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Development of an optical fiber immunosensor for the rapid and sensitive detection of phthalate esters

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ABSTRACT

A rapid and sensitive detection method for phthalate esters (PAEs) was developed by using an optical fiber immunosensor based on indirect competitive immunoassay. This sensor was constructed by covalent binding of coating antigen on the surface of optical fiber, and the inhibition signal of PAEs to immune reaction between coating antigen and fluorescent-labeled antibody was detected by avalanche photodiode. The response signal showed a negative correlation with the logarithm of PAEs concentration in the range of 0.01–100 μg/L, and the limits of detection (LODs) of eight PAEs ranged from 19 to 51 ng/L. The fluorescent-labeled antibody displayed broad cross-reactivity in the range of 16.63%–71.94% for the other seven PAEs (100% for dimethyl phthalate). The average recoveries of PAEs at the spiked levels of 5, 10 and 100 μg/kg ranged from 61.5% to 106.7% with relative standard deviation (RSD) values below 13.41%, and these results were consistent with those obtained by gas chromatography-mass spectrometry (GC–MS). The optical fiber immunosensor has good regeneration performance, reproducibility and stability, and displays wide linear range, high sensitivity, simple sample pretreatment and short detection time for PAEs detection. The new method has been successfully applied to the determination of multiple PAEs in greenhouse soils.

1. Introduction

In recent years, plasticizer contamination happened in Taiwan and China mainland has attracted much attention in the world. As a kind of synthetic chemical substances, phthalate esters (PAEs) are widely used as plasticizer to increase the fexibility and durability of industrial products, such as building materials, medical devices, furniture, cosmetics, children's toys, food packaging, and cleaning materials [1,2]. The global yield of plastics was reported to reach about 150 million tons every year [3]. Since PAEs are only physically bound to the polymer chain of polyvinyl chloride (PVC) plastics, they may release into air, water, soil and foodstuffs [4]. PAEs have been widely detected in the environment [5,6], cosmetics [7] and foods [8,9]. Due to their endocrine-disrupting and toxic effects on human body [10,11], PAEs have been listed in priority pollutants by USA, European Union and China. Therefore, it is of great significance to accurately detect PAEs for protecting ecological environment and human health.

At present, some instrumental analytical methods have been developed for the detection of PAEs, such as gas chromatography [4,12], high performance liquid chromatography [13], gas chromatography-mass spectrometry [14,15], and liquid chromatograph-tandem mass spectrometry [16]. Although these techniques are accurate and sensitive, they usually require expensive equipments and cumbersome sample extraction and cleanup procedures. Fortunately, immunoassay is generally accurate, highly sensitive, highly selective and cost-efficient. Recently, some enzyme linked immunosorbent assay (ELISA) methods have been proposed for PAEs detection [17,18]. We have also developed an indirect competitive ELISA method for sensitive detection

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of multiple PAEs [19]. However, these immunoassay techniques may consume a large amount of reagents, and require long analytical time with heavy manual labor. In recent years, some chemical sensors have been constructed to detect PAEs by using magnetic molecularly imprinted polymers [20–22], conductive polymer [23], molecular imprinted photonic crystal [24], and nanostructured nickel hydroxide [25] as sensing elements, which have displayed excellent sensitivity, especially for dibutyl phthalate. Nevertheless, it is still cumbersome and time-consuming to prepare these sensing elements and construct chemical sensors. Recently, Liu et al. [26] used AuPt-graphene sheet to immobilize dimethyl phthalate antibody (anti-DMP) and platinum-lead hollow nanoparticles as signal tags, and developed a sensitive and reliable electrochemical immunosensor to detect DMP in water samples. However, this immunosensor requires expensive materials, and the preparation of AuPt-graphene sheet and signal tags also involves many complicated operation procedures.

Recently, some photoelectrochemical immunosensors have been developed for the detection of α-fetoprotein, Aflatoxin B_1, prostate specific antibody and carcinoembryonic antigen by Tang's group [27–31]. These sensors utilized the optical and electrochemical properties of sensitive materials, and have displayed the advantages of high sensitivity, high reliability, good portability, and low cost. Kronick and Little [32] first developed an immunoassay technique which used the internally reflected light to excite the fluorescence of fluorescent-labeled antibody. Afterwards, the optical fiber immunoassay has been proposed through modifying antigen or fluorescent dye-labeled antibody on the surface of optical fiber, which is one of new immunological measurement techniques. Several investigations [33–35] have already been demonstrated to determine microcystin-LR (MC-LR), 2, 4-dichlorophenoxyacetic acid (2, 4-D), bisphenol A (BPA), and melamine in water samples using the fluorescent immunoassay. Hao et al. [36] have also constructed an optofluidics-based biosensing platform to detect sulfadimidine in dairy products. In recent years, optical fiber immunosensors have received widespread attention due to their promising advantages, such as high sensitivity, cost-effectiveness, and real-time and rapid detection [37–39]. On the optical fiber immunosensing platform, both the transmission of the excitation light and the collection of the fluorescence signal are achieved by optical fiber using a specific optical fiber coupler, and display great potential for the rapid detection of small analytes in disease diagnosis, food inspection, drug discovery and environmental monitoring. To the best of our knowledge, the detection of PAEs using optical fiber immunosensor has seldom been reported. In this study, we modified coating antigen on the surface of optical fiber to construct a simple and convenient optical fiber immunosensing platform for rapid and sensitive detection of trace PAEs. The developed immunosensor was successfully applied to the determination of multiple PAEs in greenhouse soils.

2. Materials and methods

2.1. Chemicals and materials

General reagents and organic solvents were of analytical grade unless specified otherwise. Standard substances: dimethyl phthalate (DMP, ≥ 99.5%), diethyl phthalate (DEP, ≥ 99.5%), dibutyl phthalate (DBP, ≥ 99%), butyl benzyl phthalate (BBP, ≥ 99.5%), diethylhexyl phthalate (DEHP, ≥ 98.5%), dicyclohexyl phthalate (DCHP, ≥ 99.5%), di-*n*-octyl phthalate (DnOP, ≥ 98.5%), dinonyl phthalate (DNP, ≥ 98.5%), diisobutyl phthalate (DIBP, ≥ 99%), diisononyl phthalate (DINP, ≥ 99%), and benzo[a]pyrene (BaP, ≥ 99.5%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). 3-Mercaptopropyl-trimethoxysilane (MTS) and *N*-(4-maleimidobutyryloxy)succinimide (GMBS) were purchased from Sigma-Aldrich (Steinheim, Germany), and bovine serum albumin (BSA) from Sigma (St. Louis, MO, USA). The fluorescein Cy5.5 was obtained from Tianjin Biolite Biotech Co., and pepsin from Chengdu Changzheng Chemical Reagent Co., China. All other reagents were supplied by Shanghai Chemical Reagent Co., China.

Silica fiber with the diameter of 600 μm and numerical aperture (NA) of 0.22 was purchased from Nanjing Chunhui Science & Technology Industrial Co., China. Pigtailed semiconductor pulse laser was purchased from BWT Beijing Co., and asymmetric optical fiber coupler from Beijing Xingyuan AoTe Technology Co., China. SMA905 optical fiber connector was processed by Wuxi Shunyu Optoelectronic Technology Co., Ltd., China. Glass flow cell (60 mm in length and 3.5 mm in diameter) was purchased from Jiangsu Top Quartz Products Co., Ltd., China. Avalanche photodiode was purchased from Shenzhen Bozhanxin Industrial Development Co., and lock-in amplifier from Shanghai Yuchen Photoelectric Technology Co., China.

The coating antigen and polyclonal antibody were prepared by our research group [19]. The titer of the obtained antibody was 1:25600 with the protein content of 2.959 $mg \cdot mL^{-1}$, and the affinity and specificity for various PAEs were testified by indirect competitive ELISA analysis [19].

2.2. Preparation of optical fiber immunosensor

Based on the work of Liu et al. [40], the fluorescence signal response could be effectively improved by using the combined cone type probe. The silica fiber was cut into a piece with the length of 11 cm, and then 6.5 cm of fiber coating from one end to the core was stripped to form sensing region. The bare fiber was immersed into 30% HF solution for 3 h; and then, a combined probe with the required cone angle and about 0.5 cm of conical part was obtained. The combined probe was immersed into Piranha solution (H_2SO_4:H_2O_2 = 2:1, *V*/*V*) for 30 min to remove possible contaminants and introduce hydroxyl groups on the surface. It was rinsed thoroughly with ultra-pure water, and dried in nitrogen atmosphere.

The clean probe was silanized with 2% MTS in toluene (*m*/*V*) for 2 h, and rinsed with toluene to remove excessive MTS on the surface. After being dried in nitrogen, the silanized probe was immersed into 0.02 M GMBS in ethanol for 1 h at 37 °C, and washed sequentially with ethanol and 0.01 M phosphate buffer solution (PBS, pH 7.4). Finally, the probe was placed in 0.05 mg/mL coating antigen to react for 2 h, washed with 0.01 M PBS (pH 7.4), and immersed into 2 mg/mL BSA solution for 20 min to block nonspecific binding site on the surface. The modified fiber probe was stored in 0.01 M PBS (pH 7.4) at 4 °C prior to use.

2.3. Construction of optical fiber immunosensing platform

Fig. 1 illustrates the schematic diagram of optical fiber immunosensing platform. The pulse laser beam (650 nm) originated from a pigtailed semiconductor pulse laser (8 mW in output power), and entered into the single-mode optical fiber in an asymmetry fiber coupler, and then into the multi-mode optical fiber in the coupler. Afterwards, the excitation light from the laser entered into the fiber probe (the same as the multi-mode optical fiber in the coupler) through a fiber connector, and then evanescent wave generated on the probe surface. The evanescent wave excited the fluorescein labeled on the antibody; and the partial excited fluorescence coupled back to the probe, and then, entered into the multi-mode optical fiber in the coupler. A fluorescence filter removed the reflected excitation light to let most of the fluorescence pass through it, and the fluorescence signal was detected and converted into electrical signal by an avalanche photodiode.