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Recent Advance of Biosensor Instruments in Pesticide Residues Rapid Detection

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Abstract: In recent years, efforts have been reported to develop portable biosensor instrument in the detection felid such like pesticide residues. There is a great potential in the applications of biosensor technologies for rapid detection of pesticide residues in food and environment. In other words, meantime, biosensor instrument has wide application in the instruments market.

This paper presents an overview of various transduction systems of instruments, such as based on electrochemical, optical, piezoelectric, and nanomechanics methods' biosensor instruments, which have been reported in the literature in the design and presentation of biosensor instruments for pesticide detection. Various immobilization protocols used for formation of a biorecognition interface are also discussed. Moreover, in the design of biosensor instruments, regeneration technology, include signal amplification, miniaturization, and biosensor devices are evaluated for the development and applications of these biosensors. It can be concluded that despite some limitations of the pesticide residues detection technologies, these biosensors for pesticide monitoring are becoming more and more relevant in environmental and food analysis, that is to say, with the development of means of biosensors for detection monitoring , corresponding biosensor instruments are also becoming one essential tools in society. *Copyright © 2013 IFSA.*

Keywords: Electrochenmical biosensor, Optical biosensor, Biosensor instruments, Pesticide residues.

1. Instruction

Pesticides (herbicides, fungicides, insecticides) are widely used in agriculture and industry due to their high insecticidal activity [1-3].

Pesticides derived from synthetic chemicals are essential inputs in increasing agricultural production by preventing control pest and crop losses before and after harvesting. One-third reduction in crop yield would be happened if pesticides are not used against pest [4, 5].

The pesticides is an important management tool to boost agricultural productivity-increase crop yield and reduce post-harvest losses, especially in a world facing a hunger crisis and famine. Due to high efficiency for insect elimination, easy synthesis, and low cost, different kinds of pesticides are the most widely used in agriculture [6, 7].

Numerous chemical compounds, routinely used in agriculture and chemical industry, can form persistent toxic residues in air, soil and water, thus polluting large geographical areas [8]. However, overuse of these pesticides results in pesticide residues in food, water and environment, and leads to a severe threat to human health due to their high toxicity to acetylcholinesterase (AChE), which is essential for the functioning of the central nervous system in humans [9, 10]. Numerous analysis methods such as

gas chromatography [11], high-performance liquid chromatography [12], capillary electrophoresis [13], flow injection immunoanalysis [14-16] and fluorimetry [17] have been developed for detection of pesticides residues. These analytical techniques have been described and reviewed extensively in the literature [18]. These methods are very sensitive and reliable, but they are time-consuming and expensive. Moreover, they can only be performed by highly trained technicians and are not convenient for on-site or in-field Detection. However, false positive may easily appear and this method also need some improvements (e.g. for continuous detection). These methods have some drawbacks such as poor selectivity, high cost, slow response, poor stability and time-consuming [19]. Moreover, they can only be performed by highly trained technicians and are not convenient for on-site or in-field detection, which limit their application for real-time detection.

Owing to a great mass of pesticides generally being used, there is an increased interest for developing rapid screening systems for their detection [20]. In recent years, biosensors are potentially useful as suitable complementary tools for the real-time detection of pesticides residues and have been an active research area for some years [21]. Biosensors are designed to detect and/or quantify target molecules such as those for medical use and environmental monitoring [22, 23]. Various biological recognition elements, including cofactors, enzymes, antibodies, microorganisms, organelles, tissues, and cells from higher organisms, have been used in the fabrication of biosensors [24, 25].

For that reason, they need some improvements (e.g. for continuous detection). Biosensors are potentially useful as methods to quickly detect pesticides and have been an active research area for some years [26]. Biosensors have been defined as analytical devices which comprise of two components: biomaterial and transducer. That is to say, biosensors used as a device which tightly combine biorecognition elements with physical transducers for detection of the target compounds [27, 28]. Lately, many biosensors are used for pesticide detection which are based on the inhibition reaction or catalytic activity of several enzymes in the presence of pesticides [29-33].

According to the classification of detecting signals, mainly divided as enzyme-based biosensors, immunosensors ,microbial sensors and cell-based biosensors. Enzyme-based biosensors for pesticide determination are reviewed in the literature [34, 35]. Enzyme-based biosensors (e.g. acetylcholinesterase biosensor) for pesticide determination have been widely reported in the literature [36-39]. Since a number of pesticides have a similar mode of action affecting the activity of the same enzyme, most of enzyme-based biosensors are used for screening purposes and are unspecific for individual pesticides. They can only detect total pesticide content and do not provide specific information about a particular pesticide [20]. Enzyme-based biosensors for pesticide determination have caused public interest due to their reliability, fast response, high sensitivity and selectivity. In recent years, enzyme-linked immunosorbent assays (ELISA) have grown rapidly as tools for pesticide measurement.

Immunosensors have been used to detect or quantify the specific pesticide based on the binding interactions between immobilized biomolecules (Ab or hapten) on the transducer surface with the analyte of interest (hapten or Ab), resulting in a detectable signal [41]. The sensor system takes advantage of the high selectivity provided by the molecular recognition characteristics of an Ab, which binds reversibly with a specific hapten. They consist of two process, a molecular recognition process, for sensing the specific Ag-Ab binding reaction at the surface of receptor, and a signal-transfer process, for responding to changes in an electrochemical, optical, spectroscopic, or electrical parameter of the receptor caused by the specific binding [42]. They appear to be appropriate for identification of a single pesticide or, in some cases, small groups of similar pesticides in environmental monitoring as they are rapid, specific, sensitive and cost-effective analytical devices [43].

Furthermore, with the development of biosensor detecting technologies, generating biosensor devices in biosensor instruments against pesticide molecules has been relatively mature, which has provided further impetus in this area. Excellent reviews that focused on biosensor instruments for pesticide monitoring were described in the Literatures. However, there is a time gap between current status in the field and the most recent reviews. In this review, several types of biosensor instruments developed for the different biosensors in pesticide analysis are highlighted. Various biosensor instruments used for pesticide residues in foods and vegetables are also discussed. In addition, the development of rapid determination and techniques of regeneration, signal amplification, miniaturization are evaluated for the development and applications of these biosensor instruments.

2. Classification of Biosensor

Biosensors have been defined as a device that tightly combine biorecognition elements with physical transducer by catching process reaction between sensitive element and target objects, then convert them to continuous or discrete electrical or optical signals which can become useful information. In general, immunosensors can be distinguished from immnunoassays where the transducer is not an integral part of the analytical system. The biorecognition element determines the degree of selectivity or specificity of the biosensor, whereas the sensitivity of the biosensor is greatly influenced by the transducer. Typically, biosensors classified in some ways. Depending on different kinds of biorecognition element, biosensor can be classified into enzyme sensor, microbial sensor, organal sensor, tissue sensor, immunosensor. According to the transduction mechanism, biosensors can be further classified into

electrochemical, optical, piezoelectric and nanomechanic immunosensors. Electrochemical transducers, classified as amperometric, potentiometric, conductometric, capacitive and impedimetric measure changes in current, potential (voltage), conductance, capacitance and impedance respectively [44-46].

With the development of biosensors, biosensor instruments have been appeared emerge a historic moment. Based on different biorecognition elements, biosensor instruments will be described in the following sections.

3. Instrument of Biosensor for Detection

Over the last decade, biosensors instruments based on biosensor theory have emerged as a promising technique for pesticide residues analysis, environmental monitoring, food quality control and military investigations. The sensitivity of the biosensor is greatly influenced by the transducer, and the biorecognition element determines the degree of selectivity or specificity of the biosensor [47]. These analytical instruments are designed to complement or replace the existing reference analytical methods (chromatographic and coupled chromatographic-spectrometric procedures) by simplifying or eliminating sample preparation, thus decreasing the analysis time and cost. Most biosensor instruments reported to date are utilizing different detection principle. Based on the transduction mechanism, biosensors can be further classified into electrochemical, optical, piezoelectric biosensor instruments as described in the following sections.

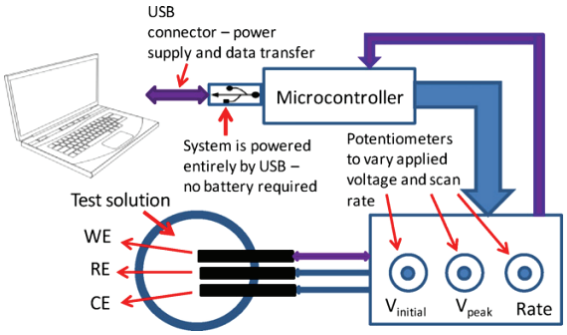
3.1. Instruments of Electrochemical Biosensors

Electrochemical biosensors have emerged the past few years as the most promising alternative to detect pesticide due to the high inherent to the electrochemical detection and the possibility of portability and miniaturization. Also the oldest and most common methods used in biosensors. They can determine the level of pesticides by measuring the change of potential, current, conductance, or impedance caused by the electrochemical reaction or immunoreaction. Thus, instruments of electrochemical biosensors also have an up and coming value of research. The instruments combine the high specificity of traditional electrochemical methods with the data acquisition, and thus exhibit great advantages. They have more advantages such like not affected by sample turbidity, quenching, or interference from absorbing and fluorescing compounds commonly found in biological samples as well as electrochemical biosensor instruments. But there still remains the big challenge in high-performance, cost-effective field analysis.

3.1.1. Instrument Based on Potentiometric Biosensors

Potentiometric biosensors are based on measuring the changes in potential induced by the label used, which occur after the specific binding of the Ab-Ag. They measure the potential across an electrochemical cell containing the Ab or Ag, usually by measuring the activity of either a product or a reactant in the recognition reaction monitored. Examples of these kinds of biosensors instrument system for the determination of the pesticides residues have been reported [48, 49]. A flow-injection system with dual amperometric and potentiometric OPH biosensors for the simultaneous and rapid measurements of OP compounds was described. The independence of the two analytical signals obtained with the dual-transducer system was illustrated [50]. The potentiometric biosensor responds favorably to all OP compounds, reflecting the pH changes associated with the OPH activity, the amperometric device displays well-defined signals only towards OP substrates (pesticides) liberating the oxidizable p-nitrophenol product. The potentiometric detection has been accomplished with a silicon-based pH-sensitive electrolyte-insulator-semiconductor (EIS) transducer, operated in the constant-capacitance (ConCap) mode. Electrolyte-insulator-semiconductor measurements were performed using the Con Cap mode, with a capacitance of 22.0 nF (in connection to total impedance of 15 kΩ). The sample injections were initiated after a 30 min stabilization of the bias voltage drift in the presence of the following carrier solution. Details of such EIS operation were described elsewhere [51]. Demonstrated a portable USB-based electrochemical biosensor prototype for point-of care testing (POCT) was designed, fabricated and tested with a model analyte. The device implements cyclic voltammetry (CV) measurements by means of a portable potentiostat in conjunction with a miniaturized electrochemical cell. The microelectronic circuit consists of a single-supply potentiostat and I/V converter, an analog-to-digital converter (ADC), and a microcontroller unit (MCU); power and controlled entirely by USB the device is suitable for field testing alongside any portable personal computer (PC). Using a simple operation protocol we can adjust the voltage range and scan rate of cyclic voltammetric measurement. The data acquisition and display can be captured using Microsoft Excel, a commonly available program on most of today's PCs. They designed a portable potentiostat that would execute cyclic voltammetric measurements. In order to create such a power Portable system, we opted for a USB powered version so there would be no need for an external supply, doubling also as the communication bus to the PC for data logging and analysis. Their system is compatible with any computer that has a USB ported is equipped with the Microsoft Excel program. However, in order to process the data and calculate the under the anodic peak, additional software is needed; we used Sigma

Plot (Systat Software, Inc.) to accomplish this task [52].



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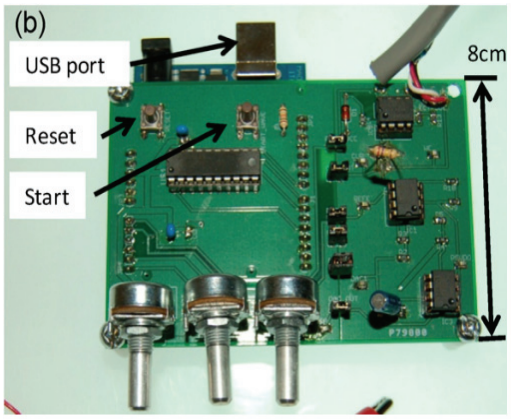

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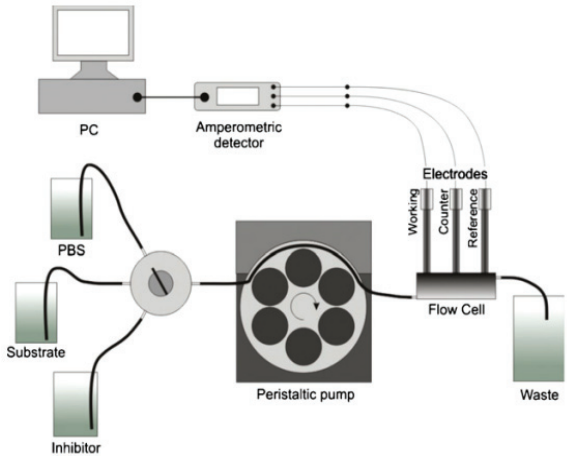
Fig. 1. (a) Overall design of the USB-powered portable electrochemical biosensor prototype; (b) Photograph of the potentiostat prototype showing the function keys/ports.

3.1.2. Instrument Based on Amperometric Biosensors

Among electrochemical biosensors, amperometric AChE biosensors as a combination of enzymatic reactions with the electrochemical methods have shown satisfactory results for pesticides analysis, where the enzyme activity was employed as indicator of quantitative measurement of insecticides [53]. When AChE is immobilized on the working electrode surface, its interactions with the substrate of acetylthiocholine (ATCl) produce the electro-active product of thiocholine [54]. The reaction equation is shown as follows [55-57]. Amperometric sensors are a subclass of electrochemical sensors. For the simultaneous analysis of several samples using only one device to developed a multichannel biosensor [58]. The detection limit for free 2,4-D in water was 0.1 ng/mL. The advantages of the presented electrochemical detector were high stability and sample through put, low detection limit, the ability to be repeatedly used, and no need for regeneration. One example is that Ivaylo Marinov et al. describe a flow-injection system with integrated amperometric biosensor featuring a replaceable AChE-immobilzed membrane with incorporated gold nanoparticles [59]. The flow-injection system comprised the following elements: three electrodes-a platinum working electrode, a standard calomel electrode (SCE) and an auxiliary platinum wire electrode; a flow-cell with a working volume of 0.275 mL; a peristaltic pump and an amperometric detector (Palm Instruments BV, The Netherlands). The flow-injection system configuration is presented in Fig. 2 (a). The biosensing device results in an integrated, automatic and portable system for environmental and Agri-food application (Fig. 2).



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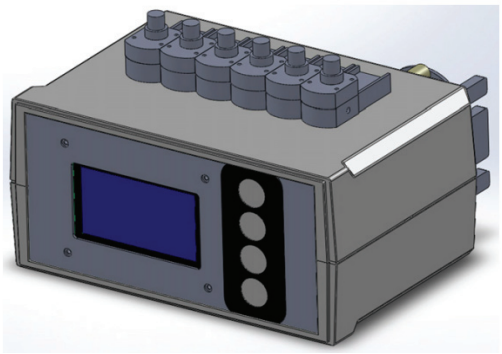

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Fig. 2. (a) Flow-injection system; (b) 3D computer aided design (CAD) view of the biosensor device.

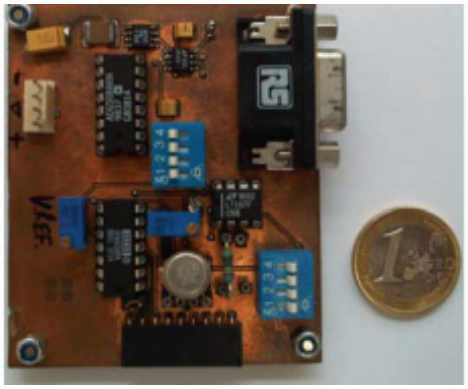
The working conditions were optimized so that the throughput of the analytical system was improved and the incubation, measurement and reactivation time of one analytical cycle was reduced twice in comparison with our previous work [60]. Where analytical measurements were conducted under static conditions. Anti-cholinesterase activities (expressed by the detection limits and the bimolecular inhibition constants) of three pesticides-paraoxon ethyl, monocrotophos and dichlorvos as well as their mixtures were assessed by the use of a flow-injection

system. This competitive behavior is worth being investigated in detail since it may present an opportunity for the discrimination between two or more pesticides present in an analyzed sample by employing chemometrics and only one type of acetylcholinesterase. The differentiation could be based on the reduction of the anti-cholinesterase activity of a supplementary inhibitor (pesticide) with known and high inhibition potency. Another example is that Viviana et al. has designed and developed one miniaturized biosensing array system was designed to be flexible, modular and small, with six independent sensing cells equipped with optical excitation and detection, current measurement and flow control systems [61]. In this study, multitask biosensor for the detection of endocrine disrupting chemicals is proposed. The sensing system employs an array of biological recognition elements. Amperometric and optical transduction methods are provided in an integrated biosensor together with flow control systems Fig. 2 (b), the biosensor system was able to detect most of the chemicals analyzed with very high sensitivity. Atrazine and diuron were detected with a limit of detection of 0.5 nM, with an RSD% less than 5 %; paraoxon and chlorpyrifos were revealed with a detection of 5 μM and 4.5 μM, respectively, with an RSD% less than 6 %; catechol and bisphenol were identified with a limit of detection of 1 μM and 35 μM respectively, with an RSD % less than 5 %.

AChE-based biosensors have a major drawback: they give a sum parameter of AChE-inhibition without any qualitative or quantitative information about the individual analytes. One approach to solve this problem involves the application of multi-sensor arrays that are combined with the data processing of artificial neural networks. Using micro or nanosensor arrays will likely become a new trend. Thus, the ability to construct arrays of enzymes will likely allow current multianalyte detection of several compounds to be expanded to accommodate the analysis of perhaps hundreds or thousands of separate compounds [62]. One of the challenges that must be met for this type of system would be the development of parallel computational methods to convert electronic responses for each analyte into meaningful concentration data. In this respect progress has been reported for artificial neural network implementation in single low-cost chip for the detection of insecticides by modeling of enzymatic sensors response [63, 64]. The design of single chip to selectively quantify mixtures of the pesticides chlorpyrifosoxon and chlorfenvinfos by an artificial neural network implementation was shown in Fig. 3B, and the dedicated system was shown in Fig. 3C [65].

3.1.3. Instrument Based on Impedance Analysis

Recent development in the area of electrochemical biosensor instruments with impedance methods of is very encouraging and offers potential advantages.



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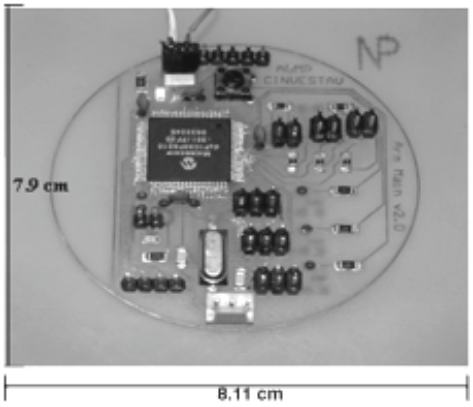

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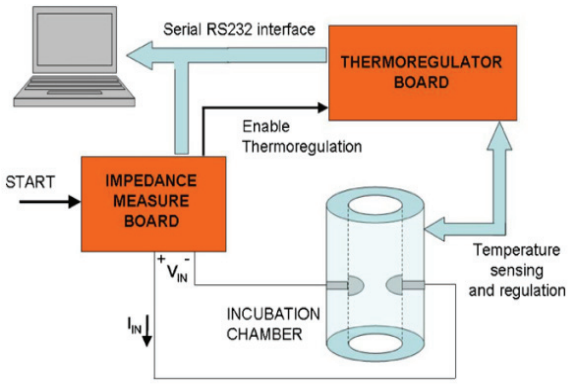
(c)

Fig. 3. Picture of the miniaturized electronic plate that functions as a potentiostat.

Usually, the concepts of impedance, conductance, capacitance, and resistance are different ways of monitoring the test system and are all interrelated [66]. Impedance biosensors instruments measure different changes of an electrical field. 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. M. Grossi et al. developed an embedded portable biosensor system for bacterial concentration. The portable biosensor system described in this work, represented in Fig. 4, features two circuit boards: one, dedicated at maintaining the SUT at the target temperature by means of an adhoc algorithm running on the microcontroller ATmega168 by Atmel (California, USA); the other, based on the microcontroller ARM STR912 by ST Microelectronics (Agrate Brianza, Italy), is used for

signal analysis and the calculation of the impedance and its components. The SUT incubation chamber features a couple of stainless steel electrodes for the electrical measures, a temperature sensor and a heating system for thermal regulation.



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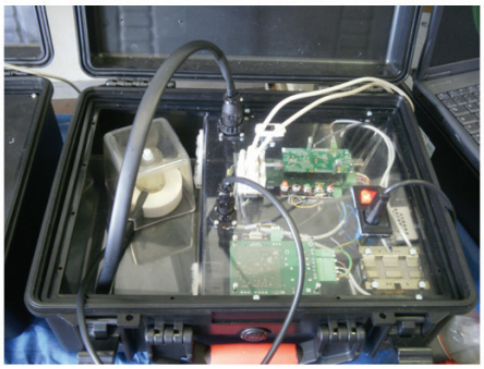


(b)

Fig. 4. Photograph (a), and schematic (b) of the biosensor system presented in the work.

The system is composed of an impedance measurement board, a thermoregulation board and an incubation chamber containing the sample under test. The system is suitable for applications in the industrial field (in particular the dairy one is of interest here), as well as for environmental monitoring (for instance for the case of water microbial screening). The greatest advantage of the presented system is that it is portable and low cost. On the contrary, most of the biosensors for microbial concentration determination on the market are essentially bench top instruments to be used within microbiology laboratories [67].

3.2. Optical Biosensor Instruments

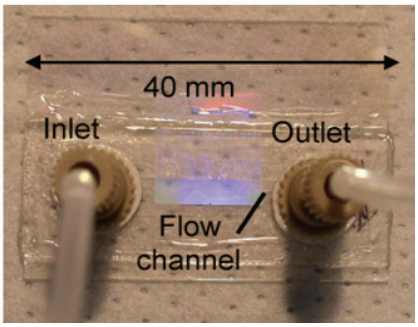
Compared with electrochemical approaches, optical devices are the second most commonly used transducers. A general optical sensor system consists of a light source, a number of optical components to generate a light beam with specific characteristics and to direct this light to a modulating agent, modified sensing head, and a photodetector [20]. Different techniques can be used for creating an optical change e.g., optical waveguide light mode spectroscopy, total internal reflection fluorescence, and surface plasmon resonance. More advantages like compactness, flexibility, resistance to electrical noise and a small probe size to be offered by optical biosensors. It is important to wash the surface before reading of the signal.

3.2.1. Optical Waveguide Lightmode Spectroscopy (OWLS)

The OWLS technique is a new sensing technique using evanescent field for the in situ and label-free study of surface processes at molecular levels. It is based on the precise measurement of the resonance angle of linearly polarized laser light, diffracted by a grating and in coupled into a thin waveguide layer. Actually, in coupling is a resonance phenomenon that depends on the refractive index of the medium covering the surface of the waveguide occurs at a defined angle of incidence**.** In the waveguide layer, light is guided by total internal reflection to the edges where it is detected by photodiodes. In recent years, efforts have been reported to develop portable sensors using fully packaged approaches based on surface plasmons [68-71] or to reduce size by integration of sample handling on-chip (e.g. in ring resonators [72]) yet these systems do not achieve a truly hand-held size ordo so at expense of lack of multiplexing or non-scalable device manufacturing [73, 74]. Sonia Grego et al. previously fabricated grating-integrated waveguide devices and characterized their Sensing performance using benchtop components including a wavelength swept laser source for telecommunication testing [75]. That work demonstrated a large wavelength scan at kHz speed driven by an external voltage source and was realized as a benchtop sensor apparatus. In 2012, another paper from Sonia Grego et al. describes a miniaturized system serving as optical reader of the waveguide devices including a new optical source and photodetector as well as multichannel capability. The configuration is designed and the components are selected with the Ultimate objective of integration into a hand-held device interrogating disposable sensor chips. The miniaturized system performance is improved compared to our previously reported values to a detection limit of 1×10^{-5} RIU and an on-chip reference configuration is demonstrated [76].

3.2.2. Surface Plasmon Resonance (SPR) Instruments

SPR has an inherent advantage over other types of biosensors in its versatility and capability of monitoring binding interactions without the need for labeling of the biomolecules.



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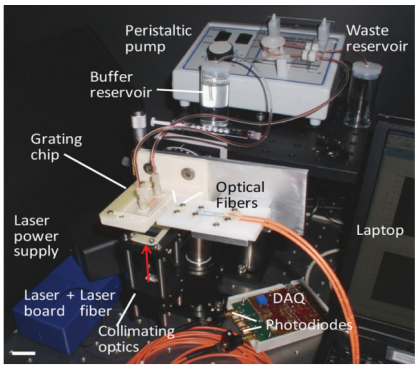


(b)

Fig. 5. (a) Photograph of the assembled cartridge, (b) Photograph of a benchtop prototype including all the components for the TWIST instrument with two sensor outputs. Scale bar is 2.5 cm. Food coloring is used to highlight the flow path.

It is versatile owing to its outstanding attributes of miniaturization, reliable portable instrumentation, and automation. Monitoring of the pesticide chlorpyrifos in water samples was performed using SPR immunosensors [77]. The chlorpyrifos derivative was immobilized onto the gold-coated sensing surface and competed with free chlorpyrifos for binding to the Ab, and as a result, increasing concentrations of chlorpyrifos will reduce the SPR signal. Other examples of single and multianalyte assays for simultaneous detection of different pesticides by SPR were reported by the same research group [78]. This portable biosensor based on SPR technology could provide a highly sensitive detection of pesticide analytes at nanogramper liter levels. The stability of the biosensor was proved by performing 15 series of daily measurements. Another sensitive and reusable SPR based immunosensor was developed for the determination of 2,4-D [79]. The SPR sensor was capable of detecting part per billion levels of 2,4-D in 20 min and the regeneration ability enabled the achievement of as many as 20 measurement cycles. SPR biosensors may suffer from disturbances caused by changes in the refractive index or temperature. The use of a reference surface makes it possible to separate signals related to binding events from signals caused by differences in refractive index between a sample and running buffer. A laboratory prototype of the SPRCD sensor has been developed. Initial version of the sensor consisted of a microfluidic cartridge incorporating the SPRCD structure and six microfluidic channels and a compact SPRCD reader [80].

Recently, capabilities of the SPRCD sensor (Fig. 6) have been further expanded. A sophisticated temperature control employing two peltier elements was incorporated to suppress the effects of the ambient temperature on the sensor response. In addition, to ensure reproducible placement of the SPRCD cartridge in the reader, a motorized system for the loading of the cartridge in the SPRCD reader was developed. Upon loading, the cartridge is automatically connected to the fluidic system of the SPRCD reader [81].

Fig. 6. A laboratory prototype of the compact SPRCD sensor.

3.3. Other Optical Biosensor Instruments

The fiber-optic biosensor consisting of AChE-immo-bilized LB film was constructed for simple and direct detection of orgarnophosphorus compounds in contaminated water. The configuration of sensor system is schematically shown in Fig. 7. The three kinds of solutions (distilled water, o-nitrophenyl acetate, and sample solution) were prepared. The absorbance change of product to be induced directly by the inhibition of organophosphorus compounds on immobilized enzyme without a pH indicator was successfully detected by the sensor system. And the proposed biosensor could successfully detect the organophosphorus compounds up to 2 ppm and the response time to steady signal of the sensor was about 10 min [82].

Fluorescence biosensor research was to develop, from first principles, a low cost, potentially portable, capable of detecting low levels of contaminants and compounds of interest in food analysis. From first principles, about L32 cm × W24 cm × H16 cm prototype biosensor detection unit was constructed that was composed of an illumination component, a sensor unit, a flow cartridge and a peristaltic pump, as illustrated in Fig. 8. The prototype fluorescent biosensor detection unit has been successfully developed that exhibits highly acceptable baseline stability, and in its current form, i.e., a manual system,

shows excellent signal repeatability. This unit has been tested using two models and has proven to be capable of detecting binding and inhibition with both compounds with a good degree of repeatability [83].

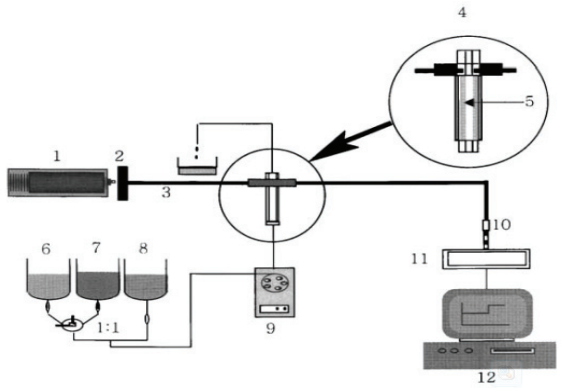
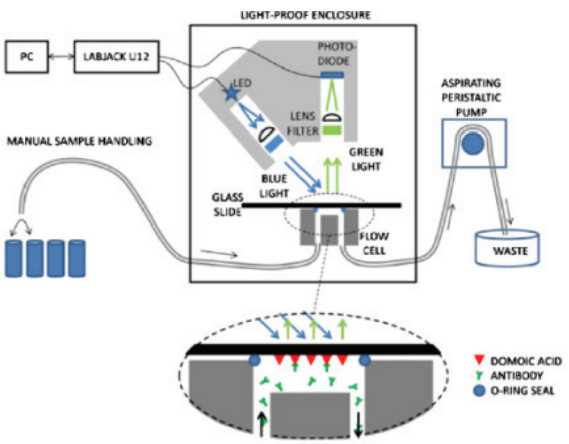


Fig. 7. The experimental set-up for biosensor: (1) xe lamp; (2) 400 nm filter; (3) optical fiber; (4) reactor containing AChE LB film; (5) immobilized enzyme layer; (6) sample; (7) distilled water; (8) sub-strate; (9) peristaltic pump; (10) photodiode; (11) A/D converter; (12) computer.



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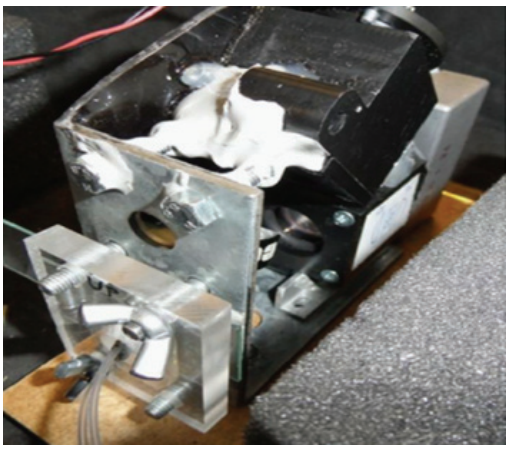

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Fig. 8. (a) Diagram of the biosensor detection unit showing light source, sensor unit, flow cartridge and glass slide integrated within a light-proof enclosure (L 32 cm ×W 24 cm × H 16 cm) and connected to a PC via a labjack U12 data acquisition device. (b) Photograph showing clamping of glass slide within the biosensor.

C. Ercole et al. describes the properties of a biosensor for the determination of E. coli cell number in water samples. The potentiometric alternating biosensing (PAB) system utilized is based on a transducing element (light addressable potentiometric sensor (LAPS)) detecting pH variations due to NH_3 production by an urease–E. coli antibody-conjugate [84, 85]. In this work, C. Ercole et al. demonstrate how an immunoassay-based PAB sensing system was able to specifically detect E. coli strains in water. This system is quite flexible, manageable and easy to construct utilising commercial electronic components and it can be further improved [86].

On top a detailed drawing of the measuring chamber is reported with the sensing electrode made of silicon, silicon dioxide and silicon nitride. In the bottom, the signal measurements performed by a synchronous demodulation techniques and the conditioning electronics blocks are shown (Fig. 9).

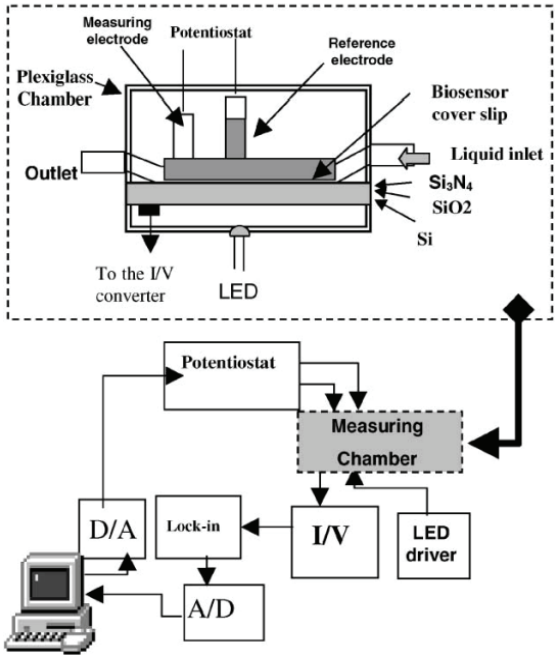

Fig. 9. Block diagram of the transducer system.

4. Immobilization Protocols

In terms of the development of electrochemical biosensors, the Ab/hapten immobilization onto a transducer or a support matrice is a key step in optimizing the analytical performance, such as response, reproducibility, stability, selectivity and regeneration. Some requirements should be met for one good immobilization method: (1) be simple and fast; (2) produce immobilized reagents that are stable and do not leach from the substrate; and (3) maintains its biological integrity flexibility, and proper active

site orientation toward the bulk solution. Thereby, Ab/hapten immobilization has been a critical issue in immunosensor technology [87-91]. Ab/hapten immobilization consists of physical adsorption and chemical binding, which depends on the driving force [92]. In general, they mostly fall into following methodologies.

1) Physical adsorption: Generally physical adsorption is based on interactions ,such as van der Wals forces, electrostatic interactions and hydrophobic interactions ,between the Ab/hapten and the transducer. Physical adsorption is simple and easy, but nonspecific attractive forces easily causes Ab/hapten desorption [93]. It is especially common on hydrophobic polymer surfaces. The main advantages of this mode of immobilization are its rapidity and simplicity, while its main drawbacks are random orientation and weak attachment. Gobi et al., created a functional sensing surface of the immunosensor by immobilizing an ovalbumin conjugate of 2,4-D (2,4-D-OVA) by simple physical adsorption on a thin-film gold chip. It has been established that the Au surface of the sensor chip was completely covered by 2,4-D-OVA up to a monomolecular layer and that the 2,4-D-OVA immobilized sensor chip was highly resistive to non-specific binding of proteins [94].

2) Covalent coupling: Ab/Ag can be covalently linked to the surfaces of a transducer through formation of a stable covalent bond between functional groups of Ab and the transducer. On the one hand, the procedure provides increased stability of the Ab ,but on the other hand covalent coupling is decreases the activity of Ab/Ag and is generally poorly reproducible. Blocking steps are usually necessary to limit the non-specific binding. An example of where this approach has been exploited is illustrated by immobilization of 2,4-D on the surface of amino-silanized optical fibers [95].

3) Self-assembled monolayer (SAM): Self-assembled monolayers (SAMs) have aroused much interest due to their potential applications in biosensors, biomolecular electronics and nanotechnology. This has been largely attributed to their inherent ordered arrangement and controllable properties. SAMs can be formed by chemisorption of organic molecules containing groups like thiols, disulphides, amines, acids or silanes, on desired surfaces to fabricate immunosensors [96]. This technique was used to immobilize analyte derivatives onto the surface of gold-coated sensors. The immunosurface formed can be used for over 120 cycles In addition to these conventional methods, new materials, such as nanoparticles have been employed in immobilizing Ab when constructing immunosensors Nanoparticles are used as solid phase due to their large surface area in a small fluid volume and good biocompatibility. Biological interactions, such as biotin/streptavid in interactions can be used to easily immobilize the Ab on the surface of nanoparticales. One of the most advantageous features of this system is that although the affinity constant between avidin and biotin is rather high, the bonding is of non-covalent nature, which allows for multiple washing and reuse of the same sensing device. Recently, describes the development of an electrochemical immunosensor for the analysis of atrazine associated to biotinylated-Fab fragment K47 antibody. The sensors are based on mixed self-assembled monolayer consisting of 1,2 dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(biotinyl) (biotinyl-PE) and 16-mercapto -hexadecanoic acid (MHDA) [97].

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5. New Trends

5.1. Miniaturization

Miniaturization is expected to have a marked impact in the development and applications of every kind of biosensor instruments. Miniaturization of a biosensor instruments not only reduces the size of detection device and integrates all steps of the analytical process into a single-sensor device, but also reduce the difficulty of experiment technology and the requirement of experimenter. Thus, it results in reduction of both the time and cost of analysis. Moreover, it is expected to lead to a further portability for in vivo sensing and in-field applications. The miniaturization trend of biosensor instruments is adapted to the miniaturization of microfabrication and nanofabrication techniques, such as micro-electromechanical systems (MEMS). MEMS combined with microelectronic circuitry are sometimes referred to as “smart sensor systems for sensor instruments”, which in turn can be configured into highly portable and inexpensive handheld instrumentation [98].

There still have some challenges remain to be overcome before miniaturized sensors instrument can be commercially applied. For one thing, miniaturization coupled with higher detection sensitivity places serious demands on both the design of instruments and methodology inherent in Ab/Ag immobilization. Furthermore, the practical application of miniaturization requires the use of complete analysis systems for sample handling, such as pumping, filtering, valving, and sample conditioning [99].

To the best of our knowledge, due to the high miniaturization and portability, immunosensors based

on SPR are now the most well known in biosensors, and then SPR instruments could faster become a popular technology in domain of detection. The combination of SPR immunosensor with flow-injection analysis (FIA) techniques offers great potential in future devices for pesticide monitoring.

5.2. High Throughput of Detection Samples

AChE-based biosensors instruments have a major drawback: they give a sum parameter of AChE-inhibition without any qualitative or quantitative information about the individual analytes. That leads to the same flaw to enzyme-based biosensor instruments. One approach to solve this problem involves the application of multi-sensor array that are combined with the data processing of artificial neural networks. Using micro or nano-sensor arrays will likely become a new trend.

5.3. Integration of Detection System

One of the challenges that some new type biosensor instruments must be met for this type of system would be of the development of parallel computational methods to convert electronic responses for each analyte into meaningful concentration data. Recently, silica based monoliths, coupled with micro-fluidic devices, have been used as an attractive alternative to packed columns for the analysis of proteins, peptides and nucleic acids with special features of low diffusion resistance during mass transfer, controllable porosity and low back pressure compared to packed columns.

5.4. Signal Amplification

Signal amplification is crucial for obtaining low detection limits in biosensors. Most amplification schemes for biosensor instruments rely on target labeling. Thus, amplification techniques lay outside the domain of label-free immunosensors. To amplify the immunoreaction signal, many methods have been proposed. Generally, methods for amplifying the immunoreaction signal are based on the detection of electro-active species, which are generated in the presence of tracer coupled to the Ag or Ab.

5.5. Real Samples Detection

The majority of biosensor instruments reported to date have been designed for detection of pesticides in environment. Application to other matrices such as food samples (fruits and vegetates) has been restricted due to the problems related to the use of these devices in the presence of organic solvents extracts. Several works reported that enzyme biosensors can function in a mixed aqueous-organic phase in low amounts of organic solvents [100, 101].

6. Conclusions

Biosensor instruments based on electrochemical, optical, and piezoelectric biosensors are strong candidates for screening pesticide residues and they become more and more relevant in environmental and food analysis, especially electrochemical biosensors. Compared to chromatography and other methods, the strengths of biosensor instruments can be described as follows:

-They are same selective and sensitive as traditional detection analytical techniques instrument, such as gas chromatography, etc.

-They can be carried out for use in the field.

-They can work with complete automation and give the results after a short period of time.

In particular, biosensor instruments based with electrochemical, optical, and piezoelectric transducers have the potential to achieve the low limits of detection imposed by legislation. Despite the promise of immunosensors, they do have certain limitations. For example, few biosensor instruments are commercially available at the present time and are yet to be established as research or routine tools, due to a lack of validated protocols for a wide range of sample matrices.

However, these biosensor instruments still face many challenges hindering their real applications.

-The data are analyzed on the electrochemical analysis instrument, and they are not enough miniaturization and portable.

-They have not specific and suitable real samples pretreatment.

-They only give a simple parameter of AChE-inhibition without quantitative information and high throughout detection.

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References

[1]. F. Arduini, F. Ricci, C. S. Tuta, D. Moscone, A. Amine, G. Palleschi, Detection of Carbamic and Organophosphorous Pesticides in Water samples Using a Cholinesterase Biosensor Based on Prussian Blue-Modified Screen-Printed Electrode, *Analytica Chimica Acta,* Vol. 580, Issue 2, 2006, pp. 155-162.

[2]. M. Adami, M. Sartore, C. Nicolini, PAB: a newly designed potentiometric alternating biosensor system,

Biosensors and Bioelectronics, Vol. 10, Issue 1-2, 1995, pp. 155-167.

[3]. D. Du, S. Z. Chen, J. Cai, A. D. Zhang, Electrochemical pesticide sensitivity test using acetylcholinesterase biosensor based on colloidal gold nanoparticle modified sol-gel interface, *Talanta*, Vol. 74, Issue 4, 2008, pp. 766-772.

[4]. M. I. Pinto, G. Sontag, R. J. Bernardino, J. P. Noronha, Pesticides in water and the performance of the liquid-phase microextraction based techniques. A review, *Microchemical Journal*, Vol. 96, Issue 2, 2010, pp. 225-237.

[5]. J. L. Tadeo, C. Sanchez-Brunete, B. Albero, L. Gonzalez, Analysis of pesticide residues in juice and beverages, *Critical Reviews in Analytical Chemistry*, Vol. 34, Issue 2, 2004, pp. 165-175.

[6]. Masumoto, Y., Sonobe, K., Size-dependent Energy Levels of CdTe Quantum Dots, *Physical Review B*, Vol. 56, Issue 15, 1997, pp. 9734-9737.

[7]. X. W. Meng, J. F, Wei, X. L. Ren, J. Ren, F. Q. Tang, A simple and sensitive fluorescence biosensor for detection of organophosphorus pesticides using H_2O_2-sensitive quantum dots/bi-enzyme, *Biosensors and Bioelectronics,* Vol. 47, 2013, pp. 402-407.

[8]. L. Pogacnik, M Franko, Determination of organophosphate and carbamate pesticides in spiked samples of tap water and fruit juices by a biosensor with photothermal detection, *Biosensors & Bioelectronics*, Vol. 14 , Issue 6, 1999, pp. 569-578.

[9]. J. L. Tadeo, C. Sanchez-Brunete, B. Albero, L. Gonzalez, Analysis of pesticide residues in juice and beverages, *Critical Reviews in Analytical Chemistry*, Vol. 34, Issue 2, 2004, pp. 165-175.

[10]. Y. Ivanov, I. Marinov, K. Gabrovska, N. Dimcheva, T. Godjevargova, Amperometric biosensor based on a site-specific immobilization of acetylcholinesterase via affinity bonds on a nanostructured polymer membrane with integrated multiwall carbon nanotubes, *Journal of Molecular Catalysis B: Enzymatic*, Vol. 63, Issue 3-4, 2010, pp. 141-148.

[11]. E. P. Syrago-Styliani, G. Evagelos, T. Anthony, A. S. Panayotis, Gas chromato-graphic-tandem mass spectrometric method for the quantitation of carbofuran, carbaryl and their main metabolites in applicators' urine, *Journal of Chromatography A*, Vol. 1108, Issue 1, 2006, pp. 99-110.

[12]. O. Eva, S. Manuel, Monitoring some phenoxyl-type N-methylcarbamate pesticide residues in fruit juices using high-performance liquid hromatography with peroxyoxalate-chemiluminescence detection, *Journal of Chromatography A*, Vol. 1007, Issue 1-2, 2003, pp. 197-201.

[13]. T. B. Jiang, H. B. Halsall, W. R. Heineman, T. Giersch, B. Hock, Capillary enzyme immunoassay with electrochemical detection for the determination of atrazine in water, *Journal of Agricultural and Food Chemistry*, Vol. 43, Issue 4, 1995, pp. 1098-1104.

[14]. J. Ramón-Azcón, E. Valera, Á. Rodríguez, A. Barranco, B. Alfaro, F. Sanchez-Baeza, M.-P. Marco, An impedimetric immunosensor based on interdigitated microelectrodes (IDμE) for the determination of atrazine residues in food samples, *Biosensors and Bioelectronics*, Vol. 23, Issue 9, 2008, pp. 1367-1373.

[15]. J. Gascón, A. Oubiña, B. Ballesteros, D. Barceló, F. Camps, M.-P. Marco, M. A. González-Martínez, S. Morais, R. Puchades, A. Maquieira, Development of a highly sensitive enzyme-linked immunosorbent assay for atrazine performance evaluation by flow injection immunoassay, *Analytica Chimica Acta*, Vol. 347, Issue 1-2, 1997, pp. 149-162.

[16]. Y. H. Zheng, T. C. Hua, D. W. Sun, J. J. Xiao, F. Xu, F. F. Wang, Detection of dichlorvos residue by flow injection calorimetric biosensor based on immobilized chicken liver esterase, *Journal of Food Engineering*, Vol. 74, Issue 1, 2006, pp. 24-29.

[17]. N. L. Pacioni, A. V. Veglia, Determination of carbaryl and carbofuran in fruits and tap water by cyclodextrin enhanced fluorimetric method, *Analytica Chimica Acta*, Vol. 488, Issue 2, 2003, pp. 193-202.

[18]. M. D. Steinberg, C. R. Lowe, A micropower amperometric potentiostat, *Sensors and Actuators B: Chemical,* Vol. 97, Issue 2-3, 2004, pp. 284-289.

[19]. Z. M. Yu, G. H. Zhao, M. C. Liu, Y. Z. Lei, M. F. Li, Fabrication of a novel atrazine biosensor and its subpart-per-trillion levels sensitive performance, *Environmental Science & Technology*, Vol. 44, Issue 20, 2010, pp. 7878-7883.

[20]. X. S. Jiang, D. Y. Li, X. Xu, Y. B Ying, Y. B. Li, Z. Z. Ye, J. P. Wang, Immunosensors for detection of pesticide residues, *Biosensors and Bioelectronics,* Vol. 23, 2008, pp. 1577-1587.

[21]. J. L. Marty, D. Garcia, R. Rouillon, Biosensor: potential in pesticide detection, *Trends in Analytical Chemistry*, Vol. 14, Issue 7, 1995, pp. 329-333.

[22]. D. R. Thevenot, K. Toth, R. A. Durst, G. S. Wilson, Electrochemical biosensors: recommended definitions and classification, *Biosensors and Bioelectronics*, Vol. 16, 2001, pp. 121-131.

[23]. T. G. Drummond, M. G. Hill, J. K. Barton, Electrochemical DNA sensors, *Nature Biotechnology*, Vol. 21, Issue 10, 2003, pp. 1192-1199.

[24]. Y. Lei, W. Chen, A. Mulchandani, Microbial biosensors, *Analytica Chimica Acta*, Vol. 568, Issue 1-2, 2006, pp. 200-210.

[25]. A. F. Collings, F. Caruso, Biosensors: recent advances, *Reports on Progress in Physics*, Vol. 60, Issue 11, 1997, pp. 1397-1445.

[26]. J. L. Marty, D. Garcia, R. Rouillon, Biosensors: Potential in Pesticide Detection, *Trends in Analytical Chemistry,* Vol. 14, Issue 7, 1995, pp. 329-333.

[27]. J. Singh, S. K. Mittal, Chlorella sp. based biosensor for selective determination of mercury in presence of silver ions, *Sensors and Actuators B,* Vol. 165, Issue 1, 2012, pp. 48-52.

[28]. G. G. Guilbault, M. Pravda, M. Kreuzer, Biosensors-42 years and counting, *Analtical Letters*, Vol. 37, 2004, pp. 14481-14496.

[29]. A. Mulchandani, W. Chen, P. Mulchandani, K. R. Rogers, Biosensors for direct determination of organophosphate pesticides, *Biosensors and Bioelectronics,* Vol. 16, Issue 4-5, 2001, pp. 225-230.

[30]. E. V. Gogol, G. A. Evtugyn, J. L. Marty, H. C. Budnikov, V. G. Winter, Amperometric biosensors based on nafion coated screen-printed electrodes for the determination of cholinesterase inhibitors, *Talanta*, 2000, Vol. 53, Issue 2, pp. 379-389.

[31]. T. M. Anh, S. V. Dzyadevych, M. C. Van, N. J. Renault, C. N. Duc, J. M. Chovelon, Conductometric tyrosinase biosensor for the detection of diuron, atrazine and its main metabolites, *Talanta*, Vol. 63, Issue 2, 2 004, pp. 365-370.

[32]. S. B. Valdez, D. E. I. Garcia, M. S. Wiener, Impact of pesticides use on human health in Mexico: a review, *Reviews on Environmental Health*, Vol. 15, Issue 4, 2000, pp. 399-412.

[33]. C. C. Liu, D. F. Cui, H. Li, A hard-soft microgfluidic-based biosensor flow cell for SPR imaging application, *Biosensors and Bioelectronics,* Vol. 26, Issue 1, 2010, pp. 255-261.

[34]. A. Mulchandani, W. Chen, P. Mulchandani, K. R. Rogers, Biosensors for direct determination of organophosphate pesticides, *Biosensors and Bioelectronics,* Vol. 16, Issue 4-5, 2001, pp. 225-230.

[35]. A. Amine, H. Mohammadi, I. Bourais, G. Palleschi, Enzyme inhibition-based biosensors for food safety and environmental monitoring, *Biosensors and Bioelectronic,* Vol. 21, Issue 8, 2006, pp. 1405-1423.

[36]. S. P. Zhang, L. G. Shan, Z. R. Tian, Y. Zheng, L. Y. Shi, D. S. Zhang, Study of enzyme biosensor based on carbon nanotubes modified electrode for detection of pesticides residue, *Chinese Chemical Letters*, Vol. 19, Issue 5, 2008, pp. 592-594.

[37]. T. Liu, H. C. Su, X. J. Qu, P. Ju, L. Cui, S. Y. Ai, Acetylcholinesterase biosensor based on 3-carboxyphenylboronic acid/reduced graphene oxide-gold nanocomposites modified electrode for amperometric detection of organophosphorus and carbamate pesticides, *Sensors and Actuators B: Chemical,* Vol. 160, Issue 1, 2011, pp. 1255-1261.

[38]. N. Chauhan, C. S. Pundir, An amperometric biosensor based on acetylcholinesterase immobilized onto iron oxide nanoparticles/multi-walled carbon nanotubes modified gold electrode for measurement of organophosphorus insecticides, *Analytica Chimica Acta*, Vol. 701, Issue 1, 2011, pp. 66-74.

[39]. P. Raghu, T. Madhusudana Reddy, B. E. Kumara Swamy, B. N. Chandrashekar, K. Reddaiah, M. Sreedhar, Development of AChE biosensor for the determination of methyl parathion and monocrotophos in water and fruit samples: A cyclic voltammetric study, *Journal of Electroanalytical Chemistry*, Vol. 665, 2012, pp. 76-82.

[40]. N. Chauhan, C. S. Pundir, An amperometric acetylcholinesterase sensor based on Fe_3O_4 nanoparticle/multi-walled carbon nanotube-modified ITO-coated glass plate for the detection of pesticides, *Electrochimica Acta*, Vol. 67, 2012, pp. 79-86.

[41]. Y. Zhu, X Sun, X. Y. Wang, Recent advance in antibody or hapten immobilization protocols of electrochemical immunosensor for detection of pesticide residues, *Sensors & Transducers,* Vol. 144, Issue 9, 2012, pp. 161-181.

[42]. X. S. Jiang, D. Y. Li, X. Xu, Y. B. Ying, Y. B. Li, Z. Z. Ye, J. P. Wang, Immunosensors for detection of pesticide residues, *Biosensors and Bioelectronicsm*, Vol. 23, Issue 11, 2008, pp. 1577-1587.

[43]. E. Mallat, C. Barzen, R. Abuknesha, G. Gauglitz, D. Barcelo, Part per trillion level determination of isoproturon in certified and estuarine water samples with a direct optical immunosensor, *Analytica Chimica Acta*, Vol. 426, Issue 2, 2001, pp. 209-216.

[44]. M. F. Yulaev, R. A. Sitdikov, N. M. Dmitrieva, E. V. Yazynina, A. V. Zherdev, B. B. Dzantiev, Development of a potentiometric immunosensor for herbicide simazine and its application for food testing, *Sensors and Actuators B*, Vol. 75, Issue 1, 2001, pp. 129-135.

[45]. K. Grennan, G. Strachan, A. J. Porter, A. J. Killard, M. R. Smyth, Atrazine analysis using an amperometric immunosensor based on single-chain antibody fragments and regeneration-free multi-calibrant measurement, *Analytica Chimica Acta*, Vol. 500, Issue 1, 2003, pp. 287-298.

[46]. E. Valera, J. Ramon-Azcon, A. Barranco, B. Alfaro, F. Sanchez-Baeza, M.-P. Marco, A. Rodríguez, Determination of atrazine residues in red wine samples. A conductometric solution, *Food Chemistry*, Vol. 122, Issue 3, 2010, pp. 888-894.

[47]. W. G. Trettnak, F. Reininger, E. Zinterl, O. S. Wolfbeis, Fiber-optic remote detection of pesticides and related inhibitors of the enzyme acetylcholine esterase, *Sensors and Actuators B: Chemical*, Vol. 11, Issues 1–3, 1993, pp. 87-93.

[48]. C. Ercole, M. Del Gallo, L. Mosiello, S. Baccella, A. Lepidi, Escherichia coli detection in vegetable food by a potentiometric biosensor. *Sensors and Actuators B,* Vol. 91, Issue 1-3, 2003, pp. 163-168.

[49]. K. A. Uithoven, J. C. Schmidt, M. E. Ballman, Rapid identification of biological warfare agents using an instrument employing a light addressable potentiometric sensor and a flow-through immunofiltration-enzyme assay system, *Biosensors & Bioelectronics,* Vol. 14, Issue 10-11, 2000, pp. 761-770.

[50]. M. J. Schöning, R. Krause, K. Block, M. Musahmeh, A. Mulchandani, J. Wang, Dual amperometric-potentiometric biosensor detection system for monitoring organophosphorus neurotoxins, *Sensors and Actuators B: Chemical*, Vol. 95, Issue 1-3, 2003, pp. 291-296.

[51]. M. I. Pinto, G. Sontag, R. J. Bernardino, J. P. Noronha, Pesticides in water and the performance of the liquid-phase microextraction based techniques. A review, *Microchemical Journal*, Vol. 96, Issue 2, 2010, pp. 225-237.

[52]. C. Loncaric, Y. Tang, C. Ho, M. A. Parameswaran, H. Z. Yu, A USB-based electrochemical biosensor prototype for point-of-care diagnosis, *Sensors and Actuators B,* Vol. 161, Issue 1, 2012, pp. 908-913.

[53]. D. Du, X. Huang, J. Cai, A. D. Zhang, Comparison of pesticide sensitivity by electrochemical test based on acetylcholinesterase biosensor, *Biosensors and Bioelectronics*, Vol. 23, Issue 2, 2007, pp. 285-289.

[54]. J. Gong, L. Wang, L. Zhang, Electrochemical biosensing of methyl parathion pesticide based on acetylcholinesterase immobilized onto Au-polypyrrole interlaced network-like nanocomposite, *Biosensors and Bioelectronics*, Vol. 24, Issue 7, 2009, pp. 2285-2288.

[55]. H. Z. Wu, Y. C. Lee, T. K. Lin, H. C Shih, F. L. Chang, H. P. P. Lin, Development of an amperometric micro-biodetector for pesticide monitoring and detection, *Journal of the Taiwan Institute of Chemical Engineers*, Vol. 40, Issue 2, 2009, pp. 113-122.

[56]. X. Sun, X. Y. Wang, Acetylcholinesterase biosensor based on Prussian blue-modified electrode for detecting organophosphorous pesticides, *Biosensors and Bioelectronics*, Vol. 25, Issue 12, 2010, pp. 2611-2614.

[57]. D. Du, S. Chen, J. Cai, A. D. Zhang, Electrochemical pesticide sensitivity test using acetylcholinesterase biosensor based on colloidal gold nanoparticle modified sol-gel interface, *Talanta*, Vol. 74, Issue 4, 2008, pp. 766-772.

[58]. S. Bhand, I. Surugiu, A. Dzgoev, K. Ramanathan, P. V. Sundaram, B. Danielsson, Immuno-arrays for multianalyte analysis of chlorotriazines, *Talanta*, Vol. 65, Issue 2, 2005, pp. 331-336.

[59]. I. Marinov, Y. Ivanov, N. Vassileva, T. Godjevargova, Amperometric inhibition-based detection of organophosphorus pesticides inunary and binary mixtures employing flow-injection Analysis, *Sensors*

and Actuators B, Vol. 160, Issue 1, 2011, pp. 1098-1105.

[60]. I. Marinov, Y. Ivanov, K. Gabrovska, T. Godjevargova, Amperometric Acetylthio-choline sensor based on acetylcholinesterase immobilized on nanostructuredpolymer membrane containing gold nanoparticles, *Journal of Molecular Catalysis B: Enzymatic,* Vol. 62, Issue 1, 2010, pp. 66-74.

[61]. V. Scognamiglio, I. Pezzotti, G. Pezzotti, J. Cano, I. Manfredonia, K. Buonasera, F. Arduini, D. Moscone, Gi. Palleschi, M. T. Giardi, Towards an integrated biosensor array for simultaneous and rapid multi-analysis of endocrine disrupting chemicals, *Analytica Chimica Acta,* Vol. 751, 2012, pp. 161-170.

[62]. K. R. Rogers, Recent advances in biosensor techniques for environmental monitoring, *Analytica Chimica Acta*, Vol. 568, Issue 1-2, 2006, pp. 222-231.

[63]. G. Istamboulie, M. Cortina-Puig, J. L. Marty, T. Noguer, The use of Artificial Neural Networks for the selective detection of two organophosphate insecticides: Chlorpyrifos and chlorfenvinfos, *Talanta*, Vol. 79, Issue 2, 2009, pp. 507-511.

[64]. G. A. Alonso, G. Istamboulie, A. Ramírez-García, T. Noguer, J. L. Marty. R. Muñoz, Artificial neural network implementation in single low-cost chip for the detection of insecticides by modeling of screen-printed enzymatic sensors response, Computers and Electronics in Agriculture, Vol. 74, Issue 2, 2010, pp. 223-229.

[65]. S. B. Valdez, D. E. I. Garcia, M. S. Wiener, Impact of pesticides use on human health in Mexico: a review, *Reviews on Environmental Health*, Vol. 15, Issue 4, 2000, pp. 399-412.

[66]. K. R. Milner, A. P. Brown, D. W. E. Allsopp, W. B. Betts, Dielectrophoretic classification of bacteria using differential impedance measurements, *Electronics Letter*, Vol. 34, Issue 1, 1998, pp. 66-68.

[67]. A. Pompei, R. Lazzarini, D. Matteuzzi, B. Riccò, An embedded portable biosensor system for bacterial concentration detection, M. Grossi, M. Lanzoni, *Biosensors and Bioelectronics,* Vol. 26, Issue 3, 2010, pp. 983-990.

[68]. T. M. Chinowsky, S. D. Soelberg, P. Baker, N. R. Swanson, P. Kauffman, A. Mactutis, M. S. Grow, R. Atmar, S. S. Yee, Furlong, Portable 24-analyte Surface plasmon resonance instruments for rapid versatile biodetection, *Biosensors and Bioelectronics,* Vol. 22, Issue 9-10, 2007, pp. 2268-2275.

[69]. M. Piliarik, M. Vala, I. Tichý, J. Homola, Compact and low-cost biosensor based on novel approach to spectroscopy of surface plasmons, *Biosensors and Bioelectronics*, Vol. 24, Issue 12, 2009, pp. 3430-3435.

[70]. M. Vala, K. Chadt, M. Piliarik, J. Homola, High-performance compact SPR sensor for multi-analyte sensing, *Sensors and Actuators B,* Vol. 148, Issue 2, 2010, pp. 544-549.

[71]. G. Suárez, Y. H. Jin, J. Auerswald, S. Berchtold, H. F. Knapp, J. M. Diserens, Y. Leterrier, J. A. E. Månson, G. Voirin, Lab-on-a-chip for multiplexed biosensing of residual antibiotics in milk, *Lab on a Chip,* Vol. 9, Issue 11, 2009, pp. 1625-1630.

[72]. C. F. Carlborg, K. B. Gylfason, A. Ka´zmierczak, F. Dortu, M. J. Bañuls Polo, A. Maquieira Catala, G. M. Kresbach, H. Sohlström, T. Moh, L. Vivien, J. Popplewell, G. Ronan, C. A. Barrios, G. Stemme, W. van der Wijngaart, A packaged optical slot-waveguide ring resonator sensor array for multiplex label-free assays in labs-on-chips, *Lab on a Chip,* Vol. 10, Issue 3, 2010, pp. 281-290.

[73]. Y. B. Shin, H. M. Kim, Y., B. H. Chung, A new palm-sized surface plasmon resonance (SPR) biosensor based on modulation of a light source by a rotating mirror, *Sensors and Actuators B,* Vol. 150, Issue 1, 2010, pp. 1-6.

[74]. B. N. Feltis, B. A. Sexton, F. L. Glenn, M. J. Best, M. M. Wilkins, T. J. Davis, A hand-held surface plasmon resonance biosensor for the detection of ricin and other biological agents, *Biosensors and Bioelectronics,* Vol. 23, Issue 7, 2008, pp. 1131-1136.

[75]. S. Grego, J. R. McDaniel, B. R. Stoner, Wavelength interrogation of grating-based optical biosensors in the input coupler configuration, *Sensors and Actuators B,* Vol. 131, Issue 2, 2008, pp. 347-355.

[76]. S. Grego, K. H. Gilchrist, J. B. Carlson, B. R. Stoner, A compact and multichannel optical biosensor based on a wavelength interrogated input grating coupler, *Sensors and Actuators B,* Vol. 161, Issue 1, 2012, pp. 721-727.

[77]. E. Mauriz, A. Calle, J. J. Manclús, A. Montoya, A. Hildebrandt, D. Barceló, L. M. Lechuga, Optical immunosensor for fast and sensitive detection of DDT and related compounds in river water samples, *Analytica Chimica Acta,* Vol. 22, Issue, 7, 2007, pp. 1401-1418.

[78]. O. S. Wolfbeis, Koller, E., Fiber optic detection of pesticides in drinking water. in: Biosensors Application in Medicine Environmental Protection and Process Control, ed. by Schmid, R. D., Scheller, F., *GBF,* New York, 1989. pp. 221-224.

[79]. K. V. Gobi, S. J. Kim, H. Tanaka, Y. Shoyama, N. Miura, Novel surface plasmon resonance (SPR) immunosensor based on monomolecular layer of physically-adsorbed ovalbumin conjugate for detection of 2, 4-dichlorophenoxyacetic acid and atomic force microscopy study, *Sensors and Actuators B: Chemical*, Vol. 123, Issue 1, 2007, pp. 583-593.

[80]. M. Piliarik, M. Vala, I. Tichy, J. Homola, Compact and low-cost biosensor based on novel approach to spectroscopy of surface plasmons, *Biosensors & Bioelectronics*, Vol. 24, Issue 12, 2009, pp. 3430-3435.

[81]. H. Šípová, M. Piliarik, M. Vala, K. Chadt, P. Adam, M. Bocková, K. Hegnerová, J. Homola, Portable surface plasmon resonance biosensor for detection of nucleic acids, *Procedia Engineering,* Vol. 25, 2011, pp. 148-151.

[82]. J.-W. Choi, Y.-K. Kim, I. H. Lee, J. H. Min, W. H. Lee, Optical organophosphorus biosensor consisting of acetylcholinesterase/viologen hetero Langmuir-Blodgett film, *Biosensors & Bioelectronics,* Vol. 16, Issue 9-12, 2001, pp. 937-943.

[83]. T. F. McGrath, K. Andersson, K. Campbell, T. L. Fodey, C. T. Elliott, Development of a rapid low cost fluorescent biosensor for the detection of food contaminants, *Biosensors and Bioelectronics,* Vol. 41, 2013, pp. 96-102.

[84]. L. Piras, M. Adami, S. Fenu, M. Dovis, C. Nicolini, Immunoenzymatic application of a redox potential biosensor, *Analytica Chimica Acta,* Vol. 335, Issue 1-2, 1996, pp. 127-135.

[85]. A. G. Gerhing, D. L. Patterson, S. I. Tu, Use of a light-addressable potentiometric sensor for the detection of *Escherichia Coli* O157: H7, *Analytica Biochemistry*, Vol. 258, Issue 2, 1998, pp. 293-298.

[86]. C. Ercole, M. Del Gallo, M. Pantalone, S. Santucci, L. Mosiello, C Laconi, A. Lepidi, A biosensor for

Escherichia coli based on a potentiometric alternating biosensing (PAB) transducer, *Sensors and Actuators B,* Vol. 83, Issue 1-3, 2002, pp. 48-52.

[87]. J. Lin, C. W. Brown, Sol-gel glass as a matrix for chemical and biochemical sensing, *Trends in Analytical Chemistry*, Vol. 16, Issue 4, 1997, pp. 200-211.

[88]. J. M. Nam, C. S. Thaxton, C. A. Mirkin, Nanoparticle-based biobar codes for the ultrasensitive detection of protein, *Science*, Vol. 301, No. 5641, 2003, pp. 1884-1886.

[89]. J. Wang, A. Ibanez, M. P. Chatrathi, Microchip-based amperometric immunoassays using redox tracers, *Electrophoresis*, Vol. 23, Issue 21, 2002, pp. 3744-3749.

[90]. J. S. Yuk, S. J. Yi, H. G. Lee, H. J. Lee, Y. M. Kim, K. S. Ha, Characterization of surface plasmon resonance wavelength by changes protein concentration on protein chips, *Sensors Actuators B*, Vol. 94, Issue 2, 2003, pp. 161-164.

[91]. X. F. Yuan, D. Fabregat, K. Yoshimoto, Y. Nagasaki, Development of a high- performance immunolatex based on "soft landing" antibody immobilization mechanism, *Colloids and Surfaces B: Biointerfaces*, 2011, in Press.

[92]. J. M. Peula, R. Hidalgo-Alvarez, F. J. de las Nieves, Covalent binding of proteins to acetal-functionalized latexes. I. Physics and chemical adsorption and electrokinetic characterization, *Journal of Colloid and Interface Science*, Vol. 201, Issue 2, 1998, pp. 132-138.

[93]. J. M. Peula, R. Hidalgo-Alvarez, F. J. de las Nieves, Covalent binding of proteins to acetal-functionalized latexes. I. Physics and chemical adsorption and electrokinetic characterization, *Journal of Colloid and Interface Science*, Vol. 201, Issue 2, 1998, pp. 132-138.

[94]. K. V. Gobi, S. J. Kim, H. Tanaka, Y. Shoyama, N. Miura, Novel surface plasmon resonance (SPR) immunosensor based on monomolecular layer of physically-adsorbed ovalbumin conjugate for detection of 2, 4-dichlorophenoxyacetic acid and atomic force microscopy study, *Sensors and Actuators B*, Vol. 123, Issue 1, 2007, pp. 583-593.

[95]. C. Wittmann, F. F. Bier, S. A. Eremin, R. D. Schmid, Quantitative analysis of 2, 4-dichlorophenoxyacetic acid in water samples by two immunosensing methods, *Food Chemistry*, Vol. 44, 1996, pp. 343-350.

[96]. S. K. Arya, P. R. Solanki, M. Datta, B. D. Malhotra, Recent advances in self-assembled monolayers based biomolecular electronic devices, *Biosensors and Bioelectronics*, Vol. 24, Issue 9, 2009, pp. 2810-2817.

[97]. S. Hleli, C. Martelet, A. Abdelghani, N. Burais, N. Jaffrezic-Renault, Atrazine analysis using an impedimetric immunosensor based on mixed biotinylated self-assembled monolayer, *Sensors and Actuators B*, Vol. 113, Issue 2, 2006, pp. 711-717.

[98]. Y. Qu, Q. Sun, F. Xiao, G. Shi, L. Jin, Layer-by-Layer self-assembled acetylcholinesterase/PAMAM-Au on CNTs modified electrode for sensing pesticides, *Bioelectrochemistry*, Vol. 77, Issue 2, 2010, pp. 139-144.

[99]. X. Sun, C. Zhai, X. Y. Wang, Recent Advances in Amperometric Acetylcholinesterase Biosensor, *Sensors & Transducers*, Vol. 137, Issue 2, February 2012, pp. 199-214.

[100]. G. Istamboulie, M. Cortina-Puig, J. L. Marty, T. Noguer, The use of Artificial Neural Networks for the selective detection of two organophosphate insecticides: Chlorpyrifos and chlorfenvinfos, *Talanta,* Vol. 79, Issue 2, 2009, pp. 507-511.

[101]. G. A. Alonso, G. Istamboulie, A. Ramírez-García, T. Noguer, J. L. Marty. R. Muñoz, Artificial neural network implementation in single low-cost chip for the detection of insecticides by modeling of screen-printed enzymatic sensors response, *Computers and Electronics in Agriculture*, Vol. 74, Issue 2, 2010, pp. 223-229.
