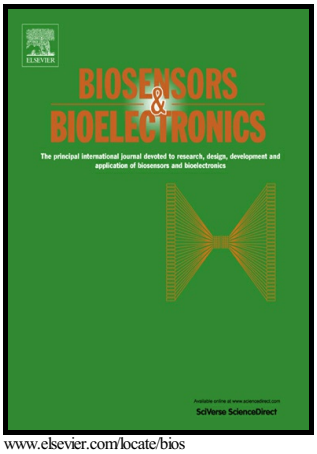


www.elsevier.com/locate/bios

NANOMATERIALS-BASED ENZYME ELECTROCHEMICAL BIOSENSORS OPERATING THROUGH INHIBITION FOR BIOSENSING APPLICATIONS

Sevinc Kurbanoglu, Sibel A. Ozkan, Arben Merkoçi

PII: S0956-5663(16)30977-0
DOI: http://dx.doi.org/10.1016/j.bios.2016.09.102
Reference: BIOS9214

To appear in: *Biosensors and Bioelectronic*

Received date: 17 June 2016
Revised date: 20 September 2016
Accepted date: 26 September 2016

Cite this article as: Sevinc Kurbanoglu, Sibel A. Ozkan and Arben Merkoçi NANOMATERIALS-BASED ENZYME ELECTROCHEMICAI BIOSENSORS OPERATING THROUGH INHIBITION FOR BIOSENSINC APPLICATIONS, *Biosensors and Bioelectronic* http://dx.doi.org/10.1016/j.bios.2016.09.102

NANOMATERIALS-BASED ENZYME ELECTROCHEMICAL BIOSENSORS OPERATING THROUGH INHIBITION FOR BIOSENSING APPLICATIONS

Sevinc Kurbanoglu[a,b], Sibel A. Ozkan[b], Arben Merkoçi[a,c*]

[a]Nanobioelectronics & Biosensors Group, Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and The Barcelona Institute of Science and Technology, Campus UAB, 08193, Bellaterra, Barcelona, Spain

[b]Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06100, Tandogan, Ankara, Turkey

[c] ICREA, Pg. Lluís Companys 23, 08010 Barcelona, Spain

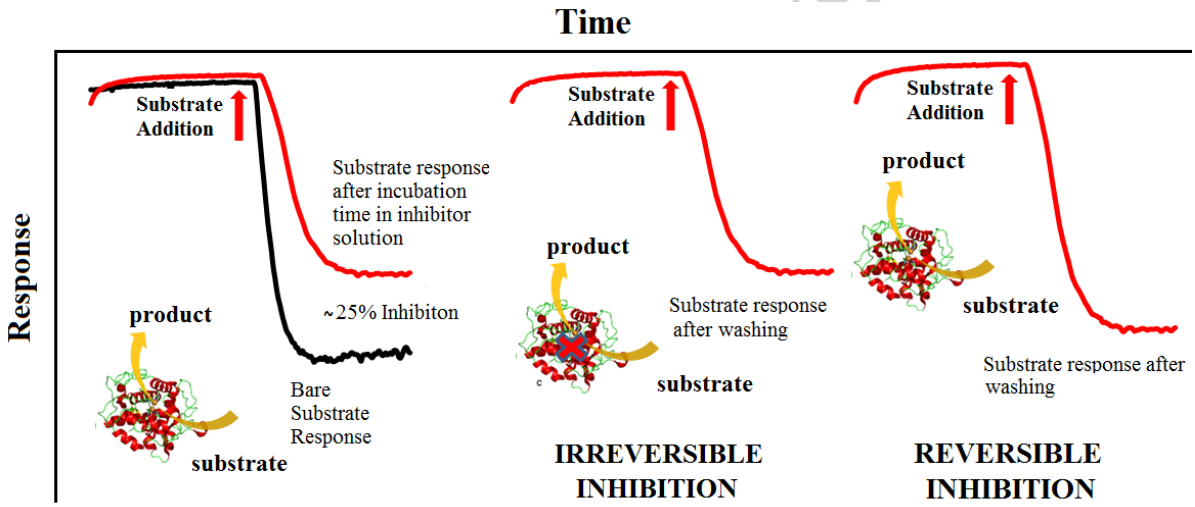
[*]Corresponding author. arben.merkoci@icn2.cat

Abstract

In recent years great progress has been made in applying nanomaterials to design novel biosensors. Use of nanomaterials offers to biosensing platforms exceptional optical, electronic and magnetic properties. Nanomaterials can increase the surface of the transducing area of the sensors that in turn bring an increase in catalytic behaviors. They have large surface-to-volume ratio, controlled morphology and structure that also favor miniaturization, an interesting advantage when the sample volume is a critical issue. Biosensors have great potential for achieving detect-to-protect devices: devices that can be used in detections of pollutants and other treating compounds/analytes (drugs) protecting citizens' life. After a long term focused scientific and financial efforts/supports biosensors are expected now to fulfill their promise such as being able to perform sampling and analysis of complex samples with interest for clinical or environment fields. Among all types of biosensors, enzymatic biosensors, the most explored biosensing devices, have an interesting property, the inherent inhibition phenomena given the enzyme-substrate complex formation. The exploration of

such phenomena is making remarkably important their application as research and applied tools in diagnostics. Different inhibition biosensor systems based on nanomaterials modification has been proposed and applied. The role of nanomaterials in inhibition-based biosensors for the analyses of different groups of drugs as well as contaminants such as pesticides, phenolic compounds and others, are discussed in this review. This deep analysis of inhibition-based biosensors that employ nanomaterials will serve researchers as a guideline for further improvements and approaching of these devices to real sample applications so as to reach society needs and such biosensor market demands.

Graphical Abstract



Keywords

Nanomaterials, enzyme, biosensors, enzyme inhibition

1. Introduction

Nanomaterials (NMs) have a length scale of approximately within the size range of 1–100 nm. At nano sizes, materials have different properties than their normal sizes. NMs have new properties and functions which are quite different from their bulk properties, because of their small structures (Niemeyer, 2001; Kittelson, 1998; Suarez-Martinez et al., 2012, Merkoçi, 2013; Tamayo et al., 2013; Barberis et al., 2015). Generally, NMs are more active than their bulk materials as a result of high surface energy (Luo et al., 2006). NMs have unique properties such as high surface/volume ratio, high reactivity, high electrical conductivity and great magnetic properties and catalytic activity and so on (Kerman et al., 2008; Niemeyer, 2001) Active sites and abundant functional groups onto NMs surface lead to high activity for adsorption and catalysis. Therefore, NMs can be used in many fields such biosensors, pharmaceuticals, cosmetics, agriculture, energy beside others (Kerman et al., 2008; Luo et al., 2006; Ansari and Husain, 2012; Marin and Merkoçi, 2012). NMs can be classified according to their chemical composition, being organic or inorganic. Inorganic materials are metals, metal oxides, and quantum dots whereas organic nanomaterials are mainly carbon based NMs such as fullerenes, carbon nanotubes, graphene etc. (Brownson and Banks, 2010; Brownson and Banks, 2011; Kerman et al., 2008; Chen et al., 2013; Aragay et al., 2012; Altavilla and Ciliberto, 2011; Valentini et al., 2004; Suarez-Martinez et al., 2012, Merkoçi, 2013; Tamayo et al., 2013; Barberis et al., 2015; Ray, 2015).

"Detect-to-protect" biosensors are compact devices and analytical tools and a type of chemical sensors converting the biochemical reaction into analytical and measureable signal (Scheller et al., 1989; Malhotra, 2005; Thevenot et al. 2001a, Gronow 1991; Edmonds, 2013; Turner, 2013). Due to their high specificity which is directly dependent on the receptor (biomolecules or synthetic compounds) that is used, their sensitivity, compact size and user friendly properties, biosensors are the main choice in detection of chemical and biological components (Mello and Kubuta, 2002; Wilson and Hu 2000). Principally, biosensors are

formed by two components named transducer (where the signal of the biosensor is obtained and changed into a measurable signal) and recognition part (consisting of a biological or synthetic receptor that utilizes a specific biochemical or chemical reaction mechanism) (figure 1). Two are the most problematic aspects in developing biosensors: a) the incorporation/immobilization of (bio)receptors in suitable matrix and b) monitoring/quantitating the interactions between the analytes and these receptors (Wilson and Hu, 2000; Malhotra et., 2005; Patel et al., 2002; Datta et al., 2013; Mello and Kubota, 2002; Grieshaber et al., 2008; Pohanka et al., 2008; Edmonds, 2013; Turner, 2013).

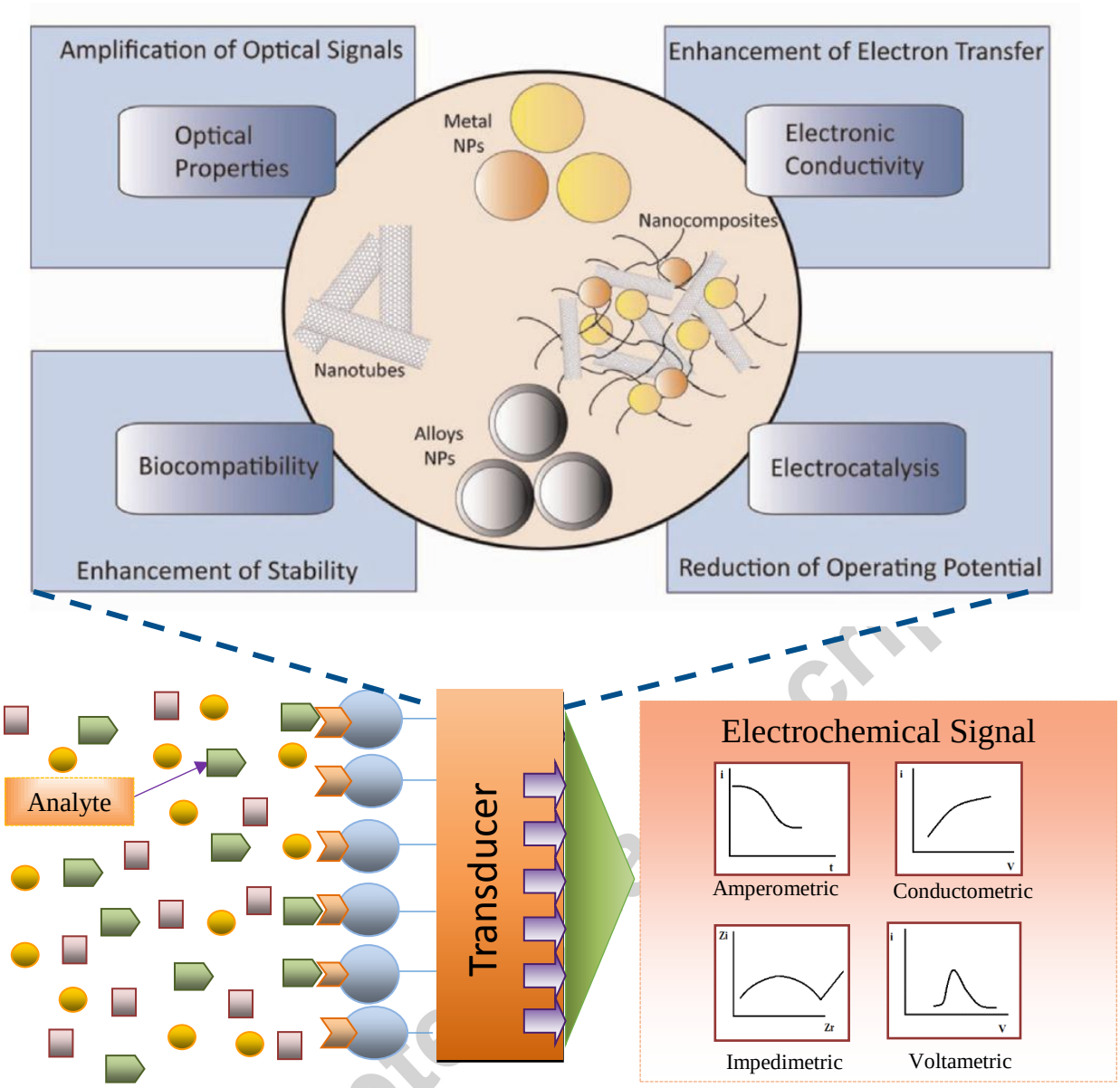


Figure 1. A) Properties and functions of nanomaterials in biosensor applications. Reprinted with permission from ref (Saxena and Das, 2016) B) Schematic presentation of a biosensor

2. Enzyme-based biosensors

Enzyme-based biosensors are the most used biosensing platforms in comparison to immunosensors (normally using antibodies), genosensors (using nucleic acids) and cell-based sensors (Thevenot et al. 2001a, Gronow 1991; Mello and Kubota, 2002). Enzymes have high affinities toward corresponding substrates being able to catalyze several biochemical reactions

without being permanently changed (Thevenot et al. 2001b, Gronow, 1991). The recognition system of a biosensor directly depends on the enzyme-substrate relation, which is measured by the transducer onto which surface enzymes are immobilized. Enzyme immobilization process is very crucial in building electrochemical enzyme-based biosensors (Datta et al., 2013). Immobilization of an enzyme enhances the shelf life of the biosensor, increases enzyme stability and reduces the time of the enzymatic response (Kennedy and White 1985; Tischer and Kasche, 1999). Enzymes can be immobilized onto the surface of a transducer via several immobilization techniques such as physical adsorption, covalent binding, entrapment, encapsulation, and covalent cross-linking (Datta et al., 2013; Kennedy and White 1985; Tischer and Kasche, 1999).

2.1 Enzymatic reaction kinetics

In low concentration of substrates, nearly all enzymes-based reactions show first-order dependence on rate of substrate which means there is a linear relation between the substrate concentration and reaction rate. As the concentration increases, the rate approaches a limit called saturation where there is no dependence on concentration. The rate is zero order in saturation area with respect to it substrate as shown in figure 2 (Cornish-Bowden and Wharton 1988).

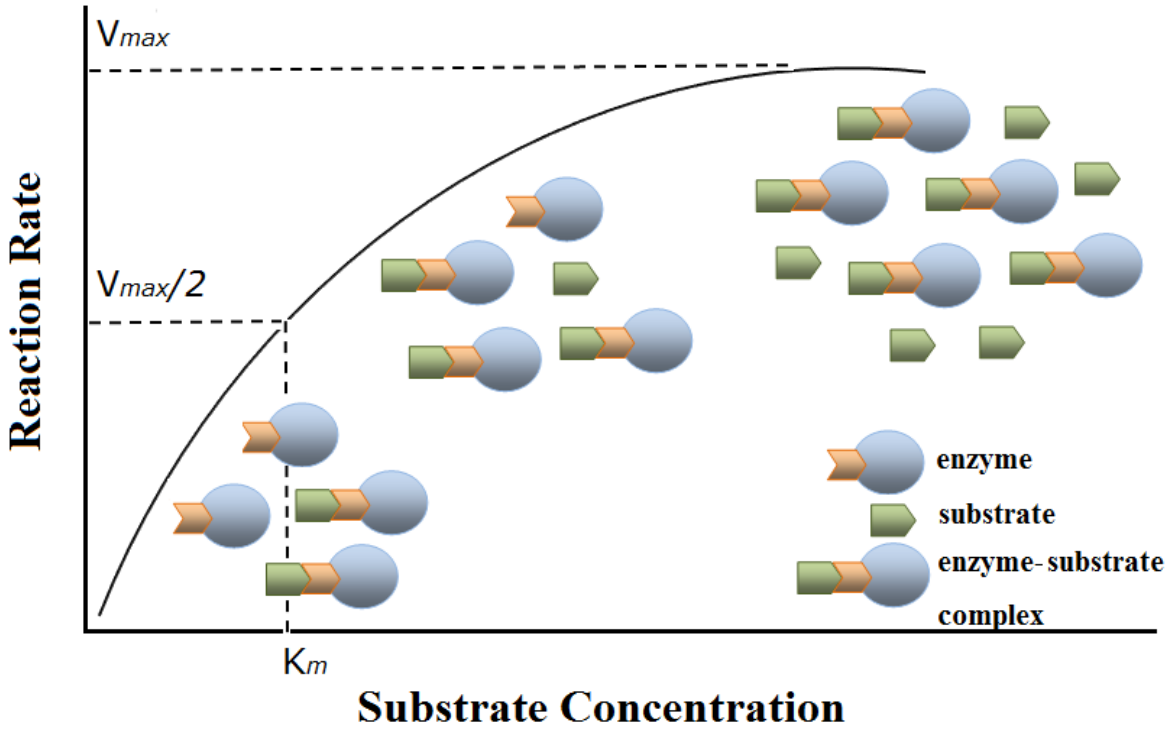


Figure 2. Dependence of enzymatic reaction rate on substrate concentration.

Michaelis and Menten suggested in 1913, a mechanism that shows how the substrate (S) is binding to the enzyme (E) to form enzyme-substrate complex (ES) (Menten and Michaelis, 1913). After that a reaction product (P) is formed due to enzymatic reaction. They come up with the following equation:

$$V_o = \frac{V_{\max}\,[S]}{K_m + [S]}$$

where $V_{\max}$ is the maximum rate, K_m is the concentration in $V_{\max}/2$. A small K_m indicates that the enzyme requires only a small amount of substrate to become saturated, and vice versa. Hence, the maximum velocity is reached at relatively low substrate concentrations. K_m and affinity are inversely proportional, in other words an enzyme with small K_m shows high affinity towards its substrate (Arduini and Amine 2013; Amine et al., 2006; El-Metwally and El-Senosi, 2010).

2.2 Enzyme inhibition

The major drawbacks in enzyme-based biosensor is that, some chemicals can behave like substrate, specifically interact with the immobilized enzyme or can bind to immobilized enzyme, causing change in its active site. In this point, enzyme inhibition is a phenomenon where the enzymatic activity can be reduced by the entrance of an inhibitor to the system; therefore detection of both inhibitor and substrate can be achieved by enzyme-based biosensors (Lu and Li, 2010). As the inhibitor introduced to the biosensor analyzing media, the residual activity of the enzyme can be lowered. The residual activity of the enzyme before and after the inhibition can be measured (Tran-Minh et al., 1990; Minh 1985; Evtugyn et al., 1998a; De Castro and Herrera, 2003). This inhibition phenomena can be induced by pesticides, derivatives of insecticides (Trojanowicz, 2002; Sol´e et al., 2003; Audrey et al., 2012), drugs (Kurbanoglu et al. 2015), food contaminants (Patel, 2002) can be detected and controlled. Some pesticides such as chlorpyrifos, dichlorvos, dimethoate, malathion, parathion, some carbamates such as carbofuran, aldicarb, pirimicarb are irreversibly inhibit enzymes, on the other hand, some drugs such as, neostigmine, rivastigmine; piperidines, such as donepezil can inhibit enzymes reversibly (Chang 2009; Liu et al., 2011b; Colovic et al., 2013). Inhibition is analytically beneficial due to its capability to ensure indirect monitoring of some analytes (inhibitors) even at very low concentration at which they alter the enzyme allowing in this way detection. Inhibition-based analytical systems have been used in the fabrication of many enzymatic biosensing devices. The inhibition can be reversible, where the enzyme can be used several times, and/or irreversible, where the inhibitor causes permanent changes in enzyme structure (Arduini and Amine 2013; Amine et al., 2006; El-Metwally and El-Senosi, 2010).

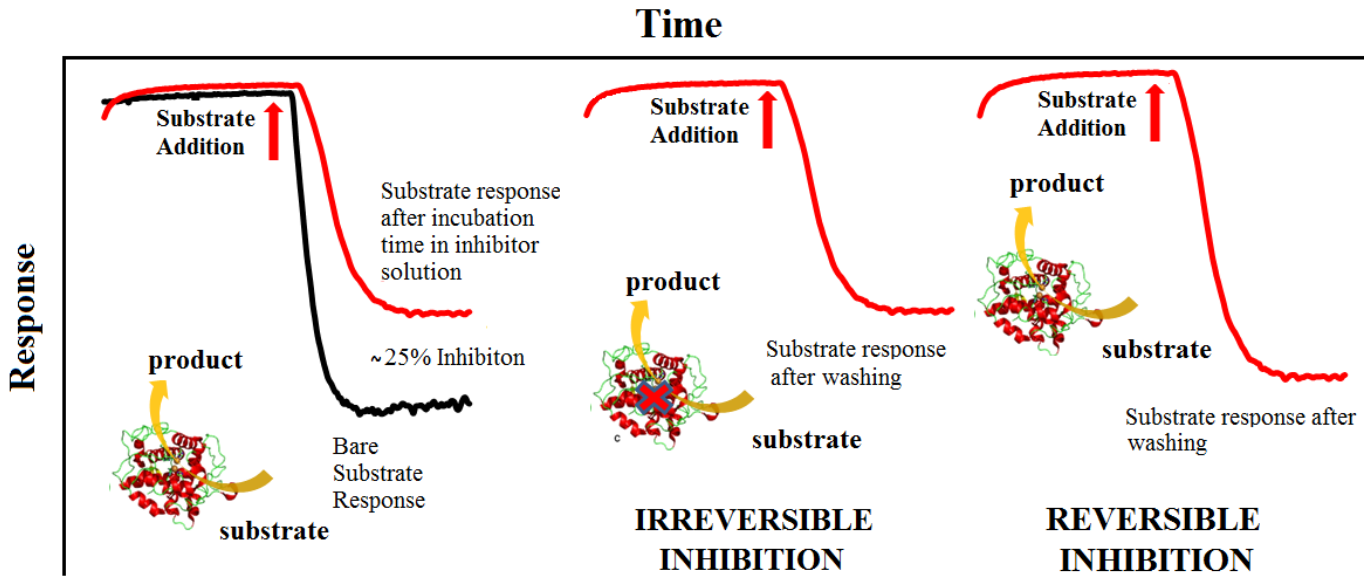


Figure 3. Irreversible and reversible inhibition using enzyme-based biosensor.

In inhibition reactions, enzyme activity is measured before (I_o) and after inhibition (I_1), and the decrease of enzyme activity is calculated as ((I_0- I_1)/I_0) x 100. After inhibition, the washed (from analyte/inhibitor) biosensor is exposed to substrate detection. If the same sensing capability is observed the process of inhibition is considered reversible otherwise the inhibition is irreversible (figure 3). In irreversible inhibition, the enzyme losses its initial activity and the original activity can never be obtained. The incubation time and thickness of the biosensing detection layer can directly affect the inhibition quality (Arduini and Amine 2013; Amine et al., 2006; Dixon 1953; Cornish-Bowden 1976).

Reversible inhibition can be divided as competitive inhibition, noncompetitive inhibition, mixed type inhibition and uncompetitive inhibition. In competitive inhibition, substrate and inhibitor are in a competition for the active site of the enzyme. In noncompetitive inhibition, inhibitor binds to other side of the enzyme than its active side. As a result of inhibitor's binding to enzyme, the active side composition of the enzyme changes, therefore substrate cannot bind to enzyme. In uncompetitive inhibition, inhibitor binds to the enzyme-substrate complex, as a result, product cannot be formed. In mixed type of inhibition, inhibitor can bind

to both enzyme itself and enzyme-substrate complex. For all type of inhibitions, the decrease in enzymatic reaction can be followed (Arduini and Amine 2013; Amine et al., 2006; Arduini et al., 2006; El-Metwally and El-Senosi, 2010).

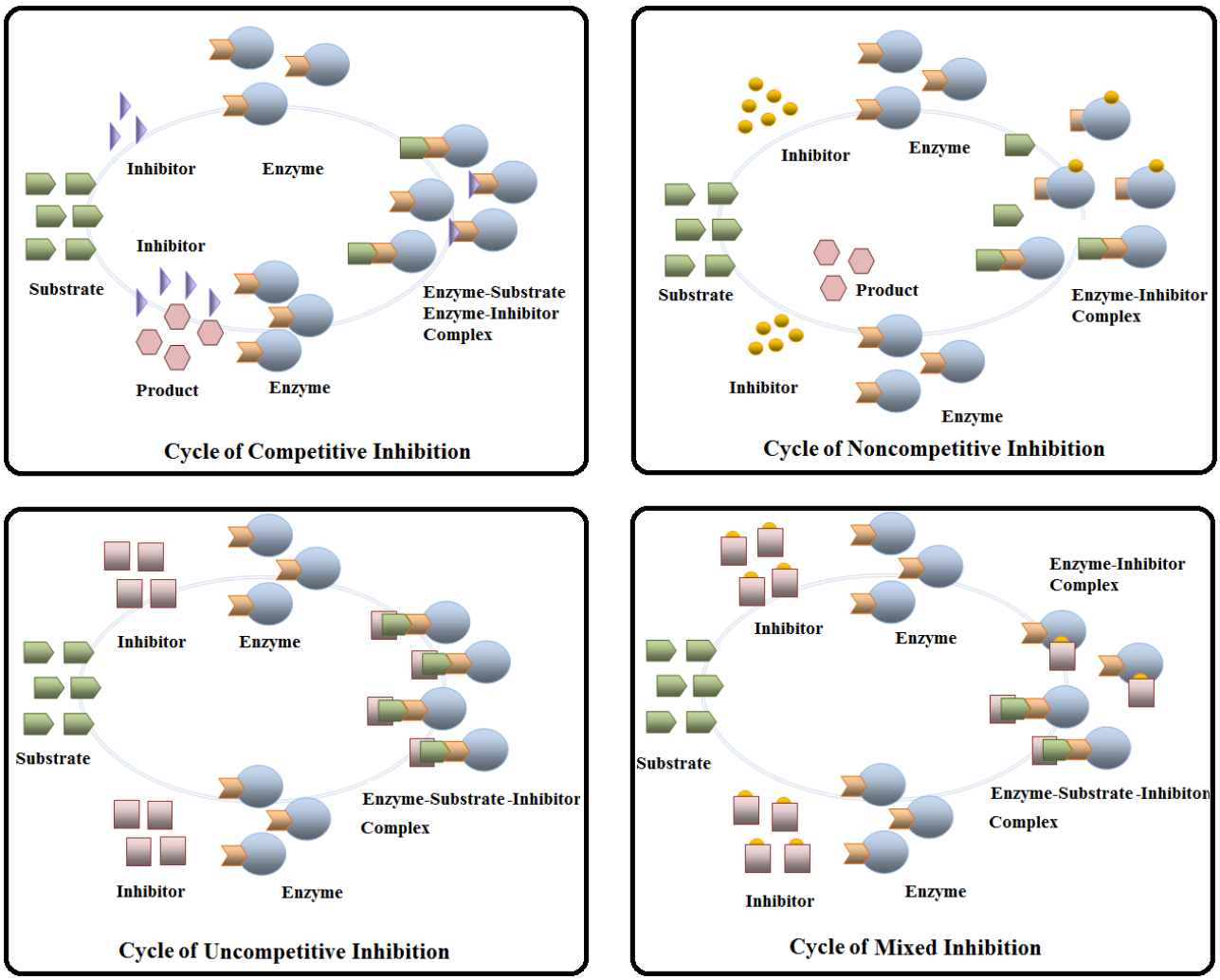


Figure 4. Cycles of reversible inhibitions competitive, noncompetitive, uncompetitive and mixed inhibitions.

From the aspect of enzyme reaction kinetics, the maximum velocity (V_{max}) of the enzyme does not change in competitive inhibition, but the Michaelis Menten constant (K_m) increases as shown in figure 2. In high concentration of substrate this inhibition cannot occur. In noncompetitive inhibition, inhibitor binds to the enzyme from a different site, and active site

of the enzyme is deformed resulted in reversible inhibition. In this inhibition substrate and inhibitor are not in a competition, V_{max} decreases whereas K_m remains constant. On the contrary of competitive inhibition, noncompetitive inhibition can be occurred in high concentration of substrate (Cornish-Bowden and Wharton 1988; Dixon 1953). In mixed type inhibition, the inhibitor binds both to the enzyme-substrate complex and enzyme itself. In both inhibitions, the mechanism is as showed in figure 4. In uncompetitive inhibition, the inhibitor binds only to the enzyme-substrate complex, and at the end of this inhibition product cannot be obtained. In this type of inhibition ES complex is always removed from the matrix therefore K_m decreases. Moreover, ESI complex is always formed and the result is a decrease in V_{max}. The mechanistic relations are summarized in figure 5. (Lineawever and Burk 1934; Dixon 1953; Cornish-Bowden 1976).

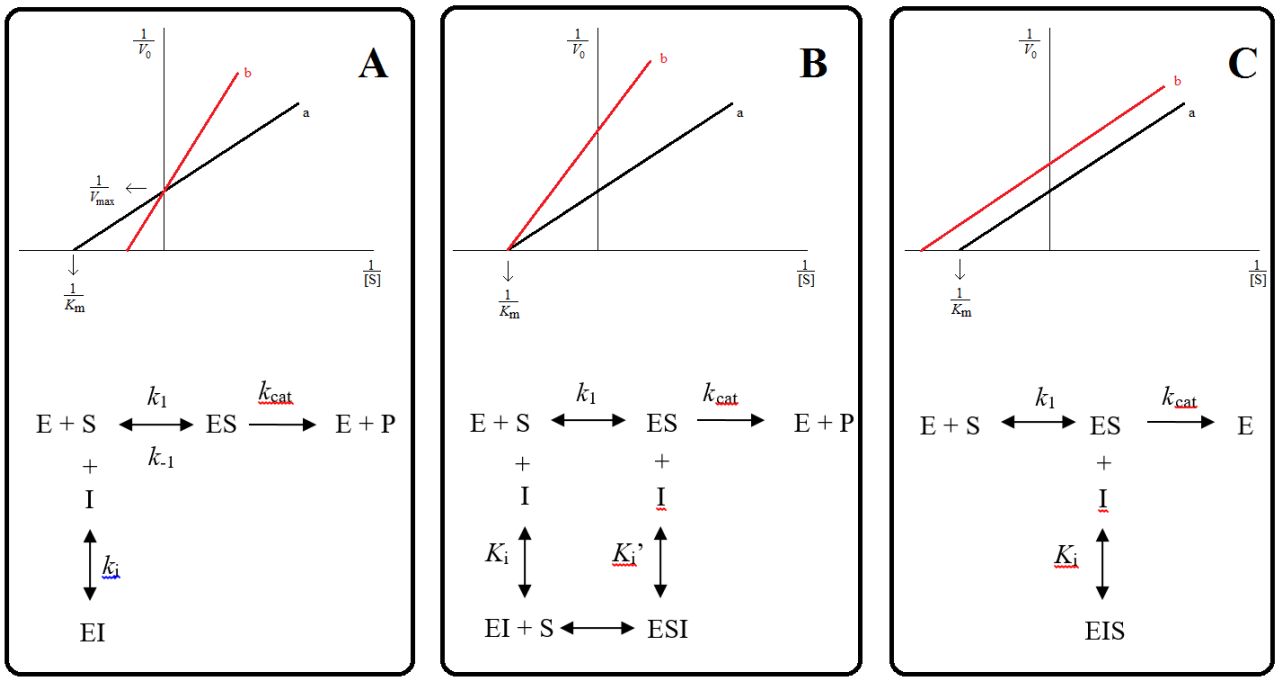


Figure 5. Lineawever-Burk plot and enzyme kinetics for a) competitive inhibition b) noncompetitive inhibition and mixed inhibition c) uncompetitive inhibitions.

In this review, our aim is to discuss the role of nanomaterials in inhibition-based electrochemical biosensors in drug analysis, safety and security applications as well as the faced problems, challenges and future perspectives.

3. Nanomaterials in enzyme inhibition-based biosensors

In modifying the biosensor, nanoparticles are the generally used NM modifier, due to their enhanced catalytic properties coming from the high surface to volume ratio. NMs exhibit important roles in biosensing. These can make easier immobilization of biomolecules, enhance electron transfer, and catalyze the enzymatic reaction beside others (Luo et al., 2006; Ansari and Husain, 2012; Mohanraj and Chen, 2007; Dubach and Clark, 2013). Some NMs can adsorb biomolecules; they are biocompatible in a considerable degree and enable enzymes to protect their activity. Enzymes can covalently bind to NMs but also there exist strong electrostatic interactions between some NMs and enzymes (Kerman et al., 2008; Luo et al., 2006; Ansari and Husain, 2012). Enhancement of electron transfer between enzyme and transducer also can be achieved by using NMs. Indeed, nanoparticles are receiving significant attention due to their ability to promote electron transfer between electrodes and the active site of the enzyme owing to their large surface to volume ratio, high surface reaction activity and strong adsorption ability (Mohanraj and Chen, 2007). Therefore the enzyme immobilization into or onto various nanoparticles including, metallic nanoparticles, such as gold nanoparticles (Castaneda 2007; Parolo et al., 2013; Pingarrón et al., 2008; Mena et al., 2005; Mukhopadhyay, et al.;2003; Tiwari, 2015), silver nanoparticles (Kerman et al., 2008; Luo et al., 2006) platinum nanoparticles (Chu et al., 2007; Yang et al., 2006), metal oxide nanoparticles such as iron oxide (Chen et al., 2010, Cevik et al., 2012), iridium oxide (Kurbanoglu et al., 2015; Mayorga-Martinez et al., 2014), magnetic nanoparticles (Mayorga-Martinez et al., 2014), carbon based nanoparticles (Pérez-López, and Merkoçi, 2012;

Merkoçi, 2006; Chen et al., 2010; Shan et al., 2009) have been proposed and reported. NMs can be divided in two categories named inorganic and organic nanomaterials. From all types of nanomaterials, carbon based and metal nanomaterials are the most used ones (Kerman et al., 2008; Luo et al., 2006; Ansari and Husain, 2012; Metters and Banks, 2014).

3.1 Carbon based nanoparticles

Carbon is one of the unique and the most abundant element, and their allotropes can be used to modify transducers in enzyme biosensors. Carbon is a multipurpose elements meaning that one can create different compounds related to its electronic configuration. Nano structured carbon based nanomaterials such as carbon nanotubes, fullerenes and graphene are promising materials for such applications. Graphene is the simplest form of carbon representing a single carbon layer of graphite (Kuila et al. 2011; Kamat, 2009; Novoselov et al., 2012; Tung et al., 2009). Graphene can be synthesized by different methods such as mechanical exfoliation (Novoselov et al., 2004; Su et al., 2011; Liu et al., 2011a), chemical vapor deposition (Sutter et al., 2008; Reina et al., 2009) and Hummers method (Hummers and Offeman, 1958; Kosynkin, 2010). By exfoliation method graphene oxide can be obtained and it can be reduced by electrochemical or chemical methods (Suarez-Martinez et al., 2012, Merkoçi, 2013; Tamayo et al., 2013; Barberis et al., 2015; Gao and Duan, 2015; Ray, 2015).

Carbon nanotubes (CNTs) are a graphene sheet in the shape of a cylinder capped by fullerene-like structures that can be created by rolling up a single layer of graphite or graphene along a certain direction into a tiny cylinder. CNTs are discovered by Iijima in 1991 and reported to have diameters from fractions to tens of nanometers and lengths up to several micrometers (Iijima, 2002) Single walled carbon nanotubes (SWCNTs), double-walled carbon nanotubes and multi-walled carbon nanotubes (MWCNTs) found a real place in biosensing applications due to their extremely large surface area, unique mechanical, thermal, electrical, and physical

properties (Merkoçi et al., 2005; Balasubramanian and Burghard, 2006; Besteman et al., 2003 Wang, 2005; Wang, 2006; Suarez-Martinez et al., 2012, Merkoçi, 2013; Tamayo et al., 2013; Barberis et al., 2015; Gao and Duan, 2015; Ray, 2015).

Fullerenes contain 12 pentagons and varying numbers of hexagons, are closed single-walled cage molecules. C_{60}, is the well-known fullerene which is made up of 60 closely packed carbon atoms (Cozzi et al., 2005; Vávrová et al., 2012; Rao et al., 1995). Using fullerene in biosensing have improvements such as long stability and wide potential window. (Hedberg et al., 1991; Dresselhaus et al., 1996; Suarez-Martinez et al., 2012, Barberis et al., 2015).

3.2 Metal-based nanomaterials

Metal nanomaterials mainly gold nanoparticles (AuNPs) are generally chosen as modifier of electrodes due to their high biocompatibility, their ability to enhance electron transfer between analytes and transducers due to the excellent conductivity (Kerman et al., 2008; Luo et al., 2006; Ansari and Husain, 2012; Tiwari, 2015). AuNPs are reason for choice due to their excellent properties such as high surface-to-volume ratio, good biological compatibility in terms of catalytic, optical, thermal, electronic stages and excellent conducting capability (Pingarrón et al., 2008; Mena et al., 2005; Chen et al., 2013; Luo et al., 2006; Shulga and Kirchhoff, 2007; Haruta and Date 2001).

Detection of some phenolic compounds through gold nanoparticles used as modifier agent in biosensing is reported. Detection of these compounds is crucial since they are poisonous being a potential hazard for human health. They can exist in natural waters coming from industrial residues. Electrochemical enzyme-based biosensors are also used in detection phenolic compounds. Vicentini et al., suggested a biosensor using a glassy carbon electrode modified with AuNPs and tyrosinase (Tyr) within a dihexadecylphosphate film for the detection of catechol in natural water. With the designed biosensor, determination of catechol

was achieved by in a linear concentration range from 2.5×10^{-6} to 9.5×10^{-5} mol L^{-1} catechol with a detection limit of 1.7×10^{-7} mol L^{-1} (Vicentini et al., 2016).

Like gold nanoparticles, silver nanoparticles, platinum nanoparticles, and copper nanoparticles, are also used in enzyme based biosensors. Metal nanomaterials can also be mixed with carbon nanotubes and used to immobilize enzymes. This can bring synergistic effects towards enzymatic catalysis. Moreover, since metals are able to form oxide compounds, oxide nanoparticles such as TiO_2, SiO_2, Ag, Pt, ZrO_2, Al_2O_3, Fe_2O_3, ZrO_2, MoO_3, CeO_2, can also enhance the electron transfer from the active centers of enzymes (Rodriguez and Fernández-García, 2007; Fernández-García et al., 2004). Due to their easy preparation, biocompatibility property, and enhancement in electron transfer, these metal nanoparticles are commonly used (Kerman et al., 2008; Luo et al., 2006). Iridium oxides are also commonly used NMs especially by our group (Kurbanoglu et al., 2015; Rivas et al., 2014; Rivas et al., 2015; Mayorga-Martinez et al., 2014). In our recent work, iridium oxides nanoparticles were used to design biosensor with the synergic properties between the high conductivity of iridium oxide nanoparticles, low-cost screen printed electrodes and the efficiency of tyrosinase for the detection of catechol and chlorpyrifos. Chlorpyrifos was also successfully detected in spiked tap and river water samples (Mayorga-Martinez et al., 2014). In our other recent work, inhibition based detection of Methimazole drug was achieved using a biosensor based on a nanocomposite of magnetic nanoparticles functionalized with iridium oxide nanoparticles and tyrosinase. The designed biosensor was successfully applied to spiked human serum and pharmaceutical dosage forms for Methimazole detection (Kurbanoglu et al., 2015). Moreover, in their work, Liu et al, developed an enzymatic biosensor based on TiO_2 nanotube-polyaniline-gold nanoparticle-horseradish peroxidase composite. TiO_2 nanoparticles were converted to titanate nanotubes by hydrothermal reaction. Using this biosensor,

chronoamperometric detection of H_2O_2, was achieved in a linear range from 1 to 1200 mM H_2O_2, with a detection limit of 0.13 mM H_2O_2 (Liu et al., 2016).

Semiconductor nanoparticles consisting of Zn and Cd with Te and Se known as Quantum Dots (QDs) are also used in enzyme based biosensor designs with the purpose of using their ability as biological fluorescent probes due to their long-term photostability, high quantum yields, tunable size-dependent emission, narrow emission bandwidth and broad excitation. (Gill et al., 2008; Lin et al., 2005; Zhao et al., 2011; Han et al., 2015; Benítez-Martínez, et al., 2016; Hu et al., 2016; Xue et al. 2016). These NMs composed of a metallic semiconductor core are most commonly coated by a polymeric shell such as phospholipid–polyethylene gylcol copolymer, amphiphilic polymers such as maleic anhydride and the alkyl-functionalized polyacrylate derivatives. (Resch-Genger et al., 2008; Lin et al., 2005; Zhao et al., 2011).

4. Applications of nanomaterial-modified enzyme inhibition based biosensors for drug analysis, safety and security applications

Biosensors based on enzyme inhibition are widely reported for detection of toxic compounds such as pesticides, organophosphorus mycotoxins and other compounds. Since, fungicides, herbicides and insecticides, would reduce the food production, their detection and control is crucial for safety and security (Trojanowicz, 2002; Sol´e et al., 2003). In food, environmental and drug analysis, when electrochemical biosensor is the concern enzyme inhibition based biosensors are mainly used (Trojanowicz, 2002; Sol´e et al., 2003; Aragay et al.2012; Van Dyk and Pletschke, 2011; Scott, 1998).

Pesticides are commonly used in food cultivation and agriculture nevertheless they can be hazardous for humans and environment (Aragay et al.2012; Van Dyk and Pletschke, 2011). Numerous electrochemical approaches using nanomaterials-based enzyme biosensor

operating through inhibition for safety and security applications are developed for the analyses of pesticides, pharmaceutical compounds, other chemicals and some selected approaches from these are shared in table 1. Different kinds of pesticides, insecticides like carbamates and organophosphate, drugs such as anti- dementia, anti-thyroid are found as analyte with different immobilization matrixes and using nanomaterials such as graphene, quantum dots, metallic nanoparticles and mainly carbon nanotubes (Aragay et al.2012; Van Dyk and Pletschke, 2011).

In their work, Du et al. suggested an amperometric biosensor for the detection of methyl parathion (MP). They, electrochemically deposited AuNPs by a multi-potential step technique at multiwalled carbon nanotube (MWCNT) film on a glassy carbon electrode. Afterwards, methyl parathion degrading enzyme was covalently attached onto the glassy carbon electrode through CdTe quantum dots used as carriers to load a large amount of enzyme (Figure 6). Use of MWCNT with AuNPs brings a synergetic effect to the biosensor. With a detection limit of 1.0 $ng.mL^{-1}$, methyl parathion was detected successfully (Du et al., 2010a).

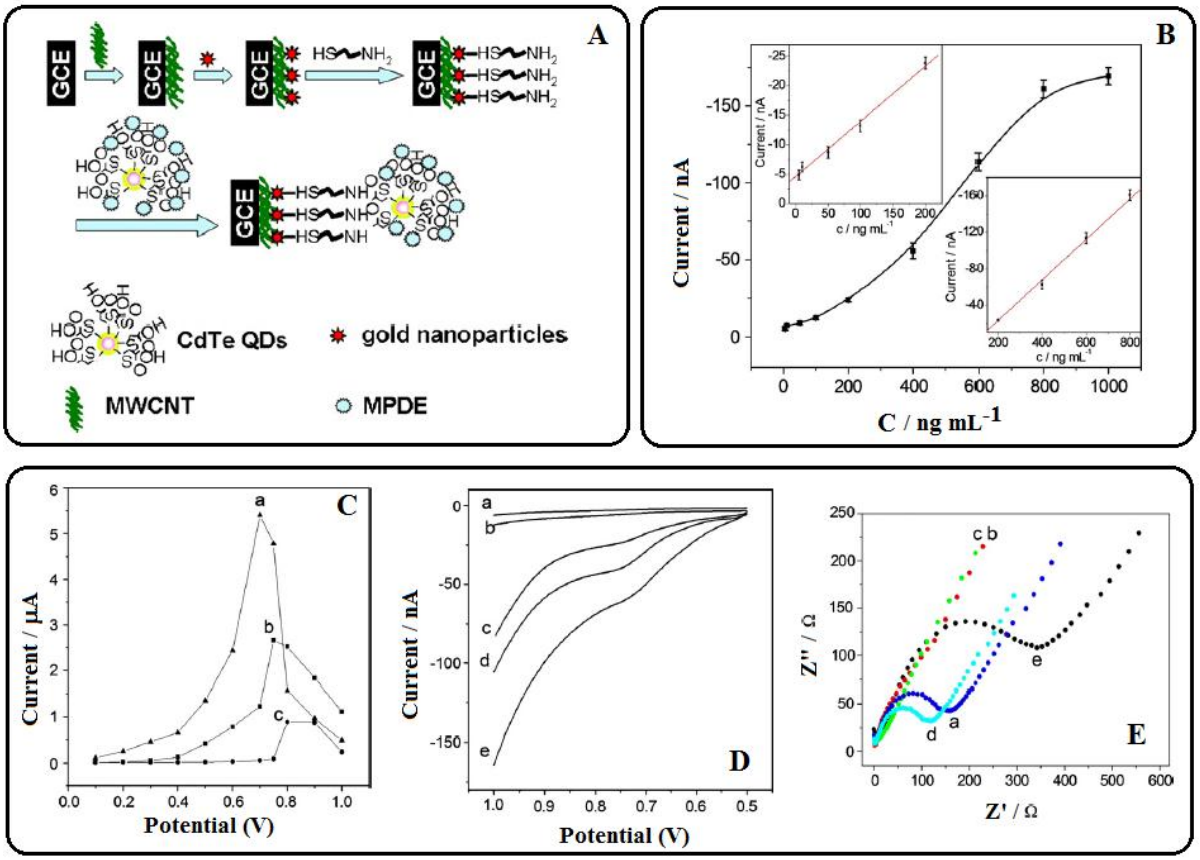


Figure 6. A) Preparation procedure for methyl parathion degrading enzyme (MPDE) biosensor B) Response curves for MP in pH 7.0 PBS at MPDE–CdTe/Cys/Aunano/MWCNT/GCE. Inset: the calibration curves for MP determination C) Hydrodynamic voltammogram of p-nitrophenol obtained at (a) Aunano/ MWCNT/GCE, (b) MWCNT/GCE and (c) Aunano/GCE. p-Nitrophenol was obtained by mixing 1mg.mL^{-1} MPDE with 100 ng.mL^{-1} MP for 10 min D) The linear scan voltammograms of 100 ngmL^{-1} MP at (a) Cys/Aunano/MWCNT/GCE, (b) QD/Cys/Aunano/MWCNT/GCE, (c) MPDE–CdTe/Cys/Aunano/MWCNT/GCE and (d) 200 ng.mL^{-1} MP and (e) 400 ng.mL^{-1} MP at MPDE–CdTe/Cys/Aunano/MWCNT/GCE. E) Electrochemical impedance spectra of (a) bare GCE, (b) MWCNT/GCE, (c) Aunano/MWCNT/GCE, (d) Cys/Aunano/MWCNT/GCE and (e) MPDE–CdTe/Cys/Aunano/MWCNT/GCE recorded in pH 7.0 PBS containing 50 mM $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$. Reprinted with permission from ref (Du et al., 2010a).

Magnetic Nanoparticles (MNPs) have ability to remove enzyme from the matrix with a help of magnet. These nanoparticles show supra-paramagnetic property below 50 nm in size. In previous work by our group, tyrosinase (Tyr) was immobilized in a matrix of magnetic nanoparticles and iridium oxide nanoparticles with the help of magnet under the screen printed electrode (figure 7) (Kurbanoglu et al., 2015).

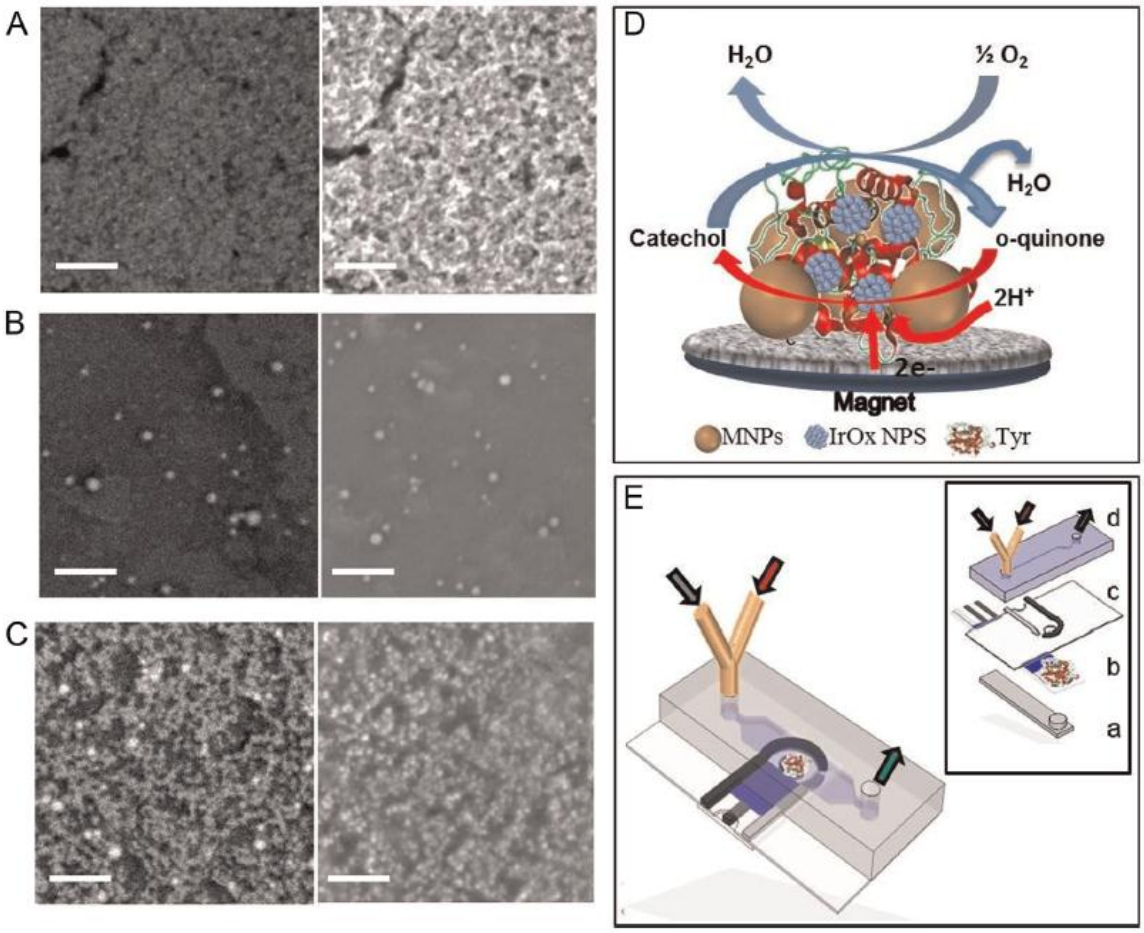


Figure 7. SEM images of MNPs and Tyr (A), IrOx and Tyr (B) and IrOx NPs–Tyr-MNPs nano composite (C). Scale bars of SEM images are 100 nm. The SEM images were obtained using backscatter electrons (BE) mode (left column) and secondary electron (SE) mode (right column). (D) Schematic representation of the proposed detection system displaying tyrosinase (Tyr) and the reaction involved in the catechol detection. (E) Lab-on-a-chip design. Reprinted with permission from ref (Kurbanoglu et al. 2015)

Zhang et al. developed a biosensor based on layer-by-layer (LbL) assembled multi-enzyme and carbon nanotubes for the determination of organophosphorus and non-organophosphorus pesticides. The author immobilized Acetylcholine esterase which is inhibited by many pesticides, with the polyethyleneimine (PEI) and DNA using their electrostatic properties (figure 8). They characterized the biosensor using surface plasmon resonance and electrochemical impedance spectroscopy and applied MWCNT–$(PEI/DNA)_2$/OPH/AChE biosensor to real apple samples (Zhang et al. 2015).

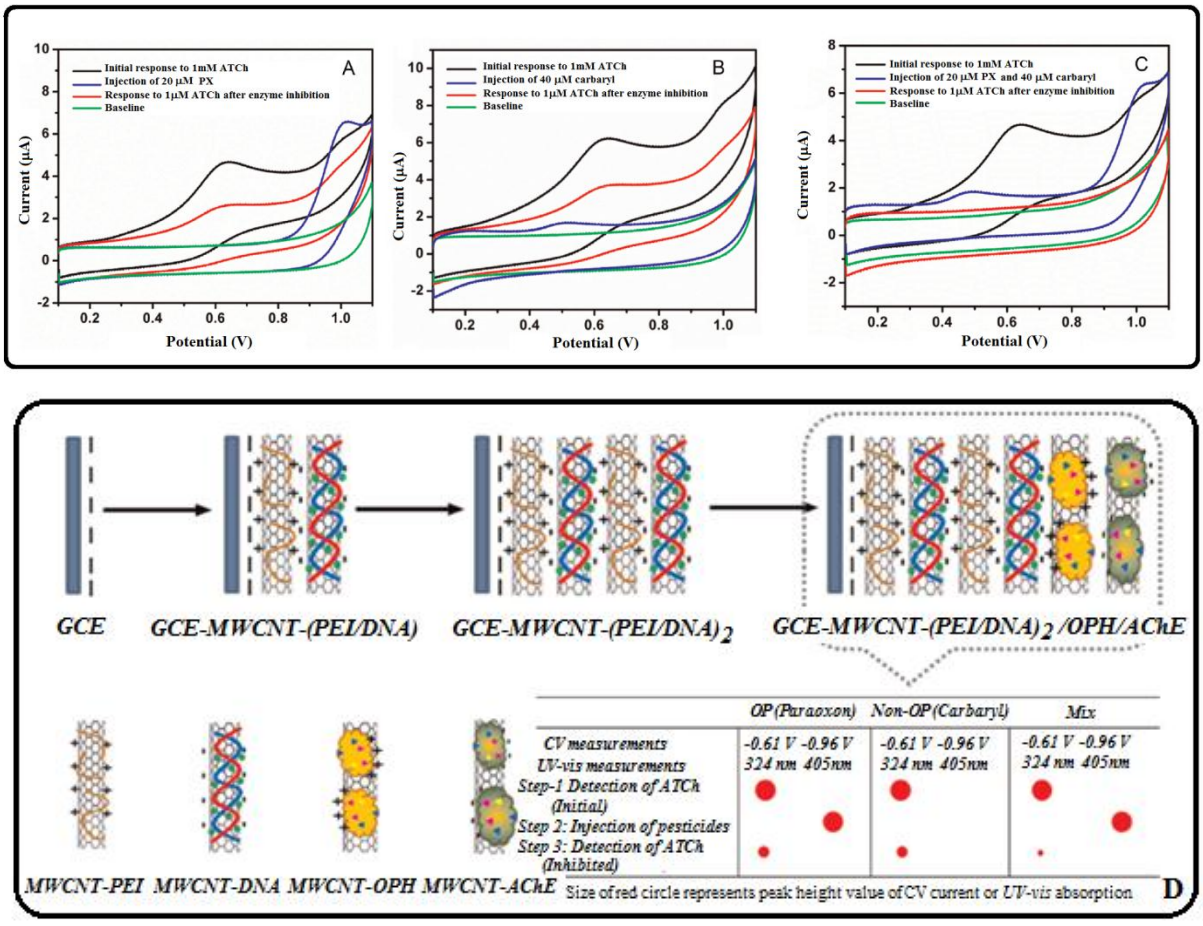


Figure 8. CV response for discriminative detection of (A) OP(20 μM paraoxon), (B) non-OP (40 μM carbaryl) and (C) mix of OP (20 μM paraoxon) and non-OP (40 μM carbaryl) D) Schematic illustration of LbL assembly and bi-enzymatic layer in biosensor interfaces

constructed on the GCE and discriminative detection of OP and non-OP using electrochemical and optical methods. Reprinted with permission from ref (Zhang et al. 2015).

In another work, Zhao et al., developed a novel biosensor using electrochemical reduced graphene oxide (ERGO), gold nanoparticles, β-cyclodextrin and Prussian blue-chitosan. They suggested that, due to the synergistic effect between ERGO and AuNPs significantly promoted the electron transfer, and remarkably improved the electrochemical detection of thiocholine. Furthermore, malathion and carbaryl inhibitory effect was followed on this designed biosensor in the range of 7.98-2.00x10^3 pg mL^{-1} and 4.3-1.00x10^3 pg mL^{-1} with low detection limits of 4.14 $pgmL^{-1}$ and 1.15 $pgmL^{-1}$ for malathion and carbaryl, respectively (Zhao et al., 2015). In our recent work, the detection of chlorpyrifos was achieved with a detection limit of 0.003 µM. Iridium oxide nanoparticles were used to enhance catechol detection and tyrosinase enzyme was immobilized through glutaraldehyde and bovine serum albumin on the surface of screen printed carbon electrode. Using proposed biosensor, both catechol and chlorpyrifos was detected, suggesting a dual biosensor (Mayorga-Martinez et al., 2014).

In another work, Huo et al, suggested a novel biosensor based on hybrid nanocomposite consisting of copper oxide nanowires (CuONWs) and single-walled carbon nanotubes (SWCNTs) for the detection of Malathion (Figure 9A). The biosensor was characterized, optimized and Malathion was detected in a wide range with a limit of detection of 0.1 ppb. The suggested biosensor was also applied to detect Malathion in spiked liquid garlic samples (Huo et al., 2014). For the detection of Malathion, in their work, Kaur et al. developed a biosensor based on carboxylated multi-walled carbon nanotubes and conducting polymer of Poly(3,4-ethylenedioxythiophene) (PEDOT) (Figure 9B). With a 10 min. incubation time, Malathion inhibition was followed towards, 0.3 mM acetylthiocholine chloride, within the

linear range 1 fM to 1 μM. The detection limit of the purposed biosensors was found as 1 fM and it was applied to spiked lettuce sample (Kaur et al., 2016). In another work, by Liu et al.,3-carboxyphenylboronic/reduced graphene oxide–gold nanocomposites modified electrode was designed for the detection of malathion, organophosphorus and carbamate pesticides (Figure 9C). The authors first modify the surface of the glassy carbon electrode with reduced graphene oxide to promoted electron transfer reaction and enhanced the electrochemical response, then gold nanoparticles were introduced to the surface. With the help of the chemistry between N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC), N-hydroxysuccinimide (NHS), cysteamine and the Acetylcholinesterase, the biosensor was constructed. Finally, the authors followed the inhibition effects of chlorpyrifos, malathion, carbofuran and isoprocarb to the biosensor response towards 0.15 mM acetylthiocholine chloride with the detection limits of 0.1 ppb, 0.5 ppb, 0.05 ppb, 0.5 ppb, respectively (Liu et al., 2011b). Zhai et al., an Acetylcholinesterase biosensor based on chitosan, prussian blue, multiwall carbon nanotubes, hollow gold nano spheres nanocomposite fabricated by one-step electrodeposition procedure was suggested (Figure 9D). Using this biosensor, inhibition studies of Acetylcholinesterase was achieved for the detection of Malathion, Chlorpyrifos, Monocrotophos and Carbofuran within the linear range and detection limit of 0.05–75nM, 0.05–75nM, 0.1–50nM, 5–80nM and 0.05 nM, 0.05nM, 0.1M, 2.5nM, respectively. This biosensor was also applied to real samples of cabbage, lettuce, leek and pakchoi (Zhai et al., 2013).

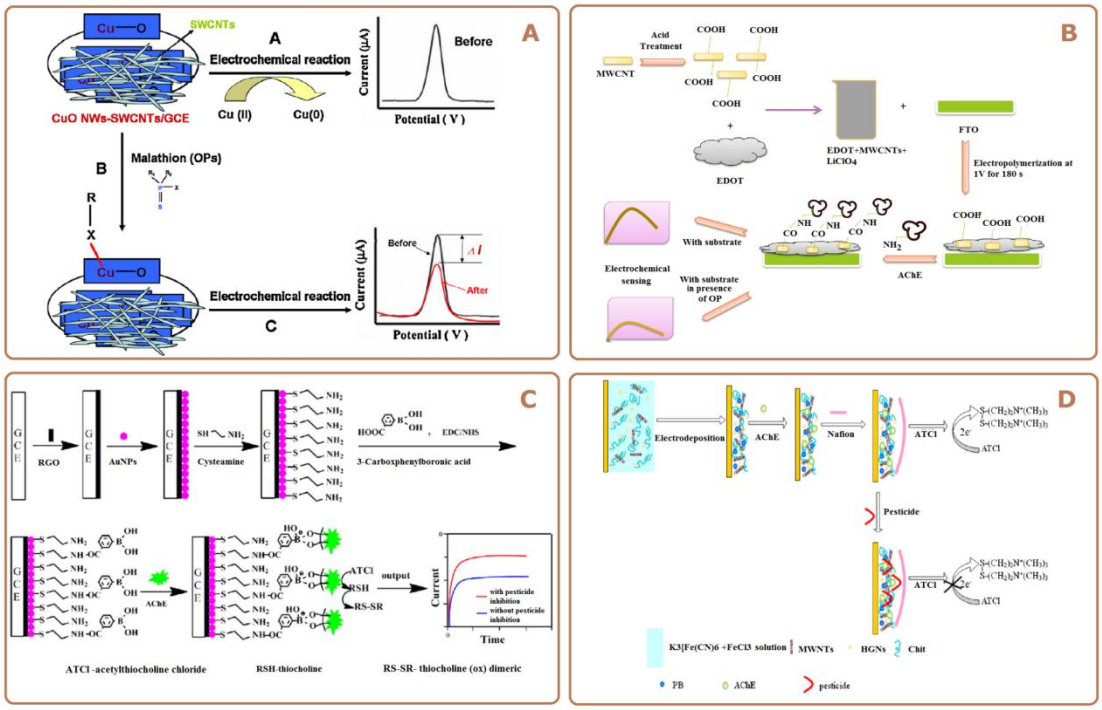


Figure 9. Examples of Malathion detection using nanoparticle-based Acetylcholinesterase biosensors operating through inhibition, modified with A) hybrid nanocomposite consisting of copper oxide nanowires (CuONWs) and single-walled carbon nanotubes (SWCNTs) Reprinted with permission from ref (Huo et al., 2014). (B) Carboxylated multi-walled carbon nanotubes and conducting polymer of Poly(3,4-ethylenedioxythiophene). Reprinted with permission from ref (Kaur et al., 2016). C) 3-carboxyphenylboronic/reduced graphene oxide–gold nanocomposites. Reprinted with permission from ref (Liu et al., 2011b). D) Chitosan, Prussian blue, multiwall carbon nanotubes, and hollow gold nano spheres nanocomposite. Reprinted with permission from ref (Zhai et al., 2013).

In another work, authors suggested a biosensor based on electrochemically reduced graphene oxide, Nafion and Acetylcholinesterase hybrid nanocomposite modified electrode. Thiocholine (TCl) was used as the substrate and the biosensor was used for the electrochemical detection of organophosphate pesticide, Dichlorvos, which irreversibly inhibits the activity of AChE. The response of the biosensor before and after 10 min

incubation in Dichlorvos solution was linear in between 5.0 and 100 ng.mL^{-1} with the detection limit of 2.0 ng.mL^{-1} (Wu et al., 2013).

In their work, Ding et al. suggested an Acetylcholinesterase/ carbon nanotubes/ nanoporous gold electrode (AChE-MWCNT-CA-NPG) based biosensor for the detection of Malathion. Carbon nanotubes were crosslinked on the surface of the electrode by the help of cysteamine with the self-assembly technique. Under optimized conditions, Acetylcholinesterase inhibition by Malathion was followed (Figure 10). Effect of the inhibition time, of 0.01 µg mL^{-1} Malathion was also studies. In the range of 0.001–0.5 µg mL^{-1} Malathion was detected with a limit of detection of 0.5 ng mL^{-1} (Ding et al., 2014).

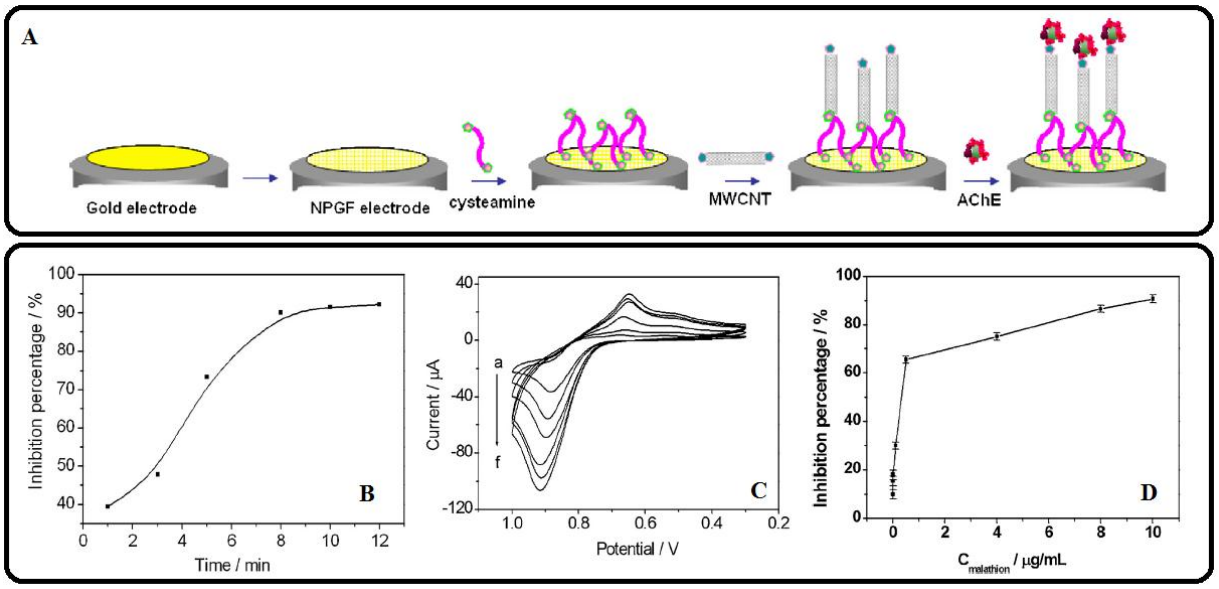


Figure 10 (A) Schematic illustration of the formation of AChE-MWCNT-CA-NPG B) Effect of the immersing time on inhibition of 0.01 µg mL^{-1} malathion. C) Cyclic voltammograms of AChE-MWCNT-CA-NPG in pH 7.0 PBS containing 0.2 mM ATCl after immersed in malathion solution with different concentrations D) Linear relationships between the inhibition percentage and malathion concentration Reprinted with permission from ref (Ding et al., 2014).

Conclusions and future perspectives

It is important to control food, environment and human from hazards, pharmaceutical compounds, pesticides and other chemicals that exert harmful effects through for example enzyme inhibition. Such control / monitoring is strongly related with the use of point of care devices in general and biosensors particularly. Biosensors represent very interesting devices for on-line monitoring beside single-detection applications. In construction of biosensors, nanomaterials are excellent building blocks that can be used as modifiers of transducers so as to enhance their electrochemical signals (case of electrochemical (bio)sensors). This review focuses on the application of nanomaterials to electrochemical enzymatic biosensors with a special attention to the various strategies and architectures reported so far and with interest in inhibition-based analytical applications. Moreover, in this review, reader can find necessary information about enzyme inhibition phenomena, nanomaterials in enzyme inhibition-based biosensors in terms of carbon-based nanomaterials and metal-based nanomaterials. The role of nanomaterials in inhibition-based biosensors for the analyses of different groups of drugs as well as contaminants such as pesticides, phenolic compounds and others, also are discussed. Recent studies, especially in last 5 years, related to organic and inorganic nanomaterials-based enzyme biosensors for drug analysis, safety and security applications are discussed. So far in the literature, carbon nanoparticles, quantum dots, gold nanoparticles are commonly used due to their various properties, mainly induced enhancement in the electrochemical signal in addition to their biocompatibility. Acetylcholinesterase, laccase, tyrosinase are the enzymes that are generally immobilized in the nanomaterials-modified transducers, for the detection of various pesticides, pharmaceuticals and hazards such as, carbaryl, paraoxon, phosmet, methamidophos, chlorpyrifos, paraoxon, methimazole, cyanide etc. in various food samples, pharmaceuticals, clinical samples etc achieving up to fM level as detection limits.

Although very interesting reports using nanomaterials-based biosensor have appeared their application in the detection of pesticides, pharmaceutical compounds, and other hazards in real samples are still in their early age. For example rapid (ex. one step assay) and high-throughput screening of pesticides, hazards and pharmaceuticals are still to-fulfill requisites for biosensors. In addition the integration of nanomaterials should be further improved on the view of the sensitivity and selectivity of the resulting biosensors to be used for pesticides, pharmaceuticals and hazards that can inhibit enzymes. Moreover the development of new functionalized nanomaterials with better and easier immobilization capability for enzymes still is necessary. Nanoparticles with improved stability, including recycled property, and overall catalytic properties are very much requested for these kind of biosensors. In addition to the conventional biosensing technologies based on the use of screen-printed and tubular electrodes there is a great demand to develop other formats such as lab-on-a-chip and paper platforms to achieve devices with less assays steps and with interest for in–situ / in-field applications. The future devices with interest for real sample applications in addition of being of a one-step procedure may also be of multi-tasks capability in terms of multidetection and easy adaption and ready to use on purpose. If more promising achievements can be successfully realized, the environmentally and human friendly biosensors can be developed for drug analysis, safety and security applications of enzyme inhibition phenomena. Hence, these biosensors can be used in green chemistry concept, with less solution consumption, with lab-on-a-chip devices. In conclusion, this deep analysis of inhibition-based biosensors that employ nanomaterials will serve researchers as a guideline for further improvements and approaching of these devices to real sample applications so as to reach society needs and market demands. More efficient devices including commercially available ones, using enzyme inhibition phenomena and nanomaterial-modified transducers will be expected in

near future so as to solve problems related to reaching the requested analytical performance for real sample applications.

Table 1. Selected recent electrochemical studies on nanomaterials-based enzyme biosensors operating through inhibition

Immobilization Matrix	Analyte	Detection Method	LOD/LOQ	Sample	Reference
AChE/GR/PANI	Carbaryl	CA	20 $ng.mL^{-1}$	NS	Li, et al., 2016
BChE/poly(TTBO)/AgNWs	Paraoxon	CA	0.212 μM	Milk	Turan et al., 2016
AChE/Pt/ZnO/Chitosan	Carbosulfan	CV	0.24 nM	Rice	Nesakumar et al., 2016
AChE/PVA-AWP/Fe–Ni NP	Phosmet	CA	0.1 nM	Olive oil	El-Moghazy et al., 2016
AChE/OMC–CS/Fe_3O_4–CS/SPCE	Methamidophos Chlorpyrifos	DPV	1 $\mu g.L^{-1}$ 0.05 $\mu g.L^{-1}$	Cabbage, Rape and Lettuce	Zhang at al., 2016
AChE/PEDOT-MWCNTs/FTO	Malathion	CA	1 fM	Lettuce	Kaur et al., 2016
AChE/OPH/MWCNT/$(PEI/DNA)_2$	Paraoxon	CV	0.5 μM	Apple	Zhang et al., 2015
AChE/GA/ILGR/Gel/GCE	Carbaryl Monocrotophos	DPV	5.3 fM 0.46 fM	Tomato juice	Zheng et al., 2015
Tyr/SPCE/MNPs/IrOxNPs	Methimazole	CA	0.006 μM	Pharmaceutical and Human Serum	Kurbanoglu et al., 2015

HRP/AuSNPs/SNGCE	Cyanide	CA	0.03 µM	NS	Attar et al., 2015
AChE/CS/PB-CS/ERGO-AuNPs-β-CD/GCE	Malathion Carbaryl	CA	4.14 $pg.mL^{-1}$ 1.15 $pg.mL^{-1}$	Vegetables	Zhao et al., 2015
AChE/[BSmim]HSO_4-AuNPs-porous carbon/BDD	Dichlorvos	DPV	0.30 pM	Lettuce	Wei and Wang., 2015
AChE/GR/CdSe@ZnS/ITO	Paraoxon Dichlorvos	PEC	0.61 fM 2.5 pM	Apple	Li et al., 2015
AChE/CChit/AgNC/RGO/GCE	Phoxim	DPV	81 pM	Water	Zhang et al., 2015
AChE/AuDMBG/RGO/GCE	Triazophos	CA	0.35 ppb	NS	Ju et al., 2015
Tyr/SPCE//GA/IrOx–BSA	Chlorpyrifos	CA	0.003 µM	Tap and river water	Mayorga-Martinez et al., 2014
AChE/CB/TC-0–AgNPs/GCE	Paraoxon Malaoxon Aldicarb Carbofuran	CA	0.05 nM 0.1 nM 0.01 µM 0.1 nM	Peanut and Grape juice	Evtugyn et al., 2014b
Lacc/AuNPs/AuE	Formetanate	SWV	0.095 µM	Mango and Grape	Ribeiro et al., 2014
AChE/Fe_3O_4–CH/GCE	Carbofuran	CV	3.6 nM	Cabbage	Jeyapragasam and Saraswathi, 2014
Lacc–TYR–AuNPs–CS/GPE	Carbaryl Formetanate Propoxur Ziram	SWV	0.02 µM 0.22 µM 0.19 µM 1.68 nM	Orange, Tangerine and Lemon	Oliveira et al., 2014
AChE/AuNPs-CSs/BDD	Methyl Parathion Chlorpyrifos	DPV	0.49 pM 0.13 pM	Cucumber juice	Wei et al., 2014

AChE-CLDH/GN-AuNPs/GCE	Chlorpyrifos	DPV	0.05 $g.L^{-1}$	Leek and Pakchoi	Zhai et al., 2014
AChE/MWCNT/CA/NPG	Malathion	CA	0.5 $ng.mL^{-1}$	NS	Ding et al., 2014
AChE/CuONWs/SWCNTs/ GCE	Malathion	DPV	0.1 ppb	Garlic	Huo et al., 2014
AChE-CS/NiO NPs-CGR-NF	Carbofuran	CA	0.5 pM	Apple and Cabbage	Yang et al., 2013
HRP/Au/GCE	4-Chlorophenol	CA	0.3 µM	Water	Qiu et al., 2013
AChE–ERGO–Nafion/GCE	Dichlorvos	CA	2.0 $ng.mL^{-1}$	River water	Wu et al., 2013
AChE–CS/SnO_2NPs–CGR–NF/GCE	Methyl Parathion Carbofuran	DPV	0.05 pM 0.5 pM	Apple and Cabbage	Zhou et al., 2013
Lacc/PB/GPE	Carbofuran Ziram	SWV	0.1 µM 5.2 nM	Tomato Potato	Oliveira et al., 2013
AChE/Chit-PB-MWNTs-HGNs/Au	Malathion Chlorpyrifos Monocrotophos Carbofuran	CA	0.05 nM 0.05 nM 0.1 nM 2.5 nM	Cabbage, Lettuce, Leek and Pakchoi	Zhai et al., 2013
AChE/CoPC/SPE	Chlorpyripho s-Oxon Ethyl Paraoxon Malaoxon	CA	5 pM 5 nM 0.5 nM	Milk	Mishra et al., 2012
AChE/Fe_3O_4NPs/c-MWCNTs/ITO	Malathion Chlorpyrifos Monocrotophos Endosulfan	CV	0.1 nM	cabbage, onion, spinach and soil samples	Chauhan and Pundir, 2012
AChE/GC/MWCNT/PANI	Carbaryl Methomyl	CA	1.4 µM 0.95 µM	Cabbage and Broccoli	Cesarino et al., 2012

AChE/ B-f-Fe@AuMNPs/ GR-SA/GCE	Furadan	CA	0.01 ppb	Tap and River water	Dong et al., 2012
AChE/SWCNTs/ Co phtalocyanine/SPCE	Paraoxon Malaoxon	CA	3 ppb 2 ppb	Sparkling and tape waters	Ivanov et al., 2011
AChE/TiO_2-G/GCE	Carbaryl	CA	0.3 $ng.mL^{-1}$	NS	Wang et al., 2011
AChE/CoPC-SPCE	Chlorfenvinphos	CA	10 μM	Wheat	Crew et al., 2011
AChE/ZnS/Pin5COOH/AuE	Malathion Chlorpyrifos	CA	0.1 nM 1.5 nM	Tap water	Chauhan et al., 2011
AChE/CPBA/AuNPs/RGO-CS/GCE	Chlorpyrifos Malathion Carbofuran Isoprocarb	CA	0.1 ppb 0.5 ppb 0.05 ppb 0.5 ppb	NS	Liu et al., 2011b
Tyr/GR/PtNPs/GCE	Chlorpyrifos Profenofos Malathion	CA	0.2 ppb 0.8 ppb 3 ppb	NS	Liu et al., 2011c
AChE/Fe_3O_4/c-MWCNT/Au	Malathion Chlorpyrifos	CA	0.1 nM 0.1 nM	Milk and Water	Chauhan et al., 2011
AChE/[BMIM][BF_4] MWCNT gel/CP	Chlorpyrifos	CA	4 nM	NS	Zamfir et al., 2011
AChE/Au–PDDA–PB/GCE	Monocrotophos	CA	0.8 $pg.mL^{-1}$	Garlic	Wu et al., 2011
AChE/PAMAM-Au/CNTs/GCE	Carbofuran	DPV	4.0 nM	Onion, lettuce and cabbage	Qu et al., 2010
AChE/ZnO/SPE	Paraoxon	CA	0.035 ppm	NS	Sinha et al., 2010
MPDE-CdTe/Cys/Aunano/MWCNT/GCE	Methyl Parathion	CA	1.0 $ng.mL^{-1}$	NS	Du et al., 2010a

AChE/MWCNTs-Au-CHIT/GCE	Malathion	CA	0.6 $ng.mL^{-1}$	NS	Du et al., 2010b
AChE/MWCNT-ß-CD/GCE	Dimethoate	CV	2 nM	Garlic	Du et al., 2010c
AChE/PANIPPy/MWCNTs /GCE	Malathion	CA	1.0 $ng.mL^{-1}$	NS	Du et al., 2010d
AChE/Mb/GA/(MWCNT-NH_2/BSA)/GA/Con A/BSA	Paraoxon	CA	1.39 pgL^{-1}	NS	Ivanov et al., 2010
AChE/CNC/GCE	Chloropyrifos	CV	15.8 nM	Water	Ion et al., 2010
AChE/Au/Chi	Methamidophos	CV	0.001 $\mu g.mL^{-1}$	NS	Li et al., 2010
AChE/NsPM/AuNPs	Paraoxon	CA	0.74 pgL^{-1}	NS	Marinov et al., 2010
AChE/Au-MWNTs/GCE	Paraoxon	CA	0.025 ppb	NS	Jha and Ramaprabhu, 2010

Abbrevations:

[BMIM][BF_4]: 1-butyl-3-methylimidazolium tetrafluoroborate
[BSmim]HSO_4-porous carbon: honeycomb-like hierarchically ion liquidsporous carbon composite
AChE: Acetylcholinesterase
AgNC: Silver nanocluster
AgNPs: Silver nanoparticles
AgNWs : Silver nanowires
ATCl: Acetylthiocholine
Au_6: Gold nanoparticles
AuE: Gold electrode
AuNPs: Gold nanoparticles
AuSNPs: Gold sonoparticles
BChE: Butyrylcholinesterase
BDD: Boron Doped Diamond
B-f-Fe@AuMNPs: boronic acid-functionalized Fe@Au magnetic nanoparticles
BSA: Bovine Serum Albumine
c- MWCNT: Carboxylated multi walled carbon nanotubes
CA:Chronoamperometry
CB: Carbon black
CChit:Carboxylic chitosan
CGR: Carboxylic graphene
CLDH: Alcined layered doublehydroxide
CNC: carbon nanostructure-chitosan composite

Con A: Concanavalin A
CoPC: Cobalt (II) phthalocyanine
CPBA: 3-carboxyphenylboronic
CS: Chitosan
CuO NWs: Copper oxide nanowires
Cys: Cysteamine
DMBG: Dimethylbiguanide
ERGO: Electrochemical reduced graphene oxide
Fe–Ni NP: Iron-Nickel Nanoparticle
FTO: fluorine doped tin oxide
GA : Glutaraldehyde
GCE: Glassy carbon electrode
Gel: Gelatin
GPE: Graphene doped carbon paste
GR: Graphene
HGNs: Hollow gold nanospheres
HRP: Horseradish peroxidase
ILGR: Ionic liquid functionalized graphene
IrOx: Iridium oxide Nanoparticles
ITO: Indium tin oxide
Lacc: Laccase
MWCNT: Multiwalled carbon nanotube
NC: Nanocomposite
NF: Nafion
NiONPs: Nickel oxide nanoparticles
NPG: nanoporous gold
NS: Not Stated
NsPM: Nanostructured polymer membrane
OMC: ordered mesoporous carbon
OPH: Organophosphate hydrolase
PAMAM: polyamidoamine
PANI: Polyaniline
PB: Prussian blue
PDDA: Poly (dimethyl diallyl ammonium chloride)
PEI: Polyethyleneimine
Pin5COOH: poly(indole-5-carboxylic acid)
poly(indole-5-carboxylic acid)
poly(TTBO): Poly(5,6-bis(octyloxy)-4,7-di(thieno[3][3,2-b]thiophen-2- yl) benzo[c] [1,2,5]oxoadiazole) /
PPy: Polypyrolle
PVA-AWP: Azide-unit waterpendant polyvinyl alcohol
SA: Sodium alginate
SNGCE: Sonogel-carbon electrode
SnO_2NPs: SnO_2nanoparticles
SPCE: Screen printed carbon electrode
TC-0–:11,17,23-Tetra-tert-butyl-25,26,27,28-tetrakis-[1-(2′-hydroxyethyl)-N-(3″,4″-dihydro xyphenyl) amidocarbonyl)-methoxy) -2,8,14,20-tetrathiacalix [4]arene in 1,3-alternate conformation
Tyr: Tyrosinase
β-CD:β-cyclodextrin

Acknowledgement

S. Kurbanoglu acknowledges the support given by Ankara University BAP 14L0237002 for her PhD thesis project. Nanobiosensors and Bioelectronics Group acknowledges the support from MINECO (project MAT2014-52485-P and Severo Ochoa Program, Grant SEV-2013-0295) and Secretaria d'Universitats i Recerca del Departament d'Economia i Coneixement de la Generalitat de Catalunya (2014 SGR 260).

References

Altavilla C., Ciliberto E., 2011. Inorganic Nanoparticles: Synthesis, Applications, and Perspectives, CRC Press, 33-68

Amine, A.; Arduini, F.; Moscone, D.; Palleschi, G. 2016. Biosens Bioelectron. 76, 180-194.

Amine, A.; El Harrad, L.; Arduini, F.; Moscone, D.; Palleschi, G. 2014. Talanta. 118, 368-374.

Amine, A.; Mohammadi, H.; Bourais, I.; Palleschi, G. 2006. Biosens Bioelectron. 21(8), 1405-1423.

Ansari, S. A.; Husain, Q. 2012. Biotechnol Advan. 30(3), 512-523.

Aragay G.; Pino F.; Merkoçi A. 2012. Chem Rev. 112, 5317–5338.

Arduini, F.; Amine, A. 2013. Biosensors based on enzyme inhibition. In Biosensors Based on Aptamers and Enzymes (pp. 299-326). Springer Berlin Heidelberg.

Arduini, F.; Amine, A.; Moscone, D.; Palleschi, G. 2009. Anal Lett. 42(9), 1258-1293.

Attar, A.; Cubillana-Aguilera, L.; Naranjo-Rodríguez, I., de Cisneros, J. L. H. H.; Palacios-Santander, J. M.; Amine, A. 2015. Bioelectrochemistry. 101, 84-91.

Audrey S. A.; Prieto-Simón B.; Marty J.L., 2012. Am J Anal Chem, 3, 210–232.

Balasubramanian, K.; Burghard, M. 2006. Anal Bioanal Chem, 385(3), 452-468.

Barberis, A.; Spissu, Y.; Fadda, A.; Azara, E.; Bazzu, G.; Marceddu, S.; Angioni A.; Sanna D.; Schirra M.; Serra, P. A. 2015. Biosens Bioelectron. 67, 214-223.

Benítez-Martínez, S.; Caballero-Díaz, E.; Valcárcel, M. 2016. Analyst, 141(9), 2688-2695.

Besteman, K.; Lee, J. O.; Wiertz, F. G.; Heering, H. A.; Dekker, C. 2003. Nano Lett. 3(6), 727-730.

Brownson, D. A.; Banks, C. E. 2010. Analyst. 135(11), 2768-2778.

Brownson, D. A.; Banks, C. E. 2011. Analyst. 136(10), 2084-2089.

Castaneda, M. T.; Merkoci, A.; Pumera, M.; Alegret, S. 2007. Biosens Bioelectron. 22(9), 1961-1967.

Cesarino, I.; Moraes, F. C.; Lanza, M. R.; Machado, S. A. 2012. Food Chem. 135(3), 873-879.

Cevik, E.; Senel, M.; Baykal, A.; Abasıyanık, M. F. 2012. Sensor Actuat B: Chem. 173, 396-405.

Chang, T.S. 2009. Int J Mol Sci, 10, 2440–2475.

Chauhan, N.; Narang, J.; Pundir, C. S. 2011. Biosens Bioelectron. 29(1), 82-88.

Chauhan, N.; Pundir, C. S. 2012. Electrochim Acta. 67, 79-86.

Chauhan, N.; Pundir, C.S. 2011. Anal Chim Acta. 701, 66–74.

Chen, D.; Tang, L.; Li, J. 2010. Chem Soci Revi. 39(8), 3157-3180.

Chen, S.; Yuan, R.; Chai, Y.; Hu, F. 2013. Microchim Acta. 180(1-2), 15-32.

Chu, X.; Duan, D.; Shen, G.; Yu, R. 2007. Talanta. 71(5), 2040-2047.

Colovic, M.B.; Krstic, D. Z.; Lazarevic-Pasti, T. D.; Bondzic, A. M.; Vasic, V. M. (2013). Curr Neuropharma. 11 (3), 315–335.

Cornish-Bowden, A.; 1976. Principles of Enzyme Kinetics, Butterworth, London, 52–70.

Cornish-Bowden, A.; Wharton. C. W. 1988. Enzyme Kinetics. Oxford: IRL Press,

Cozzi, F.; Powell, W. H.; Thilgen, C. 2005. Pure Appl Chem. 77, 843-923.

Crew, A.; Lonsdale, D.; Byrd, N.; Pittson, R.; Hart, J. P. 2011. Biosens Bioelectron. 26(6), 2847-2851.

Datta, S.; Christena, L. R.; Rajaram, Y. R. S. 2013. Biotech. 3(1), 1-9.

De Castro, M. L.; Herrera, M. C. 2003. Biosens Bioelectron. 18(2), 279-294.

Ding, J., Zhang, H., Jia, F., Qin, W., Du, D. 2014. Sensor Actuat B-Chem. 199, 284-290.

Dixon, A. M. 1953. Biochem J. 55(1), 170.

Dong, J.; Liu, T.; Meng, X.; Zhu, J.; Shang, K.; Ai, S.; Cui, S. 2012. J Solid State Electrochem. 16(12), 3783-3790.

Dresselhaus, M. S.; Dresselhaus, G.; Eklund, P. C. 1996. Science of Fullerenes and Carbon Nanotubes, Academic, New York.

Du, D.; Chen, W.; Zhang, W.; Liu, D.; Li, H.; Lin, Y. 2010a. Biosens Bioelectron. 25(6), 1370-1375.

Du, D.; Wang, M.; Cai, J.; Qin, Y.; Zhang, A. 2010b. Sensor Actuat B: Chem. 143(2), 524-529.

Du, D.; Wang, M.; Cai, J.; Zhang, A. 2010c. Sensor Actuat B: Chem. 146(1), 337-341.

Du, D.; Ye, X.; Cai, J.; Liu, J.; Zhang, A. 2010d. Biosens Bioelectron. 25(11), 2503-2508.

Dubach, J.M., Clark, H.A. 2013. Nanosensors. In: The Textbook of Nanoneuroscience and Nanoneurosurgery. Ed.: Kateb, B. Heiss, J. D. Boca Raton, FL: CRC Press. 203–216.

Edmonds T.E., 2013. Chemical sensors. Springer Science & Business Media.

El-Metwally, T.H.; El-Senosi, Y. 2010. Enzyme Inhibition. Medical Enzymology: Simplified Approach. Chapter 6, Nova Publisher, N.Y. pp 57- 77.

El-Moghazy, A. Y.; Soliman, E. A.; Ibrahim, H. Z.; Noguer, T.; Marty, J. L.; Istamboulie, G. 2016. Food Chem. 203, 73-78.

Evtugyn, G. A.; Budnikov H. C.; Nikolskaya, E. B. 1998a. Talanta. 46, 465–484.

Evtugyn, G. A.; Shamagsumova, R. V.; Padnya, P. V.; Stoikov, I. I.; Antipin, I. S. 2014b. Talanta. 127, 9-17.

Fernández-García, M.; Martínez-Arias, A.; Hanson, J.C.; Rodríguez, J.A., 2004. Chem. Rev. 104, 4063-4104.

Gao, H.; Duan, H. 2015. Biosens Bioelectron. 65, 404-419.

Gill, R.; Zayats, M.; Willner, I. 2008. Angew Chem Int Edit. 47(40), 7602-7625.

Grieshaber, D.; MacKenzie, R.; Voeroes, J.; Reimhult, E. 2008. Sensors. 8(3), 1400-1458.

Gronow, M. 1991. Biotechnol Sci Business. 20: 355-370.

Han, E.; Yang, Y.; He, Z.; Cai, J.; Zhang, X.; Dong, X. 2015. Anal biochem, 486, 102-106.

Haruta, M., Daté, M. 2001. Appl Catal A- Gen. 222(1), 427-437.

Hedberg, K.; Hedberg, L.; Bethune, D. S.; Brown, C. A.; Dorn, H. C.; Johnson, R. D.; De Vries, M. 1991. Science. 254, 410-412.

Hu, T.; Zhang, L.; Wen, W.; Zhang, X.; Wang, S. 2016. Biosens Bioelectron, 77, 451-456.

Hummers W. S., Offeman R. E. 1958. Preparation of graphitic oxide. J Am Chem Soci. 80, 1339-43.

Huo, D.; Li, Q.; Zhang, Y.; Hou, C.; Lei, Y. 2014. Sensor Actuat B-Chem. 199, 410-417.

Iijima, S. 2002. Physica B. 323, 1-5.

Ion, A. C.; Ion, I.; Culetu, A.; Gherase, D.; Moldovan, C. A.; Iosub, R., Dinescu, A. 2010. Mater Sci Eng. C. 30(6), 817-821.

Ivanov, A. N.; Younusov, R. R.; Evtugyn, G. A.; Arduini, F.; Moscone, D.; Palleschi, G. 2011. Talanta. 85(1), 216-221.

Ivanov, Y.; Marinov, I.; Gabrovska, K.; Dimcheva, N.; Godjevargova, T. 2010. J Mol Catal B: Enzy. 63(3), 141-148.

Jeyapragasam, T., Saraswathi, R. 2014. Sensor Actuat B-Chem. 191, 681-687.

Jha, N.; Ramaprabhu, S. 2010. Nanoscale. 2(5), 806-810.

Ju, K. J.; Feng, J. X.; Feng, J. J.; Zhang, Q. L.; Xu, T. Q.; Wei, J.; Wang, A. J. 2015. Microchim Acta. 182(15-16), 2427-2434.

Kamat, P. V. (2009). J Physical Chem Lett. 1(2), 520-527.

Kaur, N., Thakur, H., Prabhakar, N. 2016. J Electroanal Chem. 775, 121-128.

Kennedy, J. F.; White, C. A. 1985. Handb Enzyme Biotech. 2, 147-207.

Kerman, K.; Saito, M.; Tamiya, E.; Yamamura, S.; Takamura, Y. (2008). TrAC Trends in Anal Chem. 27(7), 585-592.

Kittelson, D. B. (1998). J Aerosol Sci. 29(5), 575-588.

Kosynkin D. V.; Berlin J. M.; Sinitskii A.; Sun Z.; Marcano D. C.; Slesarev A.; Alemany L. B.; Lu W.; Tour J. M. 2010, ACS Nano. 4, 4806–14.

Kuila, T.; Bose, S.; Khanra, P.; Mishra, A. K.; Kim, N. H.; Lee, J. H. 2011. Biosens Bioelectron. 26(12), 4637-4648.

Kurbanoglu, S.; Mayorga-Martinez, C. C.; Medina-Sánchez, M.; Rivas, L.; Ozkan, S. A.; Merkoçi, A. 2015. Biosens Bioelectron. 67, 670-676.

Li, X.; Zheng, Z.; Liu, X.; Zhao, S.; Liu, S. 2015. Biosens Bioelectron. 64, 1-5.

Li, Y.; Gan, Z.; Li, Y.; Liu, Q.; Bao, J.; Dai, Z.; Han, M. 2010. Sci China Chem. 53(4), 820-825.

Li, Y.; Zhang, Y.; Han, G.; Xiao, Y.; Li, M.; Zhou, W. 2016. Chinese J Chem. 34, 82-88

Lin, Y.; Yantasee, W.; Wang, J. 2005. Front. Biosci. 10(492-505), 582.

Lineweaver, H.; Burk, D. 1934. J Am Chem Soci. 56(3), 658-666.

Liu, Q., Shi, J., Zeng, L., Wang, T.; Cai, Y.; Jiang, G. 2011a. J Chromatogr A. 1218, 197-204.

Liu, T.; Su, H.; Qu, X.; Ju, P.; Cui, L.; Ai, S. 2011b. Sensor Actuat B-Chem. 160(1), 1255-1261.

Liu, T.; Xu, M.; Yin, H.; Ai, S.; Qu, X.; Zong, S. 2011c. Microchim Acta. 175(1-2), 129-135.

Liu, X.; Zhu, J.; Huo, X.; Yan, R.; Wong, D. K. 2016. Anal Chim Acta. 911, 59-68.

Lu C.; Li A. P. 2010. Enzyme Inhibition in drug discovery and development, Wiley, New Jersey.

Luo, X.; Morrin, A.; Killard, A. J.; Smyth, M. R. 2006. Electroanal. 18(4), 319-326.

Malhotra, B.D.; R. Singhal, A.; Chaubey, S.K.; Sharma, A.; Kumar. 2005. Curr Appl Phys. 5: 92-97.

Marin, S.; Merkoci, A., 2012. Electroanal. 24, No. 3, 459 – 469

Marinov, I.; Ivanov, Y.; Gabrovska, K.; Godjevargova, T. 2010. J Mol Catal B: Enzym. 62(1), 66-74.

Mayorga-Martinez, C. C.; Pino, F.; Kurbanoglu, S.; Rivas, L.; Ozkan, S. A.; Merkoçi, A. 2014. J Mater Chem B. 2(16), 2233-2239.

Mello, L. D.; Kubota, L. T. 2002. Food Chem. 77(2), 237-256.

Mena, M. L.; Yanez-Sedeno, P.; Pingarron, J. M. 2005. Anal Biochem. 336(1), 20-27.

Menten, L.; Michaelis, M.I. 1913, Biochem Z 49, 333–369

Merkoçi, A. 2006. Microchim Acta. 152(3-4), 157-174.

Merkoçi, A.; Pumera, M.; Llopis, X.; Pérez, B.; del Valle, M.; Alegret, S. 2005. TrAC Tr Anal Chem. 24(9), 826-838.

Merkoçi A. 2013. Electroanal, 25: 15–27.

Metters J.P.; Banks C.E. 2014. Nanoparticle Modified Electrodes for trace Metal Ion Analysis. In: Nanosensors for Chemical and Biological Applications: Sensing with Nanotubes, Nanowires and Nanoparticles, Ed.: Honeychurch, K. C., Waltham, MA: Woodhead Publishing. 54–79.

Minh, C. T. 1985. Ion Sel Electrode R. 7, 41-75.

Mishra, R. K.; Dominguez, R. B.; Bhand, S.; Muñoz, R.; Marty, J. L. 2012. Biosens Bioelectron. 32(1), 56-61.

Mohanraj, V. J.; Chen, Y. 2007. J Pharm Research, 5(1), 561-573.

Mukhopadhyay, K.; Phadtare, S.; Vinod, V. P.; Kumar, A.; Rao, M.; Chaudhari, R. V.; Sastry, M. 2003. Langmuir. 19(9), 3858-3863.

Nesakumar, N.; Sethuraman, S.; Krishnan, U. M.; Rayappan, J. B. B. 2016. Biosens Bioelectron. 77, 1070-1077.

Niemeyer, C. M. 2001. Angew Chem Int Edit. 40(22), 4128-4158.

Novoselov, K. S.; Fal, V. I.; Colombo, L.; Gellert, P. R.; Schwab, M. G.; Kim, K. 2012. Nature. 490, 192-200.

Novoselov, K. S.; Geim, A. K.; Morozov, S. V.; Jiang, D.; Zhang, Y.; Dubonos, S. A.; Firsov, A. A. 2004. Science. 306, 666-669.

Oliveira, T. M.; Barroso, M. F.; Morais, S., Araújo, M.; Freire, C., de Lima-Neto, P.; Adriana N.; Correiab, Maria B.P.P.; Oliveirac Delerue-Matos, C.; 2014. Bioelectrochemistry. 98, 20-29.

Oliveira, T. M.; Barroso, M. F.; Morais, S.; Araújo, M.; Freire, C.; de Lima-Neto, P.; Delerue-Matos, C. 2013. Biosens Bioelectron. 47, 292-299.

Parolo, C.; de la Escosura-Muñiz, A.; Merkoçi, A. 2013. Biosens Bioelectron. 40(1), 412-416.

Patel, P.D. 2002. Tr Anal Chem. 21: 96-115.

Pérez-López, B., Merkoçi, A. 2012. Microchim Acta. 179(1-2), 1-16.

Pingarrón, J. M.; Yañez-Sedeño, P.; González-Cortés, A. 2008. Electrochim Acta. 53(19), 5848-5866.

Pohanka, M.; Skládal, P. 2008. J Appl Biomed. 6(2), 57-64.

Ray, S.C. 2015. Application of Graphene and Graphene-Oxide Based Nanomaterials. Waltham, MA: Elsevier.

Qiu, C.; Chen, T.; Wang, X.; Li, Y.; Ma, H. 2013. Colloid Surface B. 103, 129-135.

Qu, Y.; Sun, Q.; Xiao, F.; Shi, G.; Jin, L. 2010. Bioelectrochemistry. 77(2), 139-144.

Rao, C. N. R.; Seshadri, R.; Govindaraj, A.; Sen, R. 1995. Mater. Sci. Eng. R: Reports. 15, 209-262.

Reina, A.; Xiaoting, J.; Ho, J.; Nezich, D.; Son, H.; Bulovic, V.; Dresselhaus, M. S.; Kong, J. 2009 Nano Lett. 9, 30–5.

Resch-Genger, U.; Grabolle, M.; Cavaliere-Jaricot, S.; Nitschke, R.; Nann, T.; 2008. Nat. Methods. 5, 763-775.

Ribeiro, F. W. P.; Barroso, M. F.; Morais, S.; Viswanathan, S.; de Lima-Neto, P.; Correia, A. N., Delerue-Matos, C. 2014. Bioelectrochemistry. 95, 7-14.

Rivas, L.; de la Escosura-Muñiz, A.; Pons, J.; Merkoçi, A. 2014. Electroanal. 26(6), 1287-1294.

Rivas, L.; Mayorga-Martinez, C. C.; Quesada-González, D.; Zamora-Gálvez, A.; de la Escosura-Muñiz, A.; Merkoçi, A. 2015. Anal Chem. 87(10), 5167-5172.

Rodriguez, J.A.; Fernández-García, M., (Eds.) 2007. Synthesis, Properties and Applications of Oxide Nanoparticles. Whiley, New Jersey.

Saxena, U.; Das, A. B. 2016. Biosens Bioelectron. 75, 196-205.

Scheller, F.; Schubert, F.; Pfeiffer, D.; Hintsche, R.; Dransfeld, I.; Renneberg, R.; Wollenberger, U.; Riedel, K.; Pavlova, M.; Kühn, M.; Müller, H. G.; Tan, P. M.; Hoffmann W., Moritz, W. 1989. Analyst. 114(6), 653-662.

Scott, A.O. 1998. Biosensors for food analysis. Elsevier.

Shan, C.; Yang, H.; Song, J.; Han, D.; Ivaska, A.; Niu, L. 2009. Anal Chem. 81(6), 2378-2382.

Shulga, O.; Kirchhoff, J. R. 2007. Electrochem Commun. 9(5), 935-940.

Sinha, R.; Ganesana, M.; Andreescu, S.; Stanciu, L. 2010. Anal. Chim Acta. 661(2), 195-199.

Solé, S.; Merkoçi, A.; Alegret, S. 2003. Cr Rev Anal Chem. 33(2), 89-126.

Su, C. Y.; Lu, A. Y.; Xu, Y.; Chen, F. R.; Khlobystov, A. N.; Li, L. J. 2011. Acs Nano. 5, 2332-2339.

Suarez-Martinez, I., Grobert, N., Ewels, C. P. 2012. Encyclopedia of Carbon Nanoforms. Advances in Carbon Nanomaterials: Science and Applications. Pan Stanford Publishing: Singapore.

Sutter P. W.; Flege J. I.; Sutter E. A. 2008. Nat Mater. 7, 406–11.

Tamayo, J.; Kosaka, P. M.; Ruz, J. J; San Paulo, A; Calleja, M. 2013. Chem Soc Rev, 42, 1287–1311.

Thevenot, D. R.; Toth, K.; Durst R. A.; Wilson G. S. 2001. Anal Lett. 34(5): 635-659.

Thévenot, D. R.; Toth, K.; Durst, R. A., Wilson, G. S. 2001. Biosens Bioelectron. 16(1), 121-131.

Tischer, W.; Kasche, V. 1999. Tr Biotechn. 17(8), 326-335.

Tiwari, P. M.; Bawage S. S.; Singh S. R.; 2015. Gold Nanoparticles and their Applications in Photomedicine Diagnosis and Therapy. In: Applications of Nanoscience in Photomedicine, Ed.: Hamblin, M. R., Avci, P., Cambridge: Woodhead Publishing. 249–266.

Tran-Minh, C.; Pandey, P.C.; Kumaran, S. 1990. Biosens. Bioelectron. 5, 461–471.

Trojanowicz, M. 2002. Electroanal. 14(19-20), 1311-1328.

Tung, V. C.; Allen, M. J.; Yang, Y.; Kaner, R. B. 2009. Nat. Nanotechnol. 4, 25-29.

Turan, J.; Kesik, M.; Soylemez, S.; Goker, S.; Coskun, S.; Unalan, H. E.; Toppare, L. 2016. Sensor Actuat B-Chem. 228, 278-286.

Turner, A.P. 2013. Chem Soc Rev, 42, 3184-3196.

Valentini, F.; Orlanducci, S.; Terranova, M. L.; Amine, A.; Palleschi, G. 2004. Sensor Actuat B-Chem. 100(1), 117-125.

Van Dyk, J. S.; Pletschke, B. 2011. Chemosphere. 82(3), 291-307.

Vávrová, J.; Řezáčová, M.; Pejchal, J. 2012. J. Appl. Biomed. 10, 1-8.

Vicentini, F. C.; Garcia, L. L.; Figueiredo-Filho, L. C.; Janegitz, B. C.; Fatibello-Filho, O. 2016. Enzyme Microb Tech. 84, 17-23.

Wang, J. 2005. Electroanal. 17(1), 7-14.

Wang, J. 2006. Biosens Bioelectron. 21(10), 1887-1892.

Wang, K.; Li, H. N.; Wu, J.; Ju, C.; Yan, J. J.; Liu, Q.; Qiu, B. 2011. Analyst. 136(16), 3349-3354.

Wei, M., Wang, J. 2015. Sensor Actuat B-Chem. 211, 290-296.

Wei, M.; Zeng, G.; Lu, Q. 2014. Microchim Acta. 181(1-2), 121-127.

Wilson, G. S.; Hu, Y. 2000. Chem Rev. 100(7), 2693-2704.

Wu, S.; Huang, F.; Lan, X.; Wang, X.; Wang, J.; Meng, C. 2013. Sensor Actuat B-Chem. 177, 724-729.

Wu, S.; Lan, X.; Zhao, W.; Li, Y.; Zhang, L.; Wang, H.; Tao, S. 2011. Biosens Bioelectron. 27(1), 82-87.

Xue, G.; Yue, Z.; Bing, Z.; Yiwei, T.; Xiuying, L.; Jianrong, L. 2016. Analyst, 141(3), 1105-1111.

Yang, L.; Wang, G.; Liu, Y.; Wang, M. 2013. Talanta. 113, 135-141.

Yang, M.; Yang, Y.; Liu, Y.; Shen, G.; Yu, R. 2006. Biosens Bioelectron. 21(7), 1125-1131.

Zamfir, L. G.; Rotariu, L.; Bala, C. 2011. Biosens Bioelectron. 26(8), 3692-3695.

Zhai, C.; Guo, Y.; Sun, X.; Zheng, Y.; Wang, X. 2014. Enzyme Microb Tech. 58, 8-13.

Zhai, C.; Sun, X.; Zhao, W.; Gong, Z.; Wang, X. 2013. Biosens Bioelectron. 42, 124-130.

Zhang, Q.; Xu, Q.; Guo, Y.; Sun, X.; Wang, X. 2016. RSC Advances. 6(29), 24698-24703.

Zhang, Y.; Arugula, M. A.; Wales, M.; Wild, J.; Simonian, A. L. 2015. Biosens Bioelectron. 67, 287-295.

Zhao, H.; Ji, X.; Wang, B.; Wang, N.; Li, X.; Ni, R.; Ren, J. 2015. Biosens Bioelectron. 65, 23-30.

Zhao, J.; Chen, G.; Zhu, L.; Li, G. 2011. Electrochem Commun. 13(1), 31-33.

Zheng, Y.; Liu, Z.; Jing, Y.; Li, J.; Zhan, H. 2015. Sensor Actuat B-Chem. 210, 389-397.

Zhou, Q.; Yang, L.; Wang, G.; Yang, Y. 2013. Biosens Bioelectron. 49, 25-31.

Highlights

- **The role of nanomaterials in inhibition-based electrochemical biosensors**
- **Inhibition phenomena and enzyme kinetics are discussed**
- **Control food, environment from pharmaceuticals, pesticides by inhibition**
- **Application of nanomaterials to electrochemical enzymatic biosensors**