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Enhancement effect of reduced graphene oxide and silver nanocomposite supported on poly brilliant blue platform for ultra-trace voltammetric analysis of rosuvastatin in tablets and human plasma

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Experimental section

1. *Reagents and chemicals*

Rousvastatin (RS) was obtained from Astra-Zeneca (purity 99 %) and used as received without further purification. Rosuvast® tablets (ChemiPharma, 5mg RS), Advochol® tablets (Advocure Pharma, 10 mg RS) and Suvikan ® tablets (Hikma, 20 mg RS) were purchased from local market. Hydrochloric, acetic and sulphuric acid were obtained from El- Nasr Company (Cairo, Egypt). Silver nitrate is obtained from Sigma Co. Cairo Egypt. Brilliant blue dye (Alliance Bio, USA) was prepared in 0.1 mol L^{-1} sodium hydroxide to obtain 3 x10^{-3} mol L^{-1} solution; further dilutions were made to obtain the optimum concentrations. All other chemicals were of analytical reagent grade and were used as received. Double distilled water was used for the preparation of standard solutions and electrolytes.

2. *Apparatus*

For the electrochemical measurements, a Princeton VersaSTAT MC (VersaSTAT 3, Model RE-1, Princeton Applied Research, AMETEK, USA) controlled by 394 software in conjunction with a PAR Model polarographic analyzer was used. A conventional three-electrode cell was employed; pencil graphite electrode PGE bare or modified is used as a working electrode, Ag/AgCl (3 mol L^{-1} KCl) as a reference electrode and a platinum wire as an auxiliary electrode. All electrochemical measurements were carried out at room temperature (25 ± 5 °C). Z-view software was used for electrochemical impedance spectroscopy calculations (Z-view version 3.5d). Rotring (HB) pencil leads with a diameter of 0.5 mm and a length of 60 mm were used. Electrical contact with the pencil was obtained using a metallic wire wrapped to the metallic part fixing the lead inside the pencil. The pencil electrode was fitted vertically and immersed into the electrolyte solution inside the electrochemical cell.

Spectrophotometric measurements were done using a double beam UV–Visible spectrophotometer (Shimadzu, Kyoto, Japan) model UV-1601 PC connected to an IBM compatible computer, with UVPC personal spectroscopy software version 3.7. The absorption spectra were carried out using 1 cm quartz cells and recorded over the wavelength range from 200 to 600 nm. All pH-metric measurements were made on Hanna pH meter (Hanna Instruments, São Paulo, Brazil) digital pH-meter with glass combination electrode. Surface morphological studies of the bare and modified electrode were carried out using scanning electron microscope (SEM), JEOL JSM-5400 LV instrument (Oxford, USA).

The FTIR spectra of graphite and GO were recorded using Fourier transform infrared (FTIR) spectrometer (Thermoscientific Nicolet 6700). The scan resolution was selected at 4 cm^{-1} with an average of 16 scans, scanned over the range 400-4000 cm^{-1}. OriginPro 8.5 software was used for graphing and analyzing the experimental data.

Raman spectra of GO and AgNPs-rGO were acquired using a Bruker Senterra Raman microscope (Bruker, Optics Inc., Germany) equipped with 785 nm excitation, a Nikon objective (x50, NA 0.75, WD 0.56) and a holographic grating with 800 lines/mm along with a charge coupled device detector (CCD). The spectra in the relevant range from 400 to 2000 cm^{-1} were recorded using a 5 s integration time.

3. Preparation of standard and reagent solutions

3.1. Preparation of RS standard solution

A stock solution of RS (1×10^{-4} mol L^{-1}) was prepared into a 25 mL calibrated flask and filled to the volume with methanol. The stock solution was further diluted with methanol to obtain working solutions in the calibrated concentration ranges. The stock and working standard solutions were preserved in light protected flasks and freshly prepared.

3.2. Synthesis of graphene oxide (GO)

GO powder was synthesized according to modified Hummer's method.[1] A stable suspension of GO was prepared by transferring 15 mg of synthesized GO powder to 10 mL volumetric flask and the volume was made using double distilled water to form GO suspension (1.5 mg mL^{-1}). The suspension was ultra-sonicated for 1 hr and further dilution was made with double distilled water to obtain the optimized concentration which transferred to the electrochemical cell for further electro-deposition to produce r-GO films over the PBB electrode.

3.3. Fabrication of AgNPs-rGO/PBB modified electrode

The PGE electrode was washed with double distilled water then immersed in $3x10^{-5}$ mol L^{-1} solution of BB dye and finally polymerized by 7 cycles over potential ranged from -0.5 V to 1.5 V at 100 mV s^{-1} producing PBB modified electrode. The modified PBB electrode was washed with double distilled water, and immersed in electrochemical cell containing mixture of silver nitrate solution (50 mmol L^{-1} dissolved in 0.5 mol $L^{-1}H_2SO_4$) and GO solution (1.5 mg mL^{-1}) at a volume ratio of 6:1 after stirring for 10 min. The electrochemical deposition was carried out using cyclic voltammetry technique, with a potential range 0 V to -1.5 V at 100 mV s^{-1} scan rate to obtain AgNPs-rGO/PBB. The modified electrode (AgNPs-rGO/PBB) was then washed with double distilled water and placed in 0.15 mol L^{-1} H_2SO_4 as a supporting electrolyte for voltammetric determination of rousvastatin (RS).

3.4. Fabrication of AgNPs/PBB modified electrode

Electrodeposition of AgNPs was carried out following Yang method.[2] Initially, PBB/PGE modified electrode was produced using the previous mentioned procedure in 3.3. Then, the modified electrode was washed with double distilled water and immersed in an electrochemical cell containing a suitable volume of 50 mmol L^{-1} silver nitrate aqueous solution containing 0.5 mol L^{-1} H_2SO_4 and the electrodeposition of AgNPs was conducted at potential -1.2 V for 60 s.

4. General Analytical Procedure

The PGE surface was polished and modified as mentioned under Section 3.3, each measurement was performed using a new pencil lead surface. An aliquot of the stock solution of RS was transferred into the electrochemical cell containing 0.15 mol L^{-1} H_2SO_4 as a supporting electrolyte. The proposed SWV method was recorded from 0.0 V to + 1.5 V under the optimized experimental conditions described later.

5. Procedure for determination of RS in pharmaceutical tablets

Twenty tablets of Rosuvast® were weighed and grounded separately with porcelain mortar. An accurately weighed amount of the finely powdered of Rosuvast® tablets equivalent to 50 mg of RS was transferred to 25 mL volumetric flask and completed to the mark with methanol. The solution was sonicated for 20 min., filtrated and the filtrate was diluted with methanol to obtain the required concentration for each experiment. The analytical procedure was continued as mentioned under general analytical procedure and the procedure was repeated exactly for Advochol® and Suvikan ® tablets.

6. Procedure for determination of RS in human plasma samples

Drug-free human plasma samples were obtained from four healthy volunteers. All samples were pooled and stored at -20°C till analysis. The plasma samples (0.5 mL) were transferred into eppendorf tubes, spiked with different concentrations of standard RS solution followed by addition with 1 mL acetonitrile. The tubes were vortex mixed, centrifuged at 5000 rpm for 10 min and filtered through a 0.45 µm nylon filter (Millipore–Whatman, Kent, UK). A suitable volume of the filtrate was transferred into the electrochemical cell and the studied analyte was analyzed using AgNPs-rGO/PBB electrode under the optimized chemical and instrumental conditions.

7. Electrochemical Impedance Spectroscopy (EIS) measurements

EIS measurements for bare PGE and AgNPs-rGO/PBB electrodes were monitored in the presence of 1.0 mmol L^{-1} $K_3[Fe(CN)_6]$ in 0.5 mol L^{-1} KCl. An excitation ac signal of 10 mV amplitude and a frequency ranged from 1 Hz to 10 kHz were used in the EIS measurements. EIS results were fitted to Randles equivalent circuit and Z-view software was used to analyze the electrochemical impedance spectroscopy results.

8. Validation study

The proposed voltammetric method was validated according to ICH guidelines[3] and different validation parameters such as linearity range, precision, accuracy, limit of detection and limit of quantitation were studied and calculated.

References of supplementary data

[1] N.I. Zaaba, K.L. Foo, U. Hashim, S.J. Tan, W.W. Liu, C.H. Voon, Synthesis of graphene oxide using modified Hummers method: Solvent influence, Procedia Eng. 2017, **184**, 469.

[2] J. Yang, X. Liu, X. Du, Electrodeposition of silver nanoparticles on ito films with different thickness and application as LSPR sensor, ECS Electrochem. Lett. 2014, **3**, B30.

[3] ICH Harmonised Tripartite Guidelines, Validation of analytical procedures: text and methodology Q2(R1) (1996) http://www.ich.org/ products/guidelines/quality/article/quality-guidelines.html. (Accessed 27 Sep 2016).

Supplementary data

Figure S1. (A) FTIR spectra of (1) graphite and (2) GO and (B) Raman spectra of (1) AgNPs-rGO and (2) GO.

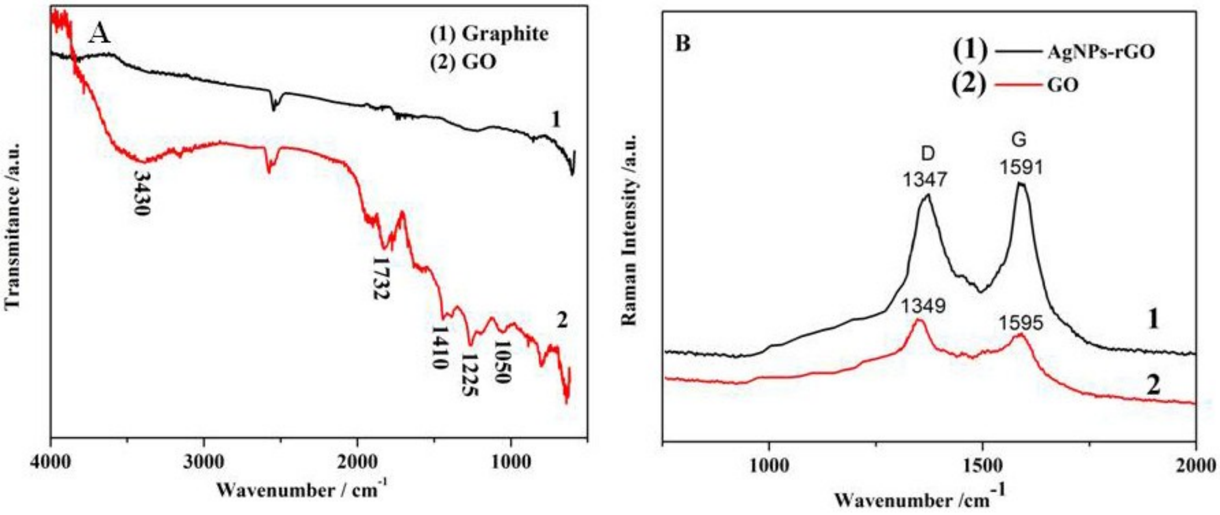

Figure S2. Mechanism of the electro-oxidation reaction of RS on AgNPs-rGO/PBB modified electrode as previously reported.[39]