



Graphene-based screen-printed electrochemical (bio)sensors and their applications: Efforts and criticisms



Stefano Cinti^{a,b}, Fabiana Arduini^{a,b,*}

^a Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", Via della Ricerca Scientifica, 00133 Rome, Italy

^b National Institute of Biostructures and Biosystems, Viale delle Medaglie d'Oro 305, 00136 Rome, Italy

ARTICLE INFO

Article history:

Received 1 December 2015

Received in revised form

22 June 2016

Accepted 3 July 2016

Available online 5 July 2016

Keywords:

Graphene

Electrochemical biosensor

Electrochemical sensor

Screen-printed electrode

ABSTRACT

K.S. Novoselov in his Nobel lecture (December 8, 2010), described graphene as “*more than just a flat crystal*” and summarized the best possible impression of graphene with (i) *it is the first example of 2D atomic crystals*, (ii) *it demonstrated unique electronic properties, thanks to charge carriers which mimic massless relativistic particles*, and (iii) *it has promise for a number of applications*. The fascinating and unusual properties of this 2D material were indeed recently investigated and exploited in several disciplines including physics, medicine, and chemistry, indicating the extremely versatile and polyedric aspect of this nanomaterial.

The utilization of nanomaterials, printed technology, and microfluidics in electroanalysis has resulted in a period that can be called the “Electroanalysis Renaissance” (Escarpa, 2012) in which graphene is without any doubt a forefront nanomaterial. The rise in affordable fabrication processes, along with the great dispersing attitude in a plenty of matrices, have made graphene powerful in large-scale production of electrochemical platforms. Herein, we overview the employment of graphene to customize and/or fabricate printable based (bio)sensors over the past 5 years, including several modification approaches such as drop casting, screen- and inkjet-printing, different strategies of graphene-based sensing, and applications as well. The objective of this review is to provide a critical perspective related to advantages and disadvantages of using graphene in biosensing tools, based on screen-printed sensors.

© 2016 Elsevier B.V. All rights reserved.

Contents

1. Introduction	108
2. Fabrication of graphene-based screen-printed electrodes	108
2.1. Screen-printed electrodes modified with graphene by drop casting	108
2.2. Screen-printed electrodes modified with graphene by ink-printing	109
2.3. Graphene based screen-printed electrode production using graphene-ink	110
2.4. Screen-printed electrodes modified with graphene by electrodeposition	110
2.5. Other methods	110
3. Biosensors based on graphene and screen-printed electrodes	111
3.1. Enzymatic biosensors	111
3.2. Immunosensors	113
3.3. DNA sensors	114
4. Electrochemical sensors	115
4.1. Hydrogen peroxide (H ₂ O ₂)	115
4.2. Ascorbic acid (AA), uric acid (UA), and dopamine (DA)	115
4.3. Glucose	115
4.4. Phenol-containing compounds	116
4.5. Heavy metals: cadmium and lead	116
4.6. Miscellaneous	118

* Corresponding author at: Dipartimento di Scienze e Tecnologie Chimiche, Università Roma Tor Vergata, Via della Ricerca Scientifica, 00133 Rome, Italy.

E-mail address: fabiana.arduini@uniroma2.it (F. Arduini).

5. Remarks and conclusions	118
Acknowledgments	121
References	121

1. Introduction

Andre K. Geim and Konstantin S. Novoselov described in their article (Novoselov et al., 2004) the remarkable observation of the electric field effect in thick monocrystalline graphitic film: the film remains metallic, continuous and of high quality down to a few atomic layers. Graphene, obtained from pyrolytic graphite by an easy exfoliation technique, was the name given to those single layers of carbon atoms. These 2D sheets, in which carbon atoms are covalently bonded into a sp²-atom network, reveal them as a cornucopia of new physics, leading to plenty of potential innovative applications (Geim and Novoselov, 2007).

Graphene possesses extraordinary properties, such as large surface area, thermal conductivity, charge carrier mobility, and mechanical strength, respectively measured equal to above 2600 m² g⁻¹ layer⁻¹, 3000 W mK⁻¹, 10,000 cm² V⁻¹ s⁻¹, and 1 TPa (Stankovich et al., 2006; Balandin, 2011; Mayorov et al., 2011; Lee et al., 2008; Geim and Novoselov, 2007).

Chosen for its outstanding properties, graphene has potential, attractive applications in many fields such as solar cells (Wang et al., 2008), photonics (Bonaccorso et al., 2010), energy storage (Yang et al., 2010), GHz transistors (Lin et al., 2010), and nanoelectronics (Eda et al., 2008). Regardless of these scenarios, the production of graphene represents a real challenge nowadays (Avouris and Dimitrakopoulos, 2012; Torres and Kaner, 2014). Depending on the specific application, there are several methods to mass-produce graphene, which allows for a wide range of graphene size, quality, and price (Novoselov et al., 2012). Mechanical and chemical graphite exfoliation (Dreyer et al., 2010), epitaxial growth on silicon carbide (Virojanadara et al., 2008), chemical vapor deposition (CVD) (Obraztsov, 2009), and “unzipping” of carbon nanotubes (CNTs) (Kosynkin et al., 2009) represent just some of the dozen methods developed to obtain graphene. In spite of the discovery of graphene sheet achieved by a mechanical cleavage, the low productivity of this approach makes it unsuitable for large-scale use. Chemically-exfoliated graphite and chemical reduction of exfoliated graphite oxide allow for an efficient and cost effective production of bulky graphene sheets (McAllister et al., 2007; Stankovich et al., 2007). A powerful feature of graphene is seen in its flexibility for the creation of dispersions and nanocomposites. However, when discussing on “graphene”, the terminology should be addressed very carefully. Depending on the number of layers (single, few, multi) and the carbon source (graphite, CNTs), it is possible to obtain various form of chemically modified graphene, including graphene oxide (GO), reduced graphene oxide (rGO) or graphene nanoribbons (GNRs) after unzipping the CNTs. With respect to considered material, many differences could be observed (Martin and Escarpa, 2014). Due to its ease of synthesis and satisfying dispersion processability, graphene becomes inexpensive and attractive for mass-scale applications in a conventional technological field, such as the development of electrochemical devices (Li et al., 2008a). However, large-scale and low-cost production of electrochemical devices is not attributable just to graphene or other innovative materials. Concerning electrochemical (bio)sensors, a critical role towards cost lowering is certainly attributable to the electrodes utilized. Economic criteria should not, in any case, reduce the reproducibility and sensitivity of the detection methods towards target analytes. Furthermore, it is also necessary to take into account the

time consumption involved in the production/modification/treatment of electrodes. Printing technology has appeared, in the last twenty years, as the most favorable methodology for the high-volume serial production of reliable single-use devices, keeping high both their affordability and analytical performance. Printed technology has contributed tremendously in the development of point-of-care (POC) devices. Taken from an IDTechEx report entitled “Printed and Flexible Sensors 2015–2025: Technologies, Players, Forecasts” released in 2015, the market of printed, flexible, and organic electronics is rapidly growing and will be worth more than \$60 billion by 2025 (Das and Harrop, 2013).

Customization of conductive inks, substrates, and patterns, has made screen-printed electrodes (SPEs) a versatile tool in accordance with the analytical requests, particularly thanks to their suitability to be modified. SPEs can be modified by incorporating enzymes, nucleic acids, metals, polymers, or electrochemical mediators, directly in the ink or subsequently by using drop-castable dispersions, as reported in a wide number of reviews (Metters et al., 2011; Li et al., 2012; Windmiller and Wang, 2013; Taleat et al., 2014; Thiagarajan et al., 2014; Arduini et al., 2016). Graphene represents one of the most used materials as electrode modifier, thanks to its excellent properties in dramatically enhancing the performance of various electroanalytical systems (Lawal, 2015).

This review will provide a comprehensive overview focused on the employment of graphene and graphene-based composites in screen-printed electrochemical sensors and biosensors. The latest developments regarding SPEs modified by means of this two-dimensional material, will be critically discussed in order to rationalize which are the advantages and drawbacks, in terms of electrocatalysis, sensitivity, inorganic and biomolecular loading, and costs for electrochemical (bio)sensor development.

2. Fabrication of graphene-based screen-printed electrodes

Depending on the specific need and economic opportunities/facilities, different strategies exist nowadays with respect to the manufacturing and modification of printed electrodes (Albareda-Sirvent et al., 2000; Laschi and Mascini, 2006; Dominguez Renedo et al., 2007; Singh et al., 2010). The widely adopted fabrication of printed sensors is based on thick-film technology, with the production of SPEs being the most used printed sensors reported in literature. The peculiarity of disposable SPEs is observed by the enormous possibility for mass utilization, allowing easy customization, coupled with effective portability, cost-effectiveness, and considerable application directly on field (Metters et al., 2011). Graphene has been involved in this kind of customization because of its prominent electrochemistry (Brownson et al., 2012).

2.1. Screen-printed electrodes modified with graphene by drop casting

The most widely used approach to modify SPEs, over the large-scale, is certainly represented by drop casting of graphene dispersion over an underlying substrate. Depending on production methods and chemical functionalizations, graphene can be obtained as dispersion by utilizing various solvents (e.g. water, ethanol, acetone, hexane, dimethylformamide (Paredes et al., 2008)). This method

facilitates the study of the effect of the dispersion composition on the detection capabilities, tailoring analytical features like sensitivity, detection limit, and reproducibility. Additionally, the study requires small amount of dispersible material. Cysteine-GO, easily dispersed in water, has been drop-cast onto SPE, producing with gold nanorods a 3D-layered gold nanorods-containing structure. These modified SPEs showed a better electrochemical performance with respect to the unmodified ones. Because of its high surface area, graphene allowed to minimize the formation of electrochemically poor catalyst-aggregates towards hydrogen peroxide detection (Xue et al., 2015). Graphene offers many possibilities in the choice of dispersing media, depending on the task it should accomplish. By dispersing graphene into poly(sodium 4-styrenesulfonate) (PSS), drop cast SPEs highlighted an increase of sensitivity towards detection of heavy metals (Huangfu et al., 2013). Even the biocompatible chitosan has been used as dispersing media to enhance the film formation of graphene onto SPE (Apetrei et al., 2016). Sometimes, as in the case of the nanocomposite formed by platinum, copper oxide, and rGO, an organic solvent such as dimethylformamide has been needed to obtain a satisfying dispersion (Dhara et al., 2014). SPEs have been quickly drop cast without any ink-related issues. Yet, particularly in laboratory-scale processes, this approach could be characterized by some drawbacks as the low homogeneity of the resulted modified-surface. A phenomena known as “coffee ring” effect was observed,

due to capillary forces present as a result of solvent evaporation which can push the modifier, e.g. graphene, platelets to the edges of the underlying electrode (Deegan et al., 1997). These devices may lack in reproducibility due to graphene agglomeration. However, the issue related to this feature can be easily addressed by using automatic dispensing system as Biodot(R) (www.biodot.com), by means of a spray deposition (Gilje et al., 2007) or by using more sophisticated instrumentations such as an inkjet printer, which allows for the deposition of picolitre drops (Calvert, 2001).

2.2. Screen-printed electrodes modified with graphene by ink-printing

Inkjet-printing has been largely utilized as a SPE-modifying approach, by using graphene as well as a variety of nanomaterials. The advantages rely on most controllable dispersion deposition because inkjetted drops rapidly evaporate. Tuantranont's group has reported on the use of a Fujifilm Dimatix inkjet-printer, to rapidly modify SPEs by-means of a graphene-poly (3,4-ethylenedioxythiophene): poly(styrene-sulfonate) (GR-PEDOT:PSS) dispersion. Many analytes have been detected with modified-SPEs, obtaining satisfactory reproducibility with the opportunity to rationally tune the performance modulating the number of printed graphene layers (Karuwan et al., 2012; Sriprachubwong et al., 2012).

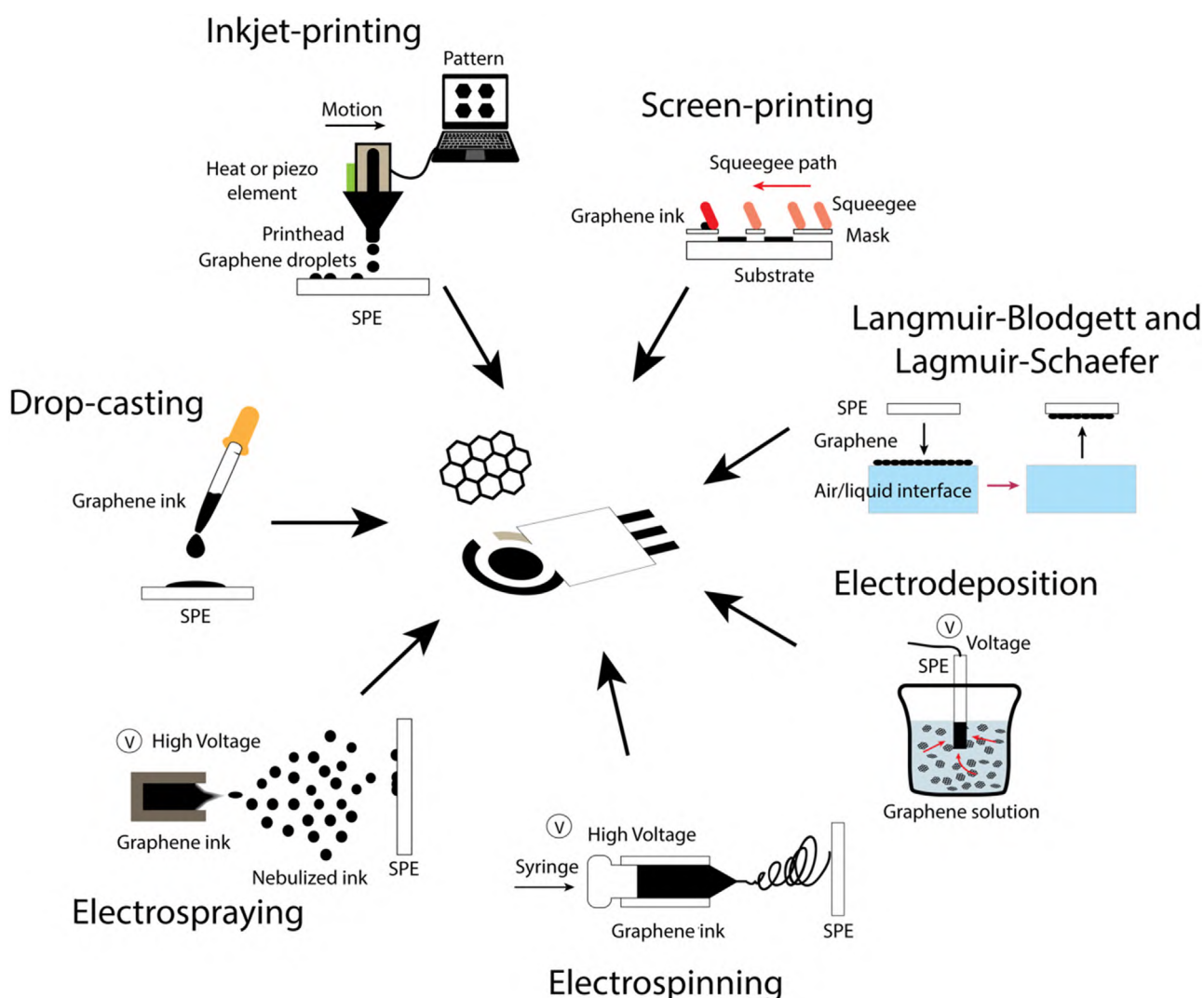


Fig. 1. Scheme of the several procedures utilized for fabrication of SPEs modified with graphene.

2.3. Graphene based screen-printed electrode production using graphene-ink

Another approach for graphene-SPE production, is thick-film technology using ink modified with graphene, since graphene can be easily incorporated into the conductive-ink (often graphite-based). Currently, many reputable manufacturing companies are leading the large-scale market of inks and SPEs, such as DropSens, Zensor, Kanichi, Gwent (www.dropsens.com; www.zensor.com.tw; www.kanichi-research.com; www.gwent.org). SPE modified with graphene (GR-SPE) manufactured by DropSens, code: DRP-110GPH, is one of the most literature-reported commercialized SPE, finding applications in several fields (Eissa and Zoroub, 2012; Antiochia and Gorton, 2014; Guiberteau-Cabanillas et al., 2015). Commercially available printable graphene inks (Randviir et al., 2014), or lab-synthesized powder mixed into conductive ink (Ping et al., 2012a), can be used to produce lab-made GR-SPE. Yet, even if screen-printing represents nowadays one of the most promising technologies, particularly towards miniaturization, the plausibility of this approach reflects in some drawbacks too. Rheological properties of the inks should be taken into account: viscosity represents the major limitation in modifying the ink with a powder.

2.4. Screen-printed electrodes modified with graphene by electrodeposition

On the other hand, SPEs can be easily modified by electrodepositing graphene on the surface of the working electrode. Many works have been reported on the use of this kind of approach, utilizing a plenty of different graphene-containing mixtures. Typically, SPEs are modified by cathodic amperometry or cyclic voltammetry, as widely reported in literature (Yang et al., 2013; Jian et al., 2013; Ping et al., 2014; Istrate et al., 2016). Electrochemical methodologies represent an accurate method to

modify the electrode surface, such as control of the film thickness, but they contain intrinsic limitations that make it difficult to extend them to a large-scale production.

2.5. Other methods

In addition to these four mainly adopted approaches, other manufacturing strategies have been employed. Langmuir-Blodgett (LB) can be utilized to manufacture an organized layer of graphene onto a vertical substrate (Li et al., 2008b). Langmuir-Schaefer (LS) is another approach in which the deposition is done by dipping the substrate horizontally, in contact with the monolayer as reported by Michopoulos et al. (2014) who modified SPE with a film containing GO-Prussian Blue (PB) hybrid nanocomposite. The recently innovative method for SPE modification with graphene involved electrospray deposition. Graphene has been efficiently electrosprayed onto SPE by means of a custom-built system as reported by Henry's group (Ruecha et al., 2015), demonstrating a low-cost alternative solution to more often-used methodologies. Electrospinning is also a recent technique employed for SPE modification. In this process, a polymer solution is injected from a needle in the presence of an electric field (Greiner and Wendorff, 2007). This is a simple and cost-effective technique, as demonstrated by Promphet et al. (2015) who electrospun a composite made by graphene, polyaniline and polystyrene to detect heavy metals. The main drawback of this approach is the necessity of a polymer matrix.

However, the suitability to modify SPEs with graphene offers a wide range of possibilities, and this paragraph includes the mainly adopted modification procedures schematized in Fig. 1. There is not a "right" method to incorporate graphene into a screen-printed electrode, but the chosen approach depends solely on the analytical requirements and on the economical possibilities.

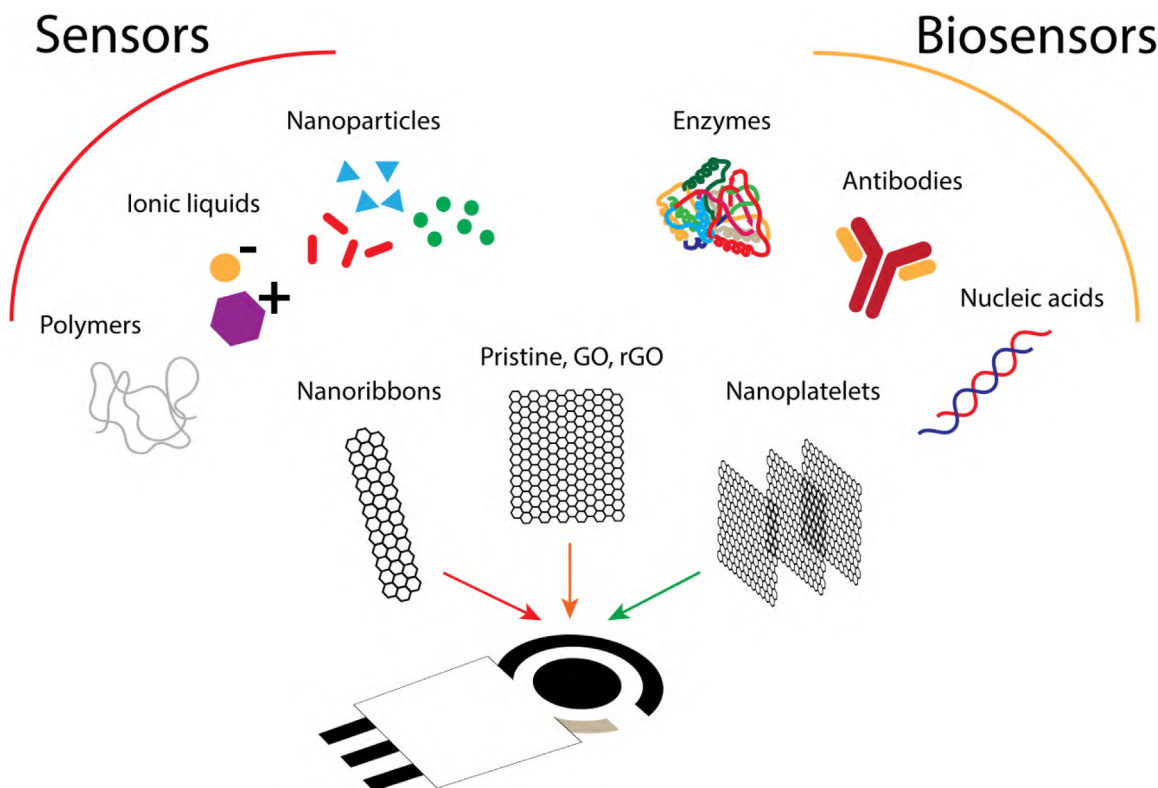


Fig. 2. Scheme of the different graphene-based screen-printed electrochemical (bio)sensors.

3. Biosensors based on graphene and screen-printed electrodes

According to the technical report published on *Biosensors and Bioelectronic* in 2001, an electrochemical biosensor is defined as a self integrated device capable of providing quantitative or semi-quantitative analytical information using biological recognition element (biochemical receptor like enzyme, DNA sequence, and antibody) in direct spatial contact with the electrochemical transduction element. Biosensors may be classified according to the biological specificity-conferring mechanism or, alternatively, to the mode of physio-chemical signal transduction (Thevenot et al., 2001). Since this review focused on electrochemical transduction using SPEs, the classification of biosensors were made as a function of biocomponent immobilized onto the surface of the working electrode, including enzymatic biosensors, immunosensors, and DNA sensors (Fig. 2).

3.1. Enzymatic biosensors

Glucose biosensors based on the use of glucose oxidase are, without any doubt, the most studied enzymatic biosensors, beginning with the first biosensor designed by Updike and Hicks (1967), to the first pen-sized biosensor launched in market by Medisense Inc. (1987) for self-monitoring of blood glucose by diabetic patients, and arriving at the recent innovative biosensors based on SPEs modified with graphene. The importance of the

glucose biosensor is related to the necessity of glucose self-monitoring by diabetic patients, and taking into account that this disease affects roughly 150 M people worldwide (Wang, 2008; Privett et al., 2008; Zhu et al., 2012; Scognamiglio, 2013). Daily, millions of diabetics test their glycemic levels by using commercial biosensor strips. 85% of the entire biosensor market is hosted by glucose biosensors (Turner, 2013).

The detection of glucose using GR-SPEs was focused on the detection of enzymatic product as well as direct electron transfer of glucose oxidase.

To our knowledge, the first glucose biosensor employing graphene and a SPE was reported in 2011 (Ping et al., 2011). Ping and coworkers deposited rGO by one-step electrodeposition of the exfoliated GO sheets onto the ionic liquid doped SPE. The modified electrode has demonstrated the ability to detect hydrogen peroxide (enzymatic product) at negative applied potential (-0.2 V vs Ag/AgCl) with a linear range comprised from 0.15 μ M to 1.8 mM. This sensor was then used as platform to develop a glucose biosensor by immobilising the glucose oxidase by using a cross-linking method using glutaraldehyde. The glucose was detected with sensitivity of 22.78 μ A mM $^{-1}$ cm $^{-2}$, linear range up to 10 mM, and detection limit of 1.0 μ M. The suitability of the biosensor was challenged in milk samples with satisfactory recovery values, indicating the potential application of this biosensor for determination of glucose in a real sample.

The detection of glucose by direct electron transfer of glucose oxidase was reported by Witsitsoraat et al. (2013) and Chia et al.

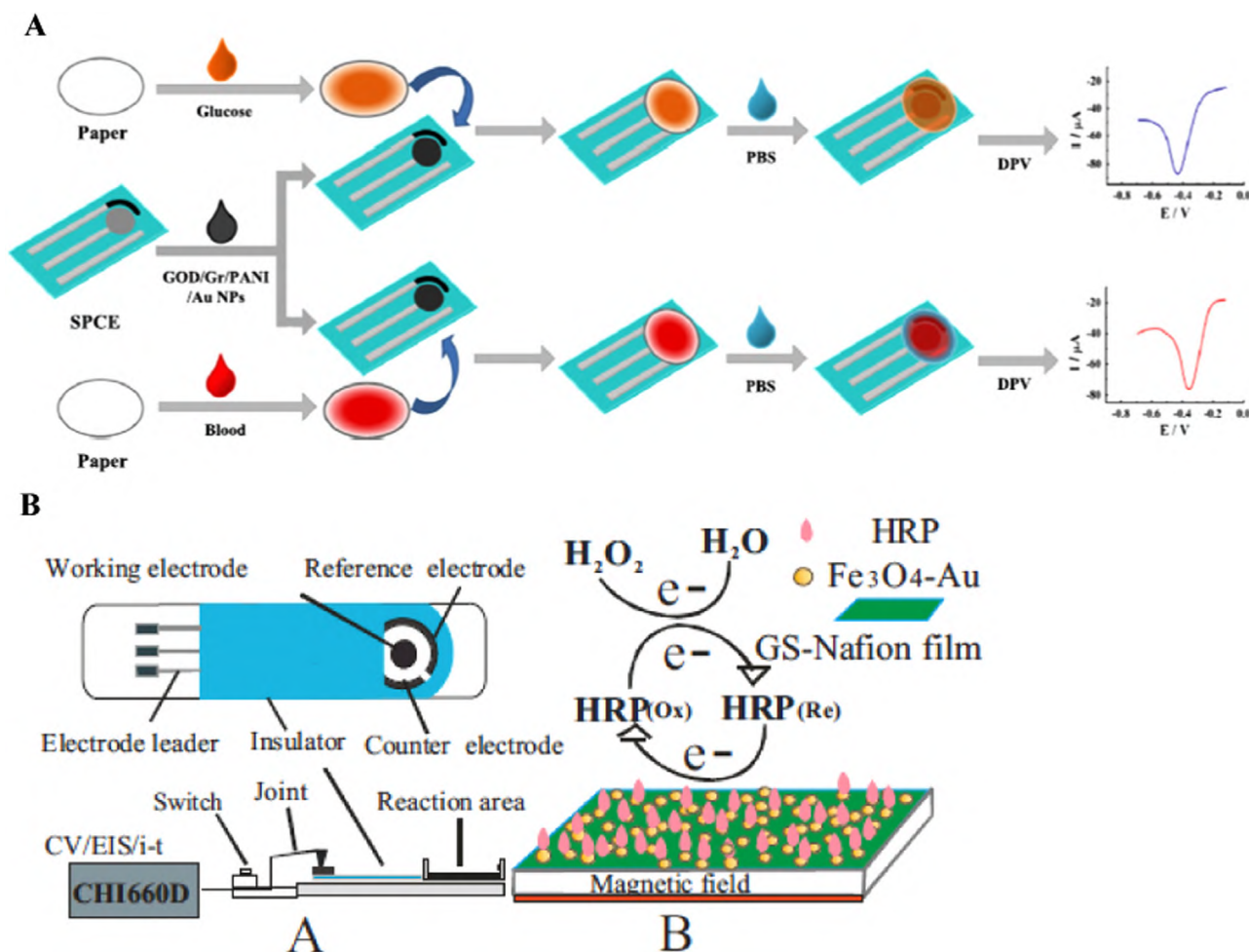


Fig. 3. Schematic representation of: (A) paper-based enzymatic glucose sensor device using glucose oxidase immobilised onto graphene modified SPE and a paper disk for sample loading (Kong et al., 2014); (B) disposable enzymatic biosensor for hydrogen peroxide determination using GR-SPE and Fe₃O₄-Au magnetic nanoparticles coated with horseradish peroxidase (Xin et al., 2013). Reprinted with permission from Ref. (Kong et al., 2014; Xin et al., 2013).