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FLUORESCENT STUDY OF ORGANIC COMPOUNDS OF ENVIRONMENTAL INTEREST IN ORGANIZED MOLECULAR SYSTEMS. ANALYTICAL APPLICATIONS

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Introduction

At present, the environmental conservation is one of the most important subjects of social and scientific interest. In this respect, the determination and control of pollutants in the environment constitutes one of the principal aspects. Because of this, in the last years important effort has been expended to optimize new and more sensitive and selective analytical methods for the determination of contaminants in environmental samples. Three important groups of these pollutants are polychlorinated biphenyls (PCBs), arochlors and polychlorinated dibenzofurans (PCDBs).

Polychlorinated biphenyls and arochlors (commercial mixtures of PCBs) are families of compounds produced commercially by the direct chlorination of biphenyl. These compounds have found application in a wide variety of industrial uses due to their chemical and thermal stability. Since their discovery in environmental samples in 1966 (1), it has been generally accepted that they are ubiquitous in every component of the global ecosystem (2-5).

It has been shown that the toxicity of PCBs varies with the degree of chlorination and the substitution pattern. The structure-activity relationships of the PCBs have shown that the most biologically active congeners have chlorine substitution of the biphenyl rings in both *para* positions, in two or more of the four *meta* positions, and in one or none of the four *ortho* positions (6).

Polychlorinated dibenzofurans are chlorinated tricyclic aromatic compounds and are emitted into the environment as unwanted by-products of anthropogenic processes. PCDBs have been globally distributed and are found in all environmental media. They are chemically stable, have low solubilities in water, and have been shown to accumulate in the foodchain. It is well known that the PCDBs with chlorines substituted in the 2,3,7,8 positions are thought to pose a risk to human health due to their toxicity, carcinogenic potential and potential effects on animal reproductive and inmunological systems (7-9)

Because of these environmental problems, the identification and quantification of these compounds is a matter of great concern for many analysts. In this work, we present new analytical methods for the determination of these organochlorinated compounds based on their fluorescent characteristics in the presence of different organized molecular systems. A systematic study of fluorescent behavior of PCBs, arochlors and PCDBs in the presence of cationic, anionic and non-ionic surfactants has been carried out. Variables, such as surfactant concentration, organic solvent concentration and temperature, were optimized and analytical parameters, such as linear dynamic range, reproducibility and detection limit, were established. The proposed methods have been applied to the determination of these pollutants in real enviromental samples.

In addition to the above mentioned studies and using the fluorescent characteristics of PCBs and PCDBs in the presence of different surfactants, we report also on the determination of these compounds by high performace liquid chromatography with fluorescent detection combined with "cloud point" methodology.

Results and Discussion

1. Fluorescence determination of organochlorinated compounds in the presence of micellar media

The use of organized media (aqueous and reverse micelles, bilayers, microemulsions, vesicles and liposomes) has been common practice in all fields of analytical chemistry for the modification on the reactivity between analytes and reagents and to improve the analytical procedures. Micellar systems can produce an increase in fluorescence intensity of organic compounds compared to that obtained in aqueous medium under identical instrumental conditions. This has led to the use of different micellar media to improve the sensitivity and selectivity of many fluorescent determinations (10-14).

We have carried out a systematic study of the following PCBs, arochlors and PCDBs: biphenyl, 4-chlorobiphenyl (4-PCB), 4,4′-dicholorobiphenyl (4,4′-PCB), 3,4,4′-tricholorobiphenyl (3,4,4′-PCB), 2,2′,5,5′-tetracholobiphenyl (2,2′,5,5′-PCB), 3,3′,4,4′-tetrachlorobiphenyl (3,3′,4,4′-PCB), 2,2′,4,5,5′-pentachlorobiphenyl (2,2′,4,5,5′-PCB), 3,3′,4,4′,5-pentachlorobiphenyl (3,3′,4,4′,5-PCB), 2,3,3′,4,4′,5-hexachlorobiphenyl (2,3,3′,4,4′,5-PCB) 2,2′,4,4′,5,5′-hexachlorobiphenyl (2,2′,4,4′,5,5′-PCB), 3,3′,4,4′,5,5′-hexachlorobihenyl (3,3′,4,4′,5,5′-PCB), 2,2′,3,3′,4,4′,5,5′-octachlorobihenyl (2,2′,3,3′,4,4′,5,5′-PCB); Arochlor 1016, Arochlor 1221, Arochlor 1232, Arochlor 1242, Arochlor 1248, Arochlor 1254, Arochlor 1268, dibenzofuran, 4-chlorodibenzofuran (4-PCDB), 2,8-dichlorodibenzofuran (2,8-PCDB), 2,4,6-trichlorodibenzofuran (2,4,6-PCDB), 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-PCDB), 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PCDB) and 2,3,4,5,7,8-PCDB), in the presence of hexadecyltrimethylammonium bromide (HDTAB), benzyldimethylhexadecylammonium chloride (BDMHDAC), cetylpyridinium bromide (CPB), sodium dodecyl-

benzenosulfonate (NaDDBS), sodium lauryl sulfate (NaLS), polyoxyethylene(10)lauryl ether (POLE), polyoxyethylene(10)oleyl ether (Brij-96) and isooctylphenoxypolyethoxyethanol (Tx-100) as surfactants.

1.1. Fluorescence characteristics

Generally, the excitation wavelengths of polychlorinated compounds present similar values in organized and aqueous (2 % ethanol) media; however, in the emision wavelengths a different behavior is observed. Thus, while for arochlors no shift is observed in emision maxima in micellar and aqueous media, for PCBs and PCDBs a shift to shorter wavelengths is observed in organized medium respect to aqueous medium (15, 16, 17).

In aqueous medium the polychlorinated compounds present a decrease of the relative intensity of fluorescence with the number of chlorine atoms on the molecule of dibenzofuran. The enhancement of fluorescence, with respect to aqueous medium, in the presence of surfactants depends on the position of the chlorine atoms: PCBs with chlorine atoms in *ortho* position do not fluoresce; however, for PCBs with chlorine atoms in other than *ortho* position, the enhancement of fluorescence increases with the number of chlorine atoms up to 3,3´,4,4´-PCB, which presents maximum fluorescence, or arochlors with 40 % chlorine. PCBs with a higher number of chlorine atoms, the relative fluorescence intensity decreases and the enhancement of fluorescence is very low. Polychlorinated dibenzofurans present a similar behavior to PCBs.

According to the results obtained, polyoxyethylene(10)lauryl ether produces the maximum enhacement of fluorescence for all compounds studied.

1.2. Optimization of variables

The influence of surfactant concentration, percentage of organic solvent, temperature and stability with time was studied for 3,4,4´-PCB, 3,3´,4,4´-PCB, 2,8-PCDB and 2,3,7,8-PCDB in the presence of POLE.

For all the compounds studied, the relative fluorescence intensity increases sharply with POLE concentration up to concentrations next to critical micelle concentration (c.m.c.). At higher surfactant concentrations, the fluorescence of polychlorinated compounds remains practically constant (Fig. 1).

The total amount of ethanol in the solution of a micellar medium of POLE affects to the fluorescence of PCBs, but no influence is observed for PCDBs. In the case of PCBs in the presence of POLE, a decrease of the relative fluorescence intensity is observed from 30 % (*V/V*) of ethanol, obtaining similar values to those obtained in aqueous medium, when high percentages of ethanol are used (Fig. 2 and 3).

The influence of temperature on fluorescence of all compounds was studied, obtaining a linear decrease between 15 and 75 °C (Fig. 4). This effect can be due to a high number of collisions between fluorophor molecules and to a decrease of solute-micelle interactions. Also, a stability of at least one hour was obtained for all compounds to the different temperatures studied.

1.3. Fluorimetric determination of 3,4,4′-PCB, 3,3′,4,4′-PCB, 2,8-PCDB and 2,3,7,8-PCDB

After optimization of conditions, new methods were proposed for the determination of 3,4,4′-PCB, 3,3′,4,4′-PCB, 2,8-PCDB and 2,3,7,8-PCDB in the presence of POLE. These studies included calibration curves, reproducibility of the methods, detection limits, as well as a study of the possible interference by other compounds of environmental interest. According to the data obtained, these methods can be considered accurate and precise. The detection limits obtained, 4.9 ng/ml for 3,4,4′-PCB, 1.4 ng/ml for 3,3′,4,4′-PCB, 1.1 ng/ml for 2,8-PCDB and 1.3 ng/ml for 2,3,7,8-PCDB are values much lower than those obtained in aqueous medium.

Due to the high environmental interest of the compounds studied, the methods established were applied to the determination of these compounds in sea water samples, obtaining very satisfactory recovery percentages.

1.4. Synchronous fluorescence determination of 3,3′,4,4′-PCB and 2,3,7,8-PCDB

3,3′,4,4′-PCB and 2,3,7,8-PCDB are highly toxic compounds that are normally found normally together in the environment. Because of this we tried the simultaneous determination of these compounds using their synchronous fluorescence characteristics in the presence of POLE. Hovewer, a high overlapping was observed on the corresponding synchronous spectra, using a range of $\Delta\lambda$ between 1 and 100 nm (18).

A study of second-derivative synchronous fluorescence spectra of 3,3′,4,4′-PCB and 2,3,7,8-PCDB indicates that both compounds can be determined simultaneously using $\Delta\lambda$ of 48 nm (Fig. 5), obtaining a detection limit of 5.3 ng/ml for 3,3′,4,4′-PCB and 2.7 ng/ml for 2,3,7,8-PCDB. This method was applied satisfactorily to the resolution of mixtures of both compounds in sea water samples.

1.5. Fluorescence quenching determination of 4-PCB and Arochlor 1221

4-PCB and Arochlor 1221 are compounds with similar percentage of chlorine and their fluorescence signal is not enhanced for none of surfactants studied. Hovewer, the presence of cetylpiridinium bromide produces a fluorescence quenching effect in these organochlorinated compounds, wich can be used from an analytical point of view (Fig. 6) (19). This quenching effect is described for the Stern-Volmer equation, the constant of which depends on fluorophor concentration (Table 1).

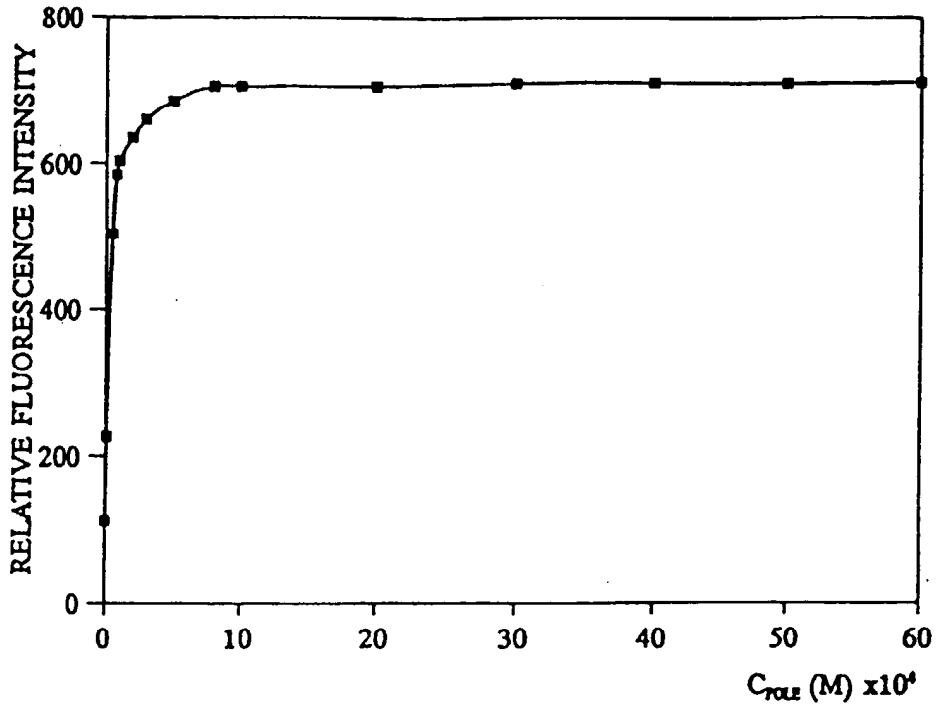

Fig. 1. Effect of POLE concentration on the fluorescence intensity of 3,4,4′-PCB in 2 % (*V/V*) ethanol-water. c_{PCB} = 1 μM.

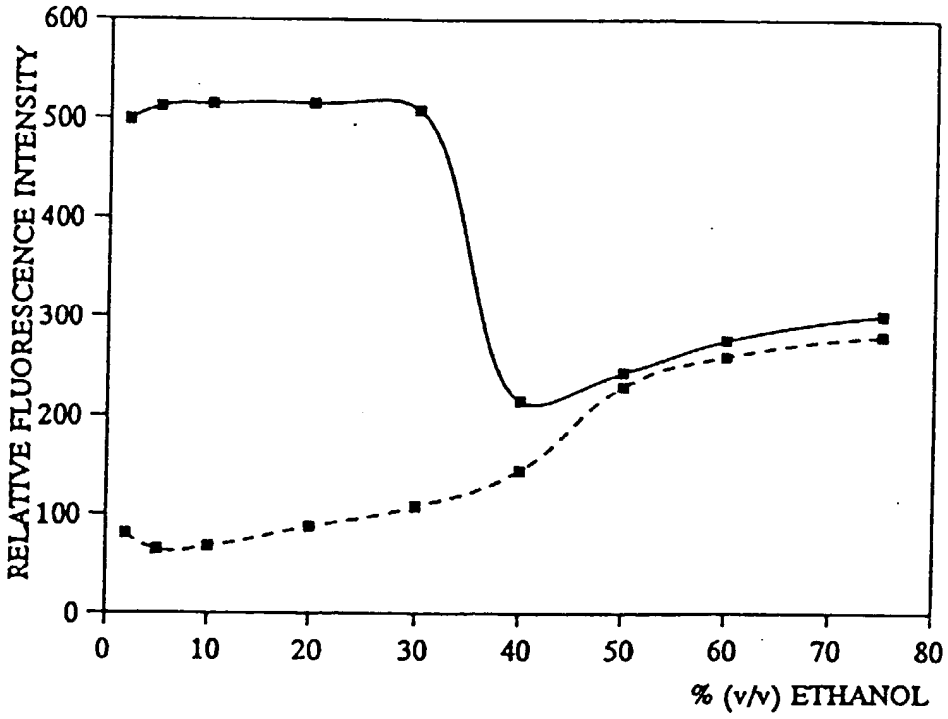

Fig. 2. Effect of ethanol on the fluorescence intensity of 3,3′,4,4′-PCB in micellar medium (solid curve) and in aqueous medium (dashed curve). c_{PCB} = 1 μM; c_{POLE} = 2 mM.

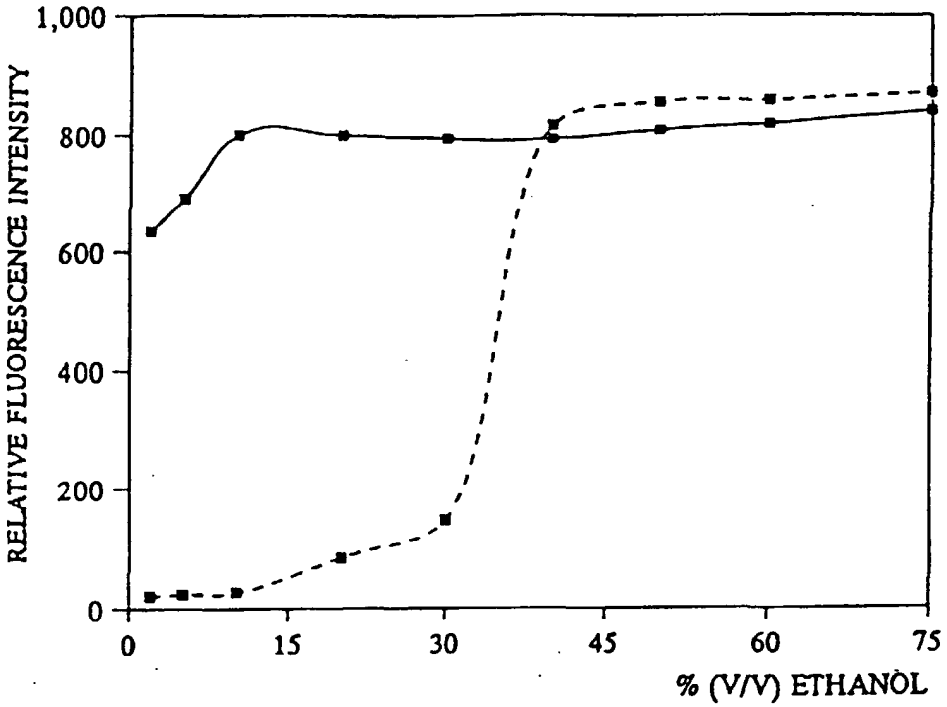

Fig. 3. Effect of ethanol on fluorescence intensity of 2,3,7,8-PCDB in micellar (solid curve) and aqueous (dashed curve) media. c_{PCDB} = 1 μM; c_{POLE} = 2 mM

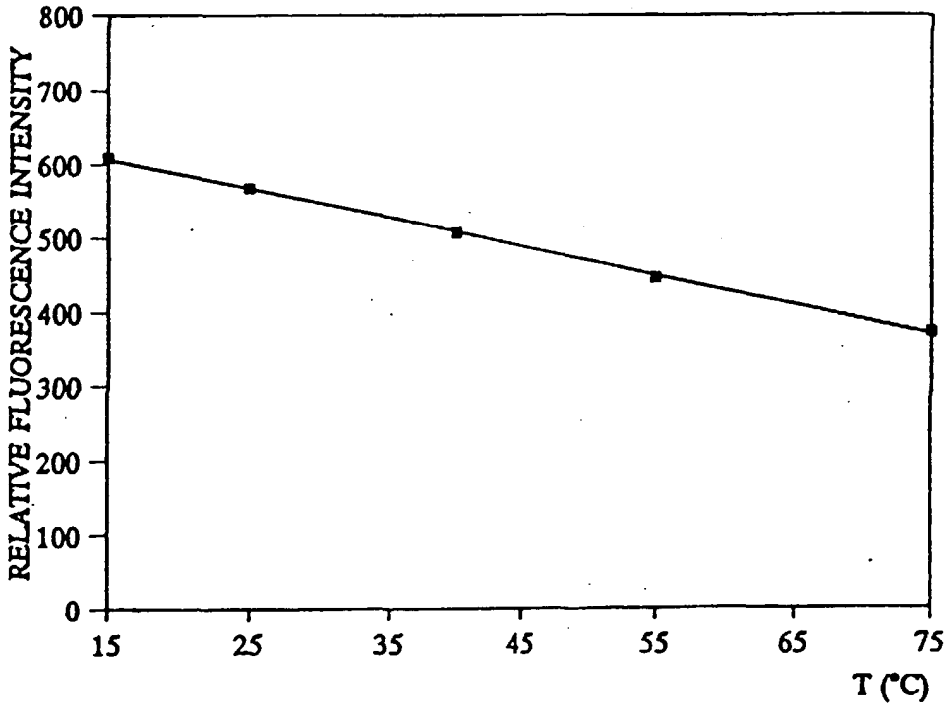

Fig. 4. Effect of temperature on fluorescence intensity of 3,4,4´-PCB in micellar medium. c_{PCB} = 1 μM; c_{POLE} = 2 mM.

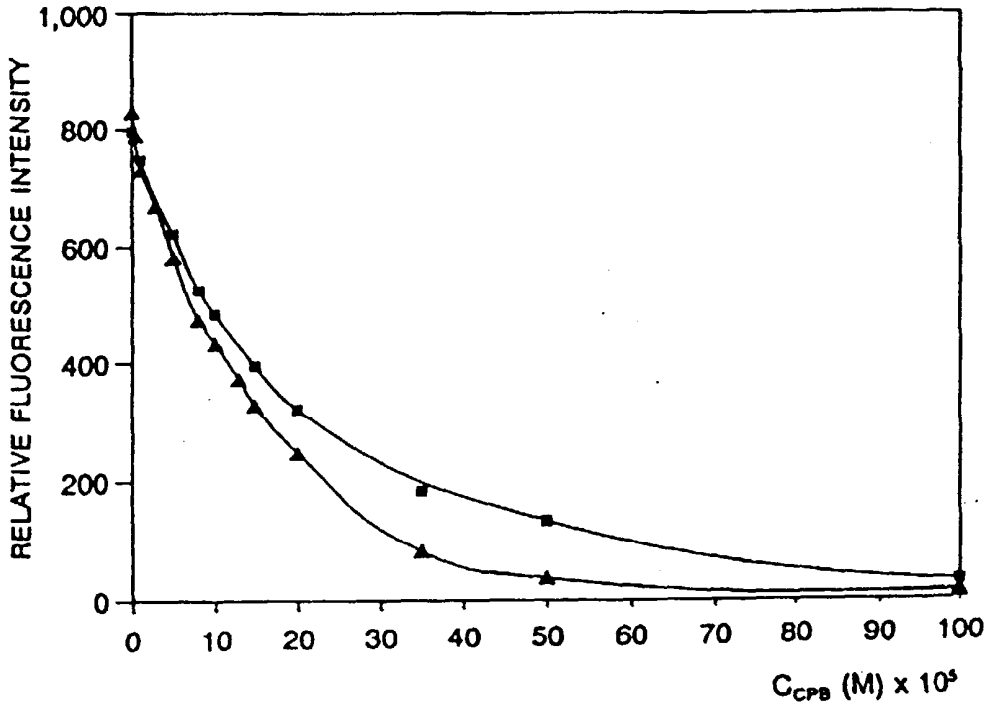

Fig. 5. Influence of concentration of CPB on the fluorescence intensity of 4-PCB (lower curve) and Arochlor 1221 (upper curve).

Table 1. Variation of Stern-Volmer constant (K_{SV}) with 4-PCB and Arochlor 1221 concentration

4-PCB		Arochlor 1221	
Concentration ng/ml	K_{SV}	**Concentration ng/ml**	K_{SV}
3.2	2985.9	5.0	1219.0
5.3	3928	8.0	3856.3
10.6	4772.1	13.6	4223.9
31.7	8951.0	25.0	4705.9
52.8	11056.3	50.0	4837.4
79.2	12142.4	60.0	5974.7
105.6	10345.2	80.0	5513.8
150.2	9158.0	90.0	5100.3
200.0	6985.9	100.0	4219.0

After optimization of variables, such as percentage of organic solvent, temperature and surfactant concentration, as well as a study of the possible interference of other polychlorinated biphenyls and arochlors, a new analytical method was established to estimate these compounds, using the quenching ef-

fect produced by CPB. High recovery percentages were obtained when the method was applied to sea- water samples.

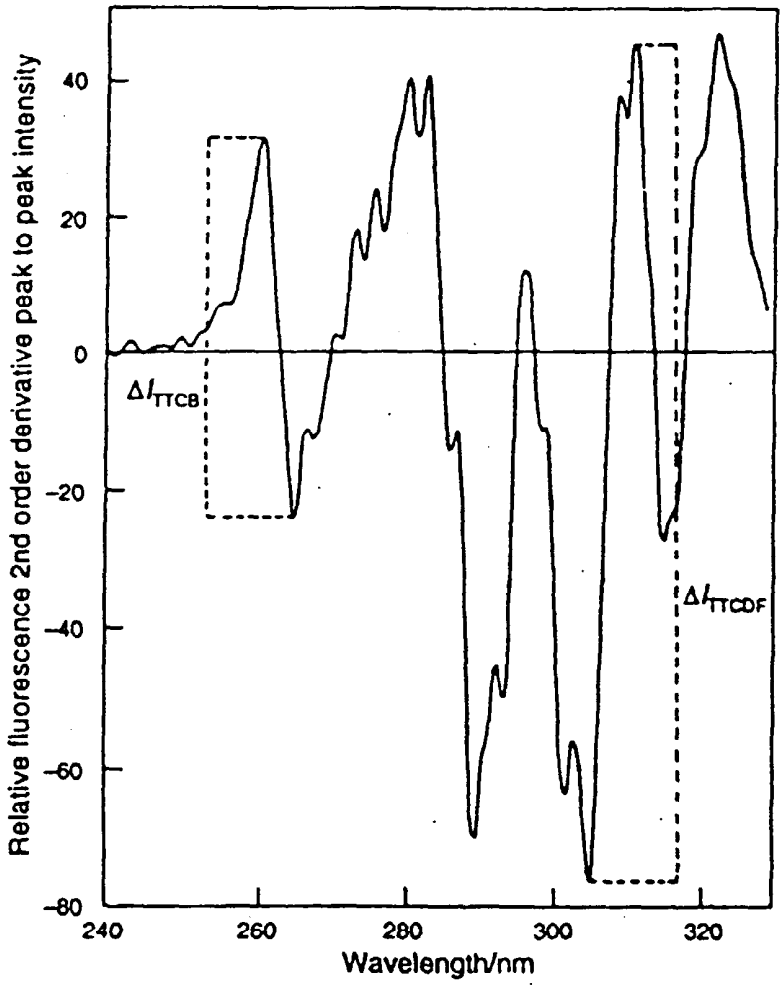

Fig. 6. Second-derivative synchronbous fluorescence spectrum of a binary mixture of TTCB (3,3',4,4'-PCB) and TTCDF (2,3,7,8-PCDB) in micellar medium. c_{TTCB} = 0.4 μM; c_{TTCDF} = 0.1 μM; c_{POLE} = 2 mM.

2. Use of organized molecular systems on the extraction/preconcentration of organo-chlorinated compounds and their determination by high-performance liquid chromatography with fluorescence detection

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 solvent. Efficient preconcentrations of organic solutes have been obtained using the cloud-point phase separation phenomena (20-22). The analytical potential of the cloud-point-phenomenon-mediated phase separations, cloud- point extraction (CPE), has been discussed by several authors (23-26). 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 part of the 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 PCBs and PCDBs previous to their determination by high performance liquid chromatography, using four non-ionic surfactants, Oligoethylene glycol monoalkyl ether (Genapol X-080), polyoxy-ethylene(10)lauryl ether (Brij-30), polyoxyethylene(10)cetyl ether (Brij 56) and polyoxyethylene(10)oleyl ether (Brij-97). This CPE methodology was applied to the analysis of mixtures of PCBs and PCDBs in sea water samples.

2.1. Phase diagrams

When we plot cloud-point values against the percentage (*W/V*) of surfactant in solution, the figure shows a co-solution curve, above which the two phases appear (Fig. 7). The behavior is similar for the four surfactants used, the first showing a sharp drop in temperature up to a concentration of 2 % (*W/V*) above which the cloud-point temperature is fairly constant. According to the results obtained, the critical temperature of the cloud-point for Genapol X-080, Brij-30, Brij-56 and Brij-97 was 80 ± 1 °C, 95 ± 1 °C, 90 ± 1 °C and 85 ± 1 °C, respectively (27, 28).

2.2. 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: concentration and volume of surfactant, equilibration time, ionic strength and analyte concentration (29).

2.2.1. Concentration and volume of surfactant

For the PCBs studied, when the concentration of surfactant changes between 0.05- and 5 % (*W/V*), keeping the volume constant, the ratio V_w/V_s (volume of aqueous phase/volume of surfactant phase) decreases with the concentration of surfactant, being practically constant from 1 % (*W/V*). When the volume of surfactant changes, keeping concentration constant, V_w/V_s remains constant for Genapol X-080 and Brij-56, and decreases linearly for Brij-30 and Brij-97. For PCDBs, these two variables do not influence the extraction process.

2.2.2. Equilibration time

The behavior of PCBs is different, depending on the surfactant. In the case of Genapol X-080, the recovery percentages of PCBs increase up to a maximum value of 15 min, decreasing after that; for Brij-30, the behavior is similar to Genapol X-080 but the recovery percentages increase again for times higher than 20 min; in the case of Brij-97, the recovery percentages decrease lightly up

to 15 min, remaining practically constant after that; for Brij-56, the time neccesary to obtain the highest recovery percentages decreases with the number of chlorine atoms on the molecule.

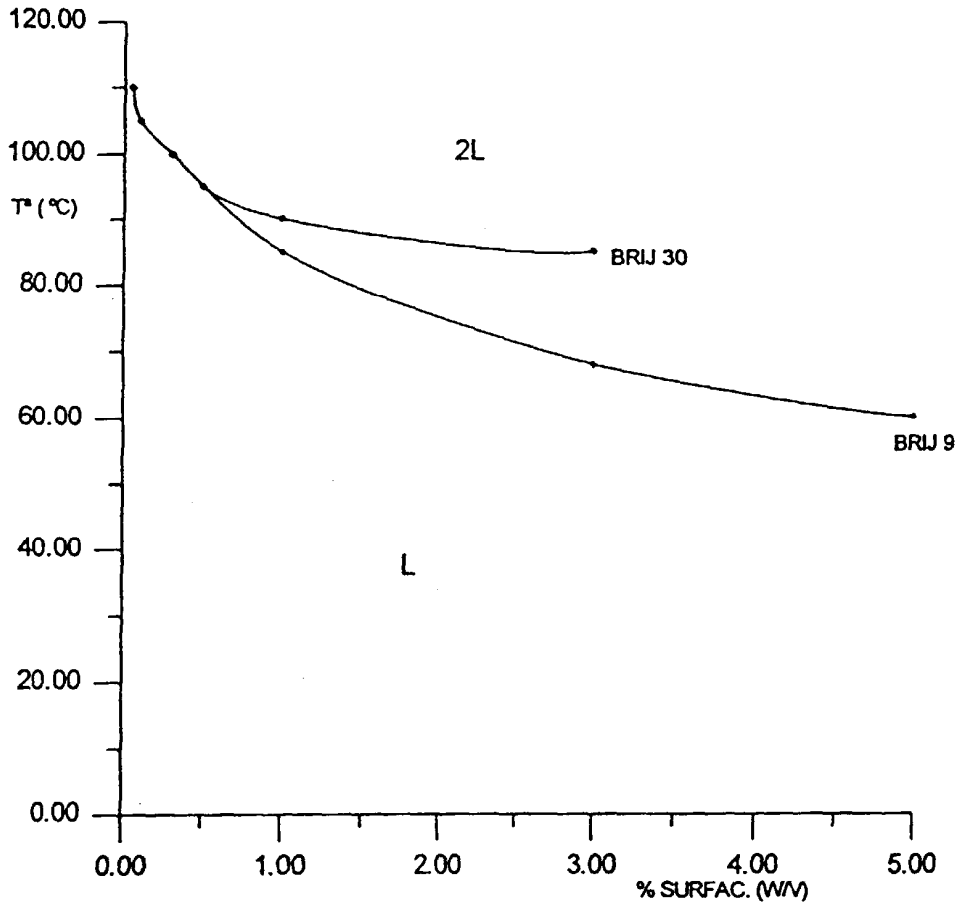

Fig. 7. Phase diagram for Brij 30 and Brij 97. The region above the cosolution curve is a two-phase region.

In the case of PCDBs, using Genapol X-080, we observed that 4-PCDB shows a similar behavior to the PCDB with an even number of chlorine atoms. The recovery percentages of these compounds decrease with time to 13 min, increasing to 15 min (maximum recovery percentage) and going down again for longer times. In the case of PCDB with odd numbers of chlorine atoms, recovery percentages increase with time to 13 min, decreasing for 15 min and increasing for longer periods. Using Brij-56, the recovery percentages decrease sharply for all the PCDB with time up to 13 min, but with longer periods two behaviors could be observed: for PCDB with even numbers of chlorine atoms, the recovery percentage increases up to 20 min, decreasing gradually over longer periods; the rest of the PCDB presents low recovery percentages for 20 min, increasing to 25 min.

2.2.3. 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 salt does not affect the extracted volume of surfactant-rich phase or the recovery percentages of all analytes studied. But the addition of inert salt increases the density of the bulk aqueous phase and facilitates the separation process of the two phases. These results are in accordance with those obtained by other authors (30).

2.2.4. Analyte concentration

To determine the effect of analyte concentration, solutions containig different concentrations of PCBs and PCDBs were subjected to the CPE procedure. The results obtained indicated that for PCBs, using Genapol X-080, Brij-30 and Brij-97, the extraction process is not affected in the concentration intervals studied; for PCDBs, neither Genapol X-080 nor Brij-56 influence the recovery percentages of these analytes.

2.3. Cloud-point preconcentration and liquid chromatographic analysis

Another important step in these studies is the optimization of the chromatographic conditions for the separation and determination of the organochlorinated compounds. When chromatography is used as a separation technique, it is necesary to obtain good relationship between the analysis time and analyte separation. The results indicated as best chromatographic conditions a mobile phase of 85:15 (*V/V*) methanol:water and a flow rate of 1 ml/min. Using these conditions and the characteristic excitation and emission wavelengths, the corresponding chromatograms indicated a satisfactory separation of all compounds (Fig. 8 and 9). After the establishment of the calibration curves and reproducibility of the methods, the detection limits (31) were calculated (Tables 2 and 3).

Table 2. Detection limits (ng/ml) for PCBs

PCB	Brij-30	Brij-97
Biphenyl	1.54	0.70
4-PCB	1.89	0.94
4,4′-PCB	2.23	1.10
3,4,4′-PCB	7.71	2.57
3,3′4,4′-PCB	2.92	2.92
3,3′4,4′,5-PCB	16.30	16.30
3,3′4,4′5,5′-PCB	10.82	18.04

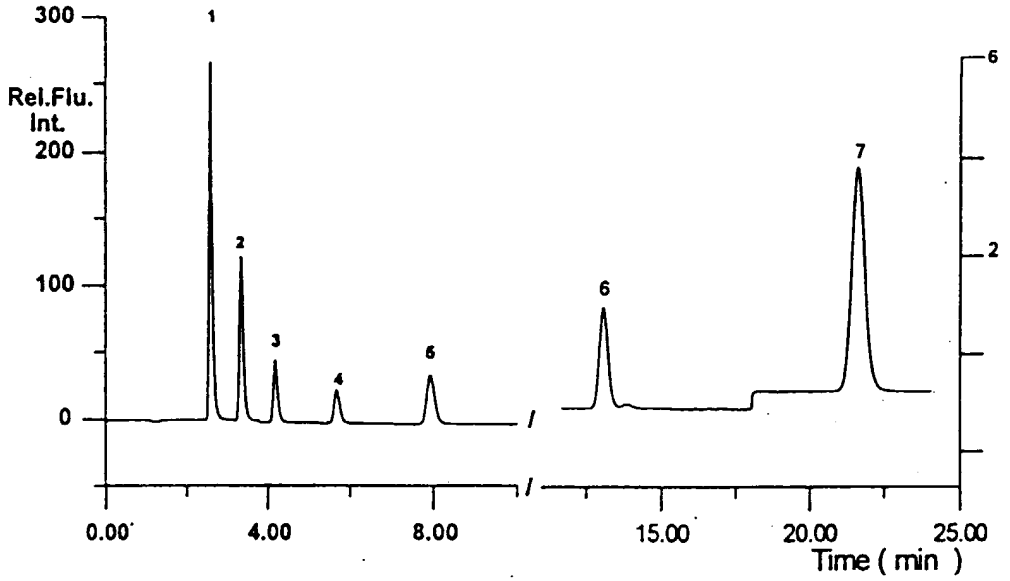

Fig. 8. Elution of a mixture of seven PCBs using 2 % (*W/V*) Brij 30 as preconcentrant. **1** - biphenyl, **2** - 4-PCB, **3** - 4,4´-PCB, **4** - 3,4,4´-PCB, **5** - 3,3´,4,4´-PCB, **6** - 3,3´,4,4´,5-PCB, **7** - 3,3´,4,4´,5,5´-PCB. Concentration 500 ng/ml; eluent was methanol-water 85:15), flow rate 1 ml/min.

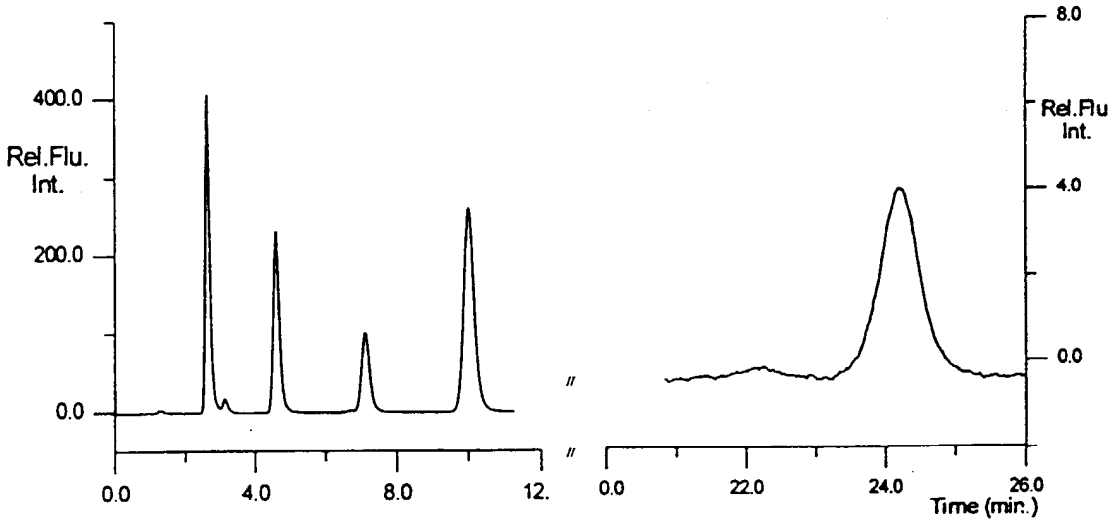

Fig. 9. Elution of a mixture of six PCBs using 2 % (*W/V*) Genapol X-080 as preconcentrant. **1** - dibenzofuran, **2** - 4-PCDB, **3** - 2,8-PCDB, **4** - 2,4,8-PCDB, **5** - 2,3,7,8-PCDB, **6** - 1,2,3,4,8-PCDB. Concentration 500 ng/ml; eluent was methanol-water 85:15), flow rate 1 ml/min.

2.4. Analytical applications

Once optimized, the proposed methods were applied to the determination of PCBs and PCDBs in sea-water samples from different Spanish areas (Arinaga and Agaete, Canary Islands and Elantxobe, Vizcaya), previously spiked with suitable amounts of PCBs and PCDBs. The results indicated recovery between 84 and 106 %.

Table 3. Detection Limits for PCDBs using Genapol X-080 and Brij-56

PCDB	Detection limit ng/ml
Dibenzofuran	0.5
4-PCDB	17.6
2,8-PCDB	13.3
2,4,8-PCDB	4.9
2,3,7,8-PCDB	0.7
1,2,3,4,8-PCDB	27.5

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