Conducting polymers in biosensors: A review

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Abstract: Biosensors have gained immense acceptance in the field of medical diagnostics, food safety, environment control and biodefence application due to high sensitivity, specificity, ability for real-time analysis coupled with speed and low cost. Application of conducting polymers (CPs) in biosensors has recently aroused much interest. CPs provide an excellent opportunity for high selective, specific, stable, economic and handy biosensing devices. This paper first introduced different types of CPs, their unique properties and their synthesis, then specific information is provided on their modification for use in biosensors applications.

Keywords: Biosensors, Conducting polymers, Polypyrrole (PPy), Polyaniline (PANI), Enzyme.

1. Introduction

The request for sensitive, specific, fast and precise analysis methods, motivated the interest for the development of the chemical biosensors as instruments of analysis. Their capacity to provide continuously and reversibly a selective and fast answer towards the presence of a specific compound from a complex mixture of compounds is explored not only in the alimentary industry, but also in medicine, agriculture and environment.

The biosensors can be classified according to the biochemical process that offers the specificity or according to the physical transducer of processing of the signal. On base of the element of translation, the biosensors are qualified as being electrochemical, optic, piezoelectric and thermic. The electrochemical transducers and especially the amperometric ones, are the ones described in specialized literature, being preferred in the applications thanks to the advantages offered: high sensitivity, low price, it offers the possibility of miniaturization and integration in mobile systems, facility of using it [1-3]. After the last definition given by IUPAC [4, 5], an electrochemical biosensor is an integrated device capable to provide analytic information, using a biologic element of recognition (biochemical receiver) in direct contact with an electrochemical transducer. Therefore, a device whose element receiver does not come from a biological body it can't be qualified as a biosensor, because it doesn't have a "biocompound". The biological element assures the phenomenon of a specific recognition of the analyte, and the transducer, under applied potential, generates a signal associated with the specific recognition.

A domain of research, more and more developed lately, is represented by the implementation of new materials used in the construction of the biosensors. Many researches are focused on finding the ideal material that can assure optimal characteristics for the biosensor. In principle, in the construction of a biosensor, there must be respected many requirements for the selection of the electrode material such as: the compatibility with the biologic element, the absence of the barriers of diffusion, the stability at the temperature modifications, pH, ionic strength, sensitivity and selectivity for the analyte of interest, low cost and possibility of production in mass. These electrode materials must also present the functional groups necessary for the atachment of the biomolecules or to allow easily the introduction of functional groups. There are situations when the biosensors are used in severe conditions and in these cases, there are necessary materials with special mechanic and chemical resistance, almost inert. An ideal electrode material must also have a good conductibility to assure a fast transfer of electrons.

A number of materials such as polymers, sol–gels and conducting polymers have been used to improve the stability of the biomolecules used in the fabrication of the desired biosensors. In this context, polymers have become the materials of choice for recent technological advances in biotechnology [6].

Conducting polymers (CPs) were first produced in the mid-1970s as a novel generation of organic materials that have both electrical and
optical properties similar to those of metals and inorganic semiconductors, but which also exhibit the attractive properties associated with conventional polymers, such as ease of synthesis and flexibility in processing [7].

Conducting polymers are a class of functional polymers that have alternating single and double carbon–carbon bonds along the polymeric chains. The highly conjugated polymer chain can be assigned reversible chemical, electrochemical and physical properties controlled by a doping/de-doping process, which makes these polymers very attractive as transducer materials in various sensing devices [8].

2. The discovery of conducting polymers

The electrically conducting polymer (CPs) polypyrrole (PPy) dates back to the 1960s, but little was understood about the polymer at this time and the discovery was essentially lost [9]. It was only in 1977, when Alan MacDiarmid, Hideki Shirakawa, and Alan Heeger reported a 10 million-fold increase in the conductivity of polyacetylene doped with iodine, that the first inherently conductive polymer was recognized [10, 11]. Although polyacetylene, a non-cyclic polylene, is still one of the most studied polymers in this field, it has significant limitations, such as difficulty with processing and high instability in air. Unlike polyacetylene, polyphenylenes, which are cyclic polyenes, are known to be thermally stable as a result of their aromaticity [12]. Consequently, the development of such aromatic CPs for different applications has received much attention. Polyheterocycles (Figure 1), such as polyaniline, polypyrrole, polythiophene, poly(paraphenylenevinylene), poly (3,4-ethylenedioxythiophene), poly(paraphenylene), developed in the 1980s, have since emerged as another class of aromatic CPs that exhibit good stabilities, conductivities, and ease of synthesis [13, 14].

Table 1. Conductivity of common CPs. [7].

<table>
<thead>
<tr>
<th>Conducting polymer</th>
<th>Maximum conductivity (S/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyaniline (PA)</td>
<td>200–1000</td>
</tr>
<tr>
<td>Polyparaphenylene (PPP)</td>
<td>500</td>
</tr>
<tr>
<td>Polyparaphenylene sulfide (PPS)</td>
<td>3–300</td>
</tr>
<tr>
<td>Polythiophene (PPv)</td>
<td>1–1000</td>
</tr>
<tr>
<td>Polypyrrole (PPy)</td>
<td>40–200</td>
</tr>
<tr>
<td>Poly(paraphenylenevinylene)</td>
<td>10–100</td>
</tr>
<tr>
<td>Polyisothionaphthene (PITN)</td>
<td>1–50</td>
</tr>
<tr>
<td>Polyaniline (PANI)</td>
<td>5</td>
</tr>
</tbody>
</table>

Fig. 1. Chemical structures of typical conducting polymers, [15].

Table 1 presents a broad range of different CPs and their conductivities.

3. Syntesis of conducting polymers

CPs can be synthesized either chemically or electrochemically, with each having advantages and disadvantages as summarized in Table 2 [16].

Different methods of chemical synthesis include either condensation polymerization or addition polymerization. Condensation polymerization proceeds via the loss of small molecules, such as hydrochloric acid or water. Radical, cation, and anion polymerizations are all examples of addition polymerization, which are distinguished by the respective radical, cation, or anion intermediate state of the live or reactive end of the polymer chain during synthesis. Chemical synthesis not only provides many different possible routes to synthesize a variety of CPs, but also permits the scale-up of these materials, which is currently not possible with electrochemical synthesis.

Electrochemical synthesis is a common alternative for making CPs, particularly because this synthetic procedure is relatively straightforward. Electrochemical preparation of CPs dates back to 1968 when “pyrrole black” was formed as a precipitate on a platinum electrode by exposing an aqueous solution of pyrrole and sulfuric acid to an oxidative potential.
Carbon atoms are in \(sp^2\) configuration in \(\pi\) bonding and orbitals of successive carbon atoms overlap providing delocalization of electrons along the backbone of polymer [17]. This delocalization provides the charge mobility along the backbone of the polymer chain and induces unusual properties such as electrical conductivity, low ionization potential, low energy optical transitions and high electron affinity. The \(\pi\) bonds in conjugated polymers are highly susceptible to chemical or electrochemical oxidation or reduction. The origin of electrical conduction in conducting polymers has been ascribed to the formation of non-linear defects such as solitons, polarons or bipolarons formed either during doping or polymerization of a monomer [6].

Prior to doping, these systems are insulative (\(\sim 10^{-10}\) S/cm; \(S = 1/\Omega\)); however, the electrical conductivity of polyheterocyclic films, for instance, can be augmented up to 12 orders of magnitude (\(\sim 12\) S/cm) depending on the polymer system and the type and extent of doping.

Conductivity is a measure of electrical conduction and thus a measure of the ability of a material to pass a current. Generally, materials with conductivities less than \(10^{-8}\) S/cm are considered insulators, materials with conductivities between \(10^{-8}\) and \(10^{-3}\) S/cm are considered semiconductors, and materials with conductivities greater than \(10^{-3}\) S/cm are considered conductors. Conductivity is the inverse of resistivity and therefore has the units of inverse ohms (\(\Omega^{-1}\)), also known as Siemens (S) [7].

Basically, doping is the process of oxidizing (p-doping) or reducing (n-doping) a neutral polymer and providing the dopant (counter-ion of opposite charge), which becomes closely associated to the charged CP backbone. The passage from an undoped to a doped state corresponds to the acquisition of a conductive ability from a semiconductive form [18]. Doping can be performed chemically or electrochemically and is dependent on oxidation potential. The oxidation potential of oligomers

### Table 2. Comparison of chemical and electrochemical CP polymerization, [16].

<table>
<thead>
<tr>
<th>Polymerization approach</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical polymerization</td>
<td>- Larger-scale production possible</td>
<td>- Cannot make thin films</td>
</tr>
<tr>
<td></td>
<td>- Post-covalent modification of bulk CP possible</td>
<td>- Synthesis more complicated</td>
</tr>
<tr>
<td></td>
<td>- More options to modify CP backbone covalently</td>
<td></td>
</tr>
<tr>
<td>Electrochemical polymerization</td>
<td>- Thin film synthesis possible</td>
<td>- Difficult to remove film from electrode surface</td>
</tr>
<tr>
<td></td>
<td>- Ease of synthesis</td>
<td>- Post-covalent modification of bulk CP is difficult</td>
</tr>
<tr>
<td></td>
<td>- Entrapment of molecules in CP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Doping is simultaneous</td>
<td></td>
</tr>
</tbody>
</table>

Today, electrochemical polymerization is performed using a three-electrode configuration (working, counter, and reference electrodes) in a solution of the monomer, appropriate solvent, and electrolyte (dopant). Current is passed through the solution and electrodeposition occurs at the positively charged working electrode or anode. Monomers at the working electrode surface undergo oxidation to form radical cations that react with other monomers or radical cations, forming insoluble polymer chains on the electrode surface. A number of important variables must be considered, including deposition time and temperature, solvent system (water content), electrolyte, electrode system, and deposition charge. Each of these parameters has an effect on film morphology (thickness and topography), mechanics, and conductivity, which are properties that directly impact the utility of the material for biomedical applications [7].

The most significant difference between electrochemical and chemical methods of CPs synthesis is that very thin CPs films on the order of 20 nm can be produced using the electrochemical technique, whereas powders or very thick films are typically produced with chemical polymerization. All CPs can be synthesized chemically, but electrochemical synthesis is limited to those systems in which the monomer can be oxidized in the presence of a potential to form reactive radical ion intermediates for polymerization. The standard CPs (PPy, PT, PANI, PEDOT) can be polymerized both chemically and electrochemically; however, several novel CPs with modified monomers are only amenable to chemical polymerization [7].

### 4. Conductivity and doping of conducting polymers

Compared to saturated polymers, conducting polymers have different electronic structures. Chemical bonding in conducting polymers provides one unpaired electron, i.e. \(\pi\) electron per carbon atom in the backbone of the polymer.
decreases as the number of monomers in the chain increases; therefore during electrochemical synthesis doping occurs because the oxidation potential for doping the CP polymer is lower than that required for polymerizing the CP.

Conductivity can be augmented by increasing the doping percentage and varying the dopant. The chemical nature of the dopant not only affects electroactivity, but also affects surface and bulk structural properties. In addition, small and large dopants can both modulate electrical conductivities and surface structural properties, but larger dopants, can change polymer density and more dramatically affect characteristics such as surface topography and physical handling properties [19]. CPs can be doped with a variety of molecules, such as small salt ions, peptides, or polymers, including polysaccharides and proteins.

5. Conducting polymer based enzyme biosensors

A biosensor is composed of a sensing element (biomolecule) and a transducer [20]. The sensing element interacts with the analyte of interest producing a chemical signal that is transmitted to the transducer, which ultimately transforms the input into an electrical signal. Enzymatic biosensors utilize the biospecificity of an enzymatic reaction, along with an electrode reaction that generates an electric current or potential difference for quantitative analysis. The biomolecules such as glucose, cholesterol, are important analytes due to their adverse effects on health. Enzymatic biosensors utilize the biochemical reactions, analyte and enzyme resulting in a product (hydrogen peroxide) that can be detected and quantified using a transducer. In general, many oxidoreductases including glucose oxidase catalyze the oxidation of substrates by electron transfer to oxygen to form hydrogen peroxide. These oxidoreductase enzymes can be immobilized on conducting polymer surfaces and the H$_2$O$_2$ formed as a result of enzyme and the corresponding analytes may be measured amperometrically [6]. However, it has not been possible to discriminate between the direct electron transfer from the oxidation of hydrogen peroxide at polymer surface and that at the underlying electrode [6]. Since conducting polymers are insoluble in aqueous solutions, electropolymerization has been frequently used to create a matrix for immobilization of enzymes at the electrode surface, and the sensor response was obtained by the oxidation of hydrogen peroxide [21], [22].

CPs are extensively used as transducers that integrate the signals produced by biological sensing elements such as enzymes (Table III).

<table>
<thead>
<tr>
<th>Table III. Examples of biosensors using conducting polymers, [23].</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analyte (sensing element)</strong></td>
</tr>
<tr>
<td>Glucose (glucose oxidase)</td>
</tr>
<tr>
<td>Cholesterol (cholesterol oxidase/esterase)</td>
</tr>
</tbody>
</table>

The most common types of transducers are amperometric and potentiometric. An amperometric biosensor measures the current produced when a specific product is oxidized or reduced (redox reaction of a substrate in an enzyme) at a constant applied potential [20]. The CPs mediate the electron transfer (via hydrogen peroxide) between an enzyme, such as an oxidase or dehydrogenase, and the final electrode [20]. Potentiometric biosensors use ion-selective electrodes as physical transducers.

The immobilization of the sensing bioelement (probe), which specifically recognizes the analyte (target), onto a transducing surface, is the key-step in the construction of biosensing devices. The choice of the immobilization method depends mainly on the bioelement to be immobilized, the nature of the solid surface and the transducing mechanism [24]. A current problem, however, is the lack of stability and activity of the bioelement in the solid–liquid interface, which is related with the need for the biomaterial to be well fixed on, or within, the substrate [25]. Regardless the immobilization method to be used, it should be simple to carry out, highly reproducible (to favour large-scale production of the biosensor) and avoid non-specific binding and extreme environmental conditions [24]. In addition, the biomolecule to be immobilized must be easily accessible after immobilization and chemically inert towards host structure [26].

Table 4 summarizes the main categories of immobilization techniques of biological sensing elements on CPs[7]. Two main classes are distinguished: non-covalent and covalent modifications. Non-covalent modifications include adsorption, physical entrapment, and affinity binding. Covalent immobilization includes all techniques that create a covalent bond between the conducting substrate and the biomolecule.
Immobilization by physical or electrochemical adsorption techniques is the simplest method to immobilize biomolecules. As an example, glucose oxidase has been adsorbed onto PPy for an amperometric sensor and was shown to detect glucose over a wide range of concentrations (2.5–30mM) using dimethylferrocene as an electron transfer mediator [27].

An alternative to adsorption is physical entrapment of the desired biomolecule during electropolymerization, which is one of the most extensively used techniques. During this process monomer, dopant, and biomolecules are mixed in a single solution used for electrochemical polymerization. This process is usually performed under mild conditions (neutral pH, aqueous, low oxidation potentials) without chemical reactions that could alter the activity of proteins, and only requires a single step for both polymerization and molecule immobilization. For this reason Cps, such as PPy, are frequently used to entrap biomolecules [28]. Many early applications successfully entrapped glucose oxidase (GOx) in PPy films [7].

Affinity binding methods are based on immobilizing molecules on the surface of CPs via strong non-covalent interactions. This technique allows control over orientation of the immobilized molecules by adjusting the location of the binding elements, which increases the activity and accessibility of the biological sensing elements.

Covalent bindings are stronger and therefore less prone to biomolecule detachment, thus increasing the stability of the linkage. Covalent linking of a biomolecule to a polymer matrix is basically a two-step process: functionalization of the analyte and covalent immobilization. This allows selecting optimal reaction conditions for each step [29]. A covalent immobilization strategy was employed to couple glucose oxidase (GOx) via acyl azide derivatives with polyacrylamide for glucose detection [30].

5.1. Polypyrrole and polyaniline

PPy is considered nowadays the most promising CP for the development of biosensor devices owing to its good biocompatibility, conductivity, stability and efficient polymerization at neutral pH. Films of PPy and derivatives can be easily formed from aqueous solutions and have a high degree of selectivity due to the inherent size-exclusion property [31]. Furthermore, the polymer is a good energy transducer (redox-activity), an efficient protector of electrodes against interfering materials and provides easy ways to immobilize biomolecules [25]. It is also an inherently biocompatible material since its high water content ensures minimum disturbance of the biologically active compound in the biosensor [25]. PPy is able to directly transducer the analytical signal generated by some redox enzymes even if the redox centre is deeply buried in the protein globule, but modification with covalently attached redox groups might facilitate electron transfer.

Table IV. Immobilization techniques of biomolecules on conducting polymers for biosensing devices, [7].

<table>
<thead>
<tr>
<th>Immobilization technique</th>
<th>Principles of immobilization</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-covalent techniques</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adsorption</td>
<td>Electrostatic forces, hydrogen bonding, Van der Waal's forces, etc.</td>
<td>Simple</td>
<td>Biomolecule loss (desorption)over time</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Limited control over immobilization</td>
</tr>
<tr>
<td>Entrapment</td>
<td>Molecule incorporation during electropolymerization</td>
<td>Simple</td>
<td>Potential loss of biomolecule activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Steric and diffusion constraints</td>
</tr>
<tr>
<td>Affinity binding</td>
<td>High affinity interactions such as avidin-biotin</td>
<td>Control over molecule orientation</td>
<td>Requires high biomolecule concentration</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Requires pre-immobilization of one of the affinity molecules</td>
</tr>
<tr>
<td><strong>Covalent techniques</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical conjugation</td>
<td>Surface chemical reaction between functional groups</td>
<td>Tighter control over immobilization</td>
<td>Complex</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Conditions are not always appropriate for biomolecules</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Potential loss of biomolecule activity</td>
</tr>
</tbody>
</table>
PPy and derivatives have a wide versatility of application and a large variety of biomolecules can covalently link their pyrrole groups. The polymer is usually generated through electropolymerization of pyrrole monomers around oligomer nucleation sites deposited onto electrode surfaces [32]. By opposition, chemically-induced polymerization results in poorer adherence of the deposited PPy onto surfaces, and with a lesser extent, since most of the PPy is formed on the bulk solution and not onto the surface. This is why electrochemical polymerization has been the preferred method when thin PPy layers are requested [25].

Potentiometric biosensors with PPy have also been designed. Enzymes, and particularly oxidases, have been the preferred biomolecules for entrapment within PPy matrices. The most successful biosensors based on oxidases entrapped within PPy were reported when redox polymers were constructed with pyrrole copolymerized with pyrrole substituted by redox mediators [25]. The successful application of enzyme-modified PPy in the design of catalytic biosensors started by entrapment of GOx and PANI within PPy for glucose biosensing [18]. Many biosensors are recently incorporating nanotechnological structures, as shown by a report of the synthesis of semi-conducting PPy/polyacrylamide microparticles and immobilized GOx before starting polymerization [18]; the microparticles with the immobilized enzymes were used as the biological layer of an amperometric glucose biosensor.

The other well-known CPs, PANI, has also received considerable attention as a transducing support in biosensors, mainly due to its remarkable stability and processability. Electrochemical biosensing is among the most promising applications of PANI [33].

Very often, PANI synthesis is carried out using aniline hydrochloride solution or a mixture of an aniline monomer and a diluted hydrochloride acid [34]. As any other CPs, PANI can function as an immobilizing substrate or an electrocatalyst, but the need for biodetection at nearly neutral pH leads to electroinactivity of deposited films and thus discourages its use as a biosensing material [25]. Biosensing applications include enzyme immobilization in PANI films for estimation of glucose, urea and triglycerides [18]. Unlike PPy, PANI cannot be easily deposited from neutral pH aqueous solutions containing the monomer [25]. PANI-modified electrodes render very high signal amplification and prevent electrode fouling. Free-PANI is usually less favorable than other materials for biosensor construction owing to its relatively lower conductivity and randomly-oriented nanofiber morphology, leading to decreased detection sensitivity [18].

6. Conclusion

CPs are organic in nature, making them more likely to be biocompatible; further, the presence of a conjugated backbone within the polymer endows it with the ability to conduct electrons, like metals/semiconductors, and unlike any other polymer. In addition to these highly desirable properties, the ease of preparation and modification of CPs have made them a popular choice for many applications.

In conclusion, CPs are inexpensive, easy to synthesize and versatile, as their properties can be tailored by a wide range of entrapped or dopant molecules; in addition, they can be tightly interfaced with biomolecules for improved transduction [20]. Their advantages for biosensors applications include biocompatibility, ability to entrap and controllably release biological molecules (reversible doping), efficient charge-transfer from a biochemical reaction and ability for tuning electrical, physical, chemical and optical properties in view of a particular application [35].

However, despite the undeniable advantages of CPs, further improvement of reproducibility, stability and easiness of fabrication must be pursued in order to support a broader range of biosensing applications.

References


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PhD student, Ștefan cel Mare University, Suceava, Faculty of Food Engineering, PhD domain: Materials engineering, PhD thesis: *Research and contributions to the development of support materials for biosensors*, PhD supervisor: Prof. Eng. Gheorghe GUT, PhD.