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# New Materials for the Construction of Electrochemical Biosensors

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Additional information is available at the end of the chapter

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## Abstract

The development of electrochemical sensors has attracted great interest due to these sensors' high sensitivity and selectivity. Here, we present the general concept and the classification of biosensors, their advantages and drawbacks, the main strategies in electrochemical biosensor technology and the materials used in electrochemical sensors, such as electrodes and supporting substrates, materials for improved sensitivity and selectivity, materials for bioreceptor immobilization, and biological recognition elements. Various nanomaterials, such as carbon-based materials (carbon nanotubes, graphene, carbon nanoparticles), inorganic and organic nanoparticles (magnetic and metal nanoparticles, nanosized clays), conductive and insulating polymers (nanosized and nanostructured polymers, molecularly imprinted polymers), and hybrid materials, etc., have been successfully applied for the enhancement of the electroanalytical performance of biosensors and for the immobilization of biorecognition elements. Among these, due to their unique physiochemical features, carbon-based materials, such as carbon nanotubes and graphenes, have received special attention in recent years, and examples of surface functionalization using various types of nanoparticles are presented. The future trends in sensor research activities and areas of development that are expected to have an impact in biosensor performance, like immobilization techniques, nanotechnology, miniaturization and multisensor array determinations, are also examined.

**Keywords:** Carbon-based nanomaterials, metal nanoparticles, magnetic nanoparticles, nanostructure, molecularly imprinted polymers

## 1. Introduction

The development of electrochemical sensors has attracted a great deal of interest due to their high sensitivity and selectivity, and they are being increasingly used in many fields, such as analytical chemistry, industrial process monitoring and control, clinical diagnostics, environmental monitoring and security, and food safety. By adding an enzyme at the electrode surface, they can also gain high specificity: thus the biosensor research area was born.

Biosensors (analytical devices coupling a transducer with a biorecognition element) are attractive for pharmaceutical and biomedical analysis due to their sensitivity (ng/mL or less) and high selectivity, and sometimes their specificity, high benefit/cost ratio, simple use and rapidity of data collection [1].

The major advantages of biosensors over traditional analytical methods, which will certainly lead in the near future to their even more pronounced use in the biomedical field, are: the fact that analyte detection can very often be made without prior separation; the short response times that make possible the real-time monitoring of biological and manufacturing processes; their ease of use, allowing in-field or point-of-care measurements; the flexibility and simplicity of preparation; the possibility of mass production and low production costs; and the possibility of miniaturization and automatization. Miniaturization is of great importance because biological samples are available in small amounts, and tissue damage must be minimized in cases of in-vivo monitoring. Therefore, the use of biosensors as components of modern medical devices has improved their portability, functionality and reliability for point-of-care analysis and real-time diagnosis [2].

However, many amperometric biosensors described in the recent literature still display a few drawbacks compared to other analytical methods. The most difficult problems to overcome for biosensors with biomedical applications are: the reduced stability, the electrochemical interferences, and the lack of or low response reproducibility. Removed from their natural environment, most biocomponents tend to rapidly lose their activity and thus limit the lifetime of the sensor. Modifying electrode surfaces in a way that exclusively favours one single electrochemical process is a difficult task and sample matrices in the biomedical field are very complex. Last but not least, in the case of in-vivo measurements biocompatibility and biofouling are critical issues [3].

The selectivity of the biosensor for the target analyte is mainly determined by the biorecognition element, whilst the sensitivity of the biosensor is greatly influenced by the transducer.

New sensing elements like genetically transformed cells and genetically engineered receptor molecules can improve affinity and specificity; therefore, genetic engineering and mass production of the molecular recognition may ultimately dictate the success of biosensor technologies. Enzyme variants that are specific for individual analytes have already been obtained by genetic modification, and novel gene fusions will lead to more sensitive biosensors [4].

As for the electrochemical transducer, important advances have been recently made thanks to the introduction of new platforms for biosensor design, such as nanotechnological

materials and nanostructured architectures (i.e., nanoparticles, carbon nanotubes and nanofibres, graphenes, nanostructured surfaces, etc.), which have improved the sensitivity of the assembly [5].

## 2. Strategies of electrochemical biosensor technology

The biosensor development and construction strategy includes five features: 1) the detected or measured parameter and the matrix, 2) the working principle of the transducer, 3) the chemical/biochemical model, 4) the field of application and 5) the technology and materials for sensor fabrication [6].

Materials generally used for electrochemical sensors are classified as: (1) materials for the electrode and supporting substrate, (2) materials for improving electroanalytical performances, (3) materials for the immobilization of biological recognition elements and (4) biological elements; the last two are applicable for electrochemical biosensors [5].

Materials used for the electrode and supporting substrate are usually conductive materials exhibiting low currents in an electrolyte solution, free of any electroactive species, over a relatively wide potential window. Among the most frequently used materials for the electrode and supporting substrate, the following may be mentioned: metals (mercury, platinum, gold, silver and stainless steel), metal oxides (indium tin oxide, ITO) carbon-based materials (glassy carbon, graphite, carbon black and carbon fibre), new hybrid materials, and organic electroconductive polymers or salts. Boron-doped diamond is a special case of material, because bare diamond is a non-conducting, allotropic form of carbon. In the last decade, in order to avoid mercury-based electrodes (which are highly toxic), different metallic films (Au, Hg, Bi, Ag, Pb, Sn, Sb, Co, Ga, Se) that are relatively simple to achieve and easy to reproduce were developed. Some strategies to develop two- and three-dimensional nanostructured electrodes with larger surfaces (mesoporous silicates, metal oxides, polymers or carbons) were also recently reported [7]. The major challenge in the field of electrochemical sensors, consisting in the improvement of their electroanalytical performances, mainly in terms of sensitivity and selectivity, was addressed by the development of nanotechnology (especially nanoparticles, carbon nanotubes and graphenes) and nanostructured architectures, and this will be detailed in section 3.

The stable immobilization of a bioelement on an electrode surface, with complete retention of its biological activity and good diffusion properties for substrates, is a crucial problem for the commercial development of biosensors. A successful matrix should stably immobilize or integrate biomolecules at the transducer's surface, it should efficiently maintain their functionality, while providing accessibility for the target analyte, and it should also ensure an intimate contact with the transducer surface [8]. The immobilization matrix may function purely as a support or may also be involved in the mechanism of signal transduction mediation. The selection of an appropriate immobilization method depends on the nature of the biological element, the type of transducer used, the physicochemical properties of the analyte and the biosensor's operating conditions [9]. The choice of materials for the immobilization of biological recognition elements strongly depends on

the employed procedure. The procedures that may be employed are as follows: 1) Entrapment behind a permeable membrane as a thin film covering the electrochemical detector (dialysis membrane) [10]; 2) Entrapment within a polymeric matrix (polyacrylonitrile, agar gel, polyurethane or poly(vinyl-alcohol) membranes, sol gels or redox hydrogels with redox centres such as  $[\text{Os}(\text{bpy})_2\text{Cl}]^+$ , conducting polymers [11]; 3) Entrapment within self-assembled monolayers (SAMs) [12] or bilayer lipid membranes (BLMs) [13]; 4) Covalent bonding on membranes or surfaces activated by means of bifunctional groups or spacers (glutaraldehyde, carbodiimide, SAMs or multilayers, avidin-biotin, silanization) [14]; 5) Bulk modification of the electrode material (graphite epoxy resin or carbon paste modified with polyoxometallates, clays and double layered hydroxides, zeolites, functionalized silica, sol-gel-derived inorganic and hybrid materials, nanomaterials, sparingly soluble or insoluble inorganic salts, molecular and macrocyclic ligands, complex compounds, organic polymers, surfactants and lipids) [7].

The **biorecognition elements** used for biosensor development are classified into biological and artificial (biomimetic) receptors. They are selected depending on the application and the desired performance criteria, based on the following interaction principles: enzyme/substrate, antigen/antibody, mRNA/DNA, PNA/DNA or RNA, microorganism/substrate or toxic molecules, cell receptor/hormone and (semi)synthetic receptor/target molecule [15]. Initially, three types of biorecognition elements were identified [16]: 1) Enzyme (mono or multienzyme), the most common and best developed recognition system, including antibodies, natural receptors and nucleic acids; 2) Whole cells (microorganisms, such as bacteria, fungi, eukaryotic cells or yeast), or cell organelles or particles (mitochondria, cell walls); 3) Tissue (plant or animal tissue slice). More recently, the range of biochemical receptors was extended with biomimetic recognition elements (aptamers, ribosymes and molecularly imprinted polymers) [17]. The biological recognition elements are based either on catalytic or bioaffinity features, whilst biomimetic ones are based on biocomplexing and bioaffinity.

The **biocatalytic recognition sensors** are based on a reaction catalysed by macromolecules, either in their original biological environment, in their isolated form, or as a product of semisynthesis. Thus, a continuous consumption of substrate(s) is achieved by the immobilized biocatalyst incorporated into the sensor, and transient or steady-state responses are monitored by the integrated detector. In the construction of biosensors, **enzymes** are the most commonly used biological elements, despite their rather high extraction, isolation and purification costs, as they rapidly and cleanly form selective bonds with the substrate. Among the enzymes commercially available the most commonly used are the oxidases, such as glucose oxidase (GOx) and horseradish peroxidase (HRP), beta-lactamase, urease, tyrosinase, and acetylcholine esterase or choline oxidase.

The **biocomplexing or bioaffinity sensors** are based on **antibodies, natural biological chemoreceptors, nucleic acids, aptamers or molecularly imprinted polymers (MIPs)**. These biosensors provide selective interactions of the analyte with a given ligand to form a thermodynamically stable complex. The analyte interacts with macromolecules or organized molecular assemblies that have either been isolated from their original biological environment or

engineered. Thus, equilibrium is usually reached and there is no further consumption of the analyte by the immobilized biocomplexing agent. These equilibrium responses are either monitored by the integrated detector or by using a complementary biocatalytic reaction. The most developed examples of biosensors using biocomplexing receptors are based on immunochemical reactions, i.e., the binding of an antigen (Ag) to a specific antibody (Ab), and will be discussed in detail in the chapter concerning immunosensors.

The *biological chemoreceptors* [18] are used for drug and neurotransmitter detection, and they are based on an affinity reaction. Using *nucleic acids* as a biocomponent, one can detect the interaction between the synthetic drug and the genetic material and also the hybridization processes by working with single-strand DNA [19, 20].

The use of *microorganisms and of whole vegetal or animal tissues* is complex and very often their selectivity is limited [21].

### 3. Nanomaterials involved in biosensor construction

Advances in nanotechnology have led to the recent development of many nanomaterials, including carbon nanomaterials, magnetic and metallic nanoparticles [22]. Nanomaterials, generally defined as materials with feature sizes smaller than 100 nm, have a remarkable impact on various fields of application due to their properties (enhanced electrical conductivity, tensile strength and chemical reactivity), which are imparted by their increased surface area per unit weight. Nanomaterials have already been applied in electronics, foods, cosmetics, electronics, drug development and sensing devices. The use of nanomaterials, especially nanoparticles and nanostructured films, offers advantageous properties that can be exploited in order to maximize the interactions with specific bioelements, to maintain their activity, minimize structural changes, and enhance the catalytic step. In the biosensor field, the analytical exploitation of such protein-nanomaterial interactions are an emerging trend that spans many disciplines [23]. Nanomaterials based on metals, semiconductors and organic compounds contribute to the enhancement of optical, electrical, chemical and magnetic properties that are relevant in the case of sensing devices, and due to these facts they have been frequently used in this research field [24, 25].

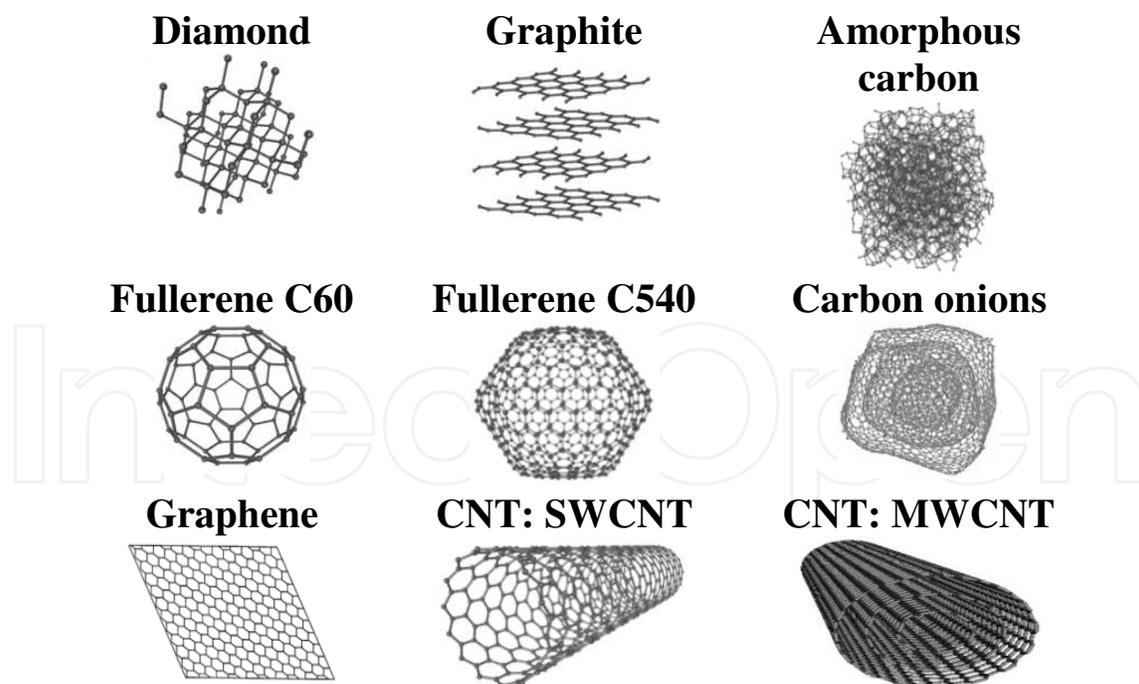
Nanomaterials are mainly used *for electrode construction or modification and as biomolecule tracers*. Nanoparticles (NPs) are very stable (compared to enzyme labels), offering high sensitivity (thousands of atoms can be released from one nanoparticle) and a wide variety are available on the market. Nanoparticles are used nowadays as electrochemical labels or as vehicles containing several hundreds or thousands of electroactive labels, pushing detection limits down to several hundreds of biomolecules [26].

Various nanomaterials have been successfully applied for the immobilization of bioelements, such as metallic nanoparticles, carbon or metallic nanotubes, nanosilver-coated magnetic beads, magnetic particles, functionalized conductive polymers, etc.

### 3.1. Carbon-based materials

Different types of carbon nanostructures are becoming more and more popular due to their specific structures, properties and the possibility of being used for many applications. In fact, a wide variety of carbon-based materials are available, such as nanoparticles, nanodiamonds, nano-onions, peapods, nanofibers, nanorings, fullerenes and nanotubes, which have been extensively used in analytical applications. The basic structure of fullerenes and nanotubes consists of a layer of  $sp^2$ -bonded carbon atoms. This configuration, which resembles that of graphene, is responsible for their good electrical conductivity and their ability to form charge-transfer complexes when in contact with electron donor groups [27]. This configuration is responsible for the development of strong van der Waals forces that significantly hamper the solubility and dispersion of carbon-based nanoparticles. In order to avoid these problems, different pretreatment methods have been proposed [28, 29, 30], such as the addition of polar groups (oxygen, hydroxyl, phenyl and polyvinylpyrrolidone). The surface defects could also affect the stability, the mechanical and electrical properties of carbon nanostructures [31, 32, 33, 34].

The carbon nanomaterials (Figure 1) cover a broad range of structures, beginning with zero-dimensional structures (fullerenes, diamond clusters), continuing with one-dimensional (nanotubes), two-dimensional (graphene), and three-dimensional structures (nanocrystalline diamond, fullerite).



**Figure 1.** Main carbon entities at nanoscale level

Carbon nanomaterials, including fullerenes, graphene and carbon nanotubes, have many technological advantages such as facile modification by functional groups, high carrier

capacity, incorporating both hydrophilic and hydrophobic substances, and high chemical stability.

### 3.1.1. Carbon nanotubes

Within the family of nanomaterials, carbon nanotubes (CNTs), described for the first time by Iijima [35], are arousing growing interest in relation to several modern devices, such as transducers, for the improvement of biosensing systems, mainly due to their unique structural, electronic and chemical properties, which include large surface area, high electrical conductivity, and excellent properties of electron transfer reactions. Furthermore, CNTs have a unique tubular structure, good biocompatibility and modifiable sidewall, making them ideal candidates for the construction of sensors with high performances [36].

Electrochemical nanobiosensors have been increasingly used in various types of nanomaterials, mainly CNTs, as electrochemical transducers, in order to improve them from the perspectives of automation, miniaturization and multiplexed analysis, but also to improve the analytical performances of such devices [37, 38]. For example, the detection of diseases at an early stage is one of the goals in developing and improving CNT biosensors, focusing on their sensitivity, fast response and small sample volume, in order to provide new strategies for the detection of specific biomarkers at low concentrations in complex sample media (e.g., serum) [39].

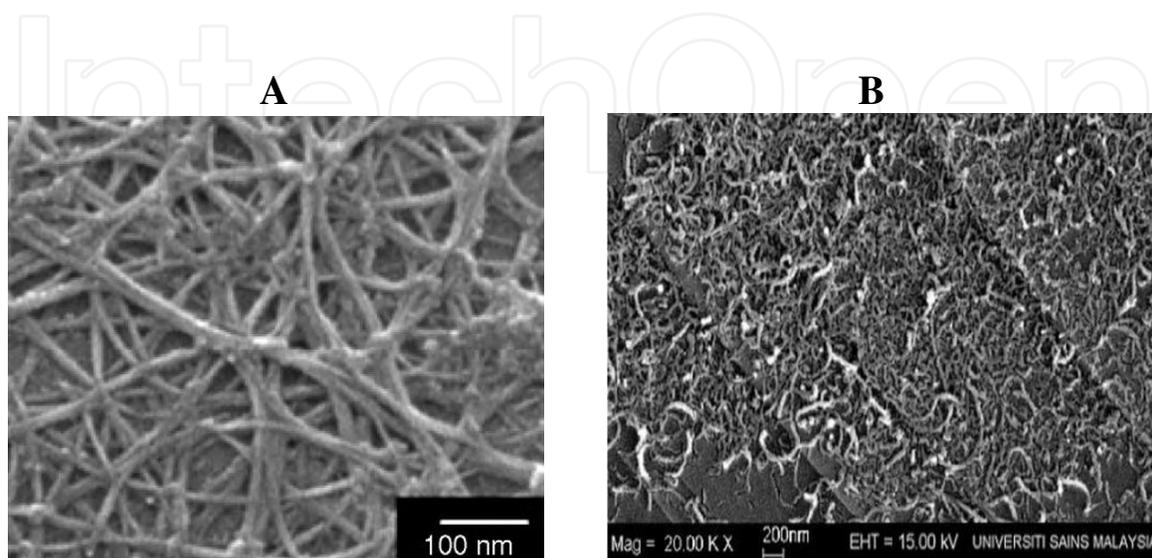
Many properties and the quality of CNTs are directly influenced by the way in which the graphene sheets are wrapped around [40], and they are affected by the operating conditions in their fabrication process. The production methods generally require transition metal nanoparticles (i.e., Fe, Co and Ni) to function as growth catalysts. When the catalysts are in contact with a gaseous carbon or a hydrocarbon source, the resulting carbon is deposited on the particle surface and the CNT grows rapidly at the surface of the catalyst. The diameter of the CNTs, their quantity and quality can be controlled by varying some reaction parameters (e.g., temperature, metal concentration, gas type and pressure). The diameter of the NPs defines the nature of the CNTs, whether single-walled (SWCNTs) with diameters in the range 0.4-3 nm, or a group of concentric CNTs sharing a common axis constituting double- and multiwalled (MWCNTs) with diameters in the range 1.4-100 nm [36].

In the case of CNT structures, the reactivity of atoms situated in the plane is different from those at the edges. For a number of biologically important compounds, the electrochemistry of atoms located at the edge-plane sites of the CNT is comparable to that of different planes of graphite [41], and the metal and non-metal impurities contained determine some of the electrochemical catalytic properties and limitations of CNTs [42, 43, 44]. It is also worth mentioning that the electrode surface modification with CNTs leads to a changed mass transport regime from semi-infinite diffusion to effective thin-layer diffusion in a porous layer, and the increased current may frequently be erroneously assigned to electrocatalysis [45, 46, 47].

Currently, SWCNTs and MWCNTs are synthesized by high-temperature processes (e.g., electric arc discharge and laser ablation, which are physical methods to convert carbon into

NTs) and chemical vapour deposition (CVD), which is performed at lower temperatures and atmospheric pressure.

Usually, the nanostructured materials obtained using CNTs were morphologically characterized using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Figure 2 presents the SEM images obtained for SWCNT [48] and MWCNT [49].



**Figure 2.** (A) SEM image of SWCNT composite film [48]; (B) FE-SEM images of MWCNT in high magnification [49]

Many papers published in recent years report CNT biosensors based on different oxidases applied for the detection of glucose [50, 51, 52, 53, 54],  $H_2O_2$  [55, 56, 57], *L*-lactate [58, 59, 60], acetaminophen [61, 62, 63, 64, 65], etc. The construction and optimization of new biosensors with HRP immobilized onto the transducer surface, either screen-printed electrode (SPE) or glassy carbon electrode (GCE), by entrapment into polymer films (polyethylenimine (PEI) and polypyrrole (Ppy)) doped with SWCNT and MWCNT, was described [61, 66].

The obtained configurations were used to monitor the signal produced by the electrochemical reduction of the enzymatically generated electroactive *N*-acetylbenzoquinoneimine (the oxidized form of acetaminophen) in the presence of hydrogen peroxide using amperometry, cyclic voltammetry (CV) and differential pulse voltammetry (DPV) in synthetic and real samples, with the best limit of detection (LOD) obtained for acetaminophen of  $1.36 \mu M$  [61].

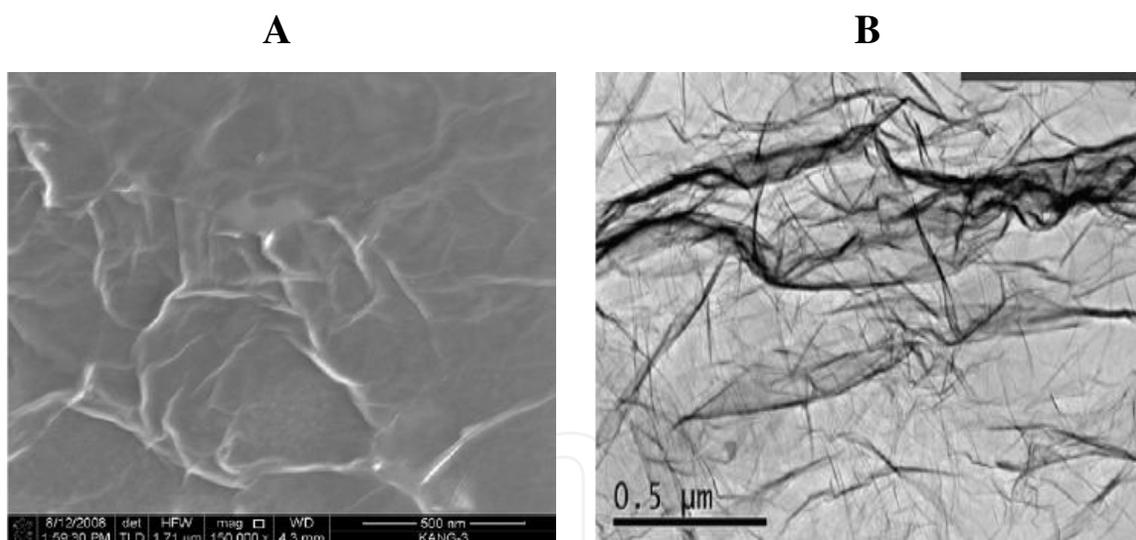
### 3.1.2. Graphene

Graphene is another material that offers great prospects for the future of analytical chemistry, consisting of a one-atom-thick sheet of  $sp^2$  hybridized carbon atom composed of six member rings that provide a surface area that is nearly twice as large as that of SWCNT [43]. This material presents high mechanical strength, high elasticity and high thermal conductivity [43], but also presents a major disadvantage: its poor dispersion in aqueous medium. Stable dispersions of graphene sheets have been achieved using amphiphilic polymers, alkylamines and hydrophilic carboxyl groups, among others, as dispersing agents [67]. The functionaliza-

tion of graphene is considered an important route for improving its dispersion. Therefore, different authors have tried producing graphene oxide, a graphite derivative with hydroxyl, epoxy and carboxyl groups covalently attached to its layers, with a better dispersion in some solvents being obtained.

Graphene can be interlinked with CNT for the fabrication of high-performance transparent electrodes, and the resulting films present features comparable to indium tin oxide [68]. The electrochemical activity of crystalline graphene is different from that of reduced graphene oxide flakes [69]. A wide variety of methods are available for graphene fabrication, the first being published in 2004 [70], and consist in the mechanical exfoliation (repeated peeling) of small patches of highly ordered pyrolytic graphite. Many other fabrication methods have been developed, including unzipping MWCNT to form graphene ribbons [71, 72], substrate-independent methods using micromoulding inside a capillary [73], and spray deposition of graphene oxide-hydrazine dispersions [74].

SEM images of a graphene film deposited on GCE revealed the typical crumpled and wrinkled graphene sheet structure on the rough surface of the film (Figure 3 A). The TEM image presented in Figure 3B shows the wrinkled graphene sheet with no aggregation, indicating that the functionalized graphene sheets were well dispersed in ethanol, forming stable suspensions [75].



**Figure 3.** (A) SEM image of graphene-film-modified GCE. (B) TEM image of graphene in ethanol [75]

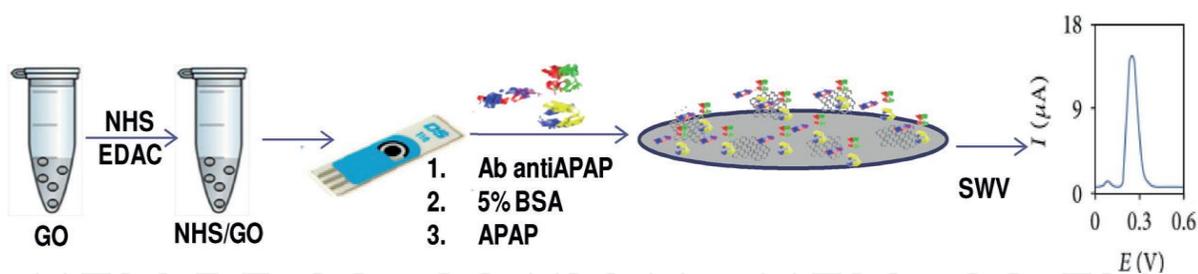
With regard to aptasensor development, graphene looks like it could have the necessary requirements to implement next-generation and high-performance aptasensors. Graphene naturally adsorbs the unfolded aptamer through the  $\pi$ - $\pi$  stacking interaction between the purine and pyrimidine bases and the graphene plane, and hinders the adsorption of the folded aptamer [76, 77].

The currently developed strategies for graphene functionalization with aptamers include covalent and non-covalent approaches. The covalent approach often relies on the oxidation or

the reduction of graphene oxide that presents carbonyl and carboxyl functional groups [78]. The density of carbonyl and carboxyl functional groups can be increased through chemical oxidation, which is widely used for the chemical modification of the inert graphitic structure [79]. Because this procedure affects the properties of graphene, the covalent modification of graphitic surface through a free radical addition reaction is used, without a preoxidation step and with less impact on the electronic properties [80]. Besides the immobilization methods mentioned above, amide [81] and phosphoramidate reactions [82] were employed towards the fabrication of graphene-based biosensors. Some graphene derivatives, such as graphene oxide, were also used as transducers in electrochemical biosensor development [83].

Graphene can also mediate electron transfer in view of the high-density edge-plane-like defects present within its nanostructure. In the absence of a target, aptamers immobilized on the gold electrode adsorb graphene nanosheets due to the strong  $\pi$ - $\pi$  interaction that accelerates the electron transfer ratio between the electroactive species and the electrode surface. In the presence of a target, the binding reaction inhibits the adsorption of graphene and blocks the electron transfer. A label-free electrochemical aptasensor was constructed by taking advantage of the ultra-fast electron transfer ratio of graphene [84]. This detection strategy was further improved by nuclease for interferon gamma, yielding an LOD of 0.065 pM [85]. Other authors [86] employed graphene oxide nanoplatelets (GONPs) as electroactive labels for thrombin detection.

A simple label-free electrochemical immunosensor was constructed by modifying a graphite-based SPE with graphene oxide after functionalization with *N*-hydroxysuccinimide in the presence of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride, Figure 4.



**Figure 4.** Steps involved in the antiacetaminophen Ab-based immunosensor development; GO=Graphene oxide; NHS=*N*-hydroxysuccinimide; EDAC=1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride; Ab anti APAP=antibody antiacetaminophen; APAP=acetaminophen; SWV=square wave voltammetry [87]

The above-described immunosensor was applied with good results for the determination of acetaminophen in synthetic and real samples by using square wave voltammetry (SWV) with an LOD of 0.17  $\mu\text{M}$  [87].

### 3.1.3. Carbon nanoparticle-based electrochemical sensor

A sensitive and selective electrochemical sensor fabricated via the drop-casting of a suspension of carbon nanoparticles (CNPs) onto a GCE was investigated in simultaneous determination of acetaminophen and tramadol in pharmaceuticals and human plasma. CV and DPV studies

of acetaminophen and tramadol were carried out at the modified and bare GCE. The results of the electrochemical investigations showed that CNPs enhanced the electroactive surface area and caused a remarkable increase in the peak currents, due to the diffusion within the porous layer. Enhanced sensitivity and a considerable decrease in the anodic overpotential leading to negative shifts in peak potentials was obtained with the modified GCE. By using DPV, the sensor showed good sensitivity and selectivity: LODs for acetaminophen and tramadol were 0.05 and 1  $\mu\text{M}$ , respectively [88].

### 3.2. Inorganic and organic nanoparticles

Nanoparticles of major environmental, pharmaceutical and biomedical importance include magnetic nanoparticles, quantum dots, metal nanoparticles, silica nanoparticles and polymeric types with intrinsic properties contributing to their use in specific applications. Typically, nanoparticles are classified into: *magnetic nanoparticles* (possessing a core of  $\text{Fe}_3\text{O}_4$ ) frequently used in drug delivery and lately in immunosensing; *semiconductor/QDs* (based on CdS, CdSe) used in bio- and immunosensing; *metal nanoparticles* (Ag, Au, Pd) used in biosensing and drug delivery; *polymeric nanoparticles* (polystyrene) mainly used in drug and gene delivery; *hybrid nanoparticles* (carbon or  $\text{Fe}_3\text{O}_4$  covered with different materials like metal oxides, polymers, amino acids, etc.) [89].

#### 3.2.1. Magnetic nanoparticles

In recent years, considerable efforts were made to develop magnetic nanoparticles (MNPs), due to their inherent advantages (magnetism, nanosize) and low cost of production. MNPs with 10-20 nm diameters exhibit their best performance, due to supermagnetism, which makes them especially suitable for a fast response. Due to their inherent dimensions, they possess a large surface area and high mass transfer capacity [90].

MNPs can be integrated into the transducer materials and/or be dispersed in the sample followed by their attraction by an external magnetic field onto the active detection surface of the (bio)sensor. Biorecognition molecules like enzymes, antibodies or oligonucleotides immobilized onto magnetic particles can be easily trapped by magnets and retained close to or on an electrode surface [90, 91]. Since the properties of MNPs depend strongly on their dimensions, their synthesis and preparation have to be designed in order to obtain particles with adequate size-dependent physicochemical properties. MNPs possessing adequate physicochemistry and tailored surface properties have been synthesized under precise conditions and applied for sensors, biosensors and other detection systems [92, 93].

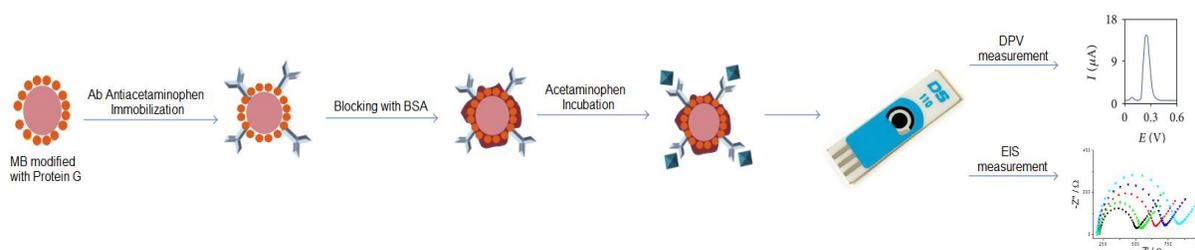
Magnetic particles consist of a magnetic core surrounded by a non-magnetic shell. Iron oxides, such as magnetite ( $\text{Fe}_3\text{O}_4$ ) or maghemite ( $\gamma\text{-Fe}_2\text{O}_3$ ), are often used as core materials instead of iron due to their higher stability [94]. For biological applications, magnetic particles generally consist of nanosized superparamagnetic iron oxide distributed through a matrix, mainly composed of silica [95] or an organic polymer [96]. Silica-based magnetic beads can be prepared in different shapes and sizes with different degrees of porosity, which are much more attractive for the immobilization of biomolecules due to their chemical and mechanical stability and their

resistance to bacterial attack [97]. Many types of magnetic particles, functionalized with different groups like carboxyl, amino, hydroxyl or epoxy, are currently available for different applications.

The majority of MNP-based systems use inorganic nanocrystals as magnetic cores. The composition of these inorganic nanocrystal ranges from metals and alloys to metal oxides. Among the metal oxide compounds, superparamagnetic iron oxide nanoparticles, including magnetite ( $\text{Fe}_3\text{O}_4$ ) and maghemite ( $\gamma\text{-Fe}_2\text{O}_3$ ), are the most popular [98]. The surfaces of the particles can be modified with organic polymer or inorganic compounds (e.g., gold, silica, alumina), suitable for further functionalization by the attachment of various bioactive molecules [99]. The functionalization of MNPs with bioelements exhibits specificity, due to both the bioreceptor and the magnetism.

MNPs amplify the electrochemical signal, improving the sensitivity of electrochemical devices [90], through their contact with the electrode surface, transport of a redox-active species to the electrode surface, and formation of a thin film on the electrode surface. The detection for MNP-based electrochemical biosensors can be performed by potentiometry [100, 101], amperometry [102, 103], electrochemiluminescence [104, 105], electrochemical impedance spectroscopy [106, 107] and especially voltammetry [108, 109, 110, 111, 112, 113, 114, 115].

Protein G-MNP-based label-free electrochemical immunosensor was elaborated for the ultrasensitive and specific detection of acetaminophen using a carbon-based SPE as immobilization platform (Figure 5). An LOD of  $1.76 \mu\text{M}$  was calculated based on the calibration data. The performances of the optimized immunoassay were tested using two pharmaceutical products containing acetaminophen with excellent recoveries [116].



**Figure 5.** Protein G-MNP-based label-free electrochemical immunosensor development [116]

Protein G-functionalized magnetic beads and graphite-based SPEs were also used to develop an HRP-labelled sandwich type immunosensor for Mucin 1 (MUC1) electrochemical detection with LOD of 1.34 ppb [117].

$\text{Fe}_3\text{O}_4$  magnetic dipolar attraction and its large surface area/volume ratio may lead to aggregation in clusters when exposed to biological solutions. Functionalization can overcome this problem and also enhance biocompatibility. Many strategies were used for the functionalization of MNPs, such as core-shell  $\text{Au-Fe}_3\text{O}_4$  [109], core-shell  $\text{Au-Fe}_3\text{O}_4\text{@SiO}_2$  [102], core-shell  $\text{Fe}_3\text{O}_4\text{@SiO}_2$  [112],  $\text{Au-Fe}_3\text{O}_4$  composite NPs [108],  $\text{Fe}_3\text{O}_4\text{@SiO}_2/\text{MWCNTs}$  [103],  $\text{Fe}_3\text{O}_4$  anchored on reduced graphene oxide [113] and  $\text{Fe}_3\text{O}_4\text{@Au-MWCNT-chitosan}$  [114]. Among all these,

core-shell  $\text{Fe}_3\text{O}_4@\text{SiO}_2$  contributes to the stabilization of MNPs in solution and enhances the binding of ligands at the surface of MNPs. Electrode surface modified with core-shell  $\text{Fe}_3\text{O}_4@\text{SiO}_2$  shows good electrical conductivity and more electroactive interaction sites, providing enhanced mass transport and easier accessibility to the active sites, thus increasing the analytical signal and sensitivity [90]. A comparison between the analytical performances of some recently developed electrochemical sensors and biosensors based on MNPs is presented in Table 1.

Detection method	MNPs type	Analyte	LOD	Reference
Voltammetry	Au- $\text{Fe}_3\text{O}_4$	Organochloride pesticides	56 pg/mL	[108]
Voltammetry	Core-shell Au- $\text{Fe}_3\text{O}_4$	Carcinoembryonic Ag	10 ng/mL	[109]
Voltammetry	$\text{Fe}_3\text{O}_4$ Au NP	Clenbuterol	220 ng/mL	[110]
Voltammetry	$\text{Fe}_3\text{O}_4$ Au NP	$\text{H}_2\text{O}_2$	20 $\mu\text{M}$	[111]
Voltammetry	Core-shell $\text{Fe}_3\text{O}_4@\text{SiO}_2$	Metronidazole	18 nM	[112]
Voltammetry	$\text{Fe}_3\text{O}_4$ -RGO	Cr(III)	-	[113]
Voltammetry	$\text{Fe}_3\text{O}_4@\text{Au}$ -MWCNT-chitosan	Streptomycin	1.5 nM	[114]
Voltammetry	Core-shell $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{MWCNT}$	Uric acid	0.13 $\mu\text{M}$	[115]
Potentiometry	MaMB Dynabeads Protein G	Zearalenone	7 ng/mL	[100]
Potentiometry	Core-shell- $\text{Fe}_3\text{O}_4$	Glucose	0.5 $\mu\text{M}$	[101]
Amperometry	Core-shell Au- $\text{Fe}_3\text{O}_4@\text{SiO}_2$	Glucose	0.01 mM	[102]
Amperometry	$\text{Fe}_3\text{O}_4@\text{SiO}_2$ -MWCNT	Glucose	800 nM	[118]
EIS	$\text{Fe}_3\text{O}_4$ -COOH/MNP	OchratoxinA	10 pg/mL	[106]
EIS	Fe@Au NP-2-aminoethanethiol-graphene NP	DNA	2 fM	[107]
Detection method	MNPs type	Analyte	LOD	Reference

**Table 1.** Selected examples of sensors and biosensors based on magnetic nanoparticles

### 3.2.2. Metal nanoparticles

Metal nanoparticles and CNTs, have been employed to immobilize biomolecules for the construction of sol-gel biosensors, due to their large surface area, high catalytic activity and good biocompatibility. Moreover, the excellent conductivity of gold nanoparticles (AuNPs) provides the potential to construct amperometric biosensors based on redox enzymes (as sensing elements) and nanoparticle arrays (as conductive matrices where enzyme molecules are immobilized) [118, 119]. Metal nanoparticles, such as Pd and Pt, were deposited on

SWCNTs and MWCNTs in order to increase the electrocatalytic activity of CNTs. An improved conductivity and electrocatalytic behaviour was obtained by combining sol-gel materials with CNTs, the electron transfer between the redox enzyme and the electrode being facilitated [120].

Gold nanomaterials have unusual optical and electronic properties, high stability and biological compatibility. Aptamer-conjugated gold nanomaterials provide powerful platforms to facilitate targeted recognition, detection and therapy applications, due to their controllable morphology, size dispersion and easy surface functionalization [121].

Many applications of nanoparticles, such as metal NPs (gold, silver) or semiconductor dots in biosensing, have been recently reported [122, 123]. A DNA array detection system based on oligonucleotide targets labelled with AuNPs was developed [124], and AuNPs or silver-enhanced colloidal gold were used as labels for electrochemical detection of DNA hybridization [125, 126, 127]. These NP-based electrochemical biosensors employed the stripping voltammetric technique as the read-out principle due to the preconcentration step resulting in ultra-trace-level LODs.

AuNPs are irreversibly immobilized on the transducer, the electrode surface being difficult to regenerate. To overcome this problem, Chen *et al.* [128] have developed a novel electrochemical system for the sensitive detection of glucose (LOD of 1 mM) as well as single-nucleotide polymorphism, which has the AuNPs directly in the supporting electrolyte. This one-pot detection system can be operated and regenerated very easily, since all the components are integrated in the electrolyte of the gold colloid and the unmodified electrode can be reused.

The sensing mechanism of potentiometric biosensors using NPs is based on a sandwich assay (Figure 6), the target being captured by the immobilized primary receptor and then attached to the NP-labelled secondary receptor. Potentiometry is finally used to detect the dissolution products of NPs after a release step with  $H_2O_2$  [123].

Miniaturized ion-selective electrodes (ISEs) designed to detect ions in microvolume samples, with a concentration of fM, were reported [129, 130], and an LOD of 12.5 pmol/50 $\mu$ L was obtained for IgG using an ISE based on silver-enhanced AuNPs [131]. Metal NPs can also be used as a catalyst for the reduction of metal ion, via the enzymatically generated reducing agent, and the biometallization processes can be monitored by ISEs in real time [132]. Thereby, an ISE based on AuNPs was developed and applied for highly sensitive detection of glucose [132, 133], while the NADH potentiometric detection was successfully obtained by monitoring the NADH-stimulated catalytic reduction of  $Cu^{2+}$  in the presence of AuNPs (which have a crucial role in improving the sensitivity) with a  $Cu^{2+}$ -ISE [134]. Wang *et al.* [135] reported a highly sensitive potentiometric biosensor for detecting DNA hybridization based on the depletion of  $Ag^+$  ions induced by the biocatalytic reaction of alkaline phosphatase, recorded in the presence of AuNPs. The sensor has been used to detect DNA and 16S rRNA of *Escherichia coli* with LOD of 50 fM, using only 4  $\mu$ L of sample solution.

Two simple electrochemical aptasensors were developed for the detection of Mucin 1 (MUC1) tumour marker based on its specific recognition by thiolated aptamers immobilized on Au NP-modified graphite and gold SPEs. The quantitative detection of MUC1 protein was electrochemically achieved by electrochemical impedance spectroscopy (EIS) and DPV. The

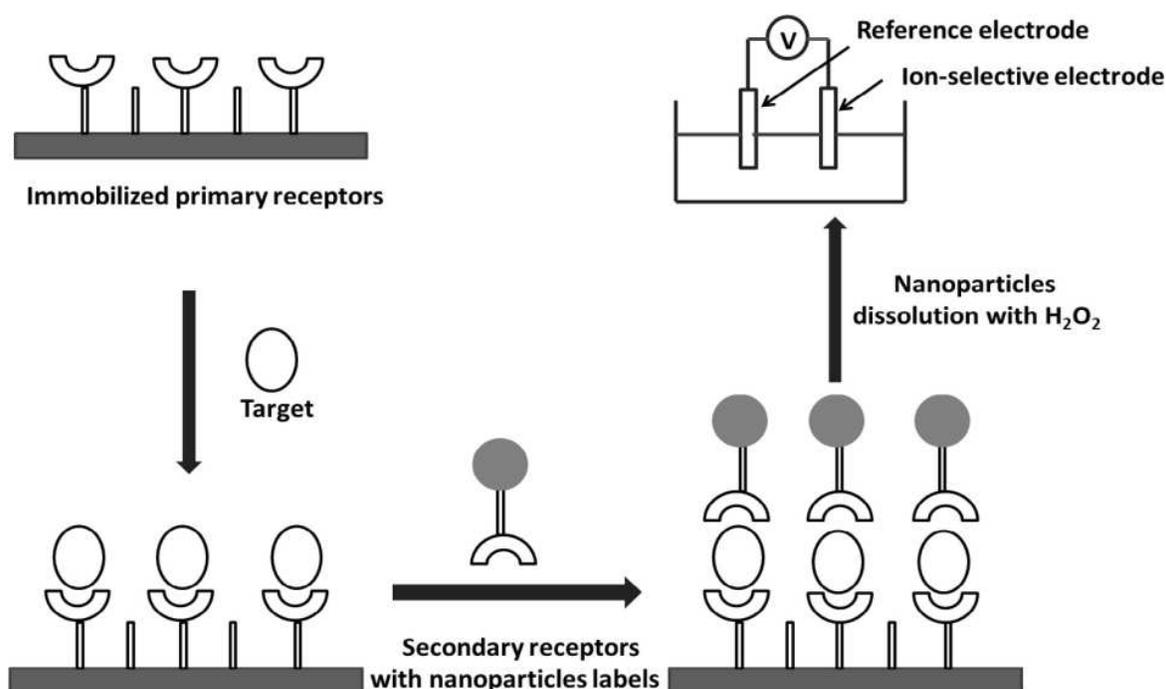


Figure 6. Potentiometric biosensors using nanoparticles as labels [123]

estimated detection limits for MUC1 were 3.6 ng/mL at AuNP modified graphite SPE by EIS and 0.95 ng/mL at AuNP modified gold SPE by DPV [136].

### 3.2.3. Nanosized clays

Nanosized clays can serve as matrices for electroactive species because they are usually able to incorporate ions by an ion exchange process. Moreover, adsorption of proteins on clay mineral surfaces plays a very important role in the development of biosensors. By modifying the electrode surface with clays, the heterogeneous electron transfer process between the protein and the electrode surface is facilitated.

The entrapment of biomolecules in the clay matrix represents an inexpensive, fast and easy method for the elaboration of enzyme electrodes. The procedure consisting in the adsorption of an enzyme/clay aqueous colloid mixture onto the electrode surface offered the possibility of entrapping a large and known amount of enzyme into the clay film, and could also be adapted for interdigitated microelectrodes [137].

Mesoporous silica nanoparticles can be easily synthesized by using surfactant templating methods [138]. Silica nanoparticles have become an important platform in biomedical applications and bioanalysis because of their high density, which facilitates separation through centrifugation during their synthesis, and their modification and detection steps [139]. The silica surface also provides a biocompatible and versatile substrate for biomolecule immobilization, required for further utilization in biosystems and for drug delivery [140]. Following typical DNA silica surface conjugate chemistry, the aptamer immobilization on silica nanoparticles has been well developed for various applications [141].

Biosensors based on clay materials can play an important role as efficient tools in the field of forensic analysis considering their well-known advantages of sensitivity, selectivity, simple functioning and affordability [142]. Thereby, a bienzyme biosensor based on acid phosphatase (AcP) and polyphenol oxidase (PPO) simultaneously entrapped in anionic clays was constructed and applied for the specific amperometric determination of As(V). The biosensor relied on the successive hydrolysis of phenyl phosphate to phenol catalysed by AcP and the oxidation of phenol to *o*-quinone catalysed by PPO. The electrochemical reduction of the formed *o*-quinone was accomplished at a detection potential of -0.2 V vs Ag/AgCl with a detection limit of 0.15 ng/mL [143]. DNA-functionalized, layer-by-layer assembled SWCNT hybrids with clay nanoparticles were proposed for the detection of As(III) achieving an LOD of 0.05 mg L<sup>-1</sup> [144]. Another simple amperometric biosensor for cyanide involved the immobilization of PPO into ion exchanger clay based on Zn-Al double-layered hydroxides (LOD of 0.1 nM). The low LOD was a consequence of the anion accumulation in the ion exchanger clay which also contained the immobilized enzyme [145].

#### 3.2.4. Nanosized and nanostructured polymeric films

One of the most important aspects of biosensor development is the bioelement immobilization platform, which needs to ensure high stability and maintain its activity as long as possible. Several types of **conductive** or **insulating polymers** were used to obtain a nano/micropatterned surface with applications in the biosensor field [146]. Electropolymerization in the presence of a template, removed after the polymer formation, is an efficient route for the synthesis of 2D and 3D conductive polymers (CPs). Among the conductive polymers, polypyrrole and polyaniline [147, 148] were extensively studied because of their electrochemical properties, aqueous compatibility and their ability to form nanostructures like nanowires, nanotubes or microcontainers [149].

Hydrogels are cross-linked hydrophilic polymer structures that can hold large amounts of water or biological fluids in their pores. As one of the newest classes of polymer-based systems, target-responsive hydrogels have found numerous biomedical and pharmaceutical applications [121, 150]. Polyacrylamide/acrylate is a copolymer of acrylamide and acrylic acid, and acrydite-modified oligonucleotides can form hydrogels by the introduction of proper complementary DNA as a cross-linker. He *et al.* [151] reported an aptamer-based reversible DNA induced hydrogel system for molecular recognition and separation.

Different types of hydrogel nanoparticles were used together with various biocomponents, such as enzymes, proteins and nucleic acids, during the development of electrochemical biosensing systems, in order to increase their sensing performances [152].

CPs and in particular functionalized CPs are useful for biosensor construction as a platform to immobilize the primary Ab on the electrode surface. Poly *o*-aminobenzoic acid (PABA) can be considered a carboxyl-functionalized aniline CP, capable of self-doping, and is also a soluble derivative of polyaniline. Its carboxyl group can be exploited as a linker, either for PABA immobilization onto the electrode, or for covalent bond formation with biomolecules such as proteins and antibodies [153].

An electrochemical aptamer-based biosensing assay for MUC1 protein detection by the modification of the graphite-based SPE's surface using a functionalized conductive polymer (PABA), and by using methylene blue (MB) as an electrochemical indicator was developed. The recognition of immunoreactions and aptamer binding events was assessed by monitoring the interfacial electron transfer resistance with EIS, while CV and DPV were employed for sensitive indirect quantification of MUC1 via MB with a detection limit of 0.62 ppb [154]. In order to establish the optimal configuration of HRP-based biosensors able to detect acetaminophen in various matrices, another conductive polymer, polypyrrole, was deposited on GCE and SPE by several methods. Chronoamperometric studies performed proved the interaction between the immobilized enzyme and the electroactive species of paracetamol, NAPQI, enzymatically generated in the presence of  $H_2O_2$  [155].

The combination of nanosphere lithography, electrodeposition and click chemistry was applied for the immobilization of ferrocene at GCE. The nanostructuring of the surface was obtained by adsorption of 100 and 900 nm diameter polystyrene nanospheres followed either by electropolymerization of *N*-(10-azidodecyl) pyrrole or by electrografting of 4-azidobenzenediazonium. This approach offered potential applications such as engineering of porous materials and was used for HRP immobilization. The nanostructured biosensor was applied for acetaminophen detection in the presence of hydrogen peroxide, and showed better performances in terms of sensitivity and LOD in comparison with a non-structured electrode [156]. Another template based on nanostructured polymeric film was developed combining latex nanosphere lithography with electropolymerization of *N*-substituted pyrrole monomer in order to immobilize both tyrosinase and GOx and to obtain a biosensor with higher sensing performances [148].

### 3.2.5. Molecularly imprinted polymers

MIPs can be successfully used in applications relying on selective molecular binding as they are fully artificial macromolecular structures with biomimetic properties, imitating receptor-ligand, Ab-Ag, or enzyme-substrate biorecognition [157].

MIPs possess several advantages over their biological counterparts, including low cost, ease of preparation, long term stability and shelf life, inherent reusability, high mechanical strength, and remarkable chemical and thermal stability under various experimental conditions, allowing the very specific binding of the analyte in "harsh" chemical media [158, 159].

The rational design of MIPs is still considered a challenge due to the numerous experimental variables to be addressed, and it has been previously reviewed in detail by several groups of authors [157, 158, 160, 161] along with their most important analytical applications.

MIPs prepared by bulk polymerization have proven to be poorly compatible with transducers in terms of efficient immobilization strategies, mass transfer and rebinding kinetics, even if they are ground before use. Therefore, several aspects related to the in-situ polymerization, and more specifically to the electrosynthesis of MIPs and hybrid technologies involving MIPs in the fabrication of biosensors, as well as their most recent advances in addressing two

important challenges for MIP-based electrochemical biosensors, the determination of biomacromolecules and enantiospecific analysis, will be very briefly presented.

Electroactive organic functional monomers can polymerize to form conducting, semiconducting or insulating polymers. Polymerization may be performed chemically (using an oxidizer as external reagent) or electrochemically (by electropolymerization). Electropolymerization is the more convenient approach, in terms of simplicity and time-effectiveness, for electrochemical biosensor fabrication as it leads to a tight binding of an MIP film onto the surface of the transducer. This procedure ensures both the synthesis and the surface immobilization of the biomimetic layer, along with the possibility of controlling polymer nucleation and growth, film thickness and morphology by the proper selection of the electropolymerization parameters. The reproducible preparation of thin MIP layers is of the utmost importance in sensing applications, because it reduces response time by significantly shortening diffusional path lengths. Solvent swelling and the inclusion of the supporting electrolyte's ions tunes the rigidity and porosity of the MIP film. Electropolymerization may be performed under galvanostatic, potentiometric or most frequently potentiodynamic conditions, in both non-aqueous and aqueous solutions. The first two techniques are able to generate well-ordered, charged polymeric layers doped with counter ions for charge neutralization, whereas the latter generates a more tangled, charged or neutral polymeric matrix due to the continuous influx and release of solvated ions during its growth upon film charging and discharging [157].

Using electroinsulating MIP films in the fabrication of electrochemical sensors carries a major disadvantage due to the high resistance to charge transfer and the lack of direct path for electron conduction from the recognition sites to the electrode transducer, suffering from an incomplete template removal upon their overoxidation. Using electronically conducting polymers for MIP synthesis avoids these undesirable effects.

Some of the most common electroinsulating layers employed in MIP technology are acrylic- and vinylic-based polymers, but the most frequently reported electrosynthesized insulating MIPs are polyphenylenediamine, polyphenol, polyaminophenol and polythiophenol. Due to their low conductivity, sensors based on such MIPs usually use optical, piezoelectric or impedimetric signal transduction. However, as long as they are electrodeposited in a thickness that does not insulate the underlying conductive electrode surface (<10 nm) [162], a successful voltamperometric signal transduction may also be performed [163].

In the case of electrosynthesis of conducting MIP layers for biosensing, electroactive functional monomers such as pyrrole, thiophene, aniline, 3-aminophenylboronic acid and porphyrin derivatives are most often reported. Analyte determination using such conducting layers may be performed by any available signal transduction procedure, such as optical, piezoelectric, voltammetric, potentiometric, chronoamperometric, impedimetric or conductometric procedures. Poly(pyrrole), poly(thiophene), poly(aniline) and their derivatives are amongst the most widely studied conducting polymers [157, 160], due to their high conductivities in their oxidized form and their ability to reversibly switch between conducting and insulating states by doping and undoping [160]. As mentioned earlier, in the non-covalent molecular imprinting, a wide variety of templates are amenable to be used with elevated imprinting efficiencies, ranging from small analytes to macromolecules

(proteins, nucleotides). Additional promising approaches include implementing surface MIP for cells (yeast), bacteria, virus or blood [164, 165, 166]. In the case of using small organic molecules as templates, MIP synthesis is well established, however the imprinting of much larger molecules is still considered to be a challenge. Larger templates are less rigid, which does not ensure the formation of well-defined binding cavities during macromolecular imprinting, and, furthermore, the secondary and tertiary structures of biomacromolecules (i.e., proteins) may be also affected under the imprinting conditions. The large size hinders the protein both in reaching and leaving the artificial binding sites, and their molecular complexity increases the chances of non-specific binding with the MIP, leading to poor selectivity and cross-reactivity. More importantly, proteins are often incompatible with the organic solvents used in MIPs synthesis. The use of an aqueous solution for macromolecular imprinting greatly restricts the choice of reactive functional monomers and cross-linkers. Additionally, water could compete for and potentially disrupt any hydrogen bonds between the template and the functional monomers. The protein as template in macromolecular imprinting may be introduced by three different approaches: it may be dissolved into the prepolymerization mixture (bulk imprinting), grafted to the surface of the transducer followed by a partial inclusion into the bulk polymer with the resulting binding sites situated on the MIP surface (surface imprinting), or only a small epitope part of the protein (epitope imprinting) may be used as a template, resulting in an MIP that is able to recognize the whole target protein. Surface and epitope imprinting enhance the imprinting effect for biomolecules, leading to higher affinity and capacity for the template, as well as to reduced non-specific binding; they improve protein binding kinetics, overcoming difficulties to mass transfer and protein removal in imprinted matrices. While surface imprinting is an efficient technique, the conditions used to accomplish the imprinting process must be carefully considered. The conformational stability of proteins is sensitive to their surrounding conditions, including temperature, pH, ion concentration, surfactant and its concentration, the ratio of template to functional monomers, the types of functional monomers used, and initiator type [167]. MIPs generated by epitope imprinting manifest minimal non-specific binding and improved affinity. Furthermore, it is less costly working with short peptides as templates, and more importantly, because of the template's higher stability, organic solvents may also be used during the imprinting process. The detection of the bound non-electroactive target macromolecule is performed indirectly, in the presence or absence of a redox probe, by various signal transduction principles, such as voltammetry, piezomicrogravimetry, impedance spectroscopy, etc.

The high selectivity of such biomimetic polymers is also demonstrated by their efficient use for the molecular imprinting of optically active templates. This special application of MIP-based chiral electrochemical sensing has not been fully exploited, even though there is a great necessity in the pharmaceutical industry for developing fast and cost-effective methods of chiral analysis applicable from the early stages of drug development. The vast majority of MIP-based electrochemical sensors were developed for non-chiral analysis. Only around 20 articles report MIP-based sensors designed for the chiral analysis of different small-molecules, but not pharmaceutically active compounds, like amino acids [168, 169, 170] or monosaccharides [171]. Moreover, enantiospecific signal transduction was only reported in the case of amino acids.

Nevertheless, very recently an MIP-based electrochemical sensor has been reported for the simultaneous enantiospecific recognition of several  $\beta$ -blocker enantiomers [163].

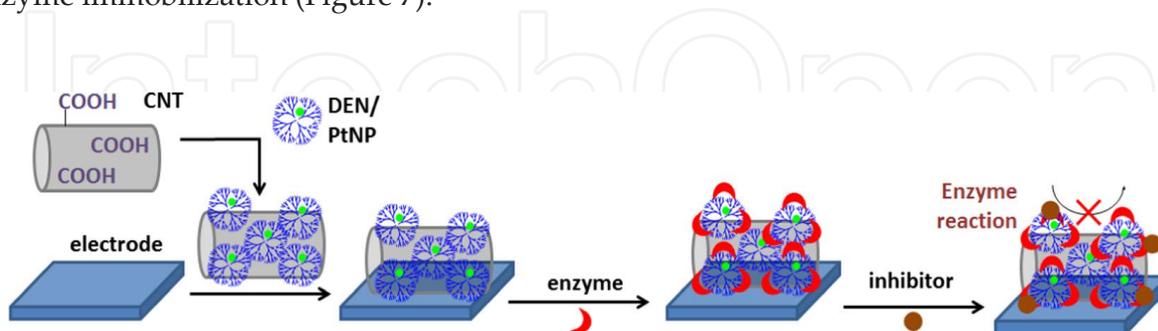
In spite of the electroinsulating properties of the R(+)-atenolol imprinted acrylate-based MIP, voltamperometric signal transduction was successfully performed, due to the resulting very thin (less than 4 nm) and porous MIP layer. The sensor exhibited distinctive enantiospecific oxidation peaks towards the R-antipodes of four  $\beta$ -blocker representatives and additional oxidation peaks common to both enantiomers of each studied  $\beta$ -blocker, thus allowing the simultaneous analysis of all of their enantiomers in a single determination.

### 3.3. Hybrid materials

Metal NPs decorating CNTs or graphene sheets, carbon-coated magnetic NPs, nanoparticles included in carbon paste electrodes, MWCNT-alumina-coated silica, and insulating MIPs combined with various nanostructures are examples of some hybrid nanomaterials used for the enhancement of biosensor sensitivity.

#### 3.3.1. CNT-based hybrid materials

Electrodes modified by hybrid materials, consisting of CNTs and metal nanoparticles, have been developed for use as fuel cell catalysts and biosensors [172]. For example, platinum nanoparticles (PtNPs) catalyse the electrochemical oxidation of  $H_2O_2$ , which is generated by the enzymatic reaction, while MWCNTs can be employed to modify the working electrode for the enlargement of the electroactive surface area [173]. A highly sensitive Pt-CNT-glucose biosensor was developed using the incorporation of GOx on a Pt-CNT electrode but with poor enzyme stability after storage [174]. Electrodes modified by CNTs/PtNPs hybrids protected by dendrimers were first developed for methanol electrochemical oxidation [175], and then adapted for the detection of inorganic phosphorous pesticide through the inhibition of the acetylcholinesterase [172]. These electrochemical biosensors are based on a hybrid material, consisting of MWCNTs, PtNPs and poly(amidoamine) dendrimer (DEN) as a binder for enzyme immobilization (Figure 7).



**Figure 7.** Biosensor preparation by loading MWCNT, PtNPs and enzyme through dendrimer binders [172]

MWCNTs increase the surface area and promote the electron transfer properties, PtNPs intensify the electron transferability in addition to catalytic performance, and dendrimers play a role in the uptake of the redox agent as well as the binder functionality.

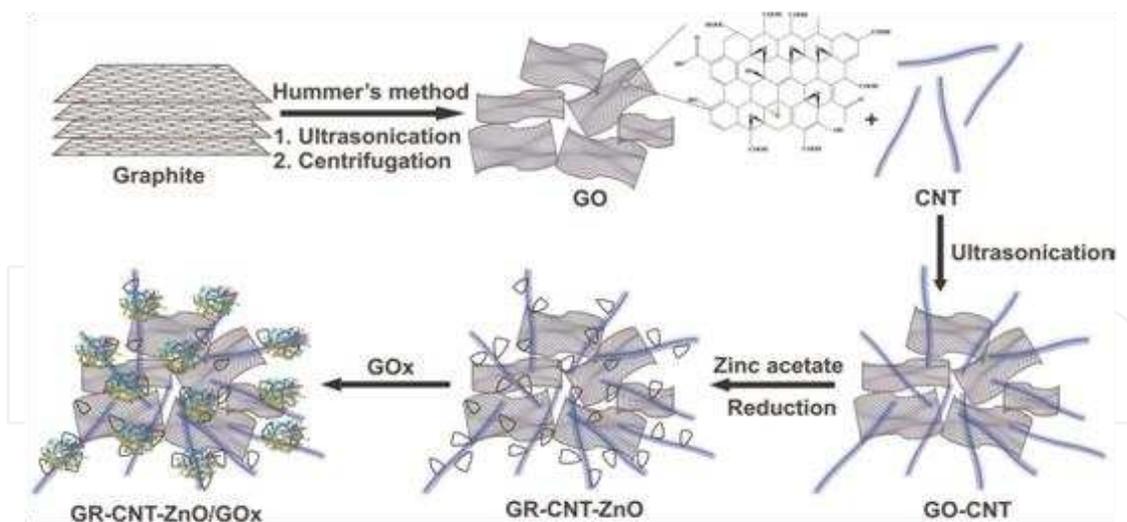
Two new xanthine biosensors, based on GC modified with chitosan (CS),  $\text{Co}_3\text{O}_4$ NPs and MWCNTs were developed [176]. Two different enzyme systems were investigated involving monoenzyme xanthine oxidase (XO) immobilization and bienzyme xanthine oxidase (XO)/HRP co-immobilization. Xanthine amperometric determination was achieved by both electrooxidation and electroreduction of the enzymatically generated hydrogen peroxide, with LODs of  $0.2 \mu\text{M}$  and  $20 \mu\text{M}$  with anodic and cathodic processes, respectively. The proposed biosensors were applied for detecting xanthine in fish with average recoveries of  $96.2 \pm 3.5 \%$  with  $\text{XO}/\text{Co}_3\text{O}_4\text{MWCNTs}/\text{CS}/\text{GCE}$  and  $95.4 \pm 2.1 \%$  with  $\text{XO}/\text{HRP}/\text{Co}_3\text{O}_4\text{MWCNTs}/\text{CS}/\text{GCE}$ , respectively.

### 3.3.2. Graphene-based hybrid materials

Graphene can be decorated with AuNPs to enhance the signal transduction or to increase the grafting area for biocomponent immobilization. AuNPs are the most commonly used metal nanoparticles in graphene-based biosensing systems, mainly due to their ability to form strong covalent bonds with the thiol groups [26].

An electrochemical aptasensor using graphene-AuNPs composite obtained by the reduction of tetrachloroauric acid with sodium citrate in a graphene water suspension was reported [109]. Direct electrodeposition technique of AuNPs was also applied to graphene-based electrochemical transducers yielding a high density of nanoparticles that provide an ultra-large specific surface area available for aptamer immobilization [177].

A graphene-CNTs-incorporating zinc oxide NPs hybrid composite (GR-CNT-ZnO) was synthesized and applied to fabricate an enzyme-based glucose biosensor (Figure 8).



**Figure 8.** The preparation of GR-CNT-ZnO composite [178]

By using CV, authors have concluded that the modified film in the presence of oxygen has high electrocatalytic ability in glucose detection with a wide linear detection range and a LOD of  $4.5 \mu\text{M}$  of glucose. The sensor was successfully applied for human serum samples, and the results were in good agreement with those determined using a standard photometric method [178].

### 3.3.3. *Molecularly imprinted polymer-based hybrid materials*

Hybrid materials combining insulating MIPs with electronically conducting polymers tend to correct the drawbacks of the former and feature networks of molecular wires connecting recognitions sites to the electrode surface [179]. Molecularly imprinted nanoparticles, nanospheres, nanoshells or nanofibres obtained by various synthesis procedures (precipitation or emulsion/suspension polymerization, mini/emulsion precipitation, etc.), along with various organic/inorganic hybrid materials [165, 180, 181], metal nanoparticles, such as Ag [182] and Au [183] or carbon nanomaterials [173], may be combined with electrosynthesized MIP films, leading to composite MIP/nanomaterial biosensing systems [160, 161] with larger specific surface area, higher sensibility and specificity, and higher biocompatibility for trace electrochemical analysis of small analytes and biomacromolecules.

## 4. Perspectives and future development

The selection of materials and fabrication techniques is crucial for adequate sensor function and the performance of a biosensor often ultimately depends upon these, rather than upon other factors. Consequently, future developments in biosensor design will inevitably focus upon the technology of new materials, especially the new copolymers that promise to solve the biocompatibility problem and offer the prospect of more widespread use of biosensors in clinical monitoring. The future trends in sensor development that are expected to have an impact on biosensor performances mainly concern immobilization techniques, nanotechnology, miniaturization and design of multisensor arrays.

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