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Electrochemical impedance studies of chitosan-modified electrodes for application in electrochemical sensors and biosensors

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ABSTRACT

Graphite-epoxy resin composite (GrEC) electrodes were modified with chitosan (Chit) films and characterised using electrochemical impedance spectroscopy (EIS). Several film modifications were made using different crosslinking agents: glutaraldehyde (GA), glyoxal (GO), epichlorohydrin (ECH) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) together with N-hydroxysuccinimide (NHS) and the characteristics of each of them evaluated in the presence of model electroactive compounds potassium hexacyanoferrate(III) and hexaammineruthenium(III) chloride. Immobilisation of functionalised carbon nanotubes into chitosan matrices (Chit–CNT) using the same crosslinking agents was also investigated. The impedance of the electrode with the best performance (GrEC/Chit–CNT/EDC–NHS) was characterised as a sensor for dipyrone and hydroquinone and for a glucose biosensor by immobilisation of glucose oxidase (GOx) on top of Chit–CNT using GA. Modelling and equivalent circuit analysis was carried out, with emphasis on diffusion characteristics and the significant features of the spectra are discussed.

1. Introduction

Electrochemical impedance spectroscopy (EIS) has been used in many fields, particularly corrosion, but only, since the 1980, has it become more widely applied, first to ion-selective electrodes [1] and then, more recently, to electrochemical sensors and biosensors in general [2–5]. The impedance experiments are now easy to perform with modern computer-controlled instrumentation. Nevertheless, good experimental protocols with an assessment of errors and reproducibility, and interpretation and modelling of the spectra, are difficult so that the spectra are often used, in the chemical sensors' field, more for diagnostic characterisation. Despite this apparent limitation, differences between sensing electrode structures can be assessed in a way that is not possible by voltammetric techniques such as cyclic voltammetry or pulse voltammetry, due to the wide range of timescales probed by EIS. Full interpretation of the impedance spectra of sensor systems often requires that the molecular arrangement and structure at the electrode surface or modified surface is predefined or organized in some way, thus avoiding non-uniformity at the nanoscale [6].

Many types of electrochemical sensor and biosensor assembly have been the object of research. Particularly interesting sensor architectures include modifiers which can interact chemically with proteins, such as enzymes, and also with substrate electrode surfaces. Amongst these, chitosan has been shown to have interesting properties. Chitosan (Chit) is a linear β-1,4-linked polysaccharide (similar to cellulose) that is obtained by the partial deacetylation of chitin, a main component of the shells of crustaceans such as crab and shrimp. Chit possesses distinct chemical and biological properties [7], because it has reactive amino and hydroxyl groups in its linear high molar mass polyglucosamine chains which are amenable to chemical modification [8–11]. Moreover, Chit is biocompatible, biodegradable, and is a non-toxic, natural and high mechanical strength biopolymer with an excellent film-forming ability and is also a very good matrix for enzyme or biomacromolecule immobilisation [8].

Carbon nanotubes (CNTs) are often used in sensors and biosensors due to their chemical and mechanical properties. CNTs have good conductivity due to defects in their structure and promote electron transfer reactions of various molecules; their use as electrode modifiers can lead to a decrease of the overpotential, a decrease of the electrode response time, and an increase of the available electroactive surface area of various electroactive substances, in comparison with conventional carbon electrodes [12–21]. Nevertheless, the low solubility of CNTs in most solvents is the major problem to control for their use as modifiers in the fabrication of chemical sensors and/or biosensors. The strategies most employed to disperse CNTs are: (i) functionalisation [16,21,22], (ii) use of surfactants with sonication [23], and (iii) polymer wrapping [24].

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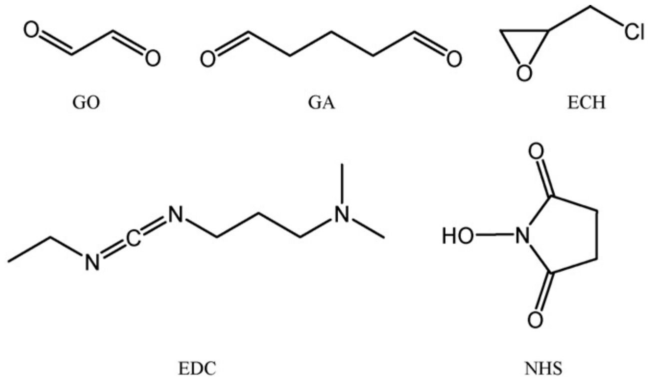

Fig. 1. Chemical structures of glyoxal (GO), glutaraldehyde (GA), epichlorohydrin (ECH), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and N-hydroxysuccinimide (NHS).

In the present work, electrochemical impedance spectroscopy was used in order to characterise graphite-epoxy resin composite (GrEC) electrodes modified with chitosan films crosslinked with glutaraldehyde (GA), glyoxal (GO), epichlorohydrin (ECH), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) together with N-hydroxysuccinimide (NHS) (EDC–NHS), structures shown in Fig. 1. The immobilisation of functionalised carbon nanotubes into chitosan matrices using the four crosslinkers was also evaluated. The best electrode composition (GrEC/Chit–CNT/EDC–NHS) was further applied to sensor and biosensor development and tested by EIS with different analytes, hydroquinone, dipyrone, and glucose. Equivalent circuit fitting was mostly carried out with the inclusion of finite Warburg diffusion elements, to reflect the shape of the spectra, and has been done in some recent diagnostic analysis of CNT-based biosensors. Characteristics of the sensors and biosensors developed are discussed.

2. Experimental

2.1. Apparatus

Electrochemical impedance measurements were carried out using a Solartron 1250 Frequency Response Analyser, coupled to a Solartron 1286 Electrochemical Interface (UK) controlled by ZPlot software. The voltage perturbation was 10 mV rms over a frequency range from 65 kHz to 0.1 Hz with 10 frequencies per decade, and integration time 60 s.

A three-electrode electrochemical cell was used for the measurements with graphite-epoxy composite, bare and/or modified, working electrode, Pt foil as auxiliary electrode and saturated calomel electrode (SCE) as reference.

The pH measurements were done with a CRISON 2001 micro pH-meter (Spain).

All experiments were performed at room temperature, 25 + 1 °C.

2.2. Chemicals

Multi-walled carbon nanotubes (MWCNTs) were obtained from NanoLab (USA). Araldit epoxy resin and Araldit hardener were purchased from Ceys S.A. (Spain). Graphite powder (grade #38) was obtained from Fisher Scientific Corporation (USA). Chitosan (Chit) of low molecular weight with a degree of deacetylation of 80%, glucose oxidase (GOx; E.C. 1.1.3.4) from *Aspergillus niger*, type II, lyophilized powder, 15,000–25,000 units/g solid, and α-D(+)glucose were obtained from Aldrich (Germany). Glyoxal (GO) (40%, v/v solution) and epichlorohydrin (ECH) (99%, v/v solution) were obtained from Aldrich (Germany). Glutaraldehyde (GA) (25%, v/v solution), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma (Germany) and N-hydroxysuccinimide (NHS) was from Fluka (Germany). Potassium hexacyanoferrate(III) was acquired from Merck (Germany) and potassium chloride was from Fluka (Germany). Hexaammineruthenium(III) chloride was acquired from Merck (Germany). Millipore Milli-Q nanopure water (resistivity >18 MΩ cm) was used for the preparation of all solutions.

For electrochemical experiments the supporting electrolyte was sodium phosphate buffer saline (NaPBS) (0.1 M Na_2HPO_4/NaH_2PO_4 + 0.05 M NaCl) pH 7.0 or 0.1 M KCl.

2.3. Pretreatment of multi-walled carbon nanotubes

Multi-walled carbon nanotubes (MWCNTs) were purified and functionalised as described elsewhere [16]. A mass of 120 mg of MWCNT was stirred in 10 mL of a 3 M nitric acid solution for 20 h. The solid product was collected on a filter paper and washed several times with nanopure water until the filtrate solution became neutral (pH ≅ 7). The functionalised MWCNTs obtained were then dried in an oven at 80 °C for 24 h. Nitric acid usually causes significant destruction of carbon nanotubes and introduces –COOH groups at the ends or at the sidewall defects of the nanotube structure.

2.4. Preparation of graphite-epoxy composite electrode

Graphite-epoxy composite electrodes were used as electrode substrates. These were prepared using graphite powder and Araldit epoxy resin/hardener by hand-mixing in the ratio 70:30 (m/m), as described previously [25]. The resulting paste was placed into the tip of a 1 mL insulin plastic syringe, and a copper rod with diameter equal to the inner size of the syringe was inserted to give external electrical contact. The resulting electrodes had 5 mm diameter, geometric area 0.196 cm^2, and their thickness was 5–7 mm. Before each use, the surface of the composite electrode was wetted with Milli-Q water and then thoroughly smoothed, first with abrasive paper and then with polishing paper, Kemet (UK).

2.5. Preparation of the film electrodes containing functionalised MWCNTs

A 1.0% m/m Chit stock solution was prepared by dissolving 100 mg of Chit powder in 10 mL of 1.0% (v/v) acetic acid solution and stirred for 3 h at room temperature until complete dissolution. The Chit solution was stored under refrigeration at 4 °C when not in use.

A dispersion of 1.0% m/v functionalised MWCNTs in 1.0% m/m chitosan was prepared by sonication of 2 mg of functionalised MWCNTs in 200 μL of 1.0% m/m Chit in 1.0 % (v/v) acetic acid solution for 2 h.

All chitosan-containing films were obtained using either 1.0% m/m Chit solution or 1.0% m/v functionalised MWCNTs in 1.0% m/m chitosan together with one of the crosslinkers placed directly onto the graphite-epoxy composite electrode; the detailed procedure is given elsewhere [26]. Briefly, the graphite-epoxy composite electrode was covered, in all cases, by first dropping 10 μL of 1% m/m Chit or 10 μL of 1% m/v MWCNTs in 1.0% m/m Chit and leaving it to dry for 1 h. After solvent evaporation, a second aliquot of 10 μL of Chit or MWCNT/Chit dispersion was dropped on the surface and the coated electrode was again left for solvent evaporation at room temperature in air for approximately 1 h. Then, 10 μL of 0.02 M NaOH solution was placed on the surface and dried for 40 min, followed by another aliquot of the same reagent, the purpose being to deprotonate the amino groups of Chit by changing the pH at the electrode surface. In the case of the EDC–NHS crosslinker, this procedure with NaOH solution was performed only once in