The Micellar Systems: an alternative to the organic solvents to the extraction and preconcentration of organic pollutants in environmental samples

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ABSTRACT

The establishment of simple, fast, low cost, sensitive and selective analytical methods to determine the presence of pollutants in the environment, is one of the main reseach lines in the environmental chemistry field. Because of the complexity of these natural samples, the analytical methodologies to be applied require several separation/extraction steps prior to the analysis, which normally becomes long and tedious.

Normally, these previous steps are carried out by using organic solvents as extractants, either alone (liquid-liquid extraction in liquid samples) or assisted by microwaves (solid samples). In recent studies, it has been demonstrated that micellar systems (surfactant solutions) constitute a real alternative to the organic solvents in order to be used as extractants of organic pollutants in liquid and solid environmental samples .

These micellar media may be used to the extraction and preconcentration of different analytes in liquid environmental samples, using the so called Cloud Point Extraction (CPE) methodology. In this methodology small volumes of the surfactant-rich phase allows the preconcentration and extraction of the analytes in one step.

In other hand, the combination of the use of surfactants as extractants and the Microwave Assisted Extraction has led to a new and efficient methodology to the extraction of different compounds in solid sampes: Microwave Assisted Micellar Extraction (MAME). In this work we present a study of the application of CPE and MAME methodologies to the extraction/preconcentration and determination of organic pollutants like Polychlorinated Biphenyls, Polychlorinated Dibenzofurans. Polychlorinated Dibenzo-p-Dioxins and Phenolic derivatives in natural, waste and sea water samples as well as in marine sediments and marine organisms samples. The obtained results are compared with those found using conventional extraction techniques like liquid-liquid extraction and soxhlet extraction. These studies show the advantages of these optimised methodologies respect to the traditional techniques.

INTRODUCTION

In recent decades the development of extraction and preconcentration steps to be implemented prior to analytical determinations of trace level compounds has been explored in considerable depth.

Commonly used methods for extraction and preconcentration in water samples, are liquid-liquid extraction (LLE) and solid-phase extraction (SPE)[1-3]. Solvent extraction methods have the disadvantage of poor recoveries, which vary from compound to compound. and there is therefore a trend to replace solvent extraction procedure in order to minimised sample manipulation. analyte looses and the use of toxic solvents [4]. Nevertheless, there is an increasing tendency to replace LLE by solid-phase extraction (SPE). SPE have certain disadvantages for water analysis: the cross-sectional area is small, sample processing rates are slow, the tolerance to blockage by particles and adsorbed matrix components is low and

channelling reduces the capacity to retain analytes. Another drawback in SPE, which is the same for LLE, is the considerable amount of time needed and manual operations involved [5].

Other methodologies have been developed in with a view to eliminating or, at least, minimising the use of organic solvents. The use of extraction and preconcentration steps based on phase separation by the cloud point methodology offers a convenient alternative to more conventional extraction systems. Aqueous solutions of some surfactants have been used in cloud point extraction (CPE) of different species prior to their determination by several techniques[6-9]. 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 types and nature.

The small volume of the surfactant-rich phase allows us to pre-concentrate and extract the analytes in one step, prior to gas or liquid chromatographic analysis. Moreover, this methodology has the advantages of safety, low cost and no toxicological effects due to their biodegradability.

The extraction of organic pollutants from soil samples requires the use of organic solvent, which compete in the release of the analytes retained owing to the high activity of the matrix. Traditional methods employ large volumes of solvents under aggressive shaking and/or temperature conditions. The most frequently used method for the extraction of organic compound from soils is Soxhlet extraction or the use of an ultrasonic bath. Soxhlet extraction, the most conventional off all methods, is particularly suitable for organic pollutants strongly adsorbed in soil matrices but requires long extraction times and the use of large volumes of frequently toxic organic solvents.[10-11].

In the last few years, the number of procedures using extraction of organic compounds from environmental matrices by microwave energy has increased[12-14]. Microwave assisted extraction (MAE) shows several advantages such as reduced extraction time and solvent consumption[15-16]. This methodology has been applied to the extraction of different organic compounds, such as organochlorine insecticides[17], polynuclear aromatic hydrocarbons,[12,18], arochlors[16], etc. with good results. In all these studies the extractant has always been an organic solvent.

A new possibility for the application of MAE is the use of micellar media as extractants. The micellar media can be applied to the solubilisation, extraction and preconcentration of several compounds present in different environments such as water samples[7-19], soils[20-21] air[22], etc., always with the benefits of low cost, easy handling and reduced toxic effects.

The aim of this work is to present the analytical possibilities of the micellar systems in the extraction and preconcentration of differents organic compounds: polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), polychlorinated dioxins (PCDDs) and phenolic derivatives in differents matrices, using cloud point methodology and microwave assisted micellar extraction.

The organochlorinated compounds above mentioned are considered hazardous pollutants, due to their widespread, persistence in the environment, and adverse effects in human health[23-25]. The analysis of these compounds is complicated due their extremely low levels of concentration in the natural samples. Therefore several clean-up steps are required prior to the analysis of these compounds[26-27].

Phenolic compounds are also widely spread in the environment and have high toxicity[28]. Efforts have been devoted to quantitating phenolic compounds from natural samples[29-30], usually determined by liquid chromatography with different detection systems such as UV and diode array detector[31-32], electrochemical[33] or fluorescence[34].

RESULTS AND DISCUSSION

Cloud Point Extraction (CPE)

It is well known that surfactants, or surface active agents, 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 number of carbon atoms and may be linear or branched, and also contain aromatic rings.

One of the most important properties of these organised structures is their good capacity to solubilise solutes of different character and nature, how to allow that sparingly-soluble or non-water-soluble materials, can be solubilized in water due to their binding to the micelles in solution. These solutes may interact electrostatically, hydrophobically or by a combination of both effects. This capacity of micelles to solubilize different compound has been used for the development in the extraction and preconcentration of organic compounds that owing to their high analytical interest continue to be the objetive of many investigations.

When a micellar solution of a non-ionic surfactant is heated, become turbid over a narrow temperature range, which is referred to as its cloud point[35-36]. Above the cloud point temperature, such solutions separate into two isotropic phases. Then the system will contain a surfactant-rich phase with a small amount of water, surfactant phase, which is separated from the bulk aqueous solution, and an aqueous phase, in which the surfactant concentration will be approximately equal to the critical micelle concentration of the non-ionic surfactant present.

The cloud point temperature depends on the structure of the surfactant and on its concentration. It has been determined the cloud point temperature of two non-ionic surfactants, Genapol X-080 and Brij 56 to be 75-80°C and 85-90°C respectively[37]. This temperature can be modified by the presence of salts, alkalis, acids, polymers, urea and other surfactants[36]. It has been shown that for Genapol X-080 the cloud point temperature obtained when NaCl is added in a 5% w/v concentration, is less than the temperature obtained when working without this salt[38].

Therefore, the solubilization of organic material in micelles and subsequent cloud point extraction technique offers a convenient alternative to conventional liquid-liquid extraction that uses organic solvents. The use of CPE for the extraction and preconcentation of organic compounds prior to their analysis by gas or liquid chromatography is relatively recent[6, 19, 36]. In CPE, it is 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 is defined by the expression $F_c = Cs/C_{in}$, where C_s is the concentration of analyte in the surfactant-rich phase after phase separation, and C_{in} is the concentration of analyte in the initial solution, before the preconcentration step. This depends on the phase relationship, on the distribution constant of the analyte between the phase and on the surfactant concentration used.

Moreover the ratio between the volume of aqueous solution to be preconcentrated and the volume of surfactant-rich phase (V_w/V_s), increases with the decrease in the concentration of surfactant. This shows that the smaller is the concentration of surfactant, the higher is the preconcentration factor; but when the volume of surfactant-rich phase is small, the extraction process become more difficult, and the accuracy and reproducibility probably suffer[37, 39].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.

The extraction process can be altered by different factors such as equilibration time, pH, concentration of surfactant and addition of salt[40]. Therefore the effect of these factors on the percentage extraction of studied analytes needs to be established.

The recovery percentage depends on the time that the analytes have to interact with the micelles and get into their core. It has been reported that longer equilibration time (more than 30 min) do not have any significant effect on the extraction parameters and that an equilibration time of 20 min is enough to obtain a good extraction[41]. In our studies, it has been applied equilibration times lower than 20 min for extract all the analytes, obtaining lower equilibration times as more hydrophobic is the compound[37,38].

The ionic form of a neutral molecule normally does not interact with the micellar aggregate as strongly as its neutral form does, and a lesser amount of the analyte is therefore extracted[40]. Influence of the pH on the recovery percentages is not important for those compounds that do not present ionic forms like are the polychlorinated aromatic compounds, PCBs, PCDFs and PCDDs. However, in the case of the phenolic derivatives, when working with pH modified by addition 1% acetic acid , the percentage extraction is better for most solutes and is the most noticeable change for more polar compounds[38].

The addition of an inert salt can facilitate the phase separation process for some non-ionic surfactant systems since it increases the density of the bulk aqueous phase[6, 36]. When the salt concentration is increased, the micelle size and the aggregation number are increased and the critical micellar concentration remains constant [7]. In addition, non-polar analytes may become less soluble in the solution at higher salt concentrations and thus contribute to higher recoveries. The results obtained indicate that the addition of salt produces an increase in the extraction of the more polar solutes while the recoveries of the less polar compounds is not affected.[37,38, 42,43].

Most analytical applications of CPE methodology for the extraction of organic compounds 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 phase usually employed in this chromatographic mode. From this, another important step is the optimisation of the chromatographic conditions. There are two important factors to be taken into account in liquid chromatography separations: the time of analysis, and a good separation of analytes. In Figure 1 we can observe the chromatogram obtained for the mixture of phenolic derivates under the optimised chromatographic conditions.

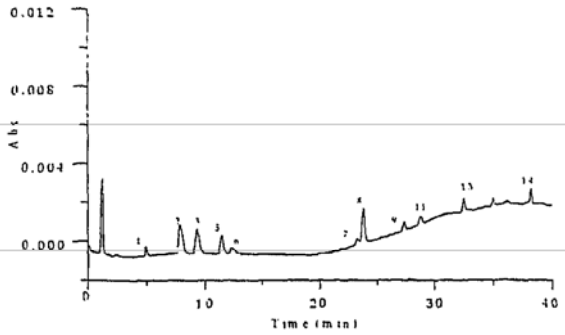

Figure1.Chromatogram of a standard solution of phenolic compounds in presence of Genapol X-080. Chromatographic conditions: 70% water (with 1% acetic acid) and 30% methanol till 16 min. and gradient up to 100% methanol in 24 min. 1._ Phenol. 2._ 4-Nitrophenol. 3._ 2,4-Dinitrophenol. 5._ 2-Nitrophenol. 6._ 2-Chlorophenol. 7._ 2,4-Dimethylphenol. 8._ 4,6-Dinitro-ortho-cresol. 9._ 4-Chloro-meta-cresol. 11._ 2,4-Dichlorophenol. 13._ 2,4,6-Trichlorophenol. 14._ Pentachlorophenol.

The extraction and preconcentration of PCBs and PCDFs using several non-ionic surfactants was applied to samples of sea water prior to liquid chromatographic analysis with fluorescence detection[37, 42, 43]. The results shown in Tables 1 and 2 indicate satisfactory data.

Table1.- Determination of PCBs in sea water samples.

Arinaga	Brij 30	Brij 97	Agaete	Brij 30	Brij 97
PCBs	recov. %	recov. %	PCBs	recov. %	recov. %
Biphenyl (B)	86.0	92.0	Biphenyl (B)	90.0	102.0
Monochloro-B	101.0	94.0	Monochloro-B	91.0	90.0
Dichloro-B	93.0	83.0	Dichloro-B	92.0	106.0
Trichloro-B	99.0	84.0	Trichloro-B	83.0	100.0
Tetrachloro-B	83.0	93.0	Tetrachloro-B	106.0	80.0
Pentachloro-B	95.0	95.0	Pentachloro-B	96.0	89.0
Hexachloro-B	87.0	87.0	Hexachloro-B	80.0	95.0

Table2.- Determination of PCDFs in sea water samples.

Arinaga	Genapol X-080	Brij 97	Agaete	Genapol X-080	Brij 97
PCDF	recov. %	recov. %	PCDF	recov. %	recov. %
Dibenzofuran (DF)	99	92.0	Dibenzofuran (DF)	86.0	92.0
Monochloro-DF	90	94.0	Monochloro-DF	101.0	94.0
Dichloro-DF	88	83.0	Dichloro-DF	93.0	83.0
Trichloro-DF	94	84.0	Trichloro-DF	99.0	84.0
Tetrachloro-DF	93	93.0	Tetrachloro-DF	83.0	93.0
Pentachloro-DF	96	95.0	Pentachloro-DF	95.0	95.0

The detection limits obtained for the different compounds are in the 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 non-ionic surfactant Polyoxyethylene 10 lauryl ether (POLE) was used for the extraction and preconcentration of PCDDs in various aqueous samples and following determination by liquid chromatographic with UV detection[44]. Recovery percentages between 70-105% were obtained for the majority of compounds (Table 3). Moreover, these results show the applicability of the methods to aqueous samples with different levels of salinity.

Table 3. Determination of PCDDs in different aqueous samples.

	Fresh water		Brackish water		Sea water	
Analyte	Purified	Drinking	Low salinity	High salinity	Aldea	Veneguera
Dibenzo-p-dioxin (DD)[a]	90,1	79,2	94.4	90.2	93.1	90.4
1Chloro-DD[a]	77.2	72.4	77.6	71.3	81.4	77.4
2Chloro-DD	101,3	99,4	103,5	98.6	100,1	99,5
3Chloro-DD	97,9	100,4	94,0	101,5	80.8	106,9
4Chloro-DD	78,9	100,1	98,5	105,0	102,6	101,7
5Chloro-DD	89,3	101,1	86,9	103,1	91,9	92,8
6Chloro-DD	56,2	66,3	73,4	75,2	68,1	66,5

7Chloro-DD	64,3	72,4	76,5	81,4	92,8	98,7
8Chloro-DD	94,1	98,6	95,4	101,4	90,2	93,3

[a] Values obtained using a preconcentration of 5 times.

The determination of phenols in water samples has also been investigated using CPE. The use of Genapol X-080X combined with liquid chromatography was applied to the analysis of those compounds in two kinds of water samples, sea water and depurated waste water[38]. The results shown in Table 4 indicate very satisfactory recoveries for all phenolic compounds present in the sample.

Table 4.- Recoveries of phenolic compounds in sea water and depurated waste water.

Compound	Sea water	Depurated waste water	Compound	Sea water	Depurated waste water
Phenol	69 ± 5	58 ± 5	4,6-Dinitro-orto-cresol	94 ± 4	82 ± 4
4-Nitrophenol	85 ± 3	70 ± 3	4-Chloro-meta-cresol	99 ± 4	92 ± 4
2,4-Dinitrophenol	71 ± 5	66 ± 5	2,4,6-Trimethylphenol	74 ± 8	67 ± 8
para-Cresol	61 ± 10	64 ± 10	2,4-Dichlorophenol	97 ± 7	101 ± 7
2-Nitrophenol	73 ± 7	65 ± 7	4-Chloro-3,5-Dimethylphenol	106 ± 13	104 ± 13
2-Chlorophenol	74 ± 7	70 ± 7	2,4,6-Trichlorophenol	107 ± 6	102 ± 6
2,4-Dimethylphenol	87 ± 11	74 ± 11	Pentachlorophenol	110 ± 5	104 ± 5

Microwave Assisted Extraction in the presence of micellar media

In the last few years the use of microwave technology for sample preparation has been widely accepted. The reasons for the large diffusion of microwave-assisted extraction (MAE) relate to its clear advantages over more traditional technology: a shorter digestion time, a supposed better recovery of volatile elements and compounds, lower contamination levels, minimal volumes of reagent are required, more reproducible procedures and a better working environment.

Ganzler and coworkers[45-46] were the first researchers ever to use a microwave oven to extract organic compounds from a contaminated soil. Since then microwave digestion methods have been developed for different types of matrices to extract organic contaminants, such as PAHs, PCBs, pesticides, phenols, etc. using an organic solvent as extractant.

It has been reported that the combination of MAE with micellar media as extractants allows the extraction of different organic compounds from solid samples. There are different parameters that can influence on the extraction process when MAE is used. The parameters most commonly studied are: solvent composition and volume, extraction temperature or extraction power and radiation time.

A correct choice of solvent is fundamental for obtaining an optimal extraction process. When selecting solvent, consideration should be given to the interaction of the solvent with the matrix, and the analyte solubility in the solvent. Another important aspect is the compatibility of the extraction solvent with the analytical method used for the final analysis step. The non-ionic surfactant solutions should be effective in desorbing and extracting organic compounds from solid matrices due to their power of solubilisation. Next, the

solution is filtered to remove the solid sample. Additionally, the surfactant phase is compatible with the hydro-organic mobile phases usually employed in HPLC.

The amount of solvent needed for a single sample is often in the range of 10-30 ml . In some cases, solvent volume may be an important parameter for efficient extraction. The solvent volume must be sufficient to ensure that the entire sample is immersed, especially when having a matrix that will swell during the extraction process. When use the surfactants solutions as extractant, only small volumes of surfactant are required to obtain good results[47-49].

The most investigated parameter in MAE is the extraction temperature or extraction power, because an increase in the power involves a corresponding increase in heat. The temperature is an important factor contributing to increased recoveries, not only for MAE, but for all extraction techniques. When MAE is conducted in closed vessels, the temperature may reach well above the boiling point of the solvent. These elevated temperature result in improved extraction efficiencies, since desertion of analytes from actives sites in the matrix will increase. However, the effects of the temperature depend on the type of analyte as well as the type of soil. Generally, the optimal temperature depended on the polarity of the analyte, but temperatures between 80-100°C gave acceptable recoveries[50]. In other cases, the extraction temperature influenced the extraction efficiencies to a very small extent as demonstrated for several organic pollutants from standard reference soil and sediments[15].

As in other extraction techniques, time is another parameter whose influence needs to be taken in account. Extraction times in MAE are very short compared to conventional techniques where extraction procedure require as much as 24 h. Often 30 min are sufficient to obtained good results in MAE. This can mainly be attributed to the difference in heating performance by the microwave technique and the conventional heating. In conventional heating a finite period of time is needed to heat the vessel before the heat is transferred to the solution. while microwave heat the solution directly. In the extraction of PCBs in marine sediments using micellar media, it was observed that the best recovery percentages were obtained for low extraction power with a long conditioning period (45 min)[51], while no improvement in the extraction efficiency was observed applying longer irradiation time. For the extraction of PCBs and PCDFs in marine organisms, with the use of non-ionic surfactant, polyoxyethylene 10 lauryl ether as extractant, at a constant microwave power, the recovery percentages decrease with the increasing in extraction time. Similar behaviour was observed for all analytes[52].

The nature of the matrix in which the analytes of interest are bound can have a strong effect on the recoveries of the compounds. MAE procedure using micellar media was applied to the determination of PCBs in two sediment samples[51] of different characteristics. sand and clay. spiked with the analytes of interest in fresh samples and aged samples (samples conditioned for ten weeks). Table 5 shows the result obtained in both types of samples. In all cases. higher recoveries were obtained from the fresh samples. while the effects of aging showed a clear decrease for many of contaminants. Decreasing recoveries resulting from aging of matrices is a well-known phenomenon, and can be explained by a more strongly bound to the matrix, due to longer contact time.

Table5.- Determination of PCBs in marine sediments: freshly spiked and aged samples.

	Canteras 24 h		Canteras 10 weeks		Taliarte 24h		Taliarte 10 weeks	
	POLE	Genapol X-080	POLE	Genapol X-080	POLE	Genapol X-080	POLE	Genapol X-080
PCBs	recov.%	recov.%	recov.%	recov.%	recov.%	recov.%	recov. %	recov.%
BP	43.0	81.0	35.0	34.7	76.0	92.0	44.3	53.0
PCB-4	55.0	92.0	48.8	35.0	86.0	95.0	52.1	47.0
PCB-15	92.0	91.0	63.6	44.4	103.0	92.0	61.2	58.3
PCB-37	90.0	90.0	75.8	62.0	92.0	95.0	86.0	61.4
PCB-77	101.0	90.0	85.8	77.2	92.0	94.0	99.6	63.0
PCB-126	97.0	95.0	93.6	76.4	101.0	98.0	98.0	69.1
PCB-169	98.0	95.0	98.5	82.3	99.0	100.0	99.1	75.2

Other examples of the influence of nature of matrix is the determination of PCBs and PCDFs using micellar medium in different matrices of bivalve molluscs (mussels, cockles and clams) previously spiked with different concentration of these compounds[52]. The results obtained showed differences between the recovery percentages found for the various samples studied. The best results were obtained for the PCDFs in blue mussel samples with recovery percentages higher than 80%. For cockles, percentages of 70-80% were obtained in most cases and finally, values between 50-60% were obtained for clams. The same trend was observed for the PCBs. Recovery percentages of between 65-80% were obtained for blue mussels, 60-80% for cockles and 47-75% for the clams.

The differences observed between the different species could be essentially due to the fact that they have different compositions and physiological characteristics. The analytes under study are compounds of a lipophilic nature, so the greatest interaction will occur with fatty tissue. In fact, it would appear that the strongest interaction is produced with triacylglycerol, as indicated by some authors[53], and it is known that mussels contain a higher concentration of lipids[54] than cockles[55] and clams[56]. However, there are numerous studies which show that this composition also has a seasonal variation related fundamentally to the reproductive cycles and feed rates[57, 58]. This variation is common in all the bivalve molluscs studied, even though these reproductive cycles are out of phase[59]. Thus, the lipid content will be different in the various organisms depending on the period of the year that the samples were taken.

In view of the results obtained, it can be accepted that the existing different composition between mussels, cockles and clams has favored a stronger interaction of PCBs and PCDFs in the latter two species. This would in turn help explain the lower recovery percentages, especially in the case of clams.

CONCLUSIONS

The results obtained with the proposed methods indicate that the CPE methodology is a good alternative extraction technique and offers a series of highly interesting advantages from an analytical point of view, such as the possibility of extract and preconcentrate the analytes of different polarities in only one step; 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; surfactants are less toxic and cheaper than the extractants used in liquid-liquid extraction. The most commonly used surfactants are commercially available and since it is not necessary to evaporate off the

solvents, no analyte is lost due to this process; the experimental operations involved in this methodology are very simple and the surfactant-rich phase are compatible with the mobile phases used in HPLC.

In another hand we may conclude that MAE procedure in presence of micellar media offers several advantages over traditional extraction techniques applied to solid samples. The major benefits are: a reduction in extraction time (typically 10-30 min) and in solvent consumption (around 25-50 ml, reduced by 5-10 fold compared to traditional extractions) as well as the opportunity to perform multiple extractions. The technique is easy to use and the systems are cheaper compared to other modern techniques. Furthermore, the use of micellar medium reduced exposure to solvent vapors and lower solvent wastes.

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