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Graphene nanoplatelets-silver nanorods-polymer based *in-situ* hybrid electrode for electroanalysis of dopamine and ascorbic acid in biological samples

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ABSTRACT

Highly sensitive and selective detection of various biomolecules is critical for biological research and diagnostics. Herein, we have fabricated an *in situ* hierarchically self-assembled hybrid electrode based on graphene nanoplatelets (GNP) functionalized with silver nanorods (AgNRs) followed by electrochemical polymerization of 4-amino-1-1′-azobenzene-3,4′-disulfonic acid dye (acid yellow 9, poly(AY)) for *in vitro* electroanalysis of biomolecules in biological samples. The UV–vis spectroscopy, FT-IR, XRD and FE-SEM were used to characterize the AgNRs and its incorporation into the graphene nano-interfaces. The GNP/AgNR/poly(AY) nanocomposite (NC) hybrid interface was introduced to modify the surface of glassy carbon electrode (GCE) to study *in vitro* electroanalysis of important biomolecules such as dopamine (DA) and ascorbic acid (AA) using cyclic voltammetry (CV) and amperometry. The GNP/AgNR/poly(AY) NC coated electrode exhibited high electrocatalytic activity towards DA and AA in physiological condition (pH~7) and separated their oxidation peaks at different potentials with reasonably good peak separation. As-prepared sensor showed linear response for the electro-catalytic oxidation of DA and AA from 1 to 200 μM with a detection limit of 0.42 μM and 0.88 μM, respectively. Furthermore, the effects of pH, scan rate and interferences on the detection of DA using human serum and urine samples are reported. Thus, the GNP/AgNR/poly(AY) hybrid film modified electrode may be utilized for the development of biomolecule transducers of various disease biomarkers.

1. Introduction

Graphene has emerged as most exciting research element due to its unique 2D structure, large surface area, unparalled flexibility, excellent electronic, mechanical and conducting properties [1] which have shown great potential in several applications like super capacitors, fuel cells, batteries, electronic devices, (bio)sensors, transistors, photo-catalysis, *etc.* [2–4]. Recent studies are focused on functionalization of graphene and its other forms namely graphene oxide (GO), graphene nanoplatelets (GNP), and reduced graphene oxide (rGO) by exploiting their hybrid nanocomposites (NCs) with conducting polymers, organic molecules, ionic liquids or metallic nanoparticles to improve the performance of devices [5]. Such hybrid NCs exhibit improved conductivity, electrocatalysis and sensitivity along with long term stability and antifouling (during electrocatalysis) characteristics compared to pristine graphene [5,6]. Restricted diffusion, decreased conductivity and slower temporal resolution are some of the drawbacks associated with use of conducting polymers [7], thus increasing focus on metal nanoparticles. Mostly reported nanoparticle/graphene NCs include gold (Au), silver (Ag), platinum (Pt) and also bimetallic hybrids [8,9]. Advantages and applications of various graphene based NC sensors have been recently reviewed in detail by Liu et al. [10] and Yang et al. [11]. Although Ag nanostructures have been used as transparent electrode materials and had shown remarkable electrocatalytic properties [12], the only drawback lies with the oxidation of Ag which decreases stability by increasing resistance. In order to overcome this, Ag nanostructures have been combined

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with 2D carbon nanomaterials which offer fast electron transfer kinetics between the electrode and adsorbed (bio)molecules via electrostatic interactions [13,14]. Many reports have been published on nanoparticle-graphene NCs for detection of heavy metal ions [15], hydrogen peroxide [16], biomolecules like DNA [17], glucose [18], dopamine (DA), ascorbic acid (AA) and uric acid (UA) [2], but there is a scarce of investigation on electrochemical detection of biomolecules using shape controlled anisotropic nanoparticles/nanorods. Nanorods exhibit two plasmon resonance bands, viz., transverse and longitudinal, out of which the latter is found to be more sensitive to local environmental changes. Therefore they prove to be more efficient for detection of biomolecules than spherical nanoparticles as the change in peak position and intensity can be easily observed [19]. Large surface to volume ratios along with excellent conducting/electronic/mechanical properties of NRs makes them suitable for biosensor applications. Few reports have reported the use of nanorods and nanowires for detection of glucose [6], hydrogen peroxide [20] and some biomolecules [21].

High conductivity and large surface area of graphene based materials usually improve stability, sensing and catalytic activity of metal nanostructures and helps in forming stable suspensions by preventing agglomeration without using the surfactants/ligands [22,23]. The oxygen containing groups of graphene derivatives serve as nucleation sites for *in situ* growth of nanostructures which in turn stabilizes and prevent restacking of graphene sheets [24]. Photosensitive zinc oxide nanorod/graphene heterostructures were formed via *in situ* aqueous seeded method on various substrates as visible-blind UV sensors [25]. Nanorods functioned as light absorbing and charge carrier generating component whereas graphene worked as conductive charge transporting component showing 45000 fold increases in photodetection. In a recent study, one pot *in situ* synthesis of gold nanorods over GO flakes was reported using UV light irradiation, for surface enhanced Raman spectroscopy measurements with the magnification factor of $\sim 10^6$ and low detection limits of 10^{-11} M for aromatic dye used [26]. Sun et al. demonstrated seed mediated method for *in situ* growth of gold nanorods with GO functionalized by polystyrene sulfonate which helped avoiding aggregation of nanorods before attachment [27]. The obtained hybrid NC exhibited good optical and morphological stability including *in vitro*, *in vivo* photothermal ability and proved to be an efficient system for precise CT-image-guided tumour photothermal therapy.

DA, a neurotransmitter present in the central nervous system, is considered one of the significant biomarker in detection of depression [28], drug addiction, diseases like Alzheimers [29], Schizophrenia [30], Parkinson's [31]; therefore its accurate detection is very important but it is also more challenging due to the presence of other biomolecules (interferences). For example, AA, an antioxidant found abundantly in food, beverages and pharmaceutical formulations, is also present with DA. In this work, for the first time, we have synthesized hybrid material of AgNR-GNP by *in situ* seed mediated growth method in the presence of cetyltrimethylammonium bromide (CTAB) stabilized GNP suspension. The AgNR-GNP film on glassy carbon electrode (GCE) was further covered with an electro-polymerized film of acid yellow 9 (4-amino-1-1′-azobenzene-3,4′-disulfonic acid, poly(AY)). This new hybrid NC biosensor showed the combined effect of AgNRs and GNP with excellent electro-catalytic activity towards selective determination of DA and AA simultaneously. Characterization studies were performed by cyclic voltammetry (CV), amperometry, differential pulse voltammetry (DPV), field-emission scanning electron microscopy (FE-SEM), X-ray diffraction (XRD), UV–vis spectroscopy and Fourier transform infrared spectroscopy (FT-IR). The selective detection of DA in real samples (human serum, urine sample and Celin-500 vitamin C tablets) was studied with satisfactory results. The results suggested that the prepared NC electrode system may be used for electrochemical biosensing of DA and AA *in vitro* applications.

2. Materials and methods

2.1. Materials

CTAB, dopamine hydrochloride, L-ascorbic acid and acid yellow 9 were purchased from Sigma Aldrich, India while silver nitrate ($AgNO_3$), sodium hydroxide (NaOH), sodium borohydrate ($NaBH_4$), sodium citrate tribasic, phosphate buffered saline (PBS, 0.1M, pH 7.4), potassium chloride (KCl), potassium ferrocyanide and potassium ferricyanide were obtained from SRL Pvt. Ltd., India. Graphene nanoplatelets were received from XG Sciences, Inc., and sulphuric acid (H_2SO_4, 98%) was purchased from Rankem, India. All chemicals were of analytical grade and used as received. Milli-Q water (18.2 MΩ cm@25°C) from Millipore water purification system was used for all experiments and stock solutions were freshly prepared. Celin-500 tablets (500 mg) were purchased from local drug store in Chennai while serum and urine samples were obtained from a running laboratory.

2.2. Synthesis of AgNRs

Synthesis of AgNR was done as reported by Jana et al. via seed mediated method using CTAB [32]. The first step included the preparation of seeds for which 20 mL solution of 0.25 mM $AgNO_3$ and 0.25 mM of sodium citrate was prepared in water. Later, 0.6 mL of 10 mM $NaBH_4$ (strong reducing agent) was added to the above solution while stirring to form Ag seeds (3–4 nm). The seeds were kept for 2 h at room temperature and thereafter used to grow into AgNRs in the second step. 10 mL CTAB (80 mM), 0.25 mL $AgNO_3$ (10 mM), 0.5 mL AA (100 mM, weak reducing agent) and 0.6 mL of seed solution was mixed to form AgNR growth solution. Lastly, 0.1 mL NaOH (1 M) was introduced to the above solution which led to colour change in 4–5 min indicating formation of AgNRs. This was centrifuged at 2000 rpm for 6 min to separate nanorods from other particles. The nanorods were then washed twice and redispersed in 0.5 mL of water for further use.

2.3. Preparation of GNP/AgNR dispersion

2-3 mg of GNP powder was dispersed in 10 mL of 80 mM CTAB by doing bath sonication for 5 min, followed by 30 min of probe sonication at 30% amplitude and 5 s on/2 s off pulse. This was centrifuged at 1000 rpm for 30 min to separate supernatant with well dispersed GNP. The GNP/CTAB dispersion was used for preparation of AgNRs as mentioned in Section 2.2 (*See Supplementary Information*).

2.4. Preparation of GNP/AgNR/poly(AY) NC coated electrode

The surface of GCE was polished with alumina powder (0.05 μm) until a mirror like finish was obtained. Further, electrochemical activation of GCE was carried out using potential cycling method between −0.4 to 2.0 V at a scan rate of 100 mVs^{-1} in 0.1 M H_2SO_4 solution. AY aqueous monomer solution (0.1 M) was mixed with 0.1 M H_2SO_4 separately which acts as an electrolyte. In order to do electrode modification, 20 μL of GNP/AgNR solution was drop casted on GCE surface and dried at 40°C in an air oven. Using CV, AY monomers were electrochemically polymerized onto the surface of GNP/AgNR/GCE by potential scanning in the range of −0.4 – 2 V at a scan rate of 100 mVs^{-1} for 20 cycles in AY monomer solution. The prepared GNP/AgNR/poly(AY) NC film was strongly adherent to the electrode surface. After several washing with water and drying method, the GNP/AgNR/poly(AY)/GCE biosensor was obtained and used for further studies.

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