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REVIEW ARTICLE

Screen-printed electrodes for biosensing: a review (2008–2013)

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Abstract Screen-printing is one of the most promising approaches towards simple, rapid and inexpensive production of biosensors. Disposable biosensors based on screen printed electrodes (SPEs) including microelectrodes and modified electrodes have led to new possibilities in the detection and quantitation of biomolecules, pesticides, antigens, DNA, microorganisms and enzymes. SPE-based sensors are in tune with the growing need for performing rapid and accurate in-situ analyses and for the development of portable devices. This review (with 226 refs.) first gives an introduction into the topic and then is subdivided into sections (a) on DNA sensors (including methods for the detection of hybridization and damage), (b) on aptasensors (for thrombin, OTA, immunoglobulins and cancer biomarkers), (c) on immunosensors (for microorganisms, immunoglobulins, toxins, hormones, lactoferrin and biomarkers), (d) on enzymatic biosensors (for glucose, hydrogen peroxide, various pharmaceuticals, neurotransmitters, amino acids, NADH, enzyme based sensors).

Keywords Screen printed electrodes · DNA sensor · Aptasensor · Immunosensor · Biosensor · Biomarker · Nanomaterials

Introduction

The interest in the fabrication of biosensors with high sensitivity, selectivity and efficiency is rapidly growing. Biosensors have recently found extensive applications in diverse industries. Currently, many analytical instruments used in environmental, food, pharmaceutical or clinical laboratories and also most of the commercial point-of-care devices work using electrochemical sensors, as a whole or a basic part. Glucose biosensors used widely in glucometers are the important and known examples of the electrochemical sensors. In recent years, screen printed electrodes (SPEs), with low cost and ease and speed of mass production using thick film technology have been extensively employed for developing novel (electrochemical) sensing platforms and improving their performances. The main advantage associated with the miniaturization of the electrochemical sensors is the reduction of sample volume required, to as low as a few microliters, which in turn helps in reducing the overall size of the diagnostic system into which the device will be integrated. The surface of SPEs can be easily modified to fit multiple purposes related to different analytes and to achieve a variety of improvements. This versatility, its miniaturized size, and the possibility of connecting it to portable instrumentation make possible highly specific on-site determination of target analytes. In addition, SPEs avoid some of the common problems of classical solid electrodes, such as memory effects and tedious cleaning processes.

The certain kind of limitation appears to be content of organic solvents in the buffer solutions used for the analyte accumulation on the electrode surface using batch voltammetric methods or in the mobile phase used in liquid flow methods. Organic solvents can be responsible for the dissolution of insulate inks and consequently the decrease of limit of detection and sensitivity [1, 2]. Naturally, the composition of the mobile phase must be compatible with the material of the detection cells housing SPEs in liquid flow methods so that their dissolution is prevented. Moreover, they are insensitive to certain important analytes due to the low rate of electron transfer. Thus, electroactive species in the samples can easily interfere with SPCE-based biosensors [3].

Screen-printed technology consists of layer-by-layer depositions of ink upon a solid substrate, through the use of a

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screen or mesh, defining the geometry of the sensor. This technology has advantages of design flexibility, process automation, good reproducibility, a wide choice of materials [4]. SPEs usually include a three electrode configuration (working, counter and reference electrodes) printed on the various types of plastic or ceramic substrates, which is easily modifiable with a great variety of commercial or self-made inks. During the printing process of SPEs, the pastes most commonly used are silver ink and carbon ink. Silver ink is printed as a conductive track, whereas the working electrodes are mostly printed using graphite inks, other materials such as gold, platinum and silver based inks are also used in the preparation of SPEs. The composition of the various inks used for printing on the electrodes determines the selectivity and sensitivity required for each analysis. Some unknown ingredients in these inks may induce unpredictable influences on the detection and analysis. Carbon inks consist of graphite particles, polymeric binder and other additives which are utilized for dispersion, printing and adhesion tasks. Carbon paste is a widespread material because it is relatively inexpensive, easy to modify and chemically inert. The exact ink formulation is regarded by the manufacturer as proprietary information and it has been shown that differences in ink composition e.g. type, size or loading of graphite particles and in the printing and curing conditions can strongly affect the electron transfer reactivity and the overall analytical performance of the resulting carbon sensors [5–7]. Gold paste is also employed in SPEs but less so than carbon because of its higher cost. However, the generation of self assembled monolayers (SAMs) through strong Au–S bonds [8] is creating an increased interest for gold to be employed in SPEs. The screen-printed gold electrodes are applied as electrochemical biosensors, including enzymatic, immune-, or genosensors [9–12].

There are very few works related to the use of unmodified SPEs in the determination of interesting analytes [13]. The extensive range of forms of modification of SPEs opens a great field of applications for these electrodes. The bulk modification of SPEs is very simple compared with ordinary glassy carbon electrodes, which needs several preparation and refreshment steps. Many kinds of modifiers of SPEs exist for easily modified to fit multiple purposes related to different analytes. These modifications give different properties to SPEs making them suitable for diverse applications. The composition of the printing inks may be altered by the addition of very different substances such as metals, metal oxides, enzymes, polymers, electrochemical mediators, complexing agents, etc. On the other hand, the possibility also exists of modifying the manufactured electrodes by means of depositing various substances on the surface of the electrodes such as metal films, polymers, enzymes, etc. [4].

In addition, great advancement has been achieved in the fabrication of SPEs by using nano structures materials. Some of these materials, such as, Au, Ag, Pt, Pd, other metal nanoparticles and carbon nanotubes (CNTs) and graphene-based inks are also used in their construction for the development of biosensors with enhanced the immobilization efficiency of biological molecules and accelerate the electron transfer rate on electrode surface.

The aim of this review is to summarize the applications of SPEs for developing various biosensors from 2008 to 2013. We discuss: (i) DNA and aptasensors constructed based on SPEs. (ii) Electrochemical immunosensors. (iii) Enzyme based biosensors. The authors sincerely apologize to anybody who feels that some key papers have been left out because only a fraction of relevant works could be covered here.

DNA sensors

Nucleic acid detection is of considerable importance to clinical diagnosis, because DNA/RNA tests can reveal genetic disorders or pathogen infection [14]. The development of inexpensive, easy to use, and rapid analytical measurement devices has thus been the focus of intensive research efforts. Advantages of electrochemical biosensing are exemplified in their application for the detection of nucleic acids. The electrochemical DNA-based biosensors are known as experimentally simple devices which require only small amount of DNA. Successful electrochemical DNA biosensors rely on the ability to monitor the binding event due to the flow of electrons, and on the immobilization of the probe onto various electrode surfaces. In recent years, an increasing effort has been expended in developing different biosensors for highly sensitive and selective DNA sensing using SPEs [14–19].

Detection of DNA hybridization

Conventional methods for the analysis of specific gene sequences are based on either direct sequencing or DNA hybridization. Because of its simplicity, most of the traditional techniques in molecular biology are based on hybridization. Most of the studies report using DNA sensors for detecting DNA hybridization [14, 17, 18]. The selective immobilization of oligonucleotides functionalized gold nanoparticles (AuNPs) probes on arrayed gold screen-printed electrodes (AuSPEs) have been described by Moreno et al. [20]. These affinity modules were able to be selectively electrodeposited on specific positions of the arrayed SPEs. The hybridization reaction of complementary oligonucleotides and PCR products was investigated on the screen-printed gold microelectrode surface with amperometric detection using horseradish peroxidase (HRP) as an enzymatic reaction. With this system, non-specific interactions between the transduction layer and the bioreceptor were avoided.

The detection of DNA using metal nanoparticles as labels is an interesting alternative to the standard fluorescence technique. Schüler et al. [21] have described the application of screen printing as a cost-efficient fabrication method for the realization of microstructured chips for the electrical DNA detection based on nanoparticle labeling and subsequent site-specific silver deposition. Their method represents an interesting alternative, especially for systems with sophisticated electrode layouts and structures. The screen printed substrates showed a high stability and the same sensitivity and specificity compared to DNA-chips produced by standard photolithography on silicon. The detection limit (50 pM) of the electrical readout system was an order of magnitude higher than the detection limit (5 pM) of the optical system.

Wang et al. [22] reported the application of disposable AuSPEs modified with the new ternary SAM interfaces to the direct measurement of target DNA in undiluted and untreated human serum and urine samples. The composition of the ternary monolayer, involving hexanedithiol (HDT), a specific thiolated capture probes (SHCP), and 6-mercapto-1 hexanol (MCH) was modified and tailored to the surface of the AuSPE. The resulted monolayer has demonstrated to be extremely useful for enhancing the performance of disposable nucleic acid sensors based on SPEs. 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was used for the electrochemical measurement of the activity of the HRP reporter. The improved capabilities of the modified disposable electrodes allowed direct, sensitive and rapid (30 min) detection of pM target DNA concentrations in undiluted biological fluids. A general schematic representation of the DNA biosensor is presented in Fig. 1.

Qiang et al. [15] successfully investigated DNA hybridization process on the screen-printed electrochemical biosensor. The compound $Co(phen)_3(ClO_4)_3$ was used as electrochemical indicator and the calibration experiment was constructed. It could determine target DNA in the linear range of 6.65×10^{-8}–4.26×10^{-6} M. Another electrochemical DNA biosensor based on SPEs was constructed for detection of the bovine papilloma virus using synthesized oligonucleotides and extracted viral DNA [23]. The probe was immobilized on an AuSPE modified with a polymeric film of poly-L-lysine and chitosan. After hybridization, the decrease in the methylene blue (MB) electrochemical reduction related to the presence of a bovine papilloma virus infection was observed.

Monitoring seawater, particularly recreational water, for indicator bacteria presence is required to protect the public from exposure to fecal pollution and to guarantee the safety of the swimming areas. Using SPCEs and DNA hybridization recognition, two colorimetric and electrochemical genosensors for the detection of *Escherichia coli* DNA in seawater was developed [24]. These assays were based on the double hybridization recognition of a single-strand DNA capture probe immobilized onto the microplate or the SPCE to its complementary ssDNA, which was hybridized with an ssDNA signal probe labeled with HRP enzyme. The proposed sensors allowed for the rapid detection (in 3–5 h) and quantification of the strain in environmental samples without any nucleic acid amplification step. The electrochemical genosensor could detect the target DNA with a detection limit of 10^{-5} mM.

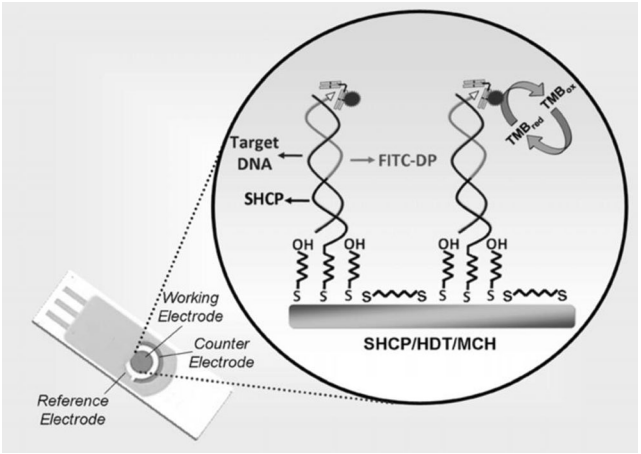

Fig. 1 Schematic representations of the Au/SPE, the SHCP/HDT/MCH interface, and the sandwich detection strategy. Reproduced from [22] with permission from Elsevier

Nanomaterials modified SPEs

Nanomaterials such as nanoparticles and CNTs are suitable candidate for bioconjugation, owing to the fact that they are compatible, and bind readily to a range of biomolecules. Generally, the immobilization of the nanomaterials on the electrode increases the surface area of the electrode and promotes the absorption capability of the electrode.

Bonanni et al. [25] demonstrated an impedimetric genosensor employing SPEs modified with carboxyl functionalized multi-wall carbon nanotubes (MWCNTs). A signal amplification of DNA hybridization detection was performed by the use of CNTs platform and streptavidin modified AuNPs and an improved limit of detection (LOD) was obtained. An ultrasensitive DNA sensor based on metallic Ag deposition on AuNP-modified SPCE has been developed by Gao et al. [26]. For the development of this biosensor, the SPCE surface was first modified by electrodeposition of AuNPs, followed by self-assembly of a hairpin probe DNA, dually labeled with thiol at its 5′ end and biotin at its 3′ end. The ultrasensitive detection of DNA hybridization was achieved by coulometric measurement of the enzymatically deposited Ag. The enhancement in the limit of detection (15 aM) was caused by the exhaustive oxidation of deposited Ag, the AuNPs modified on the SPCE that increased the surface of the electrode, as well as the efficiency of electron transfer.

Compton and co-workers [27] introduced a methodology for indirect electrochemical detection of DNA through its co-adsorption with anthraquinone monosulphonate on both MWCNT-SPEs and an edge-plane pyrolytic graphite electrode. The adsorption of the DNA to the electrode surface is effectively irreversible (over the time-scale of the experiment) and the surface coverage can be indirectly measured through the quantity of co-adsorbed anthraquinone monosulphonate. Through use of this methodology it was possible to obtain a LOD of the DNA solution phase concentration of 8.8 μM (equivalent to 5.9 μg mL^{-1}).

Ionic liquid modified SPEs

Ionic liquids (ILs), organic salts with a sufficiently low melting point, have valuable physical-chemical properties such as negligible vapor pressure, high ionic conductivity, catalytic activity, etc. Nowadays, Ionic liquids are of particular interest for electrochemical sensors due to their excellent conductivity. The ability of ILs combining with carbon materials makes them very attractive for the preparation of various electrodes. In 2010, a DNA sensor was developed based on SPEs doped with ionic liquid [C12mim][PF6] and polyaniline nanotubes to perform a DNA chronocoulometric sensing on SPEs [28]. In order to improve the conductivity of sensor, chitosan and AuNPs were covered on the electrode surface. After hybridizing with the catching probes immobilized on the sensor, the DNA target sequences were detected through chronocoulometric interrogation of $[Ru(NH_3)_6]^{3+}$ ions that bound to the DNA strands via electrostatic interactions. The target DNA quantitatively detected with the detection limit of 8.0×10^{-17} M.

The use of IL modified SPE for direct electrochemistry of DNA pioneered by the Ping group [29], who employed an ionic liquid n-octylpyridinum hexafluorophosphate (OPPF) modified graphite SPE for the electrochemical determination of herring sperm double strand DNA. The presence of ionic OPPF could remarkably accelerate the electron transfer rate and improve the stability of the SPE, which could be ascribed to the high conductivity and natural viscosity of OPPF. Two irreversible oxidation peaks corresponded to the oxidation of guanine and adenine residues were obtained at OPPF-SPE. Compared with the commercially available SPE, the modified electrode showed high electrocatalytic activity for the oxidation of dsDNA (with a detection limit of 5 μg mL^{-1}).

Detection of DNA damage

An interesting application of disposable SPE is the detection and evaluation of damage to DNA by various types of physical and chemical agents from the environment. A simple electrochemical DNA biosensor composed of commercially available SPCE and low molecular weight dsDNA recognition layer has been recently reported and applied to the detection of damage to DNA by UV-C radiation and reactive oxygen species [30]. The biosensor was used with an interface between the SPCE and DNA formed by a composite of carboxylated single-walled CNTs and chitosan to enhance the transducer conductivity. Three independent electrochemical techniques were applied to characterize time changes of dsDNA structure successfully.

Galandova et al. [31] have also shown that DNA sensors can be used to detect DNA damage after incubation with a mutagen, quinazoline. A MWCNT-polyethylenimine (PEI) polymer was deposited on a SPCE and calf thymus dsDNA adsorbed. DNA attached to the sensor facilitated detection of the $Ru(bpy)_3^{2+}$ redox indicator in solution so damage was indicated by a decrease in electrochemical signal. The investigation of the electrochemical response of DNA before and after the interaction with a DNA-targeted drug can provide an evidence for its interaction mechanism. A disposable electrochemical DNA biosensor was developed to detect of strong damage to DNA by the quinazoline derivative as a potential anticancer agent [32]. The quinazoline interaction with DNA was investigated on SPCEs without and with MWCNTs using DNA-bound electrochemical indicators present in the solution phase as well as by electrochemical impedance spectroscopy (EIS). It was found that the quinazoline derivative causes the severe damage to DNA which leads to the loss of DNA from the electrode surface.

Aptasensors

A new class of single-stranded DNA/RNA molecules, aptamers, have recently attracted a considerable attention for applications in medical diagnosis, environmental monitoring [33] and biological analysis, due to their salient advantages, such as improved temperature stability and shelf life, ease in conjugation to various molecules at desired locations without affecting the affinity, adaptability to various targets (including toxic or poorly immunogenic molecules), and inherent selectivity aptamers can rival antibodies for molecular recognition and detection. Many aptamer-based protocols have been proposed for electrochemical monitoring of DNA [34], small molecules [35, 36] and proteins [37, 38]. The key issues with any electrochemical aptasensor include the enhancement of aptamer immobilization amount [39, 40], improvement of the catalytic capabilities and conductivity of the modified electrode. To solve these problems, various types of strategies have been pursued. Modified SPEs are an excellent candidate which has been widely used as the sensoring platform to fabricate new aptasensor in various applications. To enhance the sensitivity of the aptasensors based on SPES, nanomaterials and conducting polymer has also been used [41]. Mayer et al. [42] developed an impedimetric aptasensor

for the label-free detection of lysozyme, an enzyme that hydrolyzes the polysaccharide walls of bacteria. Increased concentrations of lysozyme in urine and serum are associated with leukemia and renal diseases. An amino-modified version of the DNA aptamer-recognizing lysozyme was covalently immobilized on the surface of MWCNT–SPEs, which were employed for measurements and have improved properties compared with bare SPEs. The lysozyme detection limit was calculated as 12.09 μg mL^{-1} (equal to 862 nM).

Kanamycin is an aminoglycoside antibiotic used to treat wide variety of infections by inducing mistranslation and indirectly inhibiting translocation during protein synthesis [43]. The residual amount of kanamycin found in the foodstuff may lead to antibiotic resistance from the pathogenic bacterial strains, which can endanger the consumer. Therefore, it is critical to develop sufficiently sensitive methods to detect kanamycin residue for clinical diagnosis and food safety. Shim et al. [44] report on the highly sensitive label-free detection of kanamycin with an aptamer sensor based on a conducting polymer/gold self-assembled nanocomposite modified SPEs. A nanocomposite consisting of self-assembled 2,5-di-(2-thienyl)-1*H*-pyrrole-1-(*p* benzoic acid) (DPB) on AuNPs was prepared on a disposable SPE through electropolymerization. To fabricate the probe, a DNA aptamer was selected for kanamycin through the in vitro SELEX process and then was covalently immobilized onto the poly-DPB/AuNPs nanocomposite. The detection of kanamycin in PBS and milk sample using the probe was achieved by cyclic voltammetry (CV) and linear sweep voltammetry (LSV). The calibration plot showed a linear range from 0.05 mM to 9.0 mM kanamycin with a detection limit of 9.47±0.4 nM.

Thrombin-aptasensors

To extend the application of aptamer in the detection of thrombin, Deng and co-workers [45] made use of the advantages of aptamers together with quantum dots-coated silica nanospheres (QDs/Si) and AuSPEs to construct a novel thrombin electrochemical aptasensor. AuNPs were electrodeposited on the surface of SPE and then the sandwich format of Aptamer/thrombin/Aptamer–QDs/Si was fixed on the AuNPs-AuSPE to fabricate the aptasensor. They pointed out that the combination of nanoparticles with the SPE is favorable for amplifying electrochemical signals, and useful for large-scale fabrication of the electrochemical aptasensor. On the basis of these, the aptasensor showed a good precision and high sensitivity for thrombin concentrations.

Another thrombin detection system was developed using HRP and amino-labeled aptamer linked to apoferritin via glutaraldehyde at a high HRP/aptamer ratio [46]. Core/shell Fe_3O_4/Au magnetic nanoparticles loading aptamer1 was used as recognition elements, and apoferritin dually labeled with Aptamer2 and HRP was used as a detection probe. Sandwich type complex was formed and anchored on a SPCE. The nanoparticle-based aptasensor yielded a linear current response to thrombin concentrations over a broad range of 0.5–100 pM with a detection limit of 0.07 pM. Recently, Merkoçi and co-workers [47] reported a novel aptamer-based biosensor for diagnostic of thrombin-related diseases in human blood using anodized alumina oxide filter membranes, containing pores of 200 nm in diameter, and screen-printed carbon electrotransducers. The analytical signal, by differential pulse voltammetry (DPV) oxidation of $[Fe(CN)_6]^{4-}$, was based on the blockage in the pores which affected the diffusion of $[Fe(CN)_6]^{4-}$ to the SPCEs. The resulted biosensing system allowed detecting thrombin spiked in whole blood at very low levels (LOD 1.8 ng mL^{-1}) which are within the range of clinical analysis.

OTA-aptasensors

Most of the aptamers are known to fold into their unique three-dimensional conformation upon target binding. This change in conformation provides a great flexibility to design simple and sensitive electrochemical aptasensors particularly for small size target analyte such as ochratoxin A (OTA). OTA contaminates a variety of food commodities and has several toxicological effects. Several approaches for the construction of OTA aptasensors were recently presented.

A label free impedimetric OTA aptasensor based on the immobilization of azido-aptamer onto binary film via click chemistry was explored by Marty's group [48]. It was expected that the controlled and uniform modified SPCE surface would provide the systematic immobilization of aptamer, to improve the system reproducibility and sensitivity. The reported work highlighted the interest of using two aryl diazonium salts on same SPE surface together with click chemistry in the development of aptasensor. The increase in electron-transfer resistance (R_{et}) values due to the specific aptamer–OTA interaction was proportional to the concentration of OTA in a range between 1.2 ng L^{-1} and 500 ng L^{-1}, with a detection limit of 0.25 ng L^{-1}. Hayat et al. [49] also introduced a novel strategy for the fabrication of electrochemical label free aptasensor for OTA using polyethylene glycol immobilized on SPCE via electrochemical oxidation of its terminal amino-group. The amino-aptamer was covalently linked to carboxy end of immobilized polyethylene glycol to form two piece macromolecules. The designed immobilized macromolecules resulted in the formation of long tunnels on SPCE surface, while aptamer acted as gate of the tunnels. The aptamer gates were closed due to change in conformation of aptamer upon target analyte binding, decreasing the electrochemical signal. The decrease in electrochemical signal was used for the detection of target molecule (LOD 0.12 ng L^{-1}). In a recent study, the development of a fully automated flow-based aptasensor for the detection of OTA employing direct and

indirect competitive strategies was reported [50]. In both cases, they used SPCEs integrated into a central flow cell for on-line detection of OTA. A lower limit of detection (0.05 μg L^{-1}) was obtained with the indirect flow-based aptasensor. Finally, the flow-based aptasensor was validated with real beer samples, and the good recovery values demonstrated the efficiency of the competitive aptasensor.

Immunoglobulin-aptasensors

Human immunoglobulin E (IgE) is an important protein found in blood, where its concentration can be used as a marker for several allergic diseases through regular blood tests. Because low concentrations of human IgE (reaching over 10 times the normal level) can be found in blood during the early stages of certain diseases [51], highly sensitive methods for its analysis are required. In 2013, Hsieh's group [52] describe an ultrasensitive label-free aptamer-based electrochemical biosensor, featuring a highly specific anti-human IgE aptamer as a capture probe, for human IgE detection. Construction of the aptasensor began with the electrodeposition of AuNPs onto a graphite-based SPE. After immobilizing the thiol-capped anti-human IgE aptamer onto the AuNPs-SPE through self-assembly, and treating with MCH, a designed complementary DNA featuring a guanine-rich section in its sequence (cDNA G1) was employed as a detection probe to bind with the unbound anti-human IgE aptamer. Finally, the redox current of methylene blue was measured to determine the concentration of human IgE in the sample with the LOD of 0.16 pM. The fabricated aptasensor exhibited good selectivity toward human IgE even when human IgG, thrombin, and human serum albumin were present at 100-fold concentrations.

An interesting strategy for the amplification of signal, based on application of streptavidin functionalized silver nanoparticle/graphene hybrid for the construction of an electrochemical human IgE aptasensor, was recently introduced by Xu et al. [53]. The thiol-capped IgE DNA aptamer was used as capture probe, while the biotinylated goat anti-human IgE antibody was used as detection probe. The amount of silver nanoparticle/graphene loading on the surface of SPEs was determined by the amount IgE, and was finally quantified through square wave anodic stripping voltammetry (SWASV), leading to a low detection limit.

Cancer biomarkers-aptasensors

Since each cancer cell line has the specific intra-or extracellular biomarkers, which distinguish it from normal cell lines, therefore, methods that can enable sensitive and selective detection cancer cells through precise molecular recognition of their biomarkers are highly desired [54]. Based on this concept, Yu et al. [55] exploited modified SPCEs to develop a new electrochemiluminescence (ECL) platform for ultrasensitive detection of K562 leukemia cells. The assay principle for the ECL device is illustrated in Fig. 2. Two working electrodes were used for one determination to obtain more exact results. The SPCE was modified with nanoporous gold to provide a good pathway of electron transfer and to enhance the immobilized amount of aptamers. Then aptamers were used for cell capture and the concanavalin A conjugated ZnO@CQDs was used for selective recognition of the cell surface carbohydrate. The method showed a good analytical performance for the detection of K562cells with a detection limit of 46 cells mL^{-1}.

Mucin 1 (MUCl) is an integral membrane glycoprotein expressed by most if not all 'wet' epithelia, such as on bladder, breast, gastric, pancreas and ovary. In normal secretory epithelial cells, MUC1 is expressed at the apical plasma membrane. However, following malignant transformation, MUC1 may be expressed at high levels on the entire membrane surface as well as in the cytoplasm. In recent years, based on the discovery of DNA aptamers targeting MUC1, a few aptamer-based sensors have been developed to detect MUC1-overexpressed breast cancer cells. Recently, Sandulescu et al. [56] describe two simple electrochemical assays based on a MUC1-binding aptamer immobilized on graphite and gold SPEs modified with AuNPs. Figure 3 illustrates the principle of approaches for the detection of MUC1 on AuNPs-modified SPEs. Loosely packed aptamer were self-assembled onto SPE

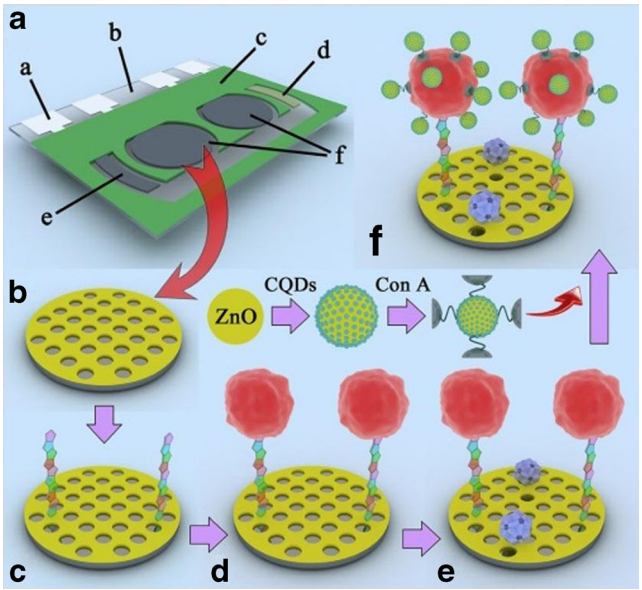

Fig. 2 Schematic representation of the assay procedure for the ECL device. **a** SPCE: (*a*) silver ink, (*b*) PVC film, (*c*) insulating dielectric, (*d*) Ag/AgCl reference electrode, (*e*) carbon ink counter electrode, (*f*) two carbon ink working electrodes; **b** NPG modified SPCE; **c** after immobilization of aptamer; **d** capture with cells; **e** blocking with BSA; and **f** immobilization with the ZnO@CQDs labeled ConA. Reproduced from [55] with permission from Elsevier

surface modified with AuNPs. The interaction between aptamer and MUC1 protein was investigated by CV, EIS and DPV techniques. The estimated detection limits of the MUC1 protein were 3.6 ng mL^{-1} at AuNPs-modified graphite SPE by EIS and 0.95 ng mL^{-1} at AuNPs-modified gold SPE by DPV methods.

Immunosensors

Immunoassay techniques are based on the ability of antibodies to form complexes with the corresponding antigens. This property of highly specific molecular recognition of antigens by antibodies leads to high selectivity of assays based on immune principles. The principle of immunoassays was first established by Yalow and Berson in 1959 [57]. Their work led to the development of the widely used radioimmunoassay to examine the properties of insulin-binding antibodies in human serum, using samples obtained from subjects that had been treated with insulin. Within unconnected work, Clark and Lyons in 1962 pioneered the concept of a biosensor [58]. The original method involved immobilizing enzymes on the surface of electrochemical sensors so as to exploit the selectivity of enzymes for analytical purposes.

Immunosensors have a high potential for many applications because various compounds of interest can be detected at a very high sensitivity. Immunosensors based on SPEs are especially attractive for point-of-care/on-site monitoring. The SPEs are mechanically robust electrochemical transducers that permit the miniaturization of sensors, making possible to integrate the reference and working electrodes in the same chip, and have a very low cost. They are also disposable devices, which are an important feature in the construction of immunosensors. The disposability of the electrodes provides a solution for problems like the electrode surface fouling by products of redox processes and an unintentional adsorption that can arise by using solid electrode materials (e.g. metal, amalgam, composite electrodes). Several SPE-immunosensors have been developed more recently, for the analysis of different substances such as enzymes, microorganisms, antigens, biomarkers and receptors [59–65].

Microorganisms

The detection of pathogenic microorganism contamination of food and water resources is an issue of great importance for ensuring food safety, security and public health [66]. In the last 20 years, *Escherichia coli* O157:H7 has been an important Food borne pathogen in a variety of foods worldwide. The monitoring of *E. coli* O157:H7 with conventional procedures could take 2–3 days. These methods are time-consuming, which delays the introduction of remedial measures [67]. Therefore, a method for rapid detection of this pathogenic microorganism in food and water would aid the prevention of infection, illness, and economic loss.

Chang et al. [67] presented a strategy for preparing a disposable amperometric immunosensor for *E. coli* O157:H7 based on AuNPs and ferrocenedicarboxylic acid (FeDC)-modified SPCEs. The immunosensor was prepared by attaching the first *E. coli* O157:H7-specific antibody, *E. coli* O157:H7 intact cells and the second *E. coli* O157:H7-specific antibody conjugated with HRP on modified SPCE surface.

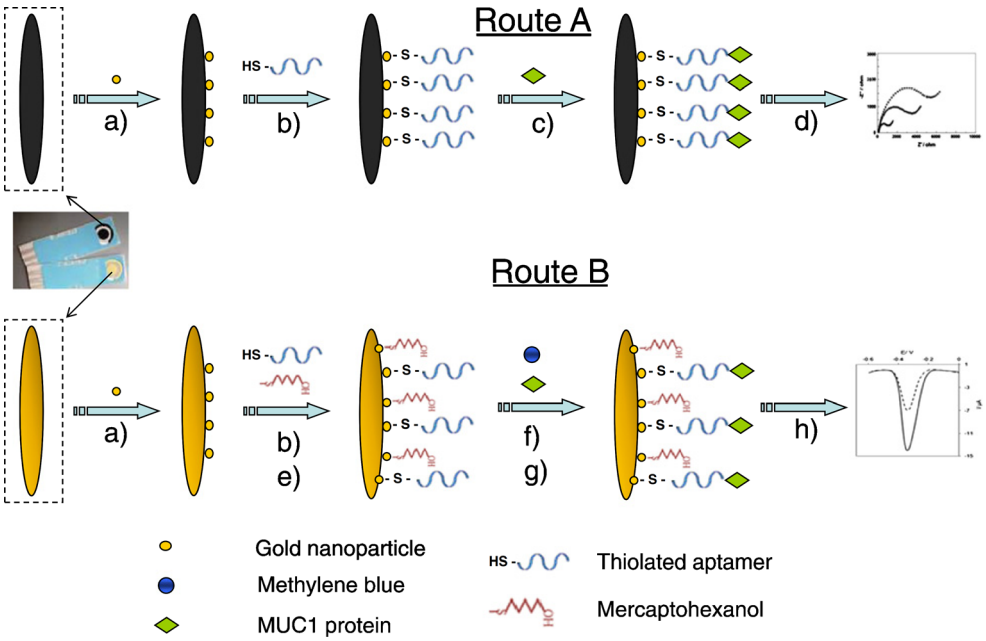

Fig. 3 Detection of mucin 1 by electrochemical impedance spectroscopy (EIS) using an AuNPs-graphite screen printed electrode (SPE) (route **a**) and by differential pulse voltammetry (DPV) on a AuNPs gold SPE (route **b**). (**a**) electrodeposition of AuNPs; (**b**) immobilization of thiolated aptamer; (**c**) affinity reaction with mucin 1; (**d**) EIS measurements; (**e**) reaction with mercaptohexanol; (**f**) accumulation of Methylene Blue; (**g**) incubation with mucin 1; (**h**) DPV measurements. Reproduced from [56] with permission from Elsevier

Hydrogen peroxide and FeDC were used as the substrate for HRP and mediator and concentrations of *E. coli* O157:H7 from 102 to 107 cfu mL^{-1} could be detected. Self-assembled monolayers- modified AuSPEs have been used by Pingarrons' group for detecting *E. coli* with two different immunosensor configurations [68]. In the first one, the immunosensing design was based on the covalent immobilization of anti-*E. coli* at AuSPEs using a homobifunctional cross-linker. The other one was based on the immobilization of the thiolated antibody onto the electrode surface. The immunosensor exhibited a highly improved analytical performance with respect to conventional bacterial plate counting and other electrochemical approaches, enabling, without preconcentration or preenrichment steps, the detection of 3.3 cfu mL^{-1} *E. coli* in river and tap water samples.

The selective interaction of lectins with carbohydrate components from microorganisms' surface was used as the recognition principle for *E. coli* detection and identification [69]. The immobilization of biotinylated lectins– bacteria complexes was performed onto AuSPEs and EIS measurements was employed for the direct label-free transduction of the bacteria–lectin binding. The electron transfer resistance varied linearly with the logarithmic value of *E. coli* concentration over four orders of magnitude, 5.0×10^{3} and 5.0×10^{7} cfu mL^{-1}. The approach can be advantageously compared with conventional bacterial plate counting methods and other electrochemical techniques, enabling the detection of 5.0×10^{3} cfu mL^{-1} *E. coli* within 1 h.

Another simple approach toward the determination of food pathogenic bacteria namely E. coli, campylobacter and salmonella using an immunosensor based on immunosensitized MWCNT-polyallylamine SPE and nanocrystal antibody conjugates was introduced [70]. The immunosensor was fabricated by immobilizing the mixture of anti-*E. coli*, anti-campylobacter and anti-salmonella antibodies with a ratio of 1:1:1 on the surface of the modified SPE. The sandwich immunoassay was performed with three antibodies conjugated with specific nanocrystal which has releasable metal ions for electrochemical measurements. SWASV was employed to measure released metal ions from bound antibody nanocrystal conjugates. Their results suggested that the assay could be directly applied for food quality control applications. Recently, Lin et al. [71] demonstrated a multiplexed immunoassay method for simultaneous detection of multiple food borne bacterial pathogens in a single piece of 4-channel-SPCE. MWCNT/sodium alginate /carboxymethyl chitosan composite films were coated on all the working electrodes to enhance the sensitization of the electrode. The immunosensor array was simple and efficiently fabricated by immobilization of HRP-anti-*E. sakazaki* and HRP-anti-*E.coli* O157:H7. Under optimal conditions, the linear range of *E. sakazakii* and *E. coli* O157:H7 were from 10^{4} to 10^{10} cfu mL^{-1}, with a detection limit of 4.57×10^{3} cfu mL^{-1} and 3.27×10^{3} cfu mL^{-1}, respectively.

Immunoglobulin

Immunoglobulins are immune proteins that bind to antigens. Immunoglobulin G (IgG) is the most abundant immunoglobulin, comprising over 75 % of serum immunoglobulins in humans. The determination of IgG in the bioclinical field is very important owing to the anti-inflammatory action exerted by these proteins contained in serum and milk [72]. In a study by Volpe et al. [73], with the final goal of making possible a single-step magneto-immunosensor based on the use of SPEs coupled with the immunomagnetic beads, the immunological interaction between IgG and its specific antibody (anti-IgG-HRP) was considered with two approaches. The first one was based on the enzyme-channeling principle which involved the use of a second enzyme, glucose oxidase (GOx), immobilized on the surface of the SPE modified with Prussian Blue. The second, more direct, approach was performed without GOx enzyme, had provided the possibility to demonstrate that the signal generated by HRP free in solution was negligible if compared to that of HRP concentrated on the sensor surface. Although both approaches allowed one to obtain a typical sigmoidal binding curve (IgG/anti IgG-HRP), the second one turned out to be easier and more practical.

In a recent study by Tomassetti et al. [74] three immunosensor methods (surface plasmon resonance (SPR), screen-printed and classical amperometric immunosensors) for IgG determination was fabricated and the responses obtained using each device were compared. Experiments were performed on human serum, powdered milks for babies and particularly on several animal milks and comparison of the results obtained by the classical amperometric immunosensor, the screen-printed immunosensor and the SPR device showed that the lower detection limit for IgG determination was of the order of 10^{-9} M in all cases, but that of the SPR method was slightly lower (i.e. 2×10^{-10} M) than those of the other two methods. The linearity range was about three decades for the classic and screen-printed immunosensors and about one and a half decades for the SPR immunodevice. A novel gold nanoprobe was prepared by Lai et al. [75] for the signal tracing of ultrasensitive nonenzymatic electrochemical immunoassay at a CNTs-based disposable immunosensor. Using human IgG as a model analyte, the method showed a wide linear range over three orders of magnitude with the detection limit down to 6.9 pg mL^{-1}.

Toxins

Biomolecule immobilization on the electrode surface is a crucial step in assembling biosensors and immunosensors. Different physical and chemical strategies are followed for the immobilization of molecular recognition agent.

Diazonium organic salt-modified electrodes are a promising alternative to conventional electrode modification schemes for immobilization of biomolecules on solids. Direct immobilization of okadaic acid, a small marine biotoxin, via diazonium-coupling reaction on SPCE has been reported by Barthelmebs et al. [76] to develop a competitive immunosensor. The immunosensor obtained using this novel approach allowed detection limit of 1.44 ng L^{-1} of okadaic acid, and was successfully validated with certified reference mussel samples.

In another study, Radi and co-workers [77] employed this strategy for sensitive detection of OTA. An AuSPE was modified with a layer of 4-nitrophenyl, assembled from 4-nitrophenyl diazonium salt synthesized in situ in acidic aqueous solution. Then, the nitro groups were electrochemically reduced to amines followed by activation with glutaraldehyde to give a stable intermediate derivative that covalently binds antibodies against OTA during the second step, thereby tailoring an immunosensor for OTA. The immobilization was demonstrated through the recognition of the immobilized antibodies by peroxidase-labeled OTA. Furthermore, an amperometric immunoassay in a competitive format for the detection of OTA was also presented. Fernández et al. [78] developed an electrochemical magneto immunosensor for the determination of OTA in red wine samples using heterogeneous competitive immunoassays. Protein G functionalized-magnetic beads (MBs) were applied as solid phase for affinity reaction between OTA and OTA monoclonal antibody. This device showed several advantages over other methods of determination of OTA in wine, such as direct measurement without any pre-treatment, using small volumes (harmful solvents are avoided), and the possibility of introducing this type of immunosensor in field measurements.

The characterization of the gold-catalyzed deposition of silver on graphite SPEs using EIS has been described by Muñoz-Berbel et al. [79]. This approach has been successfully applied to the development of an impedimetric immunosensor for aflatoxin M_1, which is a toxic fungal metabolite, produced by *Aspergillus flavis* and *Aspergillus parasiticus* with an important carcinogenic effect that can contaminate certain agricultural commodities and animal foodstuff. Good correlations found between the logarithm of the toxin concentration and the magnitude of the charge transfer resistance between 15 and 1000 free- aflatoxin M_1 ppt, with a LOD of 12 ppt.

Simplified calibration and analysis on screen-printed disposable platforms for electrochemical MBs-based immunosensing of zearalenone in baby foods has performed by Escarpa's group [80]. Remarkable LOD (0.007 μg L^{-1}) has demonstrated accordingly to the actual requirements established for this mycotoxin in baby food containing cereals as well as an excellent accuracy with recovery rate of 101–111 %.

Hormones

The method of fabrication of a prototype electrochemical immunosensor for estradiol (E2) was described [81]. Methodologies were also given for colorimetric assays, which could be used to verify and optimize reagent performance, prior to their use in the electrochemical immunoassay: these included an E2 enzyme-linked immunosorbent assay (ELISA) and a colorimetric assay performed on the immunosensor surface. The electrochemical immunosensor system was based on SPCEs which antibody against E2 was immobilized on it. Antibodies were immobilized by passive adsorption onto the working electrode surface. A competitive immunoassay was then performed using an alkaline-phosphatase-labeled E2 conjugate. The calibration plot of DPV peak current vs. E2 concentration showed a measurable range of 25–500 pg mL^{-1} with a detection limit of 50 pg mL^{-1}. The immunosensor could be applied to the determination of E2 in spiked serum, following an extraction step with diethyl ether.

Yáñez-Sedeño et al. [82] describe a direct competitive immunosensor using SPCEs and protein A-functionalized MBs for the quantification of testosterone. Under the optimized conditions, a calibration plot for testosterone was obtained with a linear range between 5.0×10^{-3} and 50 ng mL^{-1} and the detection limit of 1.7 pg mL^{-1} which were notably better than those achieved with other immunosensors.

A new immunosensing approach involves the immobilization of anti-adrenocorticotropin hormone (ACTH) antibodies onto amino phenylboronic acid-modified SPCE was used for the determination of ACTH [83]. A competitive immunoassay implying biotinylated ACTH and alkaline phosphatase labeled streptavidin was performed to detect ACTH in certified human serum sample. Under the optimized working conditions, an extremely low detection limit of 18 pg L^{-1} was obtained. Cross-reactivity was evaluated against other hormones (cortisol, estradiol, testosterone, progesterone, hGH and prolactin) and the obtained results demonstrated an excellent selectivity.

Lactoferrin

In mammalian secretions, such as milk, lactoferrin is an important iron-binding glycoprotein, present in large quantities; it is also contained in powdered milks for babies sold in drugstores. The measurement method used for determination of lactoferrin, the ELISA type or similar [84], was always competitive and separative. In 2009, Tomassetti and co-workers [85] fabricated new immunosensors for the analysis of lactoferrin protein in buffalo milk and in other commercial animal milks samples. Recently, they also developed classical and screen-printed amperometric immunosensors using an amperometric H_2O_2 electrode as transducer and the

peroxidase enzyme as marker to determine lactoferrin. Lastly, their group tested the feasibility of constructing immunosensors for lactoferrin determination based on SPR transduction [86]. The data showed a linear range of about 3 and 2 decades, respectively, for the classical and screen-printed immunosensors and about 2 and 1 decade for the SPR immunodevice working in flow or batch mode, respectively. These methods were applied to the determination of lactoferrin concentration in infant powdered milk and in the milk analysis of different animals.

(Cancer) biomarkers

The detection of cancer biomarkers has become a major focus of cancer research, which holds promising future for early detection, diagnosis, monitoring disease recurrence and therapeutic treatment efficacy to improve long-term survival of cancer patients. Tumor markers such as carcinoembryonic antigen, carcinoma antigen 125, prostate-specific antigen, CA 15–3 (MUC1) and human chorionic gonadotropin, have been widely applied for the diagnosis of colorectal cancer, pancreatic cancer, epithelial ovarian tumors and hepatocellular carcinoma, etc. [87]. In clinical assays, the conventional methods of tumor markers detection in serum have some disadvantages, such as being environmentally unfriendly, time-consuming, having poor precision, and experience difficulty in realizing automation. Therefore, there is an urgent requirement for the development of a new immunoassay method with low-cost, high speed and real-time control in large scale disease screening [88]. Electrochemical devices seem to be excellent candidates for the rapid, highly sensitive, easy-to-use and inexpensive diagnosis of diseases and for the detection of tumor markers of clinical interest. The first electrochemical immunosensor for tumor marker detection was developed in the late 1970s [89]. The device involved a competitive assay of hCG in connection with a catalase label and amperometric monitoring of the enzymatic reaction. Thereafter, many immunosensors for cancer biomarkers have been reported in the literature for cancer disease diagnosis [90–93]. Modified SPEs offer unprecedented opportunities for high sensitivity immunosensors amenable to determination of different biomolecules and biomarkers of diseases and have been employed recently for development of numerous biomarkers immunosensors [94, 95].

Human chorionic gonadotropin (hCG)

hCG is glycoprotein composed of 244 amino acids with a molecular mass of 36.7 kDa. It's most important uses as a tumor marker are in gestational trophoblastic disease and germ cell tumors. A sensitive label-free impedimetric hCG-immunosensor was constructed by using a commercial screen-printing carbon ink electrode (namely disposable electrochemical printed (DEP) chip) as the basis [96]. The carbon ink electrode of DEP chip was modified first by deposition of polypyrrole–pyrole-2-carboxylic acid copolymer and then hCG antibody immobilization via the COOH groups of pyrrole-2-carboxylic acid, which could serve as a linker for covalent biomolecular immobilization. The experimental results exposed that the designed immunosensor is more sensitive than other previously reported immunosensors.

Prostate specific antigen (PSA)

A common method to detect prostate cancer is to measure levels of PSA in blood. The presence of a tumor can elevate PSA levels, serving as a convenient blood test for cancer screening. Several biosensors are known for PSA detection. Yu et al. [97] have prepared a novel SPE on sheets of vegetable parchment for the determination of PSA in human serum samples. Vegetable parchment, also called plant-based parchment, was made by passing a waterleaf made of pulp fibers into sulfuric acid. To enhance the conductivity and sensitivity of the immunosensor, graphene and AuNPs were used to construct the immunosensor. With sandwich-type immunoreactions, the HRP-Ab/AuNPs were captured on immunosensor surface to catalyze the electroreduction process of H_2O_2, which could produce stronger electrochemical signal. This method showed wide linear ranges over 6 orders of magnitude with the minimum value down to 2 pg mL^{-1}.

A voltammetric enzyme dual sensor for simultaneous determination of free and total prostate specific antigen (fPSA and tPSA) was described [98]. Antibodies specific for free and total PSA immobilized on different SPEs–SPCEs, AuSPEs and SPCEs modified with nanogold, in order to be able to select one of the surfaces as the most adequate one to develop the dual sensor. Screen-printed carbon electrodes modified with nanogold were the SPEs with the best analytical characteristics and led to the most repeatable bioelectrodes, so they were selected for the development of the dual sensor. In fact, the integration of nanomaterials in immunosensors promotes increase of the electroactive area and the amount of immobilized molecules; in some cases, improving the electrical transfer. The nanoparticles of different sizes and nature can be easily functionalized offering reactive groups on the electrode surface, allowing a more stable and irreversible immobilization of enzymes, antigens, nucleic acid and antibodies, greatly increasing the biosensor response [99].

Carcinoembryonic antigen (CEA)

CEA, a kind of glycoprotein is found in many carcinomas such as colon cancer, lung cancer, urothelial carcinoma, ovarian carcinoma oral and breast cancer [100]. It is one of the most investigated protein markers for lung cancer. Its level is

related to lung cancer in which CEA concentration is elevated significantly in cancer cases than in healthy individuals. When the amount of CEA is higher than 3 ng mL^{-1}, lung cancer may occur. Ho te al. [101] reported on a sensitive electrochemical immunoassay system for the detection of a CEA protein, using a carbon nanoparticle/ poly(ethylene imine)-modified screen-printed graphite electrode covered with anti-CEA antibodies. This biosensor was based on a sandwich complex immunoassay, which they assembled from sequential layers of the anti-CEA antibody (αCEA) on the surface electrode, the CEA sample, and the CdS nanocrystal quantum dots sensitized with αCEA (αCEA-CdS QD). By using SWASV to amplify the signal current response obtained from the dissolved αCEA-CdS QDs, the detection limit of 32 pg mL^{-1} (equivalent to 160 fg in a 5 μL sample) was obtained.

A polyethyleneimine wrapped MWCNTs-SPE was used by Viswanathan group for direct determination of CEA level in human serum and saliva samples [102]. A sandwich immunoassay was performed with CEA and αCEA tagged ferrocene carboxylic acid encapsulated liposomes (αCEA-FCL). The square wave voltammetry (SWV) was employed to analyze faradic redox responses of the released FCL on the electrode surface. The system showed good reproducibility in human serum and saliva samples. Salivary testing is non-invasive, making it an attractive alternative to serum testing, and the possibility of developing home testing kits would further facilitate it as a diagnostic aid. Ju's group took advantage of SPCE system containing two independent working electrodes and modified them with anti-CEA and anti-AFP antibodies respectively [103]. For signal output, streptavidin functionalized AgNP-enriched CNTs were designed as trace tags and were further enlarged by a subsequent AgNP-promoted deposition of silver from enhancer solution to obtain simultaneous electrochemical-stripping signals of AgNPs on the two working electrodes. Using CEA and α-fetoprotein as model analytes, the multiplexed immunoassay showed acceptable precision and wide linear ranges over four orders of magnitude with detection limits down to 0.093 and 0.061 pg mL^{-1}, respectively.

Cancer antigen 125 (CA 125)

CA 125 is one of the most important cancer biomarkers, which found on the surface of many ovarian cancer cells. Normal blood levels are usually less than 35 UmL^{-1}. More than 90 % of women have high levels of CA 125 when the cancer is advanced. Recently, two simple and sensitive approaches for CA 125 detection by using antibody immobilized on poly-anthranilic acid modified graphite SPEs have been developed [104]. In both cases, graphite SPEs were used as solid phase to build up a label free immunosensor and a sandwich format with AuNPs silver enhancement. A high sensitivity with a detection limit of 2 U mL^{-1} of human CA 125 protein and a linear response, which matches the request of clinical needs, was achieved. This level of detection could be attributed to the sensitive electrochemical determination of silver ions and to the catalytic precipitation of a large number of silver ions on the AuNPs-labeled antibody.

C-reactive protein (CRP)

CRP, an alpha globulin with a molecular mass of ~110–140 kDa, is considered a nonspecific biomarker of inflammation and infection that can be used as a predictive risk marker of cardiovascular disease in asymptomatic individuals. Two strategies based on the use of SPEs are known for the determination of CRP in blood serum. Kumar and Prasad [105] have used an imprinted polymer modified SPCE fabricated adapting 'grafting-to' modification for the quantitative analysis of CRP in human blood serum. To mimic the biological binding between polymer and CRP, one of the monomers (AEDP) was used in the place of natural binder. DMAA, a tertiary ammonium group containing monomer, was additionally used to non-covalently interact with the negatively charged CRP molecules. Both the monomers ensured a natural binding environment for CRP and thereby made the electrode highly specific. The LOD was found to be 0.04 μg mL^{-1} (S/N=3) in aqueous environment. In the second strategy, Pingarrón et al. [106] reported an ultrasensitive magnetoimmunosensor for the determination of human CRP using MBs. They immobilized the capture antibody (antiCRP) onto activated carboxylic-modified MBs, then the antigen–antibody reaction and incubation of the modified MBs with a biotynilated antibody (biotin-antiCRP) was performed, followed by an incubation step with a Streptavidin-HRP conjugate, to allow monitoring of the affinity reaction. The electrochemical detection of the enzyme reaction product was carried out at a disposable Au/SPE using TMB as electron transfer mediator and H_2O_2 as the enzyme substrate. The sensor showed an excellent analytical performance achieving a LOD of 0.021 ng mL^{-1} and a wide range of linearity between 0.07 and 1000 ng mL^{-1}.

Cardiac troponins (I and T)

Cardiac troponins (I and T) have been recommended as the biomarkers of choice for the serological diagnosis and prognosis of acute myocardial infarction (AMI) because of their high sensitivity and specificity. In particular, the cardiac troponin T (cTnT) levels increase 2–4 h after the AMI symptoms and could be elevated up to 14 days after the acute episode of myocardial damage. The development of highly sensitive troponin assays possible to measure levels of this marker therefore presents great potential in earlier detection of AMI and in risk prediction in patients with acute coronary

syndrome [107]. In the study by Dutra et al. [108], a modified SPE was manufactured using graphite–epoxy silver composite followed by streptavidin–microsphere integration and its binding to the biotinylated monoclonal antibody for the detection of cTnT. It was observed that the use of streptavidin microspheres significantly increased the analytical sensitivity of the electrode in 8.5 times, showing a curve with a linear response range between 0.1 and 10 ng mL^{-1} of cTnT and a detection limit of 0.2 ng mL^{-1}. Their approach combines the advantages of SPEs with the stability of streptavidin–biotin interaction and the versatility to use conventional amplifiers for enzymatic reaction monitoring and shows great promise for point-of-care quantitative testing of necrosis cardiac proteins. A novel detection for cardiac troponin I (cTnI) based on the deposition of citrate-capped AuNPs on SPEs using an innovative *one-step* electrochemical technique was demonstrated by Bhalla et al. [109]. cTnI was successfully detected in a label-free manner with a LOD equal to 0.2 ng mL^{-1}. This obtained result was one order of magnitude better than that obtained with ELISA tests performed by using the same antibodies, with a detection limit of 4.3 ng mL^{-1}.

Myoglobin (Myo)

Myoglobin (Myo) is another cardiac biomarker of protein nature that changes more rapidly after cardiac injury. Myo, due to its small size (17.8 kDa), is quickly released into circulation with high sensitivity and high predictive value, and acts as a valuable early screening test for AMI. So far, different strategies for Myo detection and quantification based on surface imprinting on SPEs have been described in the literatures [110]. Sales et al. [111] have modified AuSPE by merging molecular imprinting and SAM techniques for fast screening Myo in point-of-care. The imprinting effect was produced by growing a reticulated polymer of acrylamide and N,N′ methylenebisacrylamide around the Myo template, covalently attached to the biosensing surface. EIS and CV studies were carried out in all chemical modification steps to confirm the surface changes in the AuSPE. The analytical features of the resulting biosensor were studied by different electrochemical techniques and the limits of detection ranged from 0.13 to 8 μg mL^{-1}. The same group also described a new disposable biomedical device for monitoring Myo in point-of-care, designed by coating the conductive working area of AuSPE with a PVC-COOH film and assembling the molecularly imprinted polymer (MIP) on top of it [112]. The MIP/AuSPE characteristic towards the quantitative estimation of the Myo concentration was investigated by EIS and SWV. Compared to other immunosensors made with natural antibodies, the MIP/AuSPE showed similar analytical features especially concerning the ability to show linear ranges within the biological levels of Myo and good selectivity against other concomitant proteins and seemed to be a powerful tool for screening Myo in patients with ischaemic episodes.

In order to create smart plastic antibody materials of enhanced specific binding, the recent study by Sales et al. [113] introduced for the first time charged monomers on the binding sites combined with neutral monomers in the rest of the polymeric matrix. This concept formed the basis for the design of Myo plastic antibodies over the gold surface of commercial SPEs. The resulting biosensor was evaluated by several electrochemical techniques and further applied to the analysis of biological samples.

Other biomolecules and proteins

The SPEs have been also utilized for the determination of some other biomolecules such as D-Dimer in human serum [114], β-lactoglobulin as a milk allergenic protein [115], anti-TG2 antibody in celiac disease [116], and herbicide diuron [3-(3,4-dichlorophenyl)-1, 1-dimethylurea] [117] .

Recently, improvements in immunosensor performance, including enhanced sensitivity and reduced detection time, were attributed to the use of magnetic beads [118], [119]. MBs allow easy separation and localization of target analytes by an external magnet, fast immunoreactions between antigen and antibody, and low nonspecific binding by surface modification. The development of a disposable carbon screen printed immunosensor based on MBs technology for the early detection of the Asian rust disease was described by Mendes et al. [120]. This disease is caused by Phakopsora pachyrhizi fungal which is a virulent pathogen that can quickly defoliate plants, reducing production and quality, leading to serious economic losses [121]. MBs modified with a protein G were employed as the platforms for the immobilization and immunoreaction process. The immunotest was the sandwich type, using a secondary antibody labeled with phosphatase alkaline enzyme. The disposable immunosensor presented a detection limit of 90 ng mL^{-1} and was applied for detection of the Asian rust on the soybean leaf samples.

An amperometric immunosensor for detection of chlorpyrifos-methyl has been developed by modification of the SPCE with nanocomposites made by doping of bovine serum albumin conjugated chlorpyrifos-methyl antigen and platinum colloid into silica sol–gel [122]. Chlorpyrifos-methyl is a persistent insecticide and has been widely used for control of pest insects in farming, grain storage, horticulture, forestry and public health applications for more than 30 years. By combining dual

signal amplification of platinum colloid with an enzymatic catalytic reaction, a highly sensitive method for electrochemical evaluation of chlorpyrifos-methyl concentration was presented and applied for its detection in treated soil or grape samples.

The important role that the CNTs play in the performance of electrochemical biosensors is well-known [123]. CNT-based sensors generally have higher sensitivities, lower limits of detection, and faster electron transfer kinetics than traditional carbon electrodes. Recent years have witnessed the development of highly sensitive and selective electrochemical immunosensors based on the use of CNTs. Dutra and coworkers [124] showed the advantages of using SPEs modified with caboxylated CNTs for developing highly sensitive and stable immunosensor for the non-structural protein 1 of the dengue virus. CNTs incorporated to the carbon ink improved the reproducibility and sensitivity of the CNT-SPE immunosensor. This immunosensor showed to be an innovative electrochemical method for diagnosis of early clinical phase of dengue infection. In another study, a magnetism-controlled, enzyme-free amperometric immunosensor was constructed for the determination of human immunodeficiency virus p24 antigen [125]. First, gold magnetic particle-coated MWCNTs were prepared and then, monoclonal antibody (anti-p24) was immobilized on it and adsorbed on the surface of *N,N″*-bis-(2-hydroxy-methylene)-*o*-phenylenediamine copper-modified SPCEs through external magnetic field. The immunosensor was used to determine p24 in serum samples of patients with AIDS, and the results were consistent with those of the traditional ELISA method.

A microfluidic immunosensor coupled with flow injection system for the determination of Botrytis cinerea, a plant-pathogenic fungus that produces the disease known as grey mould in a wide variety of agriculturally important hosts, has been reported [126]. It was based on an immunoreaction between both the infected plant tissue sample and the B. cinerea-specific monoclonal antibody with the B. cinerea purified antigens, using SPCE modified with MWCNTs, which showed a rapid and sensitive determination of B. cinerea in commercials apple fruits tissues samples.

Celiac disease is a gluten-induced autoimmune enteropathy characterized by the presence of tissue tranglutaminase (tTG) autoantibodies. Costa-García et al. [127] have used SPCE nanostructurized with CNTs and AuNPs for the detection of IgA and IgG type anti-tTG autoantibodies in real patient's samples. The immunosensing strategy consisted of the immobilization of tTG on the nanostructured electrode surface followed by the electrochemical detection of the autoantibodies present in the samples using an alkaline phosphatase labeled anti-human IgA or IgG antibody. The results indicated that the immunosensor can be competitive with the standard methodology.

Biosensors

The screen printing technology enables biomolecules to be immobilized onto the electrode surface, in order to fabricate selective and disposable biosensors. Many disposable (electrochemical) biosensors have been developed and applied based on monitoring the interaction between biomolecules and biological recognition elements such as antibodies, nucleic acids and enzymes. This section is, therefore, focused on the description of more recently reported disposable biosensors for the analysis of some important substances.

Glucose

The most well-known application of screen-printing technology in biomedical application has been in the clinical analysis of blood glucose levels in people with diabetes [128]. Many biosensor strategies have been devised for this application, and most of these are based on the enzyme glucose oxidase (GOx). Due to its high substrate specificity and stability in combination with its easy availability, it is still the most frequently employed enzyme for the development of electrochemical glucose biosensors based on SPEs [129–137]. Table 1 presents an overview of the glucose biosensors based on different modified SPEs.

An alternative approach involves the immobilization of glucose dehydrogenase (GDH) and the cofactor NAD^+ (nicotinamide adenine dinucleotide coenzyme) on an SPCE modified with Meldola Blue and Reinecke salt [138]. A cellulose acetate layer was deposited on top of the device to act as a permselective membrane. The biosensor was incorporated into a commercially available, thin-layer, amperometric flow cell operated at a potential of only +0.05 V versus Ag/AgCl. The mobile phase consisted of 0.2 M phosphate buffer (pH 7.0) containing 0.1 M KCl solution, and a flow rate of 0.8 mL min^{-1} was used throughout the investigation. The biosensor response was linear over the range of 0.075–30 mM glucose, with the former representing the detection limit. Zafar's group also described a third-generation amperometric glucose biosensor working under physiological conditions [139]. The glucose biosensor consisted of a recently discovered cellobiose dehydrogenase from the ascomycete Corynascus thermophilus (CtCDH) immobilized on different commercially available SPEs such as SPCEs and carboxyl-functionalized carbon nanotubes- SPCEs by simple physical adsorption or a combination of adsorption followed by cross-linking using poly(ethyleneglycol) (400) diglycidyl ether (PEGDGE) or glutaraldehyde. A drastic increase in response was observed for all three electrodes when the adsorbed enzyme was cross-linked with PEGDGE or glutaraldehyde. The linear range and detection limit of the CtCDH glucose biosensor

Table 1 Summary of some biosensors used for the analysis of glucose

Modified electrode	Linear range	Detection of limit	Sensitivity	Ref.
11-(ferrocenyl) undecyltrimethylammonium bromide (FTMA)/GOx/SWCNT-SPE	0.04–0.38 mM	–	1.73 μA mM^{-1}	[129]
Os-complex mediator-SPE	0.5–30 mM	–	10.24 nA mM^{-1} cm^{-2}	[130]
Cobalt phthalocyanine redox mediator/GOx-SPE	up to 10 mM	–	7.84 nA mM^{-1}	[131]
GOx/SWCNT/redox polymer Nanocomposite-SPCE	0.5–6.0 mM	0.1 mM	16.4 μA mM^{-1} cm^{-2}	[132]
GOx-SWCNT conjugate/redox polymer (PVI-Os)-SPCE	0.5–8.0 mM	0.07 μM	32 μA mM^{-1} cm^{-2}	[133]
polyethylenimine (PEI)/ MWCNT-SPCE	0.5–3.0 mM	–	22.18 μA mM^{-1}	[134]
snowflake-like Pt–Pd bimetallic nanoclusters(Pt–Pd BNC)-gold nanofilm SPE	0–16 mM	10 μM	–	[135]
Polyethyleneimine (PEI)/Prussian Blue (PB)-SPCE	3.5–0.15 mM		0.13 μA mM^{-1}	[136]
SPE with two working electrode	0.5–50 mM	0.05 mM	–	[137]
Meldola's Blue/ Reinecke salt coated with glucose Dehydrogenase-SPCE	0.075–30 mM	–	0.051 μA mM^{-1}	[138]
CtCDH/ SPCE, SPCE−MWCNT, SPCE–SWCNT	0.025–30 mM	10 μM	–	[139]
silica sol–gel/polyvinyl alcohol-SPE	0–4.13 mM	9.8 μM	3.47 μA mM^{-1} cm^{-2}	[140]
Preanodized-SPCE	0–900 μM	–	50.88 μA mM^{-1} cm^{-2}	[141]
Graphene/poly(3,4-ethylenedioxythiophene): polystyrene sulfonic acid (GP/PEDOT:PSS)-SPCE	~20–900 μM	~0.3 μM	7.23 μA mM^{-1}	[142]

under physiological conditions were 0.025–30 and 0.01 mM, respectively.

In recent years, extensive efforts have been made to achieve the direct electrochemistry of GOx for electrochemical detection of glucose [140, 141]. Recently, the direct electrochemistry of GOx on modified SPCE with graphene-Poly (3,4 ethylenedioxythiophene): poly-styrene-sulfonic acid (PEDOT:PSS) matrix has been investigated [142]. GP-PEDOT: PSS nanocomposite was synthesized by one-step electrolytic exfoliation method and drop-coated on SPCE. GOx enzyme was then immobilized on it by glutaraldehyde cross linking. The designated electrode exhibited a high amperometric sensitivity of 7.23 μA mM^{-1} for glucose determination, but had a relatively narrow linear dynamic range of 20–900 μM.

Hydrogen peroxide

Hydrogen peroxide (H_2O_2) has been involved in several biological events and intracellular pathways. It is not only a by-product of several highly selective oxidases, but also an essential mediator in food, biology, medicine, industry and environmental analysis [143]. Therefore, sensitive and accurate determination of H_2O_2 is becoming of practical importance. Many techniques have been developed for the determination of H_2O_2. Among these procedures, electrochemical biosensors based on SPEs are promising for the fabrication of simple, portable and low-cost biosensors. Several biosensors have been developed for electrochemical [134–136, 144] and electrochemiluminescence [145] detection of H_2O_2.

Lan et al. [146] presented a H_2O_2 biosensor based on the direct electron transfer of HRP on porous screen-printed carbon electrodes. The porous structure of SPCEs facilitated the direct electron transfer between the active sites of HRP and the electrodes due to large amounts of conductive sites available on the surface for contacting with enzyme molecules. The resulting sensor was sensitive (143.3 mAM^{-1} cm^{-2}) and could detect 0.48 μM of H_2O_2. Another disposable H_2O_2biosensor based on the direct electron transfer of myoglobin was developed using mesopores KIT-6 modified SPE [147]. KIT-6 is a new material which can absorb abundant of myoglobin molecules and also increase the affinity of enzyme and substrate. The modified electrode system showed an efficient performance to H_2O_2 detection. Recently, a new detection system was developed using Fe_3O_4-Au magnetic nanoparticles coated HRP and graphene sheets -Nafion film modified SPCE [148]. To construct the H_2O_2 biosensor, graphene sheets - Nafion solution was first dropped onto the surface of SPCE. Subsequently, the biocomposites of Fe_3O_4-Au magnetic nanoparticles coated HRP were adsorbed on the surface with the aid of an external magnetic field. The practical analytical application of the biosensor was assessed by measurement of the real samples and the results were consistent with the results obtained by KMnO4 titration method.

Pharmaceuticals

The determination of drugs in real samples such as urine and blood and other related samples is another issue which requires careful investigation since they are often electroactive species. Literature revealed that many disposable electrochemical biosensors have been reported for the detection of drugs using modified and unmodified SPEs. These biosensors were used for the determination of the quantity of codeine in

pharmaceutical commercial tablets and urine samples [149], diazepam in beverages [150], Levetiracetam in pharmaceutical drugs and spiked human plasma samples [151], Pyrazinamide [152] and isoniazid [153, 154] in human urine samples, dapsone in human urine and pharmaceutical formulation samples [155], silybin [156] and gemifloxacin [157] in pharmaceutical tablets. Table 2 gives an overview of the electrochemical biosensors based on SPEs for determination of these compounds in their related samples by different techniques.

In a study by Chai's group [158], SPEs were modified with MWCNT and molecularly imprinted membranes using ractopamine as the template were directly synthesized on the MWCNT modified SPEs using the in situ thermal polymerization techniques and then utilized for the quantification of ractopamine in pig urine with the detection limit of 6 nM.

The electrochemical behavior of colchicines was investigated using graphite-based SPEs by Bodoki et al. [159]. Colchicine is a protoalkaloid, used as a specific anti-inflammatory agent in acute attacks of gout by inhibiting the migration of leucocytes to inflammatory areas, thus interrupting the inflammatory response that sustains the acute attack. The method was validated in the concentration range of 85–1200 ng mL^{-1} with limits of detection of 41 ng mL^{-1} and was successfully applied to the determination of colchicine in tablets, without the interference of the excipients.

Cocaine is one of the most known illicit drugs and one of the most trafficked and used. It is an alkaloid that acts as analgesic and local anesthetic. It also stimulates the modulation of dopamine producing a sensation of euphoria which provokes addiction [160]. Although the trendiest analyses of cocaine are based on chromatography and immunoassays methods, electrochemical detection has been also reported. In a study by Alonso-Lomillo's group, a new electrochemical method for the determination of cocaine using SPEs modified by the enzyme cytochrome P450 2B4 (CYP450) has been described [161]. Cocaine is one of the preferred substrates of this enzyme. Chronoamperometric measurements was performed under the optimum conditions and capability of detection of 23.05±3.53 nM (n=3, $\alpha = \beta$=0.05) was obtained. Similarly, this group developed another screen-printed cytochrome P450 2B4 based biosensor, for voltammetric detection of cocaine street samples [162].

Fakhari and Rafiee [163] fabricated an electrochemical biosensor based on NiO nanoparticles modified nafion-MWCNT-SPE for methods have been explored for the quantitative analysis of insulin. It serves as a predictor of diabetes of insulinoma and trauma and is used to control glucose levels

Table 2 Summery of some biosensors used for the analysis of drugs

Electrode	Analyte	Electrochemical technique	Capability of detection	Analyzed sample	Ref.
Tetrathiafulvalene (TTF)-SPCE	Codeine	Chronoamperometry	20 μM	Codeisan®, Urine samples	[149]
Unmodified SPCE	Diazepam	Adsorptive stripping voltammetry	1.8 μg mL^{-1}	Beverage samples; Pepsi Max, Vodka Cherry alcopop	[150]
HRP/ polypyrrole (PPy)-SPCE	Levetiracetam	Chronoamperometry	9.81×10^{-6} M	KEPPRA® tablets, Human plasma samples	[151]
Poly-histidine-SPCE	Pyrazinamide	LSV, DPV, SWV	5.7×10^{-7} mol L^{-1}	Urine samples	[152]
poly-L-histidine (PH)-SPCE	Isoniazid	LSV, DPV, SWV	5.0×10^{-7} mol L^{-1}, 1.7×10^{-7} mol L^{-1}, 2.5×10^{-7} mol L^{-1}	Human urine samples	[153]
Silver hexacyanoferrates (NPAg-HCF)-SPCE	Isoniazid	FIA with amperometric detection	2.6 μmol L^{-1}	Simulated human urine samples	[154]
Platinum nanoparticles (PtNP)-SPE	Dapsone	LSV	7.6×10^{-7} mol L^{-1}	Human urine samples	[155]
MWNT/single-sided heated-SPCE	Silybin	Adsorptive stripping voltammetry	5.0×10^{-10} M	Pharmaceutical tablets	[156]
SPCE	Gemifloxacin	DPV, CV	0.15, 5.0 μM	Pharmaceutical tablets	[157]
MWCNT-/molecularly imprinted membranes (MIM)-SPE	Ractopamine	DPV	6 nM	Pig urine	[158]
SPGE	Colchicine	Anodic differential pulse voltammetry	41 ng mL^{-1}	Olchicine tablet	[159]
Cytochrome P450 2B4-SPE	Cocaine	Chronoamperometry CV	23.05±3.53 nM 0.2 mM	Cocaine street samples	[161] [162]
SPE/MWCNT/NiONs	Insulin	Amperometry	6.1 nM		[163]
SPE/CNT/$NiCoO_2$	Insulin	Amperometry	1.06 μg mL^{-1}		[164]

in blood within a narrow concentration range. The direct monitoring of insulin in a diabetic patient has a better prospect in clinical investigation rather to the glucose measurement. Modified SPEs with CNT-$NiCoO_2$ was also prepared for trace level detection of insulin [164].

Neurotransmitters/Neurochemicals

Neurotransmitters play a crucial role of endogenous primary chemical messengers to transport information among biological cells in mammalian central nervous system and are indicative of health and disease [165]. Dopamine (DA) is an important neurotransmitter molecule belonging to a family of molecules called catecholamines. Deficits in brain dopamine cause Parkinson's disease, Alzheimer's and schizophrenia in humans [166]. Since dopamine (and the like catecholamines) is an easily oxidizable compound, electrochemical methods based on modified electrodes are an ideal choice for its quantitative determination. Several examples are reported in the literature regarding on the detection of DA and other neurotransmitters in the presence of other species using different modified electrodes [167–171].

In 2010, Moreno's group [172] investigated the voltammetric behavior of dopamine on CNT-SPE with the aim to propose a new methodology for its determination at low levels in the presence of ascorbic acid (AA). A linear dependence between DA concentration and adsorptive stripping peak current was observed for the concentration range of 5.0×10^{-8} to 1.0×10^{-6} mol L^{-1} with a limit of detection of 1.5×10^{-8} mol L^{-1}. In a study by Merkoci et al. [173], a new way to entrap enzyme based on β-cyclodextrin electropolymerization onto SPE modified with MWCNT was introduced for DA quantification. The biosensor displayed good reproducibility, repeatability, and prolonged life-time under cold storage conditions. Its limit of detection was 0.48±0.02 μA with sensitivity of 0.0302± 0.0003 μA μM^{-1} that makes it comparable or even better than many other electrodes reported in the literature. Zen et al. [174] utilized screen printing technology to print a thin layer of hydrophobic room temperature ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate, onto a disposable SPCE to improve the performance of DA in the presence of high concentration of ascorbic acid. A linear calibration plot up to 100 μM was obtained. The detection limit was calculated as 0.26 μM.

Norepinephrine (NE) and Serotonin are also neurotransmitter molecules that have been target for detection with screen printed-based electrodes. Norepinephrine was detected amperometrically using low-cost mesoporous carbon inks to screen print single-use disposable electrodes [175]. To ensure NE can be effectively oxidized, an enzyme, phenylethanolamine N-methyl transferase, with a cofactor to active the enzyme was used to catalyze the reaction. The resultant sensors could detect NE at concentrations as low as 100 pg mL^{-1} in rabbit whole blood. This high sensitivity without a membrane such as Nafion was attributed to the molecular sieving capabilities of the mesopores and the large internal surface area of the mesoporous carbons.

A new rapid and convenient electrochemical method based on a Nafion membrane-coated colloidal gold SPE was described by Ding et al. [176] for the determination of serotonin (with a detection limit of 5.0 nM) in the presence of high concentration of DA, AA and uric acid (UA). SPE was used for the detection of serotonin in the platelet-rich plasma and brain homogenate of the mice. The serotonin content in the peripheral blood of the mouse can reflect the content in the brain, which suggests that the serotonin may be regarded as peripheral biochemical markers for the mice depression model.

Besides neurotransmitters, many other compounds are found in the brain. Some of these compounds are electroactive and act as interfering species for neurotransmitter detection. Therefore, many studies have concentrated on detecting neurotransmitters in the presence of ascorbic acid, uric acid, or metabolites. The use of the SPEs for simultaneous determination of ascorbic acid, dopamine and uric acid has been rarely reported, since the unmodified SPEs are unable to discriminate signals of AA, DA and UA, While several studies has detected these compounds using modified SPEs with dodecylbenzene sulfonic acid–polyaniline nanoparticles [177], cellulose acetate and ionic liquid n-octylpyridinum hexafluorophosphate [178], and iridium oxide [179]. Recently, a novel SPE prepared from graphene and ionic liquid doped screen-printing ink was presented by Ying et al. [180]. The basic characteristics of the screen-printed graphene electrode were studied in details. The sensor showed excellent electrocatalytic activity for the oxidation of AA, DA, and UA. Three well-defined sharp and fully resolved anodic peaks were found at the developed electrode. In the co-existence system of these three species, the linear dependence of peak current on the concentration was obtained in the ranges of 4.0–4500 μM, 0.5–2000 μM, and 0.8–2500 μM with the lowest detection limits of 0.95 μM, 0.12 μM, and 0.20 μM for the determination of AA, DA, and UA, respectively. Another SPCE modified with polyacrylic acid-coated MWCNTs (PAA-MWCNTs) has been prepared for the simultaneous determination of norepinephrine, uric acid, and ascorbic acid [181]. Using PAA, MWCNTs could be well dispersed and the electrode became negatively charged so that the adsorption of AA could be inhibited owing to the electrostatic repulsion. This led to the decrease in the oxidation potential of AA and the significantly enhanced oxidation peak currents of NE and UA at the PAA-MWCNTs/SPCE. As compared to SPCE, the system has found to be effective for the simultaneous determination of NE, UA, and AA in their mixture solution.

Novel combinations of polymers with CNTs have been used to enhance the response of epinephrine and norepinephrine. One such combination was MWCNT with a mixture of poly(nordihydroguaiaretic acid) and chitosan onto SPCE [182]. Epinephrine and norepinephrine were not discriminated and the lowest concentrations studied were 30 μM and 780 μM, respectively.

A few papers have studied just uric acid and ascorbic acid without also examining dopamine. One study [183] reports on an uricase biosensor based on a SPE modified with Prussian Blue, coupled with a portable instrumentation, showing a working range of 0.03–0.3 mM and a detection limit of 0.01 mM. A SPCE incorporating the electrocatalyst cobalt phthalocyanine (CoPC), has also been used for auric acid detection [184]. The sensor was the simplest reported UA biosensor operated in the chronoamperometric mode and both prevented direct oxidation of UA and solved problems from potential interferences in urine. Ascorbic acid also detected by disposable electrochemical biosensors based on SPEs, as a low-cost and disposable point of-care devices for prescreening purposes [185, 186].

Amino acids

The detection of various kinds of amino acids is an important issue in proteomics, properties of enzyme and hormone, disease diagnostics and food nutrients [187]. They can be classified as α-, β- and γ-amino acid, etc. by the distance between the carboxylic acid group and the amine group. Electrochemical detection of α-, β- and γ-Amino acids using disposable copper nanoparticle electrodeposited SPCEs was performed by a process which alternated reductive deposition and oxidative treatment over six cycles (called the RO6 method) [188]. Such disposable RO6-CuNPs/SPEs have great potential for integrating HPLC or capillary electrophoresis systems to offer sensitive detection for amino acids analysis in biomedical diagnostic and food industry applications.

Thiols are sulphur containing amino acids that build proteins and are essential in the formation and growth of tissues. Thiols such as reduced glutathione, 2-mercaptoethanol (2-ME) and cysteine have been detected by electrochemical sensors based on SPEs. Bedioui et al. [189] reported the electro-catalyzed oxidation of reduced glutathione and 2-ME by cobalt phthalocyanine-containing SPCEs. They showed electro-catalytic activity of these thiols depend on the method of electrode modification and the amount of catalyst incorporated in the ink used to fabricate the SPCEs. The electrocatalytic oxidation of cysteine at SPE modified with electrogenerated poly(3,4-ethylenedioxythiophene) film (PEDOT) was investigated [190]. Cyclic voltammetric studies showed that the electrode lowers the overpotentials and improves electrochemical behavior of cysteine oxidation, as compared to the bare SPE. Zen and Liao [191] presented a new method for total plasma thiols (cysteine, homocysteine and glutathione) measurement using a disposable SPCE coupled with a MnO_2 reactor by flow injection analysis. MnO_2 reactor was used to facilitate the oxidation of catechol (electrochemical indicator) and eliminate the interference from ascorbic acid. A detection limit of 0.1, 0.25, 0.47 μM were observed for cysteine, homocysteine and glutathione, respectively.

Methionine is a source of sulphur in the body as it is the precursor of other sulphur amino acids such as cysteine, taurine and glutathione. Banks et al. [192] reported for the first time the direct oxidation of methionine at bare graphite SPEs, allowing linear ranges over the range 0.05–5.0 mM with a detection limit of 95×10^{-6} mol L^{-1} possible in model solutions without any chemical or electrochemical pretreatments. The suitability of the use of SPEs for the measurement of the direct oxidation of methionine was demonstrated for the analysis of the content of this analyte in a pharmaceutical product.

NADH

NADH plays a central role in mitochondrial respiratory metabolism, stimulating the energy production in all living cell (brain, heart and muscles), without this coenzyme all cell processes stop. NADH detection is of a great importance because it is produced in reactions catalyzed by more than 250 dehydrogenases [193]. In 2008, an amperometric biosensors based on NADH oxidase/FMN and Prussian Blue modified SPEs have been developed in order to improve the NADH detection [193]. As NADH oxidase is an FMN dependent enzyme, an improvement of the enzyme/cofactor immobilization procedure was necessary. The detection limit was 2.6 μM NADH for FMN added in the reaction medium and 1.17 μM NADH, respectively when the cofactor was entrapped in sol–gel matrix. Doumèche et al. [194] investigated NADH oxidation on SPE modified with a new phenothiazine diazonium salt. The amperometric response for NADH oxidation showed a maximal current of 1.2 μA ([NADH] =100 μM). The sensitive layer for the oxidation of NADH was improved by electrografting the diazonium salt with a potentiostatic method. Both the surface coverage and the heterogeneous standard rate constant k_h improved and found to be $6.08\pm0.63\times10^{-11}$ mol cm^{-2} and ~5.02 s^{-1}, respectively. The amperometric response was also improved by an 8 fold factor, reaching 9.87 μA ([NADH] =120 μM). A novel hybrid platform was constructed by Pingarrón's group [195] based on electrodeposition of poly (3,4-ethylenedioxythiophene) using the ionic liquid as the electropolymerization solvent onto AuNPs-modified SPCE. The prepared modified electrodes were utilized for detection of biomolecules such as NADH. The sensitivity achieved for

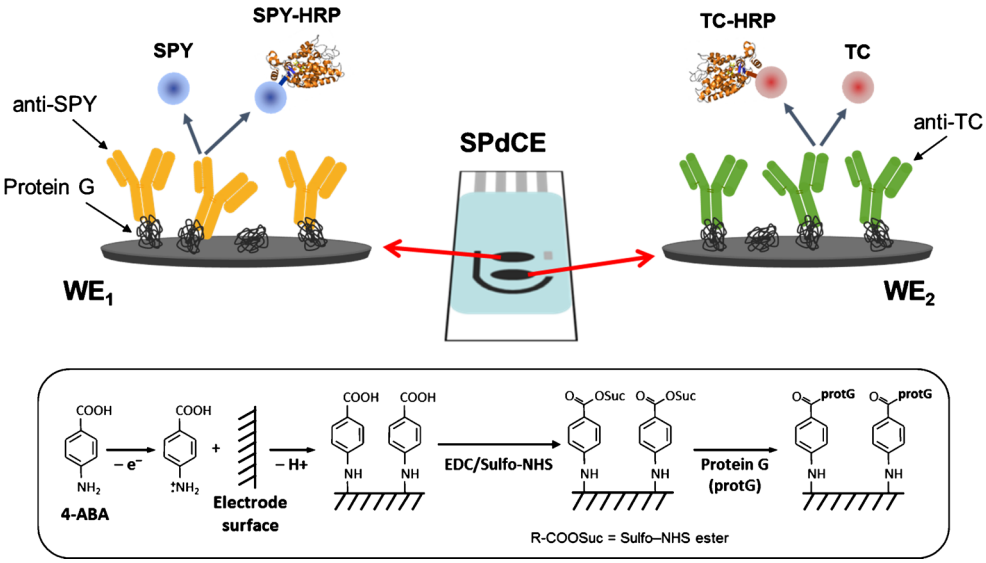

Fig. 4 Schematic display of the immunosensor for sulfonamide antibiotics, and details of the surface chemistry including covalent immobilization of the capture antibody by using a film of poly-4-aminobenzoic acid formed on a SPCE. Reproduced from [206] with permission from Elsevier

NADH using amperometry was 88 mA M^{-1} cm^{-2} with a linear calibration graph in the (1.0–100)×10^{-6} M concentration range.

Enzyme based sensors

In enzyme-based biosensors, the biological element is the enzyme which reacts selectively with its substrate. Enzymes have a high specificity and a very high affinity for specific biomolecules. Some enzyme sensors detect electrons tunneled from the enzyme directly to the electrode surface while others detect electroactive byproducts of enzymatic reactions. Examples of common compounds detected by this approach include fructose, galactose, lactose, lactate, OTA, triglyceride, cholesterol, and alcohol. The first fructose sensor using a commercial screen-printed ferrocyanide/carbon electrode was reported by the immobilization of enzyme d-fructose dehydrogenase (FDH) on the electrode surface [196]. The sensor showed a good sensitivity to fructose with a limit of detection of 0.05 mM.

Recently, development of a disposable amperometric biosensor for the measurement of circulating galactose in serum was described by Hart et al. [197]. The biosensor comprised a SPCE incorporating CoPC, which was covered by a permselective cellulose acetate membrane and a layer of immobilized galactose oxidase (GALOX). When the biosensor was used in conjunction with amperometry for the analysis of serum, the precision values obtained on unspiked and spiked serum were 1.10 % and 0.11 %, respectively.

In a study by Trashin et al. [198] modification of SPEs by polyaniline was performed to improve the electron exchange between cellobiose dehydrogenase and the SPE surface to develop a sensitive lactose biosensor. A layer of polymer in its intermediate redox state has increased the maximum response current of direct bioelectrocatalysis for lactose more than 5 times at an applied potential substantially lower than that observed when enzyme was adsorbed directly on the SPE. Another rapid and simple approach of lactose analysis proposed based on 3rd generation amperometric biosensors employing cellobiose dehydrogenase immobilized on modified SPCEs and successfully applied for the determination of lactose in dairy (milk with different percentages of fat, lactose-free milk and yogurt) [199].

Lactate detection by biosensors is of increasing importance in clinical applications to provide rapid, point of care, serial measurements for early diagnosis of disorders such as intra-abdominal sepsis to reduce morbidity and mortality. In 2009, Hart et al. [200] demonstrated for the first time that screen-printed carbon microband electrodes fabricated from water-based ink can readily detect H_2O_2 and that the same ink, with the addition of lactate oxidase, can be used to construct microband biosensors to measure lactate. Their study provided a platform for monitoring cell metabolism in-vitro by measuring lactate electrochemically over a dynamic range of 1–10 mM via a microband biosensor. Hirst et al. [201] presented another method for the quantification of lactate using pre-impregnated Prussian Blue SPCEs and polyethyleneimine polymer for lactate oxidase immobilization. The biosensor was shown to give results for lactate in post operative patient drain fluid samples that was concordant with data from a colorimetric assay for lactate.

The mycotoxin OTA, which is one of the most abundant food-contaminating mycotoxins, have been determined using HRP enzyme-biosensor based on SPEs by Alonso-Lomillo's group [202]. An HRP containing ink has been directly screen-printed onto carbon electrodes. This immobilization procedure, which is known as automated immobilization, is particularly interesting for mass production of disposable

Table 3 Enzyme based sensors based on SPEs

Sensor	Analyte	Enzyme	LOD	Linear range	Ref.
Screen-printed ferrocyanide/carbon electrodes (SPFCE)	Fructose	D-fructose dehydrogenase	0.05 mM	0.1–1 mM	[196]
Cellulose acetate-CoPC-SPCE	Galactose	Galactose oxidase	0.02 mM	0.1–25 mM	[197]
Polyaniline-SPE	Lactose	Cellobiose dehydrogenase	1 μM	1 μM–1 mM	[198]
MWCNT-SPCE	Lactose	Cellobiose dehydrogenase	250 nM	0.5–200 μM	[199]
Microband CoPC-SPCE	Lactate	Lactate oxidase	289 μM	1–10 mM	[200]
Prussian Blue impregnated poly(ethyleneimine)-SPCE	Lactate	Lactate oxidase			[201]
HRP-SPCE	Ochratoxin A	Horseradish peroxidase	26.77± 3.61 nM	23.85–203.28 nM	[202]
Polypyrrole-SPCE	Ochratoxin A	Horseradish peroxidase	0.1 ng mL^{-1}	0.24–2.06 nM	[203]
MWCNT-rhodium-graphite SPE	Cholesterol	Cytochrome P450scc			[204]
AuNPs–MWCNT–Nafion-SPE 10^{-4}–25×10^{-3} mol L^{-1}	Alcohol [205]	Alcohol dehydrogenase	$5.0\times$	10^{-5} mol L^{-1}	$2.0\times$
Au/PEDOT-PSS-SPCE	Triglyceride	Lipase, Glycerol kinase, Glycerol-3-phosphate oxidase	7.88 mg dL^{-1}	0–531 mg dL^{-1}	[207]
SPCE	Tyramine	Plasma amine oxidase	2.0±0.18 μM	2–164 μM	[208]
Prussian Blue-SPE 10^{-5}–7×10^{-4} mol L^{-1}	Trehalose [209]	Trehalase-GOx	$0.8\times$	10^{-5} mol L^{-1}	$1.5\times$
SPCE	Gluconic acid	Gluconate kinase, 6-phospho-D-gluconate dehydrogenase	7.46 μM	7.46–71.43 μM	[210]
Prussian Blue-SPE	Glucose-6-phosphate	Glucose-6-phosphate dehydrogenase, Glutathione reductase	2.3 μM	0.01–1.25 mM	[211]
PEDOT:PSS-SPE	Organophospha e insecticide chlorpyrifos-oxon	Acetylcholinesterase	4×10^{-9} M	–	[212]
SPE	Omethoate Methidathion Malaoxon	Acetylcholinesterase	10^{-9} M	7.5×10^{-5}–2.5×10^{-6} M 5×10^{-6}–5×10^{-8} M 5×10^{-7}–10^{-8} M	[213]
Au-SPE	Organophosphorus insecticide	Acetylcholinesterase	2 ppb	Up to 40 ppb	[214]
SPCE, Gold SPE, AuNPs-SPE	Phenobarbital	CytochromeP450 2B4		0.20–1.67 mM	[215]

biosensors. The same group also determined OTA using a SPCE-biosensor based on HRP immobilized by pyrrole electropolymerization [203]. The well-known mechanism of these biosensors involve the oxidation of native HRP by H_2O_2 to an intermediate compound, which is subsequently reduced by a substrate donor (OTA), regenerating the native enzyme. OTA concentration in a solution is related with the chronoamperometric current registered. The capability of detection for this method was 0.1 ng mL^{-1} (α=0.05 and β< 0.05).

In the field of cholesterol sensing, recently a cholesterol biosensor with an improved sensitivity based on rhodium–graphite SPE modified with MWCNT and cytochromes P450scc as catalytic enzyme was presented [204]. The sensitivity of the system was higher by orders of magnitude with respect to other similar systems based on cholesterol oxidase and esterase. The electron transfer improvement attained by the use of MWCNT in P450-based cholesterol biosensors was demonstrated to be larger than 2.4 times with respect to the use of AuNPs and 17.8 times larger with respect to the case of simple bare electrodes.

Alcohol is the most common poisonous substance related to clinical and forensic medicine, and it leads to a variety of health damages and traffic accidents frequently. The common methods for alcohol determination are often time consuming or require expensive instrumentation. The development of an inexpensive enzyme based electrode would be beneficial for the detection of alcohols. Generally, two kinds of enzymes, alcohol dehydrogenase and alcohol oxidase were immobilized for the construction of alcohol biosensors. An alcohol biosensor was prepared by the combination of modified SPEs with nano-materials and alcohol dehydrogenase to fabricate a novel detection strip [205]. The linear response range of the biosensor was 2.0×10^{-4} to 25×10^{-3} mol L^{-1} and the detection limit was 5.0×10^{-5} mol L^{-1} (S/N=3). In the measurement of blood samples, the biosensor had excellent detection performance for measuring blood alcohol concentration and showed a good correlation with gas chromatography.

Dual SPEs with two elliptic working electrodes are aimed at detecting two signals simultaneously, allowing differential measurement of two analytes in the solution. Disposable dual screen-printed carbon electrodes (SPdCEs) modified with Protein G, have been used for the simultaneous determination of sulfonamides (SAs) and tetracyclines (TCs) antibiotics in milk, at the low ppb concentration level [206]. The immunoreactions involved competitive binding between SAs or TCs antibiotics and HRP-labeled specific tracers for the binding sites of the capture antibodies immobilized on the working electrode surfaces. Quantification was achieved through the electrochemical monitoring of the enzyme product at the SPdCEs, using hydroquinone as electron transfer mediator and H_2O_2 as the enzyme substrate. Figure 4 shows the scheme of the disposable dual immunosensor and the details of surface chemistry involved the modification of SPdCE. The method showed very low limits of detection (in the low ppb level). The usefulness of the dual immunosensor was demonstrated by analyzing spiked milk samples as well as a reference milk containing a certified oxytetracycline content. Good recoveries were attained in an analysis time of 30 min.

Various types of biosensors based on immobilized enzymes with different modified SPEs and enzyme immobilization methods have also recently been demonstrated for determination of some compounds such as triglyceride [207], tyramine [208], trehalose [209], gluconic acid [210], Glucose-6-phosphate (G6P) [211], organophosphorus insecticides [212–214] and Phenobarbital [215]. Table 3 gives the type of electrodes and enzymes, detection limits and liner range for different enzyme based sensors.

Other biomolecules

Different modified SPEs biosensors, designed by several group, have been used for the determination of a variety of compounds, including phosphate ions in urine and pond water [216–218], pathogens [219], bacterial toxins (Microcystin) in water [220], endotoxin analysis in cell culture medium [221], and choline [222]. A study by Marty et al. [223] described the development of a superoxide biosensor based on the co-immobilization of cytochrome c and xanthine oxidase on a self-assembled monolayer-modified AuSPE and its later application to the determination of the antioxidant capacity of pure substances and several orange juices. Another new electroanalytical method for the simultaneous detection and quantification of the antioxidants *tert*-butylhydroquinone (TBHQ) and butyl hydroxyanisole (BHA) in biodiesel was developed using a voltammetric technique and MWCNT-SPEs [224]. MWCNT-SPEs have been used also for determination of arylsulphatase and phosphatase enzyme activities in agricultural soil [225]. Rodríguez-Méndez et al. have introduced [226] two multisensory systems based on carbon paste or SPEs modified with phthalocyanines to the analysis of biogenic amines and fish freshness assessment. An increase of the signals associated to biogenic amines was observed with increasing storage days.

Conclusion

Electrochemical sensors provide a crucial analytical tool as demand for sensitive, rapid, and selective determination of analytes increases. Unlike spectroscopic and chromatographic instruments, electrochemical sensors can be easily adapted for detecting a wide range of analytes, while remaining inexpensive. The recent trend to replacement of conventional electrodes by SPEs is making possible to explore other options in this field. Screening-printing technology can be easily applied to the mass production of inexpensive, reproducible and sensitive disposable electrodes, and SPEs have been applied in portable devices. Additionally, these sensors are capable of being incorporated into robust, portable, or miniaturized devices, enabling tailoring for particular applications. SPEs are gaining widespread applications in biomedical, environmental and industrial monitoring. The great versatility presented by the SPEs is based on the wide range of ways in which the electrodes may be modified (directly modifying the composition of printing ink or just depositing the substances on the surface) as demonstrated in the literature. The incorporation of biomaterials into SPEs enables the sensitivity and selectivity that are akin to nature. Major advancements in both biosensors and immunosensors revolve around immobilization and interface capabilities of the biological material with the SPEs. The use of nanomaterials and sandwich-type devices has provided a means for increasing the signal response from these types of sensors. The ability to incorporate biomaterials with the potential for direct electron transfer is another growing research area in this field. Furthermore, nanoparticles-modified SPEs are already commercially available; so,it is avoided to waste long time in the synthesis and stabilization of AuNPs on the electrode, in contrast to conventional electrodes. In general, the field of SPEs, however, continues to grow and find new areas for application. We believe that the field will focus on the incorporation and interaction of unique materials, both nano and biological, in the coming years. In this review, we have overviewed the recent developments in SPEs from their fundamental understanding through to highly novel and innovative designs which in part improved analytical performance towards target analytes.

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