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Cationic surfactant-based polyfluorate salts: Phase separation and analytical applications in the extraction and preconcentration of ionic species prior to liquid chromatography

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Abstract

The liquid–solid phase separation originating from the formation of cationic surfactant-based polyfluorate salts (CSBPS) has been explored for extracting and preconcentrating ionic species. Two cationic surfactants were tested; one with aliphatic hydrocarbon tail [Cetyltrimethylammonium bromide (CTAB)]and the other containing a heterocyclic ring [Hexadecylpyridinium bromide (HPyBr)]. Phase separation possibility was investigated with the use of hexafluorophosphates (PF_6^-) and tetrafluoroborates (BF_4^-). The effect of added acid, base and salt on the phase separation and analyte extraction was also investigated. In all cases the obtained phase diagrams consisted of two regions: a homogeneous liquid region and a solid–liquid region. Analytes of hydrophilic and hydrophobic nature such as amines, amino acids and organic chromophores were used as test compounds in both their anionic and cationic forms. The respective recoveries ranged from over 90% for anionic species and in the proximity of 50% for cationic species, remaining below 20% for neutral species. Extracts from alkaline aqueous and plasma samples spiked with tyrosine and phenylalanine were also subjected to HPLC separation with UV detection with satisfactory results. On line application was also enabled using a flow through-solid phase extraction-HPLC hyphenated apparatus, thus adding the element of automatization and increased reproducibility.

Keywords: Cationic surfactants; Polyfuorates; Phase separation; Extraction; Preconcentration; Ionic species; Molten salts; Liquid chromatography

1. Introduction

Aqueous solutions of non-ionic [1,2] as well as some zwitterionic [3] surfactants are well known for their ability to undergo clouding and liquid–liquid phase separation when heated above a certain temperature referred to as cloud point temperature (CPT) [4]. Phase separation can also be induced in an increased ionic strength environment, that is upon addition of salts [5]. Anionic surfactants [6,7] also exhibit similar behavior, in the presence of concentrated (C > 2 M) acids, that transform the anionic head groups into protonated non-ionic formations. In every circumstance the resulting surfactant rich phase creates a microenvironment consisting of a hydrophilic exterior and a hydrophobic core, where covalent, uncharged species are favorably entrapped, while ionic formations are repelled. Thus, this so-called cloud point extraction methodology has been employed, so far, for the extraction and subsequent preconcentration of hydrophobic analytes, while the extraction of hydrophilic and especially ionic species is limited, based on the neutralization of their charge.

Cationic surfactants, on the other hand, have shown limited tendency to micellization and respective phase separation through analogous schemes [8] and they have, therefore, been neglected as candidates for cloud point extraction applications. The main reason is that they require a large hydrophobic counterion (tosylate or salicylate) [9], an anionic surfactant [10], a cosurfatcant [11] or extremely large amounts (20–3 M) of common salts [12,13] to induce micellar

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growth. Moreover, cationic surfactants with promising micellization properties are limited and commercially not available [12,13]. In the case of commercially popular cationic surfactants like Cetyltrimethylammonium bromide (CTAB) and Cetylpyridinium bromide (HPyBr) more than 2 or 3 M of salt [13] was necessary for phase separation and for this reason direct analytical applications are scarce.

In these applications it became obvious that the cationic surfactant-originated rich phase, still consists of charged counter ions that may well have the ability to interact with ionic species in solution, through simple electrostatic attraction, thus entrapping them into the surfactant extract.

Under this perspective we propose the use of polyfluorates (BF_4^-, PF_6^-) in relatively low concentrations (<1 mM) to generate phase separation of CTAB and HPyBr, using them directly in cloud point extraction of charged species. Motivated by the fact that pairing of unsymetrically substituted, nitrogen-containing cations with inorganic anions has given birth to nonmolecular ionic solvents, better known as ionic liquids (ILs) or molten salts [14,15], we found that when polyfluorates are added to aqueous micellar solutions of CTAB and HPyBr, the later sustain a behavior similar to what Giokas et al. [16] called liquid coacervate extraction, formatting a surfactant-rich solid phase. Upon centrifugation this surfactant rich phase is driven either to the bottom or to the surface of the vial thus separated from the bulk aqueous phase. The ability of this solid surfactant phase – possessing both anionic and cationic components – to interact with ionic species in solution was investigated. Amines, amino acids and organic chromophores in both their anionic and cationic forms, were extracted under acidic and alkaline conditions. The obtained extracts were analyzed by high performance liquid chromatography and UV detection revealing that the proposed methodology offers high recoveries and preconcentration factors. Automatization was also performed by replacing the centrifugation step with solid phase extraction on line connected to HPLC, according to a previously developed assembly [17], thus adding the required reproducibility and handling versatility to the proposed scheme.

2. Experimental

2.1. Apparatus

A Shimadzu UV-2100 spectrophotometer with matched quartz cells of 1 cm path length was used for batch measurements. The HPLC apparatus used for chromatographic experiments consisted of a Shimadzu 10AD series for HPLC equipped with a UV–vis variable wavelength detector(Shimadzu) set at 280 nm. A LiChrospher 100 RP-18 (244 mm × 4.4 mm I.D., 5 μm) column was used for all separations. Two LiChrospher guard columns (10 mm × 4.6 mm I.D.) were also used, one as the SPE cartridge used for the on-line approach, and the other for column protection. The whole system was thermostated at 40 °C in a CTO-10A Shimadzu column oven. Data collection and manipulation were performed by means of a CLASS-VP Shimadzu automated software for chromatography. A multi-channel peristaltic pump (Ismatec, Glattburg-Zurich, Switzerland) and Tygon solvaflex tubing were used for the propulsion of reagents to the SPE cartridge in the automatized on-line application. A Vortex Velp Scientifica mixer was used for thorough mixing of solutions. Phase separation was assisted using a centrifuge (Hettich, Universal).

2.2. Reagents

All reagents were of analytical grade or of the highest grade available. Tyrosine, Phenylalanine, 8-Hydroxyquinoline, tyramine hydrochloride and 1,4-dichlorobenzene were obtained from Sigma Aldrich Chemical Company(USA). Water and Acetonitrile (Merck, Darmstadt Germany) used for chromatographic separation were HPLC grade. Cetyltrimethylammonium bromide (Aldrich, Cat. No. 85,582-0) and Hexadecylpyridinium bromide (Fluka, Cat. No. 52340) were used, without further purification, to prepare 10 g L^{-1} aqueous stock solutions. Potassium tetrafluoroborate (Aldrich, Cat. No. 45,590-3) and Potassium hexafluorophosphate (Aldrich, Cat. No. 51,597-3) were used to prepare 10 g L^{-1} aqueous stock solutions of the respective polyfluorates. As a safety note, proper precautions should be taken when handling surfactants and hexafluorophosphate (use of gloves and masks, or working under a ventilated area) because they are hazardous upon inhalation and skin contact. It should also be taken under consideration, that polyfluorate anions are slowly hydrolyzed towards the formation of hazardous and corrosive hydrofluoric acid, as well as poisonous phosphorous oxides and phosphine, especially in the presence of strong acids. Therefore the wastes produced following the proposed methodology should be collected and properly disposed off.

2.3. Procedures

2.3.1. Molten salt potential investigation

In order to test our assumption that the surfactant rich phase consists of an ionic pair with ionic liquid potential, equimolar amounts of each compound were dissolved in 50 mL of doubly distilled water. The mixture was mixed thoroughly under magnetic stirring for 1 h and left to stand for another 24 h. The resulting solids were filtered under vacuum through a Whatman No.1 filter paper and washed thoroughly with a 10 g L^{-1} NH_4NO_3 solution, until full removal of remaining bromide ($AgNO_3$ negative reaction). Then they were recrystallized from methanol and left to air-dry. Finally they were subjected to thermal analysis (DSC). Table 1 shows the melting points of the derived solids against their precursor compounds. Scanning electron microscopy (SEM) pictures of CTA-PF_6 surfactant rich phase (Fig. 1) reveal lamellar bilayers typical of ion-pairs, analogous to those obtained by Giokas et al. [16].

Table 1
Melting points of CSBPS and precursor cationic surfactants obtained after DSC analysis

Compound	Melting point (°C)
CTAB	258.8 ± 1.0
CTA-PF_6	121.0 ± 1.0
CTA-BF_4	291.7 ± 1.0
HPyBr	68.1 ± 1.0
HPy-PF_6	60.8 ± 1.0
HPy-BF_4	87.7 ± 1.0

2.3.2. Phase diagrams

The phase behavior of each surfactant system was examined under different experimental conditions by mixing appropriate volumes of the surfactant and respective polyfluorates standard solution in centrifugal vials and making a volume of 10 mL with distilled water. After 24 h of standing, the tubes were inspected to check whether the solution remained homogeneous or a solid phase was formed. Phase diagrams were obtained from these observations as a function of polyfluorates and surfactant concentration. In every case vials were centrifuged at 3500 rpm (ca. $3100 \times g$) for 5 min to verify the observations and ensure whether the observed phases were liquid or solid.

2.3.3. Cloud point extraction from aqueous samples

Although, all of the four surfactant-polyfluorate combinations were examined for phase separation and extraction of anionic species the CTAB-PF_6 system was finally adopted for producing optimum results in terms of observation, efficiency and required time. In a typical experiment 200 μL of the CTAB solution (ca. 1.6 mg CTA) were added to 10 mL of sample containing the required concentration of added electrolyte followed by another 200 μL of the $NaPF_6$ solution (ca. 0.8 mg PF_6). The mixture was stirred in a Vortex and left to stand for 30 min for complete phase formation. Separation of the two phases was achieved by centrifugation at 3500 rpm (ca. $3100 \times g$) for 5 min.

In order to evaluate the ability of cationic surfactant-based molten salts to extract and preconcentrate anionic, cationic and perhaps uncharged species from aqueous test solutions, the proposed methodology was applied to compounds, which can assume both ionic and cationic forms with simple pH manipulation. Thus, 8-Hydroxyquinoline ($pK_{a1} = 5.0$, $pK_b = 9.8$), Tyramine($pK_a = 9.5$, $pK_b = 10.7$), Tyrosine ($pK_a = 2.2$, $pK_b = 9.1$, $pI = 5.7$) and Phenylalanine ($pK_a = 1.8$, $pK_b = 9.1$, $pI = 5.5$) were chosen for containing both acidic and basic functional groups, while *p*-dichlorobenzene was also employed in the study representing the non polar (uncharged) analytes.

In order to ensure uniform conditions for the extraction of all compounds three sets of experiments were set forth.

(i) Extraction was performed from a solution containing 1 M HCl to ensure that all compound under inspection are in their cationic form.
(ii) Extraction was performed from a solution containing 1 M NaOH to ensure that all compound under inspection are in their anionic form.
(iii) Extraction was performed from a solution containing 1 M NaCl buffered to pH 7 with a 0.1 M phosphate solution, in order to ensure that all compounds under inspection have assumed their neutral form, while the increased ionic strength ensures maximum extraction efficiency.

For recovery experiments 8-hydroxyquinoline, tyramine and *p*-dichlorobenzene were examined. 10 mL of the test solution containing 0.1–1.0 mg L^{-1} of the target analyte were subjected to extraction in all three above conditions. The slurry extract (ca. 30 mg of weight) was redissolved in 1 mL of 50% aqueous acetonitril. The absorbance was measured at 280 nm against a reagent blank, in a spectrophotometer.

2.3.4. Application to HPLC

The ability of CSBPS to preconcentrate analytes prior to HPLC analysis was assessed by spiking aqueous and plasma samples with tyrosine and phenylalanine, at concentrations of 0.5 mg L^{-1}. The extraction was performed from the 1 M NaOH alkaline solution in order to ensure that both amino acids are in their anionic form and also capitalize from the acceleration of phase formation and separation induced by the presence of NaOH. The surfactant rich phase (ca. 30 mg in weight) was again diluted in 200 μL of 50% aqueous acetonitril and 20 μL were injected into the chromatographic system. For comparison the cloud point extraction of these analytes was also conducted by using a non-ionic surfactant system in which the sample was dissolved in 1% (w/v) aqueous Triton X-45 solution, which is clouded spontaneously at room temperature.

2.3.5. Liquid Chromatographic Analysis

Tyrosine and Phenylalanine extracts were separated by using a mobile phase consisting of 60% acetonitril/40% 0.005 M aqueous H_2SO_4 at a flow rate of 0.4 mL min^{-1}.

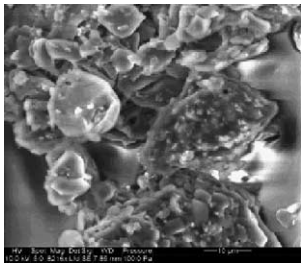

Fig. 1. Scanning electron microscopy (SEM) picture of the derived CTA-PF_6 formation.

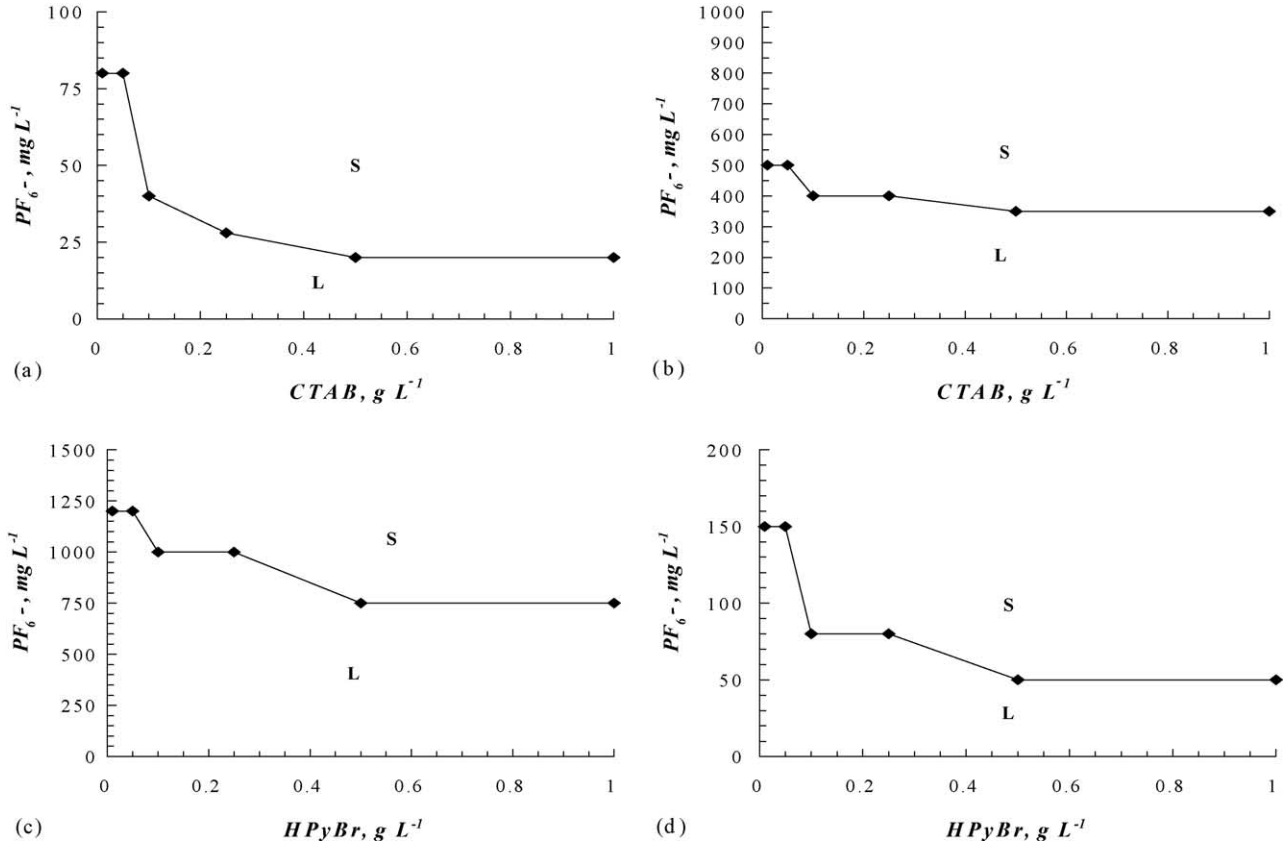

Fig. 2. Phase diagrams of surfactant vs. polyfluorate concentration. (S) denotes the solid region and (L) the liquid region. CTAB vs. $NaPF_6$, 24 h incubation. CTAB vs. $NaPF_6$, immediate phase formation. HPyBr vs. $NaPF_6$, immediate phase formation. HPyBr vs. $NaPF_6$, 24 h incubation.

3. Results and discussion

3.1. Phase diagrams of cationic surfactants upon polyfluorates addition

Fig. 2(a) and (d) shows phase diagrams of PF_6^- concentration against the respective surfactant (CTAB, HPyBr) concentration at room temperature(25–30 °C). Two regions are observed in each case; a homogeneous liquid region (L), and a solid–liquid (S) region. Other cationic surfactants like erucyl(C_{22}) trimethylammonium chloride, tetradecyltrimethylammonium bromide and dodecyltrimethylammonium bromide were also examined in preliminary experiments exhibiting similar behavior but CTAB and HPyBr were more extensively tested due to their commercial availability. Unlike non-ionic and anionic surfactants [1–7] no liquid–liquid region was observed no matter the circumstances. This could mean either that such a region is either not formed, or has a very limited boundary or that the supposedly resulting surfactant liquid is completely water miscible. It has been observed that substitution by PF_6^- induces an easier clouding than by BF_4^- and this is more pronounced with CTAB. Observation of the derived solids also leads to the deduction that PF_6^- produces more bulky and porous surfactant rich phase while BF_4^- gives finer particles. Another important observation is that by increasing the surfactant concentration over 1000 mg L^{-1} the surfactant rich phase is concentrated to the surface of the aqueous solution. This ability gives the potential of direct application of the method to solid samples like food or soil. Once formed the surfactant rich phase remains stable for over a week. Fig. 2b and c show the amount of polyfluorates required for immediate phase separation while the remaining graphs refer to observations made after a 24 h incubation period. Direct phase separation was achieved only by addition of PF_6^- at concentrations of over 350 and 750 mg L^{-1} for CTAB and HPyBr respectively, while under the influence of BF_4^- direct solid formation was not feasible even when 2500 mg L^{-1} were added.

By increasing the solution temperature the solid phase formation is delayed and hindered, while beyond 65 °C the solution remains homogeneous within the concentration range examined for surfactants and anions. On the other hand upon cooling the mixture in an ice bath phase separation became more pronounced. This results fortify the assumption that this is a clouding behavior of the upper consolute type [8].

It is obvious from the above observations that PF_6^- induced phase separation of cationic surfactants is easier especially with CTAB. Compared to previous attempts to generate micellar growth in cationic surfactant solutions phase separation is achieved in additive concentrations

200–4000 times lower (0.1–0.5 mM against 20–2000 mM) thus giving versatility and applicability to the proposed method because the up-to-date used excessive concentrations of salts could severely impair performance of flow through devices (HPLC, FIA, GC), precipitate and co-precipitate metal ions and lead to increased background signals in atomic spectrometry and plasma measurements.

3.2. Effect of HCl, NaOH and NaCl on the phase separation of CSBPS

In order to assess the effect of added electrolytes (acid, base and salt), the phase separation performance of CSBPS was examined in the presence of different concentrations of HCl, NaOH and NaCl.

It was found that the presence of these electrolytes in concentrations of 0.1–0.5 M produced similar phase diagrams with those obtained previously, but the time needed for phase separation was greatly reduced from 24 h to less than 4 h. In all cases as the concentration of the added electrolyte raised exceeding 1 M, the phase formation and subsequent separation became more evident with smaller amounts of added PF_6. This observation enhances the assumption that the driving force behind the formation of the CSBPS are salt-like electrostatic interactions that are further promoted by increased ionic strength. Another important observation is that at added electrolytes concentrations between 1 and 2 M the flocculation of the surfactant aggregates is almost spontaneous upon addition of PF_6 anions, thus allowing for direct phase separation and application of the proposed schemes in flow assemblies. Fig. 3 shows the phase diagrams of CTAB versus $NaPF_6$ concentration obtained in 1 M HCl, 1 M NaOH and 1 M NaCl which are also the conditions applied for preconcentration and extraction experiments. Fig. 4 presents the effect of increasing NaOH concentration on the incubation time required for phase separation in a solution containing 0.2 g L^{-1} CTAB and 0.2 g L^{-1} $NaPF_6$.

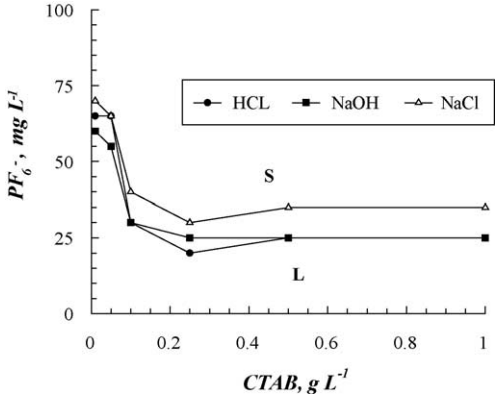

Fig. 3. Phase Diagrams of CTAB vs. $NaPF_6$ after 24 h incubation. (S) denotes the solid region and (L) the liquid region. ((●) 1 M HCl, (■) 1 M, NaOH, (△) 1 M NaCl pH 7).

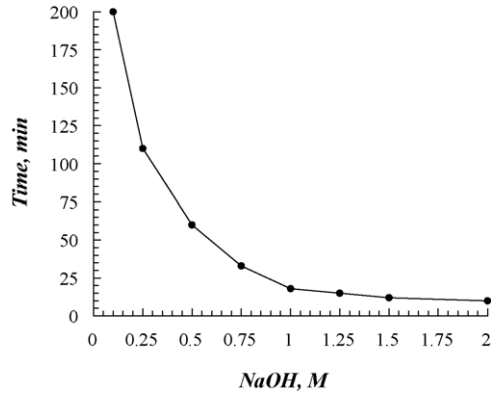

Fig. 4. Effect of NaOH concentration on the incubation time required for phase separation from a solution containing 0.2 g L^{-1} CTAB and 0.2 g L^{-1} $NaPF_6$, at ambient temperature.

3.3. Extraction efficiency of CSBPS

Table 2 shows the recoveries of 0.1–1.0 mg L^{-1} of the target compounds under acidic (1 M HCl, pH ≈ 0), alkaline (1 M NaOH, pH ≈ 14), and neutral (1 M NaCl, pH ≈ 7) conditions.

It is obvious that for both tyramine and 8-Hydroxyquinoline the extraction efficiency is more pronounced in alkaline media. Holding pK_a values in the proximity of 10 it is expected that both analytes are almost completely (99.99%) present in their anionic forms, thus having great affinity for the cationic headgroups of the surfactant, which consist the bulk of the salt aggregate.

On the other hand the extraction of cationic species generated in acidic pH is less evident especially with increasing concentrations of the target analyte. This observation

Table 2
Recoveries of tyramine, 8-hydroxyquinoline and *p*-dichlorobenzene from acidic (1 M HCl), alkaline (1 M NaOH) and neutral (1 M NaCl, pH = 7) solution after extraction into CSBPS

Concentration (mg L^{-1})	% Recovery								
	Tyramine			8-Hydroxyquinoline			*p*-Dichlorobenzene		
	Acidic	Alkaline	Neutral	Acidic	Alkaline	Neutral	Acidic	Alkaline	Neutral
0.1	75.0	102.0	30.0	60.0	99.5	20.0	5.0	7.0	6.0
0.2	60.0	99.5	25.0	55.0	95.0	18.5	5.0	6.5	5.0
0.5	55.0	98.0	20.0	40.0	93.0	15.0	4.0	5.5	5.0
0.8	55.0	95.0	18.0	35.0	90.0	12.0	3.0	4.0	3.0
1.0	50.0	92.5	15.0	35.0	84.0	12.0	1.0	2.5	1.5

denotes that the magnitude of electrostatic interactions among polyfluorate anions and cationic species in solution are limited. This can be attributed to the fact that the bulky surfactant cations restrict the polufluorate anions into the salt formation blocking their interaction with other cations, thus creating a microenvironment favorable for labile anionic species, while repelling competitive cationic formations, thus resembling the function of a molecular pump. This assumption is further assisted by the observation that in neutral pH the recovery for all species remains below 30%. This denotes that the regulating factor for an analyte to approach the CSBPS formation is the elctrostatic attraction by the positively charged quaternary ammonium, while the hydrogen bonding expected to occur among fluorates and hydroxy or amino groups has as little effect as these 30% recoveries obtained. Coming as a validation of this assumption is the fact that the extraction of p-dichlorobenzene into CSBPS formations remains in the vicinity of 5% regardless the conditions applied.

3.4. CSBPS extraction prior to HPLC

Fig. 5 shows the chromatograms obtained from injecting a blank, an aqueous and a deproteinated plasma extract from samples fortified with 0.5 mg L^{-1} Tyrosine, Phenylalanine and several other amino acids without aromatic moieties in their molecule. Extraction was performed from alkaline, 1 M NaOH solutions in order for the amino acids to assume their anionic forms. It is obvious that in the blank injection a single peak appears in the beginning ($t = 0.5$ min) of the chromatogram denoting that there is no interference even in the detection of analytes with short retention times. In the remaining chromatograms the appearance of two additional peaks corresponding to Tyrosine and Phenylalanine signals that there is a complete extraction of these 2 amino acids while no other interfering peak appears. Table 3 shows the figures of merit and the preconcentration factors obtained for the target compounds after extraction into CSBPS. The chromatograms obtained from the injecting the respective Triton X-45 extracts gave no peak of tyrosine or phenylalanine even from 5 mg L^{-1} solutions. This is expected because hydrophilic and especially charged analytes have negligible partition coefficient into non-ionic surfactant micelles.

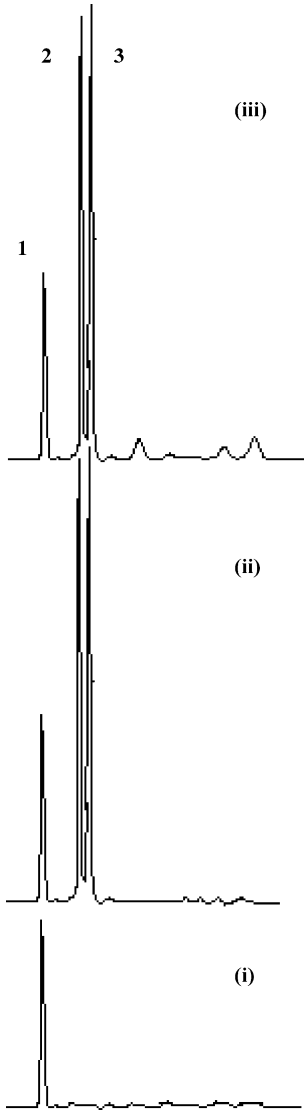

Fig. 5. Chromatograms obtained after injecting (i) a blank, (ii) an aqueous and (iii) a plasma extract into the HPLC system. Order of the chromatographic peaks 2: Tyrosine (0.5 mg L^{-1} initial concentration), 3: Phenylalanine (0.5 mg L^{-1} initial concentration). Chromatographic conditions: 60% acetonitril/40% 0.005 M aqueous H_2SO_4 ($q = 0.4$ mL min^{-1}). Detection at 280 nm.

Table 3
Figures of merit from the extraction of tyrosine and phenylalanine into CSBPS and HPLC-UV determination

Parameter	Tyrosine	Phenylalanine
Preconcentration factor	50	50
Extraction concentration factor	0.99	0.98
LOD[a] (mg L^{-1})	0.01	0.02
LOQ[b] (mg L^{-1})	0.03	0.07
RSD (%)	2.2	2.5
Regression equation	S^c = 50000 C (mg L^{-1}) + 15620	S = 32150 C (mg L^{-1}) + 15240
Correlation coefficient (r)	0.9996	0.9992

[a] Limit of detection defined as 3 times the signal to noise ratio.
[b] Limit of quantitation defined as 10 times the signal to noise ratio.
[c] Peak area (arbitrary units).

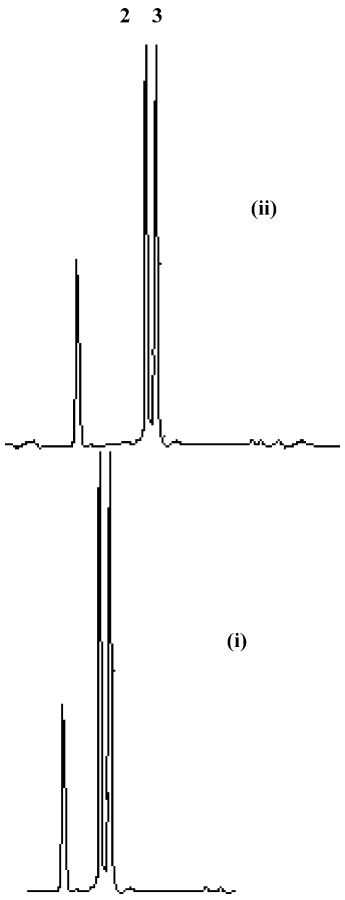

Fig. 6. Comparison of chromatograms obtained after injecting the extract obtained from deproteinated plasma fortified with 0.5 mg L^{-1} Tyrosine and 0.5 mg L^{-1} phenylalanine (i) following batch extraction and (ii) on-line approach into the HPLC system. Order of the chromatographic peaks 2: Tyrosine, 3: Phenylalanine. Chromatographic conditions: 60% acetonitril/40% 0.005 M aqueous H_2SO_4 (q = 0.4 mL min^{-1}). Detection at 280 nm.

3.5. Automatization of CSBPS extraction

Since a great deal of discussion and research is devoted nowadays to automatization and handling versatility of the surfactant mediated extraction schemes. We used a previously manufactured on-line SPE-HPLC assembly to replace the centrifugation and evaporation step that usually adds time, and loss of reproducibility to surfactant mediated approaches. Thus we replaced the HPLC loop with a C-18 guard column – which acting as a SPE cartridge – retains the surfactant rich phase from the matrix solution (driven to it by means of a peristaltic pump) allowing to be eluted later with the chromatographic program. Alternatively a cotton packed minicolumn proposed earlier by Fang et al. [18]. could be used effectively for retaining the surfactant rich phase. No optimization was performed in these approaches. Fig. 6 shows the chromatogram of the previously used amino acid solution obtained after the automatized procedure. It is obvious that sufficient extraction is achieved for both Tyrosine and Phenylalanine.

4. Conclusions

Two commmercialy available cationic surfactants were selected to examine the solid–liquid phase separation behavior of cationic surfactants upon addition of polyfluorate anions. The analogous pattern exhibited by a number of other cationic surfactants revealed that this is a general property rather than a special one applied only to selected cationic surfactants, like the one previously reported for sulfate anions [12]. Thus the derived solid phase can effectively preconcentrate ionic species especially of anionic nature and the extracts can be effectively applied to chromatographic assemblies. Automatization of the proposed technique allows for extra reproducibility and handling versatility of the CSBPS extracts.

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References

[1] W.L. Hinze, in: W.L. Hinze, D.W. Armstrong (Eds.), Ordered Media in Chemical Separations, American Chemical Society, Washington, DC, 1987, pp. 48–55.
[2] V. DeGiorgio, in: V. DeGiorgio, M. Corti (Eds.), Physics of Amphiphiles: Micelles, Vesicles and Microemulsions, North-Holland, Amsterdam, 1985, pp. 303–335.
[3] J.C. Lang, R.C. Morgan, J. Chem. Phys. 73 (1980) 5849.
[4] J. Miura, H. Ishii, H. Watanabe, Bunseki Kagaku 25 (1976) 808.
[5] H. Schott, J. Colloid Interface Sci. 192 (1997) 458.
[6] B.M. Cordero, J.P.L. Pavon, G.C. Pinto, M.E.F. Laespada, Talanta 40 (1993) 1703.
[7] I. Casero, D. Sicilia, S. Rubio, D. Perez-Bendito, Anal. Chem. 71 (1999) 4519.
[8] A.E. Vassiliades, in: E. Jungerman (Ed.), Cationic Surfactants, Marcel Dekker, New York, 1970, pp. 387–399.
[9] S.R. Raghavan, H. Edlund, E.W. Kaler, Langmuir 18 (2002) 1056.
[10] J.C.A. de Wuilloud, R.G. Wuilloud, B.B.M. Sadi, J.A. Caruso, Analyst 128 (2003) 453.
[11] X. Jin, M. Zhu, E.D. Conte, Anal. Chem. 71 (1999) 514.
[12] B.K.W. Man, M.H.W. Lam, P.K.S. Lam, R.S.S. Wu, G. Shaw, Environ. Sci. Technol. 36 (2002) 3985.
[13] J. Appell, G. Porte, J. Phys. Lett. 44 (1983) 689.
[14] J.L. Anderson, D.W. Armstrong, Anal. Chem. 75 (2003) 4851.
[15] D.W. Armstrong, L. He, L.S. Liu, Anal. Chem. 71 (1999) 3873.
[16] D.L. Giokas, G.Z. Tsogas, A.G. Vlessidis, M.I. Karayannis, Anal. Chem. 76 (2004) 1302.
[17] E.K. Paleologos, M.G. Kontominas, Anal. Chem. 76 (2004) 1289.
[18] Q. Fang, M. Du, W. Huie, Anal. Chem. 73 (2001) 3502.