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Review

Surfactant cloud point extraction and preconcentration of organic compounds prior to chromatography and capillary electrophoresis

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Abstract

The use of preconcentration steps based on phase separation by the cloud point technique offers a convenient alternative to more conventional extraction systems. It has been used successfully for the preconcentration of species of widely differing character and nature, such as metal ions, proteins and other biomaterials, or organic compounds of strongly differing polarity. Here we address the most recent analytical applications of this methodology when used as an isolation and trace enrichment step prior to the analysis of organic compounds (polycyclic aromatic hydrocarbons, polychlorinated compounds, pesticides, phenolic derivatives, aromatic amines, vitamins, etc.) via liquid and gas chromatography or capillary electrophoresis.

Keywords: Reviews; Cloud point extraction; Preconcentration; Organic compounds; Polynuclear aromatic hydrocarbons; Polychlorinated compounds; Pesticides; Vitamins

Contents

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1. Introduction

In recent decades the development of preconcentration steps to be implemented prior to analytical determinations of trace level compounds has been explored in considerable depth. With a view to eliminating or at least minimising the use of organic solvents used in conventional liquid–liquid extraction, other methodologies have been developed, such as membrane extraction [1–3], solid-phase extraction (SPE) [4–7], solid-phase microextraction (SPME) [8–10], etc.

Alternative extraction approaches that make use of liquid phases have also been proposed; among them are those that employ polymers and those that use surfactants. Extractions with polymers may be of two types: the aqueous biphasic system (ABS) [11] and extractions using thermoseparating polymer systems (TPS) [12,13]. In the case of ABS, two or more water-soluble polymers are added in the presence of a given salt concentration. When the concentrations of the polymers added are above their critical concentration, one or two phases are formed when working at room temperature. In TPS it is necessary to increase the temperature of the solution containing the polymer up to a suitable level in order to obtain two phases; this is what is known as the cloud point temperature. Both techniques have mainly been used in the separation of biomolecules [14–17].

Aqueous solutions of some surfactants are used in micellar extraction (ME) and cloud point extraction (CPE). In ME, the selective separations can be achieved owing to the fact that the micellar aggregates have a size that prevents them from crossing certain ultrafiltration membranes. This, together with the capacity of micelles to solubilise different compounds, has been used for the separation of nitrates from underground water using cellulose membranes and the surfactant cetyltrimethylammonium bromide (CTAB) [18].

The aqueous micellar solutions of some surfactants exhibit the cloud point, or turbidity, phenomenon when the solution is heated or cooled above or below a certain temperature. The temperature at which this phenomenon occurs is known as the cloud point temperature. This methodology is known as CPE or micelle-mediated extraction.

The aim of the present work is to explore the different analytical possibilities of this modality (CPE) in the extraction and preconcentration of organic compounds other than biomolecules (polycyclic aromatic hydrocarbons, polychlorinated compounds, pesticides, phenolic derivatives, aromatic amines, vitamins, etc.) that, owing to their high analytical interest, continue to be the objective of many investigations.

Different authors have offered reviews [19,20] concerning the fundamental characteristics of the CPE technique such as the basic features, experimental procedures and general applications. Here we report the most recent analytical applications of CPE when used as an isolation and trace enrichment step prior to the analysis of organic compounds by capillary electrophoresis (CE), or liquid and gas chromatography (HPLC and GC). In the final part of the work, we briefly discuss the future trends of the technique.

2. Cloud point extraction

2.1. Micellar systems

Surfactants are amphiphilic molecules, one of whose parts (the head) is polar or hydrophilic in nature and the other (the tail) hydrophobic. This latter part is generally a hydrocarbon chain with different numbers of carbon atoms and may be linear or branched. It may also contain aromatic rings.

In aqueous solution, and at low concentrations, surfactant molecules are found in monomer form, although dimers and trimers have also been detected.

When the surfactant concentration is increased above a certain threshold, called the ''critical micellar concentration'' (CMC), the surfactant molecules become dynamically associated to form molecular aggregates of colloidal size. These aggregates, which contain between 60 and 100 monomers, are called micelles and are at equilibrium with a surfactant concentration in the solution close to the CMC. Depending on the nature and concentration of the surfactant, as well as on the solvent used, another series of structures may be formed, organised as inverse micelles, microemulsions, vesicles, monolayers, bilayers [21–23].

From the analytical point of view, one of the most important properties of these organised structures is their good capacity to solubilise solutes of different character and nature. These solutes may interact electrostatically, hydrophobically or via a combination of both effects.

Quina et al. [24] found a relationship between the equilibrium constant, for the incorporation of a solute to the micellar phase, and different parameters such as hydrophobicity, the capacity to form hydrogen bridges, molar refraction, and dipolarity, among others. As well the structure of the solute, other factors are also involved in this, such as the structure of the surfactant, the presence of salts or organic additives, and temperature.

2.2. Cloud point phase separations

When a micellar solution of a non-ionic surfactant is heated above the cloud point temperature, two phases are formed. Above that temperature, the system – initially in an isotropic phase (L) – is separated into two isotropic phases (2L), one of them surfactant-rich and the other aqueous, containing a surfactant concentration close to the CMC at that temperature. The phenomenon is reversible and, upon cooling, a single phase is obtained again.

The mechanism by which separation occurs is poorly understood. Some authors have proposed that it would be due to an increase in the micellar aggregation number (an increase in micelle size) when temperature is increased [25,26]. Others have suggested that the phase separation mechanism would be caused by a change in micellar interactions, which are repulsive at low temperatures but predominantly attractive at high temperatures [27]. The fact that the presence of salts favours phase separation, when ionic surfactants are used, has been interpreted as being due to the shielding of the repulsive electrostatic effects.

Other authors have explained the cloud point phenomenon on the basis of the dehydration process that occurs in the external layer of the micelles of non-ionic surfactants when temperature is increased [28]. The dielectric constant of water decreases on increasing temperature, rendering it a poorer solvent for the hydrophilic portion of the surfactant molecule.

The cloud point temperatures of some non-ionic surfactants are shown in Table 1 [19]. The cloud point temperature depends on the structure of the surfactant and on its concentration [29–32] (Table 2).

The cloud point phenomenon is not exclusive to non-ionic surfactants. Some doubly ionic surfactants also undergo the cloud point phenomenon [19] (Table 3).

These temperatures can be modified by the presence of salts, alkalis, acids, polymers, urea [33], other surfactants [19]. It has been shown that for Triton X-100 the cloud point temperature is a function of the type of cation [34], as well as of the type of anion present in the aqueous solution [35].

From the studies conducted it can be deduced that the cloud point temperature of non-ionic and zwitterionic surfactants can be manipulated with the choice of a suitable additive.

Recently, it has been shown that at room temperature the anionic surfactants sodium dodecylsulfate (SDS), sodium dodecylbenzenesulfonic acid (SDBSA), sodium dodecanesulfonic acid (SDSA) and sodium dioctylsulfosuccinate (aerosol OT) form two phases in the presence of high concentrations of HCl [36]. To date, no analytical application of the cloud point phenomenon has been described for cationic surfactants.

The most important difference between the phase diagrams of a non-ionic and zwitterionic surfactant lies in the fact that in doubly ionic surfactants phase separation occurs when temperature decreases [37]. That is, above a given temperature these systems show only a single phase and separation into two

Table 1
Cloud point temperature of several non-ionic surfactants for a surfactant percentage of 1%

Surfactant	Cloud point (°C)
Type IGEPAL: R-$C_6H_8O(C_2H_4O)_{n-1}CH_2CH_2OH$	
CA-620 (R=C_8H_{17}; n=7)	22
CO-630 (R=C_9H_{19}; n=9)	54
CO-610 (R=C_9H_{19}; n=7.5)	26
Type AGM ACETAL: R-CH[$O(C_2H_4O)_mCH_3$]$_2$	
AGM-7(3) (R=C_7H_{14}; m=3)	34
AGM-11(3) (R=$C_{11}H_{23}$; m=3)	30
AGM-13(3) (R=$C_{13}H_{27}$; m=3)	29
Type alkylpolyoxyethylene glycol monoethers: CH_3-$(CH_2)_{n-1}O(C_2H_4O)_mH$, C_nE_m (E=oxyethylene)	
C_4E_1	44.5
C_6E_2	0
C_6E_6	83
C_8E_3	8
C_8E_4	40
C_8E_5	60
$C_{10}E_3$	0
$C_{10}E_5$	45
$C_{12}E_3$	0
$C_{12}E_4$ (Brij-30)	2
$C_{12}E_5$	31
$C_{12}E_{10}$	77
$C_{12}E_{23}$ (Brij-35)	>100
Others	
Polyoxyethylene-7.5-nonylphenylether (PONPE 7.5)	1
Polyoxyethylene-9.5-octylphenylether (Triton X-100)	64
Polyoxyethylene-7.5-octylphenylether (Triton X-114)	25
Sorbitol monooleate (Tween 80)	93

phases is caused by a cooling to below their cloud point temperature. Outstanding as exceptions to this general behaviour are dodecyl dimethylammonium dodecylmethylphosphine ($C_{12}N_{10}PPh$) and dodecylmethylphosphine oxide ($DC_{12}PO$), whose monophasic solutions at room temperature become turbid and separate into two phases when temperature is increased.

The first applications of phase separation based on the cloud point phenomenon refer to the extraction of metal ions forming complexes sparingly soluble in water. The efficiency of the process depends on the hydrophobicity of the ligand and of the complex formed, on the apparent equilibrium constants in the micellar medium, and on the formation kinetics of the complex and on the transference between the phases. This type of extraction by the cloud point method was initially described by Watanabe and co-workers [32,38] for the preconcentration of Zn(II) using 1-(2-pyridylazo)naphthol (PAN) as a ligand and PONPE 7.5 as extractant. Later, this methodology was also applied to the determination of different metal ions in different types of samples [39–42].

Another application of the CPE focuses on the isolation and purification of species of biological interest, mainly proteins. This methodology was used by Bordier [43] for the separation of hydrophilic proteins in biological membranes, using a solution of Triton X-114 as extractant. It is in this field of bioseparations that CPE currently finds one of its main areas of use, as shown by the considerable volume of literature related to the extraction and purification of membrane proteins and other biomaterials [44–47].

Table 2
Variation in the cloud point temperature with the percentage of surfactant

Surfactant	Concentration (%)	Cloud point (°C)
Triton X-100	0.25	64
	7.0	65
	33.0	76
Triton X-114	0.10	23.6
	5.0	25
	10.0	30
PONPE 7.5	0.12	1
	5.0	6
	20.0	25
C_6E_3 (E=oxyethylene)	3.0	46.9
	20	44.8
$C_{14}E_7$ (E=oxyethylene)	1	57.7
	5	58.6

2.3. Cloud point extraction of organic compounds

The use of CPE for the extraction of organic compounds other than biomolecules is relatively recent [19,37,48,49]. Fig. 1 shows a scheme of the steps that must be carried out in cloud point extraction. Usually, an aliquot of the surfactant-rich phase is introduced either directly into the separation system or after a suitable conditioning step. The addition phase may be a simple dilution aimed at decreasing the viscosity of the sample injected into the separation system or may involve a more complex treatment with the aim of removing the surfactant present in the surfactant-rich phase.

When CPE is used as a prior preconcentration step, before chromatographic separation, elution of the surfactant may interfere in the detection of the analytes of interest. This occurs when non-ionic surfactants are used, such as the polyoxyethylenated alkylphenol type (Triton X-100, Triton X-114 and

Table 3
Cloud point temperature of several doubly ionic surfactants

Surfactant	Cloud point (°C)
Ammonioethylsulfates: R_1-$(CH_3)_2$-N^+-$(CH_2)_2$-OSO_3^-, R_1AESO_4	
$C_{10}AESO_4$	77
$C_{12}AESO_4$	>120
Ammoniopropylsulfates: R_1-$(CH_3)_2$-N^+-$(CH_2)_3$-OSO_3^-, R_1APSO_4	
C_8APSO_4	32
C_9APSO_4	65
$C_{10}APSO_4$	88
$C_{12}APSO_4$	120
Ammoniopropanesulfonates: R_1-$(CH_3)_2$-N^+-$(CH_2)_3$-SO_3^-, R_1APS	
$C_{12}APS$	−0.5
$C_{14}APS$	12
$C_{16}APS$	20
Phosphobetaine	
$C_{12}PPS$, [(dodecyldimethylammonio)propyl]phosphinate	9
$C_{12}Pbu$, [(dodecyldimethylammonio)phosphonio]butyrate	<0
Dimethylalkylphosphine oxides: C_nH_{2n+1}-P-$(CH_3)_2O$, DC_nPO	
DC_8PO	41
$DC_{10}PO$	38.8; 130[a]

[a] The surfactant exhibits two cloud point phenomena: one at temperatures lower than 38.8°C and the other at temperatures higher than 130°C.

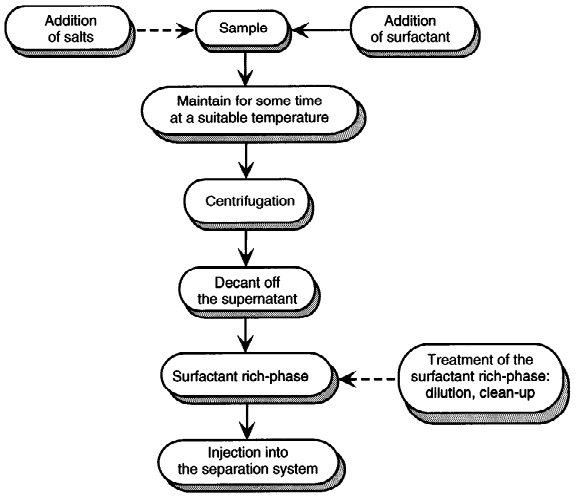

Fig. 1. Steps involved in cloud point extraction (CPE) prior to HPLC, GC and CE analysis.

PONPE 7.5), with spectrophotometric or fluorimetric detection. When non-aromatic polyoxyethylenated alcohols, anionic, or zwitterionic surfactants are used this problem is circumvented since these surfactants do not absorb above 210 nm [36,37].

Another alternative for eliminating the strong absorption of the surfactant consists of the use of electrochemical detectors [50]. This, however, has the disadvantage that many of the preconcentrated analytes may not necessarily be electroactive.

In CPE, as well as the optimisation of the detection step it is also necessary to carry out extraction under conditions in which the preconcentration factor will be maximum or the extraction yield will be 100%.

The preconcentration factor F_c is defined by the expression $F_c = C_s / C_{in}$, where C_s is the concentration of analyte in the surfactant-rich phase, after phase separation, and C_{in} is the concentration of the analyte in the initial solution, before the preconcentration step. Among others factors, this depends on the phase relationship, on the distribution constant of the analyte between the phases, and on the surfactant concentration used.

The phase ratio (V_w / V_s) is the ratio between the volume of aqueous solution to be preconcentrated and the volume of surfactant-rich phase. This ratio increases with the decrease in the concentration of surfactant [51]. However, since the volume of the surfactant-rich phase must be manageable a compromise must be reached so that the surfactant concentration will allow a high phase ratio and a manageable surfactant-rich phase; this would be around 100–250 μl.

In the case of most non-ionic surfactants the presence of salts may facilitate phase separation since it increases the density of the aqueous phase [52]. Nevertheless, it has been observed that the volume of the surfactant-rich phase does not depend on ionic strength [53]. When working with a given surfactant concentration, the volume of the surfactant-rich phase may decrease when the cloud point temperature and the phase equilibrium time are increased. At 70°C the surfactant-rich phase volume of C_8E_3 is about half of what it is at room temperature. Similar results have been reported for the surfactants PONPE 7.5 and Igepal CA-620. For Triton X-114, an increase in the cloud point temperature also leads to a slight decrease in the volumes of the surfactant-rich phase [53]. This can be interpreted in terms of the fact that as temperature is increased, the hydrogen bonds are disrupted and dehydration occurs. As temperature increases, the amount of water in a surfactant-rich phase decreases and hence the volume of that phase decreases.

The studies carried out indicate that in order to obtain a more favourable preconcentration factor CPE should be carried out at a temperature higher than the cloud point temperature and that these phases, at that temperature, should be maintained for a given time. It has also been reported that an increase in the time of centrifugation – for separating the phases – only slightly decreases the surfactant-rich phase volume [53].

Preconcentration will be favoured by the use of more hydrophobic surfactants since the distribution constant will be increased. Additionally, it has been observed that the efficiency of preconcentration for phenol and 4-chlorophenol with the surfactants C_8E_5, $C_{10}E_5$, $C_{12}E_5$ and $C_{14}E_5$ decreases with the increase in the alkyl chain length of the surfactant. This decrease can be explained in terms of the fact that the surfactant-rich phase volume increases with the increase in the alkyl chain length of the surfactant [53].

In general, it can be said that extraction efficiency

will be more efficient when more hydrophobic surfactants are used. Additionally, if a more favourable preconcentration factor is desired, a surfactant that will generate a small surfactant-rich phase should be used.

For extremely hydrophobic analytes, which show very favourable distribution constants between the micellar and aqueous phases, the maximum preconcentration factor that can be achieved – at least with this methodology – coincides numerically with the phase ratio. Fig. 2 shows the theoretical variation in F_c against the percentage of Triton X-114 for analytes with different distribution coefficients (K_D). It may be seen that when K_D tends towards infinity F_c coincides with the phase ratio and the factor decreases with the decrease in the distribution constant [55]. Moreover, in some cases apparent preconcentration factors have been found that are higher than the phase ratio. In particular, this has been observed when the signal of the analyte is sensitised by the presence of the surfactant.

When cloud point methodology is used to extract an analyte quantitatively from the aqueous phase, the factor to be optimised is the fraction extracted (E), defined as $E=Q_A/Q'_A$, where Q_A represents the number of moles of the analyte in the surfactant-rich phase and Q'_A corresponds to the number of initial moles. Fig. 3 shows the variations in F_c and E for two analytes – terbutryn and atrazine – as a function of the percentage of Triton X-114 [55]. It may be seen that in the case of terbutryn F_c is close to the phase ratio and in order to achieve a 100% recovery it is necessary to work with Triton X-114 percentages above 1%. Atrazine has a distribution constant with Triton X-114 that is less favourable than that of terbutryn and hence the F_c obtained for this herbicide is lower than the phase ratio. Furthermore, in order to achieve a recovery of 100% it is necessary to use surfactant percentages above 2%.

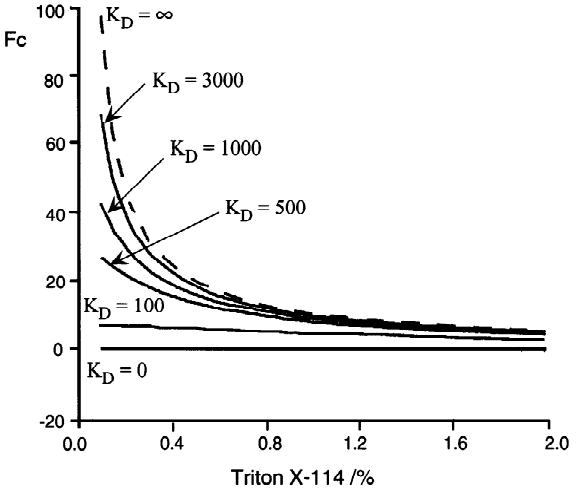

Fig. 2. Variation in the concentration factor (F_c) as a function of the percentage of Triton X-114 for different values of the distribution constant (K_D). This variation was determined for cases in which it is possible to carry out direct injection of the surfactant-rich phase. The dashed line represents the phase ratio.

3. Analytical applications

3.1. High-performance liquid chromatography

Most analytical applications of CPE methodology for the extraction of organic compounds (Table 4)

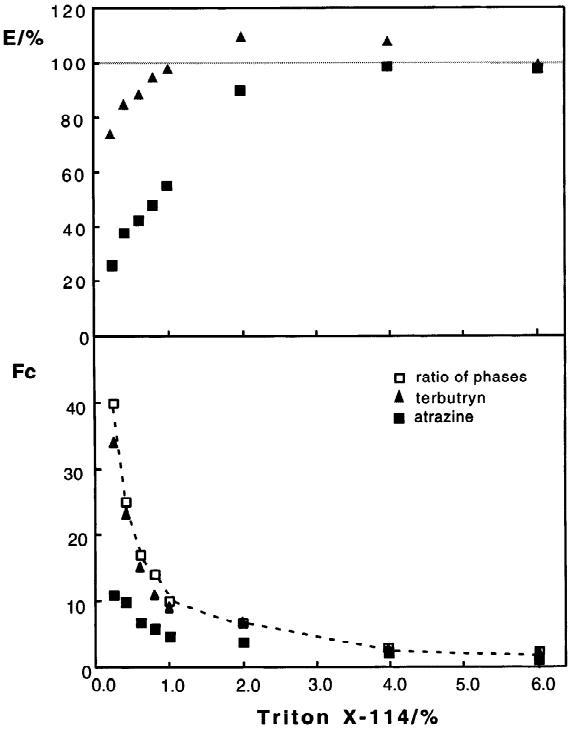

Fig. 3. Variation in the concentration factor (F_c) and extraction yield (E) for terbutryn and atrazine as a function of the percentage of Triton X-114. The dashed line represents the maximum concentration factor (phase ratio).

Table 4
Analytical applications of CPE prior to HPLC separation

Compounds	Matrix	Surfactant	LOD	Comments	Ref.
PAHs	Water	1.0% SDSA 4.2 *M* HCl		Recovery: 99.3% for pyrene at 0.1 μg ml^{-1}	[36]
	Coal	5% C_9APSO_4		No analytical data	[37]
	Dried sewage sludge	1.0% SDSA or SDS 4.2 *M* HCl		Analysis of a certified reference material	[36]
	Water	Genapol X80 2.0% NaCl	2.6–6.8 ng l^{-1}	Recoveries >90%	[48]
	Water	0.1% Triton X-114	0.002–0.12 ng ml^{-1}		[56]
	Wood ash	0.5% Triton X-114		Six PAHs found, at 3–30 ng ml^{-1} levels	[56]
	Smoke particulates	0.5% Triton X-114 without CPE		Seven PAHs found, at 9–120 μg ml^{-1} levels	[56]
	Water	1.0% Triton X-114	0.3–11.7 ng l^{-1}	Applied to river water	[58]
	Human serum	2.0% Triton X-100 4.5 *M* NaCl		Recoveries >70% at 120–180 ng ml^{-1} levels	[59]
	Water	0.1% SDSA 5 *M* HCl	0.1–7.9 ng l^{-1}	Applied to river and underground waters	[63]
Polychlorinated compounds					
PCBs	Water	2.0% Genapol X080 or Brij 56	0.7–3.6 ng ml^{-1}	Applied to sea water	[64]
PCBs	Water	2.0% Brij 30 or Brij 97	1.5–16.3 ng ml^{-1}	Applied to sea water	[65]
PCDFs	Water	2.0% Genapol X080 or Brij 56	0.5–27.5 ng ml^{-1}	Applied to sea water	[66]
PCDDs	Human serum	12% Triton X-100 4.5 *M* NaCl		Recoveries >91% at 3.9–4.4 μg ml^{-1} levels	[59]
Pesticides					
Napropamide	Water	Genapol X080	0.2 ng ml^{-1}		[67]
Organophosphorus	Water	0.25% Triton X-114	0.03–0.08 ng ml^{-1}	Applied to river water	[68,69]
Fungicides	Water	0.25% Triton X-114	4–6 ng ml^{-1}	Applied to river water	[70]
DDT	Soil	3% Igepal ICO-630 and Triton X-114 mixture		Recovery >83%	[71]
Vitamins					
E		PONPE 7.5 $C_{10}APSO_4$		No vitamin E decomposition with $C_{10}APSO_4$	[37]
E		PONPE 7.5 SDSA		No vitamin E decomposition with SDSA	[36]
A, E, D_3		1.0% Triton X-114	$3.5\cdot10^{-10}$–$1.2\cdot10^{-9}$ *M*	HPLC–ED	[50]
K_1, K_2		1.0% Triton X-114	$1.7\cdot10^{-9}$–$3.3\cdot10^{-8}$ *M*	HPLC–UV	[62]
A, E	Human serum Whole blood	Genapol X-80		Results are comparable to the reference method	[72]
Other organic compounds					
Chlorophenols		C_8E_3		Recoveries 88–99%	[53]
Chlorophenols	Water	0.5% Triton X-114	1.7–5.0 ng ml^{-1}	Applied to river water	[73]
Aromatic amines	Water Dyestuffs	0.2% Triton X-114	W: 0.1–1.4 ng ml^{-1} D: 0.5–0.9 μg g^{-1}		[74]
Hydroxyaromatic compounds	Water Dyestuffs	0.2% Triton X-114 0.25 *M* Na_2SO_4	W: 0.1–0.7 ng ml^{-1} D: 0.5–1.2 μg g^{-1}		[75]
Fulvic and humic acids	River water	4% Triton X-100		Recoveries: 82% fulvic acids; 96% humic acids	[54]

make use of reversed-phase high-performance liquid chromatography (HPLC); the surfactant-rich phase obtained in the extraction process is compatible with the hydro–organic phases usually employed in this chromatographic mode.

3.1.1. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) form a group of organic compounds that have considerable environmental impact and their determination has been addressed in many studies. Owing to their low concentrations, the analysis of PAHs in environmental samples requires a preconcentration step prior to chromatographic separation. The procedures most used involve liquid–liquid and solid-phase extraction. In the latter, one of the main problems encountered is the low recovery achieved owing to the strong interactions of the analytes with the stationary phases. Other problems related to the analysis of PAHs in water samples that most often lead to low recoveries of these compounds are the adsorption of the PAHs onto the containers used in the sampling step or during sample storage, and their interaction with the dissolved organic matter (i.e., humic acids) present in the water.

The cloud point technique has been successfully exploited for the preconcentration of PAHs as a previous step to their determination by HPLC. The use of non-ionic surfactants has been proposed by several authors [48,56–61]. One of the greatest limitations to this methodology is the high absorbance shown by many surfactants in the UV region; in most cases this prevents their use in a step prior to chromatographic separation when spectrophotometric detection is to be used unless the mobile phase employed contains a high methanol content, in which case elution of the surfactant occurs in a short time and does not hinder detection of the analytes [50,51]. One possibly way of overcoming this pitfall is to use surfactants that do not absorb at the working wavelengths normally used in chromatography [37]. Another way to circumvent this disadvantage is to use fluorescent detection [48,56,58].

Ferrer et al. [58] proposed separation of the surfactant from PAHs prior to their injection into the chromatographic system by a clean-up step using silica-gel; the PAHs were eluted with a mixture of cyclohexane–dichloromethane (80:20, v/v) while, under these conditions, the non-ionic surfactant Triton X-114 was retained on the top of the silica-gel layer.

In the determination of PAHs with Triton X-114 and fluorimetric detection García-Pinto et al. [56] reported that the interference of the surfactant can be eliminated by dilution of the surfactant-rich phase with a suitable amount of organic solvent prior to its injection into the chromatographic system.

The different types of behaviour found by Ferrer et al. [58] and García-Pinto et al. [56] seem to be related to the different fractioning that the surfactant may undergo as a function of the injection medium, the type of chromatographic column employed, and the mobile phase used. It has been reported that a surfactant-rich phase of Triton X-114 does not elicit a fluorimetric signal when injected into a Spheri-5-ODS column. However, when a Vydac 210 TP5415 column is used the surfactant gives rise to a series of signals that interfere in the detection of some PAHs. Fig. 4 shows the chromatograms obtained upon injecting 10 μl of a surfactant-rich sample of Triton X-114 into a Vydac 201TP5415 column and on injecting a surfactant-rich phase diluted with 100 μl of acetonitrile. It may be seen that the addition of acetonitrile decreases the signals of the surfactant to

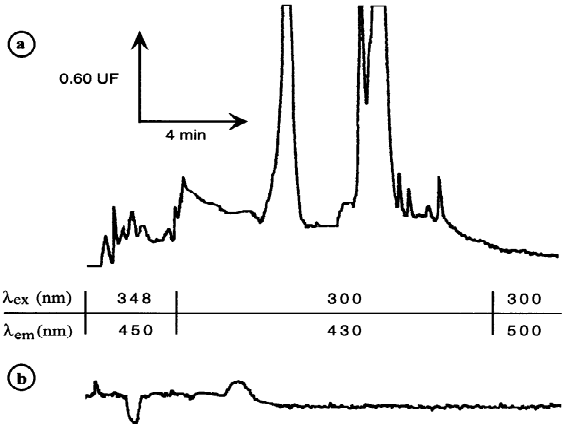

Fig. 4. Chromatograms of a phase rich in the surfactant Triton X-114 at 1% obtained with a Vydac 201TP5415 column. (a) Injection of the surfactant-rich phase; (b) injection of the surfactant-rich phase diluted with 100 μl acetonitrile. Mobile phase: acetonitrile–water (75:25, v/v); flow-rate 2.0 ml min^{-1}. The figure also shows the wavelength program used in the detection: λ_{ex} and λ_{em}.

a considerable extent. The effect produced by the addition of acetonitrile cannot be attributed to a dilution phenomenon since the acetonitrile added only produces a dilution of the surfactant-rich phase of between two- and three-fold [62].

Anionic surfactants such as SDS, SDB-SA, SDSA and aerosol OT have been proposed by Sicilia et al. [36,63] for PAHs preconcentration. These surfactants do not originate any spectrophotometric or fluorimetric signal at the retention times of the analytes, although it should be noted that the times required for phase separation to be reached are relatively long.

An additional advantage of the surfactant-based procedures for the extraction and preconcentration of these analytes from water samples is that the use of CPE, using both non-ionic and anionic surfactants, avoids the adsorption of PAHs onto the sample containers when the collection is performed in the presence of surfactants [36,56,57,63]. Moreover, the recovery of PAHs adsorbed onto the container for samples collected without preservatives is also possible when anionic surfactants are employed [63]. Likewise, it has been demonstrated [36,56] that the presence of high contents of dissolved organic matter has no detrimental effect on the recoveries of PAHs from water samples.

The determination of PAHs in more complex samples than aqueous matrices has also been described. Sirimanne et al. [59] have reported the extraction of PAHs from human serum using CPE with Triton X-100 and analysis by HPLC–UV detection. The surfactant-rich phase contains proteins that interfere in HPLC separation since it generates a precipitate with the mobile phase. Accordingly, the surfactant-rich phase is treated with acetonitrile in order to precipitate the proteins and the filtrate is then injected into the chromatograph.

The extraction and preconcentration of PAHs from solid samples has also been described, using doubly ionic surfactants [37], non-ionic [56] and anionic [36] surfactants. Using the surfactant 3-[nonyldimethylammonio]propyl sulfate it has been observed that the surfactant extracts anthracene from samples of coal [37]. The extraction of fluoranthene, pyrene benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and benzo[*ghi*]perylene from samples of smoke particulates and of wood ash has been proposed and successfully exploited using Triton X-114 as the extractant [56]. In samples of smoke particulates the extract obtained after 5 h of extraction with 0.5% Triton can be injected directly into the chromatograph. In samples of wood ash, in which the concentration of PAHs is lower, it is necessary to perform a CPE preconcentration step.

The recovery values obtained in the analysis of a certified reference material (CRM 088, which consists of PAHs in dried sewage sludge) with the surfactants SDS and SDSA show that the CPE procedure is a valid methodology for the extraction of PAHs [36].

3.1.2. Polychlorinated compounds

Polychlorinated biphenyls (PCBs) are organic compounds that exhibit very good thermostability and hence their cloud point preconcentration can be performed using surfactants with cloud point temperatures higher than 75°C.

Eiguren-Fernández et al. used several non-ionic surfactants in the preconcentration of PCBs [64,65] and dibenzofurans (PCDFs) [66] from samples of sea water prior to liquid chromatographic analysis with fluorescence detection. The detection limits obtained for the different compounds are relatively high, ranging from 0.7 to 3.6 ng ml^{-1} for PCBs, and from 0.5 to 27.5 ng ml^{-1} for PCDFs.

The preconcentration of polychlorinated dibenzo-*p*-dioxins (PCDDs) in samples of human serum using Triton X-100 at 50°C has been proposed and successfully implemented [59]. Co-extractants such as proteins were removed from the surfactant-rich phase by precipitation with 0.5 ml of acetonitrile.

3.1.3. Pesticides

The determination of pesticides in water and soils has also been investigated using CPE. The use of Genapol X-080 and Genapol 150 combined with liquid chromatography and fluorimetric detection has been applied to the detection of the herbicide napropamide [67] in natural waters.

Organophosphorus pesticides [68,69], such as methyl and ethyl parathion, paraoxon and fenitrothion have been determined in river water samples by using CPE with the non-ionic surfactant Triton X-114 prior to their separation by liquid chromatog-

raphy; electrochemical detection permits suitable detection and quantification of these pesticides. Upon preconcentrating 15.0 ml of water with a 1% concentration of Triton X-114, the detection limit is 0.5 ng ml^{-1}. This sensitivity can be increased by preconcentrating 200 ml with 0.25% Triton X-114; under these conditions, the detection limits range between 0.03 for fenitrothion and 0.08 ng ml^{-1} in the case of paraoxon. The method could also be used in the determination of these analytes in drinking water, in which the maximum concentration permitted by the European Union is 0.1 ppb/individual substance.

Another procedure based on CPE with the non-ionic surfactant Triton X-114 has been developed for the fungicides Folpet, Captan and Captafol [70] in water samples. Chromatographic elution of these fungicides requires a mobile phase with a relatively low organic solvent content (45%, v/v, acetonitrile–water). Under these conditions, not all the Triton X-114 is eluted from the chromatographic column: to remove the surfactant remaining in the stationary phase, a washing cycle with 100% acetonitrile over 10 min was performed. In addition to the extraction and preconcentration of the fungicides, the presence of Triton X-114 stabilises the fungicides and prevents their hydrolysis in aqueous medium. The addition of surfactant at the time of sample collection is a simple way to avoid losses of fungicide during the period of sample storage. Also in this case, electrochemical detection permits the simultaneous quantification of all three fungicides since spectrophotometric detection only allows the quantification of the fungicide Folpet.

Evdokimov and von Wandruszka [71] proposed a mixture of two surfactants – Igepal ICO-630 and Triton X-114 – for studying the possible elimination of DDT from polluted soils; the recovery percentage obtained proved to be greater than 83% when the polluted soil in question was treated for 2 h with a 3% mixture of the surfactants.

3.1.4. Vitamins

Vitamin E is a thermally labile compound and hence its preconcentration using surfactants with high cloud point temperatures may lead to its decomposition. A comparative study [37] of the extraction of this vitamin with the surfactant PONPE 7.5 and the zwitterionic surfactant $C_{10}APSO_4$ revealed that no vitamin E decomposition was observed if zwitterionic extraction was carried out at 12.5°C. Casero et al. [36] reported that the extraction of vitamin E with PONPE 7.5 decomposes the vitamin whereas this does not happen if the anionic surfactant SDSA is used.

The surfactant Triton X-114 has been used for the preconcentration of vitamins E and A using a cloud point temperature of 25°C; no decomposition of vitamin E was observed. For the chromatographic elution of these vitamins, it is necessary to use a mobile phase with a high percentage of methanol (methanol–water, 99:1, v/v); under these conditions, both UV and electrochemical detection are suitable [50]. Vitamins K_1 and K_2 can be preconcentrated in a similar way [62].

A procedure similar to that described for the determination of PAHs and PCCDs [59] in serum samples has been reported for the determination of vitamins A and E. The efficiency of extraction with Genapol X-080 for vitamins A and E in blood and serum samples has been shown to be comparable to that obtained using an official method, which validates this methodology for the quantification of vitamins A and E in this type of sample [72].

3.1.5. Other organic compounds

The extraction efficiency of relatively apolar organic compounds may reach 100% even when very low surfactant concentrations are used. For more polar species the surfactant concentrations to be used is a critical variable since, on one the hand, it determines the extraction yield and, on the other, it governs the volume of the surfactant-rich phase obtained, which affects the magnitude of the preconcentration factor.

CPE has been evaluated for the extraction of a series of chlorinated phenols [53] from water. The use of the non-ionic surfactant poly(oxyethyleneglycol) monooctyl ether (C_8E_3) led to good extraction efficiency, with recoveries of 88–99%, although no analytical characteristics were reported. The optimised procedure was also applied to some other substituted phenols and anilines.

The preconcentration of the five US Environmental Protection Agency (EPA) priority pollutant chlorophenols [73] has been reported using the surfactant

Triton X-114. The phase ratio, which is a function of the surfactant concentration, together with the octanol–water distribution constant (K_{ow}) of the analytes can be used to indicate the possibilities of the CPE technique for the preconcentration of relatively polar compounds. In this study [73], for the most hydrophobic analyte, pentachlorophenol (log K_{ow}= 5.24), the extraction yield was close to 100% even when low surfactant concentrations were used: 0.05% Triton X-114. In the case of 2-chlorophenol (log K_{ow}=2.15), surfactant concentrations higher than 1% are required to obtain complete extraction.

The preconcentration of hydroxyaromatic compounds and aromatic amines in water and dyestuffs with Triton X-114 has been proposed [74,75]. For their determination in water samples it is possible to perform CPE followed by HPLC with UV detection without the surfactant interfering in the chromatographic signals of the analytes, since these appear at shorter retention times than the surfactant. For determination in dyestuffs, it is necessary to conduct a prior clean-up step with a strong anion-exchange (SAX) cartridge packed with an anion-exchange resin and then carry out preconcentration with CPE.

Recently the extraction of fulvic and humic acids [54] with Triton X-100 has been described. In order to obtain a quantitative extraction of these compounds it is essential to optimise pH, temperature, and the cloud point time, as well as the presence of salts. For extraction of the fulvic acids, it is necessary to perform CPE with a surfactant concentration of 4%, with the subsequent decrease in the preconcentration factor due to the increase in the volume of the surfactant-rich phase.

3.2. Gas chromatography

The use of surfactant-based procedures as sample preparation techniques for organic compounds prior to GC is a not very well developed area and there are few references to this methodology in the literature.

Froschl et al. [76] reported a method for the determination of PCBs in water based on the combination of micellar extraction and GC–electron-capture detection (ECD).

The studies carried out to date have focused on the preconcentration of PCBs with Triton X-100. The surfactant-rich phase must be treated suitably with a view to eliminating the surfactant before its introduction into the gas chromatograph. To do so the surfactant-rich phase is passed through a column of silica gel and is eluted with *n*-hexane, after which a small volume is collected. The rest of the Triton X-100 is removed by a second column with Fluorisil. After this clean-up, the PCBs are separated and quantified by GC–ECD. The recoveries of PCBs using the CPE method have been compared with those obtained using liquid–liquid extraction in which a clean-up is also carried out with two silica gel columns. Both extraction processes give comparable results when analysing samples of ultrapure water spiked with the PCBs. On analysing water polluted with other analytes, such as that from municipal landfills and hazardous waste landfills, the recovery values are more favourable when CPE with Triton X-100 is used. Additionally, it is seen that in the liquid–liquid extraction of highly polluted water samples phase separation is extremely difficult, which is not the case when CPE is employed.

3.3. Capillary electrophoresis

The use of surfactants in CE is a normal practice either as buffer additives – to change the selectivity of the separations – or using micelle-forming surfactant solutions in the micellar electrokinetic capillary chromatography (MECC) mode. Despite this, the use of surfactant-based procedures as a sample preconcentration step prior to CE analysis has not been extensive.

Cloud point extraction with Triton X-114 has been applied as a preconcentration step for triazine herbicides prior to analysis by CE [77]. The behaviour of a surfactant-rich micellar phase injected into a capillary electrophoresis system was studied using different separation modes [MECC and capillary zone electrophoresis (CZE)]. One problem that appeared on introducing a surfactant-rich phase into a bare fused-silica capillary, using aqueous buffers, was that the surfactant was adsorbed onto the wall of the capillary, leading to a marked loss of efficiency and reproducibility. The use of dynamic coatings in the capillary, such as those obtained when the cationic surfactant CTAB is added to the separation buffer, afforded reproducible results, although the half-life of the capillary was short. The most satis-

factory results were obtained when the surfactant-rich samples were suitably diluted and injected, in the electrokinetic mode, into a non-aqueous separation medium of acetonitrile–methanol (50:50). Repeated injections of surfactant-rich samples afforded reproducible electropherograms, showing that the use non-aqueous media in the separation buffer permits the electrophoretic separation of samples with high-surfactant contents, avoiding adsorption of the surfactant onto the walls of the capillary.

Capillary electrochromatography (CEC) is a hybrid separation mode between CE and HPLC [78]. As in the case of HPLC, it has been shown that surfactant-rich phases are compatible with the mobile phases used in CEC. In this sense, it has been observed that the injection of a surfactant-rich phase of Triton X-114 containing PAHs gives rise to reproducible electropherograms when injected into a packed capillary of C_{18} [79].

The use of CPE coupled with CEC was also explored by Sirimanne et al. [80] for the analysis of environmental toxic compounds (PAHs, PCDDs and phthalates) in spiked human serum using Genapol X-080. The only post-extraction treatment required in that procedure was the addition of acetonitrile to separate unwanted co-extractants and prevent capillary clogging. The analytes within these three groups were well separated but the migration times appeared to be shifted, presumably because of the dynamic coating of the C_{18} stationary phase by the residual surfactant or the presence of co-extractants in the sample.

4. Conclusions and future trends

The use of micellar systems for the separation and preconcentration of organic compounds is a useful alternative with the following characteristics: (a) a high capacity to preconcentrate analytes of different polarities; (b) the preconcentration factor can be optimised by modifying the type and concentration of surfactant as well as the experimental conditions under which extraction and phase separation are carried out; (c) surfactants are less toxic and cheaper than the extractants used in liquid–liquid extraction; (d) the most commonly used surfactants are commercially available and other, more specific, surfactants and combinations of both make this kind of methodology very versatile; (e) since it is not necessary to evaporate off the solvents, no analyte is lost due to this process; (f) the experimental operations involved in the methodology are very simple and (g) the surfactant-rich phases are compatible with the mobile phases used in HPLC and CEC.

These characteristics have been reported by several authors, although in the future it will be necessary to study in greater depth the applications that this methodology may find in the extraction of organic compounds from solid samples. As has been shown, the extraction of oils from polluted soils may offer an attractive alternative in remediation processes [81]. Accordingly, it will be necessary to compare the extraction yields by CPE with those obtained using official methods.

The analysis of samples preconcentrated by CPE has focused on their later separation by HPLC. New studies must be conducted to expand the application of this to CE in organic media and to electrochromatography. Special attention should be devoted to applying this methodology to GC with mass detection. To do so, it will be necessary to develop rapid and simple procedures to eliminate the surfactant or to propose new surfactants or soluble polymers that are compatible with separation by GC. Another potential field of investigation will be the automation and on-line coupling of this methodology with the operation system.

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