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A sensitive chemiluminescence enzyme immunoassay based on molecularly imprinted polymers solid-phase extraction of parathion

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ABSTRACT

The chemiluminescence enzyme immunoassay (CLEIA) method responds differently to various sample matrices because of the matrix effect. In this work, the CLEIA method was coupled with molecularly imprinted polymers (MIPs) synthesized by precipitation polymerization to study the matrix effect. The sample recoveries ranged from 72.62% to 121.89%, with a relative standard deviation (RSD) of 3.74 −18.14%.The ratio of the sample matrix-matched standard curve slope rate to the solvent standard curve slope was 1.21, 1.12, 1.17, and 0.85 for apple, rice, orange and cabbage in samples pretreated with the mixture of PSA and C_{18}. However, the ratio of sample (apple, rice, orange, and cabbage) matrix-matched standard-MIPs curve slope rate to the solvent standard curve was 1.05, 0.92, 1.09, and 1.05 in samples pretreated with MIPs, respectively. The results demonstrated that the matrices of the samples greatly interfered with the detection of parathion residues by CLEIA. The MIPs bound specifically to the parathion in the samples and eliminated the matrix interference effect. Therefore, the CLEIA method have successfully applied MIPs in sample pretreatment to eliminate matrix interference effects and provided a new sensitive assay for agro-products.

Introduction

O,O-diethyl-O-p-nitrophenylthiophosphate (parathion) is a highly effective organophosphate insecticide and acaricide that is widely used to control pests and improve agricultural production [1,2]. Organophosphate pesticides (OPs) are extensively used and have replaced several organochlorine pesticides [3]. However, OPs are extremely toxic owing to the irreversible phosphorylation and inactivation of the enzyme acetylcholinesterase [4–6]. Consequently, OPs inhibit the hydrolysis of the neurotransmitter acetylcholine, leading to the accumulation of acetylcholine in vivo, respiratory tract infection and paralysis [7,8].

Traditional methods for detecting parathion residues include gas chromatography (GC) [9,10], high-performance liquid chromatography (HPLC) [11], gas chromatography-mass spectrometry (GC-MS) [12–14], and high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) [15]. Although these techniques offer powerful analyses with low limits of detection (LOD), they are time-consuming and require expensive equipment, limiting their applications in on-site screening.

Recently, various rapid methods have been developed for detecting pesticide residues, and a large number of immunoassay methods have been widely used for parathion residue detection [16–18], particularly chemiluminescence enzyme immunoassay (CLEIA) methods [19,20]. The CLEIA method has been used previously to detect parathion. Deng et al. established an indirect competitive chemiluminescence enzyme-linked immunoassay (icCLEIA) for detecting parathion. Under optimum conditions, the linear range of the developed icCLEIA assay was 0.24–15.83 μg/L, the IC_{50} was 1.14 μg/L, and the LOD was 0.09 μg/L [6]. Xu et al. [21] developed a solid-phase extraction-coupled CLEIA for the determination of organophosphorus pesticide in environmental water samples. In their study, the parathion IC_{50} was 0.7 μg/L and the linear range was 0.2–7 μg/L.

Although the method is specific and sensitive [22,23], as well as cheaper and more rapid [24] than traditional methods, most immunoassays for detecting parathion use solvent standards for

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quantification, which interferes with the LOD and assay accuracy, and leads to errors. The CLEIA method result in different luminescence signals with notable matrix effect for the same quantity in different kind of sample matrices [25]. The matrix effect heavily influences quantitative analyses [26], resulting in signal suppression or enhancement [27], and is difficult to eliminate. To analyze the interfering effects of the sample matrix on CLEIA, matrix-matched standard-MIPs curves and the matrix-matched standard curves were established to quantify in this study [28]. The MIPs technique is a promising method to prepare tailored materials for the development of specific sorbents [29]. MIPs have been extensively used for the selective enrichment and pretreatment of target compounds in complex matrix samples [30]. Recently, MIPs were used as selective matrix solid-phase dispersion (MSPD) to achieve simultaneous analyses extraction and purification, significantly reducing the labor and cost of analyses [31]. In addition, MIPs have also been gradually applied to the environmental analysis field with the rapid development of analytical methods [32]. Meanwhile, the key technique of the CLEIA is chemiluminesence system. Thus, the two most commonly used reporter systems are horseradish peroxidase–H_2O_2–luminol (HRP–H_2O_2–luminol) [33] and alkaline phosphatase in combination with 3 (2'-spiroadamantane)-4-methoxy-4-(3'-phosphoryloxy) phenyl-1,2-dioxetane (ALP–AMPPD) [34–36] was chosen to optimize to established CLEIA. Eventually, we have developed CLEIA based on the HRP-H_2O_2-luminol system to study matrix effect of samples.

In this study, samples were pretreatment by the MIPs and the mixture of octadecyl silica (C_{18}) and primary secondary amine (PSA). And then, the recovery and precision were compared in different sample pretreatment. The results revealed that parathion in the sample extracts was specifically adsorbed by the MIPs. A highly sensitive CLEIA based on extraction using MIPs was established for the trace detection of parathion.

Materials and methods

Chemicals, reagents and instruments

The blank matrix samples of apple, orange, cabbage, and rice were purchased from local supermarkets and confirmed by LC-MS to be free of parathion pesticides.

The parathion standard (Dr. Ehrenstorfer, Germany); HRP, ALP, and ethylene glycol dimethacrylate (Sigma, USA); acetonitrile and methanol (Aldrich, USA); N-dimethylformamide, N-hydroxysuccinimide and N,N'-dicyclohexylcarbodiimide (Sigma, USA) were purchased from the indicated suppliers. PSA and C_{18} solid-phase extraction packing materials were purchased from Bonna-Agela Technologies (Tianjin, China). HPLC-grade acetonitrile and methanol were obtained from Thermo Fisher Scientific (MA, USA). The HRP–H_2O_2–luminol system substrate solution was obtained from Keyue (Beijing, China). The ALP–AMPPD system substrate solution was purchased from Meite (Shenzhen, China). All other chemicals and organic solvents, including Tris, hydrochloric acid, Tween 20, peroxide, methacrylic acid, and 2, 2'-azobis (2-methylpropionitrile), were of analytical grade, or better, and were purchased from the Beijing Chemical Industry Group Co., Ltd (Beijing, China). The following materials were obtained from the indicated suppliers: Infinite F200 multifunctional plate reader (Tecan, Switzerland); a high-speed refrigerated centrifuge (Thermo, USA); 1-1000 μL pipettes (Eppendorf, Germany); white opaque 96-well flat-bottomed plates (Costar, USA); a DEM-3 washer (Beijing, China); and deionized water was purified using a Milli-Q system (Milli-Q Biocel, France).

Buffers and solution

The following buffers were used: coating buffer (Tris-HCl buffer: 50 mmoL/L pH 7.2); blocking buffer (Tris-HCl: 50 mmoL/L, pH 7.2, including 1% tryptone); and phosphate-buffered saline (PBS) (sodium phosphate: 10 mmoL/L; NaCl: 137 mmoL/L; KCl: 2.7 mmoL/L; pH 7.4) with 0.05% Tween 20 (PBST). The standard stock solutions (1 mg/mL) were prepared by dissolving an appropriate amount of each standard in 50 mmoL/L pH 7.2 Tris-HCl buffer containing 10% methanol kept at 4 °C.

The MIPs preparation

New MIPs were synthesized by the precipitation polymerization technique using 0.4 mmol parathion as the template molecule and 20 mL acetonitrile as the porogen in a flask. Subsequently, 1.6 mmol methacrylic acid was added to the flask as a functional monomer, stirred for 90 min at 200 r/min, and stored for 12 h at 4 °C. Then, 8 mmol ethylene glycol dimethacrylate, a cross-linker agent, and 7 mg of 2,2'-azobis(2-methylpropionitrile), an initiator, were added to the flask. The mixture was magnetically stirred for 15 min until fully homogenized. The reaction solution was treated with ultrasound for 15 min, purged by nitrogen for 15 min, and incubated in a water bath at 60 °C for 24 h. Non-imprinted polymers (NIPs) were synthesized in the same manner without the addition of the parathion template [37,38]. The polymer particles were first washed sequentially by methanol/acetic acid (8:2, v/v) for 24 h, followed by 200 mL of methanol for 12 h to be template free (Fig. 2). Finally, the product was dried in a vacuum oven at 40 °C for 24 h [39].

The isothermal adsorption of parathion MIPs

A 10 mg sample of MIPs and NIPs was mashed into a plastic centrifuge tube and added to 1 mL of various concentrations of parathion. After shaking for 2 h at a room temperature, the supernatant solution concentrations of MIPs and NIPs were determined by HPLC. The HPLC system (Waters, USA) was equipped with a quaternary pump, an auto sampler, a column oven, and a water 2998 photodiode array detector. A SunFire™ C18 column (150 × 4.6 mm, 5 μm), purchased from Zhongran Technology Company (Huai'an, China), was used at 25 °C for HPLC analysis. The mobile phase used the methanol (A) and aqueous (B) phases in an 80:20 (v/v, %) ratio at a flow rate of 0.2 mL/min.

Sample extraction

Sample spiking

Four kind samples (apple, orange, cabbage, and rice) were chosen to evaluate the performance of CLEIA. The samples, bought

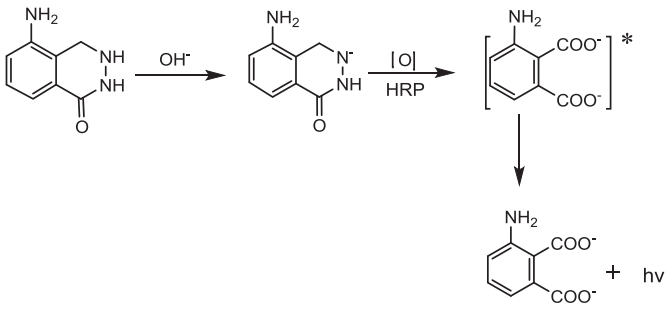

Fig. 1. The mechanism of luminol chemiluminescence immunoassay.