# Disposable electrochemical biosensors in microbiology

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## 1.Introduction

The analytical objective of developing sensitive, selective and rapid methods for the analysis of different substances in complex matrices has led to the production of several chemical sensors. Among the analytical applications of chemical sensors, the one involving the early detection of pathogen microorganisms can be considered essential in fields such as,

- Food industry
- Clinical and environmental analysis for avoidance of human health problems
- Animals and plants epidemics [1-3].

In this respect, chemical sensors can be considered as an alternative to the traditional analytical instrumentation in rapid and sensitive analysis of these species.

A chemical sensor is a device that responds to a particular analyte in a selective way through a chemical reaction. They can be used for the qualitative or quantitative determination of the analyte [4]. There are two basic components in a chemical sensor: a recognition system called receptor and, the transducer.

The receptor is the sensitive part of the sensor and it also confers the selectivity needed for a suitable analyte determination without other species interference. Thus, the receptor recognizes the chemical information present in the sample. This information is then changed into a signal in order to be recognized by the transducer. The latter finally carries out the conversion of the signal into an observable response.

Biosensors can be considered as a type of chemical sensors with high sensitivity and applicability levels. Following the IUPAC definition [5], a biosensor is an integrated receptor-transducer device, which is capable of providing selective quantitative or semi-quantitative analytical information using a biological recognition element (Fig. 1).

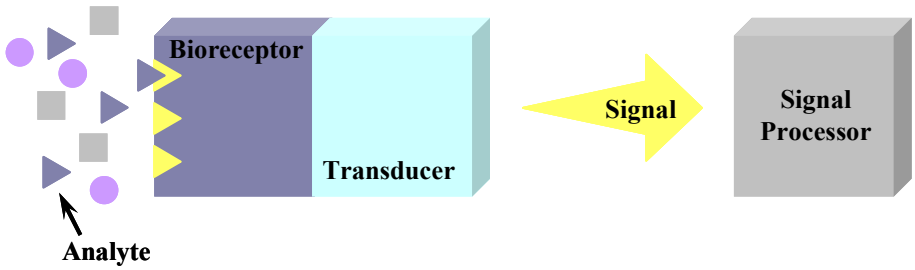



**Fig. 1** Scheme of a biosensor

The biological recognition element is generally a macromolecule, which catalyzes a biochemical reaction, immobilized on a physical transducer. Enzymes are the biological elements most often used in the development of biosensors due to their high selectivity and easy use. However, they are expensive owing to their high cost of extracting, isolating an purifying processes [4]. In this way, on many occasions microorganisms are used as alternative sources of enzymatic activity in biosensors development. Microbial biosensors have longer life times. Moreover, the many enzymes and co-factors that co-exist in the cells give them the capacity to consume and consequently detect large number of chemicals. Furthermore, the advancement in molecular biology/recombinant DNA technologies has opened endless possibilities of modification of the microorganisms to improve the activity of an existing enzyme or even express foreign enzymes in host cell. These characteristics make microbial biosensors excellent devices in the determination of a great number of substances [6].

As for the transducer, this is that part of the biosensor that converts the change observed in the system as a consequence of the bioreaction into a measurable signal related to the analyte concentration. According to the transducer type, biosensors may be classified as [7]:

- Electrochemical: potentiometry, amperometry and conductimetry
- FET (field effect transistor)-based sensors

- Optical
- Piezoelectric devices
- Surface acoustic waves
- Thermal methods

Among the various possible combinations of biocomponents and transducer techniques, the one involving electrochemical detection has been used prominently in the analysis of different substances. This fact is due to the low detection limits that can be achieved on small samples volumes with modern electrochemical techniques. Moreover, electroanalytical techniques provide other important advantages including simplicity, low cost and *in situ* analysis options.

Recently, the change of the conventional solid electrodes by screen-printed electrodes (SPEs) has increased the possibilities of electroanalytical techniques in the biosensor field. Various advantages including simple fabrication, low cost, small size, disposability, portability and easily mass-produced reinforce the use of screen-printed biosensors [8]. Moreover, SPEs avoid some common problems related to traditional solid electrodes such as the needed cleaning processes. Thus, SPEs are extensively used in the fabrication of disposable biosensors.

The fields of medicine and public health require the rapid detection of bacterial pathogens, which cause severe infectious diseases and poisonings, in food and water. Traditional techniques of analysis, including culturing methodology, remain as the preferred systems for the detection of these species. However, these techniques can be considered as time-consuming and they often lead to false negatives results [9]. Biosensors have begun to play a significant role in the determination of pathogens. Among them, electrochemical disposable devices have received considerable attention due to the combination of the high sensitivity of electrochemical transducers with their low cost and their compatibility with modern microfabrication and miniaturization technologies [10]. In this way, a great number of works describing the use of SPEs in the determination of different microorganisms can be found in the bibliography.

In the same way, microorganisms have been effectively used as the biological sensing element in the construction of disposable biosensors. They present various advantages including ubiquitously and a great capacity to adapt to adverse conditions. Moreover, they are able to metabolize a wide range of chemical compounds [11]. Microorganisms can be also considered as excellent sources of enzymes being less expensive and more stable than purified enzymes usually employed in biosensors development. Therefore, a number of papers can be found in the literature describing the successfully determination of several substances using disposable microbial biosensors.

In this chapter, a description of the above-mentioned interesting applications of SPEs in microbiology has been reported. Several important aspects related to the type of biological element and immobilization procedure used have been included. Since, the construction of the SPEs constitutes the first step in the development of disposable biosensors; a brief description of this fabrication process has been consequently included.

## 2. Screen-printed electrodes

Electroanalytical techniques are well considered in the Analytical Chemistry field. However, they have found several restrictions and practical difficulties for some applications. One of the most common problems of these techniques has been their lack of reproducibility, associated to the complexity of obtaining identical electrodes for all the measurements. Hanging drop mercury electrodes have presented fewer problems in this respect but their toxicity has lead to the development of different alternatives. In this way, the possibilities of the electrochemical techniques can be improved by means of the replacement of the classical electrodes and cell systems by disposable screen-printed devices.

SPEs add many attractive advantages to the electroanalytical techniques, including the elimination of the surface regeneration needed in solid electrodes. Moreover, SPEs can be designed according to the analytical problem characteristics by choosing the adequate fabrication materials.

Selective and disposable biosensors can be easily obtained by biomolecules immobilization on SPEs surfaces [12, 13]. Procedures based on these disposable devices have been shown as practical systems for the fast, accessible and low cost analysis of many target species, including microorganisms. In the same way, the immobilization of microorganisms on the electrode has given rise to the development of selective and sensitive disposable biosensors for the analysis of different substances.

### 2.1. SPEs fabrication

Screen-printing technology is based on the sequential layer deposition of different inks on a ceramic or plastic substrate using the appropriate screen. A typical screen is made from a finely mesh of different materials including stainless steel, polyester or nylon mounted under tension on a metal frame. The finished screen has open-mesh areas through which the desired pattern can be printed (Fig. 2) [14].

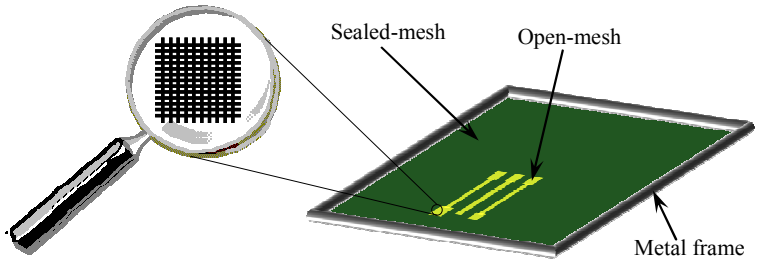



**Fig. 2** Scheme of a screen

The screen-printing ink is then poured onto the top surface of the stencil. Then, a squeegee slowly moves from the rear to the front part of the screen, forcing the ink through the open areas. The required pattern is thus deposited onto the substrate surface, as it can be seen in Fig. 3 [14].

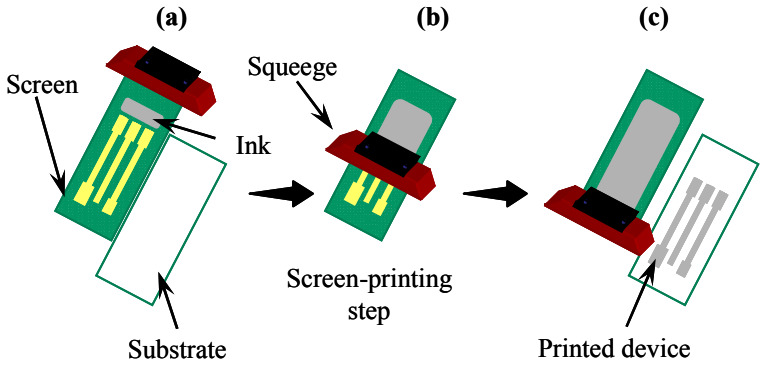



**Fig. 3** Screen-printed device fabrication process scheme: (a) Ink poured; (b) Squeegee traversing; (c) Ink deposited on the substrate surface

The next phase of the process is to dry the printed ink. Screen-printing inks usually contain various organic solvents, which are added with the aim of producing the accurate viscosity for screen printing. These solvents can be removed by drying the printed ink in an oven at an adequate temperature. After drying, the substrate retains a rigid pattern that is relatively immune to smudging [14]. The combination of different screens and inks give rise to the definition of the different electrodes (working, reference and auxiliary) in the same configuration unit.

Finally, the disposable electrochemical biosensor is generated by the subsequent modification of the working electrode with the biosensing material. This modification implies several steps in order to assure the robustness and durability of the developed biosensor.

Disposable biosensors generated by modification of SPEs have been successfully applied in the microbiology field. The next section involves the report of different disposable biosensors employed in such field, including the description of the different biocomponents and immobilization procedures used in their development.

## 3. Screen-printed electrodes biosensors in microbiology

As it has been mentioned, there is a great number of works describing the utilization of screen-printed biosensors in microbiological applications. These disposable biosensors have been developed by means of the immobilization of different nature biological elements. The immobilization procedure not only facilitates the required close proximity between the biomaterial and the transducer, but also helps in stabilizing it for reuse [11]. Thus, immobilization technology plays a very important role, being the critical step in biosensors manufacturing. Many different procedures have been thus reported for the biological material immobilization conditions onto SPEs.

A brief description of the different biological elements of the diverse immobilization procedures used in the development of disposable biosensors has been included in the following sections. This description has been focused in the applications of this kind of sensors within microbiology field.

### 3.1. Biological Element

SPEs biosensors for detecting microorganisms are mostly based on the interaction between microorganisms and biological recognition elements such as antibodies giving rise to very sensitive and selective disposable immunosensors. The fabrication of DNA electrochemical sensors has also attracted a considerable recent attention in the development of

disposable genosensors for the analysis of pathogens agents. In the same way, different microorganisms have been used in the fabrication of disposable biosensors for many interesting analytical applications.

#### 3.1.1. Antibodies and antigens

Antibodies are serum proteins endowed with the capacity to recognize, by stereospecific association, a foreign substance in the organism it has invaded. They are produced by two types of blood cells, B lymphocytes and plasma cells, in response to a foreign substance which is termed an immunogen, so-defined because it evokes an immune response. In most instances, an individual antibody will recognize only one substance and this substance is termed the antigen. For antibody-based biosensors, the analyte is either the corresponding antigen of the antibody or a part of it [7]. The use of these biological elements has given rise to very high sensitive and selective sensors named immunosensors.

Electrochemical immunosensors employs either antibodies or their complementary binding partners (antigens and haptens) as biorecognition elements in combination with electrochemical transducers. These sensors are based on the ability of antibodies to form complexes with the corresponding antigens. This property of high specific molecular recognition leads to high selectivity of assays based on immune principles. Moreover, the extreme affinity of antigen-antibody interactions results in great sensitivity of immunoassay methods. This sensitivity is increased when electrochemical transducers are used in immunosensors development [15, 16].

A great number of immunoassay disposable electrochemical systems described in the literature are based on the principle of electrochemical detection of the labelled immunoagent. Most of these systems involve the amperometric determination of the product of a reaction of a substrate catalyzed by a labeled enzyme [16].

Enzyme immunoassays combine the high sensitivity of the enzyme-catalyzed reactions with the extreme specificity of antigen-antibody interactions. These advantages are increased with the use of electrochemical transducers including disposable electrodes. Thus, disposable electrochemical enzyme-labelled immunosensors have been successfully applied in the sensitive and selective analysis of many species including microorganisms [17].

A variety of different enzyme markers have been used for substrate transformation in electrochemical immunoassays systems. Alkaline phosphatase (ALP) is one of the most popular enzymes used in imunoanalysis. This enzyme catalyzes a dephosphorylation reaction of different organic phosphates. Some of the products formed as a result of the reaction can be detected electrochemically in significantly low concentrations. Horseradish peroxidase (HRP), which catalyses the reduction of $H_2O_2$, is also a very frequently enzyme used in electrochemical immunosensors development [16].

In respect to the immunosensors mechanism of analysis, a variety of methods have been developed including direct monitoring, direct sandwich assays and indirect sandwich assays. The direct assay involves the antigen immobilization of antigen on the electrode surface, followed by detection with an excess of enzyme-labelled conjugate (Fig. 4(a)). This method presents a great simplicity and a short assay time. For instance, the analysis of *Listeria monocytogenes (L. monocytogenes)*, an important food-borne pathogen with an extremely high mortality, has been analyzed using this simple method. The proposed immunosensor design was based on the immobilization of *L. monocytogenes* antigens on SPCEs. These modified SPCEs were then incubated with goat anti-*L. monocytogenes*-ALP. Finally, the amperometric signal was registered at + 300 mV following the oxidation of p-aminophenol (p-AP), the dephosphorylation product of the substrate p-aminophenyl phosphate (p-APP) [18].

Direct sandwich assay is one of the most frequently methods selected in the development of disposable immunosensors for the determination of microorganisms. This method involves the immobilization of a capturing antibody on the electrode surface. Subsequently, an incubation process with the specific antigen takes place and the antibody-antigen formed complex is finally incubated with a labelled secondary antibody (Fig. 4(b)) [19]. This method has been successfully used in the determination of *Echerichia coli (E. coli)*. *E. coli* is an enterohemorrhagic bacterium which causes hemorrhagic colitis including symptoms such as bloody diarrhoea, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpurea [20]. The analysis of this microorganism has been realized in samples of milk using a disposable immunosensing strip based on a SPCE using HRP-conjugated polyclonal anti *E. coli* O157:H7 as the secondary antibody. Hydrogen peroxide and FeDC were used as the substrate for HRP and mediator, respectively, for the amperometric measurements at a potential of + 300 mV [20]. The electrode modification with mediators in immunosensors development reduces the working potential, avoids electrochemical interferences and increases the reversibility of electrode reactions [21].

Indirect sandwich assay is similar to the direct sandwich assay already described except that instead of the secondary antibody, two detecting antibodies are required. The first one is the specific antibody (capturing antibody) to the antigen, while the second one is an enzyme-labelled anti-species conjugate (revelling antibody) (Figure 4(c)). This system offers greater flexibility as it avoids the need to conjugate the antibody to an enzyme-label [18]. For example, *Vibrio cholerae (V. cholerae)* has been successfully determined using this indirect method of analysis. *V. cholerae* causes cholera, a serious gastrointestinal disease present in many countries [21-23] . The detection of *V. cholerae* antigen has been performed using SPCEs coated with rabbit anti-*Vibrio cholerae* IgG capturing antibody. The capturing antibody was firstly adsorbed on the SPCE surface followed by blocking the free sites with seroalbumine bovine (BSA). The blocked SPCEs were incubated with a solution containing *V. cholerae* cells. Afterward, these electrodes were further incubated with a solution containing mice serum revealing antibody. Finally, the electrodes were incubated with a rabbit anti-mouse immunoglobulin ALP conjugated (rabbit anti-mouse ALP conjugate) solution. The analytical signal

measured was the amperometric response recorded for the enzyme substrate 1-napthyl phosphate by applying a potential of + 400 mV [22, 23].

It can be found in the literature other assay methods similar to the indirect sandwich one described above, which can be called just indirect assay. In many cases, the antigen is first immobilized on the electrode surface followed by the incubation with the specific antibody solution. Finally, in a second incubation step, the enzyme-labelled anti-species conjugate is fixed (Fig. 4 (d)). This method has been used in the analysis of numerous pathogen microorganisms. One of this analyzed microorganisms has been *Salmonella typhimurium (S. typhimurium)*, one of the most common types of *Salmonella*, responsible of salmonellosis, a reported food-borne disease worldwide [24]. Therefore, the capacity to fast recognition of this pathogen is very important to protect public health safety and security. The immunosensor described by Rao *et al.* [25] for the analysis of *S. typhimurium* in serum samples was based on the immobilization of *S. typhimurium* flagellin antigen on SPCEs. After a washing step, the electrode was incubated with anti-human ALP conjugated. The amperometric response of the modified SPCE was recorded at a potential of + 400 mV in ethanoldiamine. Next, the substrate 1-naphthyl phosphate was added and the change in the amperometric current was registered.

Antigen-antibody complex formation can be also electrochemically directly detected using label-free immunosensors These biosensors display some important advantages in terms of speed and simplicity of operation [2]. The techniques are based on the detection of a change in the surface transducer physical properties as a result of the immunocomplex formation which causes a change in the transducer signal [16]. A redox probe is used in many cases in order to detect this change. The main advantage of label-free immunosensors is the single stage analysis, which leads to a lower cost and easier use than labelled-immunosensors. As an example of this type of immunosensors, $[Fe(CN)_6^{3-}]/[Fe(CN)_6^{4-}]$ has been used as a redox probe in label-free immunosensors for the successfully determination of *E. coli* by different authors. This pathogen has been analyzed using SPAuEs [2] and SPCEs modified with self-assembled peptide nanotubes (PNTs) which have functional groups on their surface that allow an easier immobilization of antibodies [26].

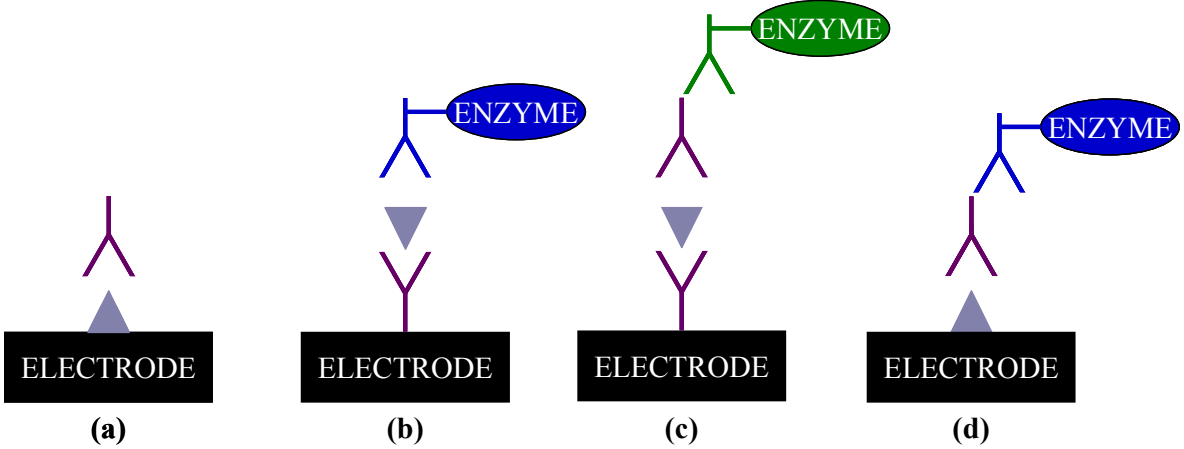
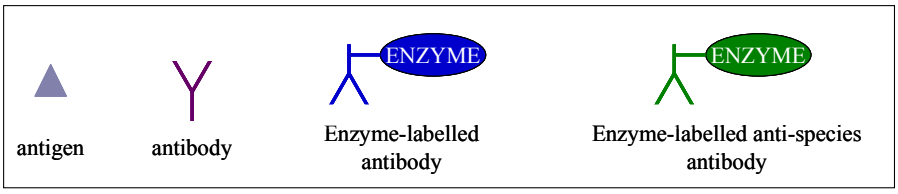



**Fig. 4** Schematic diagram of the different assay formats used in disposable electrochemical immunosensors: (a) Direct assay; (b) Direct sandwich assay; (c) Indirect sandwich assay; (d) Indirect assay




### 3.1.2. Nucleic Acids

Hybrid receptors such as DNA, which were first introduced by Millan and Mikkelsen [27], have been shown to have promising applications in different fields of analysis. Nucleic acids operate in many ways like antibodies. The principle of selective detection is based on the highly specific hybridization of complementary strands of DNA. In this way, advances in molecular biology and biotechnology have led to a great number of possibilities for DNA electrochemical biosensors development. In fact, the detection of specific DNA sequences provides the basis of many electrochemical detecting methods of microbial pathogens analysis. These methods are often based on hybridization biosensors for the detection of DNA sequences. The basics of nucleic acid hybridization devices is DNA base pairing. Thus, the immobilization of a short synthetic oligomer on the electrode surface is first carried out. The sequence of this oligomer is complementary to the target element. Then, the exposure of this DNA modified electrode to the target sample results in the formation of the hybrid on the electrode surface. Electrochemical monitoring of this duplex formation can result in a very useful transducer response. Thus, the formation of the hybrid is commonly detected by exposing it to an

electroactive indicator suitable to form strong bounds with the formed hybrid. An increase in the electrochemical response of this indicator is then related to the duplex formation [28].

Several genosensors based on the use of redox indicators have been used for the determination of pathogenic microbes including *E. coli*. The genosensor developed by Shiraishi *et al.* [29] was based on the immobilization of the probe DNA on a SPE fabricated by screen-printing a fullberene-impregnated carbon ink onto a poly(methylmethacrylate) substrate. In this case, $Co(phen)_3^{3+}$ was used as the electroactive indicator of the hybrid formation.

In many occasions, the hybridization event is detected following the enzymatic reaction of an enzyme, previously labelled on the electrode surface. Enzyme-labelled genosensors have been used, for instance, in the determination of *Salmonella* which its determination with an ALP-labelled genosensor has been successfully carried out using SPCEs [30]. In this case, the hybridization reaction was detected though the sandwich format, based on the coupled of biotin-streptavidin interaction using a streptavidin conjugated to ALP. α-naphtyl phosphate was used as substrate for the hybrid formation detection. An analogous genosensor is shown in [31] for the analysis of this pathogen using SPAuEs.

#### 3.1.3. Microorganisms

As it has been pointed up, microorganisms have been successfully used as biological elements in the development of disposable biosensors for a great number of analytical applications due to their already described great number of advantages. The microbial biosensor development includes several steps that are next summarized,

- Cultivation step, in which the microorganism growth takes place. It includes the gradually increasing microorganism inoculation with the target analyte. This is the key step in order to make the biosensor specific to the analyte of interest.
- Microorganism immobilization on the SPE surface.
- Determination of the concentration of the target compound by chronoamperometric measurements, following the oxygen consumption due to the metabolic activity of the immobilized microbe.

Microbial disposable biosensors have been successfully applied in the determination of many substances such as phenol [32], benzene [33] or the herbicide 2,4-dichloro phenoxy acetic acid (2,4-D) [13, 34, 35]. In most of the cases, the biosensor was constructed using *Pseudomonas putida* (*P. putida*) as the immobilized microorganism.

### 3.2. Immobilization of Biological Elements

The immobilization of the biological component must be carried out very carefully in order to assure the well performance of the biosensor. When selecting the most appropriate method various factors should be taken into account, amongst which it is worth highlighting the physical-chemical properties of the analyte, the nature of the biological element, the type of transducer used and the type of sample to be analyzed [7].

A large number of immobilization methods can be found in the literature for the development of disposable biosensors with different applications in microbiology. The characteristic features of the most commonly used methods will be described in the following sections.

#### 3.2.1. Adsorption

Physical adsorption is the simplest method for biological element immobilization. This procedure has been successfully used in the development of disposable immunosensors for the determination of pathogen microbes such as *Helicobacter pylori (H. pylori)* [17]. In this case, *H. pylori* antigens were immobilized by passive adsorption on the SPCE surface.

In the case of microbial disposable biosensors, often a microbial suspension is incubated with the electrode or an immobilization matrix, such as alumina and glass bead, followed by rinsing with buffer to remove unadsorbed microbe cells. The microbes are immobilized due to adsorptive interactions such as ionic, polar or hydrogen bonding and hydrophobic interaction. However, immobilization using adsorption alone generally leads to poor long-term stability because of desorption of microbes [11].

#### 3.2.2. Entrapment

Entrapment is a wide-ranging term that includes different forms of working. One of the most commonly used methods consists of the entrapment of the biological element in an inert polymer matrix on the electrode. In this case the biological component is placed in contact with a solution of a species susceptible to polymerization that forms a gel matrix in which the biocomponent is retained [7].

Alternatively, entrapment immobilization can be carried out by inclusion of the biological element by the either retention of it in close proximity of the transducer surface using dialysis or filter membrane or in chemical/biological polymers/gels such as (alginate, carrageenan, agarose, chitosan, collagen, polyacrylamide, polyvinylachohol, poly(ethylene glycol), polyurethane, and so on. The major disadvantage of this immobilization method is the additional diffusion resistance offered by the entrapment material, which will result in lower sensitivity and detection limit [11].

Some reported examples of the application of this procedure in the development of microbial biosensors include the development of amperometric systems based on the immobilization of *E. coli* [36] and *P. putida* [37] using an Anopore membrane held to the SPCE surface using a micropore tape. Others reported *E. coli* based disposable biosensors use κ-Carrageenan, as immobilization matrix for entrapment of the bacterial cells onto SPAuEs [38].

In the same way, disposable immunosensors have been developed for the analysis of the microbe pathogen *Vibrio parahaemolyticus* in food based on the immobilization of the biosensing element using the natural polysaccharide agarose [8].

#### 3.2.3. Microencapsulation.

Microencapsulation is an immobilization method which consists of bounding the enzymes using a semi-permeable membrane which allows substrate molecules and products to pass through it, but which obstruct the biocomponent [21]. There are different types of membranes (teflon, cellulose acetate (CA), polycarbonate, nafion, polyurethane) that are used in biosensor construction, the properties of which are decisive when applying the biosensor to the different types of analytes and matrices. So, as an example, CA membranes are impermeable to proteins; teflon ones are permeable to some gases, etc. The most important advantage of this method is that membranes protect the biological components diminishing its biodegradation and contamination. Moreover, the biosensor is also protected from temperature, pH and ionic force changes. However, the rate of the biochemical reaction is lower since the analyte has to pass through the membrane to reach the biocomponent, which implies a less comprehensive analysis [7].

A typical example of this immobilization procedure has been described for the bacteria *P. Putida* in the determination of benzene [33].

#### 3.2.4. Cross-linking

Cross-linking is a chemical immobilization method which involves bridging between functional groups on the outer membrane of the microbe cells by multifunctional reagents such as GA and cyanuric chloride, to form a network. Because of the speed and simplicity, the method has found wide acceptance for immobilization of microorganisms. The cells can be bounded directly onto the electrode surface or on a removable support membrane, which can then be placed on the transducer surface [11].

Several disposable biosensors have been developed for the analysis of phenol or the herbicide 2,4-dichloro phenoxy acetic acid (2,4-D) based on the immobilization of *P. putida* bacteria on SPEs by cross-linking [13, 34, 35]. Likewise, *E. coli* selective immunosesnors have been developed based on the cross-linking immobilization of bacteria antibodies on SPCEs [20].

#### 3.2.5. Covalent bounding

A highly stable binding of biological material upon the electrochemical transducer can be achieved through the formation of covalent bonds between both materials. Covalent binding methods rely on the formation of a stable covalent bond between functional groups of the biological components such as amine, carboxylic or sulphydryl and the transducer such as amine, carboxylic, epoxy or tosyl [6, 7].

This immobilization has been frequently used in the development of disposable biosensors for different pathogens analysis. Thus, *S. typhimurium* can be determined using a HRP-labelled immunosensor in which the biorecognition element was immobilized on a SPAuE surface covered with carboxymethyldextran in order to assure covalent bound formation [24].

Covalent bounding immobilization is the most frequently procedure used in the development of disposable genosensors for the determination of pathogenic bacteria. For instance, SPAuEs have been successfully used in the determination of *Salmonella* bacteria using an ALP-labelled disposable genosensor. The immobilization of nucleic acid on the electrode surface was carried out via self-assembled monolayers (SAMs) formation. The procedure involves a previous thiol-modification of the probe nucleotide [31].

A common covalent immobilization procedure for disposable genosensors and immunosensors in microbiology applications involves the use of avidin-biotin interaction to attach biotinylated biocomponents on the electrode surface. For example, *S. pneumoniae* pathogen has been analyzed using both kinds of sensors. Thus, a disposable genosensor has been developed for the determination of this microbe based on the immobilization of biotinylated oligonucleotides on the SPCE surface [10]. The analysis of this bacteria has been also performed using a disposable immunosensor fabricated by means of the immobilization of biotinylated antibodies on SPCEs [39]. In both cases, the previous modification of the electrode surface with streptavidin was necessary in order to assure the necessary avidin-biotin reaction.

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## References

[1] Oczkowski T, Zwierkowska E, Bartkowiak S. Application of cell-based biosensors for the detection of bacterial elicitor flagellin. *Bioelectrochemistry.* 2007;70:192-197.
[2] Escamilla-Gomez V, Campuzano S, Pedrero M, Pingarron JM. Gold screen-printed-based impedimetric immunobiosensors for direct and sensitive Escherichia coli quantisation. *Biosensors & Bioelectronics.* 2009; 24:3365-3371.
[3] Gamella M, Campuzano S, Parrado C, Reviejo AJ, Pingarron JM. Microorganisms recognition and quantification by lectin adsorptive affinity impedance. *Talanta.* 2009; 78:1303-1309.
[4] Eggins BR, *Chemical sensors and biosensors*. Chchester: John Willey & sons; 2002.
[5] Thévenot DR, Toth K, Durst RA, Wilson GS. Electrochemical biosensors: recommended definitions and classification (Technical Report). *Pure and Applied Chemistry.* 1999;71:2333-2348.
[6] Lei Y, Chen W, Mulchandani A. Microbial biosensors. *Analytica Chimica Acta.* 2006;568:200-210.
[7] Domínguez O, Arcos MJ. *Electrochemical Biosensors*. Encyclopedia of Sensors. Vol. 3., USA: American Scientific Publishers; 2006.
[8] Zhao GY, Xing FF, Deng SP. A disposable amperometric enzyme immunosensor for rapid detection of Vibrio parahaemolyticus in food based on agarose/Nano-Au membrane and screen-printed electrode. *Electrochemistry Communications.* 2007;9:1263-1268.
[9] Yemini M, Levi Y, Yagil E, Rishpon J. Specific electrochemical phage sensing for Bacillus cereus and Mycobacterium smegmatis. *Bioelectrochemistry.* 2007;70:180-184.
[10] Hernandez-Santos D, Diaz-Gonzalez M, Gonzalez-Garcia MB, Costa-Garcia A. Enzymatic genosensor on streptavidin-modified screen-printed carbon electrodes. *Analytical Chemistry.* 2004;76:6887-6893.
[11] D'Souza SF. Microbial biosensors. *Biosensors & Bioelectronics.* 2001;16:337-353.
[12] Domínguez-Renedo O, Alonso-Lomillo MA, Arcos-Martinez MJ. Recent developments in the field of screen-printed electrodes and their related applications. *Talanta.* 2007;73:202-219.
[13] Timur S, Della Seta L, Pazarlioglu N, Pilloton R, Telefoncu A. Screen printed graphite biosensors based on bacterial cells. *Process Biochemistry.* 2004; 39:1325-1329.
[14] White NM, Turner JD. Thick-film sensors: past, present and future. *Meas. Sci. Technol.* 1997;8:1-20.
[15] Skladal P. Advances in electrochemical immunosensors. *Electroanalysis.* 1997;9:737-745.
[16] Ghindilis AL, Atanasov P, Wilkins M, Wilkins E. Immunosensors: Electrochemical sensing and other engineering approaches. *Biosensors & Bioelectronics.* 1998;13:113-131.
[17] Messina GA, De Vito IE, Raba J. Screen-printed immunosensor for quantification of human serum IgG antibodies to Helicobacter pylori. *Sensors and Actuators B-Chemical.* 2007;128:23-30.
[18] Crowley EL, O'Sullivan CK, Guilbault GG. Increasing the sensitivity of Listeria monocytogenes assays: evaluation using ELISA and amperometric detection. *Analyst.* 1999;124:295-299.
[19] Sadik OA, Van Emon JM. Applications of electrochemical immunosensors to environmental monitoring. *Biosensors and Bioelectronics.* 1996;11:1-11.
[20] Lin YH, Chen SH, Chuang YC, Lu YC, Shen TY, Chang CA, Lin CS. Disposable amperometric immunosensing strips fabricated by Au nanoparticles-modified screen-printed carbon electrodes for the detection of foodborne pathogen Escherichia coli O157 : H7. *Biosensors & Bioelectronics.* 2008;23:1832-1837.
[21] Alonso-Lomillo MA, Domínguez-Renedo O, Arcos-Martinez, M.J. *Enzyme modified screen printed electrodes*, in *Biosensors: Properties, Materials and Applications.* Hauppauge NY: Nova Publishers; 2009
[22] Rao VK, Sharma MK, Goel AK, Singh L, Sekhar K. Amperometric immunosensor for the detection of Vibrio cholerae O1 using disposable screen-printed electrodes. *Analytical Sciences.* 2006;22:1207-1211.
[23] Sharma MK, Goel AK, Singh L, Rao VK. Immunological biosensor for detection of Vibrio cholerae O1in environmental water samples. *World Journal of Microbiology & Biotechnology.* 2006;22:1155-1159.
[24] Salam F, Tothill IE. Detection of Salmonella typhimurium using an electrochemical immunosensor. *Biosensors & Bioelectronics.* 2009;24:2630-2636.
[25] Rao VK, Rai GP, Agarwal GS, Suresh S. Amperometric immunosensor for detection of antibodies of Salmonella typhi in patient serum. *Analytica Chimica Acta.* 2005; 531:173-177.
[26] Cho EC, Choi JW, Lee MY, Koo KK. Fabrication of an electrochemical immunosensor with self-assembled peptide nanotubes. *Colloids and Surfaces a-Physicochemical and Engineering Aspects.* 2008;313:95-99.
[27] Millan KM, Mikkelsen SR. Sequence-selective biosensor for dna-based on electroactive hybridization indicators. *Analytical Chemistry.* 1993;65:2317-2323.
[28] Wang J, Rivas G, Cai X, Palecek E, Nielsen P, Shiraishi H, Dontha N, Luo D, Parrado C, Chicharro M, Farias PAM, Valera FS, Grant DH, Ozsoz M, Flair MN. DNA electrochemical biosensors for environmental monitoring. A review. *Analytica Chimica Acta.* 1997;347:1-8.
[29] Shiraishi H, Itoh T, Hayashi H, Takagi K, Sakane M, Mori T, Wang J. Electrochemical detection of E-coli 16S rDNA sequence using air-plasma-activated fullerene-impregnated screen printed electrodes. *Bioelectrochemistry.* 2007;70:481-487.
[30] Del Giallo ML, Ariksoysal D, Marrazza G, Mascini M, Ozsoz M. Disposable electrochemical enzyme-amplified genosensor for Salmonella bacteria detection. *Analytical Letters.* 2005;38:2509-2523.
[31] Farabullini F, Lucarelli F, Palchetti I, Marrazza G, Mascini M. Disposable electrochemical genosensor for the simultaneous analysis of different bacterial food contaminants. *Biosensors & Bioelectronics.* 2007;22:1544-1549.

[32] Bachmann TT, Bilitewski U, Schmid RD. A microbial sensor based on Pseudomonas putida for phenol, benzoic acid and their monochlorinated derivatives which can be used in water and n-hexane. *Analytical Letters.* 1998;31:2361-2373.
[33] Lanyon YH, Tothill IE, Mascini M. An amperometric bacterial biosensor based on gold screen-printed electrodes for the detection of benzene. *Analytical Letters.* 2006;39:1669-1681.
[34] Odaci D, Sezginturk MK, Timur S, Pazarliolu N, Pilloton R, Dinckaya E, Telefoncu A. Pseudomonas putida Based Amperometric Biosensors for 2,4-D Detection. *Preparative Biochemistry & Biotechnology.* 2009;39:11-19.
[35] Timur S, Pazarlioglu N, Pilloton R, Telefoncu A. Detection of phenolic compounds by thick film sensors based on Pseudomonas putida. *Talanta.* 2003;61:87-93.
[36] Farré M, Pasini O, Carmen Alonso M, Castillo M, Barceló D. Toxicity assessment of organic pollution in wastewaters using a bacterial biosensor. *Analytica Chimica Acta.* 2001;426:155-165.
[37] Farre M, Barcelo D. Characterization of wastewater toxicity by means of a whole-cell bacterial biosensor, using Pseudomonas putida, in conjunction with chemical analysis. *Fresenius Journal of Analytical Chemistry.* 2001;371:467-473.
[38] Held M, Schuhmann W, Jahreis K, Schmidt HL. Microbial biosensor array with transport mutants of Escherichia coli K12 for the simultaneous determination of mono-and disaccharides. *Biosensors & Bioelectronics.* 2002;17:1089-1094.
[39] Diaz-Gonzalez M, Gonzalez-Garcia MB, Costa-Garcia A. Detection of pneumolysin in human urine using an immunosensor on screen-printed carbon electrodes. *Sensors and Actuators B-Chemical,* 2006;113:1005-1011.

 