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Time-resolved immunoassay based on magnetic particles for the detection of diethyl phthalate in environmental water samples

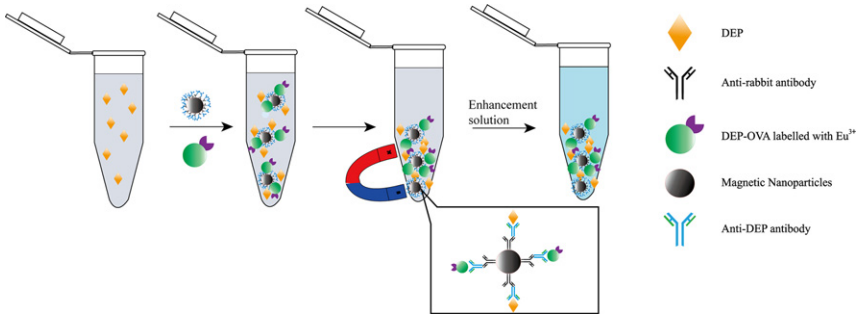
Fang Zhu [a], Chaoming Mao [b], Daolin Du [a,*]

[a] School of the Environment and Safety Engineering, Institute of Environment and Ecology, Jiangsu University, Zhenjiang 212013, China
[b] Department of Nuclear Medicine, The Affiliated Hospital of Jiangsu University, Zhenjiang 212013, China

HIGHLIGHTS

- Oriently immobilization was applied to assembly antibody on MPs, which reduced the inactivation of antibody.
- The developed TRFIA based on MPs showed high sensitivity and less time-consuming.
- DEP was widely present in environmental water from Zhenjiang City.

GRAPHICAL ABSTRACT



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ABSTRACT

Diethyl phthalate (DEP) is an extensively used phthalic acid diester (PAEs) with estrogenic activity and the potential for carcinogenic and teratogenic effects. To monitor trace DEP in environmental waters, a sensitive direct competitive time-resolved fluoroimmunoassay based on magnetic particles (MPs) as solid support was established. For the assay system, the anti-DEP antibody was oriented on the surface of the MPs using goat anti-rabbit antibody as linkers, and DEP-OVA was labeled using Eu^{3+}. Several physicochemical factors that potentially influence the assay performance of the proposed method were investigated in detail, including concentration of MPs, dilution of DEP-OVA-Eu^{3+} and incubation time. Under the optimized conditions, the method showed: (i) low limit of detection (LOD) of 5.92 ng/L; (ii) satisfactory accuracy (recoveries, 91.97–134.54%) with good reproducibility (inter-CV, 4.17–9.17%; intra-CV, 7.41–14.72%). All of which indicated that the newly established method had much higher efficiency and great potential for use in environmental water analysis for DEP. In addition, the proposed immunoassay was applied for investigation of DEP in aquatic environments at Zhenjiang City. Our results showed that DEP was detected at the concentration of 2.98–65.18 ng/mL in river samples and 46.95–306.19 ng/mL in wastewater treatment plants (WWTPs), which showed rather high concentrations compared with reported data. Our study provides background data important for risk assessment and contamination control of DEP in the aquatic environment of this area.

1. Introduction

Diethyl phthalate (DEP) is widely used as an additive in flexible polyvinyl chloride products and denaturants or solvents in perfumes, cosmetics, clothing, insect repellent, and medication coating (Schettler, 2006). As a result, this chemical is discharged into aquatic

* Corresponding author.
E-mail address: ddl@ujs.edu.cn (D. Du).

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environments and results in serious consequences to eco-environmental systems because of its estrogenic activities, and potential for carcinogenic and teratogenic effects (Al-Saleh et al., 2016; Jia et al., 2016). According to the Standards for Drinking Water Quality (GB 5749-2006) in China, the maximum limit of DEP in drinking water is set at 0.3 mg/L. Therefore, it is necessary to develop a method suitable for monitoring DEP in environmental waters.

In fact, some approaches have been established for determination of the pollutant from environmental water samples, including gas chromatography (GC) (Batlle and Nerin, 2004; Fierens et al., 2012), high-performance liquid chromatography (HPLC), (Liang et al., 2008) and gas chromatography–mass spectrometry (GC–MS) (Schecter et al., 2013). Based on these methods, DEP was found in a variety of waters, such as industrial effluent, groundwater, and drinking water (Liu et al., 2013; Net et al., 2014; Selvaraj et al., 2015). However, these instrumental methods are costly and time-consuming, and they require sophisticated sample pretreatments for purification and enrichment. As an alternative, immunoassay could be applied for rapid screening of the organic component, offering low cost, high throughput, high sensitivity, and simple preparation of samples (Sun and Zhuang, 2015; Zhang and Sheng, 2010; M.C. Zhang et al., 2013). We also established indirect enzyme-linked immunosorbent assays (ELISA) to detect DEP in river water (Zeng et al., 2016). However, the sensitivity of the common immunoassays cannot meet the requirements of analysis of trace DEP in various environmental waters. This drawback limited their application in systematic investigations of the concentration and distribution of DEP, and the related risk assessment in aquatic environments. Therefore, more sensitive immunoassays are needed.

The sensitivity of fluoroimmunoassay has been improved several orders of magnitude compared with conventional ELISA. Owing to the natural fluorescence in biological samples (e.g., proteins, solutions, and solid matrix), conventional fluorescent probes suffer from serious limitations of sensitivity. The fluorescence decay time of lanthanide chelates is on the order of 10 to 1000 μs; whereas the decay time of natural fluorescence in a biological sample is on the order of 1 to 20 μs (Yuan and Matsumoto, 1998). Therefore, time-resolved fluoroimmunoassay is considered one of the most sensitive classical immunoassays (Huang et al., 2009; Lin et al., 2014). The virtues of lanthanide chelates as labels, include long decay lifetimes, large Stokes shift, high quantum yields, and a sharp emission profile (Meriö et al., 1996). Our group also proved that the method had good application for determination of organic pollutants in water samples and adequate tolerance against environmental interference (Z. Zhang et al., 2013; Zhang et al., 2010). At the same time, to further improve the limit of detection of the immunoassay, magnetic particles (MPs) were introduced for enrichment of the target in a sample pretreatment step. One of a set of magnetic separation techniques widely used since the 1970s (Cao et al., 2006), MPs can be functionalized with different reactive groups (e.g., amines, carboxyls, epoxyls) by which biologically active molecules could be loaded onto the particles. With their paramagnetic properties, the targets can be captured and separated easily, which avoids complicated sample pretreatment and thus reduces the time required for separation (Aguilar-Arteaga et al., 2010).

The objectives of the work reported in this paper were (i) to develop a direct competitive TRFIA using MPs as solid support to detect DEP in environmental waters; (ii) to evaluate the established approach; and (iii) to investigate DEP in regional aquatic environments using the proposed method.

2. Materials and methods

2.1. Reagents and materials

Bovine serum albumin (BSA), ovalbumin (OVA), Sephadex G-50, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimidehydrochloride (EDC), and goat anti-rabbit antibody were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-DEP polyclonal antibody was developed by our lab. DEP, Dibutyl phthalate (DBP), Dimethoxyethyl phthalate (MEP), Benzyl butyl phthalate (BBP), Monoethylhexyl phthalat (MEHP), Diisooctyl(*o*-)phthalate (DiOP), Monobutyl phthalate (MBP), Di-*n*-octyl phthalate (DnOP) used was from the Beijing Beina Chuanglian Biotechnology Institute (Beijing, China). *N′*-[*p*-isothiocyanatobenzyl]-diethylenetriamine-N1, N2, N3, N4-tetraacetate-Eu^{3+} (DTTA-Eu^{3+}) was obtained from Tianjin Radio-Medical Institute (Tianjin, China). The enhancement solution for Eu^{3+} dissociation was purchased from PerkinElmer Wallac (Turku, Finland). Magnetic particles (5 μm, COOH-modified) were from Suzhou Vdo Biotechnology Co., Ltd. (Suzhou, China). Other chemicals were of analytical grade or above and were supplied by Beijing Reagent Corporation (Beijing, China). The fluorescence signal was measured with a multi-function microplate reader (BioTek Instruments, Inc. Winooski, VT).

2.2. Immuno-magnetic bead preparation

The anti-DEP antibody was immobilized onto carboxylated MPs using goat anti-rabbit antibody as linker. Briefly, 1 mg MPs was washed twice using 0.1 mol/L MES (pH 5.0) and then re-suspended in 1 mL MES buffer. Then, 5 mg EDC was added and gently stirred at room temperature for 30 min. Next, 200 μg goat anti-rabbit antibody was dropped into this mixture and incubated at 4 °C overnight. After this step, the second antibody modified with MPs was washed with 50 mmol/L Tris-HCL (pH 7.0). Finally, the anti-DEP antibody was incubated with MPs obtained above at room temperature. The final product was stored in 50 mmol/L Tris-HCL (pH 7.0) containing 0.5 mol/L sucrose and 1.5 mmol/L BSA.

2.3. Preparation of DEP-OVA

To bind with protein, DEP must be derivatized to obtain active groups, such as amino or carboxy groups. Diethyl 4-aminophthalate (DEAP) was synthesized as Yanaihara and coworkers described (Yanaihara et al., 2002). Then DEAP was conjugated to OVA via an amino diazotization linkage according to Zeng (Zeng et al., 2005). Briefly, 10 mg DEAP was dissolved in 5 mL HCL (2 mmol/L) in an ice bath and 0.1 mol/L $NaNO_2$ solution was added. After stirring for 30 min, the excess $NaNO_2$ was removed with urea, then 10 mg OVA in 0.1 mol/L borate buffer (pH 8.5) was added and stirred in the ice bath for 8 h. The brown-red product was dialyzed against 0.01 mol/L Tris buffer (pH 7.4) and stored at −20 °C.

2.4. Labeling of DEP-OVA with Eu chelate

First, 1 mg of DEP-OVA was dissolved in 1 mL carbonate buffer (0.05 mol/L, pH 8.5) and then 0.2 mg of DTTA-Eu^{3+} was added. After being incubated at 4 °C overnight, free chelates were separated from the labeled proteins using a Sephadex G-50 column. Determined by spectrophotometer at 280 nm, the concentration of DEP-OVA-Eu^{3+} was 0.55 mg/mL. DEP-OVA-Eu^{3+} was stored in 0.01 mol/L Tris buffer (pH 7.4) at 4 °C.

2.5. MP-based TRFIA procedure

The proposed TRFIA immunoassay for the quantitation of DEP was performed based on a direct competitive type immunoassay format with immuno-magnetic separation. Initially, 100 μL of standard in ultra-pure water or sample, and 100 μL of DEP-OVA-Eu^{3+} in Tris buffer (0.01 mol/L, pH 7.4) were added to a 1.5 mL tube containing 50 μL of antibody-assembled MPs in Tris buffer (0.01 mol/L, pH 7.4) and the mixtures were subsequently incubated for 45 min at room temperature with gentle stirring. Using a magnetic separation device, the immune complexes were separated and washed three times with 50 mmol/L Tris-HCl (pH 7.4) containing 0.15 mol/L NaCl and 0.05% Tween-20.