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Review

Layer-by-layer self-assembly and electrochemistry: Applications in biosensing and bioelectronics

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ABSTRACT

This paper provides an overview of different nanostructured architectures utilised in electrochemical devices and their application in biosensing and bioelectronics. Emphasis is placed on the fabrication of nanostructured films based on a layer-by-layer (LBL) films approach. We discuss the theory and the mechanism of charge transfer in polyelectrolyte multilayer films (PEM), as well as between biomolecules and redox centres, for the development of more sensitive and selective biosensors. Further, this paper presents an overview of topics involving the interaction between nanostructured materials, including metallic nanoparticles and carbon materials, and their effects on the preservation of the activity of biological molecules immobilised on electrode surfaces. This paper also presents examples of biological molecules utilised in film fabrication, such as DNA, several kinds of proteins, and oligonucleotides, and of the role of molecular interaction in biosensing performance. Towards the utilisation of LBL films, examples of several architectures and different electrochemical approaches demonstrate the potential of nanostructured LBL films for several applications that include the diagnosis and monitoring of diseases. Our main aim in this review is to survey what can assist researchers by presenting various approaches currently used in the field of bioelectrochemistry utilising supramolecular architectures based on an LBL approach for application in electrochemical biosensing.

Contents

1. Introduction

The continuous progress in the field of bionanotechnology has become crucial for the development and improvement of new and interesting architectures applicable to biosensors (Gerard et al., 2002; Decher et al., 1994; Chaki and Vijayamohana, 2002; Ricci and Palleschi, 2005; Zacco et al., 2006, 2007; Cosnier, 1999; Siqueira Jr. et al., 2010; Bossi et al., 2007). An important step in the fabrication

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of the new generation of nanostructured platforms for biosensors was achieved in the beginning of the twentieth century with the Langmuir–Blodgett (LB) method and, more recently, the layer-by-layer (LBL) method (Langmuir, 1916, 1917; Decher et al., 1998; Decher and Hong, 1991). Several works have been published about the use of the LBL method for applications involving sensors and biosensors (Crespilho et al., 2005, 2006a,b,c,d), optical devices (Lvov et al., 1997; Altman et al., 2006), immunosensors (Ou et al., 2007), electrocatalysis (Crespilho et al., 2006a,b,c,d), and other electrochemical devices (Lu et al., 2004). Also, the use of different architectures and the relative simplicity of obtaining nanostructured films on solid supports make the LBL method very attractive in the field of electrochemistry for studying biological reactions (Crespilho et al., 2009a,b,c,d; Iost et al., 2011a,b).

In recent years, oxidoreductase enzymes (Goto et al., 2011), proteins (Schimidt et al., 2009; Caseli et al., 2005), DNA (Lvov et al., 1998; Jin et al., 2003), antigens (Zucolotto et al., 2007), and many other biological molecules have been utilised in the presence of nanostructured thin films with the intent of exploring their potential for biosensor applications (Yamauchi et al., 2006). For this purpose, various electrochemical measurements such as cyclic voltammetry (Patolsky et al., 1999), chronoamperometry (Wu et al., 2007a,b,c), electrochemical impedance spectroscopy (Ben et al., 2007), and differential pulse voltammetry (Kizek et al., 2002) have been reported to be important tools for electrochemical detection. On the other hand, two of the main challenges are establishing a better correlation between sensitivity and selectivity to a specific analyte and maintaining biomolecules' integrity in order to preserve their biological properties and maximise the electrochemical signal. Another approach focused on the use of hybrids of organic molecules and nanomaterials as an interesting pathway to enhancing analytical signals on biodevices and, at the same time, preserving the molecular integrity of biomolecules (Iost et al., 2011a; Zhao et al., 2006; Alencar et al., 2007; Pumera, 2010; Allen et al., 2010).

Based on the potential application of organic films and nanomaterials in electrochemical biosensors, this review article focuses on providing an overview of various recent combinations of LBL architectures used in conjunction with biomolecules, along with their application in electrochemical biosensors. Herein, we discuss the methodologies in the fabrication of LBL films utilised in electrode preparation and the recent advances in the field of bionanoelectrochemistry, focusing on the application and development of biosensors. Electronic transport of biomolecules and charge transfer mechanisms in multilayer nanostructured films are also emphasised. Finally, the future and perspectives of the new generation of electrochemical biosensors that utilise LBL techniques and nanostructures are discussed.

2. History and methodologies of LBL films

The LBL method has received much attention in the field of electrochemistry as a very promising tool for the fabrication of nanostructured films with high organisation at the nanoscale level (Lvov et al., 1993). Maoz et al. (1988) fabricated films through specific interactions of organic functional groups for multilayer growth, and the films deposited onto a solid substrate were obtained with high stability. In an alternative approach, Decher (1997) utilised the adsorption process of organic molecules such as polyelectrolytes by electrostatic attraction for the fabrication of films. In most cases, the adsorption processes in the LBL technique are based on the attraction of opposite ionic charges located on polyelectrolyte molecular structures. For the growth of multilayer films, the adsorption occurred as a result of alternating the immersion process of a solid substrate (for example, quartz and hydrophilic or hydrophobic glasses) in a cationic and anionic polyelectrolyte solution and the excess of weakly adsorbed polyelectrolyte is washed in a solvent solution and dried with nitrogen gas/air flow (Fig. 1).

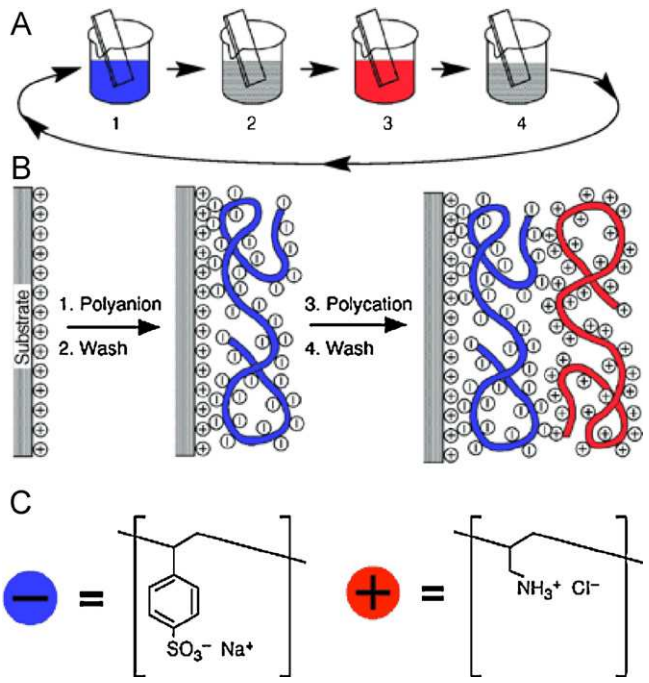

Fig. 1. (A) Schematic of the film deposition process using slides and beakers. Steps 1 and 3 represent the adsorption of a polyanion and polycation, respectively, and steps 2 and 4 are washing steps. The four steps are the basic buildup sequence for the simplest film architecture, $(A/B)_n$. The construction of more complex film architectures requires only additional beakers and a different deposition sequence. (B) Simplified molecular picture of the first two adsorption steps, depicting film deposition starting with a positively charged substrate. Counterions are omitted for clarity. The polyion conformation and layer interpenetration are an idealisation of the surface charge reversal with each adsorption step. (C) Chemical structures of two typical polyions, the sodium salt of poly(styrene sulfonate) and poly(allylamine hydrochloride).
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The LBL method becomes very advantageous because numerous different materials can be employed in the fabrication of films. Also, LBL is more versatile than other fabrication techniques and provides the possibility of using a large range of different materials in films construction, including polyelectrolytes (Clark et al., 1997), dendrimers (DENs) (Zhang et al., 2003; Astruc et al., 2010), carbon materials (Olek et al., 2004), and many others (Zhang et al., 2004). Moreover, parameters such as roughness, thickness, and porosity of films can be controlled by changing experimental conditions such as pH, temperature, polyelectrolyte concentration, and ionic strength of the media (Schonhoff et al., 2007). Another form of bilayer organisation has been reported utilising biomolecules as components for films growth (Lvov et al., 1994, 1996). Some studies describe LBL as a promising methodology and as a new path for the next generation of sensors and biosensors (Schlenoff, 2009). Before the technological applications are considered, it is quite important to analyse some theoretical aspects of nanostructured thin film formation, as described in the following sections.

2.1. LBL theoretical aspects

Some fundamental studies were conducted during the last few decades with the intent of understanding the driving forces responsible for the formation of multilayers (Farhat and Schlenoff, 2002; Dubas and Schlenoff, 1999). The compensative driving force that

governs the process of multilayer growth is based primarily on coulombic interactions between the solid substrate and the charge density of polyelectrolyte structures (Xu and Schlenoff, 1994). As been reported in several studies, hydrophobic interactions between polyelectrolyte side chains, van der Waals forces and hydrogen bonding can also be influenced in films stabilisation (Schlenoff et al., 2008; Clark and Hammond, 2000). The effect of coulombic interaction between side polyelectrolyte chains is attributed in part to the nature of the counter ion compensation. Some studies reported on the influence of extrinsic and intrinsic opposite charges responsible for this electronic compensation on polyelectrolyte side chains (Schlenoff et al., 1998). The behaviour of compensative charges between exchanged ions can be explained by the equilibrium of cationic polyelectrolyte ($Poly_1^+$) and anionic polyelectrolyte ($Poly_2^-$) salts present in solution, as shown in Eq. (1) (Farhat et al., 2002).

$$Poly_1^+A^- + Poly_2^-B^+ \rightleftharpoons Poly_1^+Poly_2^- + B^+A^- \quad (1)$$

The equilibrium of the inter-exchange of polyelectrolyte counterions in forming the associative pair and, consequently, the interaction of two polyelectrolytes with opposite charges by compensative electrostatic forces are two of the keys to multilayer growth. The process of film growth can be altered by several experimental factors, such as salt concentration on the media, hydrophobicity, and the solvent utilised. Although experimental parameters influence film growth, it is important to evaluate the kinetic and thermodynamic factors that govern the fabrication of films. These factors are associated with the charge overcompensation and the process of adsorbing polyelectrolytes between each other or in a conducting solid substrate. For the equilibrium process of doping polyelectrolytes, the expression can be described by Eq. (2) (Bucur et al., 2006).

$$K_{dop} = \frac{y^2}{(1-y)a_{MA}^2} \approx \frac{y^2}{a_{MA}^2} \quad (2)$$

where K_{dop} is the salt doping equilibrium constant, y is the compensated fraction of polyelectrolyte charge, and a_{MA} is the activity association between cation and anion.

The association between polyelectrolytes according to a more comprehensive study of multilayer thin film growth and the experimental factors that govern this process play an important role in the stability of the films. Thermal stability of LBL films has also considered an important factor in the fabrication of films (Hiroharu et al., 2010; Ahn et al., 2009; Ribeiro et al., 2006). Recent approaches shows a good thermal stability up to 200 °C of LBL films depending of molecular architecture utilised for films fabrication (Wang et al., 2004). These parameters contribute directly to film fabrication, and a rigid control of film growth becomes necessary and directly affects the film's final characteristics. In this case, the charge transfer in multilayer films is also film architecture–dependent, as we will see in the following section.

3. Charge transfer in multilayer films

An important contribution to explaining charge transfer on LBL films was reported by Laurent and Schlenoff (1997), who studied electroactive sites on the composition of multilayer films. In this case, experimental results shows the interpenetration of (PSS) and poly(butanylviologen)(PBV) bilayer with redox active centres located at the order to 2.5 layers from each redox active polyelectrolyte layer pairs. Moreover, the presence of electroactive centres on the structure of the organic films is electrochemically favourable for electron hopping between adjacent layers, as shown in Fig. 2.

The electron hopping concept is extended to several different materials that can be employed to study catalytic properties

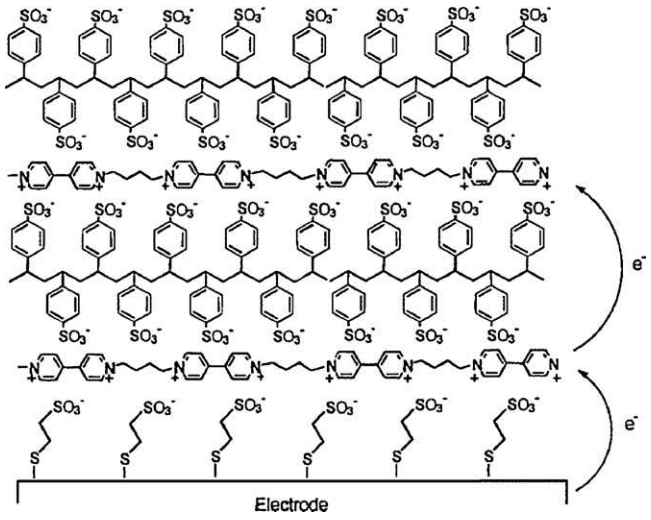

Fig. 2. Idealised PBV/PSS multilayer structure depicting electron hopping between viologen units. The actual structure is considerably more disordered.
Reproduced with permission from Laurent and Schlenoff (1997). Copyright © 1997 American Chemical Society.

and charge transfer between biomolecules and nanomaterials. For instance, using electrochemical impedance spectroscopy and cyclic voltammetry, it was observed that electrodes with LBL films comprising Au nanoparticles (AuNPs) stabilised on amine-terminated G4 PAMAM dendrimer alternated with poly(vinylsulfonic acid) (PVS) layers are efficient for oxygen reduction and diffusion and a linear increase of oxygen reduction current occurs for up to three bilayers, with no further significant increase occurring for more than three bilayers (Crespilho et al., 2007a). As consequence, the utilisation of electroactive nanostructures on LBL films becomes an interesting feature for improving enzymatic biocatalysis (Caruso et al., 2000).

4. Biosensing based on LBL films

The improvement of electrochemical properties for biosensing arises from synergistic interactions between the components on the electrode surface, and one of the main advantages is the capability of controlling film characteristics at the molecular level with experimental simplicity (Santos et al., 2010; Siqueira Jr. et al., 2010). Basically, the improvement of biosensor performance requires a good signal transducer material, and communication between the biomolecules and electrode surface must be achieved in order to intensify signal processing on the electrode surface. Although it is well known that nanostructured thin films can be an interesting tool for developing more sensitive electrochemical biosensors, many efforts have been made to establish an appropriate environment, similar to biological conditions, for the operation of biomolecules (Crespilho et al., 2009a,b,c,d; Caseli et al., 2006a,b; Zhang et al., 2009; Siqueira Jr. et al., 2006a,b; Crespilho et al., 2006a,b,c,d). As is well known, biomolecules lose part or all of their bioactivity when they are immobilised on solid surfaces owing to significant changes in their molecular structure (Crespilho et al., 2009a,b,c,d), and different strategies have been used to maintain their molecular integrity (Pyun et al., 2005; Ram et al., 2001; Stein and McShane, 2003; Qin et al., 2009; Hoshi et al., 2007). As described previously, self-assembly methodology can be applied to immobilisation of many kinds of biomolecules and detection methods. Table 1 summarises some different works involving LBL-based electrochemical biosensors.

Table 1
Summary of some biosensors and bioelectronics devices prepared using LBL for the electrochemical sensors.

Biosensor/bioelectronics	Electrochemistry	Bio-interface	References
Glucose detection	CV	PAMAM-AuNP, GOx	Crespilho et al. (2006a,b,c,d)
Urea detection	Amp	Urease, AgNP, AuNP	Crespilho et al. (2009a,b,c,d)
DNA immobilisation	VC	Gold, SAM, and DNA	Pacey et al. (2005)
DNA biosensor	DPV	DNA, SWCNT	Zhang et al. (2009)
Coase detection	CV/Amp	AuNP, Coase	Ram et al. (2001)
Lactate detection	CV	Silica, lactate oxidase	Stein and McShane (2003)
Choline detection	Amp	SWCNT, ChOD	Qin et al. (2009)
Ascorbate detection	Amp	AuNP, AOx	Hoshi et al. (2007)

AuNP: gold nanoparticles; AgNP: silver nanoparticles; DPV: differential pulse voltammetry; CV: cyclic voltammetry; SWCNT: single walled carbon nanotubes; Amp: amperometry; GOx: glucose oxidase; DNA: deoxyribonucleic acid; Coase: cholesterol oxidase; ChOD: choline oxidase; AOx: ascorbate oxidase.

4.1. Nanomaterials

Materials at the nanoscale level have received significant attention in the last few decades because of their interesting and well-known properties associated with quantum confinement and surface energy effects (Fendler, 1996). The main advantage of utilising different nanomaterial morphologies and sizes (for example, 0D, 1D, or 2D nanomaterials) is that interesting properties can be achieved (Wang et al., 2008).

Colloidal noble metal nanoparticles (NPs) have been the subject of many studies owing to their particular electronic and electrocatalytic properties (Park et al., 2002; El-Deab and Ohsaka, 2002; Bharathi and Nogami, 2001). Many electrochemical approaches utilise various types of NPs, including gold (Lin et al., 2007; Daniel and Austric, 2004), platinum (Hrapovic et al., 2004), silver (Lin et al., 2009; Liu and Hu, 2009), palladium (Lim et al., 2005), and others (Slowing et al., 2007; Fiorito et al., 2005; Zong et al., 2006); this is an important path in biosensing applications. Gold nanoparticles (AuNPs) are one of the most studied materials reported in the literature as promising diagnostic materials (Baptista et al., 2008), sensor and biosensor recognition elements (Zhang et al., 2004), electrocatalytic materials (Feng et al., 2005), and contrast agents (Moriggi et al., 2009). In spite of their electronic properties, they exhibit excellent biocompatibility with biological components (Pingarrón et al., 2008). Moreover, the obtention of colloidal solutions of AuNPs is relatively simple and much explored as components in modified electrodes (Sun and Xia, 2002; Jana et al., 2001). Other approaches have reported on carbon materials as another important class of materials utilised in the fabrication of biosensors (Siqueira Jr. et al., 2009; Wang, 2005; Besteman et al., 2003), including conducting polymers (Kros et al., 2002).

Carbon nanotubes (CNTs) have attractive conducting and electrochemical properties with several applications (Coleman et al., 2006; Keren et al., 2003; Durkop et al., 2004; Gooding, 2005). The tubular structure of CNTs is formed from sp^2 carbon atoms arranged in a hexagonal pattern with diameter on the order of nanometres and length on the order of micrometres (Tasis et al., 2006). Also, the well-known effect of electron tunnelling and the ballistic properties of carbon nanotube structures have attracted much attention in relation to optimising electronic devices (Javey et al., 2003). Furthermore, the adsorption of biological molecules such as proteins (Gooding et al., 2003), antibodies (Erlanger et al., 2001), DNA (Staii

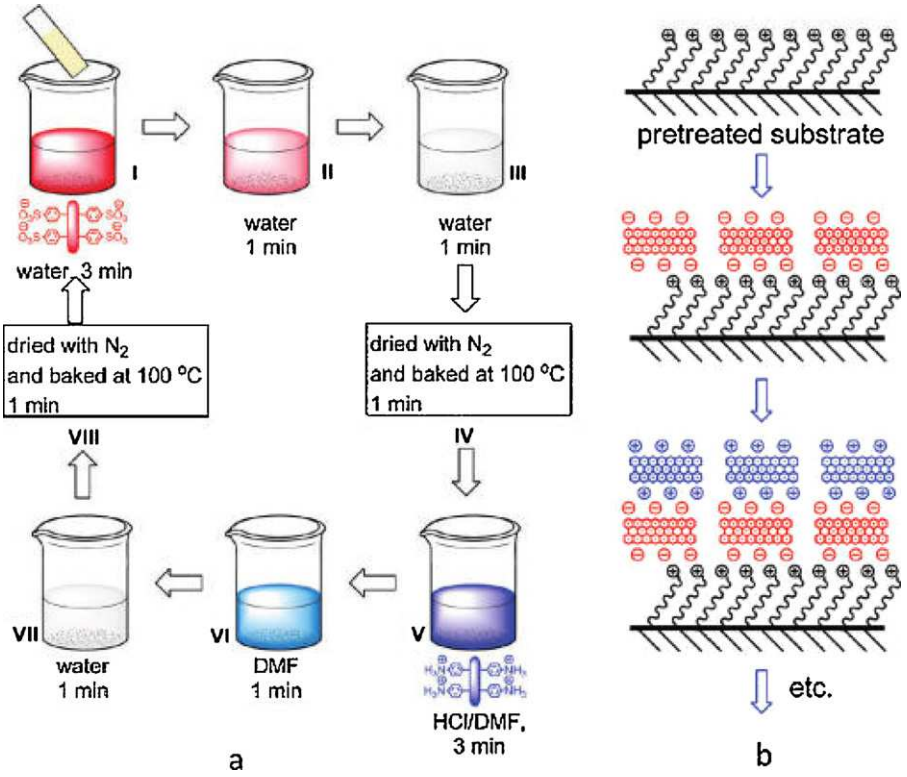

Fig. 3. (A) The layer-by-layer assembly procedure of f-GNRs and (B) an illustration of the complex formation between f-GNR1 and f-GNR2. Reproduced from Zhu and Tour (2010). Copyright © 2010 American Chemical Society.

and Johnson, 2005), and cytochrome *c* (Zhao et al., 2005) has been reported in some interesting approaches (Zhao et al., 2005).

A graphene sheet (GNs) is another type of carbon material utilised in electrochemistry (Brownson and Banks, 2010). This form of 2D carbon foil has interesting electrochemical stability (Chen et al., 2010) and is relatively easy to fabricate (Green and Hersam, 2010; Kosynkin et al., 2009; Zhou et al., 2009). According to several studies, graphene sheets have intrinsic conductive properties (Li et al., 2009), spintronic properties (Saha et al., 2010), and other properties that can be applied in technological areas (Geim and Novoselov, 2007; Stankovich et al., 2006). In an interesting approach, Zhu and Tour (2010) functionalised graphene oxide nanoribbons (GNRs) for thin film construction utilising the LBL method for many applications. The GNRs obtained using CNTs as precursors were functionalised with sulfonic and amine organic groups for precise control of film thickness. Also, the field effect transistor (FET) obtained exhibited electron mobility of 0.1–0.5 $cm^2 V^{-1} s^{-1}$ with p-type semiconductor behaviour. Fig. 3 depicts film fabrication using the LBL technique contained functionalised GNRs.

4.2. Nanomaterials and biological molecules

One of the purposes of utilising nanomaterials in conjunction with biological molecules is to evaluate the influence not only on the final biosensor properties but also on biological integrity (Pereira et al., 2011; Crespilho et al., 2009a,b,c,d; Cosnier et al., 2006; Katz and Willner, 2004). The adsorption of species (chemical, physical, or electrostatic adsorption) is an interesting approach that can be utilised to incorporate biomolecules on electrode surfaces containing nanostructured materials (Tam et al., 2009). Some studies report the utilisation of enzymes as building blocks for the growth of nanostructured films (Caruso et al., 2000; Forzani et al., 2002; Suye et al., 2005). Ferreira et al. (2004) report the utilisation of the enzyme GOx adsorbed alternately with PAH onto ITO substrates modified with Prussian blue (PB) as an amperometric glucose biosensor.

Covalent bonding of biomolecules has also been utilised for the development of modified electrodes (Masson et al., 2007). In this case, residues of amino acids present on the molecular structure of proteins such as enzymes can be loaded on a substrate through covalent attachment using specific chemical groups (Fu et al., 2011). Chemical cross-linking is one interesting example of an efficient methodology utilised for enzyme immobilisation (Fernandes-Lafuente et al., 1995; Situmorang et al., 1998; Roy et al., 2005). Thust et al. (1996) report the utilisation of a cross-linking method for the immobilisation of penicillin on solid supports containing Al/p-Si/SiO_2/Si_3N_4. Another approach uses biotin/avidin as a specific tag for the immobilisation of proteins/enzymes (Chen et al., 1998). For example, Anzai et al. (1999) showed the deposition of enzymes through avidin–biotin interaction in LBL films.

Proteins conjugated with nanomaterials have received great attention owing to the possibility of combining electrical and electrochemical properties with biological recognition in solid supports (Peluso et al., 2003; Mirsky et al., 1997). Also, some studies have explored the interaction between carbon nanotubes combined with proteins (Zhao et al., 2006; Kam and Daí, 2005), for instance the interactions of carbon nanotubes and proteins using the X-ray Absorption Near Edge Structure (XANES) technique (Zhong et al., 2009). Further, the immobilisation of biomolecules on carbon nanotubes becomes very attractive due to their unique electronic properties combined with proteins' specificity. Chen et al. (2001) reported the immobilisation of various biological molecules on carbon nanotube sidewalls with high control and specificity. In this case, the non-covalent interactions was performed using the bifunctional molecule 1-pyrenebutanoic acid and succinimidyl ester adsorbed on SWCNTs; this allows for the immobilisation of ferritin and streptavidin biomolecules (Fig. 4).

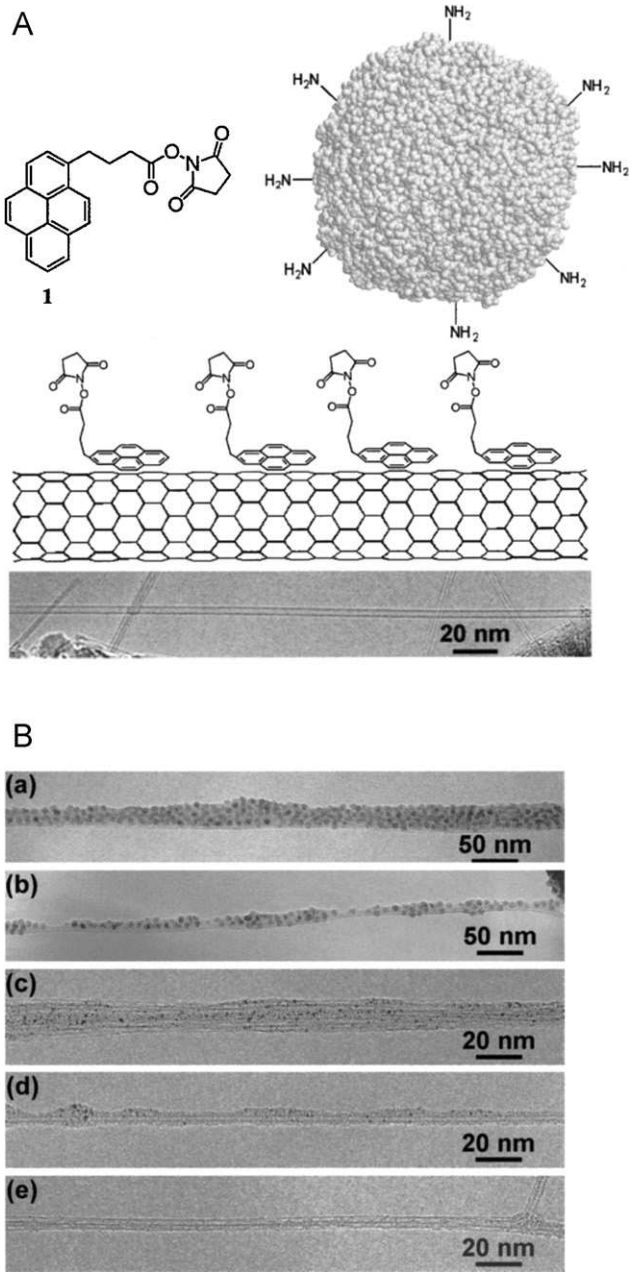

Fig. 4. (A) 1-Pyrenebutanoic acid, succinimidyl ester (1) irreversibly adsorbing onto the sidewall of an SWNT via π-stacking. Amine groups on a protein react with the anchored succinimidyl ester to form amide bonds for protein immobilisation. Lower panel: A TEM image of an as-grown SWNT on a gold TEM grid. (B) (a) A TEM image of a bundle of SWNTs functionalised by (1) followed by ferritin immobilisation. The round dark spots are the (~4 nm) iron cores of ferritin on the bundle. (b) A TEM image of ferritin immobilised onto an individual SWNT. (c) A TEM image of streptavidin–Au conjugates immobilised onto a bundle of SWNTs. The dark spots represent the 1.4 nm Au particles bound to streptavidin molecules. (d) A TEM image of streptavidin–Au conjugates immobilised on an individual SWNT. (e) A TEM image showing the absence of protein immobilisation on as-grown nanotubes without functionalisation by 1.

Reproduced from Chen et al. (2001). Copyright © 2001 American Chemical Society.

Cytochrome *c* (cyt *c*) is a heme protein component present in cytosol that is responsible for oxidative phosphorilation in mitochondria structures (Xu and Zhao, 2008). Thus, the study of the redox process of cytochrome *c* is quite important for bioelectronic devices (Bistolas et al., 2005). Dronov et al. (2008)

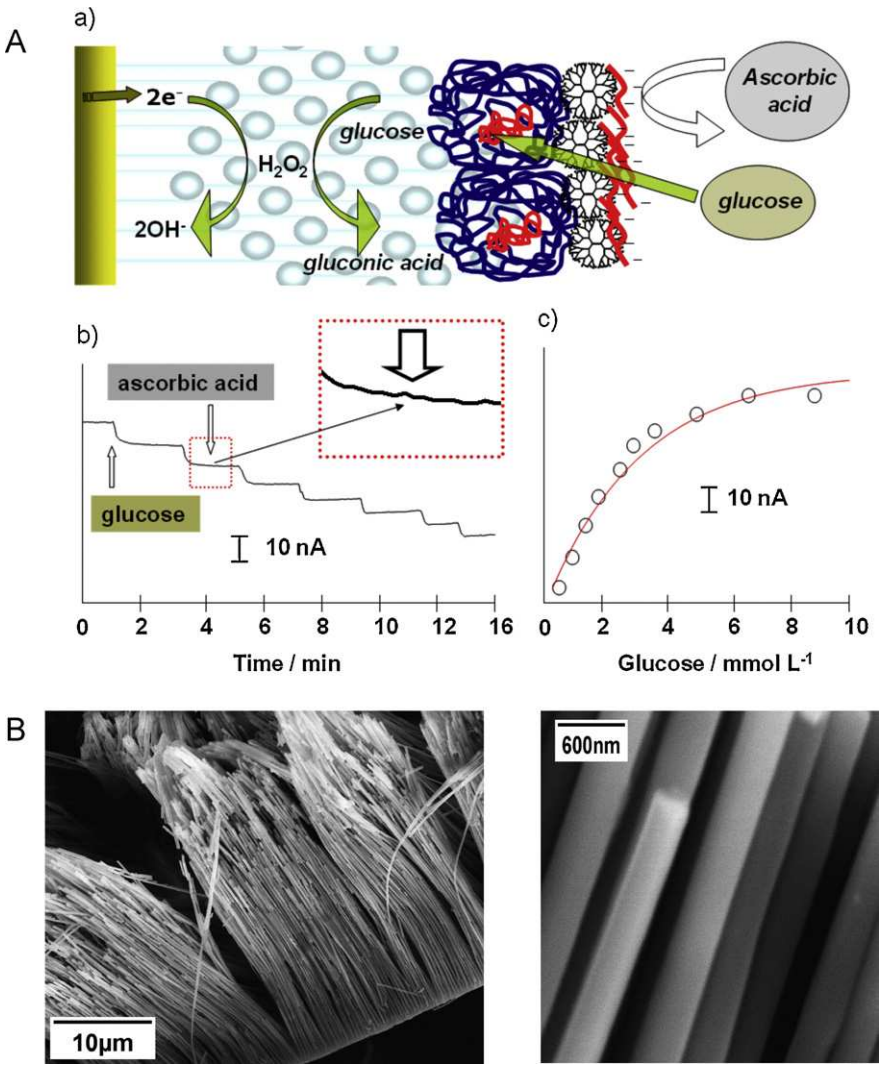

Fig. 5. (A) (a) Schematic illustration of glucose oxidation at the CoNiMo-NWs/GOx electrode after the PAMAM/PVS membrane deposition. (b) Cronoamperometry of the CoNiMo-NWs/GOx electrode after the PAMAM/PVS membrane deposition in 0.1 mol L^{-1} phosphate buffer (pH 7.0) with the addition of 0.1 mM glucose. Note that the enzyme electrode containing the PAMAM/PVS layer was subject to the glucose biocatalysis in the presence of ascorbic acid (0.5 mmol L^{-1}), and no current from the ascorbic acid addition was observed. (c) Michaelis–Menten response curves of GOx using the same system described in (b). Applied potential: 0.0 V (Ag/AgCl). (B) SEM images showing the aligned CoNiMo NWs deposited on Au-covered alumina substrates.

Reproduced from Crespilho et al. (2009a,b,c,d). Copyright © 2009 American Chemical Society.

utilised multilayers of cyt *c* and the enzyme sulphite oxidase (SOx). This self-assembly of proteins Au-MUA/MU-{(SOx/cyt *c*-cyt *c*)}8-SOx/cyt *c* exhibited better electrochemical performance during successive additions of sulphite in electrolyte media. Others interesting strategies explored the capability of different nanostructured architectures for cytochrome *c* immobilisation (Zhang et al., 2006a,b; Balkenhohl et al., 2008) for bioelectrochemical studies. Such strategies have demonstrated important steps for the fabrication of protein-based biosensors using nanomaterials as platforms for immobilisation and to improve redox processes at the electrode surface.

4.3. Enzyme biosensors

The field of enzymatic biosensors has been expanding significantly with the evolution of nanostructures for electrode preparation with the utilisation of several redox enzymes. Many efforts have been made to improve the electrochemical signal for the purpose of glucose biosensing using nanostructured thin films and hybrids of nanomaterials (Wang et al., 2008). The biocatalytic properties of the enzyme glucose oxidase (GOx) have been extensively studied in the last few decades with the aim of developing more sensitive and selective detection methods for medical applications. For this purpose, carbon nanotubes (Tsai et al., 2009), metal nanoparticles (Ren et al., 2005), and hybrids materials can be formed using the LBL approach (Wu et al., 2007a,b,c; Hodak et al., 1997; Zhao et al., 2005). The great versatility of the LBL technique makes it possible to fabricate and improve the signal detection of several other components. As an example, the determination of dopamine provides information on several biological functions and makes it possible to evaluate some important pathological conditions, as an example, Parkinson's disease (Pihel et al., 1996). Zhang et al. (2006a,b) reported multilayer films of PDDA and MWNTs on glassy carbon electrodes (GCE) as interesting tools for dopamine detection in regards to the presence of ascorbic acid (AA). The same idea can be extended for LBL films of gold nanoparticles and choline (Wang et al., 2007), PAni and SWCNT multilayer films for choline biosensor (Qu et al., 2005), horseradish peroxidase (Liu et al., 2007), thionine (Thi^+) and AuNPs on titania/AuNPs (Shi et al., 2007) and H_2O_2 biosensor formed by the LBL method

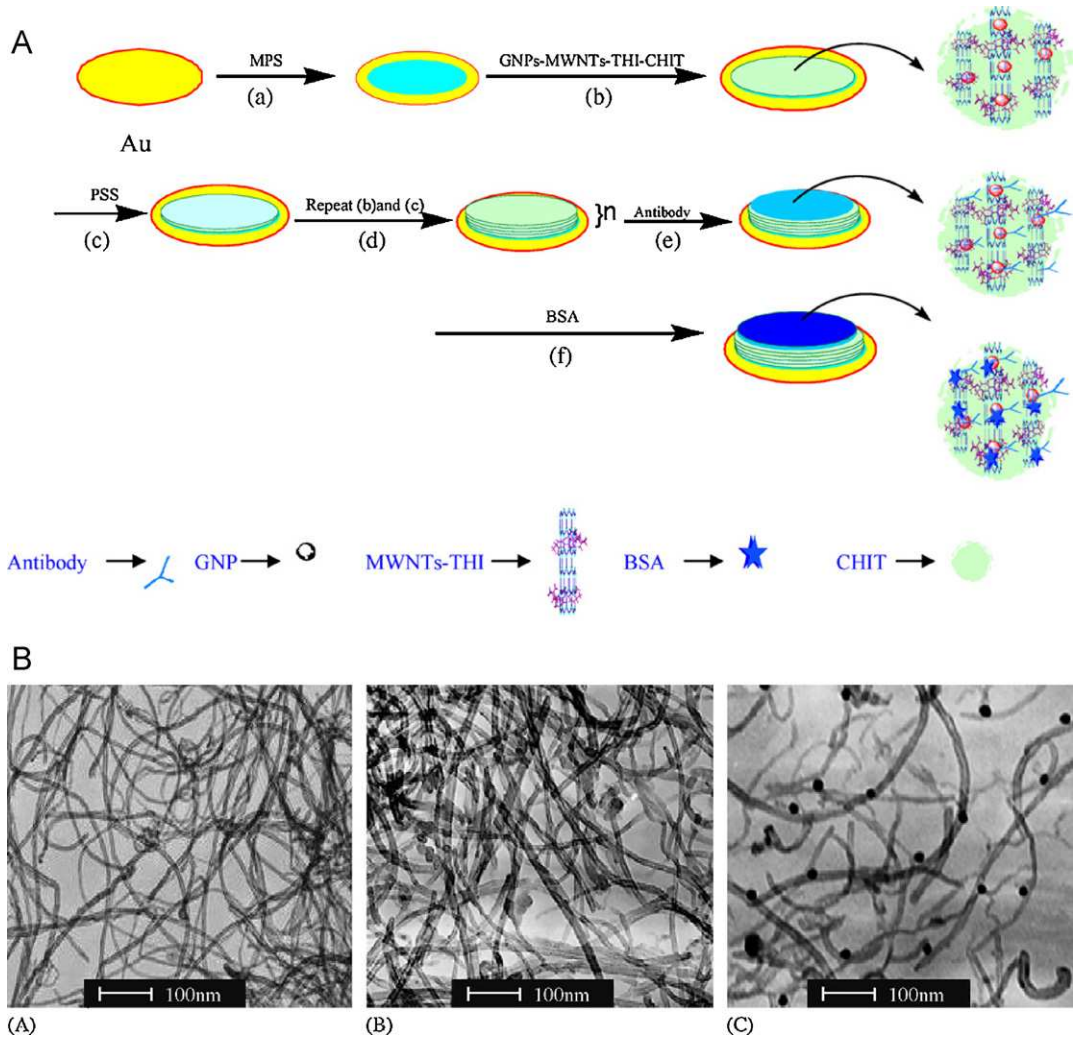

Fig. 6. (A) Scheme of alternative self-assembly procedure for the gold electrode and the electron-transfer process. (B) TEM image of MWNTs film (a), MWNTs–Thi–Chit (b) and (c) GNPs–MWNTs–Thi film.
Reproduced from Ou et al. (2007) with kind permission of Elsevier.

using toluidine blue and AuNPs (Chen et al., 2006). Moreover, the utilisation of nanotubes or nanowires (nanoscale 1D materials) has also been extensively explored in recent years (Aravamudhan et al., 2007). These nanostructure materials can play an important role in the electrochemical signal improvement of bioelectrochemical processes originated by redox enzymes. For instance, aligned and highly oriented CoNiMo metallic nanowires were used as transducing elements for GOx immobilisation (Crespilho et al., 2009a,b,c,d). Fig. 5 shows electrodeposited CoNiMo nanowires with an average diameter of 200 nm and length of 50 μm on Au-covered alumina substrates. For this purpose, alumina membranes were used as templates for electrodeposition.

4.4. Immunosensors

The arrangement of multilayer architectures has been studied to develop more sensitive and selective immunosensor devices (Ou et al., 2007), and is a promising path for developing more sensitive clinical analysis (Tang and Xia, 2008; Pan et al., 2010). Recently, several studies have focused on producing less expensive, more portable, smaller biodevices capable of rapid and sensitive diagnosis in clinical samples (Luppa et al., 2001). Ou et al. (2007) reported using MWCNT/PBNPs films as an amperometric immunosensor for α-fetoprotein detection. In another approach, a new antibody immobilisation strategy was employed as a high-sensitivity, label-free systems immunosensor for carcinoembryonic antigen (CEA) detection based on layer-by-layer assembly of AuNPs, MWCNTs, and the natural polymer chitosan (Chit). In this case, the electrode configuration of AuNPs–MWNTs–Thi–Chit was highly sensitive to CEA (Fig. 6).

4.5. DNA and oligonucleotides

Specificity in regards to recognition elements has been explored in recent years using DNA fragments on electrode-modified surfaces in conjunction with nanostructured thin films (He and Bayachou, 2005; Zhang and Hu, 2007; Wong and Gooding, 2006). DNA is known as portable of specific base sequences that determine the genomic characteristics of living organisms, and the identification of specific changes in base sequences is very important for detecting diseases (Batchelor-McAuley et al., 2009; Teles and Fonseca, 2008). Basically, a specific hybridisation event of an immobilised single strain DNA (ss-DNA) is detected using a transducer element on modified electrodes utilising electrochemical techniques (Ye and Ju, 2005; Turcu et al., 2004). He and Bayachou (2005) proposed the immobilisation of DNA on SWCNTs using the LBL technique. In this case, DNA/PDDA/SWCNTs were prepared, and DNA damage was investigated through the presence of nitric oxide

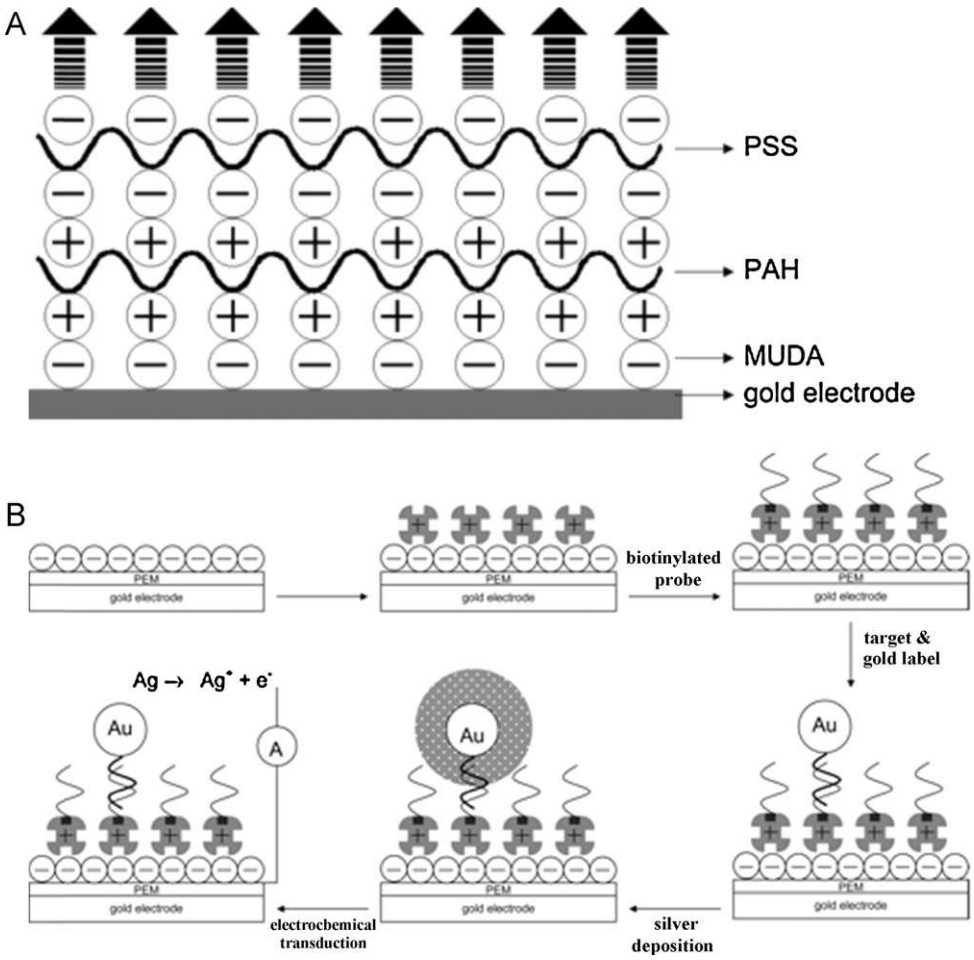

Fig. 7. (A) Illustration of the structure of electrostatic polyelectrolyte multilayer films self-assembled through the alternating adsorption of polycations (PAH) and polyanions (PSS). (B) Schematic representation of the electrochemical DNA-hybridisation detection using the silver-enhanced gold nanoparticle label on the gold electrode modified with polyelectrolytes, streptavidin, and the biotinylated probe.
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(NO). Jin et al. (2003) also reported the fabrication of multilayer films based on DNA/Mb on cysteamine-modified gold electrodes. Lee et al. (2003), who improved electrochemical detection of DNA hybridisation through the modification of a gold and ITO (indium tin oxide) electrode surface with 11-mercaptoundecanoic acid (MUDA) and the polyelectrolytes PSS and PAH on gold electrodes via avidin–biotin interaction. Fig. 7 shows a schematic representation of the electrochemical system described above. In this case, the background signal was generated through electrode modification.

5. Modern aspects of LBL and electrochemistry

The compatibility of nanostructured materials with biomolecules plays an important role in electrochemistry owing to the ability to detect biomolecules with high analytical specificity (Yang et al., 2006). Moreover, the concept of utilising metal nanoparticles stabilised on organic matrices for multilayer fabrication was also applied to developing electroactive nanostructured membranes (ENM) (Crespilho et al., 2007b, 2008). This concept can be extended to a variety of nanomaterials with combinations of different architectures that have shown their ability in the field of electrochemistry to detect small quantities of molecules. In another interesting approach, Yan et al. (2007) demonstrated a simple way to fabricate a flexible poly (ethylene terephthalate) electrode (PET) as a substrate for the self-assembly of MWCNT-GOx multilayer membranes via electrostatic adsorption with PDDA for glucose biosensor applications. On the other hand, the utilisation of metallo-phthalocyanines (MPcs) with different redox metal centres and metalloporphyrins (MP) improves the communication of enzymes when utilised in conjunction with nanostructured thin films (Sergeyeva et al., 1999). The literature has also shown the applications of MPcs for gas sensors (Lee et al., 2004), detection of the neurotransmitter dopamine (Kan et al., 2008), peroxide sensing (Alencar et al., 2009) and many others (Kang et al., 1997; Rawling and McDonagh, 2007). Also, graphene is very attractive for use in the preparation of modified electrodes (Shan et al., 2009; Kang et al., 2009).

6. Perspectives and future research

The LBL technique is considered to be a very interesting method for the fabrication of nanostructured architectures with high molecular order. Many studies have focused on maintaining the molecular integrity of biomolecules utilising several nanostructured materials as recognition elements when they are immobilised on solid supports. The good electrochemical performance obtained with the use of LBL method can be attributed in part to the possibility of developing miniaturised devices capable of more sensitive and selective analysis and of detecting small quantities of molecules. Moreover, the versatility of the LBL technique allows

for the utilisation of many materials for the fabrication of modified electrodes.

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