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Coulombimetric immunosensor for paraquat based on electrochemical nanoprobes

Enrique Valera[a,b], Raül García-Febrero[b,a], Isabel Pividori[c], Francisco Sánchez-Baeza[b,a], M.-Pilar Marco[b,a,*]

[a] CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Barcelona, Spain
[b] Nanobiotechnology for Diagnostics (Nb4D) Group, IQAC-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
[c] Unitat de Química Analítica, Grup de Sensors i Biosensors, Edifici C – Campus de la UAB, 08193 Bellaterra (Cerdanyola del Vallès), Barcelona, Spain

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This work is dedicated to the memory of Dr. Francisco Sanchez-Baeza.

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ABSTRACT

A new electrochemical immunosensor has been developed to detect paraquat (PQ) pesticide residues in food samples. The immunosensor presented uses electrochemical nanoprobes prepared by labeling the PQ specific antibodies with CdS nanoparticles (*CdS*NP) and antigen biofunctionalized magnetic μ-particles. Electrochemical measurements are performed using graphite composite electrodes (GECs). After the immunochemical reaction, the *CdS*NP are dissolved and the metal ions released are reduced at the electrode and read in the form of current or charge signal, by the well-known anodic stripping technique. Due to the amplification effect produced by the *CdS*NP on the amperometric/coulombimetric signal, a very high detectability is reached. Thus, PQ can be detected with an IC_{50} *current* of 0.18 ± 0.31 μg L^{-1} (in PBST). The immunosensor has been implemented in analyzing PQ residues in potato samples. Combined with a suitable extraction procedure PQ can be detected with a LOD *current* of 1.4 μg kg^{-1}, far below the maximum residue limit (MRL, 20 μg kg^{-1}) established by the EU for this pesticide in most crops. Likewise, the working range was in the interval between 3.08 and 67.76 μg kg^{-1}. Preliminary experiments demonstrate that the immunosensor presented here could be used as screening tool to distinguish between compliant and non-compliant food samples.

1. Introduction

The presence of potentially hazardous chemicals (e.g. pesticides, antibiotics, mycotoxins) in food products for human consumption remains a major concern in the European Community because of its serious health consequences. Ensuring safety, quality and traceability implies frequent monitoring of foodstuffs at critical steps in the food chain (recollection of the raw materials, storage, transport, final products or after food processing). To comply with these requirements there is a need for fast and efficient analytical procedures that also comply with the regulations in respect to their performance, and particularly in respect to the detectability that has to be achieved.

Amongst commercial pesticides, paraquat (N,N′-dimethyl-4,4′-bipyridinium dichloride, PQ), one of most widely used herbicides in the world, deserves special attention because of its high toxicity in humans and animals. Human epidemiological [1–4] and animals studies have indicated that PQ might be an environmental factor contributing to neurodegenerative disorders such as Parkinson's disease [3,5–10]. Similarly, accidental intoxication or exposure to PQ has been related to lung injury, inducing diseases such as adenocarcinoma or lung fibrosis [3,11,12], and oxidative stress affecting the function of other organs such as the liver [2,11,13]. PQ residues have been detected in diverse food products, such as potato, wheat, rice, corn, apple, orange, banana, coffee and tea [14]. Although the use of PQ has been banned in the EU since 2007, this rule does not apply to non-European countries exporting to the EU. Therefore, PQ is among the compounds that should be monitored in food samples to verify compliance with Good Agricultural Practices, and to ensure that residues are below the levels established by the EU [15]. Thus, a maximum residue limit (MRL) of 20 μg kg^{-1} has been established for many food products such as barley, wheat or potato [15]. Although official laboratories have implemented efficient multiresidue methods (MRMs), mainly based on HPLC–MS, PQ is among the pesticides that, due to its particular physico-chemical properties, are not covered by these programs. Its permanent ionic character, added to its high hydrophilicity and tendency to interact with surfaces, are some of the main reasons for which this pesticide has often been excluded, since it requires distinctive extraction and clean-up procedures. Alternative reported analytical methodologies include ELISA [16], flow-injection spectrophotometry [17],

* Corresponding author at: Nanobiotechnology for Diagnostics (Nb4D) Group, IQAC-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain. Tel.: +34 934006100.
E-mail address: pilar.marco@cid.csic.es (M.-P. Marco).

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gas chromatography [18], capillary electrophoresis [19], or square wave voltammetry [20]. With the idea of combining biodetection with automation arises the concept of the biosensor, miniaturized analytical devices consisting of an immobilized biological component (i.e. antibody) in intimate contact with a transducer (i.e. electrochemical) that may convert the biorecognition process into a quantifiable electrical signal. Few research papers report the detection of PQ residues using biosensors and usually analysis is only performed in water [21–23] and never in complex samples. Using specific antibodies developed against paraquat [24] our group has recently developed an amperometric magneto-immunosensor based on the use of horseradish peroxidase labeled antibodies [25]. However, the use of enzyme labels requires the use of secondary mediators and substrates and the stability and time-dependent response of the enzyme has often been reported to limit their application [26,27]. Another remarkable limitation of the use of enzymes as electroactive tags is because of the possibility of developing devices for simultaneous multi-analyte detection.

The use of inorganic semiconductor nanocrystals tracers as labels, instead of enzymes, for electrochemical immunoassays have received great attention because of the possibility to obtain simultaneous detection/measurements of different targets [28,29]. This reported electrical detection approach is based on the electrochemical anodic stripping transduction, as well as on an efficient magnetic separation. These components offer an inherently amplified signal and high selectivity. In fact, we have recently demonstrated that this approach may result in an excellent strategy to detect sulfonamide antibiotic residues in honey samples [25]. In this paper we present a coulombimetric immunosensor based on semiconductor nanocrystals for the detection of PQ pesticide residues. To the best of our knowledge, this is the first time this type of approach is reported for the detection of PQ, demonstrating its capability to be used to analyze complex samples.

2. Experimental

2.1. Instrumentation

Electrochemical measurements were made using a portable μStat 200 Potentiostat (DropSens, Spain). A three-electrode setup was used comprising a XM120 8 mm platinum plate auxiliary electrode (Radiometer Analytical SAS, France), a RE-1B Ag/AgCl reference mini-electrode (ALS, Japan), and a graphite–epoxy composite sensor (GEC) as working electrode. The pH of all buffers and solutions were measured with a 540 GLP pH meter (WTW, Weinheim, Germany). Absorbances were read on a SpectramaxPlus microplate reader (Molecular Devices, Sunnyvale, CA). The size of the *CdS*NPs was characterized by transmission electron microscopy (TEM, Jeol J2010F). The electrochemical measurements performed were analyzed using DropView 1.3 software (DropSens, Spain). The competitive curves were analyzed using a four-parameter logistic equation by means of GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA, USA). The magnetic separation during the incubation and washing steps was performed using a magnetic separation rack with a capacity of 24 microcentrifuge tubes (MagnaRack™, Invitrogen Corporation, Carlsbad, CA, USA).

2.2. Chemicals and immunochemicals

A stock solution containing PQ at a concentration of 10 mM was prepared in dimethyl sulfoxide (DMSO). The chemicals used to prepare the CdS nanoparticles were purchased from Sigma–Aldrich, Aldrich, Fluka, Merck, Panreac and Carlo Erba. The chemicals used to extract the potato samples were purchased from Merck. The chemicals used during the electrochemical measurements were purchased from Sigma–Aldrich and Merck. DMSO, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI), N-hydroxysuccinimide (NHS) and ethanolamine were purchased from Sigma. Glycine was purchased from Aldrich. Boric acid was purchased from Merck. Ammonium sulphate was purchased from Fluka. Polyvinylpyrrolidone (PVP) was purchased from Sigma–Aldrich. All the reagents were of the highest available grade. The Bradford solution (BIO-RAD protein assay cat No. 500-0006) was purchased from BIO-RAD laboratories GmbH (Munich, Germany). The graphite–epoxy composite was prepared using graphite powder with a particle size of 50 μm (BDH, UK) and Epo-Tek H77 (epoxy resin from Epoxy Technology, USA). Tosyl-activated magnetic beads (Dynabeads® Myone™ Prod. No. 655.01) were purchased from Dynal® Biotech AS (Oslo, Norway). PQ (N,N′-dimethyl-4,4′-bipyridinium dichloride), PQ1 hapten (N-(4-carboxybut-1-yl)-N′-methyl bipyridilium acid) and the immunoreagents used in this study (As198 and PQ1-BSA) were prepared and characterized in our laboratories [24]. In this study, Ab198 as a pure IgG fraction was obtained by ammonium sulphate precipitation of As198.

2.3. Buffers and solutions

100 mM PB was 0.1 M phosphate buffer (pH is 7.5). PBT was 0.01 M PB with 0.05% (v/v) Tween 20. PBS was 0.01 M phosphate buffer, 0.8% (w/v) saline solution and unless otherwise indicated the pH was 7.5. PBST was PBS with 0.05% (v/v) Tween 20. Borate buffer was 0.1 M boric acid (pH 9.5). Acetate buffer used for the electrochemical measurements was 0.2 M sodium acetate trihydrate (pH 5.6). All buffer solutions were prepared with ultrapure water (UPW, D11971 Barnstead).

2.4. Potato samples

Certified blank potato samples, free of paraquat (<0.01 mg kg^{-1}, LC–MS/MS), were kindly supplied by The Food and Environment Research Agency (FERA, York, UK). The homogenized test material was received in the form of a powder divided into sub-samples (each ~25 g) in heat sealed foil sachets and stored at −20 °C.

2.5. Fabrication of the graphite–epoxy composite (GEC) electrodes

The fabrication of the graphite–epoxy composite electrodes was the same as previously reported by Zacco [30], but without introducing the magnet inside the electrode. Before each measurement, the electrode surface was renewed by a simple polishing procedure, wetted with UPW and then thoroughly smoothed with abrasive paper.

2.6. Synthesis of the CdS nanoparticles (CdSNP)

CdS particles were synthesized by means of reverse micelles chemistry, following the technique reported by Mirgorod [31] with slight modifications [25] (see Supplementary data). Functionalized *CdS*NP as a yellow suspension (3.65×10^{16} of *CdS*NPs mL^{-1}) was obtained. The suspension was stored in 10 mM PBS (1 mL, pH 6) at 4 °C. With time, the nanoparticles were deposited on the bottom of the vial, but the resuspension could easily be reconstituted by gentle stirring for few seconds. While the size of the *CdS*NPs was characterized by TEM, in subsequent experiments, their concentration was adjusted by absorbance analysis (UV-spectrum).