

Supplementary materials

Simultaneous and sensitive determination of melatonin and dopamine with Fe₃O₄ nanoparticles-decorated reduced graphene oxide modified electrode

H. Bagheri, ^{a,*} A. Afkhami, ^b P. Hashemi, ^b and M. Ghanei ^a

^a *Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

^b *Faculty of Chemistry, Bu-Ali Sina University, Hamedan, Iran*

Experimental

Apparatus and chemicals

All voltammetric measurements were performed using a Behpajoh model BHP-2065 potentiostat/galvanostat. pH measurements were performed using a Metrohm pH-meter, Model 713, with a glass electrode (Metrohm, Swiss). A conventional three electrode system was used with a modified CPE, a standard calomel electrode (SCE) and a platinum electrode as the working, reference and counter electrodes, respectively. An Autolab electrochemical analyzer, Model PGSTAT 302N potentiostat/galvanostat (Eco-Chemie, The Netherlands), was used for electrochemical impedance spectroscopy (EIS) studies. SEM was performed on a XL30 scanning electron microscope (SEM-EDX, Philips Netherland). XRD pattern was determined by an XRD (38066 Riva, d/G.Via M. Misone, 11/D (TN) Italy) at an ambient temperature. FT-IR spectra (4000-400 cm⁻¹) in KBr were recorded on a PerkinElmer, spectrum 100, FT-IR spectrometer. Thermogravimetric analysis (TGA) was carried out with a STA 409 PC Luxx Simultaneous DTA-TGA, NETZSCH company.

All chemicals used in this work were of analytical grade and used as received without further purification. Paraffin oil and graphite powder were obtained from Merck company and used as received. DA and ML were purchased from Merck and Sigma-Aldrich, respectively. Stock solutions of DA and ML were freshly prepared as required in the appropriate buffer solutions. The commercial pharmaceuticals available from local pharmacies were subjected to the analysis. Britton-Robinson (B-R) universal buffer (0.04 M boric acid, 0.04 M acetic acid and 0.04 M phosphoric acid), acetate buffer, phosphate buffer and KNO₃ solution were prepared in double distilled water (DDW) and were tested as supporting electrolytes.

Preparation of the electrode

The unmodified CPE was prepared by mixing fine graphite powder with appropriate amount of paraffin (80:20, w/w) and thorough hand mixing in a mortar and pestle, and a portion of the composite mixture was packed into the end of a polyethylene syringe (2.5 mm diameter). The Gr/CPE was prepared by mixing the unmodified mixture with Gr (20%, w/w). The Gr-Fe₃O₄/CPE was prepared by mixing the unmodified mixture with 15% w/w Gr-Fe₃O₄ and transferred into the syringe.

Preparation of real samples

This study was conducted by the Ethics Committee and written informed consents were obtained from all volunteers. Human serum and urine sample was originally obtained from a patient (patient1: male, 58 years, 77 kg, 181 cm who underwent the treatment with ML and DA). Also, the aliquot amounts of drugs were added to biological sample which did not contain examined drugs. The samples were always collected 1 h after intake of drug. Urine samples were stored in a refrigerator immediately after their collection. A 10 mL of the sample was centrifuged for 10 min at 5000 rpm. The supernatant was filtered using a 0.45 mm filter and then diluted 5-times with B-R buffer solution of pH 5.0.

The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. Standard addition method was used for the determination of ML and DA content of the sample. Serum sample was collected. Serum sample was treated with 0.2 mL methanol as a serum protein precipitating agent. After vortexing for 30 s, the precipitated protein was separated out by centrifugation for 5 minutes at 6000 rpm. The clear supernatant layer was filtrated through a 0.45 μm milli-pore filter to produce a protein free human serum. The analysis was followed as in the general analytical procedure.

Preparation procedure of ML tablet samples (3.0 mg, Nature Made, USA), ten tablets was grinded and homogenized. Then, 100 mg of powdered tablets was dissolved in DDW with intensive stirring of magnetic stirrer for 15 min to ensure that tablets were dissolved completely. The mixture was filtered through a filter paper to obtain a clear filtrate and then quantitatively transferred into volumetric flask. To obtain final concentrations in the range of calibration curve, the sample solutions were suitably diluted with supporting electrolyte.

Injection ampule samples of DA were investigated under optimum conditions of experiment without any pretreatment.