Cloud point preconcentration of rather polar compounds: application to the high-performance liquid chromatographic determination of priority pollutant chlorophenols

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Abstract

The potential of the cloud point methodology for the preconcentration of relatively polar compounds was studied using the non-ionic surfactant Triton X-114 and five EPA chlorophenols as test analytes. Analyte determination was performed using reversed-phase gradient LC with electrochemical and spectrophotometric detection. The amount of surfactant used is a critical variable in the preconcentration factor because it determines the extraction yield and the volume of surfactant-rich phase obtained. These values were determined as a function of the Triton X-114 concentration, together with the phase ratio, which allows prediction of the maximum preconcentration factor under given conditions. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The cloud point methodology, based on the property of non-ionic and zwitterionic surfactants of separating into two liquid phases when their aqueous solutions are heated above a given temperature, has been used to separate and preconcentrate different species prior to their determination by several techniques [1–9]. Most of these species are apolar compounds for which extraction yields of about 100% can be obtained.

In this work the capability of the technique for the preconcentration of relatively polar species, such as five EPA priority pollutant chlorophenols, was assayed using the surfactant Triton X-114: poly(oxyethylene)-7,5-(*p-tert*-octylphenyl) ether. Official methods for the determination of these compounds, such as US EPA 604 [10], 625 [11], or 8041 [12], are based on liquid–liquid extraction. However, considerable research has focused on alternative preconcentration techniques that avoid the use of large amounts of organic solvents, especially solid-phase extraction [13–17], and solid-phase microextraction [18,19].

The preconcentration of several chlorophenols has been reported [20] using the non-ionic surfactant

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C_8E_3, poly(oxyethylene glycol) monooctyl ether, with good extraction yields, although no analytical characteristics were given in that work. Additionally, the ionic surfactant mixture Cetrimide has been used [21] to extract chlorophenols from river water. Phase separation is achieved by the 'salting-out phenomenon', adding salts under saturated conditions to the surfactant solution. This approach is compatible with UV–visible detection, but the limits of detection for the different chlorophenols are relatively high, ranging from 27 to 62 μg/L.

A further aim of this work was to determine the phase ratio for the surfactant Triton X-114 at different concentrations from chromatographic measurement of the analytes. Measurement of the surfactant-rich phase volume were carried out previously in a visual manner with calibrated tubes [2,3]. When the volumes are sufficiently high (>200 μL), these measurements are acceptable, but, for smaller volumes, imprecision increases substantially.

2. Experimental

2.1. Reagents

Triton X-114 was obtained from Fluka (Madrid, Spain) and was used without further purification. 2-Chlorophenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol (between 98 and 99% purity) were purchased from Aldrich (Madrid, Spain). Standard solutions of these compounds, with concentrations ranging between 490 and 565 mg/L, were prepared by dissolution of the commercial products in methanol. HPLC-grade methanol, used in the preparation of standards and the mobile phase, was from Carlo Erba (Milan, Italy).

Other reagents were of analytical grade; all solvents were filtered through 0.45 μm nylon membrane filters (Millipore, Bedford, MA. USA), and ultra-high quality water obtained from an Elgastat UHQ water purification system was used.

2.2. Instrumentation

A LC system was used consisting of a Spectra Physics SP 8800 ternary pump, an SP 8450 UV detector, and an EG&G PARC 400 electrochemical detector. The electrodes were as follows: a Ag/AgCl/0.1 *M* KCl reference electrode, a gold auxiliary electrode, and a single glassy carbon electrode MP 1305. The two detectors were connected in series, first the UV detector and then the electrochemical detector. Data collection was performed by a computer using the Chrom-card software (version 1.18, CE Instruments). In all experiments, a Rheodyne 7125 injection valve with 20 μL sample loops and a 220×4.6 mm Spheri 5 ODS stationary phase column from Brownlee Labs were used. A Jones Chromatography Model 7981 thermostatic system was employed to maintain the chromatographic column at a constant temperature. A Kokusan H-103 centrifuge was used to accelerate the separation of the two phases after cloud point preconcentration.

2.3. Procedures

2.3.1. Cloud point preconcentration

Aliquots of 10.0 mL of the solutions containing the analytes, 0.6 *M* NaCl and 0.5% Triton X-114, at a pH maintained by 0.01 *M* H_2SO_4, were kept for 20 min in a thermostatted bath at 65°C, and the two phases were separated by centrifugation for 15 min at 1400 *g*. On cooling in an ice bath, the surfactant-rich phase became viscous. The aqueous phase was then separated by inverting the tubes. Next, 50 μL of methanol were added to the surfactant-rich phase to reduce its viscosity and 20 μL were injected into the LC system.

2.3.2. Chromatographic analysis

The chlorophenols were separated using a methanol–water gradient (from 60:40 v/v to 95:5 v/v in 10 min, 10 min at 95:5 v/v and back to 60:40 v/v in 5 min) in the presence of 0.01 *M* $LiClO_4$ and 0.025 g/L H_2SO_4. The flow-rate was 1.0 mL/min and the column temperature 40°C. The electrode was pretreated electrochemically every day by keeping the potential at +1300 mV for 5 min and then applying the working potential, +850 mV. Additionally, it was polished once a week. UV detection was carried out at 290 nm.

3. Results and discussion

3.1. LC behavior of the surfactant

Triton X-114 shows absorption bands in the UV part of the spectrum. On the other hand, although the surfactant does not have electroactive groups, it may give rise to signals that will vary with the applied potential owing to the greater or lesser degree of adsorption onto the glassy carbon electrode [22]. Fig. 1 shows the chromatograms obtained for a solution of 5% Triton X-114 and a mixture of chlorophenols in methanol. Fig. 1a corresponds to UV detection at 290 nm and Fig. 1b to amperometric detection at +850 mV (optimum conditions). The largest of the peaks, coming from fractionation of the surfactant, prevents the spectrophotometric measurement of pentachlorophenol.

3.2. Influence of experimental variables in cloud point preconcentration

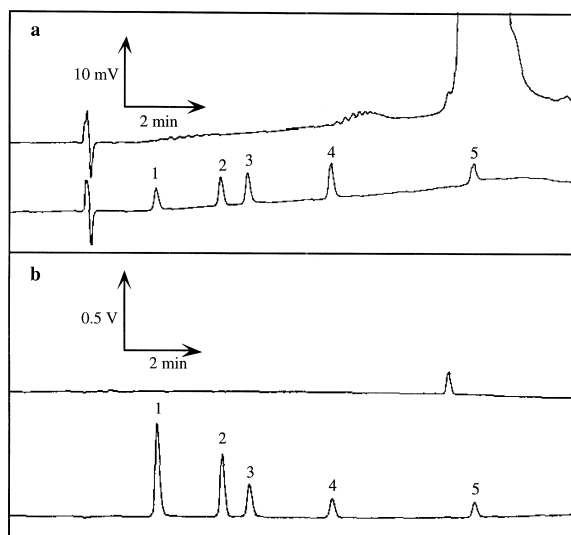To ensure that all the chlorophenols were present

Fig. 1. Chromatograms obtained for the injection of a 5% Triton X-114 solution and a solution of chlorophenols. (a) Spectrophotometric detection ($\lambda = 290$ nm). (b) Electrochemical detection ($E = +850$ mV). Peak assignment: 1=2-chlorophenol (0.36 mg/L), 2=4-chloro-3-methylphenol (0.29 mg/L), 3=2,4-dichlorophenol (0.28 mg/L), 4=2,4,6-trichlorophenol (0.22 mg/L), 5= pentachlorophenol (0.26 mg/L). Chromatographic conditions as described in the Experimental section.

in neutral form, the working pH was fixed using 0.01 M H_2SO_4, given that the most acidic analyte was pentachlorophenol (pK_a 4.74).

The influence of the ionic strength was studied by the addition of NaCl, between 0 and 1.0 M; this inert salt increases the extraction efficiency by decreasing the solubility of the organic species in the aqueous phase, particularly in the case of the more polar species. For example, a 220% increase in the 2-chlorophenol analytical signal was observed on adding the optimum 0.6 M NaCl concentration.

The temperature of extraction was changed between 30 and 80°C, with no appreciable signal alterations; an intermediate temperature of 65°C was chosen for later studies. The time of extraction was varied from 2 to 45 min; an optimum time of 20 min was chosen because, after this time, a slight decrease occurred, which could be due to loss of analytes owing to their volatility.

3.3. Parameters of the preconcentration procedure as a function of Triton X-114 concentration

To determine the extraction yield, the preconcentration factor, and the phase ratio, three chromatograms were recorded for different Triton X-114 concentrations, giving signals S_1, S_2 and S_3, respectively. The first chromatogram was obtained by injecting 20 μL of a solution containing unpreconcentrated chlorophenols. To obtain the second chromatogram, cloud point preconcentration was carried out on 10.0 mL of the initial solution and, after phase separation, methanol was added to the surfactant-rich phase up to the initial volume and 20 μL of this solution was injected into the LC system. Finally, 10.0 mL of the initial solution was preconcentrated, and 250 μL of methanol was added to the surfactant-rich phase. Although injection of the surfactant-rich phase without prior dilution is possible, the addition of methanol facilitates handling owing to a decrease in viscosity.

Extraction yields and preconcentration factors were calculated from the expressions: % recovery= $(S_2/S_1)\times100$ and preconcentration factor=(S_3/S_1), respectively. Table 1 shows the results obtained for different surfactant concentrations, together with the octanol–water distribution constants, expressed as $\log K_{ow}$ [23]. It can be observed that as $\log K_{ow}$ decreases (increase in polarity) total extraction of the

Table 1
Extraction recoveries and preconcentration factors[a] obtained for different Triton X-114 concentrations. Means of three replicates; the relative standard deviations of the recoveries ranged between ±4 and 6%

Triton X-114 (%)	2-Chloro-phenol ($\log K_{ow} = 2.15$)		4-Chloro-3-methylphenol ($\log K_{ow} = 3.10$)		2,4-Dichloro-phenol ($\log K_{ow} = 3.23$)		2,4,6-Trichloro-phenol ($\log K_{ow} = 3.72$)		Pentachloro-phenol ($\log K_{ow} = 5.24$)	
	%R[b]	PF[b]	%R	PF	%R	PF	%R	PF	%R	PF
0.05	6	2	21	7	28	8	40	12	97	28
0.10	14	5	46	14	61	17	78	21	98	28
0.20	31	10	70	19	84	23	92	23	98	22
0.30	36	10	74	19	87	21	100	22	103	20
0.50	45	11	79	19	86	19	99	21	101	21
1.00	62	13	89	16	92	17	98	16	101	18

[a] 250 µL of methanol added to the surfactant-rich phase.
[b] %R, extraction recovery; PF, preconcentration factor.

compound requires higher surfactant concentrations. Given that the preconcentration factor is a function of opposite effects, determination of polar compounds requires a compromise surfactant concentration; 0.5% Triton X-114 was chosen in this case.

The phase ratio (R_{p250}), defined as the quotient between the initial volume and the final volume, after adding 250 µL of methanol to the surfactant-rich phase, was calculated from the S_3/S_2 ratio mean value. From R_{p250}, the volumes of the surfactant-rich phase, diluted with methanol (V_{250}) and undiluted (V), were calculated. The results are shown in Table 2. Knowing the volume V, it is possible to calculate the phase ratio when no dilution is carried out (R_p) and when different volumes of methanol are added

Table 2
Phase ratios and volumes of surfactant-rich phase obtained for different Triton X-114 concentrations[a]

Triton X-114 (%)	R_{p250}	V_{250} (mL)	V (mL)
0.05	30±1	0.34±0.01	0.09±0.01
0.10	28±1	0.36±0.02	0.11±0.02
0.20	27±1	0.38±0.01	0.12±0.01
0.30	24±1	0.41±0.02	0.16±0.02
0.50	23±1	0.44±0.02	0.19±0.02
1.00	18±1	0.54±0.02	0.29±0.02

[a] R_{p250}, phase ratio (250 µL of methanol added to the surfactant-rich phase); V_{250}, volume of surfactant-rich phase (250 µL of methanol added); V, volume of surfactant-rich phase.

(Fig. 2). This allows one to design analytical schemes with a given preconcentration factor.

3.4. Analytical characteristics of the method

Calibration graphs were constructed for 10.0 mL ultrapure water samples with 0.5% Triton X-114 (in the range 5–200 or 10–500 µg/L, depending on the chlorophenol). In all cases linear relationships were obtained between peak area and concentration. The repeatability for 10 samples at a concentration of 20–46 µg/L ranged from 4 to 10% (RSD).

Table 3 shows the detection limits for a signal-to-noise ratio of 2, without preconcentration and after preconcentration. The maximum attainable preconcentration factor was given by the phase ratio, which was 40 (after the addition of 50 µL of methanol to the surfactant-rich phase). Pentachlorophenol, with a preconcentration factor of 37, almost reached this maximum, as expected from its 100% extraction yield.

Comparison of the slopes calculated for each chlorophenol in ultrapure water with those obtained with two river water samples (Tormes and Almar rivers, Salamanca, Spain) was accomplished by regression, evaluating the confidence intervals at 95%. With both electrochemical and UV detection it was observed that the intercept was equal to 0 and the slope to 1, thus indicating that no matrix effect existed. Additionally, the noise level was similar in the ultrapure and river water chromatograms, afford-
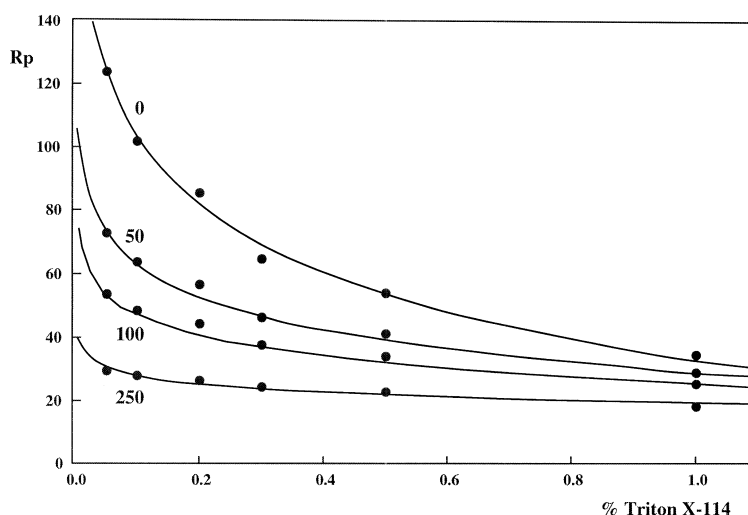
Fig. 2. Ratio of phases (R_p) as a function of the Triton X-114 concentration and of the methanol volume (in μL) added to facilitate the handling of the surfactant-rich phase.

ing comparable limits of detection. Hence, cloud point preconcentration can be used to determine chlorophenols in river water.

4. Conclusions

The cloud point methodology was applied to the preconcentration of rather polar compounds prior to

Table 3
Limits of detection of the method[a]

Compound	Limits of detection (μg/L)			
	Without pre-concentration		Preconcen-trated[b]	
	EC	UV	EC	UV
2-Chlorophenol	40	114	3	8
4-Chloro-3-methylphenol	51	76	2	2
2,4-Dichlorophenol	74	56	3	2
2,4,6-Trichlorophenol	161	71	5	2
Pentachlorophenol	172	–	5	–

[a] EC, electrochemical detection, $E = +850$ mV; UV, ultraviolet detection, $\lambda = 290$ nm; –, not determined.
[b] Conditions described in Section 2.3.1.

HPLC analysis. Five EPA priority pollutant chlorophenols were chosen as test analytes. The detection limits of the method, in the 2–5 μg/L range, do not improve the results obtained with solid-phase extraction, where enrichment makes it possible for chlorophenol determination at the part-per-trillion level, but it could be considered as an alternative in the analysis of surface waters, for which requirements are in the 1.0–10 μg/L range [24].

The phase ratio was obtained as a function of the Triton X-114 concentration; this value, together with the octanol–water distribution constant K_{ow} of the analytes, can be used to indicate the preconcentration possibilities of the method. For hydrophobic analytes the extraction yield is close to 100%, even when low surfactant concentrations are used, such that the preconcentration factor is determined by the phase ratio, which diminishes when the surfactant concentration is increased. For polar compounds, a compromise surfactant value must be chosen, considering both opposing effects.

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