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## Chapter

# Electrochemical Biosensors Containing Pure Enzymes or Crude Extracts as Enzyme Sources for Pesticides and Phenolic Compounds with Pharmacological Property Detection and Quantification

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## Abstract

Biosensors are chemical sensors in which the recognition system is based on a biochemical mechanism. They perform the specific component detection in a sample through an appropriate analytical signal. Enzyme-based biosensors are the most prominent biosensors because of their high specificity and selectivity; besides being an alternative to the common immunosensors, they are more expensive and present a limited binding capacity with the antigen depending on assay conditions. This chapter approaches the use of enzymes modified electrodes in amperometric biosensing application to detect and quantify pesticides and phenolic compounds with pharmacological properties, as they have been a promising analytical tool in environmental monitoring. These biosensors may be prepared from pure enzymes or their crude extracts. Pure enzyme-based biosensors present advantages as higher substrate specificity and selectivity when compared to crude extract enzymatic biosensors; nevertheless, the enzyme high costs are their drawbacks. Enzymatic crude extract biosensors show lower specificity due to the fact that they may contain more than one type of enzyme, but they may be obtained from low-cost fabrication methods. In addition, they can contain enzyme cofactors besides using the enzyme in its natural conformation.

**Keywords:** polyphenol oxidase, peroxidase, acetylcholinesterase, crude extracts, biosensors, pesticides, phenolic compounds, environmental enzymatic biosensors

## **1. Introduction**

Chemical sensors and biosensors are devices used in detection and quantification of an analyte by converting its concentration into an analytical signal. Advances in sensor technology have been important for the enrollment of sensing methods in several applications. Chemical sensor operates based on chemical principles, where the analytical signal emerges as a result of a chemical reaction between the analyte and a specific sensitive layer. Electrochemical sensors are able to detect H<sub>2</sub>, consisting of Pt, Pd, Au, Ag, and metal oxides, as reported by Korotcenkov et al. [1]. These capabilities are expected to be performed by biosensors as well, which are sensors that present a biological recognition element integrated with the transducer. The most popular biosensors are the enzymatic-based ones, successfully represented by the glucose biosensors. Biosensors have become an attractive analytical instrument for environmental monitoring because there still severe barriers through an effective, fast, and low-cost monitoring of harmful pollutants. Among the hazardous contaminants, phenolic compounds and pesticides represent potential human health and environmental risks. Regarding this, there are several studies reporting the use of horseradish peroxidase (HRP) for phenolic compounds and hydrogen peroxide detection [2]. Enzyme-based biosensors operate by indirectly detecting analytes, through detection of consumption or production of specific compounds in the biochemical reaction progress [3]. Phenolic pollutants are important due to their extensive use in several industrial products and their resulting negative environmental impacts. Also, enzymatic biosensors are applied to detect pesticides, particularly organophosphorus and carbamates. The operation of these devices, primarily designed to quantify those pesticides, is based on the inhibition of enzyme activity by these toxic compounds. Distinctly, the use of enzymes in biosensors for environmental monitoring brings considerable advantages, such as high selectivity and specificity, enhanced sensitivity, catalytic activity, and fast performance [4]. Nevertheless, they present some drawbacks associated with the high costs of obtainment and manipulation processes (extraction, isolation, and purification), denaturation during immobilization on transducer, and activity loss after a period (short shelf life) [4]. However, when enzymatic biosensors are compared to other sensing devices, such as immunosensors, they show superior characteristics because antibodies are more expensive, they do not present catalytic activity, and their binding ability depends on conditions of the assay, such as temperature and pH. Due to their advantages, the use of enzymatic biosensors to monitor environmental pollutants, as well as their applications in pharmacology and in pesticides monitoring will be discussed in this chapter.

## **2. Phenolic pollutants**

Phenolic compounds are present in daily activities, since they are frequently found in vegetables, materials, waste, and water, not mentioning their relevance to several applications, due to their pharmacological and antioxidant properties [4]. Beyond the natural phenolic compounds, the synthetic ones are used in many daily products, such as fragrances, moisturizers, makeup, drugs, processed foods, and plastics, among others [5]. The manufacture and use of these products result in their accumulation in the environment, mostly in water.

Several phenolic compounds have been appointed as endocrine disrupting chemicals (EDCs), defined as “chemical substances or mixtures that interfere in any aspect of the hormonal action of living organisms” [6]. EDCs comprise many chemicals used in industrial activities, such as natural and synthetic hormones,

pharmaceuticals, pesticides, and surfactants. Some examples of phenolic compounds appointed as EDCs and related products are shown in **Table 1**.

Phenolic pollutants are worldwide dispersed; they can be transported at long distances by water flows and show high persistence and penetrability [7]. Exposure of aquatic animal species, including fishes and amphibians, to EDCs has been related to be responsible for the observed feminization of many species, which in contrast, diminishes the population of these species. Studies of the exposure effects of humans to EDCs suggest a relation between the development of chronic diseases, such diabetes mellitus type II, obesity, thyroid dysfunction, poor quality sperm in males, and fertility issues [8]. Although, until now, there is no effective confirmation of the effect of EDC exposure to these metabolic anomalies, monitoring the environmental concentration of such substances had been the actual concern of the scientific community. Due to their low cost, selectivity, sensitivity, and fast response, biosensors have been considered a promising alternative to classic analytical methods, such chromatography and nuclear magnetic resonance.

## 2.1 Enzymatic biosensors

Due their complex structures, enzymes exhibit high selectivity to substrates, being able to detect one substance in multicomponent matrices. This behavior is exploited in analytical devices that present high reproducibility, sensibility, and selectivity, making use of low time-consuming analysis, low-cost equipment, and few or any sample preparation steps [9]. These advantages combined with electrochemical transducers result in cheaper portable and miniaturized biosensors, when compared to other types of transducers, such as optical and piezoelectric [10], which is a great feature for environmental applications.

The electrochemical enzymatic biosensors operate based on the electron transfer between the enzyme active site and the substrate, which is, then, transduced to generate an analytical signal. The electrochemical signal can be of three distinct types: (i) amperometric, in which the electrical current generated in the electron transfer process is measured [11], (ii) conductimetric, in which the change in the electrical conductivity of the environment is measured [12], and (iii) potentiometric, in which the electrochemical potential in the absence of measurable current is measured [13]. The amperometric biosensors are the most used ones, due to their high sensibility. These biosensors require the enzyme immobilization on the electrode surface. The most frequently used methods for enzyme immobilization are noncovalent adsorption, covalent bonding, entrapment, cross-linking, and affinity, and they are discussed below [14].

## 2.2 Enzyme immobilization on the electrode surface

The noncovalent adsorption immobilization consists of enzyme adsorption on the electrode surface by physical interactions, such as van der Waals forces,

Product class	EDC examples
Drugs (human and animal uses)	Acetaminophen, tetracyclines, salbutamol, morphine
Antimicrobials (food and cosmetics)	Chlorophenols, parabens, triclosan, propyl gallate, tert-butylhydroquinone
Plastics	Bisphenol A (BPA), bisphenol F (BPF)
Steroids	Estradiol, estrone, estriol
Surfactants	Alkylphenols

**Table 1.**  
*Phenolic compounds appointed as EDCs and their related products.*

hydrogen bonds, and electrostatic interactions [14]. In contrast, in the covalent bonding immobilization, the enzyme is anchored on the electrode surface by multiple covalent bonds between support functional groups and enzymes. The entrapment immobilization on the electrode is the enzyme inclusion in a framework, such as a polymer network, which can be organic or inorganic polymeric matrices. An additional method for enzyme immobilization that provides high stability is the application of a metal-organic framework (MOF) [15]; nevertheless, small cavities of MOFs usually result in decreased substrate affinity. Therefore, the enzymatic activity of the immobilized enzyme is decreased, when compared to native enzyme activity [15]. Cross-linking immobilization is an alternative, which requires the reaction between cross-linking protein molecules and a chemical cross-linker, usually glutaraldehyde [14]. The diversity of immobilization techniques allows the immobilization of enzymes in distinct materials, such as carbon nanostructures, (carbon black, nanotubes, and graphene and derivatives, among others), ceramic or polymeric matrices, and nanoparticles [16, 17]. It is noteworthy that the performance of an enzymatic biosensor is strongly dependent of the enzyme immobilization, which affects important parameters such as response time, stability, reproducibility, and sensitivity [18].

Another element that interferes in enzymatic biosensor response is active site location. Since proteins are molecules with a giant structure, the active center often can be closed in the molecule's center, making it a very inaccessible site and less susceptible for electron transfer processes. In these cases, a mediator can be used to facilitate the electron transfer between the active site of the enzyme and the modified active electrode. There are several mediators for that, but some are specific for only one enzyme. Regarding Barsan et al. [19], several electrodes modified by functionalized carbon nanotubes act as an alternative to promote the increase of interaction between enzymes and modified electrodes. In addition, they improve the electron transfer rate, besides the fact that phenolic molecules can be used as mediators in these processes. On the other hand, it is also common to use organic dyes such as methylene blue, safranin O, and neutral red [20] and metal complexes, for example, ferrocene [21], as mediators.

### 2.3 Crude extracts as enzyme sources for biosensing applications

Some enzymes that can be used in phenolic biosensing are peroxidases and polyphenol oxidases. Peroxidases (E.C. 1.11.1) comprise a large family of heme-containing enzymes that react with their substrates using peroxide of hydrogen ( $H_2O_2$ ) as a proton acceptor, generating water ( $H_2O$ ) and the oxidized substrate. These enzyme families have been widely used in clinical diagnostics, biosensing, and degradation of pollutants in water [22]. Polyphenol oxidase (E.C. 1.10.3.1) is another enzyme family that includes laccases and tyrosinases, also known as blue-copper oxidases. Laccase enzymes catalyze the oxidation of many phenolic substrates (most commonly ortho- and para-diphenols) with the concomitant reduction of molecular oxygen to water [23], while tyrosinases are enzymes that catalyze two distinct oxygen-dependent subsequent reactions: the hydroxylation of monophenols to ortho-diphenols and the subsequent oxidation of ortho-diphenols to ortho-quinones [24]. These enzymes are very much used in biosensor construction, being often purchased at their active lyophilized form. In the cited cases, the common commercial peroxidases are extracted from Horseradish (*Armoracia rusticana*) roots, while laccases and tyrosinases are extracted from fungi [24].

Oxidoreductases are widely distributed in the plant kingdom, being found in many vegetables. The vegetable crude extracts represent a good alternative to replace manufactured enzymes in biotechnological applications. Commercial

enzymes have the advantage of exhibiting high purity levels, which is responsible for a significant increase in selectivity of the analytical device; however, they are very expensive. The crude extracts as enzymatic sources show some advantages such as abundant and easy enzyme obtainment, low cost, and bioavailability of cofactors when necessary to enzymatic activity [25].

Usually, the crude extracts are prepared by processing vegetal tissues in a buffer solution, close to physiological pH, followed by separation of solids by centrifugation. Peroxidases and polyphenol oxidases are found in cell membranes of many vegetables and detergent solutions, such as sodium dodecyl sulfate (SDS), which are dissolved by phenolic compounds to perform the extraction and at the same time that activates the enzyme latent forms [26]. Phenolic compounds are common in vegetables and they react with peroxidases or polyphenol oxidases in the crude extract preparation. In order to preserve enzyme reactivity, phenol scavenger polymers, such as polyvinylpyrrolidones (PVPs) and their derivatives, are added to the extract. These polymers work as phenol adsorbents, interacting with phenolic compounds via hydrogen bonds, preventing these reactions [27].

Several examples of biosensors prepared with crude extracts as enzyme sources were reported [28–30]. Many studies aim to obtain less expensive biosensors with higher durability, since crude extracts mimic the natural enzyme environment. In addition, cofactors and coenzymes can be present in the crude extract. Martins et al. [28] reported the preparation of a biosensor using the crude extract of the pumpkin *Cucubita pepo* for paracetamol detection in aqueous solution, and Benjamin et al. [29] reported a biosensor prepared with a crude extract, which was a source of the polyphenol oxidase, anchored with cerium nanoparticles for rutin detection in solution, showing a limit of detection of the  $0.16 \mu\text{mol L}^{-1}$ .

The biosensor for phenolic compounds from drugs and industrial wastewater was proposed by Antunes et al. [30]. They used the crude extract from vegetal issue sources of polyphenol oxidase, which was anchored on the electrode surface, and the analysis was carried out in an electrochemical cell. The biosensor was evaluated for the quantitative determination of acetaminophen, acetylsalicylic acid, methyl-dopa, ascorbic acid, and phenolic compounds in a real sample. The limit of detection achieved was  $7 \mu\text{mol}$  of phenol, which is compared to the limit of detection of  $8 \mu\text{mol}$  for polyphenol oxidase for pharmacological samples.

There are several electrochemical biosensors to determine the pharmacological properties of phenolic compounds. Tyrosinase-based biosensor is widely used for detection of phenolic compounds [31, 32]. Its construction is based on the same approaches, such as electropolymerization and sol-gel and polymer entrapment [33]. Aranganathan et al. [33] reported the use of tyrosinase for detection of 3,4-dihydroxy-L-phenylalanine (L-DOPA), which is a preferred drug for the treatment of Parkinson's disease. Florescu and David [34] developed a tyrosinase-based biosensor for selective dopamine detection, in which its selectivity was increased by employing cobalt (II)-porphyrin (CoP) film-modified gold electrodes. It operates by enabling the direct immobilization of the enzyme layer in more available sites, acting as an electrochemical mediator during enzyme-catalyzed reaction, leading to a complete recovery of the electrode, with no effect on the detection limit [34]. Tyrosinase can be used as a pesticide detector as well. In this respect, Liu et al. [35] developed a biosensor consisting of a glassy carbon electrode modified with graphene and containing tyrosinase immobilized on platinum nanoparticles. It was for organophosphorus pesticide detection and they found that the presence of Pt nanoparticles and graphene improved the biosensor sensitivity by enhancing the efficiency of the electrochemical reduction of o-quinone. Also, in the study conducted by Everett and Rechnitz [36], the tyrosinase-based biosensor was very sensitive to pesticide in aqueous solution.

Peroxidase-based biosensors are alternatives to determine phenol and phenolic compounds. The need for a peroxidase-based material that would be more stable in aqueous media, with lower costs, leads to the use of hemoglobin in the biosensor processing [31]. Highly sensitive hemoglobin-based biosensor was obtained by the modification of a carbon-paste electrode with hemoglobin and multiwalled carbon nanotubes. It was tested in the detection of methylparaben, present in real samples of urine and human serum. It reached a detection limit of 25 nM [37]. In their work, Haijan et al. [37] also showed that the immobilization of hemoglobin onto cuprous sulfide nanorods/Nafion<sup>®</sup> nanocomposite film is an effective way to construct a biosensor for polyphenol detection. In addition to the hemoglobin immobilization, the polyphenol detection was also enhanced.

Rodríguez-Delgado et al. [23] developed laccase-based biosensors that presented high sensitivity and reproducibility for phenolic compounds *in situ* and environmental monitoring. Several others pollutants, that can be easily dissolved in water and, therefore, are considered environmental pollutants, must be monitored. It is the case of several compounds used by the food and textile industries. With this regard, tartrazine, a synthetic organic food azo dye, has its use controlled due to its potential harmfulness to human health. The first work on the use of laccase-based biosensor for the determination of tartrazine dye was recently developed by Mazlan et al. [38], which is a biosensor consisting of laccase enzyme immobilized on methacrylate-acrylate microspheres and composites with gold nanoparticles [38].

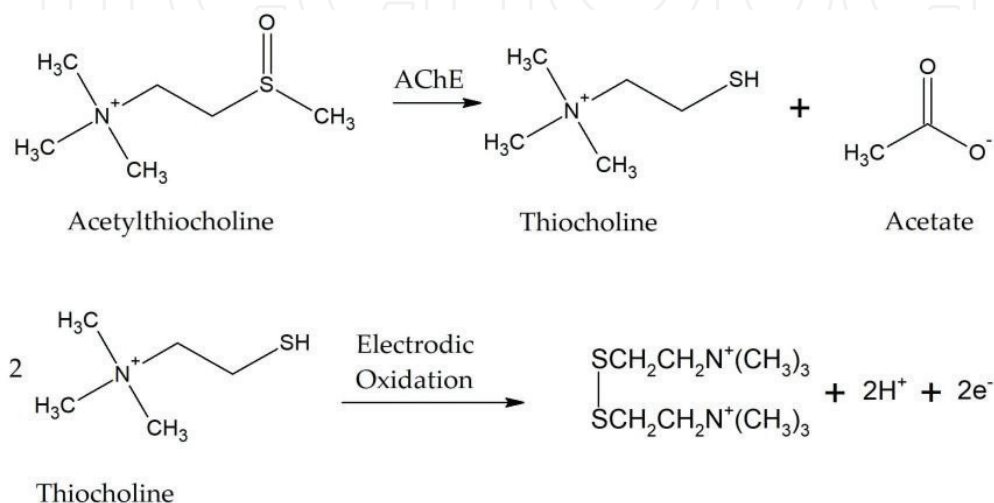
The adverse use of drugs based on morphine and narcotics causes several illnesses around the world. The development of efficient methods to detect illicit drugs in biological samples, such as urine and blood plasma, is, therefore, much required. Gandhi et al. [39] reported the advances in the field of biosensors for narcotic drug detection. Among them, they showed that the double-stranded DNA (ds-DNA) immobilized onto mercaptobenzaldehyde-modified Au electrode is an advantageous and promising biosensor to morphine detection, since it presents the advantage of no need of additional steps of extraction, cleansing, and derivatization [40].

Regarding drug detections, yet Alvau et al. [41] proposed a biosensor for therapeutic drug monitoring based on acetylcholinesterase (AChE) and choline oxidase. These are promising biosensors because they also present the possibility of distinct application, for instance, AChE-based biosensors can find application in environmental monitoring, since they can be used for the electrochemical detection of organophosphate and carbamate pesticides. The global concern over pesticide level increase rose the last decade due to the high toxicity and bioaccumulation effects of such compounds, and the significant risks that they represent to the environment and human health. Therefore, monitoring pesticide residues by sensitive analytical techniques is indispensable. In view of the harmful effects associated with pesticides, a legislative framework has been established worldwide which defines rules for the approval of active chemicals and maximum residue levels (MRLs) allowed in food and water. The legal limits for the amount of pesticides allowed in food and drinking water are set by the Environmental Protection Agency (EPA) in USA and for the European Environment Agency in European Union (EEA). These government agencies establish the appropriate pesticides levels, according to the type of crops and substance. For instance, the pesticide methomyl has the maximum tolerance established at 2.0 ppm (parts per million) in lemon in USA, whereas EEA established a MRL lower than 0.01 ppm for the same pesticide in lemon. However, in the case of the pesticide chlorpyrifos in apples, both agencies authorize the same MRL in 0.01 ppm for apples. Commonly, the MRLs are in the range of ppm to ppb (parts per billion); nevertheless, there are some pesticides that are forbidden and are illegally used. In contrast, in Brazil, the legislation regarding the use of pesticides in crops as well as the detection limit in food and water is much more permissive. For instance,

it allows a level of glyphosate in water up to 5000 times greater than that allowed in the European Union. Over the years, several biomolecules have been used as a biorecognition element in biosensors for pesticide detection, such as cells, antibodies, aptamers, and enzymes. In this section, we will focus on enzymatic biosensors for organophosphate (OP) and carbamate quantification based on electrochemical transducer. These devices use acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), in addition to alkaline phosphatase (ALP) and organophosphorus hydrolase (OPH) for OP detection, specifically. AChE-based biosensors are among the most popular electrochemical sensing platforms for the aforementioned types of pesticides [42]. AChE is susceptible to be inhibited by OPs as well as carbamate pesticides. The working mechanism of an electrochemical AChE-based biosensor is based on inhibitory effects. In the absence of OPs and carbamates (analytes), the substrate acetylthiocholine is converted into thiocholine and acetate. Afterwards, thiocholine is oxidized by the applied potential. When the analyte is present in the solution, AChE has its activity decreased by the pesticide inhibition. Consequently, the conversion of acetylthiocholine is partial or totally reduced, and the pesticides are indirectly detected [43]. **Figure 1** shows the working principle of AChE biosensor.

Selectivity is the most significant hallmark of enzymatic biosensors. In the case of AChE-based biosensors, it is only possible to detect an assortment of pesticides in a complex matrix, and no qualitative or quantitative information is obtained for a single inhibitor. Besides, AChE can be inhibited by heavy metals, drugs, and nerve agents. Therefore, the inhibition strategy to detect pesticides towards AChE implies in poor selectivity [44]. An important consideration is that AChE inhibition by pesticides may diverge according to the source of enzyme. Studies have demonstrated that AChE extracted from electric eel exhibited greater sensitivity in comparison to those from bovine and human erythrocytes [45]. On the other hand, genetically modified AChE from *Drosophila melanogaster* revealed superior results [45]. In order to address these limitations, numerous approaches have been developed, involving nanomaterial technologies to improve the transducer performance in addition to genetic engineering [46].

The design of novel AChE-based biosensors for pesticide detection concerns the application of nanomaterials offering transducing platforms with outstanding electrochemical behavior. The advantages provided by nanomaterials in electrochemical sensing are associated with large surface-to-volume ratio, controlled morphology, electrocatalytic properties, immobilization of biomolecules, and possibilities of system miniaturization [47].



**Figure 1.** Scheme of the general reaction mechanism of an electrochemical biosensor based on AChE.



Currently, the employment of screen printed electrodes (SPEs) has boosted the scenario of AChE-based biosensors. Those electrodes promote the system miniaturization addressing the sample volume issues, combining cost effectiveness and simple manipulation. Therefore, several strategies of modification have been applied to achieve high sensitivity and low limit of detection. A smart AChE biosensor approach used homemade SPE modified with single-walled carbon nanotubes (SWCNT) derivatized with cobalt phthalocyanine to detect thiocholine at a lower overpotential in comparison to bare SPE and SPE modified with nonfunctionalized SWCNT in only 80  $\mu\text{L}$  of sample [48]. Remarkably, the performance of an AChE-based biosensor was improved due to electrode modification with *N*-carbamoylmaleimide-functionalized carbon dots (*N*-MAL-CDs) as a nanostabilizer [49]. The initial electrochemical signals of thiocholine were obtained without signal loss, as a result of the Michael addition reaction functionalizing CDs with *N*-MAL. Then, *N*-MAL-CDs can react with thiol group from thiocholine, forming a thiol containing compound. The aforementioned compound cannot be easily oxidized during the detection process, avoiding the signal loss. For the fabrication of AChE/*N*-MAL-CDs/SPE biosensor, they used a commercial SPE in which all electrochemical measurements were performed in a droplet of 50  $\mu\text{L}$ . One significant breakthrough offered by SPE is the simultaneous analysis performed by an array of electrodes [50]. The multiplexed analysis integrated into an automated system enables the rapid detection of OP pesticides being convenient for commercial and routine applications. Hence, an array with 12 SPEs deposited in sequence side by side on a ceramic substrate in which the working electrode was printed with a carbon ink containing cobalt phthalocyanine and Ag/AgCl/KCl<sub>sat</sub> was used as reference/counter electrode. By means of using six types of recombinant AChE, it was possible to acquire qualitative and quantitative information through inhibition assay since the enzyme becomes selective among the OP pesticides, such as dichlorvos, malaoxon, chlorpyrifos-oxon, chlorpyrifos-methyl-oxon, chlorfenvinphos, and pirimiphos-methyl-oxon.

Despite all exceptional SPE properties, they present certain drawbacks, such as the dissolution of conductive and insulating inks due to use of organic solvents, lack of reproducibility, and need of pretreatment procedure.

The continuous progress in biosensing area leads to the development of paper-based analytical devices (PADs) with electrochemical detection. The PADs have emerged as a powerful analytical tool integrating the convenience of SPEs, i.e., portability, simplicity with easy manufacturing of paper, availability, and reduced cost. Furthermore, the PADs provide singular advantages since they can be scalable manufactured from renewable sources, biocompatibility, biodegradable, and low cost. A pioneering research involving a paper-based amperometric sensor for AChE determination was based on screen printed graphene electrodes fabricated by a wax printing method to obtain the detection area. The approach was applied for blood sample analysis, but it has potential to be used for pesticide detection.

Numerous immobilization strategies and fabrication methods have brought new perspectives to AChE-based biosensors. The investigations have focused on enzyme stability, reproducibility, miniaturization, and mass production [51]. The usage of smartphones in biosensing has played new horizons in environmental monitoring; however, it remains a challenge [52]. The electrochemical biosensors on smartphone use portable electrical detectors for amperometric, potentiometric, and impedimetric measurements, but environmental analyses are still scarce. Although great progress has been made with wireless biosensors, there is a lack of applications in pesticide detection.

### 3. Conclusions

In this chapter, we presented the state of the art of biosensors to detect phenolic compounds, with environmental and pharmacological applications. Monitoring the negative environmental impacts of phenolic compounds uses has attracted researchers' attention since these compounds are widely applied in several industrial sectors. Among the biosensors developed for environmental monitoring, enzymatic ones are the most prominent used for phenolic compound detection. By combining enzymes with electrochemical transducers, cheaper devices had been developed, which is a great advantage to environmental analytical methods. The immobilization of enzymes on the electrode surface consists in physical and chemical interactions. The location of active sites is important to biosensor response; nevertheless, mediators can be used to transpose this barrier and facilitate the electronic transfer needed for the detection process. Tyrosinase-based biosensor is the most common biosensor for phenolic compound detection, which is a precursor for drugs for the treatment of Parkinson's disease, as morphine-based drugs. Also, acetylcholinesterase-based biosensors are widely employed because they present high efficiency to detect organophosphate and carbamate compounds, which are used as pesticides. The design of novel AChE-based biosensors for pesticide detection concerns the application of nanomaterials offering transducing platforms with outstanding electrochemical behavior. The employment of screen printed electrodes promotes the system miniaturization, which is a new perspective to electrochemical biosensor application.

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### Conflict of interest

The authors state that there is no conflict of interest associated with this work.

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## References

- [1] Korotcenkov G, Han SD, Stetter JR. Review of electrochemical hydrogen sensors. *Chemical Reviews*. 2009;**109**(3):1402-1433. DOI: 10.1021/cr800339k
- [2] Pandey VP, Awasthi M, Singh S, Tiwari S, Dwivedi UN. A comprehensive review of function and application of plant peroxidase. *Biochemistry & Analytical Biochemistry*. 2017;**6**(1):308-324. DOI: 10.4172/2161-1009.1000308
- [3] Nigam VK, Shukla P. Enzyme based biosensors for detection of environmental pollutants—A review. *Journal of Microbiology and Biotechnology*. 2015;**25**(11):1773-1781. DOI: 10.4014/jmb.1504.04010
- [4] Vermerris W, Nicholson R, editors. *Phenolic Compound Biochemistry*. Dordrecht: Springer Netherlands; 2006. 276 p. DOI: 10.1007/978-1-4020-5164-7
- [5] Wells MJM, Fono LJ, Pellegrin M-L, Morse A. Emerging pollutants. *Water Environment Research*. 2007;**79**(10):2192-2209. DOI: 10.2175/106143007x218719
- [6] Nadal A, Quesada I, Tudurí E, Nogueiras R, Alonso-Magdalena P. Endocrine-disrupting chemicals and the regulation of energy balance. *Nature Reviews Endocrinology*. 2017;**13**:536. DOI: 10.1038/nrendo.2017.51
- [7] Damstra TBS, Bergman A, Kavlock R, Van der Kraak G. International Program on Chemical Safety. Global Assessment of Endocrine-Disrupting Chemicals. [Internet]. 2002. Available from: <http://www.who.int/ipcs/publications/en/ch2.pdf?ua=1> [Accessed: December 06, 2018]
- [8] Bouchard P. Endocrine-disrupting chemicals, a multifaceted danger. *Comptes Rendus Biologies*. 2017;**340**(9):401-402. DOI: 10.1016/j.crv.2017.09.001
- [9] Di Fusco M, Tortolini C, Deriu D, Mazzei F. Laccase-based biosensor for the determination of polyphenol index in wine. *Talanta*. 2010;**81**(1):235-240. DOI: 10.1016/j.talanta.2009.11.063
- [10] Wang J. *Analytical Electrochemistry*. 3rd ed. Danvers: Wiley-VCH; 2006. 272 p. DOI: 10.1002/0471790303
- [11] Sadeghi SJ. Amperometric biosensors. In: Roberts GCK, editor. *Encyclopedia of Biophysics*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013. 67 p. DOI: 10.1007/978-3-642-16712-6\_713
- [12] Dzyadevych S, Jaffrezic-Renault N. 6—Conductometric biosensors. In: Schaudies RP, editor. *Biological Identification*. Elsevier Ltd, Woodhead Publishing; 2014. 153-193 p. DOI: 10.1533/9780857099167.2.153
- [13] Mataveli LV, Antunes NJ, Brigagão MRPL, Magalhães CS, Wisniewski C, Luccas PO. Evaluation of a simple and low cost potentiometric biosensor for pharmaceutical and in vivo adrenaline determination. *Biosensors & Bioelectronics*. 2010;**26**(2):798-802. DOI: 10.1016/j.bios.2010.06.021
- [14] Pereira AR, Sedenho GC, Souza JCP, Crespilho FN. Advances in enzyme bioelectrochemistry. *Anais da Academia Brasileira de Ciências*. 2018;**90**(1):825-857. DOI: 10.1590/0001-3765201820170514
- [15] Zhang C, Wang XR, Hou M, Li XY, Wu XL, Ge J. Immobilization on metal-organic framework engenders high sensitivity for enzymatic electrochemical detection. *ACS Applied Materials & Interfaces*.

2017;**9**(16):13831-13836. DOI: 10.1021/acsami.7b02803

243-256. DOI: 10.1023/b:pyht.0000047797.81958.69

[16] Andreescu S, Njagi J, Ispas C. Nanostructured materials for enzyme immobilization and biosensors. In: Erokhin V, Ram MK, Yavuz O, editors. *The New Frontiers of Organic and Composite Nanotechnology*. Amsterdam: Elsevier; 2008. pp. 355-394. DOI: 10.1016/B978-008045052-0.50009-9

[23] Rodríguez-Delgado MM, Alemán-Nava GS, Rodríguez-Delgado JM, Dieck-Assad G, Martínez-Chapa SO, Barceló D, et al. Laccase-based biosensors for detection of phenolic compounds. *TrAC, Trends in Analytical Chemistry*. 2015;**74**:21-45. DOI: 10.1016/j.trac.2015.05.008

[17] Ansari SA, Husain Q. Potential applications of enzymes immobilized on/in nano materials: A review. *Biotechnology Advances*. 2012;**30**(3):512-523. DOI: 10.1016/j.biotechadv.2011.09.005

[24] Vicentini FC, Janegitz BC, Brett CMA, Fatibello-Filho O. Tyrosinase biosensor based on a glassy carbon electrode modified with multi-walled carbon nanotubes and 1-butyl-3-methylimidazolium chloride within a dihexadecylphosphate film. *Sensors and Actuators B: Chemical*. 2013;**188**:1101-1108. DOI: 10.1016/j.snb.2013.07.109

[18] Sassolas A, Blum LJ, Leca-Bouvier BD. Immobilization strategies to develop enzymatic biosensors. *Biotechnology Advances*. 2012;**30**(3):489-511. DOI: 10.1016/j.biotechadv.2011.09.003

[25] Fatibello-Filho O, Vieira I. Uso analítico de tecidos e de extratos brutos vegetais como fonte enzimática. *Química Nova*. 2002;**25**(3):455-464. DOI: 10.1590/S0100-40422002000300019

[19] Barsan MM, Ghica ME, Brett CMA. Electrochemical sensors and biosensors based on redox polymer/carbon nanotube modified electrodes: A review. *Analytica Chimica Acta*. 2015;**881**:1-23. DOI: 10.1016/j.aca.2015.02.059

[26] Gouzi H, Moreau T, Depagne C, Coradin T. Immobilization of a polyphenol oxidase extract from *Terfezia leonis* Tul. Desert truffle in multilayered silica films for dopamine biosensing. *Silicon*. 2013;**5**(4):241-246. DOI: 10.1007/s12633-013-9165-z

[20] Salomi BSB, Mitra CK. Electrochemical studies on horseradish peroxidase covalently coupled with redox dyes. *Biosensors & Bioelectronics*. 2007;**22**(8):1825-1829. DOI: 10.1016/j.bios.2006.08.042

[27] Hatti-Kaul R, Mattiasson B. Release of protein from biological host. In: Hatti-Kaul R, Mattiasson B, editors. *Isolation and Purification of Proteins*. New York: Marcel Dekker, Inc.; 2003. 696 p

[21] Sadeghi SJ, Gilardi G, Cass AEG. Mediated electrochemistry of peroxidases—Effects of variations in protein and mediator structures. *Biosensors & Bioelectronics*. 1997;**12**(12):1191-1198. DOI: 10.1016/S0956-5663(97)00089-4

[28] Martins TD, Ribeiro ACC, Colmati F, Souza GA, Camargo HS, Dias DL, et al. Supramolecular materials for optical and electrochemical biosensors. In: Rinken T, editor. *Biosensors*. London: IntechOpen; 2015. 138-164 p. DOI: 10.5772/60710

[22] Regalado C, García-Almendárez BE, Duarte-Vázquez MA. Biotechnological applications of peroxidases. *Phytochemistry Reviews*. 2004;**3**(1):

[29] Benjamin SR, Vilela RS, de Camargo HS, Guedes MIF, Fernandes KF,

- Colmati F. Enzymatic electrochemical biosensor based on multiwall carbon nanotubes and cerium dioxide nanoparticles for rutin detection. *International Journal of Electrochemical Science*. 2018;**13**(1):563-586. DOI: 10.20964/2018.01.51
- [30] Antunes R, Ferraz D, Garcia L, Thomaz D, Luque R, Lobón G, et al. Development of a polyphenol oxidase biosensor from jenipapo fruit extract (*Genipa americana* L.) and determination of phenolic compounds in textile industrial effluents. *Biosensors*. 2018;**8**(2):47. DOI: 10.3390/bios8020047
- [31] Kafi AKM, Lee D-Y, Park S-H, Kwon Y-S. Potential application of hemoglobin as an alternative to peroxidase in a phenol biosensor. *Thin Solid Films*. 2008;**516**(9):2816-2821. DOI: 10.1016/j.tsf.2007.04.123
- [32] Karim F, Fakhruddin ANM. Recent advances in the development of biosensor for phenol: A review. *Reviews in Environmental Science and Bio/Technology*. 2012;**11**(3):261-274. DOI: 10.1007/s11157-012-9268-9
- [33] Aranganathan V, Kavya C, Sheeja MS, Yuvraj C. Construction of biosensor for detection of phenolic compound using thermostabilized agaricus bisporus tyrosinase. *Arabian Journal for Science and Engineering*. 2017;**42**(1):11-18. DOI: 10.1007/s13369-016-2044-3
- [34] Florescu M, David M. Tyrosinase-based biosensors for selective dopamine detection. *Sensors*. 2017;**17**(6):1314-1330. DOI: 10.3390/s17061314
- [35] Liu T, Xu M, Yin H, Ai S, Qu X, Zong S. A glassy carbon electrode modified with graphene and tyrosinase immobilized on platinum nanoparticles for sensing organophosphorus pesticides. *Microchimica Acta*. 2011;**175**(1):129-135. DOI: 10.1007/s00604-011-0665-5
- [36] Everett WR, Rechnitz GA. Mediated bioelectrocatalytic determination of organophosphorus pesticides with a tyrosinase-based oxygen biosensor. *Analytical Chemistry*. 1998;**70**(4):807-810. DOI: 10.1021/ac970958l
- [37] Hajian A, Ghodsi J, Afraz A, Yurchenko O, Urban G. Nanomolar detection of methylparaben by a cost-effective hemoglobin-based biosensor. *Materials Science and Engineering: C*. 2016;**69**:122-127. DOI: 10.1016/j.msec.2016.06.061
- [38] Mazlan SZ, Lee YH, Hanifah SA. A new laccase based biosensor for tartrazine. *Sensors*. 2017;**17**(12):2859. DOI: 10.3390/s17122859
- [39] Gandhi S, Suman P, Kumar A, Sharma P, Capalash N, Suri CR. Recent advances in immunosensor for narcotic drug detection. *BioImpacts: BI*. 2015;**5**(4):207-213. DOI: 10.15171/bi.2015.30
- [40] Talemi RP, Mashhadizadeh MH. A novel morphine electrochemical biosensor based on intercalative and electrostatic interaction of morphine with double strand DNA immobilized onto a modified Au electrode. *Talanta*. 2015;**131**:460-466. DOI: 10.1016/j.talanta.2014.08.009
- [41] Alvau MD, Tartaggia S, Meneghello A, Casetta B, Calia G, Serra PA, et al. Enzyme-based electrochemical biosensor for therapeutic drug monitoring of anticancer drug irinotecan. *Analytical Chemistry*. 2018;**90**(10):6012-6019. DOI: 10.1021/acs.analchem.7b04357
- [42] Van Dyk JS, Pletschke B. Review on the use of enzymes for the detection of organochlorine, organophosphate and carbamate pesticides in the environment. *Chemosphere*. 2011;**82**(3):291-307. DOI: 10.1016/j.chemosphere.2010.10.033

- [43] Songa EA, Okonkwo JO. Recent approaches to improving selectivity and sensitivity of enzyme-based biosensors for organophosphorus pesticides: A review. *Talanta*. 2016;**155**:289-304. DOI: 10.1016/j.talanta.2016.04.046
- [44] Luque de Castro MD, Herrera MC. Enzyme inhibition-based biosensors and biosensing systems: Questionable analytical devices. *Biosensors & Bioelectronics*. 2003;**18**(2-3):279-294. DOI: 10.1016/S0956-5663(02)00175-6
- [45] de Oliveira Marques PRB, Nunes GS, dos Santos TCR, Andreescu S, Marty J-L. Comparative investigation between acetylcholinesterase obtained from commercial sources and genetically modified drosophila melanogaster: Application in amperometric biosensors for methamidophos pesticide detection. *Biosensors and Bioelectronics*. 2004;**20**(4):825-832. DOI: 10.1016/j.bios.2004.03.021
- [46] Pundir CS, Chauhan N. Acetylcholinesterase inhibition-based biosensors for pesticide determination: A review. *Analytical Biochemistry*. 2012;**429**(1):19-31. DOI: 10.1016/j.ab.2012.06.025
- [47] Kurbanoglu S, Ozkan SA, Merkoçi A. Nanomaterials-based enzyme electrochemical biosensors operating through inhibition for biosensing applications. *Biosensors & Bioelectronics*. 2017;**89**:886-898. DOI: 10.1016/j.bios.2016.09.102
- [48] Jubete E, Zelechowska K, Loaiza OA, Lamas PJ, Ochoteco E, Farmer KD, et al. Derivatization of SWCNTs with cobalt phthalocyanine residues and applications in screen printed electrodes for electrochemical detection of thiocholine. *Electrochimica Acta*. 2011;**56**(11):3988-3995. DOI: 10.1016/j.electacta.2011.01.123
- [49] Xu J, Yu C, Feng T, Liu M, Li F, Wang Y, et al. N-Carbamoylmaleimide-treated carbon dots: Stabilizing the electrochemical intermediate and extending it for the ultrasensitive detection of organophosphate pesticides. *Nanoscale*. 2018;**10**(41):19390-19398. DOI: 10.1039/c8nr05098h
- [50] Crew A, Lonsdale D, Byrd N, Pittson R, Hart JP. A screen-printed, amperometric biosensor array incorporated into a novel automated system for the simultaneous determination of organophosphate pesticides. *Biosensors & Bioelectronics*. 2011;**26**(6):2847-2851. DOI: 10.1016/j.bios.2010.11.018
- [51] Uniyal S, Sharma RK. Technological advancement in electrochemical biosensor based detection of organophosphate pesticide chlorpyrifos in the environment: A review of status and prospects. *Biosensors & Bioelectronics*. 2018;**116**:37-50. DOI: 10.1016/j.bios.2018.05.039
- [52] Zhang D, Liu Q. Biosensors and bioelectronics on smartphone for portable biochemical detection. *Biosensors & Bioelectronics*. 2016;**75**:273-284. DOI: 10.1016/j.bios.2015.08.037