Chapter 5

Phenolic compounds determination in water by enzymatic-based electrochemical biosensors

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1. Introduction

Nowadays, phenols are essential compounds broadly employed in the chemical, petrochemical, pharmaceutical, pesticide, pulp and paper, textile, metallurgic, resin and plastic, and pulp and paper industries. These chemicals are commonly used in the manufacture and processing of plastics and plasticizers, resins, explosives, drugs, detergents, paper, herbicides, insecticides, algaecides, bactericides, molluscicides, fungicides, preservatives, dyes, paints, lubricants, and fuel and solid waste combustion (Glezer, 2003; Michałowicz & Duda, 2007). In fact, the global capacity, only for phenol production, was about 9.9 million tons in 2008 (Weber & Weber, 2010). Nonetheless, most phenolic compounds are distinguished by their toxic, noxious, mutagenic and carcinogenic activity (Michałowicz & Duda, 2007). These recalcitrant pollutants accumulate over time and are found in food, potable water, sediments and soil.

Currently, some organizations have developed technical standards to determinate phenols mainly in water. The US Environmental Protection Agency (EPA), American Section of the International Association of Testing Materials (ASTM), and the International Organization for Standardization (ISO) have established several procedures for phenolic compounds determination in drinking, ground, surface, and saline waters, and domestic and industrial wastes by colorimetric, gas and liquid chromatography, capillary electrophoresis and their variations (Karim & Fakhruddin, 2012). Some of these procedures are EPA Method 420.4 (U.S. Environmental Protection Agency, 1993), EPA Method 528 (U.S. Environmental Protection Agency, 2000), EPA Method 604, (U.S. Environmental Protection Agency, 1996a, 2000) EPA Method 625 (U.S. Environmental Protection Agency, 1996b), ISO 8165-1:1992 (International Organization of Standardization, 1992), ISO 8165-2:1999 (International Organization of Standardization, 1999), and ASTM D1783-01 (ASTM International, 2012). Even though these standardized methods are able to obtain accurate results for a wide range of phenolic compounds, conventional approaches are time-consuming and cost-intensive. Furthermore, they require large volumes of toxic organic solvents such as methylene chloride, acetone, and methanol. Consequently, there is a demand for the development of reliable, portable, sensitive, simple and cost-effective methods for fast detection of phenolic compound.

Enzymatic-based biosensors have grown as a promising technology in the detection field. Enzymes are the most widely used biological sensing element in the fabrication of biosensors. The main advantages of biosensors are high catalytic activity, substrate selectivity, moderate operational potentials, high sensitivity, specificity, and applicability to multi component solutions in situ (Borgmann, Schulte, Neugebauer & Schuhmann, 2011; Karim & Fakhruddin, 2012).

This chapter seeks to provide an overview of recent advances on enzymatic-based biosensors for phenolic compound detection. In particular, specific redox-enzymes such as laccase, tyrosinase and peroxidase were described and compared. Additionally, a review of the main phenolic compounds and their toxicity for human health is highlighted to understand the importance of biosensor development.

2. Phenols

Phenolic compounds are based on organic molecules which contain a hydroxyl group bounded directly to an aromatic ring. This ring provides their characteristic stability due to electron resonance. As aromatic structure is energetically favorable, there is a preference for electrophilic aromatic substitution reactions such as chlorination, sulphonations, nitration, and nitrosation (Nguyen, Kryachko & Vanquickenborne, 2003).

The term phenols cover a very large group of chemical compounds, the most relevant and common are: (1) chlorophenols, (2) nitrophenols, (3) catechols, (4) chlorocatechols, (5) alkylphenols, (6) bisphenols, and (7) aminophenols. Figure 1 shows some of these compounds.

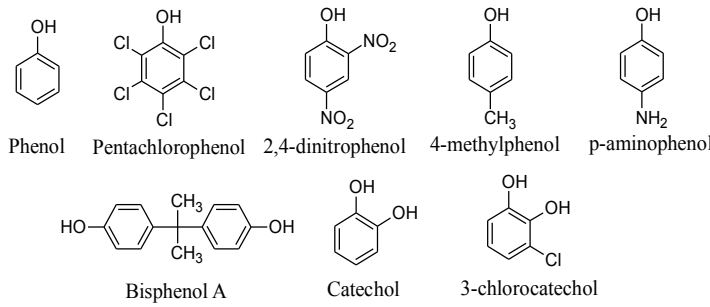

Figure 1. Examples some phenolic compounds

The US Environmental Protection Agency (EPA) as wells as The European Union (EU) have classified phenolic compounds as priority pollutants due to their strong toxic influence (Glezer, 2003). Not only chemical processing and manufacturing plant workers are exposed to phenolic compounds, but also the general population. Phenols are present in the environment due to the current industry activity, mostly in water, sewage, waste, soil, air, and even food. Moreover, their toxicity activity increases owing to their poor biodegradability (Glezer, 2003).

Phenol poisoning by skin absorption, inhalation of vapors or ingestion cause accumulation and damaging of brain, kidneys, liver, muscle, and eyes as well as necrosis. Phenol skin contact in high concentrate solutions (60%-90%) and a dose of 1 g of phenol may be lethal for an adult (Karim & Fakhruddin, 2012; Michałowicz & Duda, 2007; Services U.S. Department of Health and Human, 2008). Chlorophenols are subjected to fast skin and mucous membrane absorption. Burning pain, white necrotic lesions, vomiting, headache, irregular pulse, temperature decrease, muscle weakness, convulsions and death are typical characteristics of chlorophenols exposure. The acceptable daily intake for pentachlorophenol was established for 16 μg for a man of 70 kg of bodyweight. Nitrophenol exposure undergoes to similar consequences as chlorophenols. In fact, compounds like 2,4-dinitrophenol were used as a slimming drug and food additive until it was removed from the market because of numerous cases of chronic heat, depression and deaths. The median lethal dose of this compound is considered to be 14 to 35 mg kg^{-1} (Michałowicz & Duda, 2007). Industrial workers are highly exposed to methylphenols, especially 4-methylphenol, which causes burning pain, abdominal pain, headache, weak irregular pulse,

hypotension, fall of body temperature, dark colored urine, shock, paralysis, coma and death. The median lethal dose for rats is 207 mg kg^{-1}. Bisphenol A might be a factor of decreasing seminal fluid, increase rates of breast cancer in women and a decrease of plasma luteinizing hormone level. Furthermore, aminophenol toxicity entails semiquinones and superoxide radical generation, which damage the cell's biomolecules. For p-aminophenol, the lethal dose for an adult man is estimated to be 50 to 500 mg kg^{-1} of bodyweight (Michałowicz & Duda, 2007).

Phenol, catechols, nitrophenols, aminophenols, bisphenols show mutagenic activity as they damage and inhibit DNA synthesis and replication inside cells. In addition, carcinogenic activity, influencing tumor growth processes, has been demonstrated for some phenolic compounds such as 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, catechol, p-cresol, 2,4-dimethylphenol, 4-methoxyphenol, and bythylhydroxyanisole (Michałowicz & Duda, 2007).

3. Biosensors

The first reported biosensor was an oxygen enzyme-based electrode developed in 1962 by (Clark & Lyons, 1962). The term biosensor was introduced for the first time by (Cammann, 1977). According to the IUPAC, a biosensor is a device that transforms biochemical information into an analytical useful signal through a physicochemical transducer and a biological recognition system (receptor) that selectively reacts with the analyte of interest (Thévenot, Toth, Durst & Wilson, 2001).

A typical biosensor set-up, illustrated in Figure 2, consists on (1) a biorecongnition element (enzyme, antibody, nucleic acid, living cells or microorganism), (2) a transducer that enables to transfer the output signal from the receptor to a measurable response, mostly an electric signal, (3) an amplifier that magnifies the output of the transducer, and (4) a data acquisition element which converts the sensor signal into digital values (Grieshaber, MacKenzie, Vörös & Reimhult, 2008).

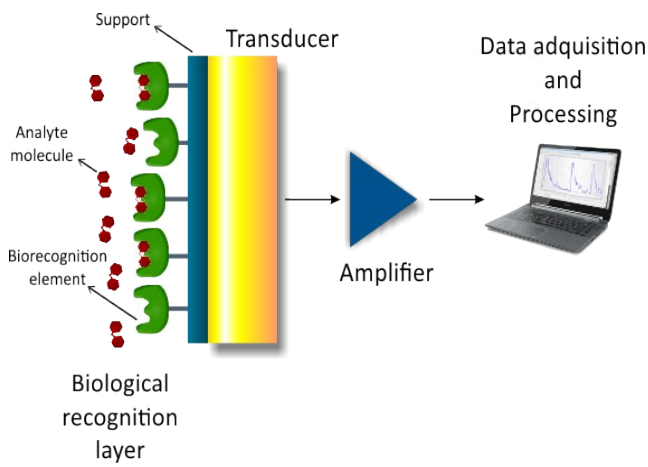

Figure 2. A typical biosensor set-up

Electrochemical biosensors can be classified according to the mode of signal transduction, such as amperometry, potentiometry or conductimetry (D'Orazio, 2003; Grieshaber et al., 2008; Mehrvar & Abdi, 2004). Amperometric approach is based on the measurement of the current resulting from the electrochemical redox reaction of electroactive species (Borgmann et al., 2011). Potentiometric biosensors involve the determination of the potential difference between two electrodes separated by a permselective membrane when zero current flows between them (Koncki, 2007). Conductometry measures changes in the ionic composition of a tested sample as a result of a biochemical reaction (Jaffrezic-Renault & Dzyadevych, 2008).

The bioreceptor and the sensor elements can be coupled together with several methods, such as, physical adsorption, entrapment, cross-linking and covalent bonding. These techniques have been summarized in many reviews (D'Souza, 2001; Sassolas, Blum & Leca-Bouvier, 2012).

4. Laccase biosensors

Laccase (p-diphenol: dioxygenoxidoreductases EC 1.10.3.2) catalyzes the oxidation of ortho- and para-diphenols, aminophenols, polyphenols, polyamines, lignins and aryl diamines with the concomitant reduction of molecular oxygen to water (Majeau, Brar & Tyagi, 2010). They are secreted by many fungi and detected in numerous plants and bacteria (Dwivedi, Singh, Pandey & Kumar, 2011). Typical fungal laccases are extracellular proteins of approximately 60-70 kDa. Laccase active site contains four copper atoms arranged in one mononuclear Type 1 site and one trinuclear cluster Type 2/Type 3. The substrates are oxidized by the Type 1 copper and the extracted electrons are transferred to the Type 2/Type 3 centre where dioxygen binds and is reduced to water (Duran, Rosa, D'Annibale & Gianfreda, 2002) (Figure 3).

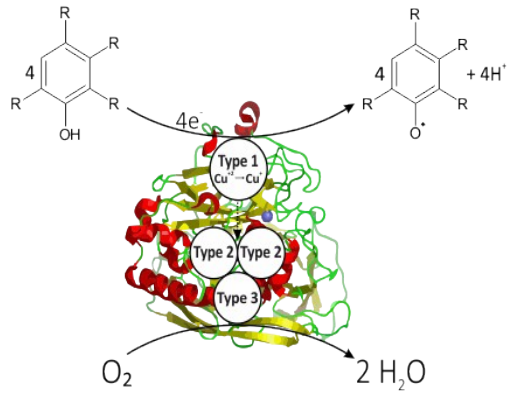

Figure 3. Catalytic cycle of laccase (Baldrian, 2006)

Laccase biosensors have been mainly focused on food industry (Chawla, Rawal, Kumar & Pundir, 2012; Di Fusco, Tortolini, Deriu & Mazzei, 2010; Gamella, Campuzano, Reviejo & Pingarrón, 2006; Martinez-Periñan, Hernández-Artiga, Palacios-Santander, ElKaoutit, Naranjo-Rodriguez & Bellido-Milla, 2011), biomedical analysis (Moccelini, Franzoi, Vieira, Dupont & Scheeren, 2011; Odaci, Timur, Pazarlioglu, Montereali, Vastarella, Pilloton et al., 2007; Shervedani & Amini, 2012)

and environmental monitoring (Jia, Zhang, Wang & Wang, 2012; Oliveira, Fátima Barroso, Morais, de Lima-Neto, Correia, Oliveira et al., 2013; Wang, Tang, Zhang, Gao & Chen, 2012). Among these applications, amperometry is the most common transducer method (Liu, Qu, Guo, Chen, Liu & Dong, 2006; Rawal, Chawla, Devender & Pundir, 2012); differential pulse voltammetry (DPV) (Shervedani & Amini, 2012) and square wave voltammetry (SWV) (Moccelini et al., 2011) have been used in less extent.

A biosensor for medical applications using DPV as a transducer method was proposed by (Shervedani & Amini, 2012). *Agaricusbisporus* laccase was immobilized on 3-mercaptopropionic acid (MPA) SAM. The amine groups of the laccase were couple to the acidic groups of MPA by formation of amide group using carbodiimide hydrochloride and N-hydroxysuccinimide. The biosensor was tested with dopamine solutions and then using blood samples. A limit of detection (LOD) of 29 nM was estimated which is lower than other works using different enzymes (Fritzen-Garcia, Zoldan, Oliveira, Soldi, Pasa & Creczynski-Pasa, 2013; Moccelini, Fernandes & Vieira, 2008). Moreover, they studied the effect of interferences using ascorbic acid and uric acid. The results showed that a 25-fold excess of uric acid and 14-fold excess of ascorbic acid did not interfere in the determination of dopamine. They achieved a repeatability and reproducibility with a relative standard deviation (RSD) lower than 5%.

A biosensor for environmental monitoring of phenols was proposed using a Black Pearl 2000 modified glassy carbon electrode with laccase immobilized (Wang et al., 2012). The biosensor was evaluated with catechol by amperometry, and the kinetic Michaelis-Menten apparent constant (K_m^{app}) obtained was 1.79 mM. The LOD, linear range and sensitivity of the biosensor were 3 µM, 3 µM - 5.555 mM and 98.84 µA mM^{-1} cm^{-2}, respectively.

Liu and co-workers designed an amperometric biosensor by entrapping laccase into a matrix of carbon nanotubes (CNTs) and chitosan on a glassy carbon electrode (Liu et al., 2006). The biosensor performance was evaluated using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS, a non-phenolic laccase substrate), catechol and O_2. For ABTS, K_m was 19.86 µM and a LOD of 0.23 µM (S/N = 3). K_m^{app} was ~ 8 fold lower compared with the previous results (Quan, Kim, Yoon & Shin, 2002) and LOD was ~ 2 fold lower than other laccase biosensor (Quan, Kim & Shin, 2004). For catechol, K_m^{app} was 9.43 µM and a LOD of 0.66 µM (S/N = 3). This K_m was between 21 and 413 fold lower than previous biosensors (Freire, Durán & Kubota, 2001; Mena, Carralero, González-Cortés, Yáñez-Sedeño & Pingarrón, 2005; Solná & Skládal, 2005; Wang et al., 2012) and LOD was ~ 20 fold lower than the screen-printed sensor based on *Coriolus hirsutus* laccase, horseradish peroxidase and mushroom tyrosinase immobilized on a 4-mercapto-1-butanol Au electrode (Solná & Skládal, 2005). In contrast with the laccase biosensor reported by Wang and co-workers (Wang et al., 2012), the LOD and K_m^{app} were 4.5 and 199 fold lower, respectively. These improvements may be attributed to the good conductivity and the enhancing effect on the electrocatalytic activity due to the presence of CNTs. Other study had shown a significant increase of the catalyzed reaction currents (around 6 fold) when multi-wall CNTs were added into a composite electrode with laccase (Oliveira et al., 2013). Besides, the reversibility was improved and the separation between peaks was lower.

This previous work pointed the current trend of taking advantage of the rapid development of nanotechnology. Currently, many biosensors are improved using nanomaterials, specially noble metals nanoparticles and CNTs.

5. Tyrosinase biosensors

Tyrosinases (E.C. 1.14.18.1) are copper proteins that catalyze two different oxygen-dependent enzymatic reactions: the hydroxylation of monophenols to o-diphenols (cresolase activity) and the subsequent oxidation of o-diphenols to o-quinones (catecholase activity) (Akyilmaz, Yorganci & Asav, 2010). Even though these enzymes are widely distributed in animals, plants, insects and microorganisms, purification and extraction of tyrosinase have focused on fungi and *Streotomyces spp*. (McMahon, Doyle, Brooks & O'Connor, 2007). In contrast to lacasse, tyrosinase molecular weight is significantly heterogeneous as several studies indicated a wide range of values (Halaouli, Asther, Sigoillot, Hamdi & Lomascolo, 2006).

Tyrosinase active sites consist on a coupled binuclear copper complex, both copper atoms are Type 3 as they are centrally located (Durán, Rosa, D'Annibale & Gianfreda, 2002). Tyrosinase catalytic cycle contains two sub-cycles (Figure 4): in the former, tyrosinase passes through several enzyme states (E_{deoxy}, E_{oxy}, E_{oxy-M}, and E_{met-D}) whilst achieving monophenol oxidation; and in the latter, as the o-diphenols are oxidized, the enzyme passes through five states (E_{deoxy}, E_{oxy}, E_{oxy-D}, E_{met}, and E_{met-D}) (Wang, Xu, Ye, Zhu & Chen, 2002). Hence, tyrosinase biosensors take advantage of the electrochemical reduction of quinones so as to measure the electric potential created.

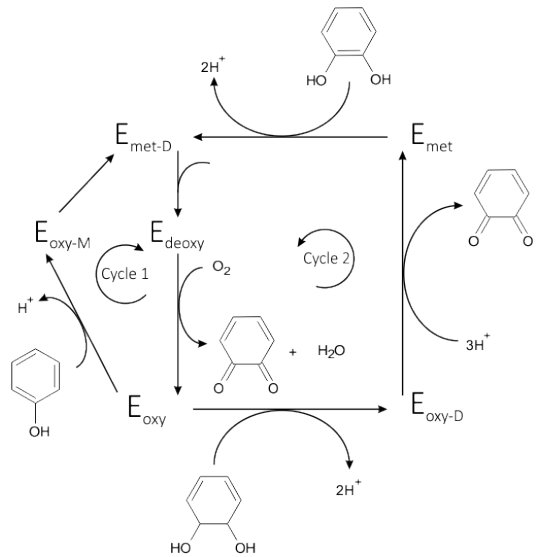

Figure 4. Catalitic cycle of tyrosinase (Seo, Sharma & Sharma, 2003)

As well as lacasse, tyrosinase has been used in biosensors principally in the food industry (Apetrei & Apetrei, 2013; Böyükbayram, Kıralp, Toppare & Yağcı, 2006; Cetó, Gutiérrez, Gutiérrez, Céspedes, Capdevila, Mínguez, et al., 2012; Ghasemi-Varnamkhasti, Rodríguez-Méndez, Mohtasebi, Apetrei, Lozano, Ahmadi et al., 2012; Sánchez-Obrero, Mayén, Rodríguez-Mellado & Rodríguez-Amaro, 2012), environmental control (Li, Li, Song, Li, Zou & Long, 2012; Mayorga-Martinez, Cadevall, Guix, Ros & Merkoçi, 2013; Moczko, Istamboulie, Calas-Blanchard,

Rouillon & Noguer, 2012; Van Dyk & Pletschke, 2011; Wu, Deng, Jin, Lu & Chen, 2012), and biomedical analysis (Apetrei, Rodriguez-Mendez, Apetrei & de Saja, 2013; Bujduveanu, Yao, Le Goff, Gorgy, Shan, Diao et al., 2013; Rather, Pilehvar & De Wael, 2013). In all different applications, the most conventional transducer method for electrochemical biosensing is amperometry (Akyilmaz et al., 2010; Apetrei et al., 2013; Apetrei & Apetrei, 2013; Bujduveanu et al., 2013; Hervás Pérez, Sánchez-Paniagua López, López-Cabarcos & López-Ruiz, 2006; Mayorga-Martinez et al., 2013; Sánchez-Obrero et al., 2012; Wang et al., 2002; Wu et al., 2012). However, cyclic voltammetry (Cetó et al., 2012; Ghasemi-Varnamkhasti et al., 2012; Li et al., 2012), and conductometry (Wang, Chen, Xia, Zhu, Zhao, Chovelon et al., 2006) have been also used.

Wu and co-workers constructed a tyrosinase biosensor for the determination of bisphenol A for rapid analysis of emergency pollution affairs, using graphene as both enzyme immobilization platform and electrode material (Wu et al., 2012). The biosensor performance was assessed and compared with multi-wall CNTs modified tyrosinase biosensors, indicating that graphene-based biosensors have significant advantages in response, repeatability, background current and LOD. These biosensors showed an analytical performance over the linear range from 100 nM to 2 µM, with LOD of 33 nM and sensitivity of 3108.4 mA M^{-1} cm^{-2}.

In parallel, a disposable, low-cost and easy to carry biosensor employing single-wall CNTs, gold nanoparticles and tyrosinase modified-screen-printed electrodes for environmental phenolic analysis in water samples was developed by (Li et al., 2012). The sensor was characterized showing a high load of tyrosinase due to the large surface area of the single-wall CNTs, as well as high tyrosinase bioactivity and enhanced sensitivity. For catechol sensing, it indicated a linear range of 80 nM to 20 µM with a LOD of 45 nM (S/N = 3) and a fast response time within 10 s. In contrast, lacasse biosensors based on a carbon nanotubes and chitosan matrix have a greater LOD for catechol detection (660 nM) (Liu et al., 2006).

Yang and co-workers constructed a tyrosinase-chitosan-carbon-coated nickel nanoparticle film for catechol detection (Yang, Xiong, Zhang & Wang, 2012). When the catechol solution was added, the biosensor cathodic current reached a 95% of steady-state current within 8 s. The linear detection range was from 0.25 nM to 27 µM, the LOD (S/N = 3) was 0.083 nM, and a sensitivity of 514 µA mM^{-1}. These results displayed an improvement in biosensor performance over those based on polyaniline-ionic-liquid carbon nanofiber composite (linear range of 0.40 nM − 2.1 µM) (Zhang, Lei, Liu, Zhao & Ju, 2009), alumina sol-gel on Sonogel-carbon transducer (linear range of 0.10 − 3.0 µM) (Zejli, Hidalgo-Hidalgo de Cisneros, Naranjo-Rodriguez, Liu, Temsamani & Marty, 2008), colloidal gold nanoparticles graphite-teflon composite (linear range of 10 nM − 2.1 µM) (Carralero, Mena, Gonzalez-Cortés, Yáñez-Sedeño & Pingarrón, 2006), Zn nanorod (sensitivity of 2.14 µA mM^{-1}) (Chen, Gu, Zhu, Wu, Liu & Xu, 2008), multi-wall CNT nafion nanobiocomposites (sensitivity of 346 µA mM^{-1}) (Tsai & Chiu, 2007), and single-wall CNTs (sensitivity of 355 µA mM^{-1}) (Zhao, Guan, Gu & Zhuang, 2005).

6. Peroxidase biosensors

Peroxidases (E.C. 1.11.1) are a large family of oxidoreductases such as NADH (E.C. 1.11.1.1), glutathione peroxidase (E.C. 1.11.1.9), and catalase-peroxidase (E.C. 1.11.1.21) among others; that catalyze several organic and inorganic compounds, using hydrogen peroxide. Most of them contain iron (III) protoporphyrin IX (heme) as prosthetic group (Conesa, Punt & van den Hondel, 2002). These enzymes, which molecular weight ranges from 30 to 150 kDa, are widely

distributed in vascular plants, animals and microorganisms (dos Santos Maguerroski, Fernandes, Franzoi & Vieira, 2009; Hamid & Khalil-ur, 2009).

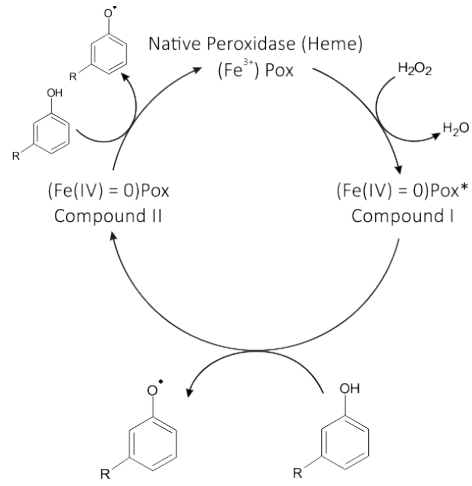

Figure 5. Catalytic cycle of peroxidase (Brill, 1966)

The classic catalytic cycle, common for most heme peroxidases, consist on the initial transfer of two H_2O_2 electrons to the enzyme state [(Fe^{3+})Pox] that leads in the formation of water and Compound I [(Fe(IV) = O)Pox*]. Then, Compound I is reduced by the oxidizing compound, in this case a phenolic compound, occurring in Compound II [(Fe(IV) = O)Pox] formation. Lastly, another one-electron transference takes place which oxidizes the oxidizing compound, resulting in the native ferric enzyme (Conesa et al., 2002; Dunford, 2010).

Peroxidases are extensively used in clinical biochemistry, aromatic chemical synthesis and peroxide removals (Hamid & Khalil-ur, 2009). In particular, horseradish peroxidase is commercially produced on large scale in clinical diagnostic kits and immunoassays (Veitch, 2004). Peroxidase-based biosensors have been also developed in environmental monitoring (Çevik, Şenel, Baykal & Abasıyanık, 2012; El Ichi, Marzouki & Korri-Youssoufi, 2009), food industry (Granero, Fernández, Agostini & Zón, 2010; Mello, Sotomayor & Kubota, 2003; Ramírez, Granero, Zón & Fernández, 2011), and biomedical analysis (Fritzen-Garcia et al., 2013; Radhapyari, Kotoky & Khan, 2013). As well as lacasse- and tyrosinase-based biosensors, the typical transducer method in peroxidase biosensors is amperometry (Çevik et al., 2012; Dai, Xu, Wu & Ju, 2005; Granero et al., 2010; Mello et al., 2003; Qiu, Chen, Wang, Li & Ma, 2013; Radhapyari et al., 2013; Ramírez et al., 2011; Rosatto, Sotomayor, Kubota & Gushikem, 2002). Nonetheless, cyclic voltammetry (Dai et al., 2005; El Ichi et al., 2009) and square wave voltammetry (Fritzen-Garcia et al., 2013) have been also used in a lower range.

Çevik and co-workers fabricated an amperometric biosensor for monitoring phenols derivates. Horseradish peroxidase was immobilized on a poly(glycidylmethacrylate) modified iron oxide nanoparticles on a gold electrode (Çevik et al., 2012). The biosensor was characterized with catechol and amperometric detection of phenols was carried out using phenol, p-crisol, 2-aminophenol and pyrogallol. Additionally, the effect of the nanoparticles was demonstrated. It

was shown for different phenols that the addition of nanoparticles increased the response between four and nine times. The LOD for p-cresol, aminophenol, catechol, phenol and pyrogallol was 26, 13, 46, 28 and 48 μM respectively. The LOD for cathecol was comparatively higher than the single-wall CNTs Au nanoparticles tyrosinase biosensor on screen printed electrodes (Li et al., 2012). The sensitivity for the phenol compounds studies ranged between 1.92 and 4.37 μA mM^{-1}.

A new peroxidase named POX_{1B} purified from *Allium sativum* L. was immobilized using chitosan microspheres cross-linked with glyozal on a gold electrode (El Ichi et al., 2009). The biosensor was tested using chlorophenols from the EPA's priority pollutant list and amperometry was used as a transducer method. The lowest measured concentration was 1 pM for 4-chlorophenol and pentachlorophenol and 10 nM for 2,6-dichlorophenol. The sensitivity for 6-dichlorophenol, 4-chlorophenol and pentachlorophenol were 1.5×10^{6}, 1.9×10^{9} and 0.9×10^{9} μA M^{-1}, respectively. The sensitivity was improved in contrast with a peroxidase and glucose oxidase based biosensor with an estimated sensitivity for 4-chlorophenol of 4.1×10^{4} μA M^{-1} and for pentachlorophenol of 4.1×10^{4} μA M^{-1} (Serra, Reviejo & Pingarrón, 2003).

A biosensor applied to dopamine determination was designed to immobilize horse radish peroxidase on dimyristoylphosphatidylcholine bilayers supported on Au (111) by dithiotreitol SAM (Fritzen-Garcia et al., 2013). Similar to the *Agaricusbisporus* laccase immobilized on MPA SAM Au electrode (Shervedani & Amini, 2012) and the carbon paste electrode with pine kernel peroxidase (Fritzen-Garcia, Oliveira, Zanetti-Ramos, Fatibello-Filho, Soldi, Pasa et al., 2009) for DA determination, a voltammetry technique was used as a transducer method. The sensor showed a linear response for dopamine concentrations from 33 μM to 1.3 mM with a LOD of 2 μM. This value was lower than the LOD using the pine kernel peroxidase (9 μM) (Fritzen-Garcia et al., 2009) but higher than the LOD using the *Agaricusbisporus* laccase biosensor (29 nM) (Shervedani & Amini, 2012).

7. Conclusion

Throughout this chapter, the principal phenolic compounds and their toxicity were overviewed. Moreover, the latest advances on lacasse-, tyrosinase-, and peroxidase-based biosensors for phenolic compound determination were described and compared. Each of these sensors are currently used and developed mainly in the food industry, environmental control, and biomedical analysis. As well as that, recently, sensor performance has been enhanced by means of the emerging science knowledge of nanotechnology.

Lacasse-based biosensors showed to be more available compared with tyrosinase and peroxidase, which are developed in less extend. Additionally, amperometry is the most conventional electrochemical transducer method in all three different enzyme biosensors because it entails higher sensitivity and allows real-time measurements.

From the reviewed biosensors, it is noticeable that enzyme-based biosensors for phenolic compound sensing can measure very low concentrations, in contrast to the conventional methods like HPLC. Hence, the development of this type of biosensor is very promising due to its several advantages.

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