Toward the development of smart and low cost point-of-care biosensors based on screen printed electrodes

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Abstract
Screen printing technology provides a cheap and easy means to fabricate disposable electrochemical devices in bulk quantities which are used for rapid, low-cost, on-site, real-time and recurrent industrial, pharmaceutical or environmental analyses. Recent developments in micro-fabrication and nano-characterization made it possible to screen print reproducible feature on materials including plastics, ceramics and metals. The processed features forms screen-printed disposable biochip (SPDB) upon the application of suitable bio-chemical recognition receptors following appropriate methods. Adequacy of biological and non-biological materials is the key to successful biochip development. We can further improve recognition ability of SPDBs by adopting new screen printed electrode (SPE) configurations. This review covers screen-printing theory with special emphasis on the technical impacts of SPE architectures, surface treatments, operational stability and signal sensitivity. The application of SPE in different areas has also been summarized. The article aims to highlight the state-of-the-art of SPDB at the laboratory scale to enable us in envisaging the deployment of emerging SPDB technology on the commercial scale.

Keywords
Biochips, biosensors, electrochemical detection, recognition receptors, screen printing

Introduction
Prevention and proper treatment of diseases require rapid but accurate diagnostics by expensive, complicated and resource hungry modern diagnostic technologies which can be available only in developed countries. Global pursuit continued over the last decade to make healthcare affordable to developing and underdeveloped countries. Accordingly, the development of rapid and accurate point-of-care (POC) diagnostics has significantly reduced the diagnosis costs. Significant development has occurred in nano-sciences and microfabrication processes along with the improvement in real-time characterizations at nano-scale (Morrin et al., 2003). Screen printed electrodes (SPE) are suitable to make effective, versatile and low-cost miniature sensors capable of giving reproducible results with high sensitivity in biochemical detection (Vidal et al., 2003). Consequently, screen-printing (SP) technology has emerged as the method of choice for mass production of POC disposable biosensors. Literature published in 1992–2011 shows an exponential growth in the numbers of papers published on SP and citations received by papers in this field. In 1983–2011, China, UK and USA contributed the most in scientific and commercial advancement of SPE. In 2012 alone, 350 papers on SP have been published and SP related articles have received 7500 citations (Figure 1). Main factor behind the growth of this field is the rapid advances in multidisciplinary sciences encompassing microfabrications, nanotechnology and biosensors (Ahmed et al., 2007, 2008, 2009, 2013, 2014).

Unlike the first-generation, second-generation SPEs contain an auxiliary electrode. SPEs have addressed memory effect and complicated pre-treatment issue which limited the application of classical solid electrodes. In addition, since we can easily modify the composition of commercial inks to print working, counter and reference electrodes, SPEs offer a greater adaptation to different fields of applications. Nevertheless, there are many critical areas, such as SPE biofunctionalization, which still require improvements. Improper functionalization affects the performance of SPEs through structural alteration of biological recognition receptor (RR) through faulty attachment on electrode surface or local perturbation in micro-environment. Consequently, optimization is essential to establish a better harmony between the support and bio-chemical receptors (Nascimento et al., 2012). Scheme-of-detection also needs
to be adapted to the circumstances of the application itself. An example is the effort to avoid or minimize the tedious labeling processes which cause destructive mutations of probes. Simpler amplification and immobilization methods are also needed to assure stability and sensitivity and to improve signal-to-noise ratio (SNR). Efforts are in place to address the effect of lower mass-transport in miniaturized devices leading to a very low microelectrode current range which gets buried under electrochemical noise of SPE devices. Microelectrodes array are emerging as a solution to lower detection limits and to favor multi-dimensional biosensing (Kadara et al., 2009). Besides, application of magnetic nanobeads, conductive nanoparticles, graphene (Lim et al., 2014; Randviir et al., 2014) and carbon nanotubes (CNTs) are enhancing electrochemical mediation to amplify signals from SPEs. In addition, progress has been made towards integration of SPEs and fluid-handling and/or sample-processing devices to ensure portable POC devices. Emergence of palm top electrochemical-readers has enhanced portability and user friendliness.

In this review, we have summarized important scientific realizations in screen printed disposable biochips (SPDB). A theoretical background on SP technology has been included to elucidate the reasons behind the large impact of SP technique in biochips market. We have also described critical technical issues related to SPE biosensor development in order to explicate possible approaches to solve problems related to device performance. Besides, some related novel subjects have been introduced, such as concept in nanoelectrodes and biochip arrays. Finally, current laboratory-scale SP biosensors platforms have been highlighted which have potential to be commercialized for clinical diagnosis, environmental protection and food control.

**Screen printing technology: fundamentals**

In SP – the ink or paste, which is a viscous fluid, is squeezed by a blade (3–10 Pa at a shear rate of 230 s⁻¹) through a mesh-screen onto the substrate (Figure 2; Lanyon et al., 2006; Metters et al., 2011; Mistry et al., 2014). The mesh-screen contains pattern that defines the dimensional characteristics of electrodes (Bigeleisen, 1972). The paste connects RR...
substrate. Alumina, ceramics and plastic (polyvinylchloride, polycarbonate) are usual substrates in SP while gold, iron, silver and fiber glass are also not uncommon (Bilitewski et al., 1992; Galán-Vidal et al., 1997; Koopal et al., 1994) and interestingly, paper based systems are also emerging (Fosdick et al., 2014; Jane Maxwell et al., 2013; Santhiago et al., 2013). The formulation of paste is usually a trade-secret since it dictates the overall analytical performance and commercial value of resulting sensors (Wang et al., 1998). Carbon inks are unpopular due to high resistivity. Best inks are ultrapure conductive crystalline substances with low residual currents (Liu et al., 2001). Adhesives (e.g. resin, cellulose acetate, cyclohexanone or ethylene glycol) are added to attach the paste to the substrate. Additives may be mixed to increase specificity, sensitivity or Signal to noise ratio (SNR). Occasionally, the ink contains powdered silver, gold or platinum to enhance electrochemical signal transduction during physical or bio-chemical interactions.

RRs are attached onto the dried and cured ink through immobilization techniques such as physical adsorption or chemical cross-linking. Recent development in immobilization includes sol–gel and electro-polymerization techniques which allow better and selective analyte diffusion. A one-step layer-biocomposite deposition technique has recently been reported where the paste was a dispersion of rabbit immunoglobulin G (Ig-G) in graphite powder and a binder (Wang et al., 1998). At times, the addition of a mediator or an electrocatalyst – either incorporated into the conductive paste or attached to the main recognition receptor – alters the detection potential to a region not interfered by signals from undesired reactions (Pandey, 1998).

**Screen printing disposable biochip (SPDB): development and applications**

**Technology and conception**

Successful biosensing depends on compatibility between biological RRs and other materials – such as biocomposite, ion-selective membrane or ionophores – that constitute the physical transducer. Beside signal transduction, it prevents denaturation of biological probes and ensures a good adhesion and a reliable expansion coefficient for solid–solid interface (Bilitewski et al., 1993). Advances are made in designs to improve biochip performance, to reduce the pre-treatment requirements and to avoid cleaning of chips. Microelectrode arrays (MEA) are under investigation as they allow significant increase in temporal resolution, current densities and faradic to capacitive current ratio (Abdellaoui et al., 2013; Song et al., 2014). MEAs overcome electrochemical noise by favoring radial diffusion leading to signals many order of magnitude higher than single electrode or classical macroelectrode systems. Examples of improvement in SPE configurations are edge band screen printed ultramicroelectrode (SPUME) which is obtained through slicing the edge of the working electrode (Chang et al., 2006; Liao et al., 2007), wall-jet screen printed ring disk electrodes (Yang et al., 2009) and hole based micro-tube electrode for 3D detection in nanolitre samples (Karousos et al 2005). A list of commercialized SPDB or SPE is given in Supplemental Table S1.

**Matrix of applications**

The available SPDBs varies widely in terms their field of applications, the nature of the RRs (enzyme, antibody, DNA and cell) as well as the methods of immobilization (adsorption, covalent interaction, affinity interaction and entrapment) as summarized in Supplemental Table 2.

**Immobilization methods**

**Physical adsorption** is a rapid and simple immobilization method which fits very well to the disposability and single-application philosophy of SPEs. Nevertheless, the lack of control over bonding forces between the biological RRs and the electrode surface made this method unpopular. In contrast, covalent and affinity interactions provides more stable attachment and better orientation of RRs to the SPE (Sassolas et al., 2002). An example of covalent and affinity interactions is the gold SPE (Tolba et al., 2012) as shown in Figure 3. Cell wall binding domain (CBD) of bacteriophage-encoded peptidoglycan hydrolases (endolysin) was immobilized onto SPE for electrochemical impedance spectroscopy (EIS) based detection of *Listeria innocua* serovar 6b from pure culture and artificially contaminated milk. Bucur et al. (2004) have immobilized acetylocholinesterase to avail high affinity interactions between mannose residues and Concanavalin A (Con-A) groups of the enzyme leading to a sensitive SPDB with high operational stability.

However, RRs may get denatured due to structural changes upon immobilization and/or local physico-chemical perturbations. Therefore, direct or arbitrary immobilization of biological RRs with or without cross linking fails to ensure ideal desirable sensor performance (Kobayashi et al., 1974; Voet & Judith, 1995). Also, during experiments at reduced working potentials in the presence of electrochemical mediators, such as the bio-organometallic ferrocene derivatives, direct, covalent or affinity interactions based immobilization fails to guarantee selectivity in biosensor response. Consequently, entrapment and/or encapsulation method has emerged as a viable alternative. In this approach, RRs are entrapped into a biocompatible polymer, sol–gel or a membrane, for instance, Shi et al. (2006) entrapped acetylocholinesterase into Al2O3 sol–gel matrix that ensured a favorable microenvironment for prolonged enzyme activity and enhanced electron transfer from thiocholine to electrode. This approach does not need electrochemical mediators, minimizes interferences from impurities and yields a significant drop in the over-potential required for thiocoline detection in conventional systems. Mersal et al. (2004) entrapped glucose oxidase into a screen printing paste polymerized by UV-irradiation for repetitive use in an automated flow injection analysis (FIA) for glucose analysis in fruit juices. Silber et al. (1996) further simplified the entrapment protocol through one-step electropolymerization of a mixture of glucose dehydrogenase (GDH) in methylene blue (MB) monomers on a thick-film gold electrode (Silber et al., 1996). Though entrapment effectively retains biological activity of RRs, signal stability and biosensor selectivity; its applicability is limited by the inherent characteristics of RRs and analytes, for which immobilization free sensors have evolved.
Immobilization free detection

Redox molecules, such as Ruthenium hexaaamine \([\text{Ru(NH}_3\text{)}_6]^{3+}\) (RuHex) interact with free, non-immobilized DNAs, constitute electrostatic redox probe for fast, easy and inexpensive sensing. This approach has been successfully used for real-time monitoring of loop mediated isothermal amplification (LAMP) amplicons of target genes from *Escherichia coli* and *Staphylococcus aureus* by square wave voltametry (SWV; Ahmed et al., 2013) by avoiding cross contamination through concurrent amplification and real-time monitoring in a single polypropylene tube with biochip (Figure 4). Defever’s group modified this approach by replacing electrostatic redox interaction with DNA-intercalating redox molecules in their disposable SPDB which has an array of eight 3-electrode electrochemical-cells assembly. The chip monitored double-stranded (ds) DNA in real-time by measuring the changes in SWV peaks in the presence of different DNA intercalators with PCR mixture (Deféver et al., 2011). A 48-electrochemical platform has been reported by Kivlehan et al. (2011) for real-time detection of isothermal nucleic acids amplicons. A measurable response was detected by SWV when a stable redox intercalator, such as Oss[(bpy)_2DPPZ]_2, was bound strongly and specifically to the PCR ds-DNA products. This approach gave results comparable to optical monitoring SYBR-Green based real-time monitoring of PCR. Miranda-Castro et al. (2012) demonstrated a target recycling strategy for label-free nucleic acid assay.

The recognition receptors in SPEs

Different biomolecules – nucleic acids, enzymes, antibodies, antigens – have been used as RRs in SPDBs. Biomolecular interactions enable faster detection than nucleic acids which offer higher specificity and sensitivity. DNA-probes are easy to prepare due to wide range of physical, chemical and biological properties of nucleic acids which makes them easy to immobilize (Dequaire & Heller, 2002; Wang et al., 1997a,b). However, these sensors exhibit lower detection limits as they need optimum pH, temperature and salinity for successful hybridization (Zhang et al., 2009). The DNA–enzyme hybrid system of Evtugyn et al.(2003) was based on DNA–enzyme–graphite SPE that detected human systemic lupus erythematosus (SLE) and bronchial asthma antibodies.
**Enzymes** are costly to extract, isolate and purify – yet they are the most popular RR in SPE biosensors owing to their specific and rapid interaction. Enzymes offer more readability and flexibility as we can genetically modify their catalytic properties and/or substrate specificity (Rogers, 2006; Tudorache & Bala, 2007). In order to reduce the cost of electrode surface functionalization in disposable electrochemical chips, we need stable enzymatic reactions which can be achieved through cross-linking (Gonzalo-Ruiz et al., 2007; Ricci et al., 2005) or probe crystallization methods (de Mattos et al., 2003). Using a water-based carbon ink, Crouch et al. (2005) has constructed a disposable amperometric glucose sensor which addressed the risk of enzyme denaturation at high temperatures. In health and environmental sensing, rapid detection at low cost using portable POC SPDB is desirable. Disposable SPEs interfaced to a small electrochemical transducer such as differential pulse anodic stripping voltammetry (DPASV) can be a good option to this end. Yan et al. (2012) demonstrated the possibility of a portable system based on enzyme immobilization on a paper cartridge for flow injection electrochemical biosensing.

Antibody–antigen based biosensors or immunosensors make use of stable and specific bioaffinity reactions having excellent sensitivity and selectivity (Yan et al., 2012). However, SPE regeneration is not possible in irreversible bio-affinity interaction between antibody and antigen while enzyme based SPEs are easy to regenerate due to reversible enzyme–substrate interaction. Electrochemical immunosensors usually require antigen or antibody labeling (Darain et al., 2003) by radioactive compounds, enzymes, fluorophores, chemiluminescence metals, latex particles and liposomes (Warsinke et al., 2000). Bagel and his co-workers developed a disposable electrochemical sensor for human chorionic gonadotropin hormone using an ion exchange film-coated SPE labeled with alkaline phosphatase (Bagel et al., 2000). In immunosensing, monoclonal antibodies usually offer greater specificity than polyclonal antibodies. It suggests the possibility of eliminating enzymatic labels using redox molecules as a probe in the case of monoclonal antibodies (Duhachek et al., 2000). Enzyme-antibody hybrid has promising biomedical POC diagnostic applications, for instance, phosphorylated proteins in human plasma by a multiplexed electrochemical immunoassay based on integrated enzyme amplification and electric field driven strategy (Du et al., 2011). The system, comparable to ELISA in performance, consisted of four separate cross-talk-proof working electrodes with four different capture antibodies immobilized onto them. Gold nano-rods enhanced the sensor sensitivity by acting as nano-carrier for multi-enzyme amplification.

**Microbial biosensors** attaches the bacteria, tissue or yeast cells on appropriate electrode surfaces to employ the whole cell as RRs. Cells are directly immobilized onto the SPE or mounted separately in a reaction chamber to record their metabolic responses upon loading the samples of interest. Success in this area is limited due to poor selectivity and difficulty in measuring electrochemical exchanges between cells and electrode surface (Lanyon et al., 2006; Rogers, 2006) where scope of improvements lies. Microbial sensors can be useful in the detection of water pollution and soil quality evaluation (Lanyon et al., 2006; Riedel, 1994). A SP whole cell biosensor has been reported for herpes simplex virus (HSV) detection (Kelso et al., 2000) and a bacteria-based sensor has been found useful for mono and disaccharide detection in an FIA (Held et al., 2002).

**Nanostructure modified SPE**

Multi-wall carbon nanotubes were used in enzyme based biosensors for higher sensitivity and faster analysis (Guan et al., 2005). Used nanoparticulate membrane on carbon electrode for glucose regulation (Gao, 2005). A nanomaterial-modified disposable potentiometric planar strip-cell has determined K⁺-concentration in saliva and beverages with precision comparable to atomic emission spectrometry (AES). SWCNT-Octadecylamine (ODA) was used as ion to electron transducer layer (Rius-Ruiz et al., 2011). Miniature poly-n-butyl acrylate (nBA) solid-contact potentiometric ion selective electrode (SCISE) and solid-contact reference electrodes were created onto a plastic matrix by SP and drop-casting techniques (Figure 5). The sensor did not require maintenance during long dry storage, ensured quick signal stabilization and
rendered the strips insensitive to light. The platform is suitable for biomedical application in hypertension, renal failure, cardiac distress etc.

Ultrasensitive multiplex immunoassay has been reported that combined alkaline phosphatase (ALP)-labeled antibody-functionalized gold-nanoparticles (ALPAb/Au-NPs) for enzyme-Au-NP catalyzed stripping analysis of deposited Ag-nanoparticles on a disposable array (Lai et al., 2011). Their sensor exhibited stability, reproducibility and accuracy for ultimate clinical applications. Figure 6 represents an all solid-state graphene-film modified electrode for Ca²⁺ detection with Ca²⁺-ion-selective membrane which showed insensitivity to oxygen, water, light and redox-species (Ping et al., 2002).

**Advances in chip configuration**

Improvement of analytical performance of the SPDB is an exciting area of research (Dequaire & Heller, 2002; Iqbal et al., 1995; Zhang et al., 2009) through standardization of electrochemical measurements or bio-functionalization or modified chip configurations for sensitive and stable SPDBs (Challier et al., 2013). Disc, ring and band are the most fundamental configurations for SPDBs (Evtyugyn et al., 2003; Rogers, 2006). Konash et al. (2009) studied that carbon-ink-based micro-tubular band-electrode in fixed-volume enzyme-reaction well can be scaled up into a strip-configuration of an array of mechanically or optically fabricated well-electrodes to be connected to a multichannel readout. MEAs offer advantages over conventional SPEs by allowing multidimensional biosensing with higher SNR, low ohmic drop, rapid mass transfer and high throughput biosensing to save time, energy and money (Bond, 1994; Corgier et al., 2007; Xie et al., 2005). In contrast, Mann & Mikkelsen’s (2008) microwell based SPDB have 16-working electrodes on a Teflon-stabilized foam pad. Recently, a wearable screen-printed electrochemical sensor on neoprene substrate has been reported that detect the presence of trace heavy metals contaminants and nitroaromatic explosives such as 2,4,6-trinitrotoluene (TNT) in seawater (Malzahn et al., 2011). The necessity to further miniaturize electrode geometry and dimensions, to monitor electrochemical phenomenon at the level of single cell, particle and molecule, has pushed forward research on nano-scale electrodes in the last three decades. Nanoelectrodes provide exceptional mass transport rates with enhanced radial diffusion to reach a steady-state voltammetric response and are especially suited to high-resistance solutions of real samples. Wu et al. (2005) has used nanoelectrodes to map exocytotic activities of cells corresponding to dopamine through amperometric method. In addition, the development of a novel SPE or SPDB device to conduct UV/vis absorption spectroelectrochemistry (SEC) has been reported by Dieguez’s group (Yamanaka et al., 2011) for quantitative determination of neurotransmitter dopamine at physiological pH in the presence and absence of interfering compounds. Palleschi’s group has developed an array immunosensor without any loss in sensitivity comparing to the 1-dimensional detection (Piermarini et al., 2007).

**SPE on microfluidics platforms**

Real-time measurements are desirable in many applications, which necessitate the integration of the sample preparation and the handling platform to the sensing unit. Microfluidic platform has emerged as the best choice to accomplish it. Delaney’s group has combined inkjet-printed paper-microfluidics with electrochemiluminescent (ECL) detection (Delaney et al., 2011) into a low cost, disposable sensor for being used with conventional photo detector or a camera phone (Figure 7).

Fantuzzi et al. (2010) combined a polymethylmethacrylate (PMMA) microfluidic system with a P450-electrode that required a mere 30 µL sample to electrochemically determine the interaction and affinity rankings of P450 and other drugs for drug discovery. The cell (Figure 8) has 2-electrode strips – gold working electrode modified with covalently bonded human cytochrome-P450-3A4 via self-assembled monolayers of (1:1) 6-hexanethiol and 7-mercaptoheptanoic acid with screen printed carbon and Ag/AgCl inks on polyethyleneterephthalate as counter/reference electrodes.
A highly specific and sensitive SPDB was developed to detect *Escherichia coli* in which the microfluidic part handled loop-mediated isothermal amplification (LAMP). LAMP amplicons were detected by linear sweep voltammetry (LSV) using their interaction with DNA groove binder Hoechst33258 redox molecule (Safavieh et al., 2012; Figure 9).

It also was integrated with a Cassette format device (Safavieh et al., 2014b,c) using OS Redox for real-time electrochemical monitoring of LAMP amplicon following by bacteria quantification (Safavieh et al., 2013, 2014a). This approach did not require DNA immobilization or DNA extraction and purification steps (Safavieh et al., 2012, 2014a). A microfluidic chip was used for sample pre-concentration in electrochemical detection of apolipoprotein E (ApoE) for diagnosis of Alzheimer’s on SPE where cadmium-selenide/zinc-sulfide (CdSe–ZnS) quantum dots (QDs) have been used as labeling carriers (Medina-Sanchez et al., 2014).

**Field of applications and analyte handling**

The nature of analytes dictates the features of the SPDB, for example, water and soil pollutants do not require highly specific probes unlike health care applications (Singh & Mittal, 2012). Real analytes need filtration to eliminate impurities. Entrapment of enzymes into polymer is an effective approach to block interfering agents. Tidineprepolymer (PAP) and polyvinyl alcohol photopolymer entrapped laccase on SPE surfaces to filter impurities in the analysis of phenolic compounds (Ibarra-Escutia et al., 2010). Similarly, pesticides sensors have been constructed by using magnetic Fe₃O₄-inorganic-core in acetycholinesterase immobilized mesoporous silica-shell (Won et al., 2009). Li et al., (2011) have used DPASV analysis to detect photocatalytical degradation of organophosphorus pesticide (OP) dichlofenthion by a photoelectrochemical sensor consisting of a nanometer-sized titania coupled with SPE. A portable, automated SP amperometric array-biosensor has been reported by Hart’s team for the detection of multiple OPs in food and environmental samples (Crew et al., 2011).

SPE immunosensor for food-allergen has been developed by Saito et al. (2008) which used differential pulse voltammetry (DPV) based label-free detection of *casein* in milk by its interaction with anti-casein antibody. Proteins are another important class of analytes which can be detected by electrochemical methods by gold-linked-electrochemical-immunoassay (GLEIA) as reported by Idegami et al. (2008). It offered a 36 pg/mL limit of detection (LOD) for human Chorionic Gonadotropin (hCG) – the pregnancy biomarker – on Carbon SPE using DPV. Viet et al. (2013) increased GLEIA’s LOD to 2.4 pg/mL by applying SWCNT microelectrodes. SPE biosensors have been applied in screening food samples for marine toxins (okadaic acid, domoic acid and brevetoxin) to nmL⁻¹ LOD (Kreuzer et al. 2002; Micheli et al., 2004), insecticides (Zhang et al., 2005) and other targets such as glycerol (Radoi et al., 2007),
dichlorophenoxyacetic acid (Shyuan et al. 2006), vitamin B1 (Kadara et al., 2006) and lean meat agents which are major food-safety concern (Li et al., 2003). A quick and portable geno-sensor has been reported which detect the meat species – pork, chicken and bovine – in raw or processed food by LSV observation of LAMP generated species specific DNA amplicons through DNA-Hoechst 33258 interaction (Ahmed et al., 2010). Tamiya and coworkers has developed the DNA stick (Figure 10) – DNA amplification system – to be integrated with SPDB (Ahmed et al., 2009).

Biomedical applications of disposable SPDBs are broad as they offer cost-effective, fast and sensitive diagnosis. SPDBs have been designed for rapid detection of glucose (Kumar & Zen, 2002), cholesterol (Foster et al., 2000), hormones (Volpe et al., 2006), cells (Crowley et al., 1999; Rao et al., 2006), viruses (Schüler et al., 2009), drugs (Luangaram et al., 2002) and tumors markers (Wu et al., 2006). Also, diffusion redox-probe has been designed for pathogenic bacteria detection (Varshney & Li, 2009). Single nucleotide polymorphisms (SNPs) sensors have also been reported (Ahmed et al., 2007). Efforts are also in place to develop a sensor to analyze serum directly, for example, Guan et al. (2004) quantified the common tumor marker α-1-fetoprotein in human serum samples. However, use of electroactive compounds such as prussian blue, cobalt-phthalocyanine (CoPC) or carbon nanotubes (CNTs) and conductive polymers are necessary to reduce the working potential and avoid interferences from serum media (Abdul-Aziz & Wong, 2011).

In order to detect heavy metals in different environmental and biological fluids, the application of a mercury film onto the working electrode surface led to electrochemical concentration by anodic stripping voltammetry (ASV; Choi et al., 2001; Zhu et al., 2005). This approach has been adapted by Parat et al. (2007) to detect trace Cd(II). However, the toxicity of Hg has led to the development of a bismuth-based novel sensor for single step Pb(II) with 5–50 ppb LOD (Fang et al., 2011). The entire field of SPEDB is moving towards POC applications where rapid improvements are discernible (Corrigan et al., 2013; Wang et al., 2013).

**Trends and future outlook**

Scientific enthusiasm towards SPDB devices is increasing. The cutting edge of SPE biosensor research focuses on the improvement of thick-film electrodes and on nanomaterials to improve electron transfer. On the other hand, integration of more components on a single SPDB through miniaturization is necessary to make disposable and scalable SP-MEAs to move toward the cost-effective “Lab-on-a-chip” strategies. However, the major hurdles remained in the immobilization of RR, redox mediators and other additives such as cofactors and functional molecules. Entrapment of biomolecules in an electro-generated polymer or within conductive paste or ink (so-called “biological ink”) is rapidly becoming a popular area of research due to its
ability in resolving issues related to automation and commercialization.

**Conclusions**

Electroanalytical methods offer simplicity, portability, lower detection limit, inexpensiveness and wide options for future improvements. This review elucidates the state-of-the-art in the field of screen printed disposable bioschips. Different areas of active research and achievements in those areas have been compared. The challenges related to SPEs in terms of substrates, screen printing pastes, types of recognition receptors and their immobilization have been discussed in the light of contemporary advances. We also have highlighted the challenges related to better association between the biological and non-biological material, structural and functional characteristics of ink and the immobilization approach required to fix the recognition receptors. SP biosensors will perform better through the adoption of new configurations such as MEA, SP-edge-band ultra-microelectrode (SPUME) and hole-based micro-tube electrodes. In the near future, SPDBs devices will undoubtedly revolutionize POC measurements.

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