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Design of a novel magnetic particles based electrochemical biosensor for organophosphate insecticide detection in flow injection analysis

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ABSTRACT

The fabrication of transducer interfaces with improved electroanalytical performance is still a challenge in the field of advanced flow based electrochemical biosensors. The use of magnetic nanoparticles for such a purpose to replace the routinely used immobilization matrix including glass, membrane, polymer, gel beads, sol–gel supports, porous silicon matrix, and porous monolithic materials is well documented in the recent literature. However, the application of magneto-based methods is restricted due to lack of reproducibility and renewal of the sensor surface. To overcome these limitations, the present work described a novel configuration strategy to integrate magnetic nanobeads into the flow based system to achieve the reproducible and renewable sensing surface. The designed flow based sensor was demonstrated for the detection of organophosphate insecticides using acetylcholinesterase (AChE) enzyme. System parameters such as optimal bead injection and flow rate were studied prior to insecticides analysis. The system can be potentially applied for on-line assessment in a sensitive, automatic, inexpensive, continuous and simple way for any other analyte of interest.

1. Introduction

Among the various electroanalytical methods, the integration of biosensing surface into flow injection analysis has gained intensive interest for rapid and automated quantitative and qualitative analysis [1]. Efforts are made to immobilize biomolecules on the various immobilization supports such as glass, membrane, polymer, gel beads, sol–gel supports, porous silicon matrix, and porous monolithic materials [2,3]. However, these methods have the limitations of difficulty in controlling the size and position of the immobilized biomolecule, time consuming preparation steps, non specific adsorption, poor reproducibility and inability to renew the sensing surface [4]. Thus establishment of electrode interfaces with a high electroanalytical performance in terms of biomolecules immobilization efficiency, sensitivity, rapidity of transducer response, and easy real applicability is still a challenge in the field of advanced electrochemical biosensors. As an attractive alternative tool, the use of magnetic nanoparticles for such a purpose to replace the routinely used immobilization matrix is well documented in the recent literature [5,6]. However, the application of magneto-based methods is restricted due to lack of reproducibility and renewal of the sensor surface. Due to ease of manipulation and separation using a magnetic field, it is possible to simply 'switch off' the magnetic effects by removing the magnetic induction field, which can be advantageous for bead recovery. Moreover, magnetic beads can be manipulated independent of normal microfluidic or biological processes which results in improved exposure of the functionalized bead surface to the surrounding viscous liquid, due to the increased relative motion of the bead with respect to the fluid [7,8]. Additionally, the application of revolving external magnetic during manipulation could also be beneficial to disperse the magnetic beads uniformly on the reactor surface to obtain the reproducible sensing surfaces. Based on the above observation, and to overcome the limitations associated with the use of magnetic beads in flow system, the present work described a novel configuration strategy to integrate magnetic nanobeads in the flow based system

Abbreviations: AChE, acetylcholinesterase; LOD, limit of detection; OIs, organophosphate insecticides; SPE, screen printed electrode; ATChI, acetylthiocholine iodide; ATChCl, acetylthiocholine chloride; DTNB, 5,5′-dithio bis (2-nitrobenzoic acid); PBS, phosphate buffer saline; CPO, chlorpyrifos oxon; HEC, hydroxyethyl cellulose.

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Organophosphate insecticides (OIs) are harmful chemicals widely employed to increase productivity in the agriculture industry worldwide [9]. As a consequence of their widespread usage, there is an indiscriminate releasing of dangerous pesticide residues which represents a major risk for the environment and human health [10,11]. Biosensors based on acetylcholinesterase inhibition have been proposed as easy, fast and accurate tools for insecticides residues assessment [12–14]. A variety of techniques have been proposed for enzyme immobilization, but the inclusion of magnetic micro and nanoparticles presents advantages over the classical immobilization techniques [15,16]. In this sense, attempts to create analytical systems using magnetic beads and enzyme have been presented for detection of carbofuran, paraoxon-ethyl, paraoxon-methyl and malaoxon [17]. The automation of the whole procedure could offer advantages over manual procedures as higher stability, lower LOD and potential implementation for automated on-line operation [18]. However, the existing flow based methods for analysis of OIs undergo the problem of reproducibility and regeneration of the transducer surface. The ultimate goal of this work was to design a regenerable magnetic nanoparticle based sensing surface and integrate it in an automatic flow based platform for the sensitive on-line monitoring of OIs. By inserting a screen printed electrode (SPE) into a custom magnetic flow through cell, the magnetic microbeads were handled to renew sensing layer. Although the specific application is demonstrated for the detection OIs, however, the system can be potentially applied for on-line assessment in a sensitive, automatic, inexpensive, continuous and simple way for any other analyte of interest.

2. Experimental

2.1. Chemicals and materials

B131 AChE from *Drosophila Melanogaster* was produced by Protein Biotechnical Instrument (Nimes, France). Acetylthiocholine iodide (ATChI), 5,5′-dithio bis (2-nitrobenzoic acid) (DTNB) and acetylthiocholine chloride (ATChCl) were purchased from Sigma–Aldrich (France). All working solutions were diluted in phosphate buffer at pH 7.4 (PBS, 0.1 mol L^{-1} K_2HPO_4/KH_2PO_4). Chlorpyrifos oxon (CPO) was supplied by Dr. Ehrenstorfer (Augsburg, Germany); stock solutions of CPO (10^{-3} M) were prepared in acetonitrile (Carlo Erba Reagenti, Italy) and stored at 4 °C. Working solutions of CPO were prepared daily by diluting stock solution in PBS buffer. Preactivated magnetic beads (diameter 300 nm) and binding buffer (20 mM Tris, 500 mM NaCl, pH 7.5 and 0.09% sodium azide) were provided together in the "Histidine Adem-kit for His-Tagged protein purification" (Ademtech S.A., France). The pastes used for screen-printing namely Electrodag PE-410, 423SS and 6037SS were purchased from Acheson (Plymouth, UK). Cobalt phthalocyanine-modified carbon paste was acquired from Gwent Electronic Materials, Ltd. (Gwen, UK). A glycerophthalic paint (Astral, France) was used as insulating layer. Hydroxyethyl cellulose (HEC) medium viscosity was purchased from Fluka, (France). Transparent PVC sheets (200 mm × 100 mm × 0.5 mm) were used as supports for printed electrodes. For real sample analysis, water from Villeneuve de la Raho Lake (42.38° N, 2.55° E, Pyrénées Orientales, France) was collected and stored at 4 °C.

SPEs were fabricated in a three-electrode configuration using a semi-automatic DEK248 printing machine accordingly to a previously described protocol [19]. The Adem-Mag SV magnetic support (Ademtech, France) was used to hold magnetic beads during the manual assays for enzyme immobilization.

2.2. Acetylcholinesterase activity

Before immobilization, AChE activity was tested by following the Ellman method [20]. For the assay, 600 μL of PBS buffer, 300 μL of 1 mg/1 mL DTNB, 100 μL of 10 mmol L^{-1} ATChI (in 0.9% NaCl) and 10 μL of enzyme were mixed together into a spectrophotometric cell. The yellow compound 5-thio-2-nitrobenzoate produced as a consequence of the reaction of DTNB with thiocoline, was spectrophotometrically detected at 412 nm. The enzymatic activity was calculated on the basis of the kinetic spectrometric measurements recorded along 1 min. The 8451A diode-array (Hewlett-Packard, USA) was used for spectrophotometric measurements.

2.3. Immobilization protocol

The previous reported immobilization technique based on metal-chelate affinity was used to bind B131 AChE to preactivated magnetic beads [21]. Here, immobilization protocol is briefly described: 30 μL of beads from Adamtech Kit stock were taken and placed in a 1.5 mL tube. Then, beads were washed twice with 300 μL of binding buffer. The tube was placed on the Adem-Mag SV magnetic support to hold magnetic beads while liquid solution was discharged. Then, a mixture of beads and 1 mL of enzymatic solution was gently mixed for 15 min and discharged by placing the tube on the magnetic support again. Unbound enzyme was eliminated by washing magnetic beads twice with 200 μL of binding buffer. These modified beads were kept in the tube and stored at 4 °C.

2.4. Design of novel flow based experimental setup

A flow injection system was used to handle liquid reagents and magnetic beads (MBs) injection. Flow stream was driven by a high precision bidirectional syringe (Cavro XLP 6000, Tecan) of 1 mL coupled with a six port rotary valve (MPV). Magnetic beads flow was handled by additional solenoid valves (NResearch, USA) inputs to avoid interaction between reagents and beads prior to CPO measurement. A 300 μL holding coil (HC) was placed between the syringe pump and the bead reservoir to avoid corruption of reagents as well. For detection, a custom flow cell (FC) with rotating magnetic field was developed to promote reproducible sensing surface. Rotating magnetic fields were produced by attaching a small neodymium magnet to a DC motor mounted on the backside of the flow cell. The rotating speed was controlled by a pulse width modulation (PWM) control with a low frequency of 2 Hz. During motor-off operation magnetic beads were injected to the flow cell and dispersedly attached to the SPE by the static magnetic field as in usual magneto based configurations. Then, PWM was activated and rotation caused a clustering of beads on the center of rotating magnetic field, which was located under the working electrode area [22]. The electrochemical response on flow cell was monitored with a PRGE potentiostat (Tacussel, France). For autonomous operation, devices (e.g. syringe, valves, motor) and operations (e.g. bead injection, flow rates, valves activation, data acquisition, speed of motor) were automatically controlled by a custom graphical user interface developed in LabVIEW 8.5®Developed flow system is schematically shown in Fig. 1a. Operation of flow cell with off and on motor operation is presented in Fig. 1b and c, while a cell phone camera captured photo for the proposed system is shown in the supporting information (supplementary Scheme 1)

2.5. Flow amperometric measurements in PBS buffer

Parameters of batch assay were taken as base for flow assays. All flow assays were carried out with a working potential of +100 mV and using cobalt phthalocyanine as mediator. The experiments