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Biointerfacial impedance characterization of reduced graphene oxide supported carboxyl pendant conducting copolymer based electrode

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ABSTRACT

We report, a comprehensive physical and biointerfacial electrochemical characteristics of electrodeposited poly(pyrrole-co-pyrrolepropylic acid) (PPy-PPa) copolymer film on the reduced graphene oxide (RGO) sheets attached over a silane modified indium-tin-oxide coated glass, for biosensing applications. The highly specific cardiac myoglobin protein antibody, Ab-cMb, has been covalently immobilized on the copolymer film through its pendent carboxyl group by carbodiimide coupling reaction. The factor 'n' describing divergence of the system from ideal capacitor characteristics exhibits a low value (n = 0.59) in a constant phase element of the impedance. This low value of 'n' showing a porous rough microstructure of PPy-PPa film on RGO exhibiting a diffusive characteristic that has been replaced by dominant charge transfer characteristic (R_{et}) with n = 0.78 on biomolecular immobilization and subsequent immunoreaction. The bioelectrode exhibits a linear impedance response to human cardiac cMb marker in the range of 10 ng mL^{-1} to 1 μg mL^{-1} in phosphate buffer solution (PBS; pH 7.4) at a low frequency region of <10 Hz with a R_{et} sensitivity of 70.30 Ω cm^2 per decade.

1. Introduction

Graphene oxide (GO), consisting of quasi-2-dimensional oxygenated sheets of sp^2-hybridized carbon, has emerged as a nanofiller for the preparation of hybrid polymer composite films due to its impressive electronic and mechanical properties, exceptional chemical stability, large surface area, absence of metal impurities and excellent biocompatibility [1,2]. The presence of highly electrically resistant surface bound groups such as carboxyl, hydroxyl or epoxy, on GO sheets is responsible for its hydrophilicity, [3] and consequent high dispersibility in both water and organic solvents by suppression of its aggregation. At the same time the enhanced interactions due to these surface bound groups facilitate processing in solution to hybrid polymer composites. Its so productive uniqueness and metal impurities free structure [4] has brought it out as the most promising carbon allotrope that an immense interest has been developed in the scientific community for its electrochemical applications such as electrical devices, [5,6] transistors, [7,8] fuel cells [9] and sensors [10]. The electrically insulating nature of GO caused by its highly electrically resistant surface reactant groups can be overcome by its electrochemical reduction to reduced graphene oxide (RGO). This is simple, fast, inexpensive and more efficient than other methods such as chemical and thermal reduction, with no additional element such as N incorporated into the obtained RGO film [11]. The RGO has been used as novel electrochemical sensing material in biological systems, such as detection of DNA, [12] protein and pathogen, [13] and drug delivery carrier [14].

Conducting polymers show extraordinary electrochemical properties such as: elevated electrical conductivity, low ionization potential, high electronic affinities and good optical properties, because of the presence of conjugated π-electron backbones [15,16]. Polypyrrole (PPy) is a widely studied conducting polymer due to its relative stability, low cost, ease of preparation, high electrical conductivity and excellent biocompatibility [15,17]. Recently composites based on PPy and different carbon materials like carbon nanotubes and graphene has gained widespread interest due to their improved electronic performances [18]. D. Zhang *et al.* [19] recently prepared a supercapacitor based on RGO doped polypyrrole (PPy) composites. The composite of ultrathin flexible graphene layers and PPy not only increased the electrical conductivity of the electrode material but also improved the stability of the polymer during doping/dedoping processes, [17,20,21] where graphene was either used as a dopant or filler in the polymer composite. Moreover, the biocompatibility of the PPy attracts its use in biomedical applications; however its hydrophobicity leads to a poor accessibility of the target biomolecules which can be overcome by the copolymerization of PPy with pyrrolepropylic acid (Pa) monomer without significantly compromising its electrical properties [22].

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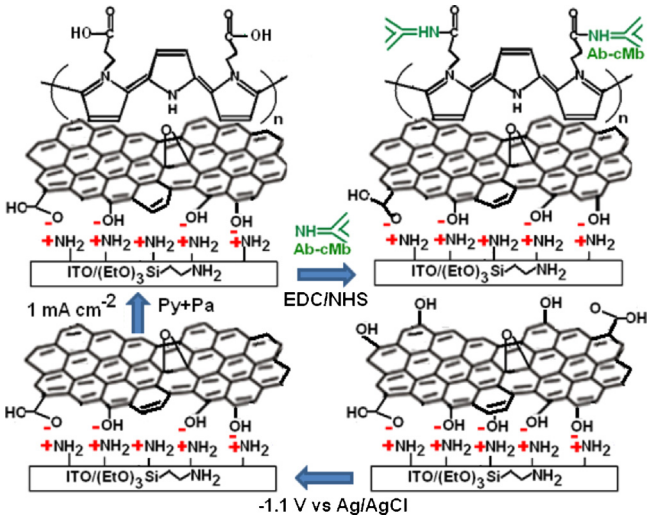
Cardiac myoglobin (cMb), a non-enzymatic cardiac protein, originates in the blood and skeletal muscle in response to damage in the heart and is being extensively used as diagnostic marker of acute myocardial infarction (AMI). During AMI, the small sized cMb (17.8 kDa) releases within 1-3 h in blood circulation and its level rises to as high as 900 ng mL^{-1} from its normal value of 30 to 90 ng mL^{-1} [23]. The high sensitivity and high predictive value of cMb makes it an important screening test for AMI. Various traditional techniques like chemiluminescent immunoassay, enzyme-linked immunosorbent assay (ELISA) and radio immunoassay (RIA) are being used for the quantification of cMb [24–26]. However; these techniques are complicate multistage processes, tedious and time consuming. Matveena *et al.* [27] has reported a silver island film (SIF) based fluorescence immunosensor for the cardiac Mb detection [15]. But, due to the non-homogeneous nature of SIF it has provided deviations in assay readings, which makes it unfavorable for the precise Mb detection.

Electrochemical impedance spectroscopy (EIS), has received considerable attention during the past decade due to its sensitive, rapid and non-destructive nature. The quantification of cMb in aqueous solution or serum is obtained by using its antigen-antibody (Ag-Ab) coupling mechanism where in these couples of cMb disturb the electrode layers by ion diffusion, causing change in electrical capacitance. These conductivity changes occurring in the electrolyte can be investigated by EIS [28,29] which is an efficient, sensitive and non-destructive tool for impedance measurement. The direct electrochemical reduction of Mb at the methylene blue-multiwalled carbon nanotubes modified electrode without going through an immunoreaction has been reported by Pakapongpan *et al.* [30]. However, this electrode has a limitation of Mb detection at high concentration range from 0.1 to 3.0 μM (~1.78 to 53.40 μg mL^{-1}) and would need sample dilution for a low level Mb detection in the physiological range.

This work demonstrates the electrochemical synthesis of conducting copolymer poly(pyrrole-co-pyrrolepropylic acid) (PPy-PPa) film on RGO matrix deposited over a silane modified indium-tin-oxide (ITO)-glass plate. Though graphene oxide and carbon nanotubes have been extensively used either as dopant or filler in the preparation of PPy composites, no one has yet reported the synthesis and physical and electrochemical characteristics of the copolymer film on RGO matrix. The RGO based PPy-PPa film possessed excellent mechanical and chemical stability together with high elecroactivity provided by the RGO sheets and excellent biocompatibility of PPy with efficient biomolecular immobilization through pendant copolymer carboxyl groups. The PPy-PPa-RGO was characterized both before and after biomolecular immobilization with myoglobin protein antibody, Ab-cMb, by fourier transform infrared microscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and electrochemical techniques. The electrochemical biosensing performance of the Ab-cMb/PPy-PPa-RGO film was studied towards the quantitative detection of Ag-cMb in phosphate buffer solution (PBS; pH 7.4). The stepwise construction of the prototype assembly is schematically represented in Scheme 1.



Scheme 1. Schematic representation of the stepwise fabrication of the bioelectrode.

2. Materials and Methods

2.1. Materials and instrumentation

Ag-cMb (Cat 8M50) and Ab-cMb (4M23) were obtained from Hytest (Turku, Finland). Mouse immunoglobulin-G (Ag-IgG) (Cat IGP3) was obtained from GENEI, Bangalore. 3-aminopropyltriethoxysilane (APTES) was purchased from Merck chemicals (Germany). Pyrrole, pyrrolepropylic acid, sodium p-Toluene sulphonic acid (PTSA), N-(3-dimethylamino propyl)-N'-ethyl carbodiimide hydrochloride (EDC) and N-hydroxy succinimide 98% (NHS), were obtained from Sigma–Aldrich chemicals. All other chemicals were of analytical grade and used without further purification.

Electropolymerization, cyclic voltammetry (CV) and EIS measurements were done on a PGSTAT302 N, AUTOLAB instrument from Eco Chemie, The Netherlands. CVs and EIS measurements were conducted in PBS (pH 7.4, 0.1 M KCl) solution containing a mixture of 2 mM $K_3[Fe_3(CN)_6]$ and 2 mM $K_4[Fe_2(CN)_6]$. The EIS experiments were carried out at a bias voltage of 0.3 V vs Ag/AgCl with an ac voltage of 0.05 V in the frequency range of 1 Hz to 100 kHz. The EIS experimental data were circuit fitted by GPES (General purpose electrochemical system version 4.9, Eco Chemie) software and values of EIS parameters were obtained. All measurements were carried out in a conventional three-electrode cell configuration consisting of a PPy-PPa-RGO based electrode/bioelectrode, as a working electrode, Ag/AgCl reference electrode and a platinum counter electrode. FTIR spectrum was taken on FTIR spectrometer, Nicolet 5700. SEM images were obtained with a LEO 440 PC; UK based digital scanning electron micrograph. High resolution transmission electron microscopy (HR-TEM) was done using FEI model: Tecnai G2 F30 STWIN with field emission gun, operated at 300 kV.

2.2. Preparation of RGO based PPy-PPa copolymer film

The ITO coated glass plates were cleaned by sequential ultrasonic cleaning for 10 min each in extran, acetone, ethanol, 2-propanol and double distilled water (DI) and thereafter dried under N_2 gas flow. The cleaned ITO glass plates were exposed to oxygen plasma for 5 minutes in a plasma chamber and then were immersed in 2% APTES solution prepared in ethanol for 1.5 h under ambient conditions, to form a self assembled monolayer (SAM) of APTES. In order to remove non-bonded APTES molecules from the surface of the substrate, the glass plates were rinsed with ethanol and dried under N_2 gas flow. These APTES modified ITO-glass plates were masked with insulated tape to open a working area of 0.25 cm^2 on each plate and immersed in a sonicated GO solution (0.3 mg mL^{-1}) for a period of 1 h to enable the electrostatic attachment of negatively charged GO sheets over the positively charged APTES modified ITO-glass, followed by washing with DI and dried under N_2 to form the GO/APTES/ITO-glass electrodes. These electrodes were electrochemically reduced by running three consecutive CV cycles, in a potential window of 0.1 to -1.1 V, at a