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Fabrification of electroreduced graphene oxide–bentonite sodium composite modified electrode and its sensing application for linezolid

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ABSTRACT

Graphene and its composites have attracted considerable attention in synthesis and electrochemical applications. In the present work, we have synthesized and characterized graphene oxide-bentonite composite (GO-BEN) and utilized it to fabricate an electrochemical sensor. For this, the solution of GO-BEN cast on glassy carbon electrode (GCE) was reduced electrochemically in phosphate buffer solution of pH 6 to obtain electrochemically reduced graphene oxide-bentonite composite (ERGO-BEN-GCE). This ERGO-BEN film was used for electrochemical investigation of an oxazolidinone class of antibiotic, linezolid (LIN) for the first time. The electrochemical sensor showed excellent enhancement and adsorptive ability towards the electrooxidation of LIN. LIN exhibited two each of oxidation and reduction peaks on ERGO-BEN film in phosphate buffer of pH 7.0. Effects of accumulation time, pH of solution and scan rate were studied and various electrochemical parameters were evaluated. The plot of pH versus E_p gave a slope of 26.2 mV/pH in the pH range of 4.2-8.0 indicating the participation of two electrons and one proton in the electrode process. An adsorptive stripping differential pulse voltammetric method (AdSDPV) was developed for the determination of LIN in bulk, pharmaceutical formulations and urine samples. Adsorptive stripping linear sweep voltammetric (AdSLSV) and differential pulse voltammetric (DPV) methods were also developed and the results were compared. LIN showed linear relationship between the current density and concentration in the range of 0.25 - 31.25 μM with a LOD of 0.0337 μM in AdSDPV method; 0.5 - 31.25 μM with a LOD of 0.100 μM in DPV method and 1.25 - 37.5 μM with a LOD of 0.5461 μM in AdSLSV method respectively. The proposed AdSDPV method was observed to be simple, fast and inexpensive and hence, could be readily adopted for quality control in pharmaceutical products.

1. Introduction

Graphene, a two-dimensional monolayer of carbon atoms arranged in honeycomb lattice, is one of the hottest subjects in current research topics because of its excellent electrical, mechanical and thermal properties. It finds extensive applications in chemical, electrochemical and gas sensors, energy-related materials and biomedical fields [1–5]. Meanwhile, graphene oxide (GO), a derivative of graphene not only owns the above mentioned properties like graphene, but also possesses other properties such as hydrophilicity, multiple oxygen moieties and controllable electronic properties [6,7]. In view of these, GO has attracted great attention in preparing advanced materials. GO has shown promising applications in electroanalysis [8,9], composites [10] and electronic devices [11], probably because of its good solution-processibility and ease of post-functionalization. Some researchers have already prepared composites of GO with various materials to achieve the desired properties [12]. The efforts were mainly made on the effective dispersibility of the composites [13], and improvement of the electrical conductivity [14], thermal stability and mechanical strength of composites [15].

Clay films are known to be potentially useful as supports for dispersed semiconductor particles, metals and metal oxides which can function as catalysts [16]. In this respect, the role of clay films is similar to that for Nafion membranes [17] used in graphene-nafion composites [18]. Electrode surfaces modified with clay or its composites offer advantages of high chemical stability, known and potentially controllable structural features, low cost, large-scale applications etc [19–21]. Bentonite is the most important type of

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clay and is extensively found in nature. The predominant property of bentonite is the huge cationic exchange capacity. Therefore, bentonite has been widely used as cation exchanger in various fields such as environmental science and electrochemistry [22,23].

Linezolid, ((S)-N-[[3-fluoro-4-(4-morpholinyl)-phenyl]- 2-oxo-5-oxazolidinyl]methyl]-acetamide, is the first of a new class of antimicrobial drugs, the oxazolidinones, used for the treatment of multidrug- resistant gram-positive bacterial infections that inhibit bacterial protein synthesis through a unique mechanism [24]. In contrast to other inhibitors of protein synthesis, the oxazolidinones act early in translation by preventing the formation of a functional initiation complex [25]. Linezolid (LIN) has inhibitory activity against a broad range of gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), glycopeptide-intermediate *S. aureus* (GISA), vancomycine-resistant enterococci (VRE) and penicillin-resistant *Streptococcus pneumoniae* [26].

Some analytical methods have been reported for the determination of LIN including voltammetric [27], HPLC [28], RP-HPLC [29,30], LC-MS/MS [31], spectrophotometric [32,33] and liquid chromatography-tandem mass spectroscopy [34]. However, the reported chromatographic and spectroscopic methods are time-consuming. In addition, the utilization of these conventional methods generates considerable waste. The reported voltammetric method [27] was less sensitive. So in order to develop a sensitive analytical method for the assay of LIN, we have developed an eco friendly, simple, rapid and cost effective electroanalytical method based on ERGO-BEN-GCE. The ERGO-BEN favored the electron transfer and also enhanced the adsorption of LIN on ERGO-BEN owing to the presence of BEN in the proposed method. Using this sensing system, greatly enhanced signals were obtained for electrochemical oxidation of LIN and hence, it was utilized for analytical applications.

2. Experimental

2.1. Apparatus

Electrochemical studies were carried out on a CHI-1110a Electrochemical Analyzer (CH Instruments Ltd. Co., USA, version 12.23) in a home-made electrochemical cell with a three electrode arrangement consisting of a GCE (3 mm diameter) or ERGO-BEN-GCE as the working electrode, a platinum wire as the auxiliary electrode and a saturated calomel electrode as the reference electrode. A magnetic stirrer (Remi Instruments, India) with a stirring bar was used to provide the convective transport of the analyte during its preconcentration onto the ERGO-BEN-GCE. For reproducible results, improved sensitivity and good resolution of voltammetric peaks, the working electrode was abraded carefully with 1.0, 0.3 and 0.05 micron Al_2O_3 slurry on a smooth polishing cloth. Then, it was thoroughly rinsed with millipore water. All the potentials reported in this paper are relative to saturated calomel electrode (SCE).

SEM studies were performed on a Hitachi SU-1500(Japan) scanning electron microscope with an accelerating voltage of 15 kV. Composition analysis of samples was carried out on a Hitachi S-3400S SEM (Japan) coupled with a Thermo Scientific EDX detector at an accelerating voltage of 15 kV and a magnification of 2 KX.

2.2. Reagents

Graphite powder was obtained from Sigma-Aldrich (<20 μM). Bentonite was purchased from Sigma-Aldrich. Clay colloids in sodium form were prepared as per the procedure described elsewhere [19,35]. In brief, sodium-bentonite sample (abbreviated as BEN) was prepared by treatment of 5 g bentonite with 1.0 M NaCl solution for 48 h. After complete exchange, it was centrifuged at 10,000 rpm for 30 min. After centrifugation, it was repeatedly washed with millipore water until it was free from chloride. The fractions were then separated by centrifugation and dried in an oven. Pure LIN was obtained as a gift sample from Biocon Limited, Bangalore, India. Tablets of LIN were obtained from commercial sources. A stock solution of LIN (2.5 mM) was prepared in a mixture of methanol and millipore water (50:50) and stored in a refrigerator at 4 °C. Working solutions were prepared freshly by diluting the stock solution as required with phosphate buffer (0.2 M) of required pH. In the present study, phosphate buffer (pH 3.0-10.6) was used. All the solutions were prepared in millipore water and all other chemicals used were of analytical reagent grade.

2.3. SEM/EDX

Films for Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) analysis were prepared by settling sample suspension on carbon film coated on sample holder and drying at room temperature.

2.4. Preparation of GO-BEN modified GCE and its electroreduction

GO was synthesized from graphite powder (Sigma-Aldrich) by Hummers method and then dried in an oven at 120 °C. [36]. The GO-BEN suspension was prepared by dispersing 7.5 mg of GO and 2.5 mg of BEN in 10 mL water using ultrasonic agitation (1 h) to obtain a relatively stable dark yellowish brown suspension. It also resulted in exfoliation of the graphite oxide to graphene oxide. It was then centrifuged to remove the unexfoliated GO, if any, by a method similar to the one reported for the preparation of GO suspension [37,38]. This stable suspension was used to modify the GCE.

Before modification, the GCE was carefully polished with 1.0, 0.3 and 0.05 μm α-alumina on a smooth polishing cloth and then washed with millipore water. The cleaned GCE was coated by casting 5 μL of the yellowish-brown suspension of GO-BEN and dried under infrared lamp to obtain a graphene oxide-bentonite sodium modified electrode, denoted as GO-BEN-GCE. Upon modification, the electrode was rinsed with water to remove the loosely adsorbed GO. The GO-BEN-GCE was then electroreduced in phosphate buffer of pH 6.0 by applying 25 cyclic potential sweeps in between 0 and -1.5 V as per the method reported recently with minor modification [39–41] to obtain ERGO-BEN-GCE. This process of preparing ERGO-BEN-GCE is illustrated in Scheme 1. The modified electrode was then transferred into another 10 mL phosphate buffer (pH 7.0) containing suitable aliquot of LIN and a preconcentration time of 150 s was maintained while the solution was stirred at 400 rpm with the magnetic stirrer. At the end of the preconcentration time, the stirring was stopped, and a rest period of 10 s was allowed for the solution to become quiescent. The voltammogram (CV, AdSDPV, AdSLSV or DPV) was then recorded with the following parameters: scan rate 0.1 Vs^{-1} and sample interval 0.001 V (for CV and AdSLSV), pulse amplitude 0.05 V, pulse width 0.06s, sampling width 0.02s and pulse period 0.2s (for DPV and AdSDPV).

Working solutions were prepared daily by diluting the stock solution as required with phosphate buffer (0.2 M). All the electrochemical experiments were carried out at 25 ± 1 °C. After every measurement, new ERGO-BEN-GCE was prepared.

The microscopic areas of the ERGO-BEN-GCE and the bare GCE were obtained by CV using 1 mM $K_3[Fe(CN)_6]$ as a probe at different scan rates. For a reversible process, the Randles–Sevćik formula as shown below was used:

$$I_{pa} = 2.69 \times 10^5 (\mathrm{A\ s\ mol^{-1} V^{1/2}}) n^{3/2} D_0^{1/2} C_0 \nu^{1/2} \quad (1)$$