Chapter

10

PERSPECTIVE POTENTIAL OF POLYMER-BASED BIOSENSOR CHIPS IN FOOD INDUSTRY AND CLINICAL DIAGNOSTICS

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10.1. INTRODUCTION

Biosensors are made of the sensing element, which responds to the substance being measured and is biological in nature. This compound has to be connected to a transducer of some sort so that a visually observable response occurs, such as in case of optical biosensors. The importance of the biological component is that its interaction with the substrate is highly specific to that substrate alone, thus avoiding interferences from other substances which plague many analytical methods. It may catalyse a reaction involving the substrate (enzyme) or it may bind selectively to the substrate. Biological elements provide the major selective element in biosensors. They must be substances able to attach themselves to one particular substrate but not to others. Four main groups of materials can do this: a) enzymes, b) antibodies, c) nucleic acids, and d) receptors. The most common component is the enzyme, although other components containing enzymes are often very suitable. These include microorganisms such as yeasts and bacteria. The most challenging step in the development of biosensors is an adequate immobilization of enzymes. This property is extremely important for stability, sensitivity, and bioanalytical functionality of the biosensor. In this chapter the role of most applicable polymers in biosensor technology, their specific properties, and application in designing biosensors will be highlighted. Application of innovative, polymer-based, biosensor strategies in medical diagnostics (*e.g.*, glucose measuring, biomarkers testing) and food industry (microbial detection) will be discussed as well. The biosensor development is one of the perspective ways to reduce costs and waiting time for many laboratory analyses and to enable highly sensitive readout system in "real-time."

10.2. POLYMER MATERIALS IN BIOSENSOR TECHNOLOGY

Polymer materials are essential components of various biosensor setups and many of them have found medical, industrial, and environmental application. Polymers are large molecules made out of hundreds, thousands, or even millions of single molecules called monomers. Since the polymers are capable of enhancing stability and sensitivity of biosensor, they are mostly used in designing the receptors for binding of biological compounds such as antigens, antibodies, microorganisms, enzymes, or some other substrates [1]. The polymer materials are characterized by biocompatibility, biodegradability, and electrical conductivity, which makes them applicable in construction of optical, electrochemical, or enzyme biosensors [2,3]. There are two types of polymers: natural and synthetic. The human body contains many natural polymers, such as nucleic acids and proteins. Other examples of natural polymers are cellulose, dextrans, collagen, and chitin. However, synthetic polymers are more often

used in biosensor construction. This enables high affinity of the biosensor to wide array of targets with high specificity. Characteristics of synthetic polymers, such as denaturation grade, can be manipulated and increase biosensor stability [4]. Several types of polymers that are commonly used in generation of biosensors are listed in Table 1.

Polyaniline (PANI) is known as conducting polymer with excellent electronic properties and ability of binding different biomolecules [2,5]. One of the PANI´s limitations is the pH sensitivity and necessity for testing to be performed in acidic environment. However, many derivates of PANI are developed to overcome these limitations and enable this polymer to provide redox activity also in neutral pH solutions [2].

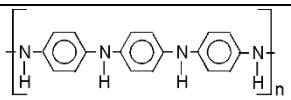
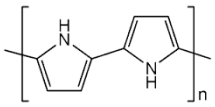
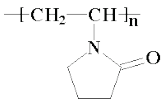
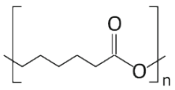
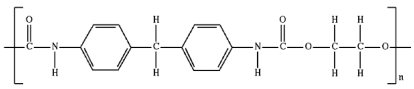
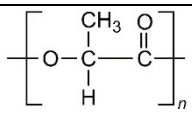
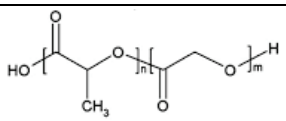
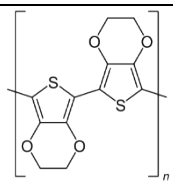
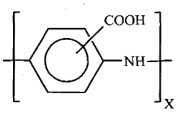
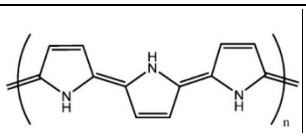
Polypyrrole is one of the conductive, conjugated polymers with broad biomedical application. Since it is characterized by a good electron transfer and biocompatibility, this polymer has been used for production of nanobiosensors for detection of hydrogen peroxide (H_2O_2), in which toxic effects in some foodstuffs, cosmetics, or plastic packaging, if not detected on time, can have some serious consequences for human health [6,7]. In some studies it was shown that the polymer polypyrrole offers some advantages, such as higher electrical response, when combined with gold nanoparticles, which, for example, can be the component of glucose biosensor design [8]. The polypyrrole belongs to a new generation of polymers that can serve as a substrate in conducting with biological cells or tissues [5,6]. The conjugated polymer acts as an electronic conductor which can be controlled by changing the redox potential of the ions entering or leaving the material in dependence on the polymer charge.

Polyvinylpyrrolidone is water soluble polymer with its applications in medicine, mostly like binder in pharmaceutical drugs. This polymer has been used in designing the paper-based biosensors showing a great conductivity and amperometric determination of the target molecule [9].

Another polymer that can be fabricated with gold nanoparticles is polycaprolactone, which is used as a substrate in specific biosensor´s setup [10].

Polyurethane is a polymer commonly used in preparation of enzyme membranes as a component of the transducer element. The polyurethane layer that can be part of glucose biosensor is responsible for biosensor sensitivity and intensity of detection signal [11,12].

Table 1. Polymers used in biosensor technology

Polymer	Structural formula	Refs
Polyaniline		[1,2,5,13]
Polypyrrole		[1,6,9,13]
Polyvinylpyrrolidone		[9]
Polycaprolactone		[3,10,14]
Polyurethane		[1,11]
Poly(lactic acid) (PLA)		[3, 15-18]
Poly(lactic-*co*-glycolic acid) (PLGA)		[4,5,18,24,27-31]
Poly(3,4-ethylenedioxythiopene)		[24-26]
Poly(3-aminobenzoic acid)		[24,27-29]
Poly(pyrrole-3--carboxylic acid)		[24,30,31]

The polymers which are distinguished by their biocompatibility and biodegradability are especially applicable in medical purposes. Here we are talking about synthetic polymers, such as poly(lactic acid) (PLA) and its copolymer (*e.g.,* poly(lactic-*co*-glycolic acid) (PLGA)), which are approved by Food and Drug Administration (FDA) and commonly used in drug targeting

and biosensor production. Both of these polymers are used in production of nanoparticles based on emulsion-solvent evaporation technique which resulted in particles with a heterogeneous size distribution [32].

PLA belongs to the family of aliphatic polyesters commonly made from *α*-hydroxy acids. PLA is a thermoplastic, high-strength, high-modulus polymer that can be made from renewable resources to yield objects for use in either the industrial packaging field or the biocompatible medical device market. It is one of the few polymers whose stereochemical structure can easily be modified by polymerizing a controlled mixture of the L- or D-isomers to yield high molecular weight amorphous or crystalline polymers that can be used for food contact and are generally recognized as safe [33]. PLA can be synthesized in a wide range of molecular weights by following two processes:

1. Direct polycondensation reaction of lactic acid which is readily obtained by the controlled fermentation of starches, usually from corn: when water is cleaved off from lactic acid, an oligomer is formed, an ester consisting of 10–30 lactic acid units. This oligomer is in equilibrium with the corresponding dilactide, an 'internal' ester consisting of two molecules of 2-hydroxypropanoic acid.
2. Ring-opening polymerization of cyclic dimers, that is, dilactide, in the presence of metal catalysts to synthesize high molecular weight polymers [34].

PLGA is copolyester of lactic and glycolic acid that can be dissolved in dichloromethane, chloroform, acetone, ethyl acetate, tetrahydrofurane, dioxane, dimethyl sulfoxide (DMSO), and toluene. PLGA is often used as a biomaterial for medical and pharmaceutical applications. Hydrolysis of the polymer chain leads to PLGA degradation and results in lactic and glycolic acid which can be metabolized in the Krebs cycle to CO_2 and H_2O.

Poly(3,4-ethylenedioxythiopene) is a synthetic polymer which in combination with some other substrates can act as binder for improvement of cathode stability and its electrochemical properties [26]. Baba and Knoll reported the possibility of using the poly(3,4-ethylenedioxythiopene) in designing DNA biosensors, where this polymer was used for modification of platinum component of the biosensor [35].

Poly(3-aminobenzoic acid) is organic compound which is partially soluble in water. It has been used in construction of conducting polymer film, as a part of the biosensor´s build [27,28]. In combination with other polymers, such as polyaniline, it can be applied for construction of immunosensors in which mechanism of action is based on the surface plasmon resonance (SPR) [29].

Poly(pyrrole-3-carboxylic acid) is another type of polymers suitable for designing of SPR-based biosensors and is applicable in food industry, pharmacy, and medical diagnostics [30].

10.3. OPTICAL BIOSENSORS AND SPR PRINCIPLE

The *electrochemical biosensors* and among them the amperometric and potentiometric ones are the best described in literature and have leading position on the biosensor market. The biosensors based on optical principles are the next most commonly used transducers. The various types of optical transducers exploit properties such as simple light absorption, fluorescence/phosphorescence, bio/chemiluminescence, reflectance, Raman scattering, and refractive index. SPR as a further transduction mechanism shows a main advantage over most optical biosensors, because of the direct determination of the analyte without the use of labelled molecules [36]. SPR is an optical biosensor technique that measures molecular binding events at a metal surface by detecting changes in the local refractive index (Figure 1).

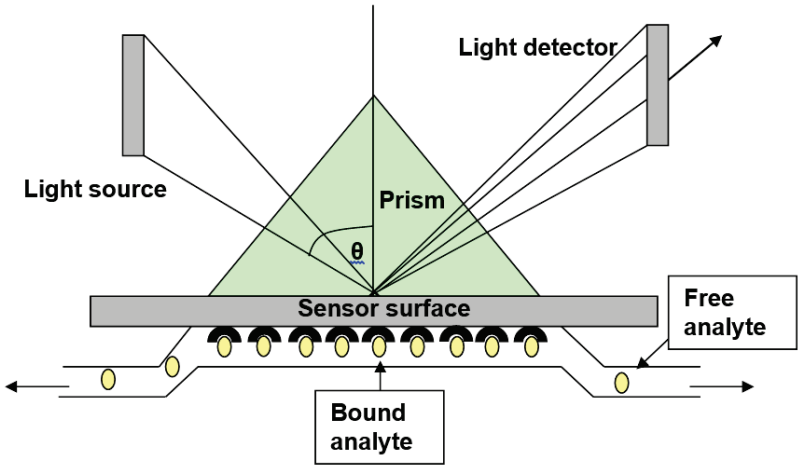


Figure 1. SPR measuring of changes in diffractive index

The phenomenon of anomalous diffraction on diffraction gratings due to the excitation of surface plasma waves was first described in the beginning of the twentieth century by Wood. Since then, surface plasmons have been intensively studied and their major properties have been assessed. When an optical beam is incident on a metal-coated glass prism its energy and momentum can be transferred into the surface of the metal to create a surface plasmon, which is a collective oscillation of free electrons that can give rise to very intense scattering and forms an electron density wave (Figure 1). The SPR technique can be upgraded electrochemically and is called the electrochemical-surface plasmon resonance (EC-SPR). EC-SPR enables monitoring of interactions with conjugated polymer films as integral part of the biosensor.

There are three types of SPR formats: scanning angle SPR, scanning wavelength SPR, and SPR imaging [37]. For all SPR formats the reflectivity of light incident on a metal/dielectric interface is monitored and correlated to changes in the local index of refraction of the dielectric layer adjacent to the metal film. The most widely used format for an SPR experiment is the scanning angle technique, in which the reflectivity of monochromatic incident light upon a metal film is monitored as a function of the incident angle. The wavelength is fixed and the incident angle is swept until resonance is observed as a dip in the reflectivity curve. Commercial application has made it possible to use SPR as a detection method for many purposes, including basic life science research, drug discovery, environmental monitoring, and process analysis. In particular the possible real-time monitoring of macromolecular interactions is of paramount importance and a great benefit [38].

In case of Biacore, SPR is evoked when light is totally internally reflected from a dielectric-dielectric interface (glass solution) into which a very thin film (10 nm) of gold (or other such metal that obeys the free electron model, *e.g.*, silver and aluminium) is interposed. The decaying evanescent wave at the interfacial region can collectively excite regions of charge in the gold film to form a surface charge density wave (the surface plasmon) parallel to the metal dielectric interface. Excitation or resonance of these surface plasmons within the metal occurs when the parallel wave vector component of the incident light becomes equal to the wave vector for the propagating surface plasmon. The resonance results in a decrease of the reflected light intensity applied as the measured parameter in this SPR device. For light of constant wavelength, the condition for resonance is a function of the light's incident angle and effective refractive index of the surface layer; thus, an SPR experiment involves varying the angle of incident light until a drop in intensity is observed [39].

If the incident angle is fixed near resonance and the wavelength swept until resonance is observed as a dip in the reflectivity curve, this technique is called scanning wavelength measurement. Both the scanning angle and scanning wavelength measurements typically provide only one or a few data points at a time. In contrast, SPR imaging measurements use the changes in reflectivity from a gold thin film that occur upon adsorption to generate difference images to simultaneously monitor tens, hundreds, or more interactions in a parallel manner. The high-throughput capabilities of SPR imaging have made it an attractive tool for screening biomolecular interactions. For example, SPR imaging has been used in an array format to study the hybridization of DNA and RNA to nucleic acid arrays fabricated on gold films [40].

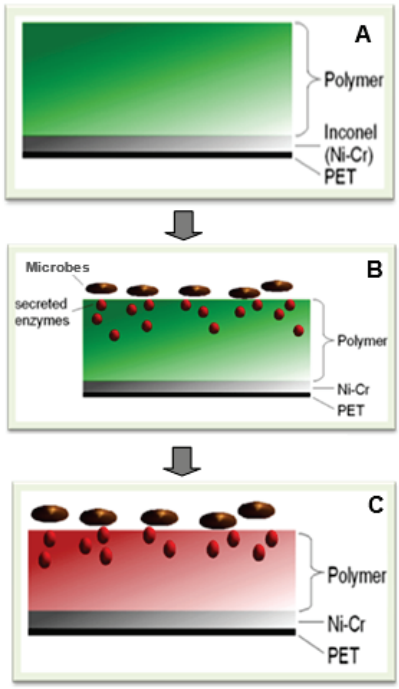
SPR offers several advantages over conventional techniques such as fluorescence or enzyme-linked immunosorbent assay (ELISA). First, because the measurements are based on refractive index changes, detection of an analyte is label-free and direct. The analyte does not require any special characteristics (scattering bands) or labels (radioactive or fluorescent) and can be detected directly, without the need for multistep detection protocols.

Second, the measurements can be performed in real-time, allowing the user to collect kinetic data, as well as thermodynamic data. Last, SPR is a versatile technique, capable of detecting analytes over a wide range of molecular weights and binding affinities. Although SPR has historically been limited by its throughput, new methods are emerging that allow for the simultaneous analysis of many thousands of interactions. When coupled with new protein array technologies, high-throughput SPR methods give users new and improved methods to analyze pathways, screen drug candidates, and monitor protein-protein interactions [41].

10.4. DEGRADATION OF POLYMER DISTANCE LAYER

Various biodegradable polymers are already well known for the production of nanoparticles aimed at drug and gene delivery applications [42]. PLGA and PLA, both of which are FDA-approved biocompatible polymers, have been the most extensively studied [21,22,32,43]. Besides for nanoparticles production these polymers have found their application in biosensor technology as well. They can be easily integrated in the matrix of optical biosensors and give a reasonable signal toward microbial contaminations [15,19]. The polymer acts as a distance layer that is usually degraded due to action of microbial enzymes, which are secreted during the cell growth. The polymer layer can be used as an attractive surface for the microorganisms which are capable of binding to the polymer, such as PLGA, and we use it as the culture growth media [22]. Typically, degradation occurs *via* the cleavage of covalent bonds within the polymer. Polymer degradation is caused by lytic enzymes secreted either from the product to be tested or from a microorganism associated with product or from both. Examples for such enzymes are phospholipases, pronases, proteinases, hydrolases, lipases, and esterases. Thus, the process of degradation is irreversible resulting in a definite “signal” of the device in form of an irreversible color change.

The color of the device is defined by remission of incident light by the reflecting layer in combination with the properties of the polymer layer. Thus, the polymer layer may display different characteristics as long as it is biodegradable and optically transparent such that incident light is able to contact the reflecting layer and remitted light is able to pass the polymer layer (wherein the polymer layer does, however, influence the remission). The thickness of distance layer is also very important, because it will be reduced through the action of microbial enzymes (lytic and lipolytic) which results in color change of the biosensor (Figure 2). In that way, an optical thin film sensor chip can detect bacterial decay of food through a specific color change.



A. General setup of a two-layer biosensor.

B. As microbes grow as a function of time and temperature, they also secrete enzymes for utilization of their nutrient sources.

C. These enzymes may degrade the polymer covering the Ni-Cr layer thus leading to a color change depending on the thickness of the biodegradable polymer layer.

Figure 2. The polymer layer degradation in two-layer PLGA biosensor setup by action of specific microbial enzymes

The intensity/degree of color change is in this respect proportional to the degree of change in the polymer layer. Incident light is remitted by the reflecting layer as described above and ultimately leads in combination with the optical properties of the polymer layer to a specific color of the device which may appear to the human eye as, *e.g.*, a red, white, blue, or green color. However, the polymer alone sometimes is not enough for induction of microorganisms to produce sufficient amount of lipolytic enzymes for its degradation. Investigating the characteristics and biology of microorganisms and their metabolic pathways, it was possible to see that not all microorganisms produce the same concentration of lipolytic enzymes under the same conditions [23].

Under addition of certain substrates in the matrix of the biosensor, it is possible to stimulate the microbes for better enzyme production, resulting in higher sensitivity of the polymer-based biosensor [23].

10.5. SELECTION OF THE CLUSTER

10.5.1. Metal clusters

Metal clusters are nanometric crystals or amorphous particles and bear unique physical and chemical behaviour. Clusters combine properties of metals and semiconductors including surface phenomena and interesting electronic and optical resonance. In the literature, metal clusters are also referred to as nanoparticles, nanoislands, or precipitates.

Atoms in metal cluster material are bound by the same forces as in bulk material, but the surface to volume ratio is quite different and therefore surface effects dominate metal cluster behaviour. Even clusters built of about thousand or more atoms contain up to 25 % of surface confined atoms. In general, surface confined energy transduction is very inefficient but in nanometer size clusters the boundary region reaches deeply beyond the surface or even penetrates the whole surface.

To describe the behaviour of clusters, classical electrodynamics are employed. Macroscopic metal objects clearly differ from nanometric metal objects in terms of electrons. Electrons in macroscopic metal materials move being unconfined by material borders. This free movement of the electron gas results in strong and unspecific reflectivity. For colloidal particles the optical properties are defined not by a freely moving electron gas but also by the oscillation of the electron gas. This collective swinging motion of electrons within the cluster is also a so-called particle plasmon. Metal clusters with a band-gap in the visible range are excited by the electromagnetic field of a light wave. Most of the novel phenomena are based on the excitation of plasmons in the visible or infrared part.

For simple consideration, the behaviour of plasmon oscillations is modelled using a quasistatic regime. It can be assumed that clusters of 10–20 nm in size behave like simple dipoles. For larger clusters and large numbers of clusters the situation becomes more complicated and the Mie function includes all electronic and atomic behaviour of the colloidal particle, which means that multipolar and magnetic modes of absorption and scattering are added compared to the simple dipolar model for small clusters.

Clusters can be formed from a wide variety of materials, but their application is limited by chemical stability in air, water, or biological environment; in particular stability against oxidation and thiol modification is a problem for many forms of clusters. Clusters of only a few atoms in size do not exhibit resonant behaviour in the visible range of the spectrum. Metal nanocluster technology is based on the fact that electrons are moving freely and are excited by an external field. Under these conditions it is clear that metals with a high amount of free electrons show the best optical effects and for theoretical studies alkali metals are ideal but they are very unstable in air and in aqueous solutions and so only noble metal clusters can be used in plasmon studies.

10.5.2. Mirror cluster

The mirror layer is of great importance for the sensor setup. It has to be very stable and to contain a smooth surface able to interact with the nanoparticles at a definite distance that results in anomalous absorption. As a mirror layer the inconel can be used. Inconel is a nickel-chromium alloy with good oxidation resistance and often used in food processing. In the majority of the cases the prefabricated inconel and gold coated PET-foils are used. Drawing a comparison between the inconel, gold, and silver mirrors requires the testing of the stability of the associated sensors (complete setup) towards high temperature and salt concentration [23].

Optical properties of mirror and metal clusters can be used to produce biomimetic devices of extraordinary functionality. The design of the sensor relates to the phenomenon of "anomalous absorption", which can best be described as a thin film enhanced absorption. A metal cluster film positioned at a well defined distance to a smooth metal surface shows that the minimum of spectral reflectivity strongly depends on the thickness of the interlayer. This setup represents a special kind of reflection interference filter. In such a sensor setup, we have integrated a biodegradable polymer as a biomimetic component which is degraded by the same enzymes and at the same rate as food decay will happen. The degradation of the polymer results in reduction of the film thickness and thus in a specific change of the color [15,16].

10.6. THE QUENCHER/LIGAND SYSTEM

The characteristic of many proteins to bind their specific ligands, which allows them to interact with some other proteins, enabled the development of biosensors with higher sensitivity toward the detection of target molecules. As transducer that must be attached to the protein, the fluorescent proteins have been used [44,45].

Fluorescent reporters that are used in labelling procedure and attached to the recognition element can be incorporated by covalent chemical modifications or site specific. We can differ between endosteric incorporation (at binding site) and allosteric incorporation (outside of the binding site). It is believed that polymer-based, fluorescent biosensors have more potential over standard methods in molecular diagnostics (ELISA, polymerase chain reaction (PCR)). The principle of their work is based on the quencher/ligand or the OFF state, where fluorescence occurs only due to binding of molecule of interest. In that case, the quencher is removed and detection signal is exhibited (Figure 3) [44].

Figure 3. Removal of quencher by an antigen results in fluorescence

10.7. MODIFICATIONS OF BIOSENSOR MATRIX AND SYSTEM

Ability of biosensors to detect or monitor specific medical, environmental, and industrial molecular targets emerged from development of various possibilities nowadays in manipulating the biosensor matrix and enabling analytical analysis of high sensitivity in real-time. The biosensor matrix is crucial for the sensor sensitivity and quality of exhibiting detection signal which can be electronic or optical. That is why the choice of components that build up the biosensor matrix must be done with highest concerns. The signal that the biosensor produces is normally proportional to concentration of target substances (chemical, toxin, enzyme, *etc.*). A biosensor has a biological sensing element either intimately connected or integrated within a transducer (Figure 4).

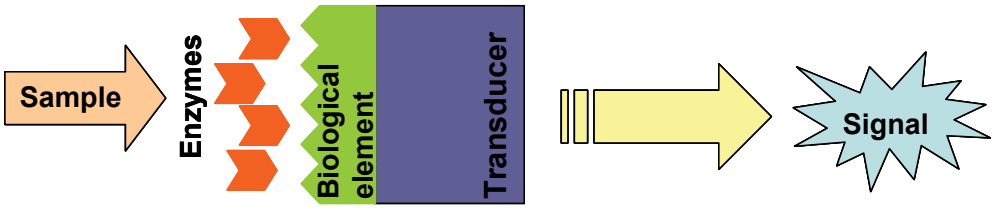


Figure 4. Major elements of an optical biosensor that gives signal when in contact with specific enzyme

Biosensors may be classified according to the biological specificity-conferring mechanism or, alternatively, to the mode of physicochemical signal

transduction. They may be further classified according to the analytes or reactions that they monitor:

a) *direct* monitoring of analyte concentration or of reactions producing/consuming such analytes.
b) alternative *indirect* monitoring of an inhibitor or activator of the biological recognition element.

Polymer-based biosensor by which the polymeric material is coating the electrode is commonly used for protein immobilization. The polymers as major component of biosensor matrix can act as electrical conductor and be applicable in many immobilization strategies [1]. As an example, there are enzyme-based amperometric biosensors which use enzymes reverse micelle membrane or polymaleinmidostyrene as immobilization stabilators [46]. Desmodur (diphenylmethane diisocyanate or triphenylmethane-4,4‘,4‘‘-triisocyanate) is an example for type of crosslinker commonly used in a way to stabilize polymer solution and the thickness of the polymer layer [20,22].

Optical properties of mirror and metal clusters (silver or gold) can be used to produce biomimetic devices of extraordinary functionality. The biomolecular environment can serve as pattern for cluster formation and generate an optical fingerprint through the specific interaction of biomolecules and chromophores [47].

We have integrated a biodegradable polymer as interlayer of food biosensor, which is degraded by the same enzymes and at the same rate as food decay will happen [15]. In some cases it is possible to increase activity of causing agents such as microorganisms, resulting in better biosensor sensitivity and readout ability. One way to do this is due to the addition of certain substrates in the matrix of the biosensor that will stimulate the microorganisms for better enzyme production, resulting in stronger signal of the biosensor [22]. Optimization of different sensor matrices enabled breakthrough in development of many immunosensors as well, making them more specific, stable, and accurate [48].

10.8. APPLICATION OF BIOSENSORS IN FOOD INDUSTRY

Monitoring of safety and nutritional quality of food is very important. Most of the microbiological methods are very expensive, demanding, and time-consuming. Having a biosensor chip that would give the signal in presence of microbial pathogens in food (fresh, canned, or vacuumed) within a short period of time (few minutes or few hours) would be of great advantage over currently used microbiological testing methods. In this connection, biosensor is an appropriate alternative to the conventional techniques. Biosensors are considered to be a revolutionary tool in combating, in some cases even life treating, medical complications as consequence of food poisoning. The

microorganisms can replicate very fast, especially in meat products. If we would not have some sensitive control system that would monitor meat decay due to the changes in storage temperature or packaging, the consumers would stay unprotected in these situations.

Microbial enzymes are major causes of quality deterioration and food spoilage. Understanding of the enzymatic processes which have taken place or may still occur in food is required for making valid, well-profound shelf-life evaluation. The range of enzyme activity can be much higher than the range of bacterial growth. A major problem is determining the relationship between microbial composition and the appearance of deterioration in food caused by physical, chemical, or enzymatic reactions. Even less is known about the activity of microbial enzymes under the effect of the intrinsic factors in food.

Biosensors are one of the foremost relevant techniques for monitoring of food decay, they are specific, and their production is considered as of a low cost. Their specificity derives from biological interactions between antigen and antibody, enzyme and substrate, receptor, and ligand or interaction between nucleic acid and transducers. Here, we would concentrate on polymer-based biosensors that are developed for food testing. Some of examples are biosensors based on PLA and its co-polymer PLGA [15,16,19,22,23]. The design of these biosensors that can be composed of two or three layers relates to the phenomenon of "anomalous absorption," which can best be described as a thin film enhanced absorption (Figure 5). A metal nanoparticle layer positioned at a well defined distance to a smooth metal surface shows that the minimum of spectral reflectivity strongly depends on the thickness of the distance layer. This setup represents a special kind of reflection interference filter. In such a sensor setup, a biomimetic polymer is integrated which is degradable by lytic enzymes excreted by microorganisms in food decay [19]. It could be shown that a meat, milk, or vegetable deterioration, which is correlated to the amount of enzymes secreted by microorganisms, can be detected by these types of biosensors [23].

Some further polymers, which can be used in sensor setup described above, may be selected from the group of polymers comprising poly-L-lactic acid (PLLA), poly(hydroxybutyrate) (PHB), and polyvinylcaprolactam (PVCL) or any other polymers, which can be classified as a biodegradable polymer. Preferably, biodegradable synthetic polymers are used, which can also include synthetic polymers of gelatine, agarose, dextrose, lipids, cellulose, starch, chitin, polyhydroxyalkanoates, poly(ε-caprolactone) (PCL) or PCL-systems, poly(ethylene/butylenes) succinate, poly(ethylene/butylenes) adipate, or polynucleic acids [38].

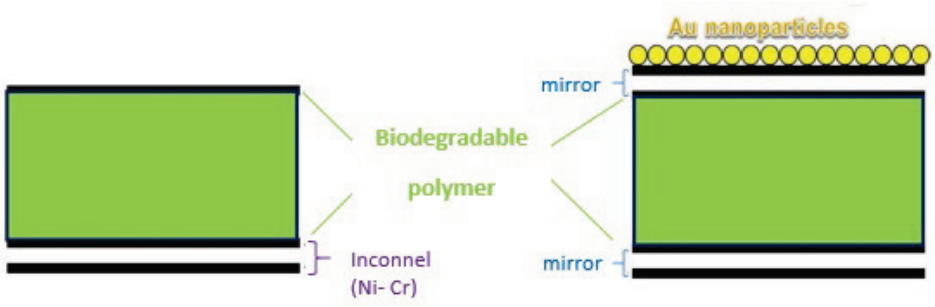


Figure 5. Two-layer biosensor (left) and three-layer biosensor (right)

The biodegradable polymer layer may additionally comprise a bifunctional crosslinking agent, such as diisocyanate, glutardialdehyde, or desmodur. These stabilizers of biosensor matrix are products based on diphenylmethane diisocyanate (MDI), toluene diisocyanate (TDI), hexamethylene diisocyanate (HDI), or isophorone diisocyanate (IPDI). This type of optic biosensors is normally constructed with reflecting layer positioned under the polymer layer and both influence a process of remission and color change of the biosensor [19,20,22].

Biosensors present a novel and cost effective method for detection not only the microbial pathogens, but also allergens and organic, toxic compounds in food, such as pesticides [49]. Fluorescence-labelled biosensor also found their application in food industry. It consists of a receptor component that binds a target ligand and a signal transduction component to convert the ligand-binding event into measurable signals, such as fluorescence, chemiluminescence, or colorimetric, electrochemical, and magnetic response [50]. Food components that can be sensed by biosensors are glucose, sucrose, fructose, lactose, glycerol, cholesterol, choline, and so forth.

One of the greatest disadvantages of biosensors is instability of their biological sensing element toward different environmental conditions (changes in pH, temperature, and ionic strength). However, due to constant novel approaches in synthesis of new polymers, many of these “weaknesses” could be overcome.

10.9. CLINICAL SIGNIFICANCE OF POLYMER-BASED BIOSENSORS

Nowadays, many medical conditions can be monitored by biosensors. They can be used for analysis of clinical samples or for *ex vivo* and *in vivo* monitoring of different physiological changes within human body [51]. Many diagnostic testing tools today include automated analyzers whose maintenance is very costly and time demanding. So, faster, smaller, and cheaper devices are highly needed for laboratory testing. Polymer-based biosensors are one of the promising ways to enable more reliable and rapid response during glucose or biomarker testing. Since diabetes mellitus as metabolic disease is present in huge part of world's population, it is necessary to have stable and rapid blood glucose tests. The blood glucose concentration is the main diagnostic criteria for patient's diabetes monitoring. For better control of diabetes, the self-monitoring of blood glucose was established as well. There are many types of biosensor capable of detecting the blood glucose levels with high accuracy. For immobilization of glucose oxidase (GOx) enzyme, the polymer polypyrrole has been used in several biosensor designs [24,52]. Besides GOx, there are two more enzymes whose mechanism of action was decisive for development of different glucose biosensor setups: hexokinase and glucose-1-dehydrogenase (GDH) [53].

Most of the glucose biosensors are electrochemical and can be potentiometric, conductometric, or amperometric in type. Development of glucose biosensors went through three generations. The first-generation glucose biosensors were based on an enzyme electrode measuring the oxygen concentration which was proportional to the glucose concentration. The second-generation glucose biosensors brought many improvements due to replacement of oxygen with some redox mediators that would transport the electrons from an enzyme to the electrode. Mostly used mediators of that kind were thionine, methylene blue, methyl viologen, ferricyanide, quinines, and many others. The third-generation glucose biosensors gave up from redox mediators and enabled direct electron transport between the enzyme and the electrode [53]. Biosensors can also be used for detection of some of the most important cancer biomarkers [54]. We know that diagnosis of the cancer in early stage increases the survival rate of the patient, and the biosensor technology enables a fast and less invasive diagnostic tool in detection of variety of pathogeneses. Since there is no single oncogen that is altered in each type of cancer, several biosensors detecting different biomarkers were developed. These biosensors are able to detect prostate specific antigen (PSA), carcinoembryonic antigen (CEA), a-fetoprotein (AFP), epidermal growth factor-2 (HER-2), interleukin-6 (IL-6), and interleukin-8 (IL-8) [55]. Respiratory insufficiency can also be monitored with help of lactate biosensor based on lactate oxidase enzyme [56]. Thanks to biosensor technology, it is possible to monitor the function of many human organs, such as kidneys, with help of urea biosensors, and to control

cholesterol levels which can be altered in course of some disorders like hypertension and anemia and can be detected by cholesterol biosensors [57]. Another type of clinically important biosensors is DNA sensors, which are applicable in diagnosis of inherited diseases. These biosensors can be made of conducting polymer that acts as transducer [58].

Even if it seems that development of biosensors was a rapid process, it was actually a long way which included many technical optimizations and requires some additional improvements even today. Starting with multiple matrix optimizations of polypyrrole based sensors or improvement of electron transfer due to properties of nanoparticles and nanotubes, all of that enabled the development of much more stable biosensors of high selectivity, specificity, and sensitivity toward various clinical targets.

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REFERENCES

1. K.E. Geckeler, B. Müller. *Naturwissenschaften* **80**(1) (1993) 18–24.
2. W.E.I. Di, A. Ivaska. *Chem. Anal. Warsaw* **51**(6) (2006) 839–852.
3. E. Llorens, E. Armelin, M. del Mar Pérez-Madrigal, L.J. Del Valle, C. Alemán, J. Puiggalí. *Polymers* **5**(3) (2013) 1115–1157.
4. P.M. Vadgama, S.M. Reddy, M.S. Kyrolainen, Membrane for Chemical and Biosensors, *U.S. Patent No. 6706532*. (2004).
5. C. Dhand, M. Das, M. Datta, B.D. Malhotra. *Biosens. Bioel.* **26**(6) (2011) 2811–2821.
6. D.D. Ateh, H.A. Navsaria, P.Vadgama. *J. Royal Soc. Interface* **3**(11) (2006) 741–752.
7. D.M. Preethichandra, M. Onoda, (2014). Polypyrrole Based Nano-Biosensor for Sensing Low Concentrations of Hydrogen Peroxide, 8th International Conference on Sensing Technology, (2014), p. 159–162.
8. N. German, J. Voronovic, A. Ramanavicius, A. Ramanaviciene, (2012). Gold nanoparticles and polypyrrole for glucose biosensor design. 26th European Conference on Solid-State Transducers, EUROSENSOR 2012 Procedia Engineering **47** (2012) 482–485.
9. N. Ruecha, R. Rangkupan, N. Rodthongkum, O. Chailapakul. *Biosens. Bioel.* **52** (2014) 13–19.
10. S.Y. Reyes-López, D. Cornejo-Monroy, G. González-García. *J. Nanomater.* **2015** (2015) 153– 159.
11. J.H. Han, J.D. Taylor, D.S. Kim, Y.S, Kim, Y.T. Kim, G.S. Cha, H. Nam. *Sensors Actuat. B: Chem.* **123**(1) (2007) 384–390.

12. J.H. Shin, S.M. Marxer, M.H. Schoenfisch. *Anal. Chem.* **76**(15) (2004) 4543–4549.
13. R. Janmanee, S. Chuekachang, S. Sriwichai, A. Baba, S. Phanichphant. *J. Nanotechnol.* **2012** (2012) 1–7.
14. A. Tiwari, M. Ramalingam, H. Kobayashi, A.P. Turner, *Biomedical materials and diagnostic devices*. John Wiley & Sons, Chapter 7, (2012), p. 217–262.
15. N. Ibrišimović, M. Bauer, F. Pittner, Sensor for real-time monitoring of food degradation, Nanotechnology 2008: Life Sciences, Medicine and Bio Materials, Vol.2, (2008), p. 98–101.
16. F. Pittner, M. Bauer, G. Bauer, R. Domnick, N. Ibrisimovic, "Device comprising two reflecting layers and a polymer layer for analyzing the age and/or quality of natural products", U7098/DB, PCT/EP2007/063895, (2008).
17. U. Bohrn. Contributions to the development of novel nanotechnological sensor chips for the detection of meat deterioration in special consideration of optimising the sensitivity with regard to chemical modification, Master thesis, University of Vienna, Vienna, (2009).
18. E. González, L.M. Shepherd, L. Saunders, M.W. Frey. *Materials* **9**(1) (2016) 47.
19. N. Ibrišimović, M. Barth, U. Bohrn, M. Ibrišimović, F. Pittner. *Monatsh. Chem. Chem. Mon.* **140**(8) (2009) 941–945.
20. N. Ibrišimović, M. Ibrišimović, M. Barth, U. Bohrn. *Monatsh. Chem. Chem. Mon.* **141**(1) (2010) 125–130.
21. N. Ibrišimović, H. Al-Dubai, V. Kerleta, F. Gabor, F. Pittner. *Acta Medica Academica* **39**(2) (2010) 118–131.
22. N. Ibrišimović, M. Ibrišimović, A. Kesić, F. Pittner. *Monatsh. Chem. Chem. Mon.* **146**(8) (2015) 1363–1370.
23. M. Ibrisimovic. Evaluation of novel, nanotechnological biosensor-chip for monitoring of bacterial spoilage, Master thesis, University of Vienna, Vienna, (2009).
24. M. Şenel, C. Nergiz. *Curr. Appl. Phys.* **12**(4) (2012) 1118–1124.
25. A. Baba, W. Knoll. *J. Phys. Chem. B* **107**(31) (2003) 7733–7738.
26. S.N. Eliseeva, O.V. Levin, E.G. Tolstopjatova, E.V. Alekseeva, R.V., Apraksin, V.V. Kondratiev. *Mater. Lett.* **161** (2015) 117–119.
27. S. Sriwichai, A. Baba, S. Phanichphant, K. Shinbo, K. Kato, F. Kaneko. *Sensors Actuat. B: Chem.* **147**(1) (2010) 322–329.
28. S. Sriwichai, A. Baba, S. Phanichphant, K. Shinbo, K. Kato, F. Kaneko. *Int. J. Polym. Sci.* **2015** (2015) 1–7.
29. S. Sriwichai, A. Baba, S. Phanichphant, K. Shinbo, K. Kato, F. Kaneko, (2008, September). Electropolymerized poly (3-aminobenzoic acid)-based surface plasmon resonance immunosensor. *International Symposium on Electrical Insulating Materials (ISEIM 2008). Electrical Insulating Materials*, (2008), p. 346–348.
30. R. Janmanee, A. Baba, S. Phanichphant, S. Sriwichai, K. Shinbo, K. Kato, F. Kaneko. *J. Appl. Phys.* **50**(1S2) (2011), p. 01BK02.
31. R. Janmanee, A. Baba, S. Phanichphant, S. Sriwichai, K. Shinbo, K. Kato, F. Kaneko. *ACS Appl. Mater. Interface* **4**(8) (2012) 4270–4275.
32. R.A. Jain. *Biomaterials* **21**(23) (2000) 2475–2490.
33. D. Garlotta. *J. Polym. Environ.* **9**(2) (2001) 63–84.
34. M. Gümüşderelioglu, G. Deniz. *Turk. J. Chem.* **23**(2) (1999) 153–162.
35. Y.Gu, M.T. Lai. *Biosens. Bioel.* **31**(1) (2012) 124–129.

36. S. Rodriguez-Mozaz, M.P. Marco, M.J. Lopez de Alda, D. Barceló. *Pure Appl. Chem.* **76**(4) (2004) 723–752.
37. B.K. Singh, A.C. Hillier. *Anal. Chem.* **78**(6) (2006) 2009–2018.
38. N. Ibrišimović. Contributions to the development of biomimetic, optical biosensors-feasibility studies and optimization of prototypes, Doctoral dissertation, University of Vienna, Vienna, (2009).
39. D. Hall. *Anal. Biochem.* **288**(2) (2001) 109–125.
40. B.P. Nelson, T.E. Grimsrud, M.R. Liles, R.M. Goodman, R.M. Corn. *Anal. Chem.* **73**(1) (2001) 1–7.
41. C. Boozer, G. Kim, S. Cong, H. Guan, T. Londergan. *Curr. Opinion Biotechnol.* **17**(4) (2006) 400–405.
42. M.N.R. Kumar, N. Kumar, A.J. Domb, M. Arora, Pharmaceutical polymeric controlled drug delivery systems. In *Filled elastomers drug delivery systems* (2002), p. 45–117.
43. R, Gref, Y. Minamitake, M.T. Peracchia, V. Trubetskoy, V. Torchilin, R. Langer. *Science* **263**(5153) (1994) 1600–1603.
44. A. Sosa-Peinado, M. González-Andrade, *Fluorescent Biosensors for Protein Interactions and Drug Discovery*, INTECH Open Access Publisher, Chapter 1, (2001), p. 3–14.
45. P.S. Heeger, A.J. Heeger. *Proc. Natl. Acad. Sci.* **96**(22) (1999) 12219–12221.
46. X. Wang, S. Uchiyama, *Polymers for biosensors construction*, State of the Art in Biosensors- General Aspects, INTECH Open Access Publisher, Chapter 3, (2013), p. 67–86.
47. G. Bauer, F. Pittner, T. Schalkhammer. *Microchim. Acta* **131**(1-2) (1999) 107–114.
48. Z. Yang, M. Lu, J. Li, Z. Tan, H. Dai, X. A. Jiao, X. Hu. *Biosens. Bioel.* **51** (2016) 356–361.
49. A. Sassolas, B. Prieto-Simón, J.L. Marty. *Am. J. Anal. Chem.* **3**(3) (2012) 210–232.
50. G. Murugaboopathi, V. Parthasarathy, C. Chellaram, T. Prem Anand, S. Vinurajkumar. *Biosci. Biotechnol. Res. Asia* **10** (2013) 711–714.
51. M. Mascini. *Sens. Actuat. B: Chem.* **6**(1) (1992) 79–82.
52. M. Singh, P.K. Kathuroju, N. Jampana. *Sensors Actuat. B: Chem.* **143**(1) (2009) 430–443.
53. E.H. Yoo, S.Y. Lee. *Sensors* **10**(5) (2010) 4558–4576.
54. Z. Altintas, I. Tothill. *Sens. Actuat. B: Chem.* **188** (2013) 988–998.
55. M. Mascini, S. Tombelli. *Biomarkers* **13**(7-8) (2008) 637–657.
56. T.J. Ohara, R. Rajagopalan, A. Heller. *Anal. Chem.* **66**(15) (1994) 2451–2457.
57. B.D. Malhotra, A. Chaubey. *Sens. Actuat. B: Chem.* **91**(1) (2003) 117–127.
58. S. Cosnier, B. Galland, C. Gondran, A. Le Pellec. *Electroanalysis* **10**(12) (1998) 808–813.