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Adsorptive stripping voltammetric determination of anticancer drug lomustine in biological fluids using in situ mercury film coated graphite pencil electrode

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ABSTRACT

In situ mercury film coated graphite pencil electrode (MF/GPE) was prepared and applied for selective and sensitive electrochemical determination of anticancer drug lomustine (LMT). Parameter optimization for the preparation of MF/GPE was examined. The bare and modified GPEs were characterized by scanning electron microscopy (SEM), cyclic voltammetry and square wave voltammetry. With pH 5.0, Britton–Robinson (BR) buffer and in the presence of 0.5 M sulfate ions as indifferent supporting electrolyte, LMT yields a well defined and sensitive reduction peak at the MF/GPE. The electrochemical parameters such as the charge value (Q), surface concentration (Γ), electron transfer coefficient (α) and the standard rate constant (k_s) for the reduction of LMT at the MF/GPE were calculated. The achieved limits of detection and quantification were 8.13×10^{-8} M and 2.71×10^{-7} M by square wave cathodic adsorptive stripping voltammetry (SWCASV), respectively. The modified MF/GPE was used as a sensor for the detection of LMT in human blood and urine samples with good accuracy and precision.

1. Introduction

Lomustine (CCNU; 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea) is an alkylating antineoplastic drug. It is an extremely potent member of nitrosourea widely used in the treatment of brain tumors, resistant or relapsed Hodgkin's disease, small cell lung cancer, lymphomas, malignant melanoma and various solid tumors [1]. Lomustine is used alone or in combination with radiotherapy and surgery for the treatment of brain tumors and brain metastasis. However, its clinical application is restricted by dose-related toxicities, including hematologic toxicity and pulmonary toxicity [2]. In addition, since it is hydrophobic drug, intravenous administration of LMT is always associated with serious side effects such as blood vessels embolization and respiratory system failure [3]. The wide use of LMT in the treatment of several diseases requires application of analytical procedures for monitoring the levels of drug in dosage form and in biological fluids. Therefore the development of new treatment strategies is imperative to improve the efficacy and safety of LMT in clinical practice. So far, the most frequented methods used for determination of LMT are chromatographic techniques with spectrophotometric detection [2,4], RP-HPLC-UV detection [5], HPLC-diode array detection [6], but there are also polarographic/voltammetric methods in classical batch are arrangements [7–9]. Recently, an application of the flow differential pulse voltammetry with tubular detector based on silver solid amalgam for determination of LMT was presented [9].

Among the conventional analytical methods, electroanalytical methods have attracted attention due to their simplicity, portability, selectivity, sensitivity, moderate cost and amenability to miniaturization [10–12]. Gold, (Au), platinum, (Pt), glassy carbon, (GC) and indium tin oxide, (ITO) electrodes are commonly used as working electrodes in electroanalytical tools, however the high cost or low signal-to-noise impose a barrier to the use of these electrodes in sensitive routine analysis instruments. The material of the graphite pencil electrode (GPE) is low in cost, easily maintained, most available and displays a wide potential window [13–18]. On the other hand, GPEs exhibit poor electrocatalytic sensitivity toward some diverse electroactive molecules [19,20]. As a result, the modification of GPEs, using a suitable electrocatalyst is crucial to the fabrication of sensitive electrochemical sensors. In this context, to improve the electrocatalytic properties of the GPEs, various approaches for either pretreatment [21,22] or modifications [23–31] were reported. In this context, gold and platinum nanoparticles modified graphite pencil electrodes have been applied for detection of some different biological molecules [27,30]. However, using of Au NPs and Pt NPs is limited due to the high cost. Moreover, these electrodes exhibit high background current and a lower hydrogen overvoltage. Thus, to fabricate sensitively electrochemical sensors, the modification of the GPE with an inexpensive electrocatalyst in a fast single step is required to obtain a high electrocatalytic property. In this respect, mercury thin film has found wide application in stripping voltammetry. This kind of electrode has the advantages of mercury electrode while a negligible amount of mercury is used [32]. In view of these benefits, modification of GPE with a mercury film is logic to make a high electrocatalytic

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electrode. The experimental procedure for the modification of GPE can be also simplified if the modifying agent is added to the background electrolyte, which is termed in situ modification. The in situ modification had the advantage of shortening or eliminating the preparation steps before the analysis [33,34]. To our knowledge, the square wave cathodic adsorptive stripping voltammetric determination of the anticancer drug LMT using GPE with in situ mercury film deposition has not been reported. Therefore, the analytical performance of in situ mercury film coated graphite pencil electrode toward LMT detection is investigated.

In the present work a sensitive square wave cathodic adsorptive stripping voltammetric method was developed for trace determination of LMT at a disposable mercury film modified graphite pencil electrode (MF/GPE) for the first time. Controlled adsorptive accumulation of LMT on MF/GPE provides the basic for the stripping measurements of the anticancer drug in biological fluids. The proposed methodology is very sensitive, free of common interferences with the molecule of interest and had a low detection limit.

2. Experimental

2.1. Instrumentation

Cyclic voltammetry (CV) and square wave voltammetry (SWV) were performed using an EG&G PAR Model 263 A potentiostat/Galvanostat controlled by an IBM micro-computer with EG&G PAR Model 270 software in conjunction with a PAR Model 303 A. The three electrode system consisted of a bare GPE or modified MF/GPE as the working electrode, an Ag/AgCl (saturated KCl) reference electrode and a Pt wire auxiliary electrode. A PAR Model 305 stirrer was used for SWV. For voltammetric measurements, the test solution was placed in a voltammetric cell (10 ml) and deoxygenated by bubbling nitrogen for 15 min to remove any oxygen interferences in the cathodic potential window. For cathodic stripping experiments an accumulation potential (E_{acc}) was applied for a certain accumulation time (t_{acc}), while the solution was stirred at 400 rev/min. At the end of the accumulation period the stirrer was stopped and the solution was allowed to become quiescent for 15 s prior to the voltammetric scan. The surface morphologies of the bare GPE and MF/GPE were analyzed through scanning electron microscopy (SEM) (JEOL, JSM-5400 LV).

2.2. Chemicals and reagents

Lomustine (LMT, Scheme 1) was purchased from Sigma–Aldrich chemicals (St. Louis, MO, USA). A stock solution of LMT was prepared by dissolving an appropriate amount of the compound in ethanol and then it was stored in the dark at 4 °C. Britton–Robinson (BR), Mcllvaine, Acetate, phosphate and citrate buffers were used as supporting electrolytes. The pH values of the buffer solutions were measured with a digital radiometer pH meter, Jenway 3310 accurate to ±0.02 units. A stock solution of 0.1 M Hg(II) was prepared by dissolving appropriate amount of mercuric chloride in 0.1 M HCl and further diluted as required. Solutions of SO_4^{-2}, NO_3^-, ClO_4^- and Cl^- were prepared from their analytical grade salts. All chemicals were of reagent grade (Merck, Darmstadt, Germany). High-quality deionized water was used to prepare the solution which was obtained by passing distilled water through a Milli-Q plus System (Millipore).

2.3. Preparation of MF/GPE

A rotring (Germany) pencil Model Tikky special 0.5 mm was used as a holder for the pencil lead (Rotring, 2B, 0.5 mm diameter). Electrical contact with the pencil lead was achieved by soldering a metallic wire to the metallic part that holds the lead in place inside the pencil. The pencil was fixed vertically with 4 mm of the pencil lead extending outside and 6 mm of the pencil lead immersed into the electrolyte solution. In the in situ modification of the GPE, the electrochemical experiments were carried by dipping the GPE in buffer solution containing 0.5 M sulfate ions and 3.34 mM mercury II. All solutions were stirred at 400 rev/min and were purged with nitrogen for 15 min before the voltammetric measurements. The mercury deposition step lasted from 1 to 5 min at deposition potential, $E_{dep.}$, with the interval 0 to −0.6 V.

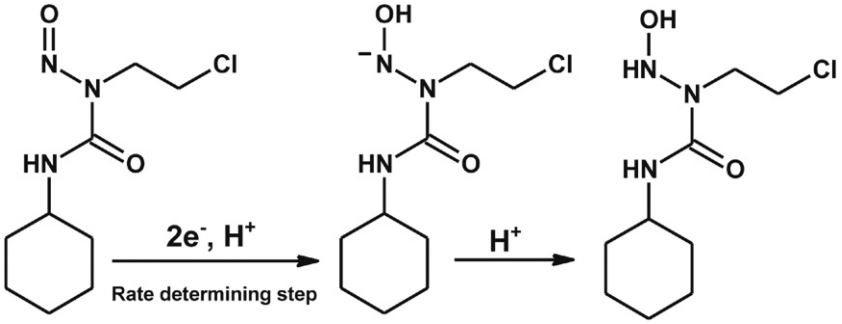
2.4. Urine and serum pretreatment

Human urine and serum samples were taken from healthy donors and used shortly after collection. Urine samples were centrifuged and filtered before use. A 0.45 ml aliquot of serum sample was treated with 0.9 ml methanol (as serum protein precipitating agent). The precipitated proteins were separated out by centrifugation for 20 min at 1400 rpm using tabletop high speed centrifuge TDZ4A-WS. The clear supernatant layer was filtered through 0.45 μm Millipore filter to obtain a protein-free spiked human serum.

3. Result and discussion

3.1. Parameters optimization for preparation of MF/GPE

To obtain best conditions for LMT reduction, we optimized the fabrication conditions of the disposable MF/GPE. Firstly, we varied the concentration of Hg(II) from 0.38 to 3.70 mM at constant deposition potential (−0.5 V) and deposition time (300 s). The SWCASV of 3.84×10^{-5} M LMT at the modified electrode in BR buffer of pH 5 shows the reduction current increases with increasing the concentration of Hg(II) up to 3.34 mM as shown in Fig. S1. At Hg concentration higher than 3.34 mM, the in situ mercury film coated GPE does not seem to grow on increasing the addition of mercury, because i_p becomes constant at high concentrations of the mercuric ion, i.e. 3.34 mM is the optimum concentration of Hg(II) to prepare MF/GPE. Secondary, in order to investigate the influence of the indifferent supporting electrolyte on the film formation process of Hg on GPE, a series of experiments was performed in BR buffer of pH 5, with and without the addition of anions such as SO_4^{-2}, NO_3^-, ClO_4^- and Cl^-. The results showed that higher peak



Scheme 1. The proposed mechanism for the electro-reduction of LMT at the MF/GPE.