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Sensitive and selective voltammetric determination of anti-cancer agent shikonin on sepiolite clay/TiO_2 nanoparticle/MWCNTs composite carbon paste sensor and investigation of its electro-oxidation mechanism

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ABSTRACT

A simple and highly sensitive voltammetric method was developed to determine the Shikonin (SHI) content in synthetic and real samples. The method is based on a carbon pate electrode (CPE) modified by the addition of sepiolite clay, TiO_2 nanoparticles and multi-walled carbon nanotubes (MWCNTs). Differential puls adsorptive stripping voltammetry (AdsDPV) technique was employed with the electrode as the anode. Furthermore, electrochemical redox properties of SHI were investigated by using cyclic voltammetry (CV) on the same electrode. The CV studies show that SHI has two quasi-reversible oxidation signals at about −0.100 V (P_1) and +0.700 V (P_2) in acidic media. To the best of our knowledge, the oxidation peak P_1 has not been observed in previous voltammetric studies on SHI. We suggest mechanisms that lead to both P_1 and P_2. The peak P_1 was selected for the quantitative analysis of SHI on the electrode concerned, because it is more intense than P_2 (higher peak current, higher sensitivity). P_1 is also the choice in terms of interference effects of several organic compounds likely to be present in biological samples. The anodic peak current of SHI in AdsDPV varies linearly with the concentration range of 0.44–1000 n mol L^{-1}. Dedection (LOD) and quantification (LOQ) limits were calculated as 0.13 n mol L^{-1} and 0.44 n mol L^{-1}, respectively. The voltammetric technique was applied to SHI analysis in human urine and serum samples and found to produce expectable quality results.

1. Introduction

Shikonin (SHI), a naphthoquinone derivate, is known to be an herbal drug isolated from the roots of the herbal plant *Lithospermum erythrorhizon*; which is native to Chine and its surroundings. The roots of the plant itself have been used in traditional Chinese medicine for the treatment of some viral disease including a type of HIV. The type compounds is also in current use for the treatment of carbuncles, burns, measles, macular eruptions and sore throats [1] The chemical structure of SHI is given in Fig. 1.

A number of pharmaceutical studies have shown that SHI has some antibacterial, antifungal, antimicrobial, anti-inflammatory and anti-thrombotic activity in humans [2]. Besides, studies in recent years indicate that the compound also has a noticeable antitumor activity. It has a cytotoxic effect on 15 different cancer cell lines, including some multi-drug - resistant ones [3]. Shikonin was also reported to be in use as a natural colorant in the field cosmetics, food and textile, etc. [2].

Taking into account all these applications, developing a sensitive and reliable analytical method appears to be worthwhile for the quick determination of SHI in various samples. Some analytical techniques already exist and among them, high performance liquid chromatography (HPLC) [4], capillary electrophoresis (CE) [5] and thin - layer chromatography (TLC) [6] are reported to be in use. These instrument based methods are fairly effective; but, the fact that the instrument involved are expensive, pre-concentration procedures complicated, the whole processes laborious and highly skill professionals are needed make hem tedious and difficult to employ.

On the other hand, quantitative electrochemical methods are reported to offer several advantages, among them high sensitivity, selectivity, shorter analysis time, low cost and better reproducibility [7–11]. In electrochemical methods various electrodes are employed. Carbon paste electrodes (CPEs) are often preferred due to their low background current, wide potential window, chemical inertness, low cost, high sensitivity, reproducibility and ease of modification [12,13].

Nowadays, some nanoparticles are used for the modification of CPEs; titanium (IV) dioxide), for example, is reported to act as a surface charge regulator, and a means of pre-concentration (adsorbent) [11,13, 14], nickel (II) oxide [15], Zinc(II) oxide [12,16] are known to act in a similar fashion. MWCNTs are also used as modifiers on CPE. This material has a highly porous hollow structure, excellent electrical conductivity, large specific surface area and good affinity toward biological

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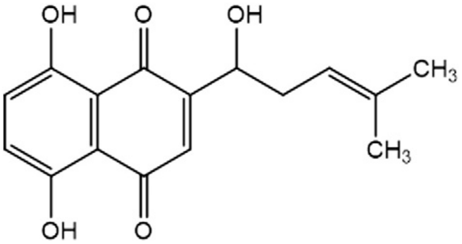

Fig. 1. Chemical structure of SHI.

molecules [17]. Sepiolite type clay minerals were also used in modifying CPEs. Sepiolites were shown to adsorb and incorporate electroactive species so as to facilitate the redox reaction involving the analyte [18].

In this study, a combination of sepiolite clay TiO_2 nanoparticles and MWCNTs was selected as the modifier. These materials are known to impart the electrode various advantages such as chemical stability and better electron transfer ability.

Here we report on the electrochemical redox properties of SHI on clay/TiO2/MWCNTs/CPE sensor system. Our investigations reveal that the redox activity SHI has two quasi-reversible oxidation peaks at about -0.100 V (P_1) and $+0.700$ V (P_2). Previous reports do not mention the existence of the peak P_1 [19–23] which suggests that the electrode system we developed is more sensitive than the reported sensors. Here, we focused on both the redox peaks and based on the data obtained now suggest an electro-oxidation mechanism which is different from the suggestions published. We also offer a new differential puls adsorptive stripping voltammeric (AdsDPV) method for the quantitative analysis of SHI in human body fluids. The method was shown to be sensitive and selective for SHI. In the development stages of the method, the peak P_1 was chosen for quantification because of its high intensity, narrower width and strong dependence on adsorption. P_1 is also superior to the other peak P_2 in the sense that the potential it is located involves less background interference from the common components of biological samples like uric acid, ascorbic acid etc. To the best of our knowledge, the proposed method has the lowest LOD value ever reached for SHI. The method was also used in the trial experiments in human body fluids; namely, recovery ratios of SHI added to urine and human serum samples were determined.

2. Material and Methods

2.1. Reagents, Solutions and Apparatus

Graphite powder, mineral oil, sepiolite clay, TiO_2 nanoparticles, Multi-walled carbon nanotubes, potassium hexacyanoferrate (II and III) and all the solvents were purchased from Sigma–Aldrich, St. Louis Missouri, USA. Herbal drug Shikonin (SHI) was also purchased from Sigma–Aldrich Company. 1.0 mmol L^{-1} stock solution of SHI was prepared by dissolving a precise amount of SHI in ethanol of known volume and kept in fridge at $+4$ °C until assay. 0.2 mol L^{-1} H_2SO_4 solution was used as the supporting electrolyte. The pH of the medium was adjusted by using 5.0 mol L^{-1} NaOH solution. The standard solution of 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ was prepared by dissolving calculated amounts of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ in double distilled water. 0.1 mol L^{-1} KCl was prepared by the same way with 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$.All other chemicals were of analytical grade.

Cyclic voltammetry (CV), Differential puls voltammetry (DPV) and Electrochemical Impedance Spectroscopy (EIS) measurements were carried out by using CHI 760B (from USA) electrochemical workstation and C3 cell stand (BASi). A traditional three electrode system was used and CPE, clay/CPE, TiO_2/CPE, MWCNTs/CPE and clay/TiO_2/MWCNTs/CPE sensors were used as working electrodes, Ag/AgCl (in 3.0 mol L^{-1} NaCl, BAS MW-1032) was used as reference and platinum wire was used as an auxiliary electrode (BAS MF-2052).

pH measurements were carried out with a HANNA Instruments HI2211 pH/ORP meter. For the calibration of the pH electrode, commercial buffer solutions at the pH values of 4.0, 7.0 and 10.0 were used. Double-distilled and deionized water was supplied from a Human Power I^+, Ultra-Pure Water System. Scanning electron microscopic (SEM) images were recorded on Carl Zeiss AG, EVO® 50 Series. All measurements were performed at room temperature.

2.2. Preparation of CPE sensors

The CPEs were prepared by hand mixing graphite powder (30 mg) and mineral oil (10 μL) in a petri plate. Before preparing the modified CPE sensors, adequate amounts of sepiolite clay, TiO_2, and MWCNT nanoparticles were mixed with each other. This mixture was then mixed with the estimated amount of graphite powder and then 10 μL of mineral oil was added. The electrode material was mixed thoroughly with a spatula and then placed into the capillary tubing of the CPE holder (Scheme 1). The electrical contact was provided by a copper wire connected to the paste in the inner tubing of the electrode body. The surface of the newly prepared electrode was flattened with a smooth-surface paper. Before all experiments, the well-made CPE sensors were cleaned by washing with water ethanol mixture (1:1).

2.3. Analytical procedure

The solutions used in the electrochemical analysis were prepared using 1.0 mmol L^{-1} stock SHI solution. Other solutions for electrochemical studies were prepared as follows;

A 7.0 mL aliquot of 0.2 mol L^{-1} H_2SO_4 was titrated with 5.0 mol L^{-1} NaOH to adjust the pH to the desire value; then mixed with 2.0 mL of ethanol and calculated volume of stock SHI solution so as to reach a predetermined concentration of the analyte. This solution was transferred to the voltammetric cell and used for all the electrochemical measurements (CV, DPV). After arranging all the electrode connections, the solution in the cell was purged with ultra-pure nitrogen (99.99% purity) for 60 s to remove oxygen; the system was kept quiet for 2 s and then the electrochemical measurements were done. The voltammograms were recorded in the potential range of (–0.7–1.1 V).

2.4. Biological sample preparation

Human urine and blood for serum samples were taken from healthy volunteers. Fresh blood samples were centrifuged to remove the clot; the supernatant-serum was then mixed with acetonitrile and centrifuged again to obtain a protein-free sample. All the samples were kept at –20 °C until assay. In all cases, calculated amount of supporting electrolyte was mixed with 10 μL of urine (or, 50 μL of serum) in the voltammetric cell. To this mixture a sufficient volume of SHI stock solution was added so as to make the final volume exactly 10.0 mL.

3. Results and discussion

3.1. Electrochemical characterization of CPE sensors

The characterization of the electrodes (bare CPE, MWCNTs/CPE, clay/CPE, TiO_2/CPE and clay/TiO_2/MWCNTs/CPE) were done by CV and EIS methods. For this purpose, a 5.0 mmol L^{-1} solution of $Fe(CN)_6^{3-/4-}$ in aqueous KCl (0.1 mol L^{-1}) was used as standard. Fig. 2A demonstrates the CVs of bare and modified CPE sensor electrodes at a scan rate of 0.1 V/s.

It can be seen that the CPE modified with only sepiolite clay and TiO_2 nanoparticles produces a voltammogram with much higher peak intensity. It is also apparent that with this electrode, the peak to peak separation (ΔEp) is smaller. The CPE modified with MWCNTs gives rise to a voltammogram with relatively lower intensity and a narrower ΔEp difference. The sensor modified with the triple composite