Application of cloud-point methodology to the determination of polychlorinated dibenzofurans in sea water by high-performance liquid chromatography

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Polychlorinated dibenzofurans (PCDF) are organic compounds with very toxic effects for humans and the environment. As they are present in low concentrations, an extraction technique is necessary prior to their determination by high-performance liquid chromatography (HPLC). In this work, the methodology of cloud-point extraction, using two non-ionic surfactants oligoethylene glycol monoalkyl ether (Genapol X-080) and polyoxyethylene-10-cetyl ether (Brij 56), is applied to the extraction and preconcentration of PCDF in sea water samples prior to their determination by HPLC with fluorescence detection.

Introduction

Interest in determining trace levels of contaminants in environmental samples has increased with the recognition that even trace levels of pollutants can pose risks for humans.

The polychlorinated dibenzofurans (PCDF) are considered one of the most hazardous pollutants of the environment.[1] It is known that isomers of PCDF having substituents at positions 2,3,7 and 8 are among the strongest synthetic poisons possessing carcinogenic and mutagenic effects.[2–4]

Various combustion processes represent major sources of these compounds in the environment. The PCDF formed are emitted to the atmosphere, and due to their hydrophobic and lipophilic properties they are dispersed onto land or into water and will adsorb strongly to organic material, both in the particulate and dissolved phases.

The analysis of PCDF is complicated due their extremely low levels of concentration in the samples. Tedious, costly and time consuming methods for extraction with an organic solvent or clean-up steps are required prior to the analysis of these compounds by gas or liquid chromatography.[5–7]

Micelles and other organized amphiphilic assembles are increasingly utilized in analytical methods.[8] Their unique microheterogeneous structures capable of selective interaction with different solute molecules, can strongly modify solubility, chemical equilibria, kinetics and the spectroscopic properties of analytes and reagents.

Separation procedures based on the peculiar properties of aqueous non-ionic and zwitterionic surfactant solutions have been also proposed as an alternative to the use of traditional organic solvents. Efficient preconcentrations of organic solutes have been obtained using the cloud-point phase separation phenomena.[9–13] The analytical potential of the cloud-point phenomenon mediated phase separations, cloud-point extraction (CPE), has been discussed by several authors.[12–17] The small volume of the surfactant-rich phase allows us to preconcentrate and extract the analytes in one step, prior to gas or liquid chromatographic analysis. Moreover, the surfactant-rich phase is compatible with the micellar and aqueous–organic mobile phase in liquid chromatography, which facilitates the application of this analytical method, with the obvious benefits.

In this work, we report the results of a study of the experimental parameters which affect the extraction efficiency and preconcentration factor of the CPE process of a series of PCDF previous to their determination by high-performance liquid chromatography (HPLC), using two non-ionic surfactants, oligoethylene glycol monoalkyl ether (Genapol X-080) and polyoxyethylene-10-cetyl ether (Brij 56). This CPE methodology has been applied to the analysis of a mixture of PCDF in sea water samples.

Experimental

Reagents

Stock solutions of 1.0×10^{-4} M of PCDFs (AccuStandard, Inc., New Haven, CT, USA) were prepared by dissolving appropriate amounts of each PCDF in ethanol (Merck, Darmstadt, Germany).

Stock solutions of the surfactants, oligoethylene glycol monoalkyl ether (Genapol X-080) (Sigma, St. Louis, MO, USA), and polyoxyethylene-10-cetyl ether (Brij 56, Sigma) were prepared in deionized water.

HPLC-grade methanol and KNO_3 were obtained from Panreac Quimica, S.A. (Barcelona, Spain).

All solvents and analytes were filtered through a 0.45 μm nylon membrane filter, and ultra-high-quality water, obtained using a water purification system, was used throughout.

Apparatus

The HPLC system consisted of a Waters pump (model 510) fitted with a Rheodyne injector valve (model 7725i) with a 20 μl sample loop and a Waters 474 scanning fluorescence detector. The system and the data management are controlled by Millennium software from Waters (Waters Cromatografia S.A., Barcelona, Spain)

The stationary-phase column was a Waters Nova-Pack C_{18}, 3.9×150 mm id, 4 μm particle diameter.

The maxima λ_{ex} and λ_{em} of each PCDF in the presence of surfactant were determined using a Perkin-Elmer Hispania, S.A. (Madrid, Spain) luminescence spectrophotometer, model LS-50. Excitation and emission slits of 5 nm were used.

A thermostatted bath (model Tectron 200) and a centrifuge (model Mixtasel), from Selecta (Barcelona, Spain), were also used.

Procedure

Preconcentration. The preconcentration step was carried out keeping aliquots of 10 ml, which contain the analytes and surfactant (2% w/v), in a thermostatically controlled bath for 15 min, at a temperature of 95 °C for both surfactants. After this time, the aliquots were centrifuged for 5 min (3500 rpm) to achieve the separation of the two phases. Previous to the centrifugation, the tube holder was heated for 15 min at a temperature close to 95 °C in an electric system.

Liquid chromatography analysis with fluorescence detection. The study of the chromatographic conditions established a methanol–water (85 + 15) mobile phase and 1 ml min^{-1} flow rate as the most adequate conditions in order to obtain an efficient separation of the six PCDF.

As the extracted surfactant-rich phase is compatible with the mobile phase, 20 μl of the extracted volume was injected directly into the liquid chromatograph and the detection of the analytes was carried out by monitoring the relative intensity of fluorescence at the maxima λ_{em} and λ_{ex} of each PCDF under study. Two different ranges were studied: 3.4–27.2 μg ml^{-1} and 168–3405 ng ml^{-1}.

The fluorescent conditions (λ_{em} and λ_{ex}) were previously determined using solutions containing a known concentration of each PCDF and a surfactant concentration above the critical micellar concentration (Table 1).

Determination of PCDF in sea water samples. Prior to the analysis, sea water was passed successively through filters of different porosity (0.45 and 0.22 μm) and ultraviolet radiation to avoid the posible interference of marine microorganisms. Solutions of 10 ml containing 5 ml sea water and 2% (w/v) surfactant concentration were spiked with suitable amounts of PCDF and analysed according to the established CPE method.

Results and discussion

The cloud-point temperature of these surfactants, necessary for the extraction step, was studied in a previous work.[12] In that study, the consolution curve of each surfactant was obtained when the temperature at which the turbidity appeared was represented against the concentration of surfactant in solution. The phase diagrams indicate a cloud-point temperature of 75–80 °C for Genapol X-080 and 85–90 °C for Brij 56. At the same time, the ratio between the concentration and volume of the added surfactant and the surfactant-rich phase volume obtained after the cloud-point process was studied for both surfactants.The results showed an increase in the extracted volume of surfactant-rich phase with the surfactant concentration in solution, but the volume of added surfactant had no influence on the volume of surfactant-rich phase.[12] A 2% (w/v) surfactant concentration and a volume of 4 ml were chosen for the study carried out in this work, obtaining in these conditions a preconcentration factor of 10.

Table 1 Fluorescent characteristics of PCDF in 2% (w/v) aqueous solutions of Genapol X-080 and Brij 56

			Genapol		Brij 56	
No.	PCDF	Abbreviation	λ_{ex}/nm	λ_{em}/nm	λ_{ex}/nm	λ_{em}/nm
1	Dibenzofuran	DBF	278	316	278	316
2	4-Chlorodibenzofuran	MonoCDF	265	333	280	321
3	2,8-Dichlorodibenzofuran	DiCDF	255	334	291	334
4	2,4,8-Trichlorodibenzofuran	TriCDF	256	335	291	337
5	2,3,7,8-Tetrachlorodibenzofuran	TetraCDF	258	335	302	337
6	1,2,3,4,8-Pentachlorodibenzofuran	PentaCDF	258	335	294	344

Optimization of the preconcentration factor

There are different factors that can alter the extraction process and it is very important to optimize them in order to obtain good recovery factors. Some of these variables are concentration and volume of added surfactant, pH, ionic strength, pressure, *etc.*[18,19] In the case of PCDF, some of the factors have no influence on the recovery percentages, thus pH is not important because these compounds do not present ionic forms; therefore, only the equilibration time, initial concentration of analyte in solution, ionic strength and surfactant concentration are the parameters which have been optimized.

Effect of the equilibration time. The recovery percentage, and because of this the efficiency of the cloud-point extraction process, depends on the time that the analytes have to interact with micelles and get into their core. For this reason, to optimise the extraction process, it is necessary to study the behavior of each surfactant when the equilibration time varies.

If we represent recovery percentages against equilibration time, two different behaviors can be observed: the behavior of the analytes with an even number of chlorine atoms and those with an odd number of chlorine atoms. This different behavior would be due to the structure of the molecules (different position of chlorine atoms) which generate different interactions between the analyte and the surfactant micelles, and therefore the solubilization effect would also be different.

For Genapol X-080, we observed that MonoPCDF presents a similar behavior to the PCDF with a even number of chlorine atoms. The recovery percentages of these analytes decrease with time, to 13 min, increasing to 15 min (maximum recovery percentage) and going down again for longer times. In the case of PCDF with odd numbers of chlorine atoms, recovery percentages increase with time to 13 min, decreasing for 15 min and increasing for longer periods (Fig. 1a).

When we used Brij 56, the recovery percentages decreased sharply for all the PCDF with time up to 13 min, but with longer periods two behaviors could be observed: for PCDF with even

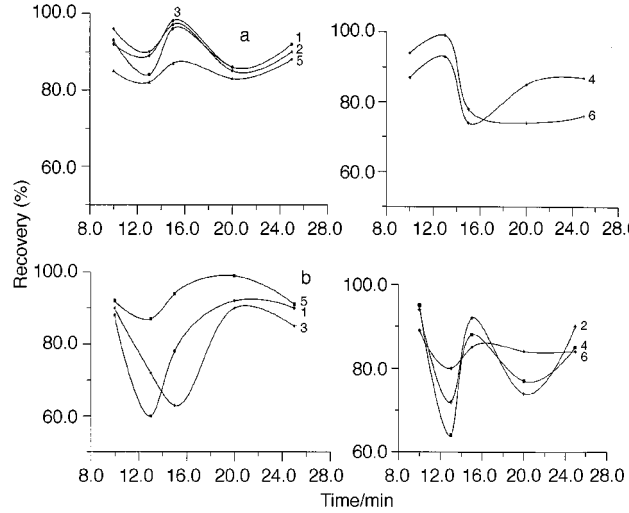

Fig. 1 Influence of equilibration time on recovery percentages of PCDF using: (a) Genapol X-080, (b) Brij 56; (1) DBF; (2) MonoCDF; (3) DiCDF; (4) TriCDF; (5) TetraCDF; (6) PentaCDF.

numbers of chlorine atoms, the recovery percentage, in all the range of concentration, increased up to 20 min, decreasing gradually over longer periods; the rest of the PCDF presented low recovery percentages for 20 min, increasing up to 25 min (Fig. 1b).

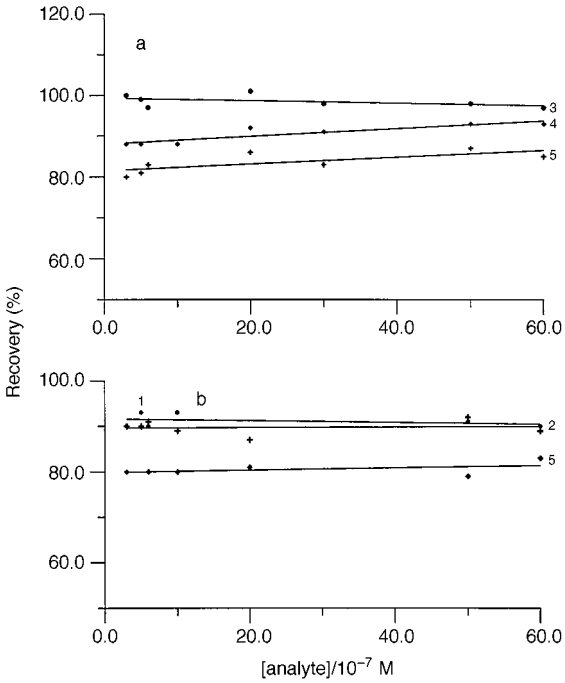

Fig. 2 Effect of the initial analyte concentration in solution on the recovery percentages: (a) Genapol X-080, (b) Brij 56; (1) DBF; (2) MonoCDF; (3) DiCDF; (4) TriCDF; (5) TetraCDF.

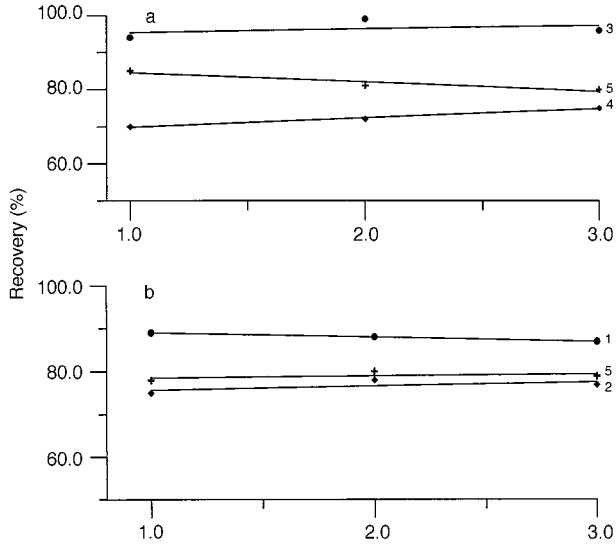

Fig. 3 Influence of added surfactant percentage (w/v) on the recovery percentages: (a) Genapol X-080, (b) Brij 56; (1) DBF; (2) MonoCDF; (3) DiCDF; (4) TriCDF; (5) TetraCDF.

An equilibration time of 15 min was chosen as adequate for all the analytes in solutions.

Effect of the ionic strength. The presence of salt in the solution can be important for the extraction process and in the extracted volume of surfactant-rich phase. The study of the influence of this parameter was carried out by adding different percentages of KNO_3 (1–10% w/v) to the solution. The results indicated that the addition of salts does not affect the extracted volume of surfactant-rich phase and as the recovery percentages obtained are similar we can conclude that the ionic strength does not have notable influence on the extraction process. But the addition of the inert salt increases the density of the bulk aqueous phase and facilitates the separation process of the two phases. These results are in accord with those obtained by other authors.[20]

Effect of the analyte concentration. The initial concentration of analyte is another factor that can affect the recovery percentages. To determine this effect, solutions containing different concentrations of PCDF were subjected to the CPE procedure. As the analyte concentration varied between 50.4–2043 ng ml^{-1} the recovery percentages remained practically constant for all the analytes studied in both surfactants, so we can conclude that the initial concentration of PCDF in solution had no influence on the recovery percentages when we used Genapol X-080 and Brij 56 as extractants (Fig. 2).

Effect of the added surfactant percentage. When the influence of the added surfactant percentage (w/v) was studied, the results showed that this parameter had no influence on the recovery percentages for the analytes under study (Fig. 3).

Cloud-point preconcentration and liquid chromatographic analysis

Another important step in this study is the optimization of the chromatographic conditions. Once the conditions of CPE were optimized to obtain good recovery percentages, it was necessary to optimize the conditions for the separation and determination of PCDF using HPLC with fluorescence detection.

When chromatography is used as a separation technique, it is necessary to obtain a good relationship between analysis time

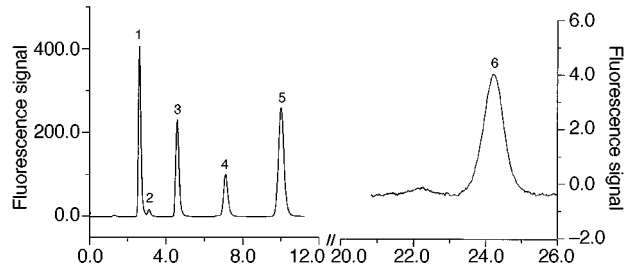

Fig. 4 Elution of a mixture of seven PCDF using 2% (w/v) Genapol X-080 as preconcentrant: (1) DBF; (2) MonoCDF; (3) DiCDF; (4) TriCDF; (5) TetraCDF; (6) PentaCDF. Concentration of each PCDF, 500 ng ml^{-1}; eluent, methanol–water (85 + 15); flow rate, 1 ml min^{-1}.

Table 2 Analytical parameters for the determination of PCDF with CPE

Calibration range	Parameter	DBF	MonoCDF	DiCDF	TriCDF	TetraCDF	PentaCDF
168–3405 ng ml^{-1}	a ($\times10^{-6}$)[a]	1.2	0.1	1.1	0.6	2.5	0.1
	b ($\times10^{-5}$)[b]	4.0	0.1	1.0	0.4	1.1	0.2
3.4–27.2 μg ml^{-1}	a ($\times10^{-4}$)[a]	12.2	0.6	10.3	58.0	23.6	0.7
	b ($\times10^{-4}$)[b]	14.8	1.5	13.9	36.6	1.1	0.7

[a] a = Slope. [b] = Intercept.

and analyte separation. An efficient separation of the six PCDF in study, in a relatively short period, was obtained when we used a methanol–water (85 + 15) mobile phase and a flow rate of 1 ml min^{-1} (Fig. 4).

The study of the analytical characteristics of the determination of PCDF using Genapol X-080 and Brij-56 showed a similar behavior for both surfactants. The calibration curves were obtained by duplicate injection of the sample containing 2% (w/v) of surfactant and the corresponding PCDF concentration. Two different ranges were studied: 3.4–27.2 μg ml^{-1} and 168–3405 ng ml^{-1}. A linear relationship between fluorescence signal and concentration of analyte was observed and high correlation coefficents were obtained for the different calibration curves (0.999–0.998). Table 2 lists the calibration characteristics of the method.

Once the methodology under study was optimized, the recovery percentages for each PCDF were determined. Different mixtures of PCDF were prepared in 10 ml of 2% (w/v) aqueous solutions of Genapol X-080 and Brij 56. After the CPE step, 20 μl of the extracted surfactant-rich phase were injected directly into the chromatographic system. The data obtained, listed in Table 3, show excellent recovery percentages for the different analytes studied in both surfactants.

The limits of detection[21] calculated for each PCDF in Genapol X-080 and Brij 56, using CPE methodology, are listed in Table 4, together with the relative standard deviation for six samples to which the complete procedure (cloud-point preconcentration and extraction and chromatographic separation) was applied for all the compounds studied in 2% (w/v) Genapol X-080 and Brij 56 solutions. The results obtained for both parameters were similar when Genapol X-080 and Brij 56 were used.

Table 3 Recovery percentages obtained for each PCDF after CPE and chromatographic determination*

	168–3405 ng ml^{-1}		3.45–27.2 μg ml^{-1}	
PCDF	Genapol Recovery %	Brij 56 Recovery %	Genapol Recovery %	Brij 56 Recovery %
DBF	100	96	96	92
MonoCDF	98	94	97	92
DiCDF	99	93	98	90
TriCDF	88	95	93	95
TetraCDF	98	92	88	99
PentaCDF	97	68	99	89

* Referred to the initial concentration of PCDF in solution.

Analytical applications

The proposed method was applied to the determination of PCBF in sea water samples from different Spanish areas (Arinaga and Agaete, Canary Islands and Elantxobe, Vizcaya). Sea water samples were previously spiked with suitable amounts of each PCBF. The results indicate satisfactory recovery percentages. (Table 5).

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Table 4 Relative standard deviation (in range 168–3405 ng ml^{-1}) and limits of detection for PCDF

PCDF	DBF	MonoCDF	DiCDF	TriCDF	TetraCDF	PentaCDF
RSD (n = 6)	0.009	0.204	0.016	0.131	0.043	0.087
LOD/ng ml^{-1}	0.5	17.6	13.3	4.9	0.7	27.5

Table 5 Recovery percentages for PCDF in sea water samples

		Genapol						Brij 56					
Origin of sample	PCDF	Added*/ ng ml^{-1}	Found*/ ng ml^{-1}	Recovery (%)	Added*/ ng ml^{-1}	Found*/ ng ml^{-1}	Recovery (%)	Added*/ ng ml^{-1}	Found*/ ng ml^{-1}	Recovery (%)	Added*/ ng ml^{-1}	Found*/ ng ml^{-1}	Recovery (%)
Agaete	DBF	84.0	83.2	99	840	789.6	94	84.0	77.3	92	840	789.6	94
	MonoCDF	101.2	91.1	90	1012	931.1	92	101.2	95.1	94	1012	991.7	98
	DiCDF	118.5	104.3	88	1185	1054.6	89	118.5	107.8	91	1185	1102.0	93
	TriCDF	135.7	127.6	94	1357	1302.7	96	135.7	119.4	88	1357	1262.0	93
	TetraCDF	153.0	142.3	93	1530	1468.8	96	153.0	137.7	90	1530	1514.7	99
	PentaCDF	170.2	163.4	96	1702	1615.0	95	170.2	115.7	68	1702	1565.8	92
Aringa	DBF	84.0	84.8	101	840	764.4	91	84.0	79.8	95	840	756.0	90
	MonoCDF	101.2	105.2	104	1012	921.0	91	101.2	93.1	92	1012	961.4	95
	DiCDF	118.5	117.3	99	1185	1149.4	97	118.5	107.8	91	1185	1208.7	102
	TriCDF	135.7	133.0	98	1357	1262.0	93	135.7	123.5	91	1357	1411.3	104
	TetraCDF	153.0	148.4	97	1530	1591.2	104	153.0	146.9	96	1530	1407.6	92
	PentaCDF	170.2	161.7	95	1702	148.9	91	170.2	127.6	75	1702	1582.9	93
Elentrobe	DBF	84.0	87.3	104	840	806.4	96	84.0	83.2	99	840	756.0	90
	MonoCDF	101.2	89.0	88	1012	971.5	96	101.2	99.2	98	1012	961.4	95
	DiCDF	118.5	111.4	94	1185	1161.3	98	118.5	106.6	90	1185	1196.8	101
	TriCDF	135.7	124.8	92	1357	1357.0	100	135.7	119.4	88	1357	1411.3	104
	TetraCDF	153.0	145.3	95	1530	1606.5	105	153.0	139.2	91	1530	1560.6	102
	PentaCDF	170.2	161.7	95	1702	1719.0	101	170.2	119.1	70	1702	1688.0	98

* Referred to the initial concentration of PCDF in solution.

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