Chapter 3

Polymers for Biosensors Construction

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Additional information is available at the end of the chapter

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1. Introduction

The unprecedented interest in the development and exploitation of analytical devices for detection, quantification and monitoring of specific chemical species has led to the emergence of biosensors. Electrochemical biosensors have gained ever-increasing acceptance in the field of medical diagnostics, health care, environmental monitoring, and food safety due to high sensitivity, specificity, and ability for real-time analysis coupled with speed and low cost and polymers are promising candidates that can facilitate a new generation of biosensors [1-7]. A biosensor is a device having a biological sensing element either intimately connected to or integrated within a transducer. The aim is to produce a digital electronic signal, which is proportional to the concentration of a specific chemical or set of chemicals (Fig. 1). A definition of biosensor is proposed by IUPAC as: "a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in direct spatial contact with a transducer element." The life time of the enzyme electrode, the rate of electron transfer between the enzymatic redox reaction and electrode, and the miniaturization of enzyme electrode are some of the critical points appeared as central to this interdisciplinary research. Biosensor has been pursued extensively in a wide range for their unparalleled selectivity and mild reaction conditions. As a coin has two sides, enzymes which are the key biological recognition element are usually costly and easy to inactivate in their free forms. The immobilization of enzymes is the main approach to optimizing the in-service performance of an enzyme, particularly in the field of non-aqueous phase catalysis. However, the immobilization process for enzymes will inevitably result in some loss of activity, improving the activity retention of the immobilized enzyme is critical. To some extent, the performance of an immobilized enzyme is mainly governed by the supports used for immobilization, thus it is important to fully understand the properties of supporting materials and immobilization processes [8-10]. The properties of immobilized enzymes are governed by the properties of both the enzyme and the support material. The interaction between the two lends an immobilized enzyme specific physico-chemical and kinetic properties that may be decisive for its practical application, and

thus, a support judiciously chosen can significantly enhance the operational performance of the immobilized system. It is widely acknowledged that analytical sensing at electrodes modified with polymeric materials results in low detection limits, high sensitivities, lower applied potential, good stability, efficient electron transfer and easier immobilization of enzymes on electrodes. In recent years, there has been growing concern in using polymeric materials as supports for their good mechanical and easily adjustable properties [11]. Of the many carriers that have been considered and studied for immobilizing enzymes, conducting polymer (CP), redox polymer (RP), sol-gel and hydrogel materials, chitin and chitosan are of interest in that they offer most of the above characteristics [12, 13].

The proper use of different compositions of binder and immobilization matrix, electron transport mediators, biomaterials and biocatalysts, and solid supports as electron collectors in the construction of enzyme electrode is critical to generate optimum current from the enzymatic redox reactions. In essence, design and fabrication of advanced materials coupled with good understanding of their behaviors when incorporated as interfacial or transducer elements would be of paramount importance. The recent advancement of polymer materials is greatly influencing the redox reactions and electron transport kinetics of the enzyme electrodes. To achieve high specificity, high sensitivity, rapid response and flexibility of use, it is clear that the research continues to focus on new assembly strategies. Polymers are becoming inseparable from biomolecule immobilization strategies and biosensor platforms. Their original role as electrical insulators has been progressively substituted by their electrical conductive abilities, which opens a new and broad scope of applications. This chapter highlights recent contributions in the incorporation of promising polymeric materials within biosensors, special emphasis was placed on different classes of polymeric materials such as nanomaterials, sol-gel and hydrogel materials, conducting polymers, functional polymers and biomaterials that have been used in the design of sensors and biosensors. We want to remind our readers that this chapter is not intended to provide comprehensive coverage of electrochemical biosensor development but rather to provide a glimpse of the incorporation of polymers within biosensors. These materials have attracted much attention to their potentials for interesting applications, broad applicability as well as tunable properties according to applications needs. In addition, the critical issues related to the fabrication of enzyme electrodes and their application for biosensor applications are also highlighted in this article. Effort has been made to cover the recent literature on the advancement of polymers to develop enzyme electrodes and their potential applications for the construction of biosensors [14-15].

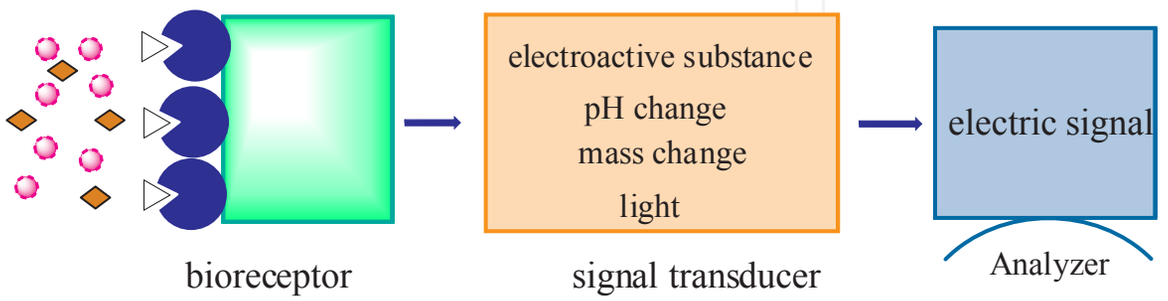

Figure 1. Principle of biosensor

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2. Construction of enzyme-based biosensors

2.1. Immobilization methods of enzyme for biosensors

Enzyme immobilization is one of the most important subjects for any enzyme-based biosensor research. Considerable efforts have been invested in this topic for a number of years [16-18]. The biosensing process of the immobilized enzyme is regarded as a heterogeneous phase reaction; thus, the main consideration for enzyme immobilization is to achieve stable and high enzymatic activity with low mass-transfer resistance. Enzymes electrodes have the longest tradition in the field of biosensors. They are one of the most intensely investigated biosensors due to highly selective and fast response. One of the key factors in developing a reliable biosensor is the immobilization of enzymes at transducer surfaces. The method of enzyme immobilization plays an important role on the performance of an enzyme electrode, such as lifetime, linear range, sensitivity, selectivity, response time, stability and anti-interferent.

Enzymes may be immobilized by a variety of methods (Fig. 2), which may be broadly classified as physical approaches and chemical approaches. To the physical methods belong: (i) physical adsorption on a water-insoluble matrix based on hydrophobic, electrostatic and van der Waals attractive forces; (ii) entrapment enzyme in sol-gel, or hydrogel, or a paste, confined by semi-permeable membranes; (iii) microencapsulation with a solid membrane; (iv) encapsulation, containment of an enzyme within a membrane reactor; (v) formation of enzymatic Langmuir-Blodgett films or self-assemble monolayer which are the spontaneous and uninstructed structural reorganizations that form from a disordered system.

The chemical immobilization methods include: (i) covalently binding enzyme to support materials immobilizing enzyme into a membrane matrix or directly onto the surface of the transducer; (ii) crosslinking enzyme employing a multifunctional, low molecular weight reagent based on the formation of strong covalent binding between the transducer and the biological material using a bifunctional agent and (iii) electrochemical polymerization based on electrochemical oxidation of a given monomer from a solution containing the enzyme obtaining a conducting or non-conducting polymer layer and (iv) Micelle: The molecule must have a strongly polar/ hydrophilic "head" and a non-polar/hydrophobic "tail". When this type of molecule is added to water, the hydrophilic head of the molecule presents itself for interaction with the water molecules on the outside of the micelle, and the hydrophobic tails of the molecules clump into the center of a ball like structure, called a micelle. Enzyme micelle membrane presented here is an innovative way and will be a well-developed biosensor technology to provide rapid and reliable measurements of food, water pollution and clinical analysis.

2.2 Recent development of biosensor researches

Electrochemical biosensors are the oldest and most widely available group in the solid-state chemical sensor field. Electrochemical sensors provide a crucial analytical tool as demand for

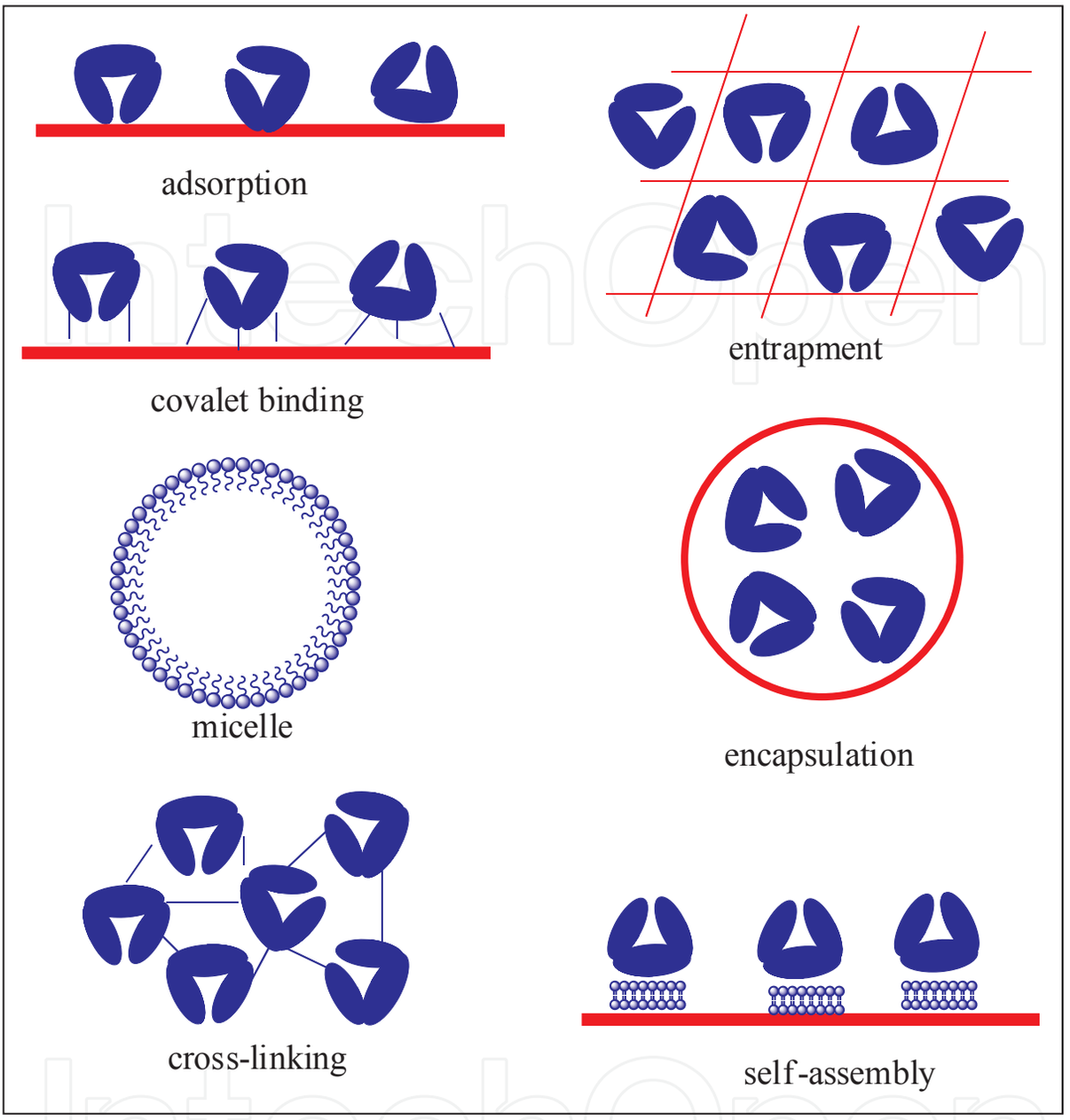

Figure 2. Illustration of enzyme immobilization methods

sensitive, rapid, and selective determination of analytes increases. Over the past two decades we have witnessed a tremendous amount of activity in the area of biosensors. Enzyme based electrodes require the immobilization of an enzyme onto an electrode surface for the quantification of an analyte and hold a leading position among biosensor systems presently available. Biosensors have found promising applications in various fields such as biotechnology, food and agriculture product processing, health care, medicine and pollution monitoring. Recent development has focused on improving the immobilization and stability of the enzymes. There have been a number of recent review articles that have focused on the development of various

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materials, techniques, and applications of biosensors [19-24]. Since there have been a wealth of biosensor developments in the past years, new approaches and materials for enzyme based sensors have been primarily focused. Strategies for incorporating materials to enhance speed, sensitivity, and stability of these sensors has been of particular interest. Major advancements in biosensors revolve around immobilization and interface capabilities of the biological material with the electrode. The use of polymer and nanomaterials has provided a means for increasing the signal response from these types of sensors. Moreover, the combination of various nanomaterials into composites in order to explore their synergistic effects has become an interesting area of research. The ability to incorporate biomaterials with the potential for direct electron transfer is another growing research area in this field [25-31]. In general, we believe that the field of electrochemical sensors will focus on the incorporation and interaction of unique materials, both nano and biological, in the coming years.

3. Polymers coating in biosensors

Enzyme immobilization using supports have been of great interest for many researches. Various supporting films on electrode surface have been developed to immobilize proteins or enzymes and many polymeric materials were used for enzyme immobilizations. Polystyrene (PS) membrane is a very promising support for the immobilization of enzymes due to its excellent biocompatibility, no toxicity, high affinity, strong adsorption ability, low molecules permeability, physical rigidity and the chemical inertness in biological processes. Its molecular structure is shown in Fig. 3. It is popular for the immobilization in enzyme-linked immunosorbent assays (ELIA) by adsorption, however, and few methods for immobilizing proteins on the PS surface by covalent bonding have been proposed, because the complicated multistep methods must be employed for introduction of functional groups that react with proteins and their procedures are tedious and time consuming. In order to solve the difficulty of introduction of functional groups, we adopted polymaleimidostyrene (PMS) to introduce maleimide group in the bulk of PS, and the coating of enzyme containing PS membrane on the electrode surface under mild conditions opens up enormous possibilities for the immobilization of biomolecules [32-40].

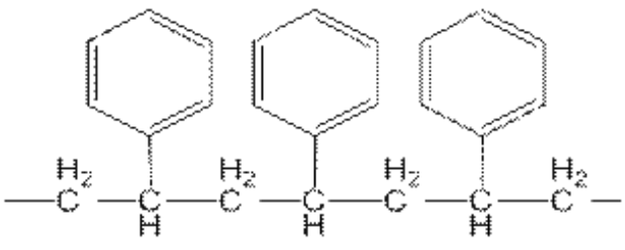

Figure 3. Molecular structure of polystyrene

The interferents and biofouling are two major problems which can affect the performance of a biosensor. Interference from electroactive substances is especially problematic when electrochemical measurements are being made in vivo. Biocompatible membranes are preferable both as a selective barrier as well as for enhancing biocompatibility within electrochemical biosensors [41]. The cellulose acetate layer permits only small molecules, such as hydrogen peroxide to reach the electrode, eliminating many electrochemically-active compounds that could interfere with the measurement. Fig. 4 depicts the molecular structure of cellulose.

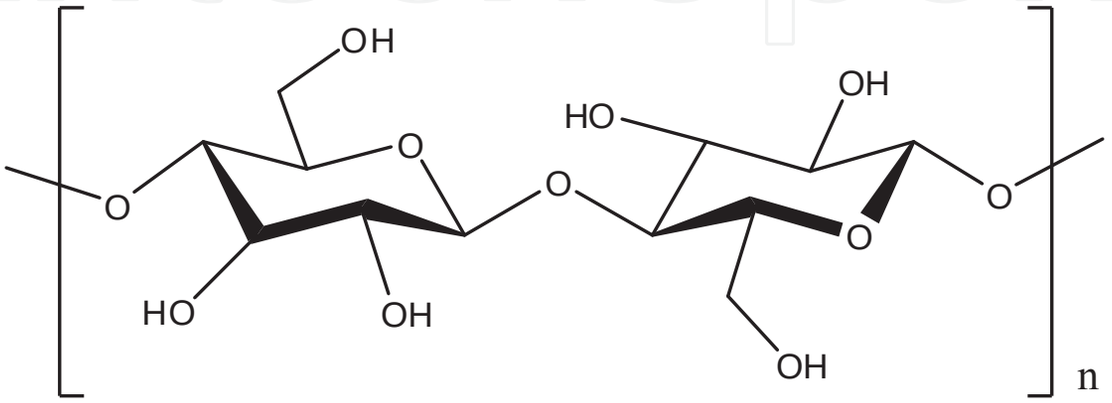

Figure 4. Molecular structure of cellulose

Nafion, a perfluorinated sulfonated cation exchanger (see Fig. 5), has been widely used as an electrode modifier due to the chemical inertness, thermal stability, mechanical strength, and antifouling properties. Nafion coated electrodes have been applied in the analysis of phenol, for the determination of parathion [42, 43]. Nafion has also been widely utilised as a coating material. The polymer displays the advantages of being chemically inert and easily cast from solution. The polymer is anionic and upon casting forms a structure with hydrophilic channels contained within a hydrophobic matrix. Films formed from this material are reasonably robust, show strong exclusion of anionic interferents and display enhanced biocompatibility.

As functional materials, chitin and chitosan offer a unique set of characteristics: biocompatibility, biodegradability to harmless products, nontoxicity, physiological inertness, antibacterial properties, heavy metal ions chelation, high affinity to proteins, gel forming properties and hydrophilicity, remarkable affinity to proteins, availability of reactive functional groups for direct reactions with enzymes and for chemical modifications, mechanical stability and rigidity, and ease of preparation in different geometrical configurations that provide the system with permeability and surface area suitable for a chosen biotransformation [44]. Owing to these characteristics, chitin and chitosan offer a unique set of these characteristics and are predicted to be widely exploited in the near future especially in enzyme immobilization supports. The most distinguishing chitosan properties are its biodegradability and biocompatibility, which makes it a green polymer. The increasing importance of materials from renewable sources has put chitosans in the spotlight, especially due to their biological

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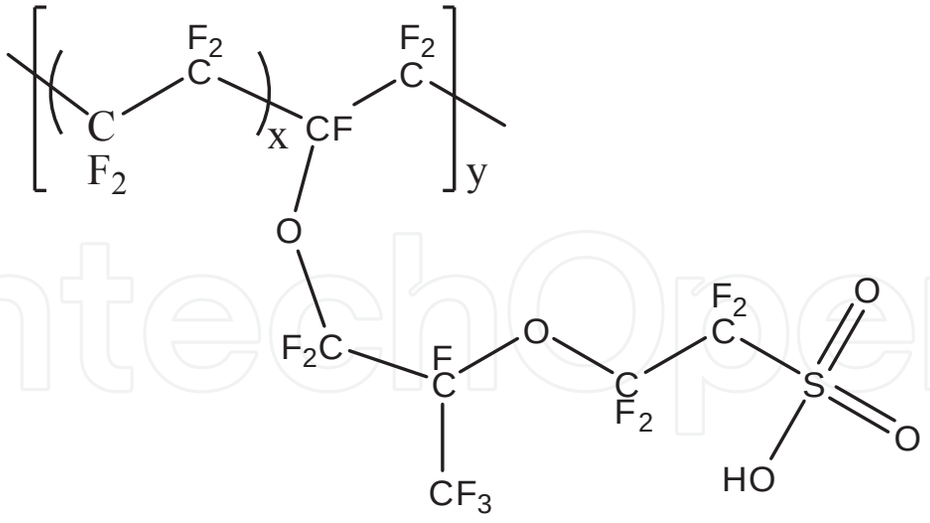

Figure 5. Molecular structure of Nafion

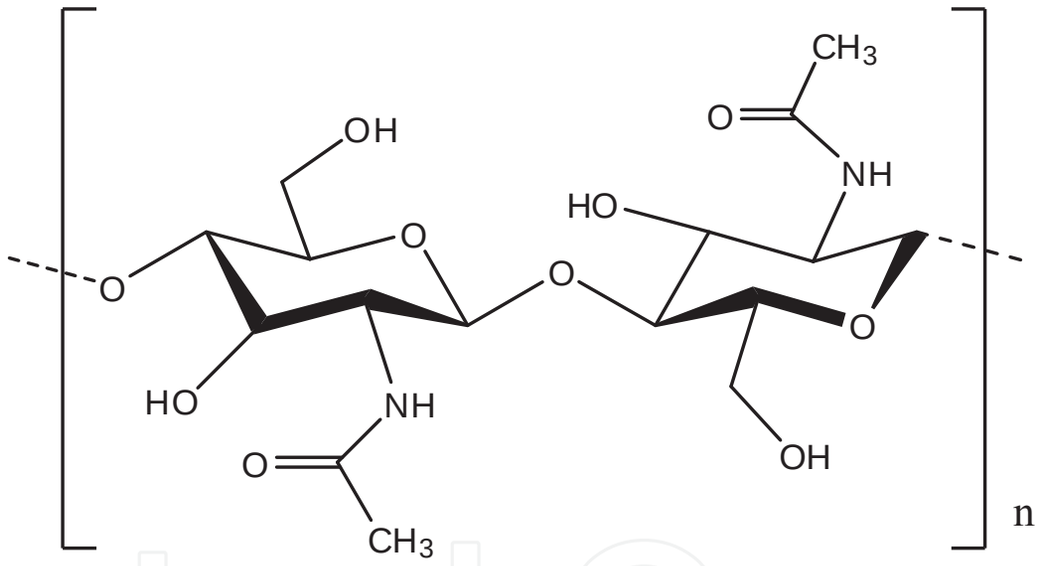

Figure 6. Molecular structure of chitin

properties, which have been exploited in many applications [45, 46]. The molecular structure of chitin and chitosan are shown in Fig. 6 and Fig. 7, respectively.

4. Polymaleimidostyrene in biosensors

Polymers are becoming inseparable from biomolecule immobilization strategies and biosensor platforms. Their original role as electrical insulators has been progressively substituted by their electrical conductiveabilities, which opens a new and broad scope of applications in both the physical adsorption and chemical coupling methods, protein molecules are immobilized on the

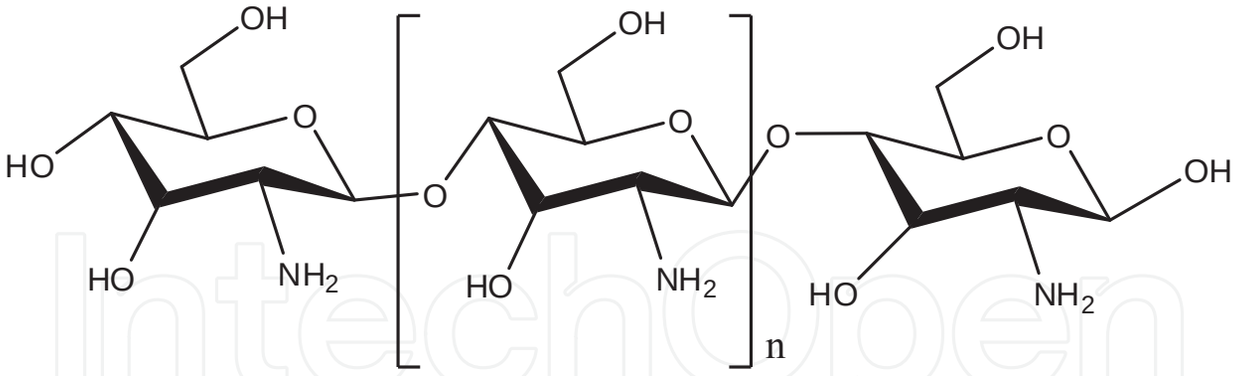

Figure 7. Molecular structure of chitosan

surface with random orientations and are likely to be denatured. In order to increase the lifetime stability of enzyme electrode, it is necessary that there should be a strong and an efficient bonding between the enzymes and immobilizing material. Hence, covalent binding of enzyme on a stabilizer or on the transducer is an efficient method of immobilization. Recently, we have developed an advanced design and preparation of enzyme-based amperometric biosensors using enzyme reverse micelle membrane, as well as the functional structure and principle. Particular emphasis is directed to the discussion and exploration of electrochemical biosensors based on novel functional polymer, polymaleimidostyrene (PMS), as effective immobilization stabilizer as support. It can be expected to be a common method for the immobilization of enzymes to fabricate various bioelectrochemical sensors [32-40].

4.1. Synthesis, structure and properties

PMS which is a polymerization of N-4-vinylphenyl maleimide (N-VPMI) is a new type of polymer mixible with polystyrene (PS). It was synthesized by Prof. Hagiwara's group in 1991 [34]. The compound was prepared by a modified method for synthesis of N-phenylmaleimide (N-PMI). N-VPMI purified by recrystallization was used as a monomer after thoroughly dried below 20 ℃ under reduced pressure. Polymerization was started by the addition of a tetrahydrofuran (THF) solution of initiator to the monomer solution at a determined temperature with stirring. The synthesis scheme and the molecular structure of PMS are depicted in Scheme. 1.

PMS possesses two polymerizable carbon-carbon double bonds with different reactivities, one of which is the vinylene group of the maleimide moiety and the other the vinyl group of the styrene moiety. The vinylene groups of a maleimide moiety react easily with sulphydryl or amino groups of enzyme by covalent bonds which prevent the unfolding of enzyme. PMS is a very effective, important and useful reagent to immobilize enzyme strongly via covalent bond, because high density of maleimide groups of PMS can catch not only exposed SH groups but also buried SH groups forming enzyme micelles [35–36]. To model the enzyme micelle structure, an illustration is displayed in Fig. 8. The hydrophobic PMS groups are outside the structure shielding the hydrophilic enzyme inside the interior. Therefore, the structure is named as reverse micelle. The reverse micelle structure tends to evolve to a lower-energy configuration under equilibrious conditions.

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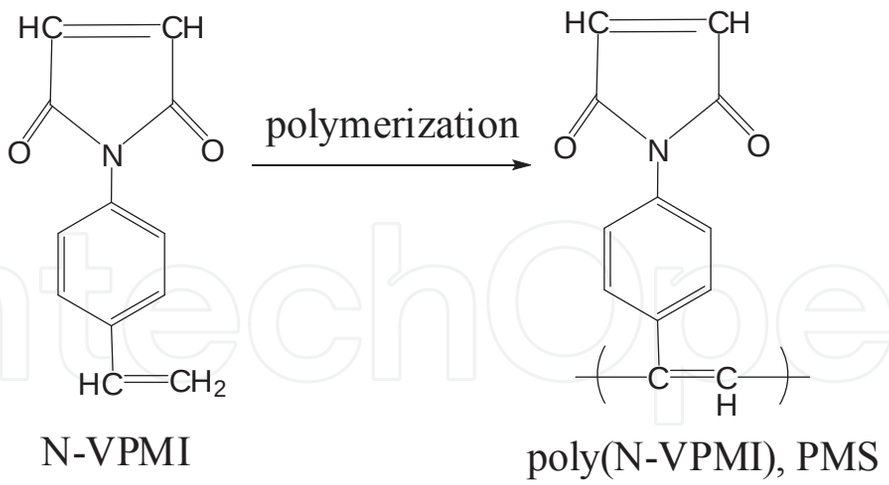

Scheme 1. Synthesis of polymaleimidostyrene

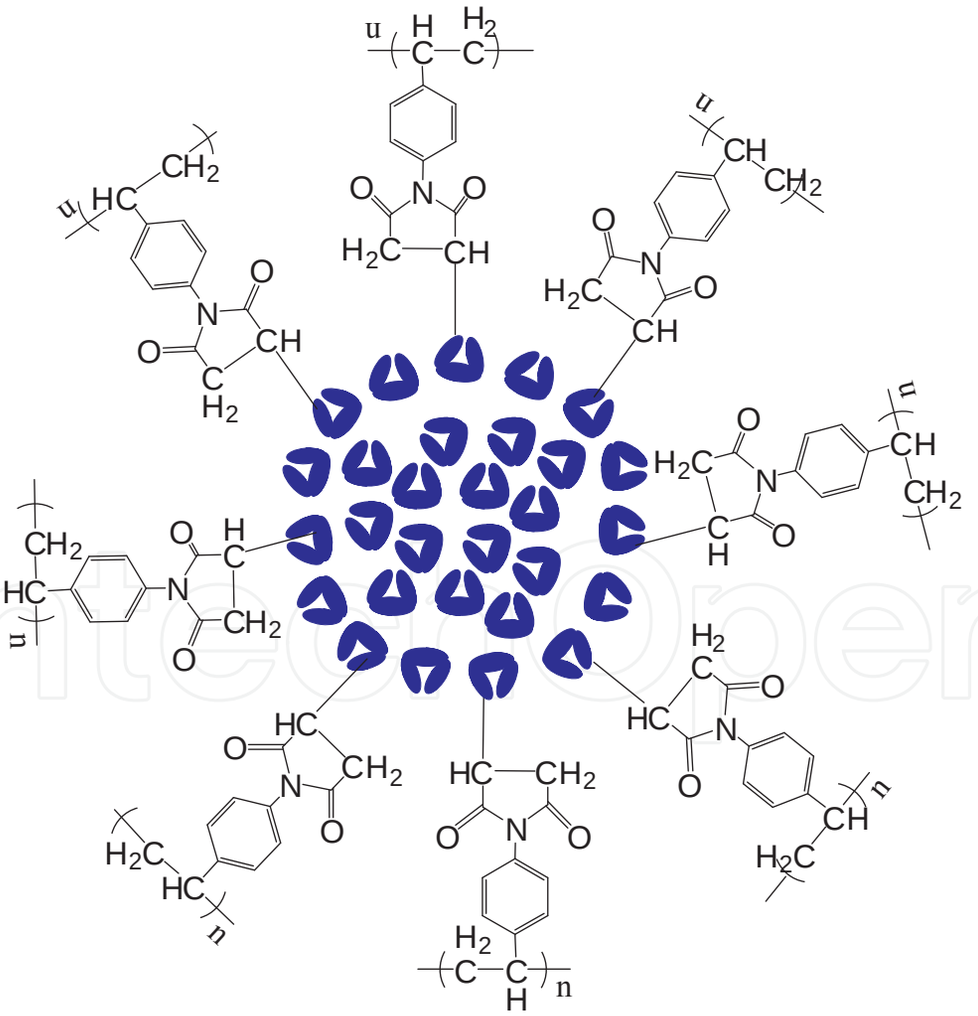

Figure 8. Illustration of enzyme reserve micelle in polystyrene film

Although, it should be mentioned that the free enzyme exists in the outer surface of micelle may lose its activity due to the hydrophobility of chloroform reagent, which was used to dissolve PS and PMS, thus there is partly loss of enzyme activity during the immobilization, the strong adsorption ability of PS membrane makes the enzyme micelle based biosensors particularly attractive for on-line analytical systems which need high stability and good endurability due to the long-time and continuous determinations in a flow system during experimentation.

Furthermore, PMS exhibits a strong affinity specific to a variety of hydrophobic materials such as polystyrene, polyethylene and polyetheretherketone due to the styrene moiety. Then PMS is considered to be an ideal stabilizer for both covalent bonding enzyme and hydrophobic affinity to PS film [30–32]. The application of PMS as a convenient immobilization reagent is summarized in table 1. PMS appears to be effective and promising for the maintenance of biological activity as well as long time stability.

4.2. Application of PMS to biosensors

It has been found that the urease reverse micelle membrane exhibits good sensitivity after stored in a phosphate buffer solution (0.1 M, PH 5.5) for one month compared with its initial sensitivity. The ideal immobilization process should be easy, quick, and enzyme friendly, result in high loading surface. In our previous study, enzyme micelle membrane, which is one-step immobilization, has been proved to be an excellent immobilization method to preserve enzyme conformation resulting in good activity and stability. To form enzyme micelle, hydrophilic PMS covalently bonded-enzyme (via both exposed and buried sulfhydryl groups and amino groups of enzyme and maleimide groups of PMS) was dispersed in hydrophobic PS solution. PMS-bonded enzyme aggregated to form micelle with enzyme inside the structure and PMS outside. The enzyme micelles were immobilized on the surface of glassy carbon electrode (GCE) utilizing PS, which has admirable properties of biocompatibility and strong adsorption ability. PMS also possess good biocompatibility, the PMS-bonded enzyme exhibits good activity due to PMS is a significantly excellent stabilizer for enzyme. On the other hand, it is amazing that the free enzyme exists in the inner part of micelle enhanced the stability of enzyme. The applications of PMS as a function polymer have been published [34-40] and were summarized in table 1.

5. Intrinsically conducting polymers in biosensors

5.1. Types, structures and features

It is generally recognized that the modern study of electric conduction in conjugated polymers began in 1977 with the publication describing the doping of polyacetylene (PA). Conductive polymers or, more precisely, intrinsically conducting polymers (CPs) having unique conjugated π-electron backbone system, are organic conjugated polymers that conduct electricity and are one of the more promising biocompatible materials [48]. Professor Alan Heeger along with Prof. Alan G. MacDiarmid and Prof. Hideki Shirakawa shared the 2000 Nobel Prize in

Electrode configuration	Enzyme	PH	Potential (V)	Linear range	Response time (min.)	Stability (days)	Ref.
OE/PCS	GOD	5.50		0.1– 3.0 mM	12	< 5	36
	ASOD	7.00	–0.7	0 – 1.5 mM	4	150	35
AGCE/PCS	Urease	7.00	1.2	0.5 – 21 mM	< 10	3	37
Gold/ERMM	Uricase	8.5	–0.7	0.005 – 0.105 mM	< 1	7	39
AGCE/ERMM	ASOD	5.50	–0.30	0.01 –0.6mM	< 1	15	38
	GOD	6.50	–0.45	0.2 – 2.6 mM	< 2	"/> 60	40
	Urease	7.00	1.2	0.5 – 16 mM	< 10	"/> 60	34

OE: oxygen electrode; PCS: porous carbon sheet; AGCE: aminated glassy carbon electrode; ERMM: enzyme reverse micelle membrane; GOD: glucose oxidase; ASOD: ascorbate oxidase.

Table 1. Amperometric sensors using PMS as a stabilizer

Chemistry for their seminal contribution to the discovery and development of conductive polymers. A substantial account of the electronic properties was studied. From the point of versatility of synthesis techniques, properties, and broadness of the scope of application, CPs have raised a great deal of scientific and technological interest and have led the research in materials science in a new direction. The last comprehensive reviews devoted to CP were published and are excellent summary of earlier work [49-52].

Since the beginning of conductive polymer research, it has witnessed the emergence of CPs as an intriguing class of organic macromolecules that offer high electrical conductivity and optical properties of metals and semiconductors and, in addition, have the processability advantages and mechanical properties of polymers, in particular, are especially amenable to be further exploited to develop a new form of electrochemical biosensor either as sensitive components or as a matrix for providing biomolecule immobilization, signal amplification and for rapid electron transfer for the fabrication of efficient biosensor devices. A variety of monomers can be electropolymerised on an electrode surface and under correct conditions form stable conductive films. Structures of some CPs commonly used in biosensors are described in Fig. 9.

CPs like polypyrrole (PPy), polyaniline (PANI), polythiophene (PT) can be obtained by electrochemical polymerization either potentiostatically, galvanostatically or by means of multi-sweep experiments. The thickness of the polymer films can be defined by measuring the charge transferred during the electrochemical polymerization process and by controlling parameters like temperature, monomer concentration, polymerization potential or current, as well as the concentration and nature of the supporting electrolyte. Moreover, CPs films exhibt interesting properties concerning the decrease of the influence of interfering compounds due to their size-exclusion and ion-exchange characteristics.

The π-electron backbone which is an extended conjugated system having single and double bonds alternating along the polymer chain is responsible for their unusual electronic properties

such as electrical conductivity, low energy optical transitions, low ionization potential and high electron affinity [53]. Scientists from many disciplines are now combining expertise to study organic solids that exhibit remarkable conducting properties. A key requirement for a polymer to become intrinsically electrically conducting is that there should be an overlap of molecular orbitals to allow the formation of delocalized molecular wave function. Besides this, molecular orbitals must be partially filled so that there is a free movement of electrons throughout the lattice [54]. The electronic conductivity of conducting polymers changes over several orders of magnitude in response to changes in pH and redox potential of their environment. The electrical properties can be fine-tuned using the methods of organic synthesis and by advanced dispersion techniques. Polymeric material containing interesting electrical properties is a step forward for research in materials. CPs has the ability to efficiently transfer electric charge produced by the biochemical reaction to electronic circuit. Moreover CPs can be deposited over defined areas of electrodes. The study of unique property of CPs has resulted in fundamental insights into the understanding of the chemistry and physics of this novel class of materials, and it has been exploited for the fabrication of amperometric biosensors.

5.2. Application of CPs in biosensors

Polymers are being discarded for their traditional roles as electric insulators to literally take charge as conductors with a range of novel applications. The electronic CPs has an organised molecular structure on metal substrates, which serves as proper and functional immobilizing platforms for biomolecules. These matrices provide a suitable environment for the immobilization and preserve the activity for long duration. This property of the conducting polymer together with its functionality as a membrane has provided opportunities to investigate the development of biosensors. Application of organic CPs in biosensors has recently aroused much interest as potential candidates to enhance speed, sensitivity and versatility for electrochemical biosensors due to their easy preparation methods along with attractive unique properties such as high stability at room temperature, good conductivity output and facile polymerization and being compatible with biological molecules in a neutral aqueous solution. Moreover, the CP film provides a suitable environment for the immobilization of biomolecules. Thus, CPs have been studied extensively for the development of biosensors. The electrochemically prepared conducting polymers used for the biomolecule immobilization are polyacetylene (PA), polypyrrole (PPy), polythiophene (PT), polyaniline (PANI) etc because of their good electrical properties, environmental stability. Many applications of conducting polymers including analytical chemistry and biosensing devices have been reviewed by various researchers [55, 56].

Enzyme immobilization onto the electrode surface is a crucial step in assembling amperometric biosensors. The CPs have attracted much interest as suitable matrices for biomolecules due to that the extended conjugation along the polymer backbone provides unusual electrochemical properties such as low energy optical transitions, high electrical conductivity, low ionization potential, high electronic affinities. Polymer matrices can be used either in the sensing mechanism or in the immobilization of the bioelement responsible for sensing the analyte. The

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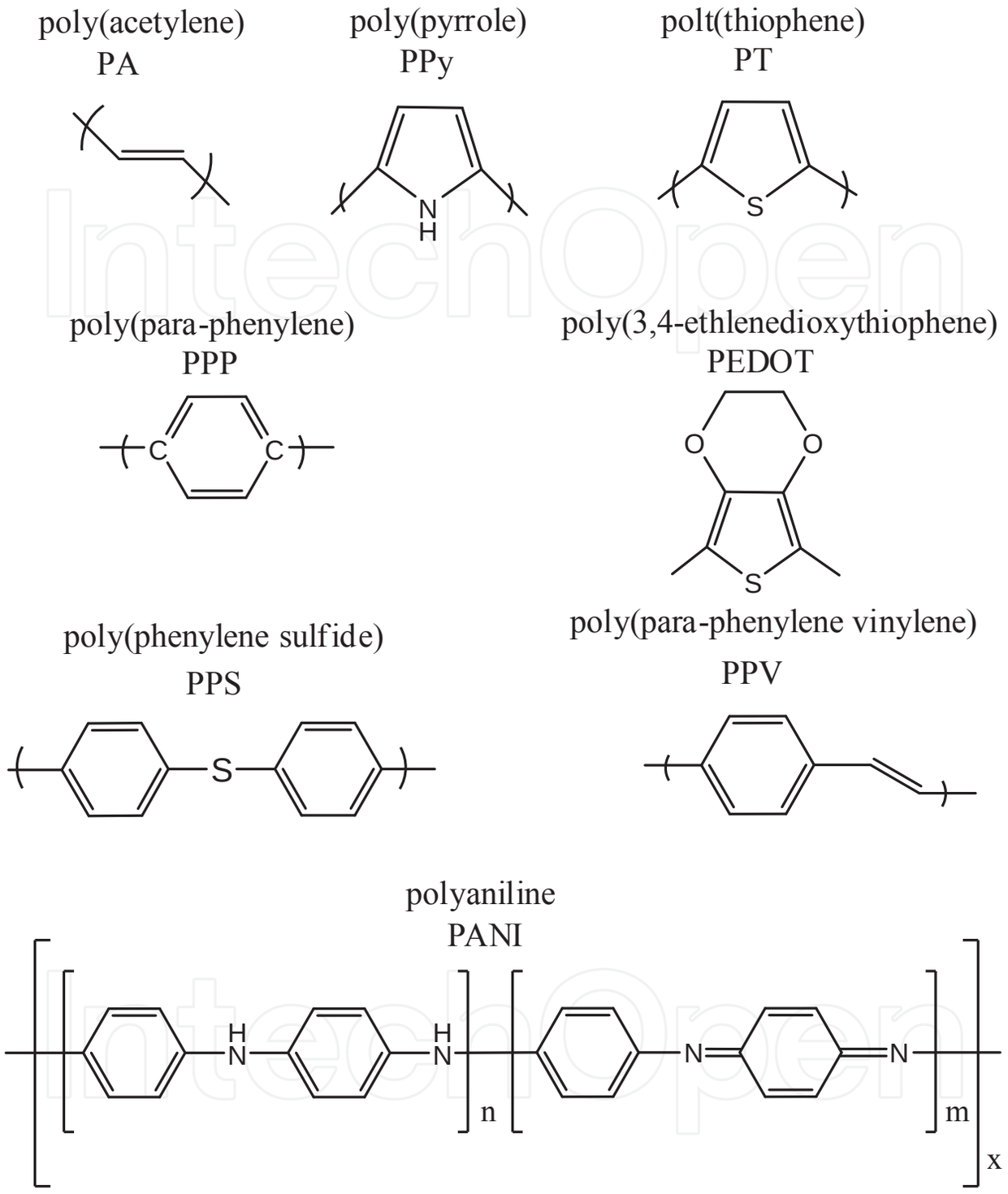

Figure 9. Structures of some conducting polymers commonly used in biosensors.

empolyment of promising CPs in electron transfer as an appropriate surface for enzyme immobilization provides rapid response encourages the coexistences of biomolecules and raises the stability of the biosensors. Numerous papers have been published indicating organic

CPs as a convenient component, forming an appropriate environment for the immobilization of enzyme at the electrode surface. Stable immobilization of macromolecular biomolecules on conducting microsurfaces with complete retention of their biological recognition properties is a crucial problem for the commercial development of miniaturized biosensor. Most of the conventional procedure for biomolecule immobilization such as cross-linking, covalent binding and entrapment in gels or membrane suffer from a low reproducibility and a poor spatially controlled deposition.

Due to that CPs have considerable flexibility in the available chemical structure, which can be modified as required, CPs have attracted much interest to serve as good matrices for the immobilization of enzymes. The techniques of incorporating enzymes into electro-depositable conducting polymeric films permit the localization of biologically active molecules on electrodes of any size or geometry and are particularly appropriate for the elaboration of multi-analyte micro-amperometric biosensors. Another advantage offered by CPs is that the electrochemical synthesis allows the direct deposition of the polymer on the electrode surface, while simultaneously trapping the protein molecules. In addition, the electrochemically prepared CPs can be grown with controlled thickness using lower potential and they also provide an excellent enzyme-entrapping property. The polymerization through electrochemical oxidation provides greater control over the process and enables control over the thickness of the polymer layer and even small electrode substrate to be coated. This technique offers a suitable way to make a homogeneous film that adheres strongly with the electrode surface.

Another important advantage of using CPs is that the biomolecules can be immobilized onto the nanowire structure in a single step rather than the multiple steps that are required when other non-polymeric materials are used. Nanostructured conjugated polymers and their nanocomposites represent new advanced materials that are key issues for the development of new devices and structures offering the association of the various properties required in advanced applications. As conducting polymer nanomaterials are light weight, have large surface area, adjustable transport properties, chemical specificities, low cost, easy processing and scalable productions, they are used for applications in nanoelectric devices, chemical and biological sensors [57]. There are extensive studies in the literature concerning the synthesis, characterization, and application of these CPs.

Among the CPs, PPy is one of the most extensively used conducting polymers in design of bioanalytical sensors. PPy and its derivatives play a leading role due to several interesting properties such as electroactivity, ionic exchange properties, supercapacitors for energy storage, secondary batteries, and elastic textile composites of high electrical conductivity, as well as good stability. PPy have the most versatile applicability for the construction of different types of bioanalytical sensors. The background presented illustrates that PPy is a very attractive, versatile material, suitable for preparation of various catalytic and affinity sensors and biosensors. The immobilization of biologically active molecules into PPy can be obtained during electrochemical deposition during which either some undesirable electrochemical interactions can be prevented or the electron transfer from some redox enzymes can be facilitated. The developments in nano-structured conducting polymers and polymer nano-composites have large impact on biomedical research. Significant advances in the fabrications

of nanobiosensors/sensors using nano-structured CPs have been reviewed [58]. Recent advances in application of PPy in immunosensors and DNA sensors and recent progress and problems in development of molecularly imprinted PPy have been presented. The use of PPy in conjunction with bioaffinity reagents has provident to be a powerful route that has expanded the range of applications of electrochemical detection and its future development is expected to continue [59].

Among various CPs, PANI which can be directly and easily deposited on the sensor electrode and has controlled, high surface area, chemical specificities, long term environmental stability and tuneable properties has attracted much attention to be a suitable candidate to be used in various applications in biosensors due to its unique and controllable chemical and electrical properties, its environmental, thermal and electrochemical stability, and its interesting electrochemical, electronic, optical and electro-optical properties. PANI has gained much popularity in biosensor applications, partially due to its favourable storage stability, simple synthetic procedures with good processibility, rapid electron transfer and direct communication to produce a range of analytical signals and new analytical applications. Efforts have been made to discuss and explore various characteristics of PANI responsible for direct electron transfer leading towards fabrication of biosensor interfaces and can also be used as a suitable matrix for immobilization of biomolecules [60, 61]. Moreover, PANI exhibits two redox couples in right potential range to facilitate an enzyme–polymer charge transfer and thereby acts as self-contained electron transfer mediator. In particular, PANI's transport properties, electrical conductivity or rate of energy migration, provide enhanced sensitivity. In addition, Nanostructures of PANI can offer the possibility of enhanced performance and also helps to overcome the processibility issues associated with PANI. In a conclusion, the various remarkable characteristics of PANI matrix make it a novel platform for fabrication of variety of biosensors interface.

6. Sol-gel and hydrogel material in biosensors

The terminology sol-gel is used to describe a broad class of processes in which a solid phase is formed through gelation of a colloidal suspension. A typical hydrogel network involve poly (vinyl alcohol) (PVA) or poly(acrylic acid) (PAA). Sol-gel is gradually attracting the attention of the electrochemical community as a versatile way for the preparation of modified electrodes and solid electrolytes. Sol-gel electrochemistry is rather young, and often researchers are still excited by the mere feasibility of realizing an application by sol-gel technologies due to its better processibility, improved diffusion rate, large ion-exchange capacity, fast proliferation of organic-inorganic hybrids, and other application specific chemical properties. The types of sol-gel materials that are useful for electrochemistry and the recent advances in the various fields of sol-gel electrochemistry have been reviewed by a few research groups [62, 63]. The ease of preparation and the wide ranging flexibility of incorporating desired functionalities by careful selection and design can be readily applied to different types of electrode materials without being restricted by electrode shapes and designs, which are suited for the immobilization of biomolecules. However, sol–gel matrices, despite being also biocompatible, are

traditional fragile nature, similarly to biological membranes, and suffer from low sensitivity and reproducibility which hampered their application in biosensor. The organic–inorganic material prepared by sol–gel method can yield a highly sensitive, robust and stable biosensor.

Hydrogels have also been extensively investigated as coatings support for immobilization of enzymes. Enzymes can often denature and lose their efficiency; however this effect can be mitigated by encapsulating it inside a hydrogel, because hydrogel is a type of waterswollen and cross-linked polymer formed by the gelling process and features a highly hydrophilic structure of three dimensional networks. And the consequent swelling of the polymer matrix provides a biocompatible microenviroment for the enzyme to maintain its natural configuration, thus is an ideal matrix for the massive entrapment of cell and enzyme. Moreover, the biosensors based on the hydrogel have high sensitivities. Although it exhibits a high affinity for water, it does not dissolve, and provides sufficient permeability for both solvent and substrate molecules so that they are capable of diffusing quickly through the water-swollen polymer which has reasonably high water content. In addition, the external hydrophobic organic solvent is unable to distort the native conformation of the entrapped enzyme in the sol-gel, and the hydrogel is soft and of rubbery consistence which closely resemble living tissues. Consequently, the widely utilised application for hydrogels has been as enzyme stabilising agents [64-68].

7. Conclusion and future perspectives

Proper electrode fabrication using different materials for efficient electron transport has recently aroused much interest as a versatile tool for the constructing biosensors. Biosensors designed employing polymeric materials results in low detection limits, high sensitivities, lower applied potential, reduction of background, efficient electron transfer and easier immobilization of enzymes on electrodes. Application of organic CPs in biosensors has recently aroused much interest as potential candidates to enhance speed, sensitivity and versatility for electrochemical biosensors due to their easy preparation methods along with attractive unique properties such as high stability at room temperature, good conductivity output and facile polymerization and being compatible with biological molecules in a neutral aqueous solution. However, there are verious of challenges to be addressed in order to fulfill the applications of polymers. In addition, long laboratory synthetic pathways and costs are also involved in the production of the functional polymers. The aforementioned disadvantages of the above polymeric materials call for search for low cost biomaterials as alternative for the development of novel electrochemical sensors and biosensors. Those focuses towards designing smart polymers such as nanostructure doped polymers are the most promising for polymers to be further investigated. By combination of the unique properties of nanostructured material and various polymers, it is possible to develop novel enzyme-based bioelectronic devices with particular advantages. In particular, the integration of nanotechnology, with novel polymeric materials should lead to very sensitive and fast assays.

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