



Cloud point extraction coupled with HPLC-UV for the determination of phthalate esters in environmental water samples

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Abstract

A method based on cloud point extraction was developed to determine phthalate esters including di-ethyl-phthalate (DEP), di-(2-ethylhexyl)-phthalate (DEHP) and di-cyclohexyl-phthalate (DCP) in environmental water samples using high-performance liquid chromatography separation and ultraviolet detection (HPLC-UV). The non-ionic surfactant Triton X-114 was chosen as extraction solvent. The parameters affecting extraction efficiency, such as concentrations of Triton X-114 and Na₂SO₄, equilibration temperature, equilibration time and centrifugation time were evaluated and optimized. Under the optimum conditions, the method can achieve preconcentration factors of 35, 88, 111 and detection of limits of 2.0, 3.8, 1.0 ng/ml for DEP, DEHP and DCP in 10-ml water sample, respectively. The proposed method was successfully applied to the determination of trace amount of phthalate esters in effluent water of the wastewater treatment plant and the lixivium of plastic fragments.

Key words: phthalate esters; cloud point extraction; Triton X-114; non-ionic surfactant, HPLC-UV

Introduction

Phthalate esters (PAEs) are a class of chemical compounds primarily used as plasticizers for polyvinyl chloride resins, cellulose film coating, and adhesives (Prokupková *et al.*, 2002). It is estimated that the production of these compounds in the world is several million tons per year (Polo *et al.*, 2005). During the process of their production, manufacture, usage and disposal, significant migration of them into the environmental compartments is possible. Certain phthalates and/or their metabolites are suspected to be human endocrine disruptors, and carcinogens (Wezel *et al.*, 2000). Several of them have been included in the priority pollutants of endocrine disrupter compounds, due to their potential risks for human health and environment (Li *et al.*, 2004). Environmental researches are mainly focused on the aquatic environments adjacent to or downstream from industrial sites. It was reported that PAE concentrations are in the range of 0.1–300 µg/L for surface marine water and freshwater sites, and 0.1 ng/g–100 µg/g for river sediments (Yuan *et al.*, 2002).

The most widely used methods for analyzing phthalate esters are chromatographic techniques such as GC or HPLC, but their sensitivity and selectivity limit their

direct use for determination of these contaminants at a very low levels of concentration in environmental samples with complex matrix. Therefore, a sample pretreatment prior to chromatographic analysis, such as liquid-liquid extraction and solid-phase extraction, is usually necessary. Unfortunately, all of these methods are time-consuming and need a large sample volume. In particular, the traditional liquid-liquid extraction method is also dangerous to analysts because of the large volume of volatile organic solvent used. As a result, the green liquid-liquid extraction method—cloud point extraction (CPE) has been employed in analytical chemistry to preconcentrate organic compounds (Bai *et al.*, 2001; Casero *et al.*, 1999; Carabias-Martinez *et al.*, 1999) and metal ions (Chen and Teo, 2001; Manzoori and Karim-Nezhad, 2003; Yuan *et al.*, 2005) in the last decades. Compared with the traditional organic liquid-liquid extraction, cloud point extraction requires a very small amount of relatively nonflammable and non-volatile surfactants that are friendly to the environment. Another important merit is that no solvent concentration procedures, which may cause analytes loss, are needed under appropriate conditions such as temperature, concentration of surfactant and Na₂SO₄, equilibration time, the solution containing the surfactant becomes turbid and separates into two phases: a surfactant-rich phase (at a very small volume) and a larger volume aqueous solution phase (bulk amount) with a diluted surfactant concentration, which approximates to its critical micelle concentration

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(CMC). The hydrophobic analytes of the solution are extracted into the small volume of surfactant-rich phase with a high enrichment factor. As a promising alternative to traditional solvent extraction, CPE, especially the extraction of environmental pollutants is still at its initial stage. Only a few reports can be found on the extraction of polycyclic aromatic hydrocarbons (PAHs) (Rodenbrock *et al.*, 2000; Collen *et al.*, 2002; Sirimanne *et al.*, 1996), polychlorinated biphenyls (PCBs) (Manzoori and Karim-Nezhad, 1998), dibenzofurans (PCDFs), polychlorinated dibenzop-dioxins (PCDDs), pesticides (Fernández *et al.*, 1999), vitamins (Pinto *et al.*, 1992) and other organic compounds such as chlorinated phenols (Saitoh and Hime, 1991). These reported results indicate that cloud point extraction has a great analytical potential as an effective enrichment method. However, no researches focused on the extraction of phthalate esters from the water by CPE, have been published.

In the present study, a method was developed for the determination of trace levels of PAEs in water by CPE using Triton X-114 as the extraction solvent.

1 Materials and methods

1.1 Materials

All reagents used were HPLC grade, and purified water from a Milli Q system was used throughout the experiments. Standard stock solutions containing phthalate esters compounds were prepared by dissolving an appropriate amount of these compounds in methanol. Working solutions were prepared daily by an appropriate dilution of the stock solutions. The non-ionic surfactant Triton X-114 was from Acros Organics, New Jersey, USA. Na₂SO₄ (Beijing Chemical Factory, China) was prepared immediately before each experiment.

Analysis of phthalate esters in environmental samples may pose a serious problem because high blanks are often encountered due to PAEs presence in many laboratory products, including chemicals and glassware. Therefore, the vessels used for trace analysis were washed with methanol and purified water before use.

1.2 Instrumentation

The HPLC system includes an Agilent 1100 series binary pump, an Agilent 1100 series VWD detector and a Rheodyne 7225i injector. The separations were performed on an ODS-C18 column (250 mm × 4.6 mm, particle size 5 μm). A mixture of acetonitrile and water was used as mobile phase with the gradient: 0–10 min, 75:25 (v:v; acetonitrile:water); 10–11 min, the organic phase increased from 75% to 100%; 11–22 min, 100% acetonitrile. A flow rate of 1 ml/min was selected. The 226 nm wavelength of the UV detector was selected for all of the three kinds of phthalate esters. A personal computer equipped with an Agilent Chemstation program for LC systems was used to acquire and process chromatographic data. Peak area was used as the analytical measurement. A thermostatic bath (TB-85Therma Bath, Shimadzu, Japan), maintained at the

desired temperature, was used to obtain cloud point pre-concentration. Centrifugation with calibrated centrifuge tubes (Beijing Medicinal Instrument Company, China) were used to accelerate the phase separation process. Easypure deionized water was used in this study (Model D7382-33, Barnstead Thermolyne Corporation, Dubuque, IA, USA). The injector volume was 20 μl.

1.3 Cloud point procedure

For the pre-concentration of phthalate esters, aliquots of 10 ml of the sample solution containing the analytes, 0.25% Triton X-114 and 0.4 mol/L Na₂SO₄, were kept for 60 min in the thermostatic bath at 45°C. Then the phase separation was accelerated by centrifugation for 5 min at 3500 r/min. After phase separation, the bulk aqueous phase was removed, and 20 μl of the remaining surfactant-rich phase was injected directly in the HPLC loop for subsequent analysis.

1.4 Sample preparation

Sample 1: since the phthalate esters are not chemically but only physically bound to the polymer chains, they may be leached into food and beverages from the plastic containers (Li *et al.*, 2004). It was assumed there are free phthalate esters in the lixivium of plastic fragments. Broken down 30 g plastic package into fragments and mixed with 50 ml purified water. The sample was subjected to the ultrasonic bath for agitation for 5 h.

Sample 2: the effluent water from Gaobeidian Wastewater Treatment Plant (WWTP), Beijing, China.

1.5 Extraction of phthalate esters in real samples

The real water samples were filtered through a 0.45-μm pore-size membrane filter to remove the suspended particular matter. A 10-ml real water sample was submitted to the cloud point extraction procedure using 0.25% Triton X-114 and 0.4 mol/L Na₂SO₄. After phase separation, 20 μl of surfactant-rich phase was injected directly to the loop of HPLC coupled UV detector for the analysis. Standard solutions containing 40, 60, 50 ng/ml of di-ethyl-phthalate (DEP), di-(2-ethylhexyl)-phthalate (DEHP) and di-cyclohexyl-phthalate (DCP) were added to a 10-ml real water sample for the recovery test, respectively.

2 Results and discussion

Triton X-114 with a cloud-point temperature of 23°C (Toerne *et al.*, 2000; Delgado *et al.*, 2004) is one of the most common nonionic surfactants used in the bibliography in the cloud point extraction (Delgado *et al.*, 2004). There are several different parameters that may influence the extraction efficiency. They were investigated in this experiment excluding the initial concentrations which were proved to no contribution to pre-concentration factor (Li and Chen, 2003).

2.1 Effect of the concentration of surfactant

The theoretical pre-concentration factors depend on the volume of the surfactant-rich phase, which varies with the

surfactant concentration in solution at the same time (Delgado *et al.*, 2004). The nonionic Triton X-114 surfactant was chosen for its low cloud point temperature and high density, phase separation was facilitated by centrifugation (Shemirani *et al.*, 2005). The effect of the surfactant concentration ranging from 0.125%–0.75% was examined and the results are shown in Fig.1. It can be found that the highest extraction efficiency is obtained at 0.25%. When the concentration of surfactant is below 0.25%, the volume of surfactant-rich phase is too small to be separated from the bulk solution, Moreover, such small volume is not enough for triple injections. Based on these experimental results, 0.25% Triton X-114 was adopted as the optimum amount to achieve the best analytical signals and highest extraction efficiency.

2.2 Effect of equilibration time, temperature and concentration of Na_2SO_4

Surfactant can exhibit different behaviors when the equilibration time varies (Manzoori and Karim-Nezhad, 1998). Triton X-114 surfactant exhibits a similar behavior for all the phthalate esters under the given concentration range. Fig.2a shows an increase in the enrichment effect from 27 to 70 min, and then a decrease as the time goes. As a consequence, 60 min was adopted as the optimum equilibration time.

The addition of salt to the solution may influence the extraction process. In the case of most non-ionic surfactant, the presence of salts may facilitate phase separation since it increases the density of the aqueous phase (Carabias-Martinez *et al.*, 2000). Available elec-

trolytes can also change the cloud-point temperatures of nonionic surfactant. The relevant electrolytes are usually in high concentrations (exceeding 0.1 mol/L) (Purkait *et al.*, 2004). The salting-in and salting-out effects can be used to interpret the electrolyte effects on the cloud-points of nonionic surfactant (Pino *et al.*, 2002). In this work, different concentrations of Na_2SO_4 , ranging from 0.2 to 0.6 mol/L were added to the solution. The results are in agreement with other studies. The final surfactant-rich phase volume was not noticeably influenced by the increased ionic strength (Pino *et al.*, 2002). When the concentration is higher than 0.4 mol/L, the surfactant-rich phase will be on the surface of the solution, which may make it more difficult to separate the extraction system into two phases and the accuracy and reproducibility were probably not satisfactory. As shown in Fig.2b, the highest extraction efficiency can be obtained at a concentration of 0.4 mol/L.

When the cloud point extraction procedure was processed at equilibration temperature of the surfactant, the best extraction efficiency was achieved (Raymond *et al.*, 1994). If the temperature is lower than the cloud point, the phase separation is difficult to be formed. In order to obtain the maximum phase separation, the lowest equilibration temperature need to be examined. Theoretically, the optimal equilibration temperature of the extraction occurs when the equilibration temperature is 15–20°C higher than the cloud point temperature of surfactant (Raymond *et al.*, 1994). Fig.2c shows the effects of equilibration temperature on the extraction efficiency. The maximum signals were presented between 40–50°C. Therefore, 45°C was selected as the working equilibration temperature.

2.3 Effect of centrifugation time

The effect of centrifugation time on phase separation was studied in the range of 2–20 min at 3500 r/min. The results showed 5 min is available for a complete phase separation.

2.4 Characteristics of analytical method

Table 1 shows some characteristics of the proposed method. The linearities of the three kinds of phthalate esters were in the range of 8–200 ng/ml for DEP, 10–200 ng/ml for DEHP, 5–200 ng/ml for DCP, respectively. The detection limits based on 3 levels of the ratio of signal to noise were 2.5 ng/ml for DEP, 3.8 ng/ml for DEHP, 1.0 ng/ml for DCP respectively. There is significant

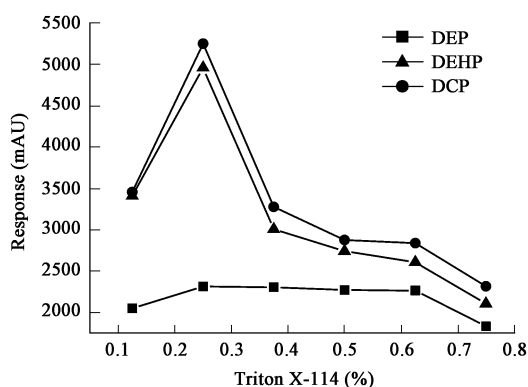


Fig. 1 Extraction effect of the phthalate esters as a function of Triton X-114 concentration.

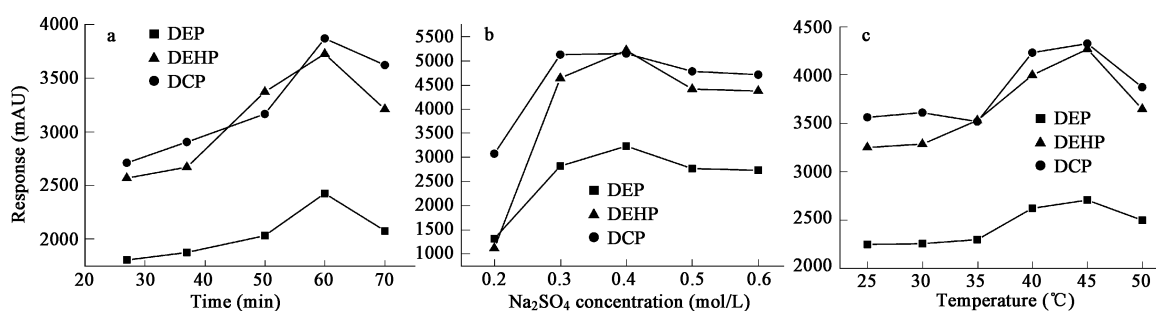


Fig. 2 Effect of the equilibration time (a), ionic strength (b) and temperature (c) on the extraction efficiency.

Table 1 Analytical characteristics of the method

Compounds	Enrichment factor	Detection limit (ng/ml) ^a	RSD (%)	R ²	Linear range (ng/ml)
DEP	35	2.5	3.89	0.9982	8–200
DEHP	88	3.8	3.71	0.9964	10–200
DCP	111	1.0	1.87	0.9998	5–200

^a. Determined as 3 levels of the ratio of signal to noise.

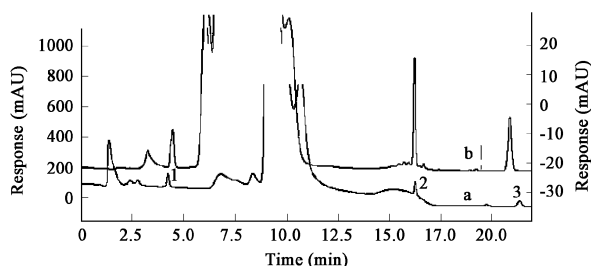


Fig. 3 Chromatogram of the standard solution of the three kinds of phthalate esters. (a) standard solution in the bulk aqueous before enrichment (y-axis on the right). (b) standard solution in the enrichment-phase (y-axis on the left). (1) DEP; (2) DEHP; (3) DCP.

difference on the preconcentration factor between DEP and DEHP or DCP. This can be explained by their hydrophobic properties $K_{o/w}$ (Saitoh *et al.*, 2004). Fig.3 has compared the enrichment efficiency of phthalate esters before and after enrichment as the concentration in aqueous solution is 2.5 $\mu\text{g/ml}$ for DEP, 3.74 $\mu\text{g/ml}$ for DEHP, 3.2 $\mu\text{g/ml}$ for DCP, respectively.

2.5 Analysis of real samples

In order to validate the accuracy and precision of the proposed method under the selected conditions, WWTP effluent water sample, the lixivium of plastic fragments and spiked samples had been tested by HPLC-UV. The results are shown in Table 2.

No PAEs were detected out in the selected real WWTP

Table 2 Determination and recoveries of three compounds in spiked water samples

Compounds	Added conc. (ng/ml)	Detected conc. ^a (ng/ml)	Recovery ^b (%)
Sample 1	DEP	–	nd
		40.0	39.5
			98.7±1.9
DEHP	–	nd	–
	60.0	51.9	86.5±5.0
DCP	–	nd	–
	50.0	49.4	98.8±5.9
Sample 2	DEP	–	nd
		12.5	12.9
		40.0	40.9
		125.0	107.3
			85.8±4.5
	DEHP	–	nd
	18.7	18.9	101.1±9.8
	60.0	58.4	98.0±6.7
	150.0	151.9	101.3±5.3
DCP	–	nd	–
	16.0	16.2	101.3±11.0
	50.0	51.8	101.5±9.0
	128.0	127.4	99.5±4.9

^a Mean for three determinations; ^b mean and standard deviation for three determinations; nd: not detected.

samples. It may be that the PAE concentrations in the real samples were below method detection limits. In all cases, the spiked recoveries were satisfied, showing no obvious matrix interferences.

3 Conclusions

The cloud point technique was applied as an effective method for the extraction of three kinds of PAEs (DEP, DEHP, DCP) in aqueous samples. Experimental results showed that high recoveries and precision can be obtained at the optimized parameters: concentration of Triton X-114, 0.25%; for Na_2SO_4 , 0.4 mol/L; equilibration temperature, 45°C, equilibration time, 60 min and centrifugation time, 5 min. Furthermore, the proposed method is a simple, rapid, and effective for the simultaneous determination of three kinds of phthalate esters with low concentration levels in environmental water.

References

- Bai D S, Li J L, Chen S B *et al.*, 2001. A novel cloud-point extraction process for preconcentrating selected polycyclic aromatic hydrocarbons in aqueous solution[J]. *Environ Sci Technol*, 35: 3936–3940.
- Carabias-Martinez R, Rodriguez-Gonzalo E, Dominguez-Alvarez J *et al.*, 1999. Cloud point extraction as a preconcentration step prior to capillary electrophoresis[J]. *Anal Chem*, 71: 2468–2474.
- Carabias-Martinez R, Rodriguez-Gonzalo E, Moreno-Cordero B *et al.*, 2000. Surfactant cloud point extraction and preconcentration of organic compounds prior to chromatography and capillary electrophoresis[J]. *J Chromatogr A*, 902: 251–265.
- Casero I, Sicilia D, Rubio S *et al.*, 1999. An acid-induced phase cloud point separation approach using anionic surfactants for the extraction and preconcentration of organic compounds[J]. *Anal Chem*, 71: 4519–4526.
- Chen J R, Teo K C, 2001. Determination of cobalt and nickel in water samples by flame atomic absorption spectrometry after cloud point extraction[J]. *Anal Chim Acta*, 434: 325–330.
- Collen A, Persson J, Linder M *et al.*, 2002. A novel two-step extraction method with detergent/polymer systems for primary recovery of the fusion protein endoglucanase I-hydrophobin I[J]. *Biochim Biophys Acta*, 1569: 139–150.
- Delgado B, Pino V, Ayala J H, 2004. Nonionic surfactant mixtures: a new cloud-point extraction approach for the determination of PAHs in seawater using HPLC with fluorimetric detection[J]. *Analy Chim Acta*, 518: 165–172.
- Fernández A E, Ferrera Z S, Rodríguez J J S, 1999. Application of cloud-point methodology to the determination of polychlorinated dibenzofurans in sea water by high-performance liquid chromatography[J]. *Analyst*, 124: 487–491.
- Frankewicht R P, Hinze W L, 1994. Evaluation and optimization of the factors affecting nonionic surfactant-mediated phase separations[J]. *Anal Chem*, 66: 944–954.
- Li J L, Chen B H, 2003. Equilibrium partition of polycyclic aromatic hydrocarbons in a cloud-point extraction process[J]. *J Colloid Interf Sci*, 263: 625–632.
- Li X J, Zeng Z R, Chen Y *et al.*, 2004. Determination of phthalate acid esters plasticizers in plastic by ultrasonic solvent

- extraction combined with solid-phase microextraction using calix[4]arene fiber[J]. *Talanta*, 63: 1013–1019.
- Manzoori J L, Karim-Nezhad G, 1998. Determination of polychlorinated biphenyls by liquid chromatography following cloud-point extraction[J]. *Anal Chim Acta*, 358: 145–155.
- Manzoori J L, Karim-Nezhad G, 2003. Selective cloud point extraction and preconcentration of trace amounts of silver as a dithizone complex prior to flame atomic absorption spectrometric determination[J]. *Anal Chim Acta*, 484: 155–161.
- Pino V, Ayala J H, Afonso A M *et al.*, 2002. Determination of polycyclic aromatic hydrocarbons in seawater by high-performance liquid chromatography with fluorescence detection following micelle-mediated preconcentration[J]. *J Chromatogr A*, 949: 291–299.
- Pinto C G, Luis J, Pavon P *et al.*, 1992. Cloud point preconcentration and high-performance liquid chromatographic analysis with electrochemical detection[J]. *Anal Chem*, 64: 2334–2338.
- Polo M, Llompert M, Garcia-Jares C *et al.*, 2005. Multivariate optimization of a solid-phase microextraction method for the analysis of phthalate esters in environmental waters[J]. *J Chromatogr A*, 1072: 63–72.
- Prokupková G, Holadová K, Poustka J *et al.*, 2002. Development of a solid-phase microextraction method for the determination of phthalic acid esters in water[J]. *Anal Chim Acta*, 457: 211–223.
- Purkait M K, DasGupta S S, De S, 2004. Separation of congo red by surfactant mediated cloud point extraction[J]. *Dyes and Pigments*, 63: 151–159.
- Rodenbrock A, Selber K, Egmond M R *et al.*, 2000. Extraction of peptide tagged cutinase in detergent-based aqueous two-phase systems[J]. *Bioseparation*, 9: 269–276.
- Saitoh T, Hime W L, 1991. Concentration of hydrophobic organic compounds and extraction of protein using alkylammonio-sulfate zwitterionic surfactant mediated phase separations (Cloud Point Extractions)[J]. *Anal Chem*, 63: 2520–2525.
- Saitoh T, Matsushima S, Hiraide M, 2004. Aerosol-OT- γ -alumina admicelles for the concentration of hydrophobic organic compounds in water[J]. *J Chromatogr A*, 1040: 185–191.
- Shemirani F, Baghdadi M, Ramezani M *et al.*, 2005. Determination of ultra trace amounts of bismuth in biological and water samples by electrothermal atomic absorption spectrometry (ET-AAS) after cloud point extraction[J]. *Anal Chim Acta*, 534: 163–169.
- Sirimanne S R, Barr J R, Patterson D G, 1996. Quantification of polycyclic aromatic hydrocarbons and polychlorinated dibenzo-*p*-dioxins in human serum by combined micelle-mediated extraction (cloud-point extraction) and HPLC[J]. *Anal Chem*, 68: 1556–1560.
- Toerne K, Rogers R, Wandruszka R, 2000. Fluctuating clouding behavior in mixed micellar solutions[J]. *Langmuir*, 16: 2141–2144.
- Wezel A P, Vlaarding P, Posthumus R *et al.*, 2000. Environmental risk limits for two phthalates, with special emphasis on endocrine disruptive properties[J]. *Ecotoxicol Environ Saf*, 46: 305–321.
- Yuan S Y, Liu C, Liao C S *et al.*, 2002. Occurrence and microbial degradation of phthalate esters in Taiwan river sediments[J]. *Chemosphere*, 49: 1295–1299.
- Yuan C G, Jiang G B, He B *et al.*, 2005. Preconcentration and determination of tin in water samples by using cloud point extraction and graphite furnace atomic absorption spectrometry[J]. *Microchim Acta*, 150: 329–334.