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MIP-MEPS based sensing strategy for the selective assay of dimethoate. Application to wheat flour samples

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ABSTRACT

The aim of this work was to demonstrate the potentialities of the use of a molecularly imprinted (MIP) sensor coupled to a microextraction by packed sorbent (MEPS) strategy for the selective and sensitive detection of dimethoate in real samples. A dimethoate-polypyrrole MIP film was realised by cyclic voltammetry (CV) on the surface of a glassy carbon electrode (GCE). Being dimethoate electro-inactive, $K_3[Fe(CN)_6]$ was used as probe for the indirect quantification of the analyte via the decrease of redox peaks observed upon binding of the target analyte. Detection of dimethoate at low nanomolar range was achieved with linearity in the 0.1–1 nM range. Relative standard deviation calculated for different electrodes at 0.5 nM of dimethoate was < 3% and selectivity was very satisfactory being the response for omethoate only 23% of dimethoate. A MEPS strategy for the extraction of dimethoate from a challenging matrix as wheat flour was then used in conjunction with the MIP electrochemical sensor. The procedure applied to flour samples spiked with dimethoate at 0.5 MRL, MRL, and 1.5 MRL gave very favourable comparison with a validated UHPLC-MS/MS method with deviations in the −21% /+17% range, demonstrating the feasibility of the approach as screening assay. This work clearly shows that the sequential use of a microextraction based procedure and electrochemical sensing system is low cost, easy to realise and use and can open new perspectives for the development of selective sensing system to be used in field or decentralised lab testing for the selective screening of target analytes.

1. Introduction

Molecularly imprinted polymers (MIPs) have received considerable attention in analytical chemistry, primarily because specific recognition sites are formed in the MIP matrix, and excellent selectivity toward the analyte can be achieved [1]. The fascinating concept by which selected functional monomers are polymerized around a target analyte (template) and, after removal of the template, a polymer matrix complementary in shape and functionality works as selective recognition element has been exploited both for solid phase extraction and for sensing purposes [2–5]. In fact, the chemical and mechanical stability, the facility of preparation and the relatively low cost of polymers make them attractive for several analytical applications and, in some cases, they can even replace natural receptors and enzymes [6]. Particularly attractive is the electropolymerization process that has been frequently used in the development of molecularly imprinted electrochemical sensors. Advantages rely on the control of the polymer thickness, which can be regulated by the electrochemical conditions, simple preparation procedure and formation of very thin films that are beneficial to rapid response [7]. The electropolymerization of polypyrrole (PPy) is very well known and has been widely used for the preparation of molecularly imprinted electrochemical sensors. Polypyrrole, indeed, presents excellent biocompatibility, electrical conductivity, stability and facility for the immobilization of different compounds [6,8,9]. MIP electrochemical sensors have been developed for the analysis of some organophosphate pesticides, such as chlorpyrifos (molecularly imprinted polypyrrole) [10], triazophos (molecularly imprinted polyhydroxyphenol) [11], parathion (molecularly imprinted polyethyleneimine/silica gel; molecularly imprinted phenol) [12,13], methidathion (molecularly imprinted polymers/sol-gel) [14]. However, despite the ease of the approach, the use of such sensors for real samples analysis is still very limited. In fact, not all the analytes are electrochemically active and can be easily detected at electrodes surfaces or nanostructured

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materials; thus, sensing should be often obtained indirectly via a redox probe [9,15–17]. In addition, direct "extraction" of the analyte using the MIP sensing surface is possible only for liquid samples and direct extractions of real matrices can give rise to further problems of variability and sensitivity depending on sample complexity. The coupling, then, of the sensing system with an appropriate extraction procedure appears ideal providing that the pre-treatment is not time-consuming, labor-intensive, complex and, in some cases, expensive as in the conventional extraction procedures [18]. There is a growing demand to develop new technologies to minimize sample preparation, reducing costs, times and waste. Microextraction by packed sorbent (MEPS) is a miniaturised version of classical solid phase extraction [19]. It is fast, it requires minimal volumes of solvents and samples, it facilitates the enrichment of the analytes and it is simple and inexpensive [20]. In MEPS the sample preparation takes place on the packed bed containing 1–2 mg of sorbent. The extraction is performed by drawing sample through the syringe manually or by an auto-sampler. When the sample is passed through the solid support, the analytes are adsorbed to the extracting media. Finally the analytes are eluted with an organic solvent [21]. The target selected for this study was dimethoate. Dimethoate is a phytochemical belonging to the class of organophosphate pesticides (OPs) and has an inhibitory effect on the enzyme acetylcholinesterase (AChE). This enzyme hydrolyses the neurotransmitter acetylcholine and this effect leads to a pathologic excess of acetylcholine in the body with severe nerve function disorders [22]. Additionally, OPs may cause negative effect on the visual system, sensory function, cognitive function; they may cause respiratory dysfunction, delayed polyneuropathy, immunotoxicity, carcinogenesis and endocrine dysfunction, developmental and reproductive toxicity which result in severe health problems and even death in both animals and humans [23–25]. The detection of organophosphate pesticides in food samples has already been achieved using biosensors based on AChE, for example in egg, bovine meat, milk and honey [26], in durum wheat [27], in water [28], in apple juice [29] and olive oil [30]. This systems are quite sensitive but they have some drawbacks such as the poor selectivity. Because of wide use and acute toxicity of OPs, it is important to develop rapid, sensitive, selective and portable detection methods to accurately monitor their concentration levels for the protection of ecological systems and food supplies [31]. Wheat is exposed to phytosanitary treatment during planting, growing, harvesting and storage. The European Union, with the Regulation (EC) 396/2005 [32] of the European Parliament and the Council, has provided restrictions on the use and applicability of pesticides, imposing Maximum Residue Limit (MRL) values of pesticides in food and feed to protect human and animal health and the ecosystem. The monitoring of pesticide residues in wheat flour is important to ensure food safety because wheat flour provides the basis for many processed consumer products, which are among the most consumed foods worldwide [33].

The aim of this study was the development of a rapid, selective and potentially portable screening method for the detection of dimethoate residues in wheat flour. The method consists of a microextraction by packed sorbent (MEPS) that allows the analyte extraction and preconcentration [34], followed by MIP-glassy carbon electrode (MIP-GCE) detection. The dimethoate-polypyrrole MIP films were electropolymerized by cyclic voltammetry (CV) on the surface of glassy carbon electrode (GCE), with pyrrole (Py) serving as the monomer and dimethoate (dim) as the template [35]. Dimethoate is electroinactive, therefore an electroactive $K_3[Fe(CN)_6]$ solution was used as the probe in the electrochemical measurements for the indirect quantification of dimethoate observing the diminishment of the oxidation peaks. So far, to best of our knowledge, this is the first work about a molecularly imprinted polypyrrole based sensor or biosensor for determination of an analyte in a challenging sample matrix such as wheat flour.

2. Materials and methods

2.1. Chemicals and apparatus

All the chemicals were obtained from Sigma-Aldrich (Johannesburg, South Africa; Milan, Italy) Pyrrole was distilled under vacuum until a colorless liquid was obtained, purged with argon and kept in darkness at −30 °C. Electrochemical measurements (CV and SWV) were carried out using an Autolab potentiostat-galvanostat controlled by a GPES 4.9.007 Software. A three-electrode system was used for all measurements: a MIP-glassy carbon electrode (3 mm diameter) as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl/KCl (3 M) as the reference electrode. The selected pesticides were analysed by an UHPLC Nexera LC20AD XR from Shimadzu (Kyoto, Japan) equipped with autosampler, vacuum degasser and column oven. The chromatographic separation was carried out using a Kinetex XB-C18 column (100 × 2.1 mm) packed with 1.7 µm particles obtained with core-shell technology from Phenomenex (Torrance, CA, USA); a guard column was also included. Identification and quantification of the analytes were carried using a triple quadrupole mass spectrometer 4500 Qtrap from Sciex (Toronto, ON, Canada) equipped with a V-Spray source operating in positive ionization (PI) for all analytes.

2.2. Preparation of MIP and NIP film electrodes and electrochemical measurements

A bare glassy carbon electrode (GCE) was polished using 1, 0.3 and 0.05 µm alumina paste on microcloth pads and rinsed thoroughly with distilled water until a mirror-like surface was obtained. Finally the electrode was washed with distilled water and allowed to dry at room temperature before use. The scheme of the assay is reported in Fig. 1. The GCE was, then, immersed in a pH 6.8 PBS solution 0.1 M (Phosphate Buffer Solution) containing 30 mM pyrrole and 10 mM dimethoate for the electropolymerization step by using cyclic voltammetry (CV) in the potential range between −0.4 and +1.5 V for 10 cycles at a scan rate of 50 mV s^{-1}. The dim-PPy MIP electrode, obtained after electropolymerization, was immersed in HCl solution pH 2 with stirring for 30 min at room temperature to remove dimethoate from the imprinted polymer (dim-free MIP electrode). Finally, for the rebinding step, the dim-free MIP electrode was dipped into dimethoate solutions (100 µL) at different concentrations (0.075–2 nM) for 15 min (dim-rebinding MIP electrode) at room temperature. No significant changes were observed using from 30 min to 60 min for the template removal and from 15 min to 30 min for the rebinding step.

Electrochemical measurements were carried out, using CV and SWV in a pH 7 PBS (0.1 M) containing 10 mM $K_3[Fe(CN)_6]$ and 0.1 M KCl solution in the potential range from −0.3 to +0.8 V at a scan rate of 0.01 V s^{-1} (CV) and in the potential range from −0.3 to +0.8 V, step potential 0.0051 V and amplitude 0.01995 V (SWV). $K_3[Fe(CN)_6]$ was used as the probe because the dimethoate is electro-inactive. All measurements were performed at room temperature. All these conditions were selected as the optimal after having tested acetate, phosphate, and TRIS buffer at different pHs, differential pulse and square wave voltammetry and different amounts of K3[Fe(CN)6]. Quantitative detection of dimethoate was achieved plotting the ΔIpa (%) vs. concentration. ΔIpa was defined as the difference between the SWV oxidation peak current of probe at the dim-free MIP electrode and that at the dim-rebinding MIP electrode. This decrease on the response (ΔIpa%) can be used to indirectly detect the analyte quantitatively.

A control electrode (non-molecularly imprinted polymer electrode, NIP) was prepared under the same conditions but with no dimethoate during the electropolymerization.