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Electrochemical Immunosensor for Pesticide Residues Detection in Food Analysis

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Abstract: Electrochemical immunosensors have emerged as a highly sensitive and rapid technique for chemical contaminants detection in environmental monitoring, food safety and quality control. This review concentrates on recent advances made in detection and quantification of chemical contaminants such as pesticides and antibiotics residues with electrochemical immunosensors. In this paper, the preparation of immunosensors, the selection of analysis methods and real samples detection are introduced in detail. Future prospects toward the development of selective, sensitive immunosensors systems are also discussed. *Copyright © 2013 IFSA.*

Keywords: Electrochemical immunosensors, Chemical contaminants, Pesticides residue, Detection.

1. Introduction

Pesticides are widely used in agriculture produce, and antibiotics are also being used on a large scale in livestock production. These lead to the excessive chemical residues in foods and cause severe impairment of human health. The analysis of foods to assess the presence of chemical contaminants is a practice of crucial importance for ensuring food safety and quality. Currently, the majority of chemical contaminants are commonly analyzed by various analytical techniques such as gas chromatography (GC), high-pressure liquid chromatography (HPLC), capillary electrophoresis (CE) and mass spectrometry (MS) [1-4]. These conventional methods are very sensitive and reliable, but have disadvantages such as complexity, extensive time consumption, and the need for costly, bulky instrumentation. For these reasons, the development of rapid and efficient monitoring methods becomes more and more important.

In recent years the generation of antibodies against pesticides residues has seen significant progress leading to the introduction of several immunoassays for environmentally sensitive small toxic molecules [5-9]. As consequence, immunochemical methods, such as enzyme-linked immunosorbent assays (ELISAs), have already gained a place in the analytical benchtop as alternative or complementary methods for routine pesticide and veterinary drugs analysis. They are fast, economic, and at least as sensitive as usual chromatographic techniques. However, the analyte detection in ELISAs is always indirect because one of the immunoreagent is labeled. Moreover, that need extensive sample handling such as rather large number of washing steps. On the contrary, in immunosensors the detection is direct: one of the immunoreagents is immobilized on the surface of the transducer, and a direct physical signal is produced when the immunochemical interaction occurs. This label-free direct detection represents an essential

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advantage of immunosensors as compared to label-dependent immunoassays [10-13]. As consequence, a great number of research papers have appeared over the last years describing the development of novel immunosensors for detecting trace amounts of chemical residues in environmental and food samples.

Among the immunosensors, the electrochemical immunosensors for pesticide residue detection have attracted extensive interest in recent years [14-16]. Fig. 1 is the schematic diagram of electrochemical immunosensor for analyte [17].

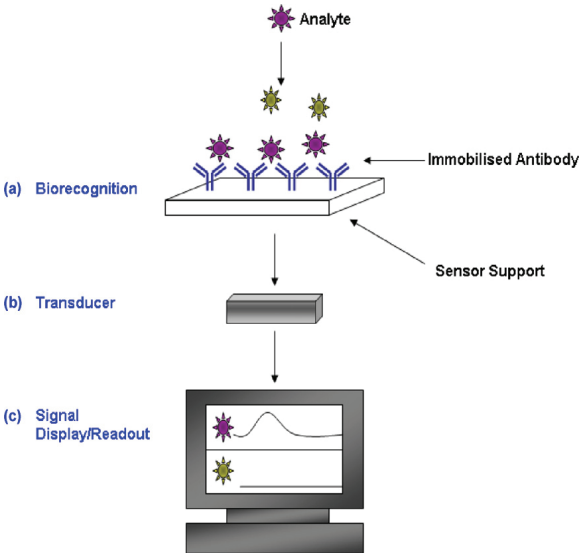

Fig. 1. The schematic diagram of electrochemical immunosensor for analyte.

Electric impedance spectroscopy (EIS) is a sensitive technique, which detects the electrical response of the system studied after application of a periodic small amplitude AC signal. Impedance immunosensors detect the pesticide residue concentrations by the measurement the changes of electrical conductivity of the solution and capacity due to the Ab-Ag interaction on the electrode surface, which also can be reflected in impedimetric response. Enrique Valera's group [18-23] has reported series methods about impedance immunosensor for atrazine detection based on the interdigitated microelectrode array. The complete assay process and the mechanism of the immunosensing reaction are shown in Fig. 2 and Fig. 3, respectively. However, the detection of pesticide residue such small molecule compound is usually performed under competitive conditions involving the competition between the free antigen (analyte) and a fixed amount of coated antigen for a limited amount (low concentration) of antibody (Ab). At the end of the reaction the amount of Ab captured on the electrode surface and hence the free antigen (analyte) is determined, as schematically shown in Fig. 3.

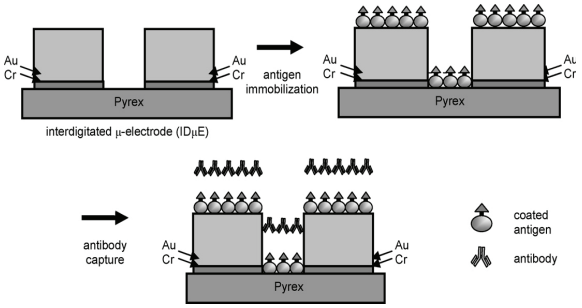

Fig. 2. Schematic diagram of the complete assay system performed on the IDμE's.

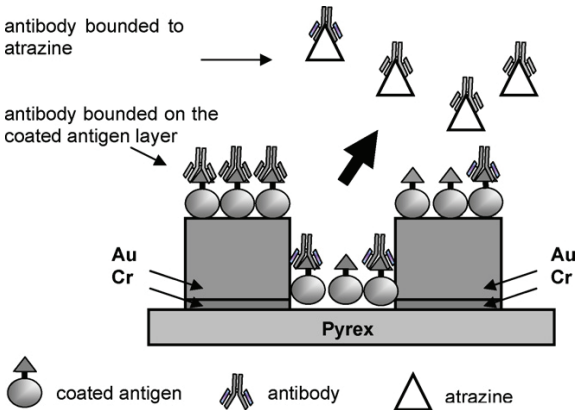

Fig. 3. Immunosensor reaction. An amount of the specific antibody is bounded on the coated antigen layer. Other amount is evacuated of the IDμE's, this amount is related to the atrazine concentration.

Except for few transducing principles already well established (i. e., SPR, surface plasmon resonance) [24-26], most of the immunosensors reported until now, rely on the use of labels to reach the necessary detection limits required by the legislation. As it is well known, the competitive assay is complex. In order to obtain simpler and faster immunosensing methodology, direct detection of pesticide residues in food products without the use of labels (fluorescent compounds, enzymes, etc.) are increasingly meeting these challenges. Different sensor approaches have been published to avoid the use of labels when the target analyte is a biomacromolecule, a bacteria or a virus, but only a few immunosensors are used to detect haptens which are difficult to immobilize and have little effect on electron transfer of the electrochemical mediator in solution, since haptens usually are small molecular compounds. For the detection of electroactive hapten, it also has been reported that the antibody was immobilized on the electrode to capture the hapten, then the hapten adsorbed on the electrode showed well-shaped redox responses [10] (Hu *et al.*, 2003). It can suffer from steric hindrance between the small antigen and large antibodies. However, it is difficult to detect non-electroactive small molecules. Therefore, it requires offer highly sensitive small

molecule immunosensor technologies through careful consideration of sensor interface design and signal enhancement [24].

However, there is a time gap between current status in the field and the most recent reviews. Thus, in this review, we specifically provide an overview of the research carried out during the last 5 years relative to electrochemical immunosensor for food and environment safety. We will review the key steps to construct an immunosensor including the immobilization protocols of antibod, the formation of a bio-recognition interface, and the electrode modification. We also will discuss the trends and challenges associated with designing a reliable immunosensor for practical applications in detail.

2. The Antibody Immobilization Methods

Using nanomaterials as electrode modification materials, the antibody is immobilized the modified electrode surface. This method is the most of reported antibody immobilization methods. Sun *et al.*, fabricated amperometric immunosensor for carbofuran detection using deposited gold nanocrystals/4,4′-thiobisbenzenethiol multilayers membranes to modify Au electrode [14]. Suri *et al.*, immobilized diuron antibody onto electrode modified with Prussian blue-gold nanoparticle film to prepare immunosensor for diuron detection [27].

Another immobilization strategy is based on the antibody bonding through Fc fragment to Protein A or G. The bond strength from strong to weak between Protein A (or G) and an antibody are greatly affected by the antibody classes and subclasses. The binding involves the formation of multiple non-covalent bonds between the Protein A and amino acids of the binding Fc site [28].

Antibodies or antigens are also immobilized at the surface of magnetic beads and all immunological steps are performed in micro-tubes using a rotation sample mixer. After each incubation or washing step, the magnetic beads are concentrated on the side wall of the micro-tubes by placing the tubes in a specially designed magnetic particle separator allowing the supernatant to be discarded. Finally, the magnetic beads are concentrated onto the magnetized working electrode surface and the electrochemical measurements are carried out. Using this approach, which combines the selectivity of the antibodies with the sensitivity of the electrochemical detection and the possibility of concentrating magnetic particles on the electrode surface, it is possible to achieve remarkable enhancement in the performance of classical immunoassays [29].

3. The Selection of Electrode Materials

At the present, the most of reported immunosensors use normal three electrode system, such as glass carbon electrode, gold electrode and so on. Usually, before immobilizing antibody onto electrode surface, the electrode is first modified with nanomaterials to enhance electron transfer between electrode and testing solution.

To overcome this problem, in recent years the application of single use screen-printed electrodes (SPEs), characterized by low-cost fabrication and mass production, has attracted an increasing interest for the development of immunosensors (especially enzyme immunosensors). Tothill *et al.*, have developed an immunosensor for herbicide detection with membrane-based screen printed electrode [30]. Using screen printed electrode, the detection process is simplified, shortened, which can realize portable and miniaturization. However, the signal of immunosensor with screen printed electrode is instability, and low sensitivity.

Another kind of electrode is microelectrodes. The use of an array of microelectrodes can overcome the disadvantagement, because one of the main benefits of using a microelectrode in a sensor application is the greater sensitivity that arises from the enhanced mass-transport at these small electrodes. Hemispherical diffusion layers are formed at such electrodes and a much faster diffusion of electroactive substances occurs due to the multi-dimensional nature of this process, resulting in sigmoidal (or steady-state) cyclic voltammograms (CVs). The advantages are in the improved response time (faster response), greater sensitivity and increased response per unit electrode surface area (greater current density, increasing the signal-to-noise ratio) [31].

4. Trends and Challenges of Immunosensor for Pesticide Residues Detection

In any case, the general strategy for immunosensor construction is to place the biological material in close contact with the transducer in order to obtain high sensitivity and to minimize the time of measurement. Several immunosensors for the detection of biological and chemical contaminants are reported in the literature, but only a few have actually been applied to food analysis.

Electrochemical biosensors have revolutionized modern chemical analysis because of their technical simplicity and fast response due to the direct transduction of the biomolecular recognition event into electronic signals [19]. Mass fabrication, low cost, and decentralized infield analysis are other important features of these electrochemical sensors.

A disposable amperometric immunosensor for 2, 4-D was described by Kaláb and Skládal [32, 33], who could measure concentrations in the range of 0.1 ug/l but required an assay time of 60 min per test due to a long preincubation step. Compared to optical detection systems, amperometric sensors have several advantages. They are very sensitive and usually exhibit a wide linear range. The transducers

are easily prepared and quite inexpensive; portable devices are also available.

Ciumasu *et al.* reported a versatile, portable miniaturized flow-injection immunosensor instrument for TNT, diuron and atrazine detection (Fig. 4) [34].

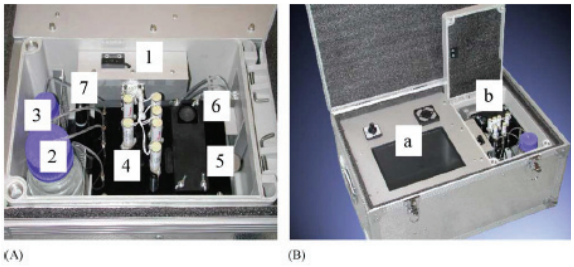

Fig. 4. The portable miniaturized flow-injection immunosensor instrument.

The applicability and advantages of immunosensors have been particularly demonstrated in the field of pesticide analysis. Nonetheless, biosensors cannot compete with HPLC or GC (with selective detection), when it comes to detailed sample composition analysis. In most environmental applications, multi-analyte determination is needed, and because biosensors can only normally detect single analytes, chromatographic methods are preferred. These techniques generally feature the immobilization of antibodies or other receptor molecules in discrete locations of a sensor surface. A planar array immunosensor equipped with a chargecoupled device (CCD) as detector have been developed and applied to the determination of different bacteria, viruses, and toxins (some of them of particular concern because of their potential use as biological warfare agents) [35].

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