Electrochemical Biosensors Based on Polyaniline

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Electrochemical biosensors based on conducting polymers offer many advantages and new possibilities to detect biologically significant compounds. Various biosensors, such as enzyme sensors, DNA sensors and immunosensors based on polyaniline are reviewed in this paper. Besides electrochemical detection methods, some new trends with the combination of other techniques are also shortly discussed.

Biosensory elektrochemiczne z zastosowaniem polimerów przewodzących oferują liczne zalety i wiele nowych możliwości w oznaczaniu związków o dużym znaczeniu biologicznym. W niniejszym artykule przedstawiono przegląd sensorów enzymatycznych, DNA oraz immunosensorów, stworzonych z użyciem polianiliny. Poza elektrochemicznymi metodami analizy, przedyskutowano również nowe kierunki wykorzystania innych technik analitycznych.

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A biosensor is an analytical device that converts the concentration of the analyte into a signal (*e.g.* electrical signal) by integrating biological sensing element into a transducer [1]. Biosensors are portable, simple-to-use, and high specificity analytical tools, which are compatible with data-processing technologies. Therefore, biosensors will have promising applications in various fields, such as pharmacy, health care, pollution monitoring, food and agricultural product processing *etc.*
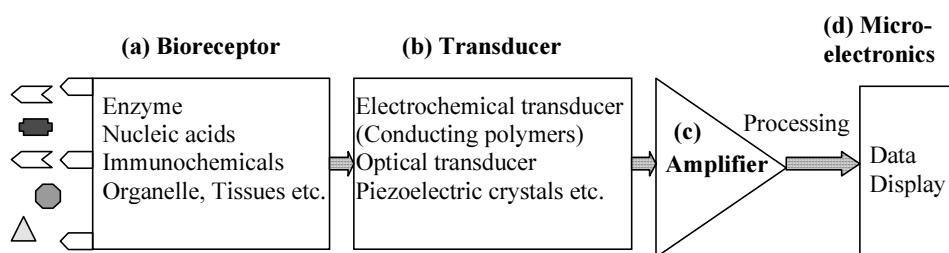

Figure 1. Basic structure of biosensors: (a) bioreceptor, (b) transducer converts biological recognition to an electrical signal, (c) amplifier enhances the output from the transducer, and (d) microelectronics for data display

Figure 1 shows the main components of a biosensor. Enzymes, nucleic acids, antibodies, tissue slice, binding protein, whole cells or other bioreceptors have been used as sensing probes in biosensor fabrications [2]. They have an intimate contact to the transducer. The transducer can be an electrochemical, optical or piezoelectrical device. Bioreceptor (a) and transducer (b) together are sometimes referred to as biosensor membrane. A very important advancement in producing biosensor membranes is the use of electrically conducting membranes that contain the enzyme, cofactor, and mediator. There are many different kinds of conducting polymers that can be chosen. The conducting polymer can act as the electrochemical transducer to convert the biological information into an electrical signal. It has been used in analytical applications already for a while [3]. There are some comprehensive reviews on the use of conducting polymers in electrochemical sensors [4–9].

PANI is one of the most intensively investigated conducting polymers, particularly due to its excellent stability in different solutions, good electronic properties, and strong biomolecular interactions [10]. The emeraldine salt (ES) form, which can be obtained in acidic conditions (pH = 2.5 ~ 3.0), is the only conducting state among the four basic states of PANI as shown in Scheme 1. Although PANI has been successfully applied in gas sensors [11–13], the pH sensitivity seems unfavorable for its application in biosensors, because most bioassays must be performed in neutral or slightly acidic conditions.
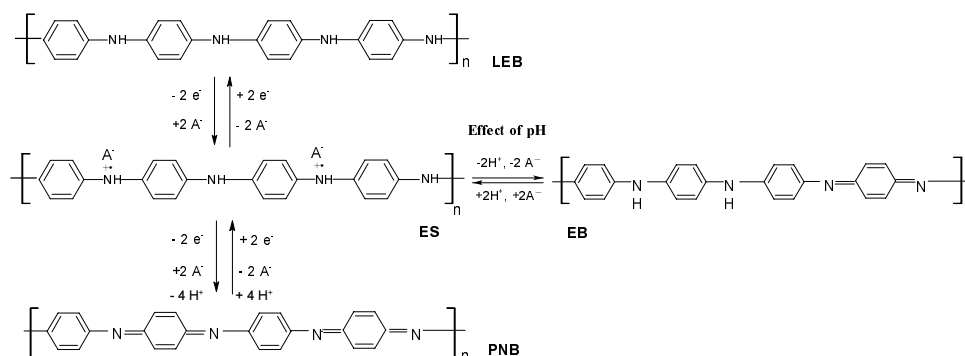
Scheme 1. Transitions between the four basic states of polyaniline: LEB, leucoemeraldine base (fully reduced form); ES, emeraldine salt (the only conducting form); PNB, pernigraniline base (fully oxidized form). Transitions between them are controlled by oxidation/reduction reactions. EB, emeraldine base (deprotonated form). Transition between ES and EB is controlled by pH

Recent research reveals that the N-substituted anilines do not have pH sensitivity [14]. This is due to that the alkyl chain, which is covalently bounded to the nitrogen atom, prevents formation of the EB form. Another derivative of PANI, self-doped PANI, which is usually referred to sulfonated PANI, shows redox activity even in solutions with neutral pH [15]. Sulfonated PANI was used in amperometric enzyme sensors with a sensitivity of 24.91 uA cm^{-2} to detect H$_2$O$_2$ in pH 6.4 buffer solutions [16]. It has also been demonstrated that the PANI blends that included negatively charged co-components such as sulfonic acid or polyacrylic acids exhibit redox activity in neutral aqueous solutions [17–19]. Shi *et al.* reported poly(aniline-aniline boronic acid) wires generated on double-strand (ds)DNA templates [20]. These wires are deposited on an electrode by means of electrostatic interactions, in which PANI exhibits redox activity also in solutions with neutral pH. The redox activity behavior at neutral pH values was also observed by Granot *et al.* [21]. They have developed PANI/single walled carbon nanotube hybrid systems, and these composites as matrices give enhanced bioelectrocatalytic activation of enzymes. These modifications are potentially useful in electroanalytical applications of PANI in biosensors. However, PANI has already been used to detect different biological compounds by using its properties such as pH sensitivity [22], electrochromism [23] and conductivity [24]. A nanobiodetector has been developed based on the changes of conductivity of PANI micro- and nano-fibrils in the presence of micro-organisms [25]. It was found that the electrical response depends on the number of cells deposited on the PANI nano-network, and is specific to different kinds of micro-organisms. The minimum number of cells to be detected by this sensor is below 100.
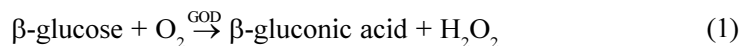
ENZYME SENSORS

Potentiometric technique relates the potential to the analyte concentration (activity) when the electrode reaction is at equilibrium and no current flows through the electrodes. Whereas in amperometric biosensors the current is based on heterogeneous electron transfer reaction *i.e.* the oxidation or reduction of the electroactive substances, and is proportional to their concentration.

The enzyme electrode is a combination of any electrochemical probe (amperometric, potentiometric or conductimetric) with a thin layer (10–200 mm) of immobilized enzyme. There are many methods to immobilize the enzymes, such as adsorption, physical entrapment in gels or membranes, crosslinking, covalent binding, and doping *etc.* [26]. PANI has been used not only for the physical entrapment of the enzymes during electrodeposition, but also as a matrix for covalent enzyme immobilization [27].

Glucose biosensor

For determination of glucose, the reaction with glucose oxidase (GOD) enzyme electrode is shown with the following equations:

$$\beta\text{-glucose} + O_2 \xrightarrow{\text{GOD}} \beta\text{-gluconic acid} + H_2O_2 \tag{1}$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \tag{2}$$

The amount of hydrogen peroxide produced in equation (1) is usually determined by amperometric method by oxidation at the working electrode according to equation (2). This is referred as an amperometric biosensor.

Equation (2) also shows that protons are produced in the follow-up oxidation reaction. If pH is measured by potentiometric method, and then related to the amount of glucose, the biosensor is referred to as a potentiometric biosensor. There are pH transducers that have been developed for potentiometric biosensors [22, 28]. Although the response of these types of biosensors is interfered by the buffer capacity of the sample, they, however, allow the direct, reagentless detection of the analyte, while the amperometric method requires several consecutive reactions.

Potentiometric glucose biosensor. The potentiometric detection of enzyme activity is based on the measurement of the change in pH in the enzymatic layer on the sensor surface. The pH change (*e.g.* release of acid) is caused by oxidation of H_2O_2 produced by the enzymatic reaction as indicated in equation (2). The enzyme catalyzes the conversion of the target analyte in reactions consuming or releasing protons. The sensitivity limit of a potentiometric enzyme electrode is the sensitivity of

the pH transducer. Conventional pH transducers, *e.g.* glass electrode or pH sensitive field effect transistors, can be used for this purpose. The advantages of PANI as an advanced potentiometric pH transducer that can be used in biosensors, have been reported by Karyakin *et al.* [28]. The chemically synthesized PANI (doped with camphorsulfonic acid) showed reversible potentiometric response of approximately 90 mV per pH unit over the pH range from 3 to 9. Some other processible PANI and self-dope PANI have also been used for this purpose. The pioneer potentiometric glucose biosensor based on PANI is shown in Figure 2 [22].
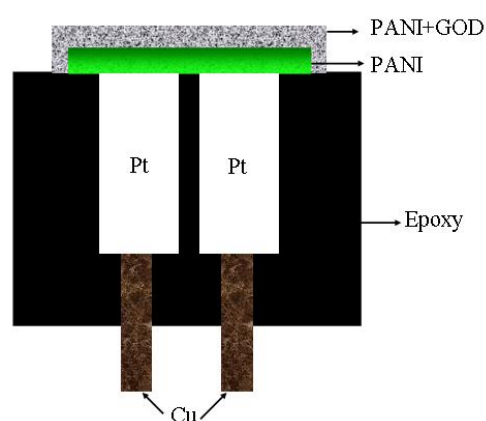

Figure 2. Prototype structure of potentiometric PANI-glucose oxidase (GOD) sensor [11]. Reproduced by the kind permission from American Chemical Society

The same strategy as with glucose biosensors has also been used in urea sensors, which reach a detection limit of 10^{-5} mol L^{-1} [28]. The urea detection can also be done *via* pH sensors made by PANI doped with tetraphenylborate [29]. On the surface of this PANI layer, urease was immobilized within either a poly(vinyl alcohol) matrix or modified sol-gel glass. It was shown that the sensor response from the latter is better and more stable.

Amperometric glucose biosensor. In amperometric glucose biosensors, the amount of hydrogen peroxide is usually detected by measuring the current during the oxidation reaction in equation (2). The GOD, which carries a negative charge, is normally immobilized (doped) in PANI during the polymerization reaction when the PANI membrane is formed. The fibril of a PANI film is about 2000 Ĺ [30], which is enough for immobilization of GOD into the film. The enzyme can also be adsorbed on the PANI film, but the doped film has better stability [31]. It has been shown that the glucose biosensor based on PANI–polyisoprene composite film has a high permselectivity for hydrogen peroxide compared to ascorbic acid, therefore dimini-

shing the interference of ascorbic acid in detection [32]. An amperometric glucose biosensor has also been made by depositing the horseradish peroxidase (HRP) on a PANI–modified Pt electrode. HRP was electrostatically immobilized on the surface of the PANI film, and the electrocatalytic reduction of hydrogen peroxide under diffusion controlled conditions was monitored by a voltammetric method [33]. Amperometric glucose biosensors based on Prussian Blue and PANI–GOD modified electrodes have also been developed [34].

The electron transfer pathways between immobilized enzyme molecules which were integrated with conducting polymers and the modified electrode have been reviewed [35]. The electrons can transfer *via* redox mediators as well as the conducting polymer wires directly. The understanding of these pathways is very important to construct the conducting polymer based amperometric enzyme sensors. Raitman *et al.* [36] have covalently attached N-6-(2-amiboethyl)flavin adenine dinucleotide as a linker between glucose oxidase and PANI–poly(acrylic acid) composite. This direct electrical contact yielded very high electron transfer rates. It was shown that the glucose concentration controls the steady-state concentration ratio of oxidized and reduced form of PANI. Yu *et al.* [37] have constructed an efficient electrical wiring of enzymes for biosensors with improved sensitivity. A film containing poly(styrenesulfonate) and enzymes (HRP) covered a thin layer (4 nm) of sulfonated PANI, underneath which is a polycationic layer. It was shown that at least 90% of proteins were electrically coupled to this kind of self-assembly electrode, and it has a detection limit of 3 nmol L^{-1} for hydrogen peroxide. Compared to classic PANI-based biosensors, an increase in linear response range with shorter response time was observed when entrapping enzymes into poly(styrenesulfonate)–PANI composites [38]. The composites were synthesized within the pores of track-etched polycarbonate membranes, which resulted in immobilizing the enzymes during polymerization. Pan *et al.* [39] reported a method to prepare PANI glucose oxidase sensor *via* template process. The process contains three procedures. First, enzymes (glucose oxidases) were entrapped into polymer film directly during electropolymerization of aniline. Second, the electrode is hydrolyzed in hydrochloric acid solution to remove the trapped enzymes from the PANI film. Finally, fresh glucose oxidase is immobilized again to obtain an active PANI glucose oxidase sensor. The stability and response was much enhanced in the sensor made by this process, since the denatured and inactive glucose oxidase affected by aniline monomer during electropolymerization has been removed during hydrolysis. Amperometric enzyme biosensors based on PANI nanoparticles have also been reported [40]. HRP immobilized on the electrodeposited PANI nanoparticle film showed improved signal to noise ratio compared to HRP deposited directly in a PANI film.

Amino acid biosensor

Amino acids are basic components in living organism. The amino acid biosensor is widely used in many areas. Choline is a very important amino alcohol in the functions of human organism. Detection of choline is frequently needed in food and clinical analysis. Langer *et al.* have developed a choline sensor by immobilization of choline oxidase (ChO) to nanoporous PANI layers [41]. In this construction, PANI behaves as a transducer. The oxidize nanoporous PANI layers trap and bound enzyme units due to coulombic interactions between positively charged PANI and negatively charged polar groups in the enzyme molecules. It was found that the electrical response of this sensor was substantially increased when PANI was charged and discharged several times in the presence of enzyme molecules by applying an oscillating potential. The structure of this PANI–ChO biosensor is shown in Figure 3. Its sensitivity is 5 μA mmol L^{-1} in the amperometric mode and 10 mV mmol L^{-1} in the potentiometric mode of measurements. At the constant potential 0.4 V (*vs* Ag/AgCl), the measured current response is linear vs. the choline concentration. It can detect choline with concentrations below 20 mmol L^{-1} in the potentiometric mode.
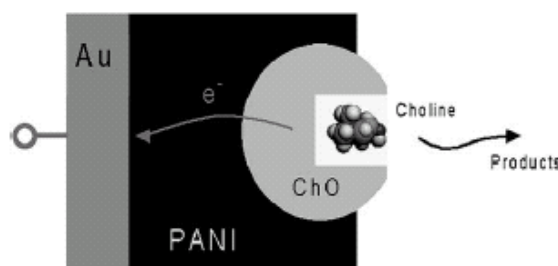

Figure 3. The processes leading to generate an electrical response of Langer's sensor owing to enzymemediated oxidation of choline. ChO: choline oxidase [23]. Reproduced by the kind permission from Elsevier Science Ltd

Cholinesterase-based potentiometric biosensors for pesticide determination was also reported by Ivanov *et al.* [42]. It is based on PANI doped with camphorsulfonic acid. Cholesterol oxidase has been immobilized in PANI film by electrochemical doping method [43]. A PANI-based cholesterol biosensor was reported to have a detection limit of 25 mg dL^{-1} with sensitivity of 0.042 uA mg dL^{-1} [44]. A comprehensive review on the determination of α-amino acids and vitamins by amperometric biosensors has been published by M.S. Alaejos *et al.* [45].

Vitamin sensor

Ascorbic acid (Vitamin C) is an essential vitamin for human life. Dr. Linus Pauling, the double Noble Prize winner, convinced that vitamin C can help to prevent the cardiovascular disease, inhibiting the formation of disease-promoting lesions on blood vessel walls. Vitamin C plays an important role as a cofactor of enzymes involved in the synthesis of collagen and carnitine. Its most vital role, however, is a water-soluble antioxidant in our body [46, 47].

The redox transition potential for PANI from its leucoemeraldine form (fully reduced light yellow color) to emeraldine form (half oxidized green color) is about 0.3 V (*vs* Ag/AgCl), which is more positive than for ascorbate/dehydroascorbate redox couple. Thus, it is possible to reduce PANI by ascorbate. Since both of these redox forms of PANI differ greatly in their light absorbance, this reaction can be followed by photometric means. The electrochromism properties of PANI can therefore be used in sensor application. It was found that optical absorbance at 650 nm proportional to ascorbate concentration within the range of 2.5 ~ 250 mg L^{-1} [48], and the detection limit can reach 1 mg L^{-1} [49]. This kind of PANI optical sensor has been used to determine ascorbic acid in a capillary electrophoresis system [48]. Several PANI based biosensors for ascorbic acid have been discussed by A. Malinauskas *et al.* [23]. Besides these optical methods, an amperometric ascorbate sensor based on PANI-modified electrode has also been reported [50]. It was shown that ascorbate assay can be conducted by PANI-modified electrode within the pH range of 5.5–7.2. It showed a linear range of response from a low detection limit of 0.05 mmol L^{-1} to 0.6 mmol L^{-1} in a potential window of 0.1–0.3 V (*vs* Ag/AgCl).

DNA SENSORS

There is extensive and continuous interest in the development of DNA sensors for analysis of unknown mutant genes, diagnosis of infectious agents in different environments and detection of drugs, pollutants, etc. Electrochemical DNA sensors are typically more portable and inexpensive than the current instruments with fluorescence detection. Many important reviews and papers have been published in this area [51–58]. In these DNA sensors, the single-strand DNA (ss)DNA probes are immobilized by techniques of adsorption, direct covalent binding, and entrapment in a polymer matrix [59-61].

A biosensor monitoring DNA hybridization based on PANI intercalated graphite oxide (PANI/GO) nanocomposite has been developed by Wu *et al.* [62]. This PANI–GO nanocomposite was mixed with graphite powder to make a carbon paste electrode, which displayed electrochemical activity and two sharp current peaks in square wave

voltammometry (SWV). Wu *et al.* used this electrode to investigate the interaction between DNA and PANI–GO. Their results showed that the PANI–GO-modified carbon paste electrode possessed strong catalytic effect on the redox reaction of single-strand (ss)DNA and double-strand DNA (ds)DNA, and therefore it can be utilized to monitor hybridization of complementary ssDNA by measuring the current at the peak of –270mV (*vs* SCE) when immobilized with ssDNA. This sensor exhibited a good stability and reproducibility in detecting ssDNA. More recently, Gu *et al.* [63] have electropolymerized a ultrathin layer of PANI–polyacrylic copolymer film on the boron doped diamond surface. The copolymer film contains high density of functional groups which link to ssDNA probes. This kind of impedimetric dimamond based DNA sensor was reported to have a detection limit of 2×10^{-8} mol L^{-1} at 1000 Hz.

IMMUNOSENSORS

Immunosensors are based on the specific recognition in the coupling reaction of an antigen with an antibody. In these devices, antibodies are immobilized in a polymer matrix, such as PVC, *etc.* Due to the high binding affinity between antibody and its target antigen, changes in conductance, mass or optical properties, are detected directly with different transducers. The scheme of a conductimetric biosensor for the electrochemical immunoassay is illustrated in Figure 4 using PANI as a conductance label [24].
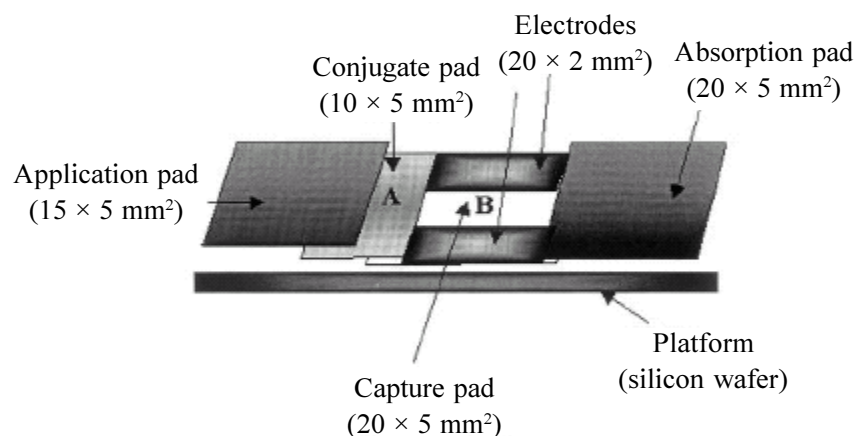

Figure 4. Schematic diagram of an immunosensor. (A) Conjugate pad for Antibody-PANI absorption. (B) Capture pad coated on each side with silver electrodes. Gap between electrodes is the site for antibody immobilization [13]. Reproduced by the kind permission from Elsevier Science Ltd.

This conductimetric biosensor uses lateral flow to enable the liquid sample to move from one membrane to another through capillary action. Figure 5 illustrates the concept. Prior to the application of the sample, the gap between the electrodes in the capture pad is open (Fig. 5A). Immediately after applying the sample, the solution carrying the antigen flows to the conjugate pad and dissolves the antibody-PANI by forming antigen-antibody-PANI complex (Fig. 5B). When this complex is carried into the capture pad containing the immobilized antibody, a second antibody-antigen reaction occurs to form a sandwich structure (Fig. 5C). PANI in the sandwich structure forms a molecular wire, and bridges the two silver electrodes for signal generation. The unbound non-target organisms are then separated by capillary flow to the absorption membrane. In this conductimetric immunosensor, the signal (change in conductance) is due to the presence of PANI that is conjugated to the antibody and forms a complex with the antigen. Therefore, an increase in the bounded antigen-antibody-PANI complex should lead to an increase in signal.
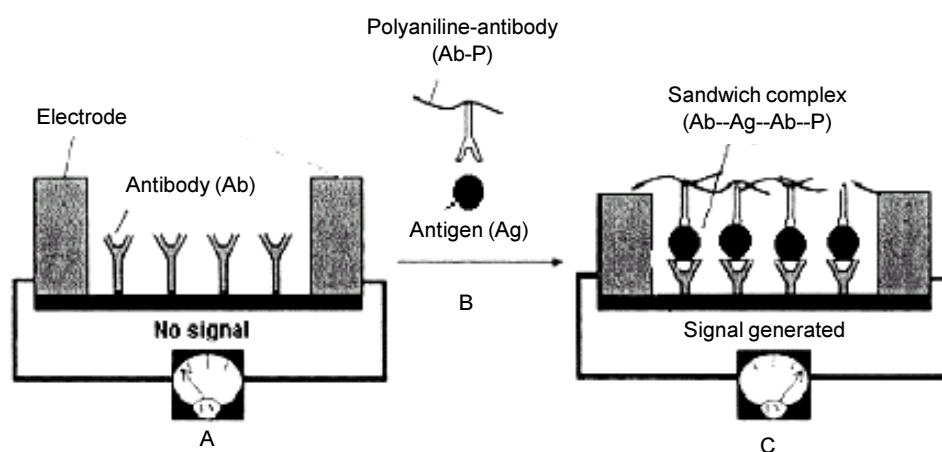

Figure 5. Cross-section of a capture membrane (A) before and (C) after analyte application [13]. Reproduced by the kind permission from Elsevier Science Ltd.

Alocilja *et al.* [24] used this biosensor to detect food-borne pathogens. The results show that the biosensor is specific to target bacteria even in the presence of other non-target organisms. The low detection limits were obtained for Salmonella, E. coli O 157:H7, and E. coli biosensors with $8.3 \pm 0.6 \times 10^1$, $7.9 \pm 0.3 \times 10^1$, $7.5 \pm 0.3 \times 10^1$ CFU mL^{-1} (colony forming units per milliliter) respectively. By changing the specificity of the antibody, the biosensor can be made to detect other analytes of concern to biosecurity. This type of sensors has the applications in medical diagnostics and bio-defense related measurements. Kim *et al.* [64] reported a conductimetric immunosensor with PANI-bound gold colloids as signal generator. They introduced

PANI as a conductivity-modulating agent on the gold surface after immobilizing an antibody (specific to human albumin as a model analyte) on the surface of membrane strips. This innovating signal generator amplified the conductimetric signal 4.7 times compared with the plain gold.

OTHERS

Besides traditional electroanalytical methods, other techniques, such as biotechnological methods (*e.g.* polymerase chain reaction (PCR)) and optical method (*e.g.* surface enhanced Raman, surface plasmon resonance) provide assistant or alternative ways to electrochemical biosensors. Ye *et al.* coupled PCR to electrochemical analysis. This pretreatment of the DNA samples enhanced the signal when studying the electrochemical behavior and recognition of DNAs [65]. Kang *et al.* [66] demonstrated that the transition in the conductivity of PANI film on gold electrode surface leads to a large change in surface plasmon resonance (SPR) response due to the change in the imaginary part of the dielectric constant of PANI film upon electrochemical oxidation/reduction. An innovative PANI-mediated HRP sensor has been fabricated based on the amplifying response of SPR to the redox transformation of PANI film as a direct result of the enzymatic reaction between HRP and PANI in the presence of hydrogen peroxide.

Paul *et al.* [67] have reported fabrication of PANI-based microelectronic devices. An analytical interrogation methodology based on small-amplitude, pulse DC has been developed and incorporated into the Electrocondcutive Polymer Sensor Interrogation System (EPSIS). Electroconductive PANI within highly hydrophilic poly(hydroxyethyl methacrylate)-based hydrogels have been synthesized [68]. The composite structure provides stabilization and bioactive recognition of molecules in EPSIS. This novel sensor technology platform provides possibility to grow conducting polymer membranes on microfabricated interdigitated microsensor electrodes.

CONCLUSIONS AND OUTLOOK

A sensor market survey by Frost & Sullivan [69] identified that medical applications are the major driving force for the development of sensor technologies, such as smart sensors, fiber-optical sensors, and thin film devices, *etc.*

The enzyme sensors developed from PANI have been intensively investigated by using potentiometry or amperometry as the electrochemical detection technique. Most immunosensor derived from PANI are based on conductimetry. As far as the electro-

chemical techniques used in the DNA sensors are concerned, square wave voltammetry (SWV) has mostly been used [62, 70]. The use of stable polymers with a specific receptor structure is important for their bioanalytical purpose. A relatively durable conjugated polymer such as PANI is considered as a three-dimensional network of intrinsically conducting macromolecular wires, which are able to transfer electrical signals. The advantage of using PANI for the biosensor development lies in its capability as a biomolecule (*e.g.* enzyme) entrapment matrix. Its transducer characteristics to convert a biochemical signal into an electric signal result into signal amplification. Further functionalization of such polymers with biospecific agents will provide more efficient biosensor membranes. PANI is a stable, electrochromic, processible conducting polymer, and it provides and will provide larger scope of usefulness in biosensors and micro-fabrications. As one of the main components in organic electronics, PANI will contribute to the development of new generation of portable and flexible analytical devices to be used in biological applications.

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