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In-Situ polymerization and FT-IR characterization of poly-glycine on pencil graphite electrode for sensitive determination of anti-emetic drug, granisetron in injections and human plasma

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Supplementary file

1. Experimental

1.1. Reagents and chemicals

Granisetron hydrochloride (GRN) standard (99.20%) was kindly provided by from GlaxoSmithKline, Cairo, Egypt. Injection containing granisetron hydrochloride (Em-Ex ampoule manufactured by Mankind Pharmaceuticals Pvt. Ltd. (Discovery)) was obtained from commercial source. Britton Robinson, phosphate and borate buffer were prepared in de-ionized water and used without filtration. All chemicals used were of analytical reagent grade and employed without further purification. Glycine, graphite and paraffin oil were obtained from Sigma Aldrich (Germany).

2. Instrumentation

Electrochemical measurements were performed using Princeton VersaSTAT MC (*VersaSTAT* 3, Model RE-1, Princeton Applied Research, AMETEK, USA). Z-view software was used to calculate the electrochemical impedance spectroscopy calculations (Z-view version 3.1c). The electrochemical cell in this method used three electrodes, Ag/AgCl (3M KCl) as a reference electrode, the auxiliary electrode from platinum wire and glassy carbon electrode (GCE), carbon paste electrode (CPE), pencil graphite electrode bare (PGE) or poly-Gly/PGE were used as a working electrode. 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 modified electrode were carried out using scanning electron microscope (SEM), JEOL JSM-5400 LV instrument (Oxford, USA). A Nicolet 6700 FTIR advanced Gold Spectrometer, supported with OMNIC 8 software (Thermo Electron Scientific Instruments Corp., WI USA) for data processing.

3. Analytical procedures

3.1. Preparation of standard solutions

A weight portion of GRN powder equivalent to 1.0×10^{-3} M was transferred to a 100-mLvolumetric flask and completed to the volume with de-ionized water. Working solutions were prepared by taking suitable aliquots from this stock solution and diluting them with de-ionized water.

3.2. Preparation of the different working electrodes

a) *Glassy carbon electrode (GCE):* Prior to each experiment, a bare GCE was carefully polished to a mirror finish using aqueous slurry of 0.05 mm alumina and rinsed thoroughly with de-ionized water.

b) *Carbon paste electrode (CPE):* the carbon paste was prepared by mixing of 0.5 g graphite powder with 0.3 mL of paraffin oil in a mortar using a pestle to obtain a homogeneous paste. Then a portion of carbon paste was packed into an insulin syringe with a 3.0 mm diameter. Insert a copper wire to connect the electrode with the external circuit.

c) *Pencil graphite electrode (PGE):* Rotring (2H) pencil leads with a diameter of 0.5 mm and a length of 60 mm were used. Electrical contact with the pencil was obtained by connecting a metallic wire to the metallic part fixing the lead inside the pencil. The pencil electrode was fitted vertically and immersed into the solution inside the electrochemical cell.

d) *Polyglycine modified pencil graphite electrode (Poly(Gly)/PGE):* The surface of PGE was activated electrochemically by applying multiple cyclic scans for 5 cycles at a potential between -0.2 and 2.0 V *vs* Ag/AgCl with a scan rate of 100 mV s^{-1} in 0.2 M phosphate buffer solution (pH 7.0). Then glycine was electro-polymerized as a film using cyclic voltammetry for 7 cycles at a potential between -0.5 and 2.0 V *vs* Ag/AgCl electrode with a scan rate of 100 mV s^{-1} in 0.2 M phosphate

buffer solution (pH 5.0). After polymerization, the electrode was washed with de-ionized water and then used for the subsequent studies. A new pencil lead is used for each experiment throughout the whole study.

3.3. Electrochemical procedures

Square wave voltammetry (SWV) and cyclic voltammetry (CV) were used for determination of GRN. The CV and SWV measurements were performed in Britton Robinson buffer solution BR (pH=7) and the electrode potential was between -0.2 and 1.5 V at pulse height of 10 mV, frequency of 250 Hz, step height of 10 mV and 120 s as a deposition time. In this work, all reported potentials were referenced to (Ag/AgCl) reference electrode.

3.4. Electrochemical Impedance Spectroscopy (EIS) measurements

EIS measurements were conducted using an excitation ac signal of 10 mV amplitude and a frequency range from 1 Hz to 100 kHz in presence of 1.0 mM $K_3[Fe(CN)_6]$ in 0.5 M KCl adjusted to pH 2.5. EIS results were fitted to a Randles equivalent circuit. Z-view software was used to analyze the electrochemical impedance spectroscopy results (Z-view version 3.1c).

3.5. Characterization of the modified poly (Gly)/PGE

FTIR spectroscopy study was carried out on a Nicolet advanced spectrometer using the KBr pellet method for the powder of glycine or for poly (Gly) polymer. The formed poly (Gly) polymer film was separated by cutting of nearly 10 mm of the electrode length after glycine electro-polymerization on the surface of the electrode, and scratching the outer layer deposited on the surface of the electrode by a thin small spatula. Characterization of bare and poly (Gly)/PGE electrode were done also

using scanning electron microscopy (SEM). After cutting 10 mm of the electrode, it was fixed on a conducting carbon tape and sputtered with a thin layer of gold to avoid charging during the analysis. Surface morphological studies were carried out on JEOL JSM-5400 LV instrument.

4. Applications

4.1. Application to GRN ampoules

A volume portion equivalent to 1.0×10^{-3} M of GRN was transferred from an ampoule into a 50-mL calibrated flask completed to the mark with deionized water and then stored in dark at 4 °C. Further dilutions were performed with de-ionized water and the voltammetric procedures were carried out as described in **3.3.**

4.2. Analysis of GRN in spiked human plasma samples

Drug free plasma samples were obtained from Drug Bank, Assiut University Hospital. All plasma samples were poured together, frozen and stored at - 4 °C till analysis. A volume of 0.5 mL of plasma sample was spiked with different aliquots of GRN standard solution and treated with 1.5 mL of methanol to remove plasma protein effectively. Afterwards the solution was centrifuged at 5000 rpm for 30 min at room temperature then filtered through a 0.45 μm nylon filter (Millipore–Whatman, Kent, UK). Use the filtrate for further work as described before in section **3.3.**

4.3. Analysis of GRN in real human plasma samples

Blood samples were collected from five healthy volunteers administered GRN pharmaceutical ampoule (Em-Ex, 3mg/ampoule) IV as a single dose. They were collected into heparinized tubes and then centrifuged at

3000 rpm for 30 min to separate plasma. A volume of 0.5 mL of plasma sample was treated with 1.5 mL of methanol to precipitate plasma protein, after that the solution was centrifuged (at 5000 rpm for 30 min) at room temperature then filtered through a 0.45 μm nylon filter (Millipore–Whatman, Kent, UK). The filtrate was used for further work as described before in section **3.3.**

The experimental protocol was performed according to the Egyptian regulations and was approved by the Institutional Human Ethics Committee, Assiut University, Assiut, Egypt.