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Poly(brilliant green) and poly(thionine) modified carbon nanotube coated carbon film electrodes for glucose and uric acid biosensors

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ABSTRACT

Poly(brilliant green) (PBG) and poly(thionine) (PTH) films have been formed on carbon film electrodes (CFEs) modified with carbon nanotubes (CNT) by electropolymerisation using potential cycling. Voltammetric and electrochemical impedance characterisation were performed. Glucose oxidase and uricase, as model enzymes, were immobilised on top of PBG/CNT/CFE and PTH/CNT/CFE for glucose and uric acid (UA) biosensing. Amperometric determination of glucose and UA was carried out in phosphate buffer pH 7.0 at −0.20 and +0.30 V vs. SCE, respectively, and the results were compared with other similarly modified electrodes existing in the literature. An interference study and recovery measurements in natural samples were successfully performed, indicating these architectures to be good and promising biosensor platforms.

1. Introduction

The selection and development of active sensing materials for electrodes is a big challenge for the construction of electrochemical biosensors. By using nanotechnology, a large number of new materials and devices of desirable properties can be designed and it is possible to control the fundamental properties of materials without changing the chemical composition. Complex nanobiosensor architectures can aid in performing continuous monitoring as implantable devices and in high throughput analysis such as lab-on-chip devices for rapid and low-cost screening of physiological metabolites [1].

Carbon nanotubes (CNT) have been extensively used in recent years due to their low cost, excellent chemical stability, good mechanical strength and electrical conductivity, good electron transfer kinetics and biocompatibility [2]. CNT can improve electrochemical properties, provide electrocatalytic activity and minimise electrode surface fouling, reasons that make them excellent materials for the development of electrochemical sensors and biosensors [3–5], generally leading to higher sensitivities and lower detection limits than traditional electrode materials.

Conducting polymers (CP) have also been extensively studied as electroactive materials during recent decades. Among them, redox dye polymers, especially phenazine derivatives, have had many applications in sensors and biosensors (e.g. [6–9]).

CP/CNT nanocomposite modified electrodes have received significant interest because the incorporation of conducting polymers into CNT can lead to new composite materials possessing the properties of each component, with a synergistic effect that would be useful in specific applications [10]. Carbon nanotubes can improve the conductivity of conducting polymer matrices and form a three-dimensional network which can facilitate access to the analyte and increase the rate of electron transfer [11].

The determination of both glucose and uric acid is of great clinical importance. Uric acid is related to gout, cardiovascular and renal diseases, leukaemia and pneumonia [12] and a high glucose level is associated with diabetes, which is related to complications to retina, the circulatory system and kidneys [1]. New strategies based on different nanomaterials, nanostructures or nanotechnologies for the development of biosensors have been explored for the determination of these compounds, electrochemical ones often being preferred [1,13–16].

In the present work, nanostructured composites have been prepared by electropolymerisation of brilliant green (BG) and thionine (TH) (see Scheme 1), onto CNT-modified carbon film electrodes (CFE). The modified electrodes PBG/CNT/CFE and PTH/CNT/CFE served as platforms for the immobilisation of glucose oxidase (GOx) and uricase (UOx) which were used for sensing glucose and uric acid. To our knowledge, thionine monomer and carbon nanotubes have been only used once to develop uric acid [17] and glucose [18] biosensors; however, in both studies thionine

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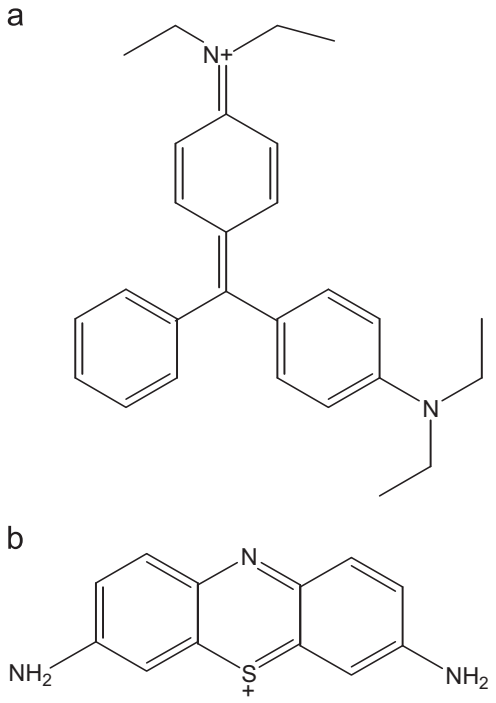

Scheme 1. Chemical structure of monomers (a) brilliant green and (b) thionine.

was not polymerised and, in the case of glucose, the biosensor also contained platinum nanoparticles. Regarding carbon nanotubes and poly(brilliant green), there is no report up until now either for glucose, or for uric acid determination. A comparison between the performances of the developed biosensors under the same conditions was performed, the results are discussed with respect to other sensors in the literature and natural samples are analysed.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade and were used without further purification. Glucose oxidase (GOx, E.C. 1.1.3.4, from *Aspergillus niger*, 24 U/mg), uricase (UOx, E.C. 1.7.3.3, from *Bacillus fastidiosus*, 16.2 U/mg), phenol and brilliant green (BG) were acquired from Fluka. α-D(+)-glucose, uric acid (UA), L-ascorbic acid (AA), glutaraldehyde (GA) (25% v/v in water), bovine serum albumin (BSA) and urea were purchased from Sigma. Citric acid, creatinine and ammonia were from Merck. Multi-walled carbon nanotubes (MWCNT) were from NanoLab, U.S.A., with ~95% purity, 30 ± 10 nm diameter and 1–5 μm length. Chitosan (Chit) of low molecular weight with a degree of deacetylation of 80% and thionine (TH, dye content 90%) were obtained from Aldrich.

All solutions were prepared using Millipore Milli-Q nanopure water (resistivity > 18 MΩ cm). The supporting electrolyte for biosensors evaluation was sodium phosphate buffer saline, NaPBS (0.1 M NaH_2PO_4/Na_2HPO_4 + 0.05 M NaCl), pH 7.0. For BG electropolymerisation, universal buffer McIlvaine (0.1 M citric acid + 0.2 M Na_2HPO_4) pH 4.0 was used and for TH polymerisation the buffer was sodium tetraborate (0.025 M $Na_2B_4O_7$) + 0.10 M KNO_3, pH 9.0.

2.2. Methods and instruments

All measurements were performed in a 15 mL, one-compartment, cell containing a carbon film electrode (CFE) as working electrode, a platinum wire auxiliary electrode and a saturated calomel electrode (SCE) as reference.

Voltammetric and amperometric experiments were carried out using a CV-50 W Voltammetric Analyser from Bioanalytical Systems, controlled by BAS CV-2.1 software.

The pH measurements were performed with a CRISON 2001 micro-pH-meter. All experiments were performed at room temperature, 25 ± 1 °C.

2.3. Carbon film electrode preparation and pre-treatment

The working electrodes were made from carbon film resistors (2 Ω nominal resistance, 15 μm film thickness) of length 6 mm and 1.5 mm in diameter; the detailed preparation is described elsewhere [19]. The cylindrical resistor, a carbon film pyrolitically deposited on a ceramic substrate, has two tight-fitting metal caps, one at each end, linked to an external contact wire. In order to make the electrode one of them was removed and the other shielded in plastic and protected by normal epoxy resin. The exposed geometric area of the electrodes is 0.20 cm^2.

Since carbon film electrode surfaces cannot be renewed by polishing or other mechanical methods, electrochemical pretreatment was always performed before use in order to achieve a reproducible electrode response. This consisted in potential cycling between −1.0 and +1.0 V vs. SCE, at 100 mV s^{-1}, until a stable voltammogram was obtained.

2.4. Carbon nanotube functionalisation and deposition

Multi-walled carbon nanotubes (MWCNT) were purified and functionalised as previously described [20]. A mass of 120 mg of MWCNT was stirred in 10 mL of a 5 M nitric acid solution for 24 h, in order to cause partial destruction of the CNTs and introduce –COOH groups at the ends and sidewall defects of the CNT [21]. The solid product was collected on a filter paper and washed several times with nanopure water until the filtrate solution became neutral (pH ≅ 5). The functionalised MWCNT were then dried in an oven at 80 °C for 24 h.

In order to prepare a 1.0% w/v chitosan solution, 100 mg of Chit powder was dissolved in 10 mL of 1.0% v/v acetic acid solution and stirred for 3 h at room temperature to ensure complete dissolution. The chitosan solution was stored at 4 °C.

A 1.0% w/v MWCNT solution was prepared by dispersing 3 mg of functionalised MWCNT in 300 μL of 1.0% w/v Chit in 1.0% v/v acetic acid solution and sonicating for 3 h. For CNT deposition a 10 μL drop of the 1% w/v MWCNT solution was placed on the surface of the CFE, left to dry in air at room temperature and this step was then repeated.

2.5. Brilliant green and thionine polymerisation

Poly(brilliant green) (PBG) and poly(thionine) (PTH) films were formed by electropolymerisation using potential cycling.

Prior to polymerisation of BG, the electrode was activated, as described in [22] for malachite green, by cycling in 0.1 M sulphuric acid between −1.0 and +2.0 V vs. SCE at 100 mV s^{-1} until a stable voltammogram was obtained. Polymerisation of BG was carried out in an aqueous solution containing 1 mM brilliant green in McIlvaine buffer, pH 4.0, sweeping the potential between −1.0 and +1.2 V at a scan rate of 100 mV s^{-1} during 5 cycles [23] at CFE and 20 cycles at CNT/CFE.

For TH polymerisation, a solution of 0.025 M $Na_2B_4O_7$ + 0.10 M KNO_3, pH 9.0 and 1 mM thionine was used. Polymerisation of thionine can occur from different media [24,25]; these studies point to a higher pH value for better film growth, as occurs with other phenothiazines [26]. Potential cycling polymerisation was