Talanta 82 (2010) 1629–1636

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Review

# Screen-printed biosensors in microbiology; a review

M.A. Alonso-Lomillo, O. Domínguez-Renedo, M.J. Arcos-Martínez*

*Department of Chemistry, Faculty of Sciences, University of Burgos, Plaza Missael Bañuelos s/n, 09001 Burgos, Spain*

ARTICLE INFO

*Article history:*
Received 29 April 2010
Received in revised form 20 August 2010
Accepted 20 August 2010
Available online 27 August 2010

*Keywords:*
Microorganism
Screen-printed electrodes
Electrochemical biosensors

ABSTRACT

Disposable screen-printed biosensors have been successfully employed in the development of analytical methods that respond to the growing need to perform rapid "in situ" analyses. Thus, the early detection of microorganisms, which plays an important role in the prevention of human health problems, animals and plants epidemics, has been carried out using this kind of devices. Moreover, microorganisms have been used as biological sensing elements in the development of sensitive microbial biosensors for the analysis of different analytes of interest. This review presents the electrochemical application of disposable screen-printed biosensors in the microbiology field.



**Contents**



## 1. Introduction

One of the main objectives for an analytical chemist is the development of methods which allow the rapid, selective and sensitive determination of the target analyte. In this way, sensors and particularly biosensors, which involve the use of biological substances, can be considered as very useful tools for various sample analysis.

A biosensor is an analytical device which uses biologically sensitive material to detect biological or chemical species directly without the need for complex sample processing. An essential part of a biosensor is the transducer, which function is to transform the rate of the biochemical reaction that takes place during biological recognition into a measurable response.

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. Electroanalytical techniques provide important advantages such as low detection limit, relative simplicity, low cost of equipment, automatic on-line and portable options. These advantages have been increased with the advent of the screen-printing technology.

Screen-printed electrodes (SPEs) avoid some of the common problems of classical solid electrodes, such as memory effects and tedious cleaning processes. Moreover, the extensive range of forms

* Corresponding author.
*E-mail address:* jarcos@ubu.es (M.J. Arcos-Martínez).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.08.033

of modification of SPEs opens a great field of applications for these electrodes. These modifications include for example the immobilization of a biological component for the development of selective and sensitive biosensors.

Screen-printed disposable biosensors have been developed for the analysis of microorganisms such as *Escherichia coli* (*E. coli*), *Salmonella*, etc. In the same way, the incorporation of microorganisms in the SPE can be used for the analysis of different substances of analytical interest.

This review paper is, therefore, focused on (i) the description of the different applications of electrochemical biosensors based on SPEs for the analysis of microorganisms; (ii) the use of microorganisms for the development of disposable electrochemical biosensors for the analysis of different substances.

## 2. Screen-printed biosensors

Screen-printed technology consists of layer-by-layer depositions of ink upon a solid substrate, through the use of a screen or mesh, defining the geometry of the sensor [1]. This technology has advantages of design flexibility, process automation, good reproducibility, a wide choice of materials [2]. The construction of SPEs for the development of disposable sensors has been well-documented [1,2]. It includes a series of basic stages, namely selection of the screen, selection and preparation of the inks, selection of the substrate and the printing, drying and curing stages. In this way, several SPEs configurations based on different materials can be built. Compared to other technologies that are available for manufacturing electrodes, such as thin-film, thick-film electrodes are relatively inexpensive, simple to fabricate and are congruent for mass production [3].

In addition to these very attractive advantages, the technology enables biomolecules to be immobilized onto the electrode surface, in order to fabricate selective and disposable biosensors. The sensing element is the analyte-specific part of biosensors. Enzymes, microorganisms, antibodies, nucleic acids and receptors have been commonly employed in the construction of screen-printed biosensors. They can be immobilized onto the surface of the working electrode, which can be of different nature such as carbon or gold, through adsorption, entrapment, microencapsulation, cross-linking or covalent attachment [3,4].

Procedures based on SPEs biosensors are shown as practical devices for the rapid, easy-to-use and low cost determination of many substances of analytical interest, among them microorganisms.

## 3. Screen-printed biosensors for the determination of microorganisms

One of the main challenges in food industry, environmental monitoring and clinical diagnosis is the development of fast, reliable, sensitive and accurate methods for the detection of pathogen microorganisms. The early detection of low microorganism amount plays an important role in the prevention of human health problems and animals and plants epidemics [5–7]. Traditional microorganism detection methods (culture tests, biochemical tests, microscopy, flow cytometry), are time-consuming, inconvenient and require several handling steps [7]. Electrochemical biosensors present a practical solution to these problems.

Disposable electrochemical biosensors reported in the literature for detecting microorganisms are mainly based on monitoring the interaction between microorganisms and biological recognition

**Table 1**
Summary of some biosensors used for the analysis of microorganisms.

| Biological element | Enzyme-label | Immobilization procedure | Analyte | Electrochemical technique | Analyzed sample | LOD | Reference |
|---|---|---|---|---|---|---|---|
| IgG/anti-IgG | ALP | Adsorption | *H. pylori* | Square wave voltammetry | Serum | 0.5 U $mL^{-1}$ | [9] |
| IgG/anti-IgG | ALP | Adsorption | *V. cholerae* | Amperometry | | $10^5$ cells $mL^{-1}$ | [11] |
| IgG/anti-IgG | ALP | Adsorption | *L. monocytogenes* | Amperometry | Milk | $9 \times 10^2$ cells $mL^{-1}$ | [13] |
| IgG/anti-IgG | ALP | Streptavidin-biotin reaction | *S. pneumoniae* | Cyclic voltammetry | Urine | | [14] |
| IgG/anti-IgG | HRP | Adsorption/covalent | *S. typhimurium* | Amperometry | Meat | $5 \times 10^3$ cells $mL^{-1}$ | [16] |
| IgG/anti-IgG | ALP | Adsorption | *S. typhimurium* | Amperometry | Serum | | [17] |
| IgG/anti-IgG | HRP | Adsorption | *E. coli* | Amperometry | Milk | $5 \times 10^3$ cells $mL^{-1}$ | [18] |
| IgG/anti-IgG | HRP | Covalent | *S. aureus* | Amperometry | Milk | $3.7 \times 10^2$ cells $mL^{-1}$ | [19] |
| IgG/anti-IgG | HRP | Entrapment | *V. parahaemolyticus* | Cyclic voltammetry | | $7.4 \times 10^4$ cells $mL^{-1}$ | [20] |
| IgG/anti-IgG | | Covalent/thiolated antibody immobilization | *E. coli* | Electrochemical impedance spectroscopy | River and tap water | 5–1 $\times 10^8$ cells $mL^{-1}$ | [6] |
| IgG/anti-IgG | | Peptide nanotubes | *E. coli* | Cyclic voltammetry | | | [23] |
| IgG/anti-IgG | | Electropolymerization | *L. monocytogenes* | Electrochemical impedance spectroscopy | | | [25] |
| IgG/anti-IgG | | Cross-linking | *S. pyogenes/S. aureus* | Amperometry | Serum | $5 \times 10^{-9}$ mg $mL^{-1}$ of bacterial antigens | [27] |
| DNA | | Electrochemical deposition | *E. coli* | Chronopotentiometry | Environmental water | 50 ng $mL^{-1}$ of the DNA target | [29] |
| DNA | | Adsorption | *E. coli* | DPV | | | [30] |
| DNA | | Electrochemical deposition | *Cryptosporidium* | Chronopotentiometry | Drinking and river water | ng $mL^{-1}$ levels of the DNA target | [31] |
| DNA | | Magnetic beads | *E. coli* | Amperometry | | 5.7 fmol | [32] |
| DNA | HRP | SAMs/avidin-biotin reaction | *E. coli* | Amperometry | | 0.002 ng $\mu L^{-1}$ | [33] |
| DNA | ALP | SAMs | *S. enterica/L. monocytogenes* | DPV | | | [34] |
| DNA | ALP | Covalent/adsorption | *Salmonella* | DPV | | 0.3 nM | [35] |
| DNA | ALP | Avidin-biotin reaction | *S. pneumonae* | Cyclic voltammetry | | 0.49 fmol | [36] |
| DNA | | Avidin-biotin reaction | *S. pneumonae* | Amperometry | | 24.5 fmol | [37] |
| DNA | HRP | Avidin-biotin reaction | *V. cholerae* | Amperometry | | 10 CFU $mL^{-1}$ | [40] |
| Lectin–bacteria complexes | | Biotynilation | *E. coli* | Electrochemical impedance spectroscopy | | $5 \times 10^3$ CFU $mL^{-1}$ | [7] |

elements such as antibodies, nucleic acids and enzymes (Table 1). Several works, which show the analysis of different microorganisms using lectin-based SPEs and bacteriophage-modified SPEs, are also described in the bibliography.

### 3.1. Immunosensors

When antibodies or antibody fragments are used as molecular recognition element for specific analytes (antigens) to form a stable complex, the device is called immunosensor. Electrochemical immunosensors combine the specificity inherent to antigen–antibody interactions with the high sensitivity of electrochemical transduction. For this reason, these devices have been an attractive subject for clinical diagnosis, environmental monitoring and food analysis.

Most of the literature detailing electrochemical immunosensors is based on the immobilization of antibodies onto an electrode surface, which react with free antigens in competition with labeled antigens. Anti-IgG–enzyme conjugate (commonly named secondary antibody) is the most frequently species used as labeled antigens [8]. The antibody–antigen interaction is then followed by the enzyme activity measurement involving the addition of a specific enzyme substrate.

Recently labelless immunosensors, in which the immune interaction between antibody and antigen is directly monitored, have been also reported. These biosensors exhibit some important advantages in terms of speed and simplicity of operation [6].

Here, we present several examples of the use of both types of immunosensors for the analysis of different microorganisms using SPEs.

### 3.2. Enzyme-labeled immunosensors

Enzyme-labeled immunosensors combine the specificity of the antigen–antibody reaction with the sensitivity and signal amplification of enzyme-catalyzed reactions. Numerous works describing the use of this type of immunosensors in the analysis of microorganisms can be found in the literature. Alkaline phosphatase (ALP) and horseradish peroxidase (HRP) are frequently used as label in the elaboration of these immunosensors [9,10].

A typical ALP-labeled electrochemical immunosensors has been described by Messina et al. [9] for the determination the Gram-negative pathogen *Helicobacter pylori* (*H. pylori*), which infection can cause peptic ulcer and chronic gastritis. The analysis of this pathogen has been carried out in serum samples using a disposable immunosensor based on the immobilization of *H. pylori* antigens on a screen-printed carbon electrode (SPCE) surface by passive adsorption. Antibodies in the serum sample were then allowed to react immunologically with the antigens. The bound antibodies were quantified by ALP enzyme-labeled second antibodies specific to human IgG. Finally, *p*-aminophenyl phosphate (*p*-APP) was converted to *p*-aminophenol (*p*-AP) by ALP and was quantified using square wave voltammetry.

One of the microorganisms frequently analyzed by ALP enzyme-linked disposable immunosensors has been *Vibrio cholerae* (*V. cholerae*). This bacteria causes cholera, a severe gastrointestinal disease which represents a serious human health problem in many countries [11,12]. The detection of *V. cholerae* (antigen) has been performed by Rao et al. [11] using SPCEs coated with rabbit anti-*V. cholerae* IgG (capturing antibody). The capturing antibody was firstly adsorbed on the SPCE surface followed by blocking with bovine serum albumine (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 1-napthyl phosphate (enzyme substrate) by applying a potential of +400 mV. A similar procedure has been described by Sharma et al. [12] for the analysis of *V. cholerae* in environmental water samples.

There are several works described in the literature that also use ALP enzyme labeled immunosensors for the analysis of other microorganisms. In this way, *Listeria monocytogenes* (*L. monocytogenes*), an important food-borne pathogen with an extremely high mortality, has been analyzed using a disposable immunosensor [13]. The proposed immunosensor design was based on the immobilization of *L. monocytogenes* antibodies on SPCEs. After blocking with BSA, the electrodes were exposed to solutions containing *L. monocytogenes*. Anti-*L. monocytogenes*–ALP were subsequently added. Finally, the amperometric signal was registered at +300 mV following the oxidation of *p*-AP, the dephosphorylation product of the substrate *p*-APP.

ALP enzyme immunosensors have been also used in the determination of *Streptococcus pneumoniae* (*S. pneumoniae*). This bacterium has been consistently shown to be the most common cause of community-acquired pneumonia in humans [14]. Immunosensor devices for the analysis of this bacterium were based on pneumolysin (PLY) determination. PLY, a toxin produced by *S. pneumoniae*, is a an antigenic marker of *S. pneumoniae* found in all the pneumococcal strains. Moreover, PLY encoding gene exhibits very limited sequence variability thus making this molecule perfect immunodiagnostic target. Díaz-González et al. [14] have described an immunosensor for the analysis of this bacterium based on the adsorption of streptavidin on the surface of SPCEs and using BSA for blocking free surface sites of streptavidin modified SPCEs. Then, biotinylated monoclonal mouse antibodies against PLY (anti-Ply-bio) were immobilized through the streptavidin-biotin strong interaction. The next step consisted of a first incubation of the resulted electrode with the sample containing PLY and, on a second incubation with rabbit IgG anti-PLY. Finally, the immunosensor was incubated with goal IgG-ALP. Cyclic voltammetry (CV) was used as the electrochemical technique for recording analytical signal using 3-indoxyl phosphate (3-IP) as substrate. The above described immunosensor was improved by previous modification of the SPCE surface with multiwalled carbon nanotubes which augment the electron transfer rate increasing the sensitivity of the immunosensor [15].

*Salmonella typhimurium* (*S. typhimurium*), one of the most frequent type of *Salmonella*, has been also analyzed using ALP-labeled immunosensors. Salmonella serotypes are among the most common bacteria responsible of salmonellosis, a worldwide reported food-borne disease worldwide [16]. Therefore, the capability to rapidly detect this pathogen is extremely important to preserve public health safety and security. The immunosensor described by Rao et al. [17] for the analysis of *S. typhimurium* in serum samples was based on the immobilization of *S. typhimurium* flagellin antigen on SPCEs. This antigen was prepared using recombinant DNA technology. After blocking with BSA, the electrode was incubated with serum samples from patients. 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.

*S. typhimurium* has been also analyzed by Salam and Tothill [16] using a disposable amperometric immunosensor labeled, in this case, with HRP. The capture antibody (mouse monoclonal antibodies raised against *S. typhimurium*) was placed onto a gold screen-printed electrode (SPAuE) using two different immobilization procedures; passive adsorption and covalent immobilization. Passive adsorption was carried out by covering the electrode

surface with the antibody solution and subsequently incubating under controlled humidity. Covalent immobilization involved the use of carboxymethyldextran and N-hydroxysuccinimide (NHS) for antibody immobilization. Once the antibody was immobilized, *Salmonella* samples were placed on the electrode and incubated. The assay was then completed using rabbit polyclonal antibody-HRP. The detection of the enzyme label was then conducted using an amperometric electrochemical system comprising an electron transfer mediator, 3,3′,5,5′-tetramethylbenzidine dihydrochloride (TMB) with hydrogen peroxide as the enzyme substrate.

Other bacterium analyzed with HRP-labeled amperometric immunosenors has been *E. coli*. *E. coli* is classified as an enterohemorrhagic bacterium with the capability to cause hemorrhagic colitis with symptoms such as bloody diarrhoea, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpurea [18]. This microorganism has been analyzed in samples of milk using a disposable immunosensing strip based on a SPCE. The working electrode surface was modified in a first step with gold nanoparticles (AuNPs) which were immobilized on the electrode surface with glutaraldehyde (GA). The AuNPs–SPCEs were subsequently modified with monoclonal anti-*E. coli* antibodies by incubation during 15 min at 37 °C and coated with ferrocenedicarboxylic acid (FeDC). Then, the electrode was incubated with a small volume of the sample containing *E. coli*. Finally, the resulted modified SPCE was incubated during 15 min at 37 °C with HRP-conjugated polyclonal anti *E. coli* O157:H7 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 [18].

HRP-labeled immunosensors have been also used for the determination of *Staphylococus aureus* (*S. aureus*), one of the major pathogens for humans presented in food samples. Escamilla-Gómez et al. [6,19] have described an immunosensor based on the covalent immobilization of the antibody (RbIgG) on a SPAuE surface using 3,3-dithiodipropionic acid di(N-succinimidyl ester) (DTSP) as cross-linker. Once the antibody was immobilized, the electrode was modified with tetrathiafulvalene (TTF) by means of cross-linking with GA. The TTF–RbIgG–DTSP–SPAuE was first incubated in a solution containing *S. aureus* and then in a RbIgG–HRP solution. The analytical measurements were carried out by amperometry using hydrogen peroxide as substrate. In this case, TTF played the role of the electron transfer mediator.

Zhao et al. [20] have described a method for the analysis of the food-borne pathogen *Vibrio parahaemolyticus* (*V. parahaemolyticus*) based on a HRP-labeled immunosensor. In this case the SPCE was modified with an agarose/nano-Au membrane and the *V. parahaemolyticus* detection was based on the inhibition of the enzymatic activity to the oxidation reaction of thionine by hydrogen peroxide. This inhibition was due to the formation of an immunocomplex on the surface of the immunosensor.

Another microorganism analyzed by HRP-labeled immunosensors has been *Botrytis cinerea* (*B. cinerea*). This plant–pathogenic fungus produces the disease known as grey mould in a wide variety of agriculturally important hosts in many countries. The determination of this species was carried out by Fernández-Balbo et al. [21] using a disposable immunosensor based on the immobilization of *B. cinerea* antigens on SPCEs modified with carbon nanotubes (CNTs). In this case 4-tertbutylcatechol (4-TBC) and hydrogen peroxide were used as mediator and substrate, respectively.

### 3.3. Label-free immunosensors

The labeling process in the construction of electrochemical immunosensor involves tedious procedures, expensive sophisticated experimental techniques and frequently leads to the denaturation of the modified biomolecules. Therefore, there is still a great demand for sensitive and disposable label-free immunosensors [22]. Some labelless immunosensors for the analysis of microorganisms are described below.

*E. coli* has been analyzed in river and tap water using a label-free electrochemical impedance disposable immunosensor [6]. The analysis of this microorganism was carried out by means of SPAuEs using two different immunosensor configurations. In the first one, anti-*E. coli* was covalently immobilized on the SPAuE surface using 3,3′-dithiobis[sulfosuccinimidylpropionate] (DTSSP) as cross-linker. The other configuration tested implied the immobilization of thiolated antibodies. Antibodies were thiolated using sulfosuccinimidyl 6-[3′-(2-pyridyldithio)propionamide] hexanoate (sulfo-LC-SPDP) as thiolation reagent. Finally, the analysis of *E. coli* was performed by electrochemical impedance spectroscopy in the presence of $[Fe(CN)_6^{3-}]/[Fe(CN)_6^{4-}]$ as redox probe. This last configuration presented a higher sensitivity than other impedimetric biosensors reported in the literature.

$[Fe(CN)_6^{3-}]/[Fe(CN)_6^{4-}]$ have also been used as redox probe by Cho et al. [23] for the determination of *E. coli* bacteria using a disposable immunosensor. In this case, SPCEs were modified with self-assembled peptide nanotubes (PNTs). PNTs have functional groups on their surface that allow an easy immobilization of antibodies. Finally, the antigen–antibody interaction was analyzed by CV. Another label-free biosensor for the determination of *E. coli* implied the use of antibody-coated SPCEs that specifically binds the target bacteria. The system did not require the use of a secondary antibody because detection was based on the measurement of the intrinsic β-D-galactosidase enzyme activity by amperometry using *p*-aminophenyl β-D-galactopyranoside (PAPG) as substrate [24].

An electrochemical labelless disposable immunosensor was also used in the determination of *L. monocytogenes* [25]. In this case, SPCEs modified with polyaniline were used. Water-soluble biotin-sulfoNHS and neutravidin were placed on the polymer-coated working electrode surface. Finally, anti-InlB polyclonal antibodies were immobilized onto the netravidin-modified polymer films. Antibodies were previously biotinylated with biotinamidohexanoicacid-3-sulfo-N-hydroxysuccinimide (BAC-sulfo-NHS) to facilitate the immobilization. Antibody-loaded sensors were then exposed to increasing concentrations of the target antigen. Finally, complex plane impedance analyses were used to relate the changes in sensor output to the antibody–antigen binding events at the electrode surface.

*L. monocytogenes* have been analyzed using a labelless immunosensor based on the immobilization of antibodies on a thiol modified SPAuE surface using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and NHS. In the presence of analyte, a change in the apparent diffusion coefficient of the redox probe ($[Fe(CN)_6^{3-}]/[Fe(CN)_6^{4-}]$) was observed, attributable to impedance of the diffusion of redox electrons to the electrode surface due to the formation of the antibody-bacteria immunocomplex [26].

*Streptococcus pyogenes* (*S. pyogenes*) and *S. aureus* have been analyzed using label-free disposable immunosensors [27]. The sensing part of the immunosensor was prepared by the jointly immobilization of cholinesterase and antibodies in the presence of BSA. Butyrylthiocholine iodide was used as substrate for the amperometric measurement of the antigen–antibody interaction. The presence of the antigen raised the amperometric signal. This increase was due to the formation of an immune complex that facilitates the attraction of additional amounts of the positively charge substrate to the electrode.

### 3.4. DNA biosensors (genosensors)

DNA electrochemical biosensors, coupling the high specificity of DNA hybridization events with the inherent sensitivity, portability and low cost of electrochemical transducers, hold great promise

for the rapid screening of genetic or infectious diseases, as well as for the monitoring of virus and bacterial pathogens in different samples [28,29]. Most of these biosensors are based on the immobilization of a DNA probe on the working electrode surface using different methods (covalent binding, adsorption, etc) followed by a hybridization step with the DNA target. The hybridization event is often detected using a redox indicator or following the enzymatic reaction of an enzyme, previously labeled on the electrode surface. Different microorganisms have been analyzed using disposable genosensors which are below briefly described.

*E. coli* bacterium has been often analyzed using DNA disposable biosensors. One of the first papers describing its analysis [29] involves the immobilization of a 25-mer probe, from the *E. coli lacZ gene*, onto a SPCE. The immobilization was carried out by applying a potential of +1.8 V for 1 min followed by 2 min at +0.5 V in a stirred acetate buffer solution. Then, the probe-coated electrode was immersed into a stirred hybridization solution containing the *E. coli* DNA target for the desired time, while holding the potential at +0.5 V. The response of the developed device was based on the increased peak area of a redox indicator after the duplex formation. In this case, the chronopotentiometric detection of $Co(bpy)_3^{3+}$ was used as indicator for monitoring the hybridization event. For this reason, the electrode was subsequently immersed into a stirred buffer solution containing $Co(bpy)_3^{3+}$ for 2 min while holding the potential at + 0.5 V. Finally, the surface accumulated $Co(bpy)_3^{3+}$ was measured, after transfer to a blank solution, using constant-current chronopotentiometry with an initial potential of +0.5 V and a constant current of −3 μA.

The determination of *E. coli* was carried out by Shiraishi et al. [30] using a biosensor based on the immobilization of the probe DNA on a SPE fabricated by screen-printing a fullerene-impregnated carbon ink onto a poly(methylmethacrylate) substrate. The electrode was activated with air plasma in a preliminary step. In this case, $Co(phen)_3^{3+}$ was used as redox indicator and its accumulation in the duplex was measured by differential pulse voltammetry (DPV). The results pointed out a high improvement in the surface coverage of the immobilized DNA probe, and of the reduction peak of the redox indicator due to the incorporation of fullerene.

$Co(phen)_3^{3+}$ has been also chosen as redox indicator by Wang et al. [31] in the development of a disposable genosensor for determination of DNA fragments specific to the deadly waterborne pathogen *Cryptosporidium*. The genosensor design was based on the immobilization of 38-mer oligonucleotide unique to the *Cryptosporidium* DNA onto the SPCE following of a hybridization reaction using a similar procedure described as in [29].

*E. coli* bacteria are often analyzed using enzyme-labeled genosensors as well. In this way, recent DNA disposable biosensors for analysis of *E. coli* has been developed by Loaiza et al. [32,33] using SPAuEs. One of the genosensors developed by these authors was based on the immobilization of biotinylated 25-mer capture probe on a tetrathiafulvalene (TTF) modified SPAuE [32]. The immobilization was carried out using magnetic beads (MBs) captured by a magnetic field on the working electrode surface. MBs were first modified by streptavidin and then biotinylated oligonucleotide probes were captured onto the modified beads. Subsequently, the hybridization process took place using a solution with the biotinylated target. The hybrid-attached beads were labeled with the streptavidin–HRP polymer enzyme. Finally, amperometric measurements were obtained at −0.15 V using hydrogen peroxide as enzyme substrate. The same authors have performed the analysis of *E. coli* using similar genosensors based on HRP-labeling [33]. In this case, two different immobilization procedures of the DNA probe were tested. In a first approach, direct coupling of thiolated probes to bare SPAuEs was investigated; the efficiency of the hybridization process was enhanced by employing 6-mercapto-1-hexanol (MCH) for the displacement of nonspecifically adsorbed oligonucleotide molecules, while methylene blue (MB) was used as the electrochemical indicator to monitor DNA hybridization process. The second immobilization procedure tested consisted of the coupling of DNA biotinylated probes to avidin-coated DTSP-modified SPAuEs. An enzyme amplification detection scheme was employed, based on the coupling of streptavidin–HRP to the biotinylated complementary target, after the hybridization process, and immobilization of TTF as redox mediator atop the modified electrode. Finally, the electrode was exposed to a hydrogen peroxide solution in order to obtain the analytical signal by amperometry.

SPAuEs have been used in the construction of DNA biosensors for the analysis of *Salmonella enterica* (*S. enterica*) and *L. monocytogenes* [34]. For the development of these biosensors, the gold electrode surface was first modified with thiol-tethered oligonucleotide probes. This immobilization step was then followed by treatment with a spacer thiol. The modified working electrode was then immersed in the hybridization solution containing surface-tethered probes and biotinylated signaling probes. The resulting biotinylated hybrids were coupled with a streptavidin–ALP conjugate and then exposed to α-naphthyl phosphate and the oxidation signal of the enzymatic product (α-naphthol) was measured by DPV taking its peak height as the analytical signal.

α-Naphtyl phosphate has been used as substrate in the development of disposable ALP-labeled genosensors for *Salmonella* bacteria determination [35]. Two immobilization procedures of the DNA probe on the SPCE surface were investigated; covalent and adsorption immobilization. In the first case, the immobilization was carried out by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and NHS. After immobilization, the hybridization process with the biotinylated signaling probe was carried out. Finally, the biotinylated hybrid obtained on the electrode surface was reacted with a solution containing a streptavidin-ALP conjugate. The enzyme-modified surfaces were then incubated with α-naphthyl phosphate and DPV was used to detect the α-naphthol signal.

Moreover, *S. pneumoniae* bacteria have been analyzed using ALP-labeled genosensors [36–38]. Hernández-Santos et al. [36] have described the development of an enzymatic genosensor on SPCEs for the identification of nucleic acid determinants exclusively present on the genome of the pathogen *S. pneumoniae*. The SPCE surface was first modified with streptavidin by physical adsorption. Free surface sites were blocked using a BSA solution. The formation of the sensing phase was performed by the immobilization of 3′-biotinylated oligonucleotide probes on the streptavidin-coated electrodes. Then hybridization was performed by immersing the electrode in a solution containing oligo nucleic target probes. These targets were randomly labeled with the universal linkage system (ULS) [39]. The labeling system consisted to use a platinum (II) complex acting as a coupling agent between DNA strands and a label molecule, frequently fluorescein (FITC) [37]. Following the hybridization process, the genosensor device was immersed in a solution containing the anti-FITC ALP conjugate. Then, the enzymatic reaction was carried out dropping a 3-IP solution on the electrode surface. The analytical signal was finally recorded using CV. A similar work using the same genosensor development for the analysis of *S. pneumoniae* has been performed by Fanjul-Bolado et al. [38]. In this case a new substrate solution that combined an indoxyl compound (3-IP), and silver ions was used, resulting in a high sensitivity improvement. A parallel procedure has been described by Hernández-Santos et al. [37] for the analysis of virulence nucleic acid determinants of pneumolysin and autolysin genes exclusively present on the genome of the human pathogen *S. pneumoniae*. In this case, the analytical signal was achieved by measuring chronoamperometrically the current generated by the hydrogen evolution catalyzed by the platinum (II) complex. Thus, no enzyme-labeled was necessary.

The ULS linkage system via FITC has been employed in the development of a disposable genosensor for the detection of *V. cholerae* using intermittent pulse amperometry on a streptavidin-modified SPCE [40]. The analytical signal was achieved though the incorporation of anti-FITC-HRP using hydrogen peroxide and 3,3′5,5′-tetramethylbenzidine as substrate and mediator, respectively.

### 3.5. Other biosensors

It is possible to find in the literature disposable biosensors of different designs from the one described above, suitable for the analysis of several microorganisms. Thus, biosensors based on the selective interaction of lectins with carbohydrate components from microorganisms surface membranes have been development for the determination of *E. coli* by electrochemical impedance spectroscopy [7]. Lectin–bacteria complexes were formed in solution and biotinylated using concavalin A (Con A). Then, these complexes were immobilized onto a SPAuE surface. The electron transfer resistance varied linearly with the logarithmic value of *E. coli* concentration over four orders of magnitude. In order to further increase the selectivity of the development biosensor, the electrochemical monitoring of β-galactosidase activity of the bacteria attached to the surface, via biotinylated-Con A, was carried out. The measurement of the enzyme activity allowed distinguishing between *E. coli* and *S. aureus*, which exhibited a similar activity towards biotinylated-Con A.

Immobilization of living cells on SPCEs surface can be used for the development of useful biosensors in the determination of human pathogens. By using living cells as sensing component, biosensors are able to respond to many biological active agents [41]. Moreover, such cells are highly sensitive to environmental conditions and this oscillation can elicit cellular responses contributing to the electronic signals in biosensor assay [42]. This kind of sensors can then be used in the analysis of pathogens or pathogen-derived molecules (elicitors) which can cause changes in cellular properties. A well-characterized elicitor is bacterial flagellin, which induces changes in cellular concentration of $H^+$ and the accompanying plasma membrane depolarization [43]. In the work described by Oczkowski et al. [5], a biosensor based on the immobilization of tobacco plant cells on SPCEs for the detection of flagellin-containing bacteria has been developed. The electrochemical detection of proton gradient across the plant membrane was less time consuming and more sensitive than the methods usually employed in suspension culture cells.

Bacteriophages are small viruses that have recently been postulated as promising recognition components for use in bacterial biosensors [44] due to their high specificity towards bacteria. In addition, they are harmless to humans and less expensive to produce than antibodies. A novel method has been described by Shabani et al. [45] for the specific and direct detection of bacteria using bacteriophages as recognition receptors immobilized covalently onto functionalized SPCEs. The SPCEs were first functionalized via electrochemical oxidation in acid media of EDC by applying a potential of +2.2 V. Then the immobilization of T4 bacteriophage was performed through amide bonds formation between the protein coated of the phage and the electrochemically generated carboxylic groups at the carbon surface. Subsequently, the modified electrodes were immersed in a solution containing the target bacterium. Finally, impedance measurements were carried out which provided a rapid and low-cost method for the determination of *E. coli* using a simple phage-based approach.

## 4. Microbial screen-printed biosensors for analytical applications

Microorganisms, as biological sensing elements, either intimately connected to or integrated within a suitable transducing system, can specifically recognize species of interest [3,46–48].

Over 90% of the enzymes known to date are intracellular. Therefore, the utilization of whole cells as a source of intracellular enzymes provides a multipurpose catalyst, especially when the process requires the participation of a number of enzymes in sequence [46]. In this way, all substances that influence the turnover of one of the enzymes could influence the measured cellular metabolic activity [49,50].

Thus, microbial sensors, which are inexpensive and easily produced, posses the sensitivity and stability comparable with those of enzyme sensors and no additional efforts are needed for purification of enzyme, but usually they lack of selectivity [51]. Obviously, the characteristic substrate spectra of the specific microbial species used in biosensors must correspond to the spectrum of compounds present in the sample. This fact highlights the importance of the appropriate culture selection [46,51,52].

The use of SPEs for the fabrication of microbial biosensors is quite attractive, bearing in mind the above-mentioned characteristics of this kind of electrodes. However, the combination of bacteria with SPEs has not been reported widely in the literature, perhaps due to difficulties in observing efficient electrochemical exchanges between bacteria and the electrode surface [50].

Different procedures reported for the biological material culturing and immobilization conditions onto SPEs, which can be regarded as the key step in the fabrication of such devices [1], for analytical applications are detailed below (Table 2).

### 4.1. Adsorption

Adsorption is probably the preferred method of immobilization of biomolecules, due to its simplicity. In the case of SPEs, the direct absorption of whole cells onto the working electrode is not viable [49]. Therefore, the SPEs are usually modified prior to the cells immobilization, as it can be seen with the following microbial biosensors.

**Table 2**
Summary of some microbial biosensors used for the analysis of different compounds.

| Biological element | Immobilization procedure | Analyte | Operating potential | Analyzed sample | Reference |
|---|---|---|---|---|---|
| *A. nicotianae* | Adsorption | Free fatty acids | −600 mV | Milk | [53] |
| *P. putida* | Adsorption | Phenol, benzoic acid and their monochlorinated derivatives | −600 mV | | [54] |
| *P. putida* | Entrapment | Several toxic chemicals to activated sludge | | Wastewater | [57] |
| *P. putida* | Microencapsulation | Benzene | −700 mV | | [50] |
| *P. putida* | Cross-linking | 2,4-dichloro phenoxy acetic acid | −700 mV | Herbicide | [46] |
| *P. putida* | Cross-linking | Phenol | −700 mV | Wastewater | [3,52] |
| *Pseudomonas* | Adsorption | Phenol | 50 mV | | [51] |
| Activated sludge | Entrapment | Toxic chemicals to activated sludge (3,5-Dichlorophenol) | 550 mV | Wastewater | [55] |
| *E. coli* | Entrapment | Phenols | 550 mV | Wastewater | [56] |
| *E. coli* | Entrapment | Mono- and disaccharides | −600 mV | | [48] |

A microbial sensor based on the microorganism *Arthrobacter nicotianae* (*A. nicotianae*) has been applied for the determination of free fatty acids in milk, using silanized SPEs [53]. Silanization prevents any leakage of oxygen and increases the adhesion of the microbial layer [53,54]. *A. nicotianae* exhibited enzymes of the β-oxidation pathway, such as acyl coenzyme A (CoA), with a high specificity for short-chain fatty acids. This microorganism was cultivated under aerobic conditions at 30 °C for 16 h in a medium consisting of yeast extract, peptone, $K_2HPO_4$, $KH_2PO_4$, butyric acid and KOH to adjust the pH to 7.0. After cultivation, the microbial cells were immobilized in calcium alginate over screen-printed Pt electrodes. The respiratory activity of the microorganisms was detected by measuring the oxygen concentration at −600 mV vs. Ag/AgCl reference electrode [53]. Thus, a steady baseline was recorded at first which represents the endogenous respiration rate of the immobilized microorganism. An increase of the respiratory activity and oxygen consumption was observed after addition of the substrate, resulting in a decrease of oxygen concentration near the electrode surface. This was accompanied by a corresponding decrease in the output sensor signal until a secondary baseline was reached, representing the maximum respiratory activity for the concentration of substrate injected.

Another microbial sensor based on the direct immobilization of *Pseudomonas putida* (*P. putida*) DSM 548 onto silanized carbon electrode surfaces has been reported for phenol, benzoic acid and their monochlorinated derivatives [54]. The microorganism was grown at 30 °C in a mineral medium containing phenol or benzoic acid as sole carbon source. The cells were further cultivated for 12 h with the desired monochlorophenol as carbon source, in order to induce them. Then, cells were harvested and centrifuged. The pellet was resuspended in NaCl and mixed with aqueous sodium alginate solution for immobilization. The sensor response was finally monitored at −600 mV. The combination of suitably engineered microbial strains with overlapping specificities and disposable SPCEs might eventually result in multi-analyte microbial sensors which allow for the onsite analysis of a wide range of xenobiotics in the environment using pattern recognition techniques.

Different *Pseudomonas* strains have been studied for development of biosensors for amperometric detection of phenols. In order to reduce the possibility of interfering reactions and thus enhancing the selectivity, mediators have been applied. Electron mediators perform a special function in biosensors; their role is to replace the electron transfer performed by oxygen, in consequence, preventing the process from the problem of having a low oxygen concentration [51]. Analogously to the *P. putida* DSM 548 cultivation [54], eight different *Pseudomonas* strains were cultivated in solid agarized medium M9 at 30 °C for 48 h, using vapors of phenol as source of carbon and energy. The sediment obtained after cultivation, washing and centrifuging, was re-suspended in 50 mM phosphate buffer pH 7 to obtain the bacterial suspensions [51]. Then, 24 types of modified graphite composite electrodes based on them and three types of mediators, namely ferrocene, dimethylferrocene and duroquinone, in all combinations have been tested. The sensors were based on a SPE system, in which the original platinum-based measuring electrode was covered with a graphite/acetyl cellulose layer containing the mediator and, subsequently, with a mixture of the cells suspension and graphite powder. In this way, the operating potential was reduced to 50 mV. The performance of the sensor was reported to be 10 consecutive measurements without any decrease in signal, for all types of sensor.

The construction of biosensors based on adsorption of bacterial cells has been shown as a very simple procedure with excellent performances. However, the procedure of immobilization has been further addressed to increase the stability of the biosensors.

### 4.2. Entrapment

Entrapment, which consists of the physical retention of the enzyme in the inner cavities of a porous matrix deposited onto the electrode surface, has been also used as immobilization technique in microbial biosensors. In this way, the analysis of toxicity of wastewaters to activated sludge has been carried out.

Activated sludge comprises a mix of population of microorganisms, largely bacteria and protozoa, that is capable of degrading the major organic constituents. The presence of chemicals which are toxic to the microorganisms of the activated sludge may impair the extent and rate of degradation [55–57]. Rapid and easy-to-use methods for the assessment of toxicity to activated sludge are important for the protection of the treatment process. Biosensors incorporating a cellular biocatalyst intimately linked to SPEs offer an alternative approach to other cell-based bioassays.

With this aim, amperometric biosensors based on the immobilization of activated sludge [55], *E. coli* [56] and *P. putida* [57] have been reported. The biocatalyst-loaded SPEs were prepared by immobilizing the corresponding bioactive component in an Anopore® membrane held to the carbon surface using a micropore tape. These biosensors used ferricyanide to divert electrons from the respiratory system of the immobilized living bacterial cells to the electrode. The resulting current at 550 mV was, thus, a measure of bacterial respiratory activity and the perturbation by pollutants can be detected as a change in the magnitude of the current.

Others *E. coli* K12 based biosensor have been reported for the simultaneous determination of mono- and disaccharides in various media [48]. As usual, the bacterial cells were grown in a specific medium containing the mono- or disaccharide for which the function of the carbohydrate transport system should have been induced. After 20 h at 29 °C the cells were harvested by centrifugation and washed in saline. The obtained cell pellet was re-suspended in saline and mixed with κ-Carrageenan, which was used as immobilization matrix for entrapment of the bacterial cells onto SPAuEs previously platinised. The decrease of the diffusion-limited oxygen reduction current recorded at −600 mV vs. Ag/AgCl reference electrode was related to the presence of the nutrient.

### 4.3. Microencapsulation

An alternative technique has been reported for the immobilization of *P. putida* ML2 onto the surface of SPAuEs for the analysis of benzene [50]. *P. putida* ML2 were grown in a minimal liquid medium, containing $K_2HPO_4$, $KH_2PO_4$, tryptone, $MgSO_4{\cdot}7H_2O$, $FeSO_4{\cdot}7H_2O$ and benzene in deionized water, adjusted to pH 7.2, for 18 h at 30 °C.

For immobilization, 1 mL of bacterial culture was centrifuged, and the pellet re-suspended in 30 μL of potassium phosphate buffer (50 mM, pH 7). The cell paste was then entrapped between the electrode surface and a cellulose acetate (CA) membrane attached onto an adhesive ring around the working electrode.

The measurement scheme, as it has been mentioned above, was based on the respiratory activity of the bacterial cells, which was recorded by chronoamperometry at a fixed potential of −0.7 V.

### 4.4. Cross-linking

*P. putida* cells have been furthermore used as bioactive material of different biosensors [3,46,52]. Substrate specificity was yielded by bacterial cell adaptation, which in some cases may eliminate the necessity of using genetically manipulating microorganisms [52].

Control cells of *P. putida* DMS 50026 were sub-cultured on nutrient agar, inoculated into mineral salts medium containing glucose as carbon source and incubated at 28 °C. After 20–24 h the biomass was harvested by centrifugation. The supernatant was removed and

the cellular paste was used for making biosensors [3,46,52]. Induction of the cells to 2,4-dichloro phenoxy acetic acid (2,4-D) or to phenol was performed with gradually increasing 2,4-D [46] or phenol [3,52], respectively, and decreasing glucose concentrations by daily inoculations.

Then, *P. putida* was mixed with gelatin in phosphate buffer (pH 7.5, 50 mM) or in mineral salts medium at 38 °C in the step of preparation of the bioactive layer material for the analyzed of 2,4-D or phenol, correspondingly. The mixture was placed on SPCEs [3,46] or on the gold working electrode of ceramic thick films [52]. After drying, the sensors were immersed into GA solution for the bioactive layer cross-linking.

It has been reported that the immobilisation on the working electrode with a CA membrane, prior the bioactive layer, allows to obtain stable signals without drift and to increase the signal to noise ratio. The controlled diffusion of oxygen by the CA membrane seemed to be the main reason that enabled better results to be obtained [3].

Finally, the concentration of the herbicide 2,4-D and phenolic compounds were determined by chronoamperometric measurements at −0.7 V in phosphate buffer pH 7, following the oxygen consumption due to the metabolic activity of the bacteria.

## 5. Conclusion

The great growth in the use of SPEs observed in recent years, involves the development of disposable biosensors for the analysis of different microorganisms. With this aim, enzymes, nucleic acids and antibodies have been immobilized using different techniques onto SPEs, especially for the detection of human pathogens. *E. coli*, *Sallmonella*, *S. Pneumonia*, *L. monocytogenes* have been detected in different matrices using screen-printed biosensors. These electrochemical biosensors have been shown as an efficient alternative to the traditional methods of detection of microorganisms.

Moreover, microorganisms have been successfully used in the fabrication of screen-printed biosensors for the determination of different analytes of interest, such as phenols and fatty acids. The advantage of these biological sensing materials lies in the imported source of intracellular enzymes they possess. Microbial biosensors can be employed for the determination of any substance that influences the metabolic activity of one of the enzymes in the whole cell.

## Acknowledgements

Authors would like to acknowledge funding via Junta de Castilla y León (GR177) and Spanish Ministry of Science and Innovation (TEC-2009/12029). M.A. Alonso-Lomillo is funded by a Ramón y Cajal fellowship from the Spanish Ministry of Science and Innovation.

## References

[1] M.A. Alonso-Lomillo, O. Domínguez Renedo, M.J. Arcos-Martínez, in: R.C.A.P. Novotny (Ed.), Biosensors: Properties, Materials and Applications, Nova Publishers, Hauppauge, NY, 2009, pp. 1–52.
[2] J.M. Cooper, A.E.G. Cass, Biosensors: A Practical Approach, Oxford University Press, Oxford, 2004.
[3] S. Timur, L. Della Seta, N. Pazarlioglu, R. Pilloton, A. Telefoncu, Process Biochem. 39 (2004) 1325–1329.
[4] O. Domínguez-Renedo, M.A. Alonso-Lomillo, M.J. Arcos-Martínez, Talanta 73 (2007) 202–219.
[5] T. Oczkowski, E. Zwierkowska, S. Bartkowiak, Bioelectrochem 70 (2007) 192–197.
[6] V. Escamilla-Gómez, S. Campuzano, M. Pedrero, J.M. Pingarron, Biosens. Bioelectron. 24 (2009) 3365–3371.
[7] M. Gamella, S. Campuzano, C. Parrado, A.J. Reviejo, J.M. Pingarron, Talanta 78 (2009) 1303–1309.
[8] F. Ricci, G. Volpe, L. Micheli, G. Palleschi, Anal. Chim. Acta 605 (2007) 111–129.
[9] G.A. Messina, I.E. De Vito, J. Raba, Sens. Actuators B: Chem. 128 (2007) 23–30.
[10] C.M. Ruan, Y.B. Li, Talanta 54 (2001) 1095–1103.
[11] V.K. Rao, M.K. Sharma, A.K. Goel, L. Singh, K. Sekhar, Anal. Sci 22 (2006) 1207–1211.
[12] M.K. Sharma, A.K. Goel, L. Singh, V.K. Rao, World J. Microbiol. Biotechnol. 22 (2006) 1155–1159.
[13] E.L. Crowley, C.K. O'Sullivan, G.G. Guilbault, Analyst 124 (1999) 295–299.
[14] M. Díaz-González, M.B. Gonzalez-Garcia, A. Costa-Garcia, Sens. Actuators B: Chem. 113 (2006) 1005–1011.
[15] P.J. Lamas-Ardisana, P. Queipo, P. Fanjul-Bolado, A. Costa-Garcia, Anal. Chim. Acta 615 (2008) 30–38.
[16] F. Salam, I.E. Tothill, Biosens. Bioelectron. 24 (2009) 2630–2636.
[17] V.K. Rao, G.P. Rai, G.S. Agarwal, S. Suresh, Anal. Chim. Acta 531 (2005) 173–177.
[18] Y.H. Lin, S.H. Chen, Y.C. Chuang, Y.C. Lu, T.Y. Shen, C.A. Chang, C.S. Lin, Biosens. Bioelectron. 23 (2008) 1832–1837.
[19] V. Escamilla-Gómez, S. Campuzano, M. Pedrero, J.M. Pingarron, Talanta 77 (2008) 876–881.
[20] G.Y. Zhao, F.F. Xing, S.P. Deng, Electrochem. Commun. 9 (2007) 1263–1268.
[21] M.A. Fernández-Balbo, G.A. Messina, M.I. Sanz, J. Raba, Talanta 79 (2009) 681–686.
[22] J. Okuno, K. Maehashi, K. Kerman, Y. Takamura, K. Matsumoto, E. Tamiya, Biosens. Bioelectron. 22 (2007) 2377–2381.
[23] E.C. Cho, J.W. Choi, M.Y. Lee, K.K. Koo, Colloids Surf.: Physicochem. Eng. Aspects 313 (2008) 95–99.
[24] A.S. Mittelmann, E.Z. Ron, J. Rishpon, Anal. Chem. 74 (2002) 903–907.
[25] E. Tully, S.P. Higson, R.O. Kennedy, Biosens. Bioelectron. 23 (2008) 906–912.
[26] S. Susmel, G.G. Guilbault, C.K. O'Sullivan, Biosens. Bioelectron. 18 (2003) 881–889.
[27] G.R. Safina, E.P. Medyantseva, O.N. Bazarnova, N.I. Glushko, G.K. Budnikov, J. Anal. Chem. 61 (2006) 912–916.
[28] S.R. Mickelsen, Electroanalysis 8 (1996) 15–19.
[29] J. Wang, G. Rivas, X.H. Cai, Electroanalysis 9 (1997) 395–398.
[30] H. Shiraishi, T. Itoh, H. Hayashi, K. Takagi, M. Sakane, T. Mori, J. Wang, Bioelectrochem 70 (2007) 481–487.
[31] J. Wang, G. Rivas, C. Parrado, X.H. Cai, M.N. Flair, Talanta 44 (1997) 2003–2010.
[32] O.A. Loaiza, S. Campuzano, M. Pedrero, M.I. Pividori, P. Garcia, J.M. Pingarron, Anal. Chem. 80 (2008) 8239–8245.
[33] O.A. Loaiza, S. Campuzano, M. Pedrero, J.M. Pingaron, Electroanalysis 20 (2008) 1397–1405.
[34] F. Farabullini, F. Lucarelli, I. Palchetti, G. Marrazza, M. Mascini, Biosens. Bioelectron. 22 (2007) 1544–1549.
[35] M.L. Del Giallo, D. Ariksoysal, G. Marrazza, M. Mascini, M. Ozsoz, Anal. Lett. 38 (2005) 2509–2523.
[36] D. Hernández-Santos, M. Díaz-González, M.B. Gonzalez-Garcia, A. Costa-Garcia, Anal. Chem. 76 (2004) 6887–6893.
[37] D. Hernández-Santos, M.B. Gonzalez-Garcia, A. Costa-Garcia, Anal. Chem. 77 (2005) 2868–2874.
[38] P. Fanjul-Bolado, D. Hernández-Santos, M.B. Gonzalez-Garcia, A. Costa-Garcia, Anal. Chem. 79 (2007) 5272–5277.
[39] F.M. Van der Berg, J. Reedijk, M.J. Bloemink, E.L.M. Lempers, Pt-containing compound, process for its preparation, and application of such compounds, US5580990 (1996).
[40] C.Y. Yean, B. Kamarudin, D.A. Ozkan, L.S. Yin, P. Lalitha, A. Ismail, M. Ozsoz, M. Ravichandran, Anal. Chem. 80 (2008) 2774–2779.
[41] D.A. Stenger, G.W. Gross, E.W. Keefer, K.M. Shaffer, J.D. Andreadis, W. Ma, J.J. Pancrazio, Trends Biotechnol. 19 (2001) 304–309.
[42] H.M. McConnell, J.C. Owicki, J.W. Parce, D.L. Miller, G.T. Baxter, H.G. Wada, S. Pitchford, Science 257 (1992) 1906–1912.
[43] G. Felix, J.D. Duran, S. Volko, T. Boller, Plant J. 18 (1999) 265–276.
[44] S. Balasubramanian, I.B. Sorokulova, V.J. Vodyanoy, A.L. Simonian, Biosens. Bioelectron. 22 (2007) 948–955.
[45] A. Shabani, M. Zourob, B. Allain, C.A. Marquette, M.F. Lawrence, R. Mandeville, Anal. Chem. 80 (2008) 9475–9482.
[46] D. Odaci, M.K. Sezginturk, S. Timur, N. Pazarliolu, R. Pilloton, E. Dinckaya, A. Telefoncu, Prepar. Biochem. Biotechnol. 39 (2009) 11–19.
[47] B.R. Eggins, Biosensors: An Introduction, Wiley Teubner, Chichester, 1996.
[48] M. Held, W. Schuhmann, K. Jahreis, H.L. Schmidt, Biosens. Bioelectron. 17 (2002) 1089–1094.
[49] S. Heim, I. Schnieder, D. Binz, A. Vogel, U. Bilitewski, Biosens. Bioelectron. 14 (1999) 187–193.
[50] Y.H. Lanyon, I.E. Tothill, M. Mascini, Anal. Lett. 39 (2006) 1669–1681.
[51] P. Skladal, N.O. Morozova, A.N. Reshetilov, Biosens. Bioelectron. 17 (2002) 867–873.
[52] S. Timur, N. Pazarlioglu, R. Pilloton, A. Telefoncu, Talanta 61 (2003) 87–93.
[53] A. Schmidt, C. Standfuß-Gabisch, U. Bilitewski, Biosens. Bioelectron. 11 (1996) 1139–1145.
[54] T.T. Bachmann, U. Bilitewski, R.D. Schmid, Anal. Lett. 31 (1998) 2361–2373.
[55] M.R. Evans, G.M. Jordinson, D.M. Rawson, J.G. Rogerson, Pesticide Sci. 54 (1998) 447–452.
[56] M. Farré, O. Pasini, M. Carmen Alonso, M. Castillo, D. Barceló, Anal. Chim. Acta 426 (2001) 155–165.
[57] M. Farre, D. Barcelo, Fresenius J. Anal. Chem. 371 (2001) 467–473.