Chapter 24
Electrochemical Formation of 'Synthetic Receptors' Based on Conducting Polymers

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Abstract Analysis of pharmaceuticals and biologically active materials recently can be performed by biosensors, which can be based on conducting polymers. Therefore, in this Mini Review some achievements in the synthesis and application of conducting polymer—polypyrrole (Ppy), which is often used in the design of sensors and biosensors, are overviewed. Some perspective methods of conducting polymer synthesis are outlined. Significant attention has been paid to electrochemical, chemical and biochemical synthesis of conducting polymers (CPs) , which were developed by authors. The applicability of polypyrrole based functional layers in the design of electrochemical biosensors is overviewed. The adaptability of enzyme—glucose oxidase (GOx), which can be applied as (i) biological recognition element—in the design of glucose biosensors, (ii) a biocatalyst—in the synthesis of some above mentioned conducting polymers, is discussed. Part of biocompatibility related

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aspects of some conducting polymers are also discussed and some insights in the application of polypyrrole-based coatings for implantable sensors are outlined.

24.1 Introduction

Demands for advanced pharmaceutical and biomedical analysis are evolving very rapidly. Therefore, various new and advanced technologies based on sensors and biosensors [1] are finding the application in the solution of challenging problems of pharmaceutical and biomedical analysis [2]. Various polymer-based nanomaterials recently are applied in the design of sensors and biosensors in order to advance their sensitivity and selectivity and in this way to extend their bioanalytical applicability [3, 4]. It should be noted that conducting polymers are among the most promising materials, which can extend analytical characteristics of sensors [5–7], including their sensitivity and selectivity [8]. These achievements are possible due to electrical conductivity, electrochemical activity, mechanical elasticity and environmental stability of conducting polymers [9–11]. In addition, unique capabilities of transferring electric charge from biological objects towards electrodes are also very attractive property for sensorics related application of conducting polymers [12]. Simple doping and dedoping of conducting polymers enables to manipulate the properties of material makes conducting polymer ideal material for sensing applications [13, 14]. Among a variety of conducting polymers polypyrrole (Ppy), polyaniline (PANI) and polythiophene (PTH) are of the greatest interest due to their high technological potential, which has been exploited in the design of super capacitors [15], rechargeable batteries [16], corrosion prevention [17], sensors [18, 19] solar cells [20] and biosensors [21]. Technologically it is advantageous that various conducting polymer based nanocomposites with entrapped enzymes [22], receptor-like proteins [13], antibodies [23] and DNA [24] can be formed by different methods overviewed and discussed in this review. Therefore, such conducting polymer based composite materials can show unique sensing properties, which are determined by entrapped materials and dopants.

The aim of this 'Mini Review' is to evaluate the methods of synthesis and bioanalytical application of some conjugated polymers including polypyrrole, polythiophene and polyaniline.

24.2 Synthesis of Conducting Polymers

24.2.1 Synthesis of Conducting Polymers

It was demonstrated that conducting polymers might be produced by chemical [25], electrochemical [13, 26] and even by biotechnological approaches [3].

Therefore, these conducting polymers synthesis methods are taken for consideration in this review.

Chemical synthesis is one of the most popular methods to form conducting polymers. This method is relatively cheap and simple based on usage of strong oxidators such as $FeCl_3$ or H_2O_2 [17, 27]. Spherical particles of polypyrrole can be formed using H_2O_2, which has oxidation potential sufficient to initiate the polymerization of pyrrole and other monomers that are forming conducting polymer [28], this method is very attractive because excess of H_2O_2 can be easily degraded into H_2O and O_2, therefore pure particles of the conducting polymer can be formed, which possesses a good biocompatibility with living stem cells [29, 30] and even when injected in mice peritoneum [31].

The advantage of chemical synthesis is that using this method can be formed large quantities of nanoparticles, based on the conducting polymer, which are suspended in a solution. During the next technological steps these nanoparticles eventually can be modified and applied for biomedical purposes. However, this method is not always well suited for the formation of films, because chemically formed conducting polymer layers mostly are sparse and not stable enough for further technological applications.

Some authors presented in their work where polypyrrole nanostructures such as nanowires and nanotubes were chemically synthesized inside aluminium template [32]. The response surface methodology based on central composite design was used to determine the relationship between the morphology of the nanostructures (nanowires or nanotubes) and synthesis conditions [32]. Other authors showed that polypyrrole nanostructures can be gained chemically synthesizing Ppy within the pores of microporous and nanoporous particle track-etched membranes [33]. Chemical synthesis of polypyrrole nanoparticles is possible using facile one-step chemical oxidative polymerization method at room temperature. Using this method doping of conjugated-polypyrrole nanoparticles into electrically conductive adhesives (ECAs) can be obtained. The purpose of such polymerization procedure is to prepare low-electrical resistivity interconnecting materials [34].

Biochemical synthesis of conducting polymers can be performed in several ways: (i) enzymatic—using redox enzymes, e.g. glucose oxidase or (ii) microbiological—using whole microorganisms [35, 36].

Enzymatic synthesis of conducing polymers. Catalytic reactions of Glucose oxidase (GOx) E.C. 1.1.3.4. from *Penicillium vitale* can be applied for the synthesis of various conducting polymers such as: polypyrrole [3, 13, 28, 29], polyaniline [37] and polytiophene [38], polyphenanthroline [39] and some other conducting polymer based layers and nanoparticles. This is a unique approach, which is based on catalytic action of GOx. Actually polymerization of monomers, which are forming conducting polymer, is initiated by hydrogen peroxide that is formed during the catalytic action of GOx. Immobilized and in water dissolved enzymes have been successfully applied in the enzymatic synthesis of conducting polymer based layers or particles with entrapped enzymes, which are producing hydrogen peroxide. Due to remaining activity of entrapped enzyme, such particles and layers are well suitable for the creation of amperometric glucose biosensors and biofuel cells.

Microbiological synthesis of polypyrrole. Some redox processes, which are part of metabolism occurring in living cells can be adapted for the synthesis of Ppy within cell wall of living cells: yeast cells [22].

Electrochemical synthesis is also very attractive method used for the deposition of conducting polymer based layers. The adjustment of various electrochemistry-related parameters such as polymerization-inducing potential, current, scan rate and polymerization duration enables to create polymeric layers of different characteristics [13, 40, 41]. This method enables to manipulate properties of conducting polymers for particular application by controlling the process parameters [42]. In addition, electrical conductivity and some electrochemical properties of conducting polymers can be tailored and controlled by variation of polymerizable monomer concentrations, pH of polymerization bulk solution and different dopant concentrations [43–45]. Electrochemical formation of conducting polymer based layers is attractive because both morphology and thickness of formed layer can be controlled by the adjustment of (i) potential-profile and the duration of potential pulses or (ii) potential sweep rate [13], pH of the electrolyte [46].

24.2.2 Some Chemical and Physical Properties of Conducting Polymers

In our researches we have determined that conducting polymer—polypyrrole—exhibit unique electrochemical, affinity [32] and/or optical [33] properties. Therefore, changes of one or more of these physicochemical properties (e.g. resistance of CP-based biological recognition layer, variation of electrical capacitance, changes of optical properties etc.) can be determined by particular signal transducer and can be precisely monitored by registration device. It should be noted that among number of CPs—polypyrrole mostly has been used in the design of enzymatic biosensors as enzyme immobilization matrixes [3].

24.3 Conducting Polymers for Biosensor Design

Conducting polymers in the design of amperometric biosensors. Due to relatively low permeability of Ppy layer for substrates and reaction products, apparent Michaelis constant (K_{Mapp}) of immobilized enzymes increases significantly. This effect enables to extend linear range of such enzymatic sensors [14], which can be used for biosensors based on enzymes, which have low K_{Mapp} and therefore are not suitable for the investigation of samples with high substrate concentration, e.g. glucose concentration in blood is significantly higher in comparison with K_{Mapp} of the most popular glucose oxidases [34]. This methodology enables to tune analytical characteristic such as lower and upper limits of detection and linear range of biosensor [3, 23, 24]. In addition to the above mentioned properties, some conducting polymers are capable to transfer charge directly from redox enzymes towards electrodes [39]. Therefore, electrochemically generated conducting polymers are very promising as advanced immobilization matrixes of amperometric catalytic biosensors [14] biofuel cells and other bioelectronic devices [3, 47–49]. However, most of enzymes have redox centers deeply buried within protein 'shell'. Therefore, charge transfer from these enzymes is complicated even if they are entrapped within conducting polymer. In order to solve this problem in some of our researches we have demonstrated that charge transfer capabilities of electrochemically formed conducting polymers can be improved by grafting to the surface of carbon-based electrodes [39]. Glucose biosensors, which are mostly based on GOx, are important tools, which are used not only for biomedical purposes, but also as a model system in the development of enzymatic sensors. Therefore, very different conducting polymer and GOx based structures have been formed and applied in glucose sensor design [14, 50]. Single-step procedure has been established for the modification of electrode by composite material consisting of polypyrrole, Prussian blue (PB) and glucose oxidase (GOx) [31].

Conducting polymers in the design of affinity biosensors can serve as (i) immobilization matrixes [13, 31], signal transduction systems [13, 39] and even analyte recognizing components [28]. Electrochemically deposited layers of conducting polymers modified with entrapped proteins (antibodies or antigens) have been applied in the design of various types of ***immunosensors*** [13], in such sensors conducting polymer layer is enhancing potentiodynamically generated electrochemical signal [13] or reducing the influence of interfering materials if photoluminescence based analytical signal is registered [51]. The most promising among affinity sensors are ***molecularly imprinted polymers (MIPs) based sensors***, because they do not need very expensive biological recognition materials [52–54]. Overoxidized polypyrrole seems to be one of the most promising among all MIPs because it can be prepared in very simple electrochemical way using single polymerizable monomer. Polypyrrole can be easily imprinted by large [28, 29, 55–57] and low [7, 10, 58, 59] molecular weight molecules (e.g. by Caffeine [10], theophylline [58], L-aspartic acid [59] and histamine [7]) and even by large molecular weight biomolecules (e.g. by DNA and proteins [28, 29, 55–57]).

24.4 Conclusions

Conducting polymers have found application in various sensors and biosensors, which can be used in the analysis of pharmaceuticals and biologically active materials. Electrochemical synthesis of conducting polymers seems promising, because it can be controlled by adjustment of electrical current profile. Characteristics of conducting polymer based sensors are determined by the thickness of polypyrrole layer. Enzymatic and microbial synthesis of polypyrrole demonstrated that in such way formed polypyrrole/bio-composite materials are also suitable for the development of biosensors and biofuel cells. Also chemical synthesis of polypyrole reveal possibilities to form different nanostructures that can be used as biosensing platforms. The applicability of similar methods for the synthesis of other conducting polymers such as polyaniline, polythiophene, and some others conducting or π-π conjugated polymers is predicted.

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