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# Coacervative Extraction of Phthalates from Water and Their Determination by High Performance Liquid Chromatography

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A simple, rapid and low cost method for determination of phthalic acid esters (PAEs) including Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Di-n-butyl phthalate (DBP) and Butylbenzyl phthalate (BBP) in water samples was investigated. The method is based on the extraction of PAEs with coacervate made up of decanoic acid reverse micelles and the subsequent determination by HPLC-UV. Effect of parameters such as concentration of tetrahydrofuran (THF) (2-40% v/v) and decanoic acid (20-400 mg in 40 ml total volume), ionic strength (0.0-0.1 M NaCl), pH (1-4) and stirring time (2-60 min) on recoveries (Rs) and enrichment factors (EFs) were investigated and optimized. The optimum condition for extraction was the stirring of 36 ml of water sample with 4 ml of THF containing 100 mg of decanoic acid for 10 min and its centrifugation (10 min, 3500 rpm). Recoveries and enrichment factors of PAEs mainly depended on the amount of decanoic acid and THF making up the coacervate and were not affected by ionic strength of the sample solution (up to 0.1 M of NaCl), pH (1-4), and stirring time (2-60 min). Recoveries, enrichment factors, LODs and relative standard deviations (RSD%) for PAEs were between 87-94%, 187-202, 0.22-0.30 μg $l^{-1}$ and 2-5%, respectively. This method was applied to determine PAEs in tap water, river water, and sea water samples. No PAEs were found in tap water. The amount of DMP and DEP in the Babolrood River was 0.87 and 0.67 μg $l^{-1}$, while in the Caspian Sea was 0.49 and 0.52 μg $l^{-1}$, respectively.

**Keywords:** Coacervation, High performance liquid chromatography, Phthalate, Reverse micelle, Water analysis

## INTRODUCTION

Phthalic acid esters (PAEs), simply known as phthalates, are chemical compounds widely used as plasticizers giving plastics flexibility and durability. World production of these compounds is estimated to be several million tons per year. A vast amount of this, about 90%, is used for polyvinyl chloride (PVC)-based plastics [1,2]. Industrial applications of PVC-based plastics include coatings, plumbing, construction materials and the manufacture of common plastic products such as vinyl upholstery, tablecloths, and shower curtains. PAEs are also present in plastic products for human use, *e.g.* teething rings, pacifiers, soft squeeze toys, plastic bottles, and enclosures for food containers and in medical products, e.g. flexible devices for administering parenteral solutions, blood bags, and vinyl gloves [3]. Phthalates can be easily released and transferred from plastics to the environment because they are not chemically bound to the plastics. Hence, significant migration of them into the environmental compartments is possible during their production, manufacture, use and disposal [4]. Taking into account all these considerations, the development of reliable analytical methods to analyze phthalates from different water samples is necessary. The most common technique used for determination of PAEs in

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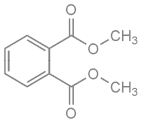
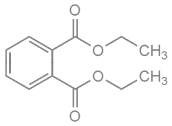
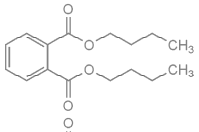
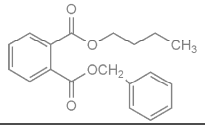
environmental samples has been gas chromatography with electron capture [5,6] or mass spectrometry detection [7-12]. Trace level existence of PAEs in the complex matrix of environmental samples makes the sample preconcentration step cricial for a reliable determination of these compounds. The preconcentration techniques which are commonly applied to determine phthalates in water are liquid-liquid extraction (LLE) [13,14], and solid-phase extraction (SPE) [12,15-23]. Solid-phase microextraction (SPME) has been extensively investigated to simplify sample treatment prior to GC analysis of PAEs [6-8,24-26]. Reversed-phase liquid chromatography with UV or MS detection combined with SPE [18-21] and liquid-phase microextraction (LPME) [27] have been used for PAEs analysis. Materials used as sorbents include silica-based $C_{18}$ [16] and $C_8$ [17], organic polymers [18,19], carbon nanotubes [20], microorganisms (*Saccharomyces cerevisiae*) immobilized on silica gel [21] and supramolecular assemblies called hemimicelles and admicelles [22,23]. Coacervates are water immiscible liquids that are separated from colloidal solutions by the action of a dehydrating agent, namely temperature, pH, electrolyte or a non-solvent for the macromolecule [28]. After separation phase, the coacervate contains most of the colloid and is in a dynamic equilibrium with the initial solution. In micelle-mediated extractions, the aqueous sample solution is made colloidal by the addition of surfactants at concentrations above their critical micelle concentration (CMC). So, the coacervate, that is, the extractant, is produced in situ in the bulk sample solution. The most frequently used surfactant aggregates in micelle-mediated extractions have been aqueous non-ionic [29-31], amphoteric [32], anionic [33] and cationic micelles [34]. Recently, coacervates made up of vesicles [35,36] and reversed micelles of alkyl carboxylic acids [37] have been reported, which permit the extraction of analytes in a wide polarity range. This work deals with the extraction of four phthalates, characterized in Table 1, using coacervate made up of decanoic acid reverse micelles and their subsequent determination by HPLC-UV in water samples. Parameters affecting the extraction recovery and the enrichment factor were investigated and the method was applied to the determination of phthalates in different real water samples.

## EXPERIMENTAL

### Reagents and Sample Preparation

All standards of phthalates (purity range 98-99%) were supplied by Alfa Aesar (Karlsruhe, Germany). Analytical grade tetrahydrofuran (THF) was supplied by Merck (Darmstadt, Germany) and was distilled before use to remove the BHT stabilizer. Decanoic acid and HPLC-grade

**Table 1.** Physicochemical Properties of the Selected Phthalates

| Name | Structure | Molecular weight | Solubility in water (mg $l^{-1}$) | $logK_{ow}$ [a] |
|---|---|---|---|---|
| Dimethyl phthalate (DMP) |  | 194.2 | 4200 | 1.61 |
| Diethyl phthalate (DEP) |  | 222.2 | 1100 | 2.38 |
| Di-n-butyl phthalate (DBP) |  | 278.4 | 11.2 | 4.45 |
| Butylbenzyl phthalate (BBP) |  | 312.4 | 2.7 | 4.59 |

[a]Octanol-water partition coefficient.

acetonitrile were obtained from Fluka (Buchs, Switzerland). Deionized doubly distilled water was used throughout the experiment. A stock standard solution containing a mixture of PAEs, 1000 mg $l^{-1}$ each, was prepared in acetonitrile and stored under dark conditions at 4 °C. Working solutions were made by the appropriate dilution of the stock solution.

River and sea water samples were taken from the Babolrood River and the Caspian Sea respectively (Babolsar, Mazandaran province) in the north of Iran. Tap water sample was taken from our lab in Babolsar. All water samples were filtered through a Millipore membrane filters (0.45 µm pore size) immediately after sampling in order to remove suspended solids. The filtered samples were adjusted to pH 2 with 1 M $HNO_3$ and stored under dark conditions at 4 °C until analysis.

### Apparatus

The chromatographic measurements were carried out with HPLC system equipped with a series 10 LC pumps, UV detector model LC-95 set at 286 nm, and model 7125 manual injector with a 10 µl sample loop all from Perkin-Elmer (Norwalk, CT, USA). Separation was done by an isocratic elution on a $C_{18}$ (250 × 4.6 mm, 5 µm) column from Waters (Milford, MA, USA). Mobile phase was a mixture of acetonitrile and water (65:35, v/v) with flow rate of 1.0 ml $min^{-1}$. A Hettich Rotanta centrifuge (Tuttlingen, Germany) was used for sample preparation. Adjustment of pH was made by model 3030 Jenway pH meter (Leeds, UK). Handmade centrifuge tubes with narrow necks (~7 mm i.d.), which were specially designed for easing of withdrawing coacervate phase after measuring its volume, were used for extraction. Measurement of coacervate volume at the narrow neck of the tube was made with a digital caliper.

### Extraction Procedure

Water sample (36 ml) adjusted at pH 2 was added into the handmade centrifuge tube containing 100 mg decanoic acid dissolved in 4 ml THF. Addition of water sample induced formation of water immiscible coacervate made up of decanoic acid reverse micelles. The mixture was stirred for 10 min with a magnetic stir bar to enhance the extraction rate of phthalates, and then centrifuged (3500 rpm, 10 min) to speed up the separation of the coacervate phase from the bulk solution. The height of coacervate standing at the top of the solution in the narrow neck of the tube was measured by a digital caliper for the subsequent calculations of the volume of the coacervate and phase volume ratio (the ratio of the water sample volume over the coacervate volume). Eventually, aliquots of the coacervate were withdrawn using a microsyringe and directly injected into the HPLC-UV system for analysis.

## RESULTS AND DISCUSSION

### Investigation of Parameters Affecting Extraction Recovery and Enrichment Factor

Effects of experimental parameters including concentration of THF (2-40% v/v) and decanoic acid (20-400 mg in 40 ml total volume), ionic strength (0.0-0.1 M NaCl), pH (1-4) and extraction time (2-60 min) on recoveries (Rs) and enrichment factors (EFs) were evaluated. All the extractions were carried out according to section 2.3 using aqueous standard solution containing 100 µg $l^{-1}$ of PAEs. The recovery percentage can be expressed by

$$R(\%) = \frac{D \times 100}{D + (V_{aq}/V_c)} \quad (1)$$

where D is distribution coefficient and $V_{aq}$ and $V_c$ are the volumes of aqueous solution and coacervate phase obtained after the extraction step, respectively. Enrichment factor (EF), defined as the ratio of analyte concentration in coacervate to original sample was used as a criterion for the selection of the experimental conditions as follows:

$$EF = \frac{R(\%)V_o}{100V_c} \quad (2)$$

where $V_o$ is the volume of original aqueous solution prior to the extraction step.

**Effect of THF and decanoic acid concentration.** Concentration of both decanoic acid and THF as the main components making up the coacervate was found to be a highly influential parameter on recoveries and enrichment factors. For all the PAEs good recoveries were obtained at decanoic acid amounts between 90-400 mg in 40 ml of the total volume (Fig. 1). Since the volume of coacervate increased with decanoic acid concentration, a 100 mg of

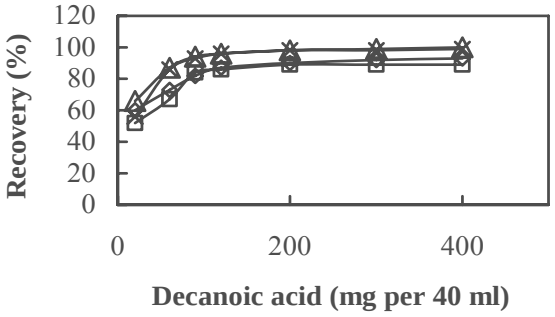



**Fig. 1.** Effect of decanoic acid concentration on recovery of PAEs. THF = 20%; pH = 2; stirring time: 10 min (700 rpm); centrifugation: 3500 rpm, 10 min. (◇) DMP, (□) DEP, (△) DBP, (×) BBP.

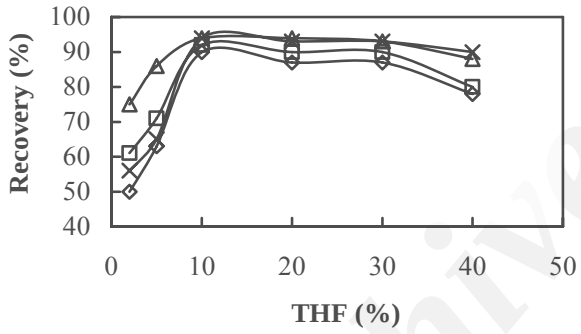



**Fig. 2.** Effect of THF percentage (v/v) on recovery of PAEs. Decanoic acid = 100 mg; other conditions as Fig. 1. (◇) DMP, (□) DEP, (△) DBP, (×) BBP.

decanoic acid was selected as the optimal condition for further experiments.

The influence of THF concentration (2-40% v/v) on recoveries of PAEs (Fig. 2) shows that the maximum recoveries were obtained at 10% of THF for all PAEs and was selected as the optimal condition for the extraction of the compounds from water samples. Dissolution of a portion of coacervate phase in the THF/water bulk solution occurs at high THF percentages [37]. So the lower recoveries at percentages higher than 10% THF are probably due to a change in the composition of coacervate.

**Effect of ionic strength, pH and extraction time.** The influence of ionic strength was examined by determining recoveries and enrichment factors of PAEs at different concentrations of NaCl (0.0-0.1 M). Results indicated that the addition of salt did not affect the volume of coacervate phase and recovery percentages.

Coacervation process occurs only in solutions containing protonated decanoic acid molecules ($pK_a$ = 4.8 ± 0.2) [37]. Thus the effect of pH was examined by varying pH between 1 and 4. Recoveries of PAEs and phase volume ratios obtained were not affected at pH range of 1-4. So pH 2 was used for the extraction of PAEs from water sample solutions.

The efficiency of micelle-mediated extractions based on non-ionic surfactants has been reported to depend on the time that analytes interact with micelles and get into their core [38]. In order to determine the effect of this parameter on recoveries and enrichment factors of PAEs the following experiment was carried out. The mixture containing the bulk sample solution and the coacervate phase were mixed by magnetic stirrer (700 rpm) before centrifugation (3500 rpm, 10 min). Changing the stirring time between 2 and 60 min had no significant effect on recoveries and enrichment factors for all the PAEs.

## Figure of Merits and Application of the Method

Limits of detection (LOD), linear range and relative standard deviation (RSD%, n = 6) were obtained in terms of peak area for PAEs in water samples (Table 2). Limit of

**Table 2.** Analytical Performance for Determination of PAEs in Water Samples

| PAEs | LOD ($\mu g\ l^{-1}$) | Linear range ($\mu g\ l^{-1}$) | Linear equation | $R^2$ | (RSD%) (n = 6) |
|---|---|---|---|---|---|
| DMP | 0.22 | 0.5-100.0 | y = 27.454x + 0.0109 | 0.9993 | 2.1 |
| DEP | 0.23 | 0.5-100.0 | y = 24.470x + 0.1263 | 0.9969 | 3.7 |
| BBP | 0.28 | 0.5-100.0 | y = 24.476x + 0.0877 | 0.9977 | 4.9 |
| DBP | 0.30 | 0.5-100.0 | y = 20.036x + 0.5339 | 0.9991 | 5.1 |

detection was calculated on the basis of $3S_b/m$, where $S_b$ is the standard deviation of blank and is equal to P-P noise when only mobile phase was passing through the column for 45 min and $m$ is the slope of calibration curve. All measurements were made by standard addition method using calibration curves of 0.5-4.0 μg $l^{-1}$ spiked samples. Determinations of phthalates in different water samples were assessed whose results are shown in Table 3. The accuracy of the method was evaluated by a recovery test carried out with PAEs-spiked water samples. Recoveries were between 87 and 94% for the four PAEs. Figure 3 shows the chromatograms obtained for standard solution, river water and sea water samples.

**Table 3.** Recoveries and Concentrations of PAEs in Real Water Samples (n = 3)

| Water sources | Analytes | Original sample (μg $l^{-1}$) | Spiked sample | | |
|---|---|---|---|---|---|
| | | | Added (μg $l^{-1}$) | Found (μg $l^{-1}$) | Recovery ± SD (%) |
| Tap water | DMP | nd[a] | 1.0 | 0.89 | 89 ± 3 |
| | DEP | nd | 1.0 | 0.92 | 92 ± 3 |
| | BBP | nd | 1.0 | 0.94 | 94 ± 5 |
| | DBP | nd | 1.0 | 0.93 | 93 ± 4 |
| River water | DMP | 0.87 | 1.0 | 1.74 | 87 ± 2 |
| | DEP | 0.67 | 1.0 | 1.57 | 90 ± 3 |
| | BBP | nd | 1.0 | 0.90 | 90 ± 5 |
| | DBP | nd | 1.0 | 0.91 | 91 ± 5 |
| Sea water | DMP | 0.49 | 1.0 | 1.40 | 91 ± 2 |
| | DEP | 0.52 | 1.0 | 1.42 | 90 ± 4 |
| | BBP | nd | 1.0 | 0.92 | 92 ± 5 |
| | DBP | nd | 1.0 | 0.93 | 93 ± 5 |

[a]Not detected.

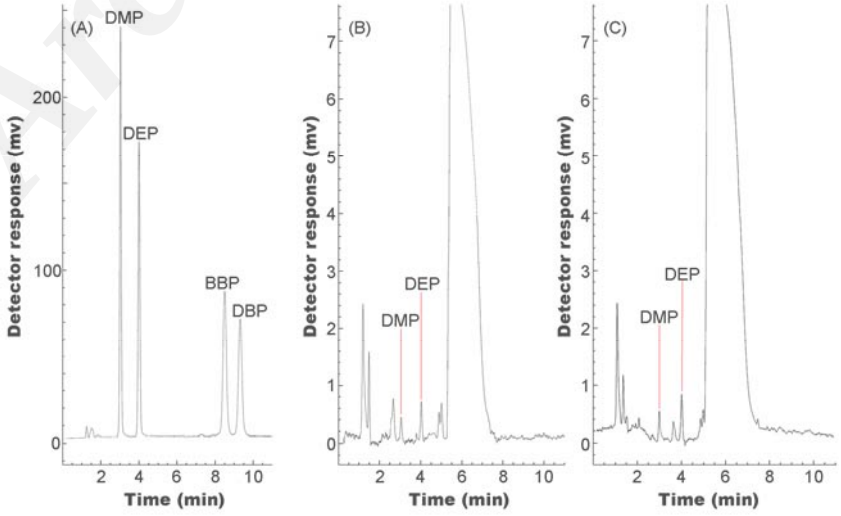



**Fig. 3.** Chromatograms of (A) standard solution of PAEs, (B) the Caspian Sea water and (C) the Babolrood River water samples. Column: $C_{18}$ (250 × 4.6 mm, 5 μm); Mobile phase: acetonitrile/water (65:35, v/v), Flow rate: 1.0 ml $min^{-1}$; λ = 286 nm; room temperature.

## CONCLUSIONS

Extraction of PAEs from water samples prior to their determination by HPLC was performed using coacervate made up of decanoic acid reverse micelles. The most influential parameters on the extraction recovery and enrichment factor were the concentrations of decanoic acid and tetrahydrofuran. The method has advantages that make it robust in routine monitoring of phthalates in water samples. The procedure is simple (treatment of samples only require the extraction of PAEs for 10 min and no clean-up of extracts or solvent evaporation are necessary) and rapid (each complete extraction procedure takes about 15-20 min and several samples can be simultaneously extracted, so sample throughput will be dependent mainly on the chromatographic analysis of the target compounds). It also requires low volume sample (36 ml), features low cost (no special equipment is required for extraction), and achieves enrichment factors of 187-202 with the detection limits around 0.22-0.30 μg $l^{-1}$ for PAEs which is comparable with the other pre-concentration methods like SPE.

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