Sensors and Actuators B 202 (2014) 129–136

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Enhanced detection of the potential electroactive label methylene blue by electrode nanostructuration with carbon nanotubes

Raquel García-González, Agustín Costa-García, M. Teresa Fernández-Abedul*

Departamento de Química Física y Analítica, Universidad de Oviedo, 33006 Oviedo, Asturias, Spain

ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form 17 April 2014
Accepted 30 April 2014
Available online 10 May 2014

Keywords:
Methylene blue
Carbon nanotubes
Nanostructured electrodes
Gold screen printed electrodes
Electrochemical sensor

ABSTRACT

Electrochemical sensors are developing very fast in the last decades due to the possibilities of generating miniaturized and simplified low-cost analytical devices. However, the search for highly detectable electroactive labels and sensitive detection strategies is still required. In this work, the electrochemical signal of a very promising molecule that can be employed as biolabel, methylene blue, is enhanced by recording it on nanostructured (MWCNTs-NH_2) gold screen-printed electrodes. A dispersion of amino-functionalised CNTs in Nafion®/ethanol:water (1:1) is employed for modification by depositing a drop on the surface of a screen-printed gold working electrode. This produces a change in the electrochemical behavior of MB, with increase of both, reversibility and peak current. On the other hand, unlike what happens on bare electrodes where MB presents a diffusion controlled process, adsorption occurs on modified electrodes. This allows higher enhancement of the signal by introducing an accumulation time. In this way, and once parameters that affect the modification are optimized, improvements up to fifteen times are obtained. This is the first time such enhancement is produced for this molecule based on its adsorption on nanostructured electrodes. In this way, a detection limit of 100 nM for differential pulse voltammetry or square wave voltammetry is obtained after 120 s of accumulation time. The sensitivity increases almost by seven times (from 0.711 to 5.029 $\mu A\,\mu M^{-1}$) when calibration curves obtained from SW voltammograms recorded on bare and modified electrodes are compared. Modification is stable for at least 10 days.

1. Introduction

Methylene blue (MB) is a thiazine type cationic dye, which was used for dying silk, leather, plastics, paper and cotton, as well as for the production of ink and copying paper in the office supplies industry [1]. MB has long been used for staining in medicine, bacteriology and microscopy [2,3]. Although it is not considered to be a very toxic dye, it can cause some harmful effects such as vomiting, increased heart rate, diarrhea, shock, cyanosis, jaundice, quadriplegia, and tissue necrosis on human beings [4]. Consequently, MB containing wastewater should be treated before discharge [5] and its determination is justified. Furthermore, the reversible equilibrium between the reduced and oxidized forms of MB renders this compound useful as redox indicator [6,7]. In this context, MB main uses are related to the determination of glucose, oxygen or ascorbic acid [8,9] and also as redox marker for electrochemical genosensors. The hybridization event has been reported by means of a differential non-covalent interaction between MB and single or double DNA-strands [10,11] or by using oligonucleotides labeled covalently with MB [12,13]. Carbon paste electrodes [10], sputtered [12,13] or screen-printed [11] gold electrodes were employed for voltammetric detection, in the last case modified with polymeric films of poly-L-lysine and chitosan.

Due to their unique structure, high chemical stability and high surface-to-volume ratio, the use of carbon nanotubes (CNTs) has been extremely attractive in electrochemical sensors [14–17]. Multiwalled carbon nanotubes (MWCNTs) electronic properties and catalytic activity makes them very interesting for developing promising applications in electrochemistry as novel electrode materials [18,19]. Their electrical properties and biocompatibility [20] can help to improve the biosensing performance on the electrode surface and to amplify the signal [21]. Preliminary studies performed in our research group have demonstrated the increase in the electrochemical signal of methylene blue, dopamine or iron sulfate [22] on nanostructured gold screen-printed electrodes with carbon nanotubes in Nafion® as dispersing agent. Disposable screen-printed electrodes (SPEs), fabricated *via* thick-film technology, provide attractive opportunities for the development of

* Corresponding author. Tel.: +34 985102968; fax: +34 985103125.
E-mail address: mtfernandeza@uniovi.es (M.T. Fernández-Abedul).

http://dx.doi.org/10.1016/j.snb.2014.04.104
0925-4005/

miniaturized low-cost electrochemical sensors for the detection of different analytes [23–25]. The high precision attained in the manufacturing processes allows performing parallel assays which in turns produces a significant decrease on analysis time. Therefore, SPEs are becoming an essential tool in the development of electrochemical sensors.

Most of the electrochemical immunosensors are based on enzymatic detection principles due to the amplification possibilities of enzymes, which convert several molecules of substrate in electroactive product. On the other hand, most of DNA biosensors are based on the detection of indicator molecules that interact selectively with DNA single or duplex strands in different parts of the DNA molecule. However, in both cases (enzymes [26] or indicators [27]) a new interaction has to be included and therefore complexity is added to the system. In this context, MB could be considered as a molecule of high-interest in the development of biosensors based on a direct electroactive labeling. Since in this approach amplification is not possible, enhancement of sensitivity is needed. This could be achieved by using highly detectable labels (as is MB) or through nanostructuration of the electrode surface, which is made in this paper through aminated multiwalled carbon nanotubes (MWCNTs-NH_2). A notorious improvement in the electrochemical signal of MB with a clear enhancement of the sensitivity on nanostructured screen-printed gold electrodes is described for the first time. Not only electrocatalysis but also adsorption of MB on modified electrodes is here considered as responsible for these beneficial effects.

2. Experimental

2.1. Reagents and solutions

Nafion® (perfluorinated ion-exchange resin, 5 wt. % solution in a mixture of lower aliphatic alcohols and water), methylene blue and tris-hydroxymethyl-aminomethane hydrochloride (Trizma), were obtained from Sigma–Aldrich (St. Louis, MO, USA). Sulfuric acid (95–97% purity) and ethanol were purchased from Merck (Darmstadt, Germany) and iron(III) sulfate was purchased from Panreac (Barcelona, Spain). Finally, amine-functionalized carbon nanotubes, MWCNT-NH_2, were obtained from Belgium Nanocyl (Auvelais, Belgium). CNTs were produced *via* catalytic carbon vapor deposition (CCVD) process. They have 9.5 nm of average diameter, <1 μm of average length, >95% in carbon purity and <0.5% of functionalization.

2.2. Apparatus

Voltammetric measurements were performed with an Autolab PGSTAT 10 (ECO Chemie) potentiostat interfaced to a Pentium 120 computer system and controlled by Autolab GPES software version 4.8. An Elma ultrasonic bath, a Nahita centrifuge with interchangeable car, a Mettler Toledo (AB54) balance, a Crison Micro-pH 2001 pH-meter, a magnetic stirrer Asincro (J.P. Selecta), a Sanyo refrigerator and a Sanyo (MIR-162) incubator were also used.

2.3. Gold screen printed electrodes (AuSPE)

Gold screen-printed electrodes purchased from DropSens (Llanera, Spain) include a traditional three system electrode configuration printed on the same strip. The format of these SPEs includes a gold disk electrode (12.6 mm^2) as working electrode, a silver pseudo-reference electrode and a gold counter electrode using the same ink of the working electrode. All of them are screen-printed on a ceramic substrate (3.4 cm × 1.0 cm × 0.05 cm) and subjected to high-temperature curing (AuSPE-AT). An insulating layer serves to delimit the working area and electric contacts. The production characteristics of commercial SPEs are regarded by the manufacturers as proprietary information. A specific connector supplied by DropSens allows their connection to the potentiostat.

2.4. Dispersion of carbon nanotubes

Generally, carbon nanotubes are presented in the market as a black solid powder and therefore, most of the applications of CNTs, electrode modification among them (especially with screen-printed electrodes), require a pre-solubilisation for obtaining a homogeneous suspension. Achieving this step is not easy because CNTs are not dispersed in many solvents. The CNTs weight/solvent volume ratio and the dispersion procedure are very important. In this case, an ultrasonic bath and a centrifuge with interchangeable car for 1.5 and 5 mL were employed for dispersion, following a procedure previously optimized [14]. Briefly, 1 mg of MWCNTs was solved in 1 mL of 0.5% Nafion® (in EtOH) and 4 cycles (2 h under sonication and 10 min of centrifugation) were applied. After each cycle the precipitate is discarded and supernatant is subjected to a new cycle. Finally, a completely homogeneous dispersion was obtained.

2.5. Nanostructuration of screen printed electrodes

Nanostructuration of electrodes with CNTs was carried out by evaporation at room temperature of a drop of CNTs dispersion deposited on the working electrode. Volumes between 1 and 4 μL were used. The electrochemical characterization of gold screen printed electrodes nanostructured with carbon nanotubes (CNTs-AuSPE) was carried out by recording voltammograms in methylene blue solutions.

2.6. Voltammetric measurements

In all cases, voltammograms were recorded in a 10 or 20 μM MB solution in 0.1 M Tris–H_2SO_4 pH 8.0 buffer solution [14]. Different techniques were employed: cyclic (ν = 0.25 V s^{-1}), square wave (f = 50 Hz, A = 0.05 V, s = 0.008 V) and differential pulse (A = 0.05 V, s = 0.008 V) voltammetry, scanning the potential from 0.0 to −0.7 V. Due to its low cost and since possibility of adsorption exist, electrodes were considered as single-use. All measurements were recorded on 3 electrodes and error bars are included in the corresponding graphics.

3. Results and discussion

MB presents a well-defined two-electron redox process [28] with cathodic (conversion to leucomethylene blue (LB)) and anodic (oxidation to MB) peaks. The electrochemical behavior of this molecule converts it into a suitable candidate for covalent electrochemical biolabel. Since direct signaling is employed, enzymatic conjugation and further substrate to product conversion is not needed. Similarly, covalent labels allow eliminating interaction with indicator molecules and further separation of free molecules is not required. Analysis time is then reduced with a simpler procedure. However, sensitivity is needed, overall when decreasing detection limits is being continuously required. Two different strategies, one focusing on the label itself and other on the detection surface could enhance it. Even when the label is highly detectable, a covalent attachment does not have necessarily to produce a high label density. Therefore, the detection surface has to be carefully designed for favoring charge transfer. Based on a previous gold screen-printed electrodes characterization [29], AuSPE-AT has been chosen as very adequate transducers. Nanostructuration has been performed with MWCNTs-NH_2 dispersion and voltammograms have been recorded in MB solutions in a pH 8.0 buffer