT-MS has been developed to monitor alkylammonium surfactants in sludge samples. The mixed aggregate-based ACPE technique was found quantitative, fast and simple for the isolation of cationics from sludge. Ideal hydrophobic and nonideal electrostatic interactions between the extractant and the target compounds lead to the formation of mixed aggregates, which facilitate the breakdown of sludge–cationic interactions and provide high extraction yields. The formation of extractant–analyte mixed aggregates is a valuable strategy for the extraction of amphiphilic substances from solid matrices using the cloud-point technique.

Several operational parameters associated with ACPE compare favorably with those used in conventional liquid extraction and SFE for the isolation of cationic surfactants from sludge. Thus, ACPE uses water as extractant, whereas an organic solvent is required in liquid extraction (550 mL) and SFE (7 mL). ACPE total sample preparation takes 90–100 min. This time compares very favorably with that required to treat four samples in conventional extraction (about 1 week). SFE reduces the total sample preparation time for a single sample to 30–40 min, however we have routinely carried out the simultaneous treatment of eight sludge samples in that time. On the other hand, ACPE, like SFE, does not require clean-up steps, thus minimizing the risk of laboratory contamination. To sum up, the main advantage of ACPE compared to SFE is the possibility of performing simultaneous treatments with no special extraction equipment. The main drawback is that operations are not automated. Experiments in progress are being addressed to solve this problem.

The use of LC–ESI-IT-MS permitted the sensitive determination and identification of homologues belonging to four groups of cationic surfactants. The methodology proposed is therefore a valuable tool in

the trace-level monitoring of alkylammonium surfactants in sludge samples for both control purposes and for studies directed towards the understanding of the fate of cationics in the environment.

Acknowledgements

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