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# Electrochemical Molecularly Imprinted Polymer Based Sensors for Pharmaceutical and Biomedical Applications (Review)

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## Abstract

Recent challenges in the pharmaceutical and biomedical fields require the development of new analytical methods. Therefore, the development of new sensors is a very important task. In this paper, we are outlining the development of molecularly imprinted polymer (MIP) based sensors, which belongs to important branch of affinity sensors. In this review, recent advances in the design of MIP-based sensors are overviewed. MIPs-based sensing structures can replace expensive natural affinity compounds such as receptors or antibodies. Among many different polymers, conducting polymers show the most versatile properties, which are suitable for sensor application. Therefore, significant attention is paid towards MIPs based on conducting polymers, namely polypyrrole, polythiophene, poly(3,4-ethylenedioxythiophene), polyaniline and ortho-phenylenediamine. Moreover, many other materials, which could be imprinted analyte molecules, are overviewed. Among many conducting polymers, polypyrrole is highlighted as one of the most suitable for molecular imprinting. Some attention is dedicated to overview polymerization methods applied for the design of sensing structures used in various affinity sensors. The transduction of analytical signal is an important issue, therefore, physicochemical methods suitable for analytical signal transduction are also outlined. Advances, trends and perspectives in MIP application are discussed.

**Keywords:** Molecularly imprinted polymers (MIPs); Affinity sensors; Immunosensors; Conducting polymers (CPs); Electrochemical deposition; Electrochemical sensors.

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## List of abbreviations

Conducting polymers	CPs
Density-Functional-Theory	DFT
Electrochemical QCM	EQCM
Glucose oxidase	GOx
Gold nanoparticles	AuNPs
Human hemoglobin	HbA
Molecularly imprinted polymer	MIP
Poly(3,4-ethylenedioxythiophene)	PEDOT
Poly(styrenesulfonate)	PSS
Polyaniline	PANI
Polypyrrole	Ppy
Polythiophene	PTH
Quartz crystal microbalance	QCM
Quartz crystal microbalance with dissipation	QCM-D
Surface plasmon resonance	SPR

## 1. Introduction

Among various types of systems, the pharmaceutical formulations and biomedical matrixes are the most complex. Consequently, the design of sensitive and selective analytical system is still rather challenging task. Various affinity sensors are a good choice in the purpose of simplifying the analysis and reducing expenses [1]. The combination of the different sensor types with different analytical signal registration methods leads to the sufficient sensitivity [2, 3]. The most common sample types in biomedical applications are: saliva, urine, blood serum, and some other biological liquids. Such samples contain biomarkers, which are marking some diseases. These biomarkers can be detected by sensors and biosensors, but the most of these biomarkers are determined or detected by affinity sensors [4, 5].

Various semiconductors-based structures are developed to increase the selectivity and sensitivity of chemical sensors and biosensors [6, 7], but conducting polymers (CPs) are used for this purpose the most frequently. The research articles describe many different approaches to deposit the conducting polymers on the electrode surface. Recently the conducting polymers are used for the development of sensing-structures useful for electrochemical applications, which increases the selectivity of the analytical system toward selected analytes [2]. Some polymers have several features that make them especially interesting for the design of sensors. These features are electrical conductivity [8], high electrical capacitance [9–11], good adherence on the surface of the electrodes, and the ability to form physically and chemically stable coatings [12, 13]. Ability to transfer electrical charges is characteristic for some CPs, therefore, they are used for charge transfer from some redox enzymes and some other biomolecules [14]. Due to aforementioned properties, CPs are used in the development of sensing structures in combination with various signal transducers. Polyaniline (PANI), polypyrrole (Ppy), poly(3,4-ethylenedioxythiophene) (PEDOT) and polythiophene (PTH) are CPs, which are the mostly applied in sensor and biosensor design [15–19]. These polymerization methods, which are the most commonly used for the formation of CPs, can be divided into four main types: chemical synthesis [20], enzymatic formation [21], electrochemical deposition [9] and/or microorganism assisted polymerization [22–25]. It is notable that only electrochemical deposition is the most preferred method when thin polymer film deposited on an electrode is needed. Meanwhile some other methods (chemical polymerization induced with hydrogen peroxide, FeCl<sub>3</sub>, etc., enzymatic polymerization or microorganism assisted polymerization) have advantages for the production of polymer based particles. Moreover, CPs can be used as immobilization matrixes for biomolecules that can selectively bind selected analytical-targets including DNA [26], receptors [27], antibodies [2], antigens [28], antibodies [21] and enzymes [29–31]. However, some of these

immobilized biomolecules are very expensive and are unstable, therefore, some alternatives are required. One of the most promising alternatives to native biomolecules is the design and exploitation of various ‘artificial receptors’ and/or molecularly imprinted polymers (MIPs) [32, 33]. It should be noted that conducting polymers can be used for the design of these structures [32–35]. In some investigations, it was shown that MIPs can be used for the design of biosensors for the detection of infection agents [36].

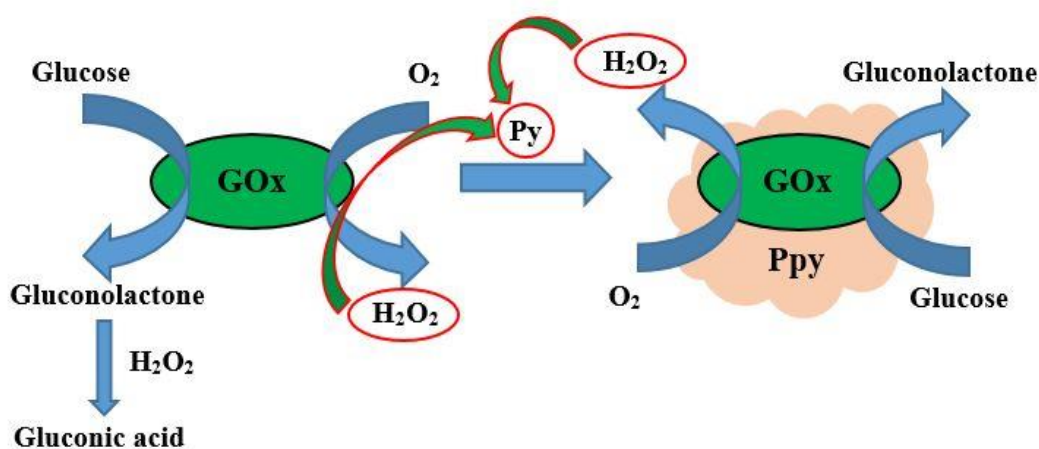
In this paper methods used for the development of CP-based sensors and the formation of molecularly imprinted sites within these polymers are overviewed. It is very usual to classify MIPs according to the detection principle in the review articles of MIPs applications. Principally, MIPs are formed in such steps: (i) chemical or electrochemical polymerization, (ii) deposition on the electrode of the MIP structure, (iii) extraction of the template molecule. In the case of electrochemical polymerization of conducting polymer with imprints of template molecule, the deposition of polymer on the electrode is obtained simultaneously. After the last step of template molecule extraction, the MIP has the shape and structure, which is ready for interaction with target molecules. Such MIPs can be used in the design of optical, electrochemical, electrochromic, magnetic and other sensors. On the basis of the here-mentioned attitude, this review confines the description and considerations about MIPs in the polymerization conditions, template molecule, and signal transduction in sensors. The first part of the review is dedicated to the description of polymerization conditions with an overview of chemical and electrochemical methods. Next part of the review is dedicated for the description of application of MIPs template with high and low molecular weight molecules. In the same part of the review, we found reasonable to summarize the findings about signal transducers applied in sensors based on molecularly imprinted polymers. The last part of the review is dedicated for the aspects of compatibility. We find particularly interesting the compatibility aspects regarding potentially growing interest of scientific community to the potential application of MIPs in the field of wearable sensors.

## **2. Formation of conducting polymers for MIPs by chemical or electrochemical methods**

### ***2.1. Chemical formation of conducting polymers based on redox processes***

Chemical synthesis is one the most suitable polymerization method for the formation of CP-based nano- or/and micro-particles. Such nano-or/and micro-particles of conducting polymer with molecular imprints further are used in the design of chemical sensors, chromatographic systems and some other technological purposes [37, 38]. The application field of electro-active polymers is not limited with sensor design [39, 40] but also are applied for various biomedical purposes including tissue regeneration [41].

In order to fulfill recent technological demands, various conducting polymer synthesis methods have been elaborated. Chemical methods can be used for the formation of large amounts of CPs. Chemical polymerization is initiated using oxidants such as  $\text{FeCl}_3$ ,  $\text{H}_2\text{O}_2$  etc. [42–44]. The application of  $\text{H}_2\text{O}_2$  enables the synthesis of rather clean conducting polymers, while all excess of  $\text{H}_2\text{O}_2$  turns into water and oxygen. Many types of monomers were polymerized in presence of  $\text{H}_2\text{O}_2$  including polypyrrole [20, 44, 45], (Fig. 1), polythiophene [44, 46], poly-phenanthrenequinone [47], poly(pyrrole-2-carboxylic acid) [48], polyphenanthroline [14], azobenzene [49] and carbazole [50].



**Figure 1.** Glucose oxidase (GOx) assisted formation of polypyrrole  $\text{H}_2\text{O}_2$  formed during enzymatic reaction initiated this polymerization reaction. Adapted from reference [51].

Chemical polymerization enables to form large amounts of CPs. During chemical polymerization, different nanostructures, biomolecules, organic and inorganic molecules and even various ions can be entrapped formed polymeric structure [31, 48, 55–57].

Some redox enzymes (e.g. glucose oxidase (GOx)) can be used for the synthesis of conducting polymers [21, 31, 48, 55–57]. Such enzyme assisted formation of CPs can be performed in an aqueous media at ambient conditions [58]. Dissolved [55, 57] and immobilized [29, 31, 48, 56] enzymes can be applied for the synthesis of CPs and after encapsulation of enzyme within formed CP-based structure enzyme often retain some enzymatic activity. Such method can be applied for the adjustment of enzymatic characteristics (e.g. Michaelis constant ( $K_M$ )) that is dependent on diffusion of reacting materials towards active site of enzyme embedded within CP. CP/enzyme-based structures are used in biosensor and biofuel cell design [2, 5, 59, 60].

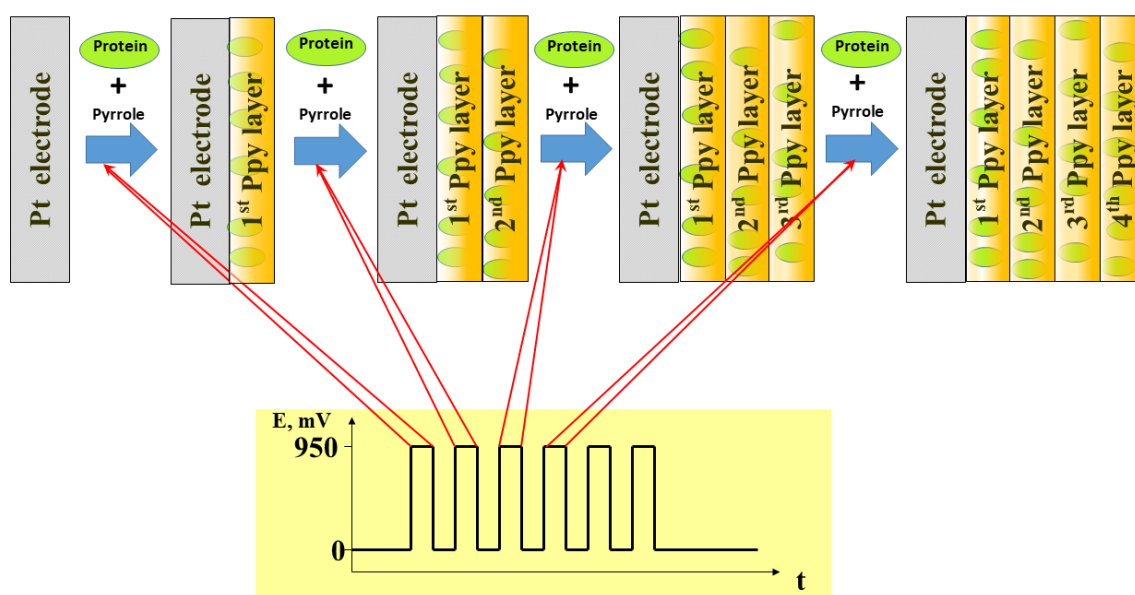
Some conducting polymers (e.g. polypyrrole) can be formed by oxidizing chemicals (e.g.  $\text{Fe}^{3+}$  or  $[\text{Fe}(\text{CN})_6]^{3-}$  ions) [61], which can be generated by metabolic processes that are occurring in living cells, therefore, some cell-induced redox processes can be used for the synthesis of CPs [22–25]. Structures based on PANI, Ppy with entrapped GOx and gold nanoparticles (AuNPs) PANI/AuNPs&GOx can be formed [62], which is suitable for the mass production of MIPs [63, 64]. Transducer surface can be covered by such conducting polymer based MIPs by solvent casting or some other reliable method [65]. It should be noted, that CPs can be not dissolved in conventional solvents, therefore, the deposition of CP-based structures on analytical signal transducer is rather challenging. Electrochemical deposition of conducting polymers can be applied in order to overcome this obstacle.

## ***2.2. Electrochemical methods applied for the formation of conducting polymer based structures***

Many different approaches are applied for the electrodeposition of CP-based layers [66]. Parameters used for the electrodeposition are significantly affecting the most important characteristics of deposited conducting polymer based structures. The electrodeposition is mostly controlled by: (i) the application of particular potential or current control techniques (e.g. potentiodynamic methods such as potential pulses, linear or cyclic potential sweeping can be applied), (ii) potential variation rate and critical voltages, [67, 68], (iii) the composition of polymerization- solution [69–71], and (iv) additional treatment by other conditions (e.g. application of ultrasound) [72]. Here mentioned factors are affecting the density, thickness, permeability and some other characteristics of electrodeposited CP-layers [28, 73, 74]. The analytical characteristics of CP-based layer is affected by the porosity of formed conducting polymer that can be tailored by the variation of above mentioned electrochemical setups [75–77]. It is remarkable that, the electrodeposition of CPs can be well tailored by the assessment of electrical current applied to electrode [28]. Polypyrrole [1, 9, 12, 15, 16, 28, 32, 45, 78], polyaniline [62], poly-9,10-phenanthrenequinone [47] and polythiophene derivatives [42, 79], are these conducting polymers, which are mostly used for the electrodeposition.

Different materials can be incorporated within electrodeposited layer of CP (Fig. 2) [51]. In order to design MIPs these materials can be removed from polymeric matrix by various solvents. Various polymerization methods are applied for the design of MIPs, but among them electrodeposition is the most beneficial [80, 81], because it enables to vary the thickness, morphology and doping/de-doping of formed CP-based structures. In addition, the overoxidation at electrode potentials, which are more positive than that required for electrochemical polymerization of corresponding monomers [51], is useful for the development of MIPs, due to formation of oxygen containing groups such as carboxyl,

carbonyl and hydroxyl, which all are able to form hydrogen bonds and to attend in various electrostatic interactions with imprinted molecules. After the removal of these molecules, formed carboxyl, carbonyl and hydroxyl groups are creating a complementary site, which selectively ‘recognizes’ imprinted molecule. Sometimes, overoxidation can be applied to facilitate the extraction of imprinted materials and/or regeneration of sensing structures after the measurement [82]. Overoxidized polypyrrole deposited on glassy carbon electrode was used for the design of sensors sensitive to Adefovir [83] and Pemetrexed [84].



**Figure 2.** Electrochemical deposition of polypyrrole with simultaneously entrapped proteins using potential pulse-based technique, figure adapted from [51].

In previous studies, the polypyrrole was electrochemically deposited from pyrrole-based aqueous solutions for different electrochemical approaches [85]. The numerous researches on electrochemically formed polypyrrole based MIPs confirms significant advantages of the Ppy-based MIPs in sensor design. Moreover, the electrochemical polymerization is strictly controllable, and the predictability of the final structure provides opportunity for the modification with dopants or ‘decoration’ with various types of nanoparticles and/or other nanostructures. This process can be performed by computerized potentiostats [28]. Polypyrrole-based MIPs were applied in the design of sensors for the determination of dopamine [86, 87], theophylline [32, 88], caffeine [15, 16, 89], histamine [90], quercetin [91], gallic acid [92], bilirubin [93], sarcosine [94], tetracycline [85], microcystin-LR [95], sulfanilamide [96], adrenaline [97], ganciclovir [98], uric acid [99], serotonin [100], L-aspartic acid [101], cysteine enantiomers [102], kanamycin [103], dibutyl phthalate [104],



epinephrine [105, 106], tryptamine [107], testosterone [108], fenvalerate [109] and  $\text{NO}^3$ - ions [110].

Phenylenediamine-derivatives [111–113] are frequently applied in the formation of MIPs that are used for the determination of anticancer-drugs, e.g. ortho-phenylenediamine was molecularly imprinted by pemetrexed [114] and butyrylcholinesterase [115]. Electrodeposited MIP based on poly-meta-phenylenediamine molecularly imprinted by erythromycin was applied for the determination of erythromycin in water-based samples [111]. Poly-nicotinamide based electrochemical sensors for the determination of dopamine were developed [116]. Moreover, polyresorcinol molecularly imprinted by sulphanilamide [117], triphenylamine molecularly imprinted by poly(1-naphthylamine) [118] and azorubine imprinted 1-naphthylamine [119] have been developed. It was reported that, MIPs can be used for the determination of various medications [120].

Structures, which are based on CPs, which can be further modified in many different ways [29], e.g. by organics [121], some inorganic compounds [31, 48, 55–57], ions [125] and various biomolecules [44, 55, 57, 122, 123]. Such modified structures can be used in the design of sensors and biosensors [124].

### **3. The applications of MIPs based sensors for pharmaceutical and biomedical applications**

#### ***3.1. Polymers imprinted by proteins and large biological compounds***

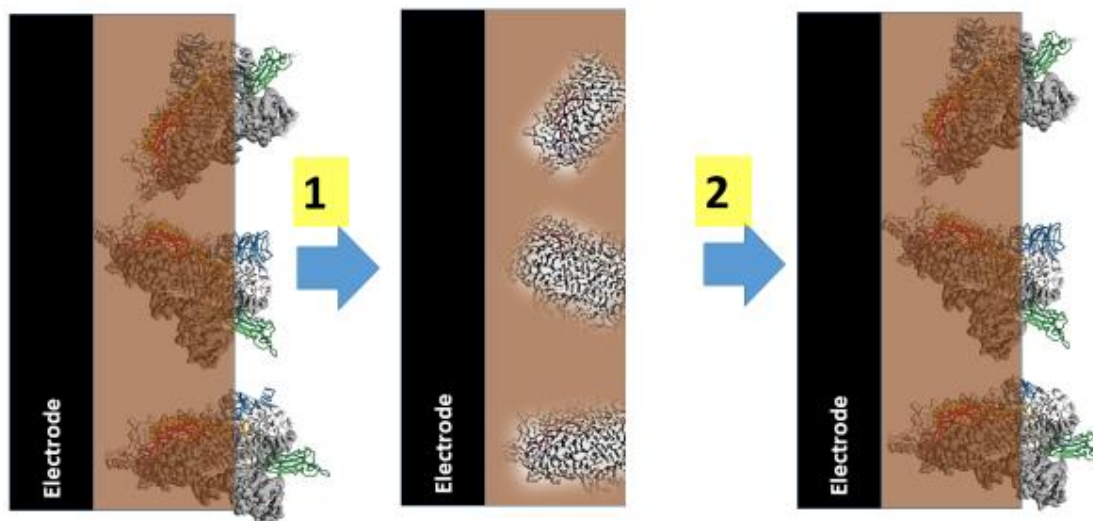
The health system has improved significantly due to innovative solutions and inventions in the field of pharmaceuticals and their monitoring by biomedical devices. However, the benefits of pharmaceuticals are effective if they are free of impurities and used in the right way. The biocompatibility is one of the most important aspect of pharmaceuticals before their application. Various chemical and instrumental methods, which are used for the evaluation of pharmaceuticals before assess their intended application, are regularly developed. Impurities in pharmaceuticals can appear during development, transportation, and storage stages. Therefore, pharmaceuticals and their components need to be detected and quantified during all these stages. Some of these goals can be achieved using MIP-based sensors. High number of immunosensors have been developed by immobilizing proteins within polymeric layers. The performance of immunosensors depends on the orientation of immobilized proteins, which recognizes the analyte, which in most cases is also a protein that is binding with immobilized one by forming corresponding immune-complex, and in such way it induces analytical signal [126]. Therefore, proper orientation of immobilized protein molecules is among key issues during the design of affinity-based immunosensors [27, 127,

128], because it is critical to achieve efficient target-protein binding [127]. Hence, analytical performance of immunosensors and some other affinity sensors depends on the orientation of immobilized antibodies [129], fragments of antibodies, which are generated by the reduction of disulfide bounds holding together polypeptides that are forming antibodies [127] or receptors [27]. Proper entrapment of the proteins in the assembled polymer layer matrix can be achieved by the electrochemical methods, which can be used to form the conducting polymer based layers [28]. Molecular imprinting technology enables to design MIPs that have properly oriented binding sites [99, 130, 131]. Therefore, the application of polymers imprinted by proteins is very promising [132, 133] (Fig. 3), because it enables to replace very expensive antibodies [28] and receptors [27], which are used in affinity sensors, therefore, protein imprinted MIPs are frequently applied for the bioanalytical purposes [134–137]. During the entrapment and extraction procedures, proteins can undergo some conformational changes [138], and/or proper orientation of formed cavity within polymer can be achieved [139]. Therefore, MIPs often are called as ‘synthetic receptors’ or ‘artificial receptors’ [135] and ‘plastic antibodies’ [140, 141]. Various conducting polymers prepared by electrochemical deposition can form a polymeric backbone suitable for MIP formation, e.g. polypyrrole imprinted bovine leukemia virus glycoprotein was designed [135]; Electrochemically formed poly-o-phenylenediamine/hydroquinone imprinted by human serum albumin (HSA) was applied for the determination of HSA in urine [142]; the surface of polydopamine layer was imprinted by immunoglobulin G [143]; molecularly imprinted hetero-structure based on PEDOT/PSS was also used for the detection of proteins [139]; synthetic receptor based on electrochemically formed polydopamine was applied for the determination of a prostate specific antigen in human blood plasma [144]; electrodeposited composite based on polypyrrole/(carbon nanotube) was imprinted by S-ovalbumin and was used for the detection of this protein in egg’s white [145]; electrochemically formed poly(o-phenylenediamine) was imprinted by myoglobin [146]; electrodeposited MIPs based on polyscopoletin were exploited for the detection of HSA [147]; poly-scopoletin imprinted by cytochrome c (Cyt-c) was applied for the determination of Cyt-c [148]; copolymer based on hydroxyethyl acrylate and ethylene glycol dimethacrylate imprinted by lysozyme was developed [149]; poly(2-hydroxyethyl methacrylate-N-methacryloyl-(L)-histidin-Cu(II)) imprinted by ceruloplasmin was synthesized by radical polymerization [150]; the SARS-CoV-2 protein imprinted poly-m-phenylenediamine based electrochemical sensor was used for the determination of infection by SARS-CoV-2 [113]; MIP-based sensor for detection of follicle-stimulating hormone was designed [151]; electrodeposited ortho-polydopamine imprinted by alpha-fetoprotein, which was temporarily covalently immobilized on gold nanoparticle covered substrate, was applied in sensor design [152]; acrylamide/N,N<sub>0</sub>-methylenebisacrylamide copolymers imprinted by both prostate-specific antigen and

myoglobin were applied for the determination of both these proteins [153]; sensor based on polyacrylamide imprinted by hemoglobin was developed [154]; and o-phenylenediamine was used for the determination of imprinted troponin T [155].

Conducting polymer – polyaniline (PANI) is also rather often used in sensor design [Erreur ! Signet non défini.]. However, in the research based on molecularly imprinted polymers only a few reports related to PANI-based MIPs can be found: PANI-based MIP was applied for the determination of antibiotic azithromycin [156] and for some hydroxy acids and saccharides [157]. It should be noted that even inorganic compounds such as titanium dioxide (TiO<sub>2</sub>) can be molecularly imprinted by proteins, e.g.: TiO<sub>2</sub> was imprinted by urease [158]. In some researches it was shown that peptides, which are serving as epitopes of some proteins, can be imprinted and such MIPs can be used for the determination of the ‘parent proteins’ and this technology was applied for the design of electrochemical sensor based on MIP imprinted by N-terminal pentapeptide VHLTP-amide, which is an epitope of human hemoglobin (HbA) [159].

MIP-formation needs knowledge in organic and polymer chemistry [160, 161]. It was demonstrated that DNA [29] can be entrapped [26] and molecularly imprinted [162–164] within CP-based layers. Therefore, some investigations are dedicated to replace direct application of DNA-based sequences in analytical systems [165]. It should be noted that even relatively large objects such as whole bacteria [166, 167] (e.g.: *Escherichia coli* [168]) or spores (e.g.: *bacillus cereus*) [169] were imprinted within electrodeposited polypyrrole. Some polymers were imprinted by viruses [170] and bacteria [168, 171–174] and other living cells [175]. Such MIPs can be used for the determination of bacteria in various environments [36, 176].



**Figure 3.** 1) The formation of molecularly imprinted polymer based sensor, 2) Molecularly imprinted polymer based layer in action. Figure adapted from [2].

### ***3.2. Signal transducers applied in sensors based on molecularly imprinted polymers***

Some polymeric structures can selectively recognize and bind target molecules quite selectively and have much better resistance towards harmful environmental factors in comparison to that of native biomolecules, which are commonly used in the design of biosensors. Therefore, some molecularly imprinted polymers seem very promising [177, 178] even if the application of molecularly imprinted polymers is still rather challenging [179]. The most stable at room conditions are MIPs based on acrylic acid, methacrylic acid and acrylamide [180–184].

The selection of the most suitable polymer for the design of MIP is an important technological issue [185]. Formed MIP should be able to establish hydrogen bonds, electrostatic and/or  $\pi$ - $\pi$  interactions [186]. Moreover, all these interactions should be capable to dissociate easily [3, 183, 187], because it is important for both MIP-formation and the regeneration of sensor after the measurement. Solution theories, which were derived by Flory and Huggins [188, 189], Hansen [190, 191] and Hildebrand [192, 193], well explain the formation and action mechanism of MIPs. Shape of cavities formed within MIPs' are changing during the swelling in applied solvents [194], and it significantly affects the 'shape memory' behavior of these MIPs [195]. In order to predict the most efficient structure and composition of MIPs molecular dynamics [196] and Density-Functional-Theory (DFT) [197, 198] calculations have been applied.

The determination of analyte binding with MIPs can be performed by direct and indirect electrochemical approaches. For direct assessment of analyte binding to MIP

potentiodynamic electrochemical methods [199], which are based on the determination of restricted diffusion of some ions [200], are applied. On the other hand formation of complex between analyte and MIP can be rearrange the electronic structure of polymeric backbone and this effect induces changes of electrical conductivity of MIP-layer [199]. Due to the above-mentioned effects, MIPs can be applied for the design of organic electrochemical transistors [201]. Doping compounds can induce p- or n-type conductivity to polyaniline [202], polypyrrole [28], and poly(3,4-ethylenedioxythiophene)/poly(styrenesulfonate) (PEDOT/PSS) [203]. However, doping and/or 'de-doping' of CP by various ions and materials in most cases is a reversible [202], therefore, it can be also exploited for the generation of analytical signal. However, mostly direct electrochemical detection has a major drawback because in this case, various nonspecific interactions are negatively affecting registered signal. For this reason, redox-probes are used, because they can and significantly increase the sensitivity of MIP-based electro-analytical systems [204, 205]. Moreover, the enhancement of analytical signals can be achieved using enzymes, namely tyrosinase [206], glucose oxidase [207], acetylcholinesterase [208], creatine kinase [209], cytochrome P450 [210], hexameric heme protein [211], laccase [212], microperoxidase [213], horseradish peroxidase [213–215] and lactoperoxidase [213]). Some other catalytic features like catalysis by Pt/Cu-based nanoparticles [216] or the inhibition of enzymatic activity [217] can be also adapted for the amplification of registered sensor response.

Signal transducing systems with quartz crystal microbalance (QCM) can also be applied in MIP-based sensor design, which are capable to detect: (i) low molecular weight chemicals [218, 219], namely: naproxen [220], histamine [221], *S*-propranolol [222], ibuprofen [223]; (ii) proteins [167, 224–226], trypsin [227], ribonuclease A [228] and oxidized-low-density lipoprotein [229]; and (iii) DNA [226, 230]. QCM-based determination of mass variations of MIP-based structures can be combined with some electroanalytical techniques (EQCM) [16, 99, 231]. Recently the most advanced QCM method – QCM with dissipation (QCM-D) has been also applied in MIP-based sensors [232].

Some optical techniques such as photoluminescence [233, 234] and surface plasmon resonance (SPR) [235] have been also used in MIP-based analytical systems. Remarkable optical characteristics of CPs can be well applied in the design of sensors based on optical transducers [236, 237] and photoluminescence sensors [234, 238, 239]. Studies affirmed that conducting polymer – polypyrrole – has great photoluminescence quenching ability [234, 238], which can be well exploited in the design of sensing devices and improve sensitivity and selectivity of biosensors [128, 240].

Optical analytical registration methods are used widely: MIPs were applied for the determination of organics such as estradiol and derivatives of this compound [241–243] were exploited. MIPs-modified by quantum dot nanoparticles modified by poly(ethylene-co-vinyl

alcohol) heterocomposite was used for optical detection of some salivary proteins [244]. MIP-based on  $\text{Cu}^{2+}$ -metalorganic-framework, which was imprinted by tetrabromobisphenol A, exhibited enzyme-like catalytic activity towards the oxidation of tetrabromobisphenol A by hydrogen peroxide [245]. Microarrays based on poly-scopoletin imprinted by ferritin – have been electro-spotted on a gold-modified substrate and applied in surface plasmon resonance (SPR) based ferritin detection [246]. To improve optical capabilities MIPs can be combined with photonic crystals [247] and liquid crystals [248]. Very useful optoelectrochemical property of some conducting polymers is an electrochromic effect, which can be exploited in the development of sensing devices [202]. Electrochromism is a reversible change of optical absorbance during oxidation/reduction of electrochromic material (e.g.  $\text{WO}_3$  [249], PEDOT/PSS, Ppy, PANI, etc.) layer by the variation of electrical potential. Such electrochromic sensors based on conducting polymers can be applied for the determination of some ions (e.g.  $\text{Cu}^{2+}$  [202] or  $\text{NH}_4^+$  and  $\text{CO}_3^{2-}$  ions) [79, 250, 251].

#### **4. Compatibility of conducting polymers with various biological compounds and immune system of mammals as of forecasting application in the wearable sensors based on MIPs**

Implantable sensors and other biomedical tools are demanded for rapidly evolving field of biomedicine. Therefore, good compatibility of sensing elements is important for the development of implantable bioanalytical devices. However, since now, in almost all researches in this area, the biocompatibility of these structures is investigated rarely. In some researches, the biocompatibility of conducting polymers, which are forming sensing structures, only towards rather basic biological molecules (enzymes, DNA, etc.) is evaluated [29, 31, 48, 55–57]. It should be noted that such evaluation does not provide an estimation of the complex biocompatibility of these polymers, which is required for safe biomedical application [252]. Therefore, cell line and/or laboratory animal-based experiments are necessary for the evaluation of advanced biocompatibility. In several researches, it was shown that conducting polymers have a good biocompatibility with entrapped proteins [28, 29, 31, 48, 55–57]. Research have demonstrated the biocompatibility of polypyrrole with stem cells derived from bone marrow [52], primary mouse embryonic fibroblast (MEF) and human T lymphocyte Jurkat cells [53], and differentiated neuronal cell [253]. It was also acknowledged that polypyrrole is not affecting the immune system of mammals and their hematological parameters [54]. Among many composite structures hydrogels, which are based on conducting polymers, show a good biocompatibility due to the significant amount of water confined within the structure of these polymers [254]. It was demonstrated that, the biocompatibility of conducting polymer based structures can be advanced by incorporation of chitosan [255] and/or some other biocompatible polymers [256–258]. Moreover, some of

these additionally used polymers (e.g. chitosan) are suitable for the design of MIPs [259]. Outstanding biocompatibility of Ppy [52–54] and hydrogel-based polymers pave a way to exploit composite structures based on these materials in the development of attachable [260], wearable [261], and other [262, 263] sensors and biosensors. Hence, conducting polymer based composites are suitable for the design of scaffolds [264–266], incorporation of living cells and some other biomedical applications [267–270].

## 5. Conclusions

Conducting polymers are frequently used in the design of chemical sensors and biosensors, as well as for many other technological approaches. Sensors based on MIPs are providing fast analytical responses, are operating at ambient conditions, and are characterized by good sensitivity and selectivity. Conducting polymers are appropriate for the formation of MIPs and these polymers can be designed by different polymerization methods. Electrochemical formation of CP-based structures can be controlled in many ways and enables to design of very different CP-based structures even from the same composition of polymerization-bulk solution, therefore, they are suitable for the development of a great variety of MIPs. Some conducting polymers can be overoxidized after the formation; this treatment is especially eligible for the development of MIP-based sensors because it can be applied for (i) the formation of oxidized radicals, which are increasing sensitivity/selectivity towards imprinted target molecules within MIP-based structure and (ii) the facilitation of template removal and/or regeneration of MIP-based layers.

Polypyrrole is the most used conducting polymer and it is often applied in the formation of MIPs. Moreover, the advantages of the overoxidation of this polymer are the most frequently reported. Yet this application of overoxidized polypyrrole still has a lot of room for improvement and extension in the application of polypyrrole based MIPs, because polypyrrole can be easily synthesized by chemical and electrochemical methods from various solutions based on the most frequently used solvents and overoxidation of polypyrrole can be easily performed during the synthesis and/or after formation of Ppy-based layer. Moreover, polypyrrole shows great compatibility with various biological compounds and do not irritate the immune system of mammals, therefore, is suitable for the development of implantable biomedical tools, such as sensors, biosensors and biofuel cells.

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