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Abstract

A fast and facile electrochemical sensor for detection of important anti-cancer drug 5fluorouracil (5-FU) is fabricated using a copper-nanoparticles decorated multi-walled carbon nanotube and chitosan composite modified electrode. Copper nanoparticles/multi-walled carbon nanotubes/ionic liquid/chitosan modified glassy carbon electrode (CuNPs/MWCNTs/IL/Chit/GCE) prepared by the consecutive coating of MWCNTs/IL/Chit nanocomposite on the GCE, followed by the electrodeposition of copper. Surface characteristics of the modified electrode were studied by scanning electron microscopy (SEM). The electrochemical capability of the fabricated modified electrode for the detection of 5-FU is examined by cyclic voltammetry, differential pulse voltammetry (DPV). The nano Cu decorated MWCNTs/IL/Chit/GCE is found to be efficient for the electrocatalytic oxidation of 5-FU. The peak current of the DPV exhibited a linear



relationship against 5-FU over a wide concentration range of 1-110 μM with a low detection limit (0.15 μM). Additional, the sensor was successfully applied in biological fluid sample analysis.

Keywords: 5-Fluorouracil, Carbon nanotubes, Copper nanoparticles, Chitosan.

Introduction

F luorouracil (5-FU) is a chemotherapy drug used to treat different cancers including breast, bowel, skin, stomach, esophageal (gullet), and pancreatic (Scheme 1). 5-FU is effectively used in the treatment of solid tumors of breast and rectum.¹ The cytotoxicity of 5-FU is towards to MDA-MB-231 cell line,² WiDr, HT-29, and SW620 cell line.³ Also, 5-FU exhibited various unpleasant conditions like cardiotoxicity, nephrotoxicity, hepatotoxicity, and marked organ toxicity coupled with increased oxidative stress and apoptosis.⁴



Scheme 1. Chemical structure of 5-fluorouracil.

In recent years, metal nanoparticles have been used in wide applications in electrochemical sensors. Electrodes modified with gold, platinum, palladium, copper, nickel, and silver nanoparticles have revealed good performances in catalysis, through facilitation of mass transport and increase of effective surface area.⁵⁻⁷ Among these, considering the operability, availability, and cost, copper is

Corresponding author: Mahmoud Roushani, Email: <u>mahmoudroushani@yahoo.com</u>, and m.roushani@ilam.ac.ir of particular interest. Therefore, studies related to the preparation and applications of copper-nanoparticles modified electrodes are very important. Multi-walled carbon nanotubes (MWCNTs) have exceptional electrical, thermal, and mechanical properties. Thus, they considered being an ideal filler for making high-performance nanocomposites.⁸ Carbon nanotubes have become extremely attractive for the modification of chemical sensors, biosensors, and more applications due to their high electrocatalytic effect, fast electron transfer rate, high chemical stability, sensitivity, lower detection limit,⁹ and excellent biocompatibility.^{10,11}

lonic liquid (IL) also as new green media with high ionic conductivity have been used recently as a new kind of binder for the preparation of nanocomposites.¹² The combination of the carbon nanotubes and ILs can also improve the electrochemical properties of the modified electrodes.^{13,14}

Chitosan (Chit) is a polysaccharide derived by deacetylation of chitin. It possesses many advantages such as excellent membrane forming ability, high permeability toward the water, good adhesion, biocompatibility, and high mechanical strength.¹⁵

Another interesting nanomaterial for the modification of the electrode surface is copper nanoparticles (CuNP). CuNPs, a kind of soft metal nanomaterial was paid much attention as lubricant additive because of its low cost, easy preparation, and excellent lubricating performance.^{16,17}

Various analytical methods have been aimed for the detection of 5-FU based on spectroscopic,^{18,19} chromatography capillary,^{20,21} electrophoresis,²² and electrochemical methods.²³⁻²⁵ Electrochemical transduction methodologies are advantageous because they offer miniaturization portable analysis, rugged instrumentation with no movable parts, high sensivity, on site analysis, and etc.

In the present work, an electrochemical sensor has been successfully fabricated by the use of CuNPs/MWCNTs/IL/Chit nanocomposite. The prepared sensor has shown excellent catalytic activity toward the electrochemical oxidation of 5-FU, which has been studied by cyclic voltammetry. The modified electrode has been used in voltammetric detection of micromolar concentrations of 5-FU in blood serum samples.

Experimental

Materials and reagents

5-FU, copper nitrate, sodium chloride, chitosan, 1-methyl-3octylimidazolium tetrafluoroborate ionic liquid (IL), and MWCNTs were ordered from Merck, Sigma-Aldrich Co. LLC (USA), and Aldrich. Doubly distilled water was used throughout the experiments. The 0.1 M PBS (pH 9.0) was applied as a supporting electrolyte. All other chemicals were of analytical reagent grade and doubly distilled water was used in the experiments.

Apparatus

Cyclic voltammetry and differential pulse voltammetry measurements were performed on a μ -AUTOLAB electrochemical system type III and FRA2 board computer controlled Potentiostat/ Galvanostat (Eco-Chemie, Switzerland) driven with NOVA software in conjunction with a conventional three-electrode system. CuNPs /MWCNTs/IL/Chit/GCE, an Ag/AgCl (Sat'd 3.0 M KCl), and a platinum wire electrode respectively as working, reference and counter electrodes, was used to carry out electrochemical studies. To obtain information about the morphology and size of the particles, scanning electron microscopy (SEM) images were taken by using an X-30 Philips instrument. Also, the energy-dispersive X-ray (EDS) and wavelength-dispersive Xray (WDX) spectroscopy were recorded by a Vega-Tescan electron microscope.

Preparation of the CuNPs/MWCNT/IL/Chit/GCE

In order to remove adsorbed particles, GCE was subsequently polished with 0.03 μ M alumina powders on polishing cloth and sonicated successively in a mixture of water and ethanol at a ratio of 1:1. The nanocomposite was prepared by mixing 1 μL of IL, 0.2 mg of Chit and 2 mg of MWCNTs. The working surface area of a bare GCE was modified by placing some of MW-IL-Chit nanocomposite paste on the electrode surface to form a homogeneous layer and dried at room temperature. Then 10 µL solutions of CuNO₃ (0.1 M) was directly dropped onto the surface of MWCNT/IL/Chit/GCE at room temperature. For preparation copper nanoparticles, 0.1 M copper nitrate dissolved in sodium acetate/acetic acid buffer solution (pH 4.5) and deposit by NaCl (0.5 M). It was carried out by immersing the Cu2+chelated electrode in 0.5 M NaCl aqueous solution at -0.9 V vs. Ag/AgCl (sat'd 3.0 M KCl) for 60 s. Then the electrode was placed in a 0.1 M PBS (pH 9.0) and the potential was sweeped in the range of -0.2 to 1.5 V for 20 cycles at a scan rate of 100 mV s⁻¹.

Preparation of human blood serum sample

Human blood samples were collected in dry and evacuated tubes

from a healthy volunteer. The blood was allowed to clot by leaving it undisturbed at room temperature and were centrifuged for 15 min at 1200 rpm for the separation of the clot. The 5 ml portion of the resulting supernatant (serum) was diluted to 50 ml in a volumetric flask with 0.1 M PBS buffer (pH 9.0) and it was transferred into the voltammetric cell for recording the DPV.

Results and discussion

Characterization of CuNPs/MWCNT/IL/Chit/GCE

The SEM image of MWCNTs/IL/Chit/GCE was previously reported.²⁶ Figure 1A shows the SEM image of CuNPs/MWCNTs/IL/Chit/GCE. As can be seen, when the copper ions are electrodeposited on the MWCNTs/IL/Chit/GCE surface, the nanoparticles of the copper are observed clearly on the electrode surface. The elemental analysis of the mentioned nanocomposite was also investigated by EDS measurement (Figure 1B). As shown, the peaks corresponding to C, and Cu elements were observed, indicating the presence of CNTs, Chit, and CuNPs in the nanocomposite. To further confirm the presence of CuNPs on the surface of the electrode, WDX analysis for Cu was taken and the distribution of Cu was shown in Figure 1C.



Figure 1. (A)Typical SEM image of CuNPs/MWCNTs/IL/Chit nanocomposite at the surface of modified electrode, (B) EDS spectrum of the CuNPs/ MWCNTs/IL/Chit nanocomposite, and (C) WDX analysis for CuNPs.

Electrocatalytic oxidation of 5-fluorouracil

Cyclic voltammograms of 5-FU were recorded using three different electrodes GCE, MWCNT/Chit/IL/GCE, and CuNPs/MWCNTs/IL/Chit/GCE in PBS buffer (pH 9.0) containing 250 μ M 5-FU and the CVs are shown in Figure 2. In the absence of 5-FU, no characteristic peak was observed at any of the electrodes from the PBS buffer. In the presence of 5-FU, an irreversible anodic peak was observed for all electrodes. The anodic peak potential for the oxidation of 5-FU over GCE is located at +1.22 V, while a well-defined anodic peak was observed at a less positive potential (+1.10 V) for both MWCNT/IL/Chit/GCE and CuNPs/MWCNTs /IL/Chit/GCE electrodes (Scheme 2).



Scheme 2. Possible reaction mechanism.

Cyclic voltammetric studies showed that the catalytic peak current of CuNPs/MWCNTs/IL/Chit/GCE was amplified approximately up to 5 times in comparison with the bare GCE, indicating a strong catalytic effect. Comparison of the electrochemical behavior of modified and unmodified electrode shows drastic changes in the behavior of 5-FU. Due to the increase in the electro-active area of the electrode, there was an appreciable increase in the sensitivity.



Figure 2. CVs recorded at (a) bare GCE, (b) MWCNT/IL/Chit/GCE, and (c) CuNPs/MWCNT/IL/Chit/GCE (200 μ M 5-FU in PBS buffer (pH 9.0), Scan rate: 50 mV s⁻¹).

Effect of pH on the electrochemical characteristics of 5-FU was investigated at CuNPs modified electrode in PBS buffer at different pH values from 5.0 to 11.0 by cyclic voltammetry (Figure 3). The pH of the buffer solution obviously influences on the oxidation of 5-FU. The peak currents are high in the pH range of 8.0–11.0. The slope of the E_p vs. pH plot is 0.057 V and is very close to the theoretical Nernstian value 0.059 V, which indicates that the number of protons and the number of electrons transferred in the electrochemical oxidation of 5-FU are equal to each other. The peak current was very high at pH 9.0. Considering these observations, PBS buffer of pH 9.0 has been chosen for further analysis. The effect of 5-FU addition to the system was investigated in the range of 50–550 μ M (Figure 4), fitting a linear dependence of currents vs. concentration of 5-FU which fitted the following equation:

$$I (\mu A) = 0.0578 (\mu A \text{ mM-1}) - 1.72 (\mu A), R^2 = 0.99$$
 (1)

Cyclic voltammograms of 75 μ M 5-FU at CuNPs/MWCNTs /IL/Chit/GCE were recorded with different scan rates and are shown in Figure 5A. The peak currents for the oxidation of 5-FU gradually increased with the scan rate. In scan rate investigation, we observed a linear variation of the peak current with the square root of scan rate (v^{1/2}, R² = 0.9917). This result clearly indicates a diffusion-controlled electrooxidation process (Figure 5B).

For an irreversible electrochemical reaction, the relationship between the peak potential (E_p) and the scan rate (v) are expressed in equation (2) by Laviron²⁷:

$$E_P = E^0 + \frac{RT}{\alpha n F} ln \left[\frac{RT k^0}{\alpha n F} \right] + \left[\frac{RT}{\alpha n F} \right] ln(v)$$
(2)

where α is the charge transfer coefficient, k° is the standard rate constant for the heterogeneous electron transfer, n is the number of electrons involved in the reaction and E° is the formal potential. According to equation (2), the n value can be determined from the slope of Ep vs. In(v) plot (Figure 5C), and k° can be calculated from the intercept of the plot. From the slope of the Ep vs. In(v) plot, n was calculated to be 0.49. Generally, could be assumed to 0.5 for an irreversible electrode process. So the electron transfer number (n) for the electrochemical oxidation of 5-FU becomes 1, and the k° value is determined from the intercept to be 2.69 s⁻¹.



Figure 3. CVs recorded at CuNPs/MWCNTs/IL/Chit/GCE in the presence of 250 μ M 5-FU in PBS buffer of different pH values (4.0–11.0). Scan rate: 50 mV s⁻¹. Inset shows Plots of ip vs. pH.



Figure 4. CVs recorded for CuNPs/MWCNTs/IL/Chit/GCE in PBS (pH = 9.0) contains various concentrations of 5-Fu (50 to 550 μ M), at the scan rate of 50 mV s⁻¹. Inset shows the calibration curve of the reduction peak current versus the concentrations of 5-Fu.



Figure 5. (A) CVs of 75 μ M 5-FU in PBS buffer (pH 9.0) recorded at CuNPs/MWCNTs/IL/Chit/GCE with different scan rates (10, 20, 30, 40, 50, 60, 80, 100 mV s⁻¹), (B) Plot of the anodic peak current against the square root of scan rate, and (C) Plot of Ep vs. In (scan rate)

Differential pulse voltammetry (DPV)

Differential pulse voltammograms of 5-FU at CuNPs/MWCNTs/IL/ Chit/GCE were recorded in PBS buffer (pH 9.0). As illustrated from Figure 6, two linear ranges were found; one from 1 to 8 μ M and the other from 8 μ M to 20 μ M and the detection limit was estimated to be 0.15 μ M. A well defined anodic peak was observed at +1.01 V. The peak current increased gradually with the increase in the concentration of 5-FU. A linear plot with a regression coefficient of 0.99 was obtained, as shown in inset Figure 6. Low-detection limit of the sensor system is defined as the change in the peak current by three relative standard deviations, and it is determined from the plot to be 0.15 μ M. The obtained linear determination range is 1-550 μ M.



Figure 6. DPVs of 5-FU in PBS buffer (pH 9.0) at different concentration over CuNPs/MWCNTs/IL/Chit/GCE, and inset plot of i_p vs. 5-FU concentration.

The detection limits reported at different methods for 5-FU is listed in Table 1. Based on reported and obtained data could say this method was better than other reported methods.²⁸⁻³¹

| Table1: | Comparison | of | detection | limits | of | 5-fluorouracil | by | different |
|----------|------------|----|-----------|--------|----|----------------|----|-----------|
| reported | l methods. | | | | | | | |

| Electrode | LOD (µM) | Linear range (µM) | Ref. |
|--|-------------|----------------------|--------------|
| GCE/BTB ^a -MWCNT ^b | 0.26 | 0.8 to 5000 | 23 |
| GCE/CTAB ^c | 0.02 | 0.2 to 0.6 | 28 |
| AuNPs/SPE ^d | 0.76 | 0.153-384 | 29 |
| IL/CPE ^e | 0.13 | 0.5-800 | 30 |
| MB ^f /CPE | 0.002 | 0.1-40 | 31 |
| CuNPs/MWCNTs/IL/Chit/GCE | 0.15 | 1-550 | This work |

^aBTB: Bromo thymol blue, ^bMWCNTs: Multi-walled carbon nanotubes, ^cCTAB: Cetyltrimethyl ammonium bromide, ^dSPE: Screen printed electrode, ^eCPE: Carbon paste electrode, ^fMB: Methylene blue.

Interferences study

The electrochemical response of 5-FU in the presence of potentially electroactive biological compounds such as ascorbic acid (AA), uric acid (UA), aspartic acid, citric acid (CA), glucose has been investigated at the CuNPs/MWCNTs/IL/Chit/GCE by DPVs. The experimental results showed which these substances did not interfere with the voltammetric signal of 5-FU. The data is given in Table 2.

Table 2. Influence of potential interferents on the voltammetric response of 100 μ M 5-FU.

| Interferents | Concentration (M) | Signal change (%) | | |
|---------------|-------------------|-------------------|--|--|
| Ascorbic acid | 0.1 | 0.6 | | |
| Uric acid | 0.1 | 0.3 | | |
| Aspartic acid | 0.1 | 0.2 | | |
| Citric acid | 0.1 | 0.1 | | |
| Glucose | 0.1 | 0.4 | | |

Determination of 5-FU in the blood sample

In order to evaluate the applicability of the proposed sensor determination of 5-FU in human blood serum samples was performed. The concentration of 5-FU in an artificially prepared specimen, by adding known amounts of 5-FU to serum samples was measured. The standard addition method was used for determination of 5-FU in this sample. The obtained recovery percent by this method reveal the capability of the method for determination of 5-FU in human blood serum samples. The data is given in Table 3.

 Table 3. Application of DPV for the determination of 5-fluorouracil in human

 blood.

| Sample | Injection Added | Found | Average | RSD |
|----------------|----------------------|----------------------|----------|------|
| | (10 ⁻⁵ M) | (10 ⁻⁵ M) | Recovery | (%) |
| Blood sample 1 | 2.1 | 1.87 | 89.05 | 2.7 |
| Blood sample 2 | 3.1 | 2.85 | 91.94 | 1.5 |
| Blood sample 3 | 4.1 | 3.76 | 91.71 | 2.7 |
| Blood sample 4 | 5.1 | 5.35 | 104.90 | 3.1 |
| Blood sample 5 | 6.1 | 6.25 | 102.45 | 1.35 |

Reproducibility and Reusability

The repeatability and stability of the CuNPs/MWCNTs/IL/Chit/GCE for the detection of 5-FU was investigated by CVs analysis. The relative standard deviation (RSD) in responses for six measurements was found 2.5%. When the electrode was stored in the laboratory, the modified electrode retains 95% of its initial response after two weeks and 93% after 30 days. These results indicate that the reusability and reproducibility of the CuNPs nanocomposite electrode are quite satisfactory.

Conclusions

In this study, a new sensor has been successfully fabricated with the use of a GCE modified with CuNPs/MWCNTs/IL/Chit. The nanocomposite film remarkably enhanced the current response and decreased the oxidation overpotential of 5-FU. The excellent electrocatalytic activity of CuNPs/MWCNTs/IL/Chit/GCE toward 5-FU is attributed to the synergistic effect of MWCNTs/IL/Chit nanocomposite and Cu nanoparticles. According to these results, the proposed electrode showed high current sensitivity, low detection limit, high stability, and good reproducibility which proved its potential sensing for the measurement of this drug.

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