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REVIEW ARTICLE

BIOPRODUCTION AND RELEVANCE OF CONDUCTING POLYMERS: POLYPYRROLE

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Abstract: Conducting polymers (CPs) have been under extensive research and development during the past decades due to their inherent electric conductivity. Responsive to electrical fields and stimuli, they are suitable for numerous biological and medical applications. Hence, an attempt has been made to review the current understanding of conductive materials regarding both synthesis and utilization outlining biocatalytic pathways. In this paper, perspectives for modification and design along with appliance as biomaterial have been presented for polypyrrole as it is one of the most studied CPs.

Keywords: conductive materials, polypyrrole, biocatalysis, modern applications

Discovery and properties of conducting polymers

Conducting polymers (CPs) are defined as organic materials that possess electrical and optical properties similar to metallic conductors and semiconductors while their synthesis and processability are characteristic to conventional polymers (Heeger, 2001). The importance of their discovery relies in the possibility of substituting metallic conductors and semiconductors with polymeric materials designed for specific applications. While CPs render the opportunity of being tailor-made in respect to their electrical, mechanical, optical and thermal properties, their solubility in most common solvents still represents a challenge (Kumar and Sharma, 1998).

The discovery of conducting polymers started with polypyrrole in 1960s (Feast 1986, Street 1986), followed by Shirakawa *et al.* (1977) that proved that halogen doping of polyacetylene leads to a superior conductivity. Subsequently, Diaz *et al.* (1979) reported a major progress obtaining pyrrole black as a highly conductive, stable and manageable film.

Polyacetylene represents one of the most studied CPs even though its non-cyclic polyene structure causes significant limitations, such as environmental instability and poor processability (Su *et al.*, 1979).

Unlike polyetilene, polyheterocycles exhibit superior stability and conductivity due to their aromatic characteristics which made them available for numerous different applications. The reader is referred to Heeger (2001) for a comprehensive review on the generation of aromatic conducting polymers, developed in 1980s such as polypyrrole (PPy), polythiophene (PT), polyaniline (PANI), and poly(3,4-ethylenedioxythiophene) (PEDOT) (Figure 1) (Ravichandran *et al.*, 2010).

Conjugated polymers represent sequences of consecutively alternating single and double carbon–carbon (carbon–nitrogen) bonds. Their structure entails a singular σ-bond backbone with overlapping sp^2 hybrid orbitals that allow delocalization of electrons over the entire molecule (Ramanavičienė and Ramanavičius, 2004a).

This structure allows low-energy optical transitions and ionization potentials, detection and undergoing of redox processes as well as polymer charge. Owing to their high-electron affinities, conducting polymers can act as electrode materials while redox activity can switch at specific potentials (Adeloju and Wallace, 1996; Ateh *et al.*, 2006).

The redox switching constitutes a remarkable asset and is the basis of many applications. It can be accompanied by the movement of dopant ions into or out of the material, process called doping (Ahuja *et al.*, 2007). Mostly CPs are insulators in their neutral state (e.g. polypyrrole) and through oxidation (p-doping) or less frequently reduction (n-doping) they attain carriers for conductivity. Their conjugated structure is acquired by formation of nonlinear defects such as solitons, polarons or bipolarons during either polymerization or doping of polymeric chain regions owning unpaired electrons. PPy at different oxidation states is illustrated in Figure 2 (Conzuelo *et al.*, 2010). It was demonstrated that the polymer does not engage in the charge transfer (Saxena and Malhotra, 2003; Otrokhov *et al.*, 2013).

For PPy, charging is achieved in oxidized state which implies the diffusion of a counterion into the polymer. In its reduced state the counterion is removed and the backbone becomes neutral (Skotheim and Reynolds, 2007).

If it is doped with small counter ions such as (-Cl, -ClO_4, -NO_3) its behaviour will be of anion exchanger due to the high mobility of this ions in the polymer matrix.

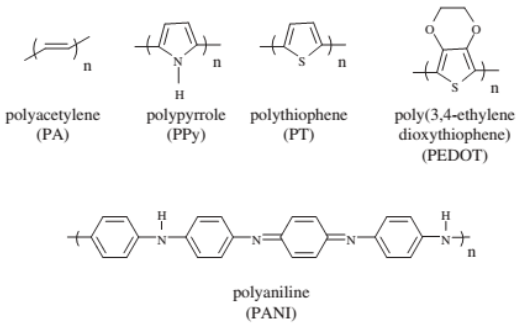

Figure 1. *Chemical structure of the most commonly studied CPs* (Ravichandran *et al.*, 2010)

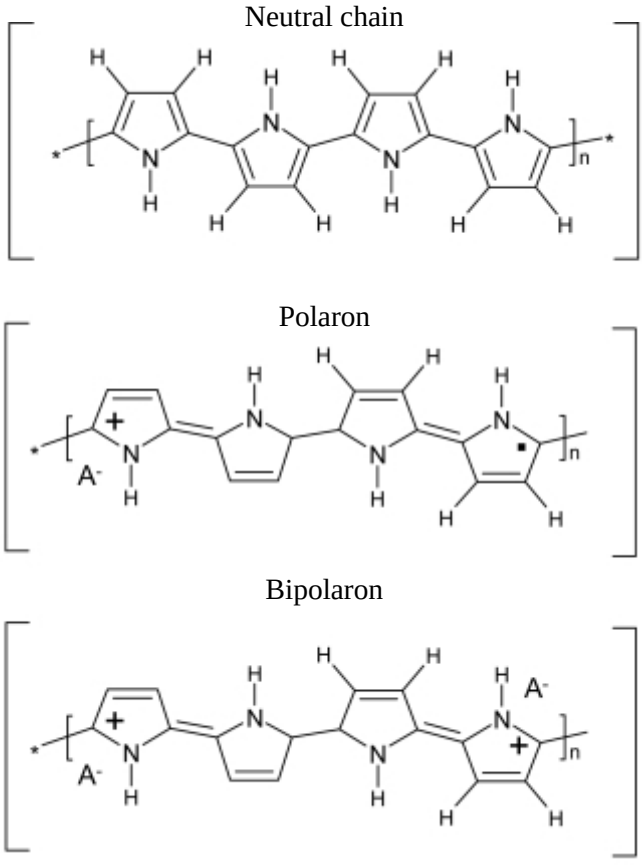

Figure 2. *Polypyrrole at different oxidation levels* (Conzuelo *et al.*, 2010)

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In order for the polymer to exhibit cation exchanger behaviour there have to be incorporated large anionic molecules such as polystyrene sulfonate (PSS) due to their immobility within the polymer matrix (Şahin *et al.*, 2008).

The relevance of doping consists in the opportunity to select counterions and functional groups to induce specific functionality to the polymeric material tailoring it for different applications (e.g. actuator applications benefit from the significant change in polymer volume due to the diffusion of mobile counter ions species) (Smela, 2003; Geetha *et al.*, 2006).

Methods of CPs synthesis

The method used for the synthesis of CPs represents a key aspect in designing for specific applications. It has been an extensively researched area seeing that it has a direct impact on the final product.

Electrochemical synthesis

'Pyrrole black' was firstly synthesized in 1968 using the electrochemical procedure, specifically an aqueous solution of pyrrole and sulfuric acid was exposed to an oxidative potential which led to the formation of polypyrrole precipitate on the platinum electrode (Dallolio *et al.*,1968).

The basic electrochemical synthesis entails the dissolution of the monomer in an appropriate solvent, followed by an anodic potential at the working electrode surface leading to the oxidation of the monomer. The monomer generates radical cations (several resonance forms, Figure 3a) that owning an unpaired electron density in the α-position dimerize (loss of two protons, Figure 3b). The dimers react with another monomer radical cation and forms a trimer dication that loses a proton resulting a neutral trimer (Figure 3c). The propagation continues by the same process: oxidation, coupling, deprotonation until insoluble polymer chains are attained (Figure 3d) (Sadki *et al.*, 2000).

This type of polymerization implies a three-electrode configuration (working, counter, and reference electrodes) in a solution of the monomer, an appropriate solvent and an electrolyte, that plays also the role of dopant and is carried out using a potentio-galvanonstat.

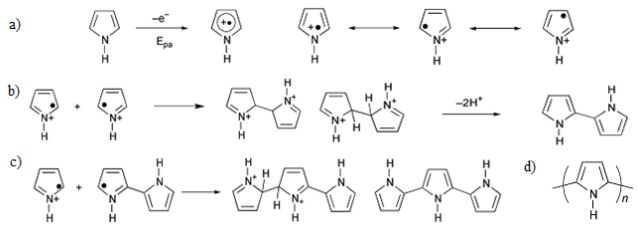

Figure 3. *Electrosynthesis of polypyrrole (adapted by* Sadki *et al.*, 2000*)*

The techniques used for obtaining the polymeric film can vary between cycling potential of fixed potential methods, pulsed potential approaches or galvanostatic conditions. While galvanostatic conditions are recommended to obtain thick films, potentiostatic conditions are required for thin films (Li, 2002). Important parameters to be considered include: deposition time and temperature, solvent and electrode systems, as well as electrolyte and deposition charge, each of them having its own influence on the morphology, mechanics and conductivity of the resulting polymer (Guimard *et al.*, 2007).

Thus, it can be established that the electrochemical polymerisation presents both advantages such as simplicity of synthesis allowing simultaneous doping, as well as limitations, such as limited amount of final product and its poor processability (Ramanavičius *et al.*, 2005).

Chemical synthesis

Succeeding the electrochemical method, chemical polymerization stimulates the oxidation of the proposed monomer employing oxidative compounds (Henry *et al.*, 2001).

Chemical synthesis proceeds through condensation or addition reaction with the use of strong oxidants such as Fe^{3+}, Ag^{+}, I_2, Br_2, AsF_5 or Cu^{2+}. Condensation or step-growth polymerisation involves the loss of small molecules, such as hydrochloric acid or water, while addition is achieved by radical (cation or anion) intermediate

state of polymer chain (Malinauskas, 2001). Figure 4 describes an example for the chemical process of polymerization of polypyrrole using iron (III) chloride along with overall stoichiometry of reaction (Ansari, 2006).

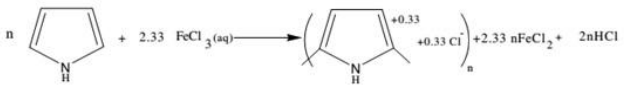

Figure 4. *Chemical polymerization of polypyrrole (Ansari, 2006)*

The main distinction between the electrochemical and chemical method of synthesis consists in the quantity and dimension of the resulting polymer because electrochemical yields thin CP films (20 nm) while chemically, thicker films or powders. Also, large-scale production has not been successfully achieved with electrochemical synthesis (Ansari, 2006). However, not all monomers are able of oxidizing in the presence of an electric potential. Standard conducting polymers such as PPy, PT, PANI, PEDOT are amenable to both synthesis methods (Guimard *et al.*, 2007).

As an improvement, chemical polymerization allows a variety of possibilities of synthesis for different CPs along with a certain degree of flexibility and processability which contribute to tailoring the materials for specific applications (Ramanavičius *et al.*, 2005). The impediments posed by the chemical approach consist in the high amount of oxidants required. For example, a molar ratio of 1:2.5 to 1:3 monomer: oxidant (Fe^{3+}:Cu^{2+}) is needed for pyrrole polymerization. Moreover, due to inter-chain strong links insolubility in common solvents still represents an issue (Dias *et al.*, 2006).

There is a strong necessity for the widespread utilization of CPs, thus, there have been attempted many different polymerization techniques. Investigations include photochemistry, metathesis, chemical vapour deposition, solid-state polymerization, soluble precursor polymer preparation, concentrated emulsion synthesis etc. (Kumar and Sharma, 1998). The main aim of this extensive research was the finding of a synthesis method that provides high processability and biocompatibility while being economically and environmentally benign (Zhou *et al.*, 2002).

Biocatalytic synthesis

The employment of biotechnology in polymer synthesis had a tremendous impact as it represents an asset in various scientific and technological fields including pollutant assaying agents, antibiotics, recombinant proteins, vaccines, antibodies (Loos, 2011; Miletić *et al.*, 2012, Rao *et al.*, 2014).

Enzymes represent bio-catalytic compounds with the ability to regulate the kinetics of biochemical reactions without being altered in the process. They behave as catalysts in living organisms displaying outstanding features such as activity, selectivity and specificity. The specificity can refer to differentiation between substrates (substrate specificity), similar molecule segments (regiospecificity) or optical isomers (stereospecificity) (Yildiz *et al.*, 2013). Therefore, they are considered appropriate for a wide range of applications such as chemical and pharmaceutical synthesis, biosensor applications, bioremediation etc. (Miletić *et al.*, 2012).

Enzyme-catalyzed synthesis has been investigated for CPs production as a way to address environmental and biocompatibility issues associated with traditional methods for polymerization (i.e. chemical, electrochemical) (Ramanavičius *et al.*, 2008).

While chemical polymer catalysis involves rigorous conditions of pH, temperature and strong oxidative compounds, biocatalysis is usually carried out in aqueous organic environments at average pH and temperature (Kaušaitė-Minkštimienė *et al.*, 2011) proving to be a reliable and environmentally friendly alternative (Shumakovich et al., 2012a).

Loos (2011) presents multiple areas of research involving enzyme-catalysed polymerizations.

Enzyme-catalyzed synthesis has been widely reported for the polymerization of advanced π-functional materials. The reaction is biocompatible, does not produce oxidation by-products and the employed catalysts are derived from sustainable sources. In addition to being environmentally benign, it can provide a higher degree of control regarding kinetic conditions

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rendering the opportunity of observing monomer-dopant interactions, which is usually a difficult parameter to observe. There is also potential for obtaining high yields of product (Bouldin *et al.*, 2011; Kaušaitė-Minkštimienė *et al.*, 2011).

Oxidoreductases are highlighted as they constitute widely studied bio-initiators used for bioremediation (Whiteley and Lee, 2006; Popa *et al.*, 2014) lignin degradation (Popa *et al.*, 2010), defence against pathogens (Kumar *et al.*, 2010) etc.

Peroxidases (EC 1.11.1) and laccases (EC 1.10.3.2) prevail in the biocatalytic polymerization field even if they exhibit multiple differences concerning catalytic mechanism and active site structure. The electron transfer mechanism catalyzed by these enzymes as described by Hollmann and co-workers (Hollmann, 2010; Hollmann and Arends, 2012) is based on the electron abstraction/deprotonation from the enzymatic substrate in order to produce a new radical, the hydrogen is subsequently transferred to a suitable acceptor with water as final by-product. Hydrogen peroxide provides a suitable electron acceptor for peroxidases, while laccases use molecular oxygen (Cojocaru *et al.*, 2007).

Examples of peroxidases applications include generation and lignification of cellular walls tissue protection against pathogenic factors, oxidation of indolyacetic acid etc. (Hofrichter, 2002).

Heme-peroxidases, namely horseradish peroxidase (HRP) and soybean peroxidase (SBP) have been preferred for the enzymatic induction of radical processes due to their ability to oxidize a wide variety of organic and inorganic compounds. The active center of the enzyme is the prosthetic group ferriprotoporphyrin IX (heme) whose ferric ion is able to reduce hydrogen peroxidase with oxidation of different substrates. The reaction mechanism of HRP is presented in Figure 5 (Rich and Iwaki, 2007). H_2O_2 contributes to the peroxidase-initiated polymerizations in a dual manner. While it is essential for the catalytic action serving as substrate, a high amount of it plays an inhibitive role (Hofrichter *et al.*, 2010; Hollmann and Arends, 2012).

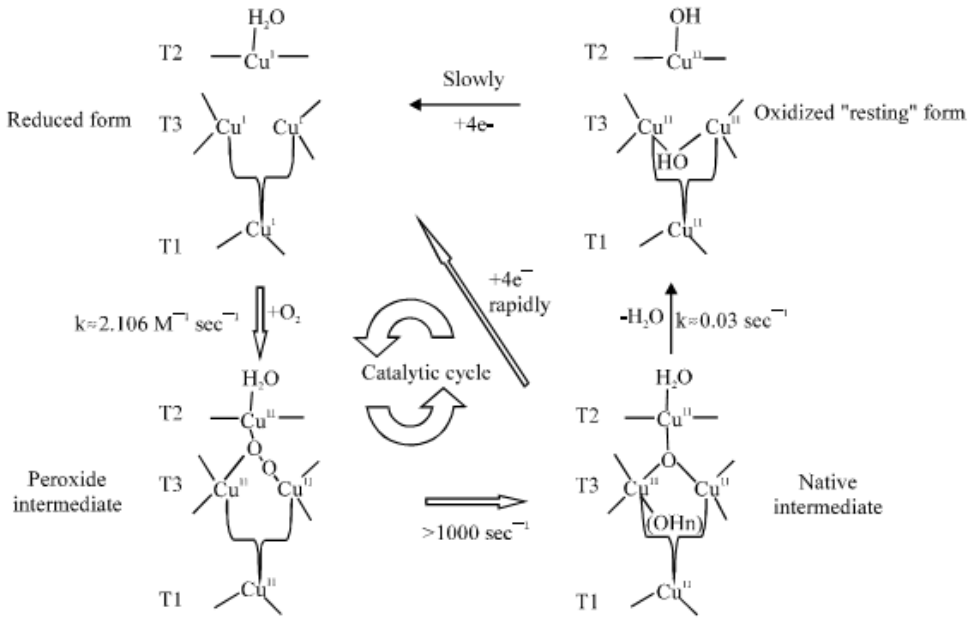

Figure 5. *Catalytic cycle of HRP-peroxidase (Rich and Iwaki, 2007)*

Laccases are known to be involved in many bioconversions with practical applications (Mayer and Staples, 2002). The so-called blue-copper oxidases are part of a larger group of enzymes termed the multi-copper enzymes. They are predominantly found in fungi but also in bacteria, higher plants and insects. These copper-containing enzymes catalyze the oxidation of various substrates with the simultaneous reduction of molecular oxygen to water. Because of their high stability,

selectivity for phenolic substructures, and mild reaction conditions, laccases are attractive for fine chemical synthesis (Kunamneni *et al.*, 2008; Witayakran and Ragauskas, 2009).

Laccases contain four copper ions classified in three types termed Type 1 copper ion (T1), Type 2 (T2) and Type 3 (T3). Optical and electron paramagnetic resonance (EPR) spectroscopy techniques reveal the distinctions between types. T1 copper represents the primary redox center or the site where the reducing Examples of enzymes employed for bio-oxidation include: peroxidases (horseradish peroxidase, soybean peroxidase, palm tree peroxidase) or bilirubin oxidase, glucose oxidase, laccase. Since polyaniline (PANI), polypyrrole (PPy) and polyethylenedioxythiophene (PEDOT) have been the most extensively studied conducting polymers, most of the initiating studies exploring enzymatic pathways of polymerization were focused on them.

Enzymatic synthesis of conducting polyaniline has been attempted since 1990 by Aizawa *et al.* using the copper-containing enzyme, bilirubin oxidase (BOD). The polymerization took place at the contact surface between BOD-impregnated solid matrix and buffer solution containing aniline. The polymeric film obtained retained part of the enzymatic activity of the catalyst conferring promising potential. Kobayashi *et al.* (1995) and Wang *et al.* (1999) reported peroxide-catalyzed oxidation for various aniline derivatives as well as the usage of negatively charged polymer sulfonated polystyrene (SPS) as a template in the synthesis of PANI resulting in PANI/SPS complexes with medium electrical conductivity and processability.

PANI/SPS complexes have been also enzymatically synthesized by Sakharov *et al.* (2003) involving royal palm tree peroxidase (RPTP) that proved to have very high stability at extreme temperatures and under strongly acidic and alkaline conditions unlike HRP which is inactivated at pH 4.5 or lower.

Cruz Silva and co-workers (Cruz-Silva *et al.*, 2005; Cruz-Silva *et al.*, 2006) proposed template-free soybean peroxidase (SBP) - catalyzed synthesis of

Copolymers of poly (aniline-co-3-aminobenzene-boronic acid) [poly(aniline-co-AB)] have been developed by Huh *et al.* (2007) with the usage of horseradish peroxidase together with SPS as anionic

substrate binds electrons from the electron donors while at the T2/T3 copper cluster is the site where oxygen binds and the reduction to water takes place (Claus, 2004; Desai and Nityanand, 2011). Thus, fully oxidized laccase is transformed via four successive, fast single electron transfer steps into the fully reduced laccase. The description of the catalytic cycle of laccase is given in Figure 6 (Roriz *et al.*, 2009).

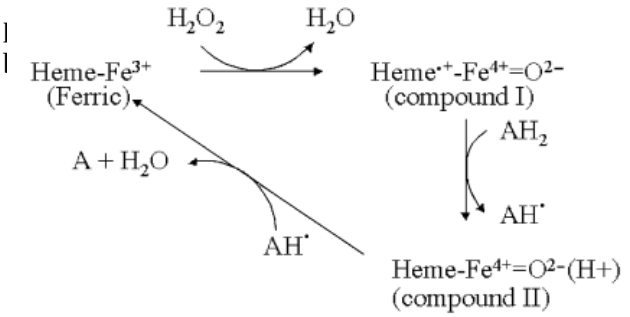

Figure 6. *Catalytic mechanism and Cu-coordination network of laccase (Roriz et al., 2009)*

PANI and horseradish peroxidase (HRP) for bioproduction of PPY (Cruz-Silva *et al.*, 2008).

Caramyshev *et al.* (2005) carried out a comparison of five plant peroxidases (horseradish, royal palm tree leaf, soybean, cationic and anionic peanut peroxidases) concerning their stability for the synthesis of PANI complexes. The research proved that palm tree peroxidase has the best performance under acidic conditions and a new complex PANI - poly(2-acrylamido-3-methyl-1-propanesulfonic acid) (PAMPS) was generated. Subsequently, they examined the catalytic efficiency of horseradish and palm tree peroxidases involving dodecylbenzene-sulfonic acid (DBSA) as a dopant for obtaining chiral PANI (Caramyshev *et al.*, 2007).

Xu *et al.* (2006) presented a comprehensive review on enzymatic mechanisms for aniline and aniline derivatives polymerization including peroxidases in different environments in order to optimize polymer characteristics.

 Received July 10, 2015 / Revised August 1, 2015/Accepted August 2, 2015

Aniline derivatives monomers that are not amenable to chemical polymerization have also been polymerized with the aid of horseradish peroxidase. It has been identified that monomers with methoxy The polyanilines resulted proved increased solubility in water and organic solvents such a dimethyl-formamide (DMF), ethanol and dimethyl sulfoxide (DMSO) and they are available for further functionalization in ortho and meta positions (Kim *et al.*, 2007).

HRP-catalyzed polymerization in presence of polyanionic template SPS was also attempted for conducting PPy by Nabid and Entezami (2004) and the result was outstanding providing a water-soluble polymer that required minimal purification. Consequently, Nabid *et al.* (2007) enzymatically synthesized different ring-substituted polyalkoxyanilines with variation of groups, such as ortho-methoxy, meta-methoxy, ortho-ethoxy and meta-ethoxy using horseradish peroxidase in order to form polyalkoxyanilines/SPS complexes.

Water-soluble PANI has been enzymatically prepared using HRP with an innovative polyanionic template, namely sodium dodecyl diphenyloxide disulphonate (DODD) by Rumbau *et al.* (2007a). Additionally, Rumbau and co-workers (Rumbau *et al.*,2007b) introduced HRP-catalyzed polymerization of water-soluble PEDOT (poly(3,4-ethylene-dioxythiophene)) in the presence of SPS template.

Sikora *et al.* (2009) reported a biphasic catalytic system involving horseradish peroxidase encapsulated within 3,4-ethylenedioxythiophene droplets at interface with aqueous phase poly (sodium 4-styrenesulphonate) template that resulted in PEDOT polymer with high conductivity values of $2 \cdot 10^{-3}$ S/cm. Additionally, this method provides a facile manner of encapsulating enzyme within CPs.

Another enzyme employed for the development of CPs is glucose oxidase (GOx). The catalytic mechanism of GOx is considered different in the sense that instead of requiring the presence of hydrogen peroxide it generates it using both glucose and dissolved oxygen as substrates. The H_2O_2 resulted initiated the polymerization of both aniline (Kaušaitė *et al.*, 2009) and pyrrole (Ramanavičius *et al.*, 2006).

and methyl blocking groups in orto/meta position can lead to the conducting form of para-linked polyaniline without the use of an anionic template.

Laccase can also oxidize various phenolic compounds. Its mechanism consists in initiating the polymerization process by producing cation radicals which coupling contribute to the growth of polymeric chain (Mazur *et al.*, 2009). Laccase isolated from fungi *Trametes hirsuta* has been mostly employed ***in biocatalytic polymerizations.***

Karamyshev *et al.* (2003) reported laccase-catalyzed synthesis of water-soluble conducting polyaniline in presence of SPS template. Lacasse produced by *Coriolus hirsutus* strains, proved impressive advantages compared to horseradish peroxidase such as high activity and stability under acidic conditions. Optically active PANI was synthesized by laccase-catalyzed reaction in presence of chiral dopant, *S*- or *R*-camphorsulfonic acid by *in situ* deposition method (Vasil'eva *et al.*, 2007).

Laccase biosynthesized by *Trametes hirsuta* strains was also used for the polymerization of aniline in micellar solutions of sodium dodecylbenzen-sulfonate (SDBS) by Streltsov *et al.* (2009). The anionic surfactant was employed in order to improve solubility and processability of the electroactive PANI/SDBS complexes. Likewise, Schumakovich and co-workers employed a high redox potential laccase to produce conducting PANI (Shumakovich *et al.*, 2012a) as well as conducting PEDOT (Shumakovich *et al.*, 2012b). Inorganic redox mediator potassium octocyanomolybdate was used in both polymerizations being able to increase the synthesis speed by switching from octocyanomolybdate^{4+} ion to octocyanomolybdate^{5+}.

As shown above, both hydrogen peroxide and oxygen can adequate oxidizers owing to their wide availability and low toxicity. Peroxidases and laccases have had successful effects in oxidizing monomeric molecules. However, there still is the necessity for redox mediators in order to increase the polymerization rate and for dopant templates that confer the desired features for the resulted polymer (Cruz-Silva *et al.*, 2008).

Insights for conducting polymers as biomaterials

Since CPs exhibit electrical and optical properties similar to metallic conductors and semiconductors, their applications are widely diversified. Originating in microelectronics industry as rechargeable batteries, photovoltaics, electronic and optical displays, molecular transistors, solar energy convertors, anti-static and anti-corrosives materials (Gurunathan *et al.*, 1999) their appliance progressed toward doping with biological macromolecules or even whole cells (Ateh *et al.*, 2006), electrical stimulation of biological tissues involving nerve, bone, muscle and cardiac cells, modulation of cell adhesion, DNA synthesis, protein secretion etc. (Shi *et al.*, 2004; Bidez *et al.*, 2006; Svirskis *et al.*, 2010).

Bioanalytical fields of applications such as biosensors, monitoring and controlled release of drug or metabolites have substantially employed conducting polymers due to their high sensitivity and selectivity and biocompatibility towards biological molecules (Ahuja *et al.*, 2007; Kaušaitė-Minkštimienė *et al.*, 2011). Recent developments include nanostructured biological systems (Ramanavičienė and Ramanavičius, 2004a), tissue-engineering scaffolds, neural probes, bio-actuators.

Richardson-Burns *et al.* (2007) investigated the possibility for a functional contact between a bio-electronic device and target tissue involving PEDOT, synthesized around living neuronal cells. The aim was to create a cell-patterned conducting polymer for expecting contact with electrically-responding tissues such as the brain and heart. It was stated that the resulted cell-polymer-electrode interface would represent an ideal candidate material for the development of a new generation of biomaterials.

Guimard *et al.* (2007) provided a state of the art review paper regarding biomedical applications for conductive materials. Gerard et al. (2002) and Malhotra *et al.* (2006) addressed biosensing applications while Geetha *et al.* (2006) focused on drug-delivery mechanisms. The employment of CPs in immunological, DNA and cell-based sensors have been reviewed by Andreescu and Sadik (2004), while Smela (2003) discussed the potential for actuator devices.

Biosensors and enzyme immobilization

Extensive research has been focused on extracting enzymes from tissues or producing them from bacterial cultures in order to immobilize them without loss of biological integrity (Ramanavičius *et al.*, 2005). Foremost, enzyme immobilization represents an accessible method for reusability of enzymes increasing their activity, selectivity as well as long-term stability.

The characteristics of immobilized enzymes systems are strongly related with the characteristics of both the enzyme and the immobilization material. Chemical, biochemical, mechanical and kinetic properties are influenced by the properties of the support material as well as its interaction with the immobilized enzyme (Miletić *et al.*, 2012). Furthermore, the underlying mechanism in biosensing devices consists in immobilization of bioactive molecules within polymeric structures and providing in-depth contact between the two elements (Gerard *et al.*, 2002).

Different techniques can be applied for immobilizing enzymes onto supports even by covalent links or non-covalent techniques such as adsorption, affinity binding, entrapment or encapsulation within a polymeric film (Guimard *et al.*, 2007).

A biosensor consists of a sensing element such as a biomolecule and a transducer that is able to transform the biochemical input received from the sensing molecule into electrical signal (Ahuja *et al.*, 2007). CPs are considered efficient transducers integrating the biochemical signal sensed from elements such as enzymes, microbial cells into electrical input so a target analyte can be monitored in a biological fluid considering the specific biomolecule that has been immobilized. The mechanism of recepting the chemical signal can be amperometric, potentiometric, conductometric, optical, calorimetric or piezoelectric (Gerard *et al.*, 2002).

Tailoring CPs for bio-sensing applications consists in adjusting the functionality, hydrophobicity and conductivity in order to incorporate the desired biomolecules rendering superior detection (Guimard *et al.*, 2007). The immobilization of biomolecules can proceed by electrochemical means, where the

 Received July 10, 2015 / Revised August 1, 2015/Accepted August 2, 2015

enzyme represents a dopant for the polymer matrix or recently, enzymatic synthesis allows the encapsulation of enzymes during the polymerization process (Song and Palmore, 2005; Ramanavičius *et al.*, 2006; Mazur *et al.*, 2009).

The underlying principle of enzymatic biosensors is that of the biochemical reaction between the substrate and the enzyme that generates a specific chemical compound (e.g. H_2, H_2O_2, NH_3) which can be detected and quantified by various techniques (Malhotra *et al.*, 2006).

Since Foulds and Lowe (1986) entrapped GOx in CP films for amperometric glucose sensors tremendous progress in the field has been made.

Trojanowicz *et al.* (1990) and Fortier *et al.* (1990) developed sensors for glucose detection by immobilizing GOx in electrosynthesized polypyrrole films showing that the key aspect in obtaining a high-magnitude response is the level of enzyme loading within the polymeric film. Later on, Zhu *et al.* (2005) reported a GOx/PPy sensor prepared onto a gold microelectrode that proved enhanced sensitivity. Wang *et al.* (2005) tested different surface morphologies for the Pt electrode involved in a GOx/PPy biosensing system.

Recent progress led to self-encapsulation of GOx within PPy (Ramanavičius *et al.*, 2005) respective PANI (Kaušaitė-Minkštimienė *et al.*, 2010) performing glucose detection. The reported bioanalytical devices displayed superior detection range and stability.

PANI derivatives such as polynitroaniline and poly(2-fluoroaniline) as well as [poly(An-FAn)] films have been used for glucose sensing by immobilization of GOx by Sharma and co-workers (Sharma *et al.*, 2001; Sharma *et al.*, 2003; Sharma *et al.*, 2004). Entrapment of GOx within polyaniline and polyaniline-co-poly(o-toluidine) (PANI co-POT) has been reported by Borole *et al.* (2004) showing fast response in glucose biosensing.

Horseradish peroxidase has been the subject of research in biosensors as well. The entrapment of HRP was reported by Ngamna *et al.* (2005) in order to render a polyaniline-derivative/polylysine complex water-insoluble for developing aqueous-based biosensors. Chen *et al.* (2006) exploited HRP-immobilized PEDOT films for amperometric sensors and Åsberg and Inganäs (2003) reported the design of a hydrogel bio-electrode consisting of an aqueous dispersion of PEDOT/PSS incorporating HRP enzyme.

Védrine *et al.* (2003) reported tyrosinase entrapment in electrochemically synthesized PEDOT in order to develop an amperometric biosensor for detecting phenolic compounds and herbicides. Consequently, Böyükbayram *et al.* (2006) used a pyrrole – thiophene copolymer to immobilize tyrosinase for the detection of phenolic substances in red wines and polypyrrole functionalized with polydimethylsiloxane (PDMS/PPY) matrixes entrapped tyrosinase in order to develop enzyme electrodes able to detect green and black tea compounds (Arslan *et al.*, 2005).

Singh *et al.* (2006) reported the co-immobilization of three enzymes (cholesterol oxidase, cholesterol esterase and peroxidase) onto PANI films for a cholesterol biosensor, while Chaubey *et al.* (2000) produced a biosensor able to detect low concentrations of L-lactate by physical adsorption of both lactate oxidase and lactate dehydrogenase on PANI films.

PANI-uricase amperometric biosensors with suitable characteristics have been reported by Kan *et al.* (2004) and Arora *et al.* (2007). Composite films of conducting polyaniline were also employed for electrochemical oxidation of β-nicotinamide adenine dinucleotide (NADH) for dehydrogenase-based biosensors (Bartlett *et al.*, 2002). Nanostructured PANI of controlled porosity and thickness was generated by Langer *et al.* (2004) for the development of a choline sensor by choline oxidase incorporation.

Whole cells have also been utilized in order to produce the necessary enzymes for biosensing systems on account of providing assets such as over-coming enzyme purification step, multi-enzyme behaviour without the need for cofactor/coenzyme.

For instance, the entrapment of banana pulp cells has been reported in an attempt to detect the conversion of dopamine to quinone catalysed by polyphenol oxidase (Sidwell and Rechnitz, 1985). Microbial cells of *Brevibacterium ammoniagenes* strain have been entrapped in conducting polymer complex

PSS-PANI as urease source in order to develop a conductometric urea biosensor (Jha *et al.*, 2009).

CP-based biosensors have been thoroughly researched seeing that they meet the requirements of modern bioanalytical analysis including multi-analyte multi-amperometric assays, high information density and miniaturization (Geetha *et al.*, 2006; Malhotra *et al.*, 2006). Additionally to the biosensing applications, other important fields of research for conducting polymers include drug delivery systems and bio-actuators.

3.2 Other applications

Concerning drug delivery systems (DDS), the main purpose is to develop a self-adjusting drug rate in response to modifications in local environment. Herein lies the necessity for a biosensing material that allows the adjustment of drug-dose according to external stimuli whether physiological or chemical (Svirskis *et al.*, 2010). In addition, safe release of drugs in specifically targeted areas of the body such as stomach (where low pH can degrade medication) or affected bone and tissue has been attempted.

Following tremendous efforts in the field of micro- and nanotechnology, polymeric structures (microspheres, micelles, hydrogel) have proven adequate for increasing drug targeting specificity, absorption rates, decreasing systemic drug toxicity and preventing biochemical degradation (Geetha *et al.*, 2006).

The underlying principle behind applying CPs in drug-delivery and bio-actuators applications is that of change in redox state (oxidation/reduction) which triggers the expulsion of dopant ions from the polymeric material. This is associated with change in volume (actuators) and release of medication (drug-delivery).

For DDS, the redox state of this materials modified by electrical stimulation, in turn, modifies the release rate of the drug. In this manner, patient adherence and benefit: side effect ratio should be improved (Kaušaitė-Minkštimienė *et al.*, 2011; Entezami and Massoumi, 2006). Leonavicius *et al.* (2011) proposed hollow PPy containers as encapsulation material or for controlled drug delivery systems.

Wadhwa *et al.* (2006) reported the incorporation of the anti-inflammatory drug, dexamethasone (Dex) in PPY coatings for controlled release. The same drug was tested for delivery from PEDOT-nanotubes (PEDOT-coated nanofibers of biodegradable poly(l-lactide) (PLLA) or poly(lactide-*co*-glycolide) (PLGA)) by electrical stimulation of the nanotubes by Abidian *et al.* (2006). Controlled release of heparin was attempted by Li *et al.* (2005) using covalent immobilization technique for poly(vinyl alcohol)-heparin hydrogel on PPy film. Heparin release was triggered by an electric current of 3.5 mA. Along with the involvement of nano-technology, applications such as preparation of artificial muscle (robots, artificial limbs) are available (Entezami and Massoumi, 2006).

Both drug-delivery systems and "artificial-muscle" devices based on PPy films have been developed by Kulinsky *et al.* (2006). Negative small potentials applied to PPY induce flexing of PPy/Au bi-layer actuator and drug-releasing.

Otero and co-workers described artificial muscle applications for CPs (Otero and Sansiñena, 1997; Otero and Cortés, 2003) PPy was employed in a triple layer actuator consisting of PPy film/non-conducting and adhesive film/PPY film that proves both sensing and actuating capabilities when current is applied across the conducting films. As a result, one of the PPy films is oxidized while the other one is reduced. As reported, the oxidation occurs along with dopant ions intrusion and expansion while reduction expels the ions while contracting.

Tahhan *et al.* (2003) reported the development of an actuator composite consisting of a single-wall carbon nanotube (CNT) and conducting polyaniline CNT/PANI that has been triggered by Non-Faradaic electrochemical charging and redox reactions. Likewise, both PANI and PPy have been tested by Spinks and co-workers (Spinks *et al.*, 2005a; Spinks *et al.*, 2005b) for actuator applications in the form of layer composites involving CNTs such as PANI/PPy, PANI/CNT/PPy and neat PPy. It was reported that the PPy/PANI composite presented the highest work-per-cycle.

Additionally, CPs started being explored as scavengers of harmful free radicals from environment due to their facility to oxidize. Research in this field has particular relevance for

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biomedical applications involving tissues affected by oxidative stress (Gizdavic-Nikolaidis *et al.*, 2004).

Biocompatibility along with good conductivity and redox stability are the main requirements for biomedical applications of CPs. Table 1 presents some of the most studied biomedical field that have employed CPs and specific requirements necessary for each type of application.

Table 1. *Biological applications of conducting polymers (adapted from* Guimard *et al.*, 2007)

Application	Applicability of CPs	Adjustments of CPs
Biosensors	Incorporation of biomolecules Improved detection Efficient signal transduction	Diffusion Hydrophobicity
Drug Delivery	Incorporation of biomolecules Controlled release - redox process	Hydrophobicity
Tissue engineering	Biocompatibility Conductivity	Biomolecule functionalization Redox stability Degradability
Neural probes	Increased surface area Decreased impedance Conductivity Stability Biocompatibility	Cell specificity Electrical contact
Bio-actuators	Biocompatibility with body temperature and fluids Conductivity Control volume (dopant uptake/release) Lightweight	Response limited by ion mobility Delamination of films

Polypyrrole: bioproduction and applications

Polypyrrole is perhaps one of the most studied amongst CPs, its relevance consists on its superior properties such as inherent electrical nature, facility and flexibility of synthesis, good mechanical properties, environmental, chemical and thermal stability (Ramanavičius *et al.*, 2005). It has been employed in various application fields from electronic and electrochromic devices (Rowley and Mortimer, 2002), counter-electrode in electrolytic capacitors, chromatographic stationary phases (Ge *et al.*, 1991), light-weight batteries (Nyström *et al.*, 2009) to chemiresistors (Mabrook *et al.*, 2006), biosensors (Ramanavičienė and Ramanavičius, 2004a), actuators and other biomedical devices (Ateh *et al.*, 2006).

There are various methods to obtain electrically conductive PPy from basic electrochemical and chemical synthesis to UV-induced radical polymerization, plasma polymerization, chemical vapor deposition (CVD) (Eofinger *et al.*, 1998; Liu *et al.*, 2002; Xu *et al.*, 2005) etc. Ansari (2006) provided a review on chemical and electrochemical preparation methods for PPy.

Bioproduction of polypyrrole and modifications

The present section will focus on describing enyzmatic pathways of synthesizing polypyrrole (PPy), methods of design and adjustment as well as its appliance in different fields.

It is considered that enzymatic synthesis of PANI was introduced previously to PPy due to difficulties related to oxidation potential (Ep). Pyrrole has higher Ep (1.2 V) (Ag/AgCl) than aniline (0.9V) (Ag/AgCl) while most oxidoreductases employed for catalytic polymerization have slightly lower values of oxidation potential (Claus, 2004; Veitch, 2004; Matsushita *et al.*, 2005).

An attempt towards a greener method for PPy synthesis has been made by Dias *et al.* (2006) by adding catalytic amounts of oxidant and H_2O_2 in aqueous HBF_4 medium. It was reported that acidic media was proven necessary in order to prevent the nucleophilic attack of water on the polymer. Hydrogen peroxide was used as regenerator of catalyst and even if it was thermodynamically able to initiate the polymerization reaction on its own, it presented kinetic limitations, hence small concentrations of oxidant (molar ratio of 1:0.06 pyrrole: Fe^{3+}) were preferred.

The straightforward enzymatic oxidation of pyrrole is a problematic matter since it is considered a poor substrate of most oxidoreductases. In this sense, redox mediators such as ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) coupled with above mentioned enzymes have been employed to initiate the oxidation mechanism.

Cruz-Silva *et al.* (2008) reported the HRP-catalyzed polymerization of pyrrole in mild aqueous media. ABTS was used as initiator of enzymatic reaction being rapidly oxidized by horseradish peroxidase and, in turn, generating a radical cation able to oxidize the pyrrole monomer. The polymer obtained was spectroscopically similar to chemically prepared PPy. The HRP-catalyzed polymerization of pyrrole in presence of ABTS was also investigated by Kupriyanovich *et al.* (2008).

Although PPy was successfully synthesized in these conditions, redox mediators have their own influence on the resulted polymer, thereby Bouldin *et al.* (2011) investigated the soybean peroxidase-catalyzed polymerization of pyrrole at low-temperature in aqueous media without redox mediators. The experiment had successful results as a result of an oxidation potential of 1.2 V (Ag/AgCl) of SBP, which appoints it as adequate for pyrrole enzymatic synthesis. The resulted polymer presented fewer structural defects due to kinetic conditions (slower and controlled release of radicals by enzymes) that favored α–α linkages in polymeric structure.

Owing to its successful applications in nanocomposites field, innovative biocatalytic synthesis of nanocomposite PPY - lactate oxidase - CNT was demonstrated by Cui *et al.* (2007). Lactate oxidase (LOD) with lactate as substrate catalytically produced hydrogen peroxide in order to initiate pyrrole polymerization. Firstly, enzyme molecules served as template for polymerization resulting in PPY-LOD nanoparticles followed by introduction of CNT as template which attained a three - component nanocomposite. The nanocomposites were employed for lactase biosensor, showing superior capacity.

Laccase-catalysed reactions are more attractive considering that beside from being environmentally friendly the reaction does not require extra addition of hydrogen peroxide to the reaction medium. While H_2O_2 influences in a great deal the peroxidases activity (at concentration above 1mM they start losing catalytic activity) for laccase-catalysed systems molecular oxygen serves as oxidant rendering a more facile synthesis. In addition, horseradish peroxidase shows low stability at low pH due to acidic dissociation of holoenzyme (Shumakovich *et al.*, 2012a; Streltsov *et al.*, 2009).

Nonetheless, there is an issue regarding kinetic conditions for enzyme-initiated polymerizations because the reaction proceeds slowly.

Song and Palmore (2005) reported an environmentally friendly route for the polymerization of pyrrole involving laccase. The polymerization reaction was initiated by the oxidation of pyrrole monomer to radical cation while reducing one of the four Cu (II) ions to Cu (I) in the active site of laccase. After 4 pyrrole units have been oxidized, four Cu (I) ions with the corresponding electrons are available to reduce 1 unit of dioxygen to water, completing the biocatalysis cycle. In order to increase the synthesis rate, ABTS mediated the electron transfer between pyrrole and enzymatic active center. In addition, it improved the physicochemical properties of the resulted polymer playing the role of redox-active dopant.

The laccase-catalysed polymerization of pyrrole was also reported by Mazur *et al.* (2009) in an innovative manner. Laccase obtained from fungi *Cerrena unicolor* strain embodied in aqueous droplets was absorbed onto a solid surface and PPY layer was disposed at the droplet's surface. The droplets had also the role of templates and as a result, the water-dispersable hemispherical capsules of polymer were encapsulated with enzyme. The enzyme-filled microcapsules represent a tremendous technological progress rendering applications in building oxygen sensors and biofuel cells.

Thus, the biocatalyzed polymerization of conducting polypyyrole remains a valuable approach for innovative and productive application. A promising feature of the resulting polymer has been ascertained in many of the catalytic methods of synthesis, namely the absorption of a significant amount of biocatalyst. Enzymes are encapsulated within the polymer matrix which leads to increased biocompatibility and biosensing characteristics (Ramanavičius *et al.*, 2006; Cui *et al.*, 2007; Mazur *et al.*, 2009).

Nonetheless, there are several important parameters to be accounted for when tailoring a polymeric structure for a specific biomedical application (Wang *et al.*, 2010) in as much as CPs reactions with biological tissues can be modulated by a variety of factors which are interconnected with the choice in method of synthesis and dopants, factors with direct

 Received July 10, 2015 / Revised August 1, 2015/Accepted August 2, 2015

impact on the properties of the final product such as film morphology (thickness, surface chemistry, topography) (Ateh *et al.*, 2006).

Pyrrole rings modified with alkyl groups or N-substituted monomers have been polymerized in order to increase polymer processability. Gursoy *et al.* (2010) chemically synthesized three PPy derivatives: (p-benzoic acid) PPy (NpbPPy), N-(o-aminophenyl) PPy (NoaPPy), N-(m-nitrophenyl) PPy (NmnPPy) from which NpbPPy matrix conferred best biosensing features. Co-polymeric composite has also been achieved by Rivers *et al.* (2002). Pyrrole and thiophene oligomers linked by ester groups rendered a bioconductive material with biocompatibility and biodegradability features.

Concerning the doping of enzymatically synthesized PPy, there have been reported several attempts either by redox mediators (i.e. ABTS) that improve both reaction speed and mechanical properties of the conducting polymer (Song and Palmore, 2005) either by charge balancing dopants that serve as "soft" templates.

In order to increase solubility characteristics, co-doping of PPy was attempted with dodecylbenzenesulfonic acid (DBSA) and tetraethylammonium tetrafluoroborate (TEABF4) by Lee *et al.* (2002). Bouldin *et al.* (2011) reported doping with various compounds including polystyrene sulfonate (PSS), camphorsulfonic acid (CSA), poly (n-vinylpyrrolidone) (PVP) and citric acid. The highest conductivity was achieved with CSA (3.8 S/cm). Overoxidation of PPy in presence of 1-naphthalenesulfonate (1-NS) has resulted in excellent selectivity of structural enantiomers and structural isomers of naphthalene (Shiigi *et al.*, 2003).

Molecular imprinting has become a versatile technique for tailoring polymers with predetermined biomolecular recognition features. The cavities resulted in the polymeric matrix are complementary to the desired compound providing specific biological response (Malhotra *et al.*, 2006).

The preparation of PPy-based molecularly imprinted polymer was investigated for recognition of virus (BLV) glycoprotein gp51 (Ramanavičienė and Ramanavičius, 2004b). Caffeine-imprinted PPY development was proposed by Ebarvia *et al.* (2005) using electro-synthesis. The caffeine molecules remained entrapped within the polymer matrix leaving behind caffeine-recognizing cavities for piezoelectric sensor. Namvar and Warriner (2007) reported the production of glassy carbon electrodes coated with PPY poly(3-methylthiophene) imprinted with Bacillus subtilis strain endospores. The template was removed with DMSO and the recognition of endospores was assayed via impedance spectroscopy.

On account of the numerous methods of synthesis described in literature, PPy stands out as one of the most investigated CPs. Concerning its employment in biomedical applications the modification by organic, inorganic or molecular imprints in order to enhance both its solubility and its biological recognition for the required applications has been a key aspect. Enzyme-catalyzed polymerizations render even more suitable prospects.

Biosensing devices

PPy has been widely used to immobilize molecules for biosensor developments. PPy films have been applied in coating electrodes for urea-detecting biosensors by Pandey and Mishra (1988) followed by Tamiya *et al.* (1989) that described adsorption of GOx within PPy-modified micro-electrode.

Urease and glutamase dehydrogenase enzymes were immobilized in polypyrrole-polyvinyl sulfonate PPy-PVS materials (Gambhir et al., 2001).

Razola *et al.* (2002) reported in situ entrapment of horseradish peroxidase within PPy film for H2O2 detection. Likewise, Ekanayake *et al.* (2008) immobilized HRP in nanoporous PPY in order to develop a novel biosensor with enhanced properties able to detect H2O2 at low concentrations.

Llaudet *et al.* (2003) attempted co-entrapping of xanthine oxidase, purine nucleoside phosphorylase and adenosine deaminase within PPY modified matrix in order to identify purine production from central nervous system.

Cristea *et al.* (2005) developed an organic phase biosensor entrapping polyphenol oxidase within electro-generated hydrophilic PPY film. Ameer and Adeloju (2009) immobilized tyrosinase within polymeric film for potentiometric detection of catechol. Amperometric detection of tyramine in

food samples was accomplished by Apetrei and Apetrei (2013) by doping the PPy film with phosphate anion followed by cross-linking the tyrosinase.

Zhu and Lu (2005) presented the electrochemical embedding of lactate dehydrogenase (LDH) in polymeric film to test pyruvate concentrations as well as the detection of NADH coenzyme using PPy-LDH-NADH/Au system. Carelli *et al.* (2006) reported the development of an alcohol biosensor based on immobilized alcohol oxidase on overoxidized PPy film.

Redox mediators have been incorporated in order to improve sensor features such as sensitivity and selectivity. Arslan and co-workers (Arslan *et al.* 2006a, Arslan *et al.* 2006b, Çete *et al.* 2006) tested the sensitivity of Pt/PPy-Fc electrode towards H2O2 by immobilizing uricase for detection of uric acid. In addition, immobilized xanthine oxidase on PPy by crosslinking with glutaraldehyde/bovine serum albumin was used for xanthine detection. Prussian blue/polypyrrole (PB/PPy) composite film was employed by Li and Gu (2006) for immobilization of cholesterol oxidase in order to detect cholesterol concentrations in blood.

PPy has been also employed in the development of DNA biosensors as following: DNA biosensor designed to detect the human immunodeficiency virus (HIV) (Fu *et al.*, 2006), DNA/RNA electrochemical microsensor (Chen *et al.*, 2006), overoxidised PPy-DNA composite for dopamine and serotonine detection (Jiang and Lin, 2005), electrosynthesized PPy/oligonucleotide samples applied for real-time detection of DNA hybridization (Lassalle *et al.*, 2001).

Whole living cells have also been immobilized within PPy films. PPy was considered for generating an erythrocytes-doped PPy biosensor in order to detect blood Rhfactor via the Rhesus factor antigens on the cell surface (Campbell *et al.*, 1999).

The covalent attachment of Listeria ssp. antibodies to PPy backbone for amperometric identifying of bacterial strains has been reported by Minett *et al.* (2002). Anti-rabbit IgG antibody has been entrapped within electro-synthesized PPY membrane for amperometric detection of rabbit IgG antigen (Gooding *et al.*, 2004).

Ionescu *et al.* (2006) reported the entrapment of Chlorella vulgaris algal cells in both alginate gel and pyrrole-modified alginate matrix setting up two comparative biosensors for amperometric detection of algal alkaline phosphatase activity. The stability of pyrrole-alginate coating has been proven.

Tissue engineering and neural applications

Along with many satisfactory properties such as various facile methods of preparation and modification, surface charge, humidity and stability, PPy has proved impressive biocompatibility towards biological tissues. This characteristic makes it available as substrate material for neural scaffolds, electrodes and devices (George *et al.*, 2005).

Similar to biosensor investigations, the research on CPs appliance to tissue engineering was initially focused on PPy. Ateh *et al.* (2006) illustrated an extensive research in this particular field.

Due to the fact that polypyrrole has the ability of triggering specific cellular response from biological tissues (Mattioli-Belmonte *et al.*, 2005) it has been shown to improve the regeneration of several tissues including bone and nerve (Collier *et al.*, 2000).

Biomedical applications of PPy started since the early nineties when it was studied as cellular substrate for protein adsorption and mammalian cell culture by Wong *et al.* (1994). It was considered as a very promising biomaterial, whose properties and surface binding features are reversed by electrical potentials. It was recommended as an adequate substrate for cell culture since it conferred a noninvasive manner of cellular regulation.

Garner *et al.* (1999) developed a suitable heparin-PPy composite as substrate for human umbilical vein endothelial cell growth followed by Collier et al. (2000) that synthesized polypyrrole – hyaluronic acid composites. The bio-composites induced enhanced vascularization due to electrical stimulation.

George *et al.* (2005) cultivated cerebral cortical cells on PSS/ NaDBS - doped PPy proving biocompatibility of the polypyrrole implants.

Mattioli-Belmonte *et al.* (2003, 2005) studied cellular behaviour in respect to oxidative state of PPy films as well as tissue tolerance and cellular

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interactions between non-resorbable (polypyrrole, polyaniline) and resorbable materials.

A dependence between PPy film thickness due to pyrrole concentration during admicellar polymerization and the viability and differentiation of mesenchymal stem cells towards the osteoblastic phenotype has been proved by Castano *et al.* (2004).

Ateh *et al.* (2007) proved the feasibility of dermatan and chloride-loaded PPY films for supporting keratinocyte growth, while Wang *et al.* (2004) tested the biocompatibility of PPy with nerve tissue in vitro and in vivo showing that cells had better survival and proliferation rate in contact with PPy. Thus, the polymer presents biocompatibility towards peripheral nerve tissue having potential for bridging the peripheral nerve gap.

In vivo study on mice was reported by Ramanavičienė *et al.* (2007) proving a high degree of biocompatibility of polypyrrole particles since they did not exhibit any cytotoxic effect towards mouse cells. PPy particles did not generate any allergic response, neither did they affect organs.

Many of the advancements rendered by CPs in tissue engineering applications, are relevant for progress in the neural electrodes field. A key aspect in neural interfaces is the finding of conductive substrate that has the ability to promote neural interactions. Biological moieties have been prevalent in designing PPy for neural probe applications.

Gomez *et al.* (2007) reported patterning PPy for embryonic hippocampal neurons culture. PPy microchannels were generated with electron beam litography and electropolymerization.

Fibronectin fragments (SLPF) and nonapeptide CDPGYIGSR biomolecules have been combined with PPy rendering enhanced density of bioactive sites for interaction with neural cells. While rat glial cells attached to PPy/SLPF-coated electrodes, neuroblastoma cells preferred PPy/CDPGYIGSR-coated electrode sites (Cui *et al.*, 2001). Subsequently, Stauffer and Cui (2006) have reported several pursuits in combining the conductivity of polypyrrole with specific biomolecules that promote cellular growth and attachment. Laminin fragments (CDPGYIGSR (p31) and RNIAEIIKDI (p20)) have been used as dopants and the combination of the two peptides proved enhanced neuronal density.

Even now, there are still many unanswered questions regarding CPs's appliance in biological fields. PPy has been thoroughly researched in biomedical fields and its redox characteristics have been influencing biomolecules in various manners. For example, as much as the reduction of charged PPy that is associated with polymeric contraction causes the expulsion of small ions (widely applied in actuators and drug-delivery systems), it can also compel the incursion of small positive ions. As reported by Wong *et al.* (1994), Na+ ions from medium were integrated within polypyrrole – polystyrene sulfonate (PPy/PSS) which had its own effect in protein adsorption and cellular activity.

Thus, although extensive investigations have been devoted to determine the influence of electric potential over cellular activity there are still many answered questions. The possibilities for synthesis and modification of conducting materials are progressively advancing and biotechnology represents an indubitable resource.

Conclusions

The purpose of the present review was to outline the importance along with the applicability of conductive materials. The present studies highlight the versatile methods of synthesis and the numerous fields of appliance. Biocatalysis has proven a constructive influence towards the biocompatibility of CPs rendering tremendous opportunities in the biomedical department. Brief descriptions of the enzymes involved in polymeric synthesis were given in order to stimulate further investigations.

Polypyrrole was intended to be the focus of this paper since most initial studies were focused on it being acknowledged as one of the most widely used CPs. Enzymatic pathways of polymerization for PPy were described as well as modification and design in order to attain the required polymeric material.

The preliminary studies establish the possibility of in vitro and in vivo polymerization of CPs using different enzymes or even whole cells. Furthermore, enzymatic synthesis in the presence of the cellular components that produce the necessary enzymes for the bioproduction of conductive materials will soon be considered.

As ultimate goal this review outlines current achievements in innovative domains such as biosensors, tissue engineering, neural probes, drug-delivery systems and actuators with an assessment of future opportunities.

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