Fabrication of a new electrochemical sensor based on Au–Pt bimetallic nanoparticles decorated multi-walled carbon nanotubes for determination of diclofenac

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A B S T R A C T

A new electrochemical sensor was developed for determination of diclofenac based on functionalized multi-walled carbon nanotubes (f-MWCNTs) and gold–platinum bimetallic nanoparticles (Au–PtNPs) modified gold electrode. The Au–PtNPs were deposited electrochemically on the surface of the f-MWCNTs modified electrode. The surface morphologies of the modified electrodes were investigated by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to evaluate the electrochemical properties of the constructed sensor. Under the optimized experimental conditions, the calibration curve was linear in the concentration range of 0.5 μM to 1000 μM of diclofenac and the detection limit was found to be 0.3 μM. The developed electrode also showed a high selectivity for diclofenac in the presence of the interfering species. The reproducibility, repeatability and stability of the electrode were satisfactory. The proposed electrochemical sensor was successfully employed for determination of diclofenac in real samples such as tablet and human urine samples.

1. Introduction

Diclofenac sodium (sodium [o-(2, 6-dichloroanilino) phenyl] acetate, DS) which is classified as a nonsteroidal anti-inflammatory drug (NSAID), possesses analgesic, anti-inflammatory and antipyretic activity [1]. It is widely used for treating a variety of inflammatory and painful diseases including rheumatoid arthritis, osteoarthritis, soft tissue disorders, renal colic, acute gout, dysmenorrhea and migraine [2,3]. On the other hand, diclofenac may cause life-threatening problems such as heart attack and stroke, especially if patient uses it for a long time. It can also cause adverse effects on humans such as aplastic anemia, gastrointestinal disorders and agranulocytosis and changes in renal function [4]. Therefore, for drug development and treatment purposes, it is important to determine the trace amounts of diclofenac in biological and pharmaceutical samples. So far, a variety of methods have been employed for determination of diclofenac in various types of samples. These include spectrophotometry [5,6], fluorimetry [7], high performance liquid chromatography [8,9], gas chromatography–mass spectrometry [10,11], liquid chromatography–mass spectrometry [12] electrokinetic chromatography [13], thin layer chromatography [14] and also some electrochemical methods [15,16]. Among these different analytical methods, the electrochemical techniques which are selective, sensitive, inexpensive and also simple, are suitable methods for diclofenac quantification in solutions.

It is well known that the electrochemical performance of the electrode is influenced by its electron conductivity and accessible surface areas. Therefore, in order to enhance the electrochemical performance of the sensors; the effective strategy is the design of composites by combining highly electrocatalytic active materials with excellent conductivity substances to modify the electrode surface.

Bimetallic nanoparticles have attracted increasing attention as electrode materials because of their unique electronic, catalytic and mechanic properties [17]. Compared with the individual metals, bimetallic nanomaterials display better catalytic activity due to the synergistic effects of the two constituent metals [18]. Recently, bimetallic nanoparticles modified electrodes have been used for the electrochemical detection of different analytes [19–21].

Among the various bimetallic materials, gold–platinum (Au–Pt) bimetallic nanoparticle is a promising system as it shows excellent electrocatalytic activity towards many electrochemical reactions [22–24]. However, the high surface energy of the nanoparticles and the large surface to-volume ratio results in their aggregation. Therefore, the catalytic activity of the nanoparticles decreases. In order to improve the catalytic activity, carbon materials have been used as the support for nanoparticles [25,26].
Fig. 1. The SEM images of (A) f-MWCNTs/AuE, (B) Au–PtNPs/AuE, (C) Au–PtNPs/f-MWCNTs/AuE and (D) EDS analysis of Au–PtNPs/f-MWCNTs/AuE.
Multi walled carbon nanotubes (MWCNTs), as a kind of novel carbon materials, are widely used in electrochemical sensing field due to their superior properties such as good biocompatibility, high surface area, strong mechanical strength and high electrical conductivity [27–29]. Several techniques have been developed for decorating f-MWCNTs with nanoparticles [30–33]. Among them, the electrochemical deposition methods possess some advantages such as rapidness, simplicity, repeatability and low cost. In addition, one of the most important advantages of electrodeposition is the ability to control the size and distribution of nanoparticles by varying potential, time or solution concentration [34].

In the present paper, we report a simple electrochemical procedure for the decoration of Au–Pt bimetallic nanoparticles on the carbon nanotubes, which are pre-casted on the surface of gold electrode. The electrochemical behavior of diclofenac sodium at the Au–PtNPs/f-MWCNTs/AuE was investigated using cyclic voltammetry (CV) and differential pulse voltammetry (SWV) techniques. This modified electrode was used as a new voltammetric sensor for the determination of diclofenac sodium in solutions.

2. Experimental

2.1. Chemicals

Diclofenac sodium and hydrogen tetrachloroaurate trihydrate (HAuCl₄.3H₂O) were purchased from Sigma-Aldrich Company (USA). Diclofenac sodium in solutions.

2.2. Instrumentation

The voltammetric measurements were performed using a µ-Autolab TYPE III potentiostat/galvanostat (Netherlands). A platinum wire, a saturated Ag/AgCl electrode and a gold electrode were used as, counter, reference and working electrodes, respectively. A pH meter (Metrohm, Model 827) with a glass electrode (Corning) was used to control the pH of the solutions. The morphological characterization of the modified electrodes was performed using a scanning electron microscope (LEO 1450 VP, Germany).

2.3. Preparation of the modified electrode

At first, in order to increase the edge sites and better dispersion of the carbon nanotubes, the multi-walled carbon nanotubes were functionalized under concentrated nitric acid treatment process [35]. 0.3 g of crude MWCNTs was added in to 50 mL nitric acid and homogenized by ultrasonication. The resulting mixture was refluxed at 120 °C under stirring for 24 h. After cooling to room temperature, it was filtered and washed with deionized water successively until the pH of the filtrate solution was reached to 7.0 and then dried under vacuum. Then, the gold electrode surface was polished with a slurry of alumina and then ultrasonicated in water and ethanol for 2 min, respectively. After that, the electrode surface was washed through immersing it in piranha solution (1:3 mixture of 30% H₂O₂ and concentrated H₂SO₄) and then washed with water. For preparation of f-MWCNTs suspension, 1.0 mg of f-MWCNTs was dispersed in 1.0 mL of dimethylformamide (DMF) solvent under sonication for 15 min. Then, 7 µL of this suspension was dropped directly on the electrode surface by a micropipette and the solvent was evaporated under an infrared lamp. The electrodeposition of Au–Pt bimetallic nanoparticles on the surface of f-MWCNTs modified electrode, was carried out by cyclic voltammetry in the potential range of –0.4 V to +1.2 V for 20 cycles with a scan rate of 50 mV s⁻¹ from an aqueous solution of 0.3 M HClO₄ containing 1 mM HAuCl₄.3H₂O and 1 mM H₂PtCl₆.3H₂O. The modified electrode is denoted as Au-PtNPs/f-MWCNTs/AE.

3. Results and discussion

3.1. Characterization of the modified electrode

The surface morphology of the modified electrodes was specified using scanning electron microscopy (SEM) and the corresponding images are shown in Fig. 1. As is evident in these images, a homogeneous layer of f-MWCNTs with well dispersed Au–Pt bimetallic nanoparticles has been coated on the surface of the electrode. The presence of C, Au and Pt on the electrode surface has been proved by energy dispersive X-ray (EDX) analysis results (Fig. 1D).

Cyclic voltammetry (CV) measurements were used to investigate the modification process of the electrode. Fig. 2, shows the cyclic voltammograms of the bare and modified electrodes in 5.0 mM [Fe(CN)₆]³⁻/⁴⁻ containing 0.1 M KCl solution. As shown in curve a, a pair of well-defined redox peaks for [Fe(CN)₆]³⁻/⁴⁻ probe are appeared at the surface of the bare electrode. After modification the surface of the gold electrode with f-MWCNTs, a significant increase in the peak current of the [Fe(CN)₆]³⁻/⁴⁻ with a decrease in the peak separation (∆E) is observed compared to the bare electrode (curve b). This behavior can be attributed to the large specific surface area and high electrical conductivity of f-MWCNTs. When the Au–Pt nanoparticles are deposited on the surface of the f-MWCNTs modified electrode, the peak current increases sharply which indicates that the Au–Pt nanoparticles can significantly facilitate the electron transfer process of the redox probe on the electrode surface (curve c).

3.2. Electrochemical behavior of diclofenac

The electrochemical behavior of diclofenac at the surface of the fabricated electrodes was studied using cyclic voltammetric technique and the results are shown in Fig. 3. The cyclic voltammograms were obtained in 100 µmol L⁻¹ DS solution (pH 7.0) at the potential range of 0.2 to 1 V and scan rate of 50 mV s⁻¹. As is evident in curve a, a weak oxidation peak of DS around 0.66 V is observed at the surface of the bare gold electrode. Comparison of the CV curves which were recorded with f-MWCNTs/AuE and Au–PtNPs/f-MWCNTs/AuE, reveals a higher peak current for Au–PtNPs/f-MWCNTs modified electrode, which is due to the synergistic effect of incorporation of the multi-

![Cyclic voltammograms (CVs) of (a) bare AuE, (b) f-MWCNTs/AuE and (c) Au–PtNPs/f-MWCNTs/AuE in 5.0 mM [Fe(CN)₆]³⁻/⁴⁻ containing 0.1 M KCl solution at scan rate of 50 mV s⁻¹.](image-url)
walled carbon nanotubes and Au–PtNPs which increases the specific surface area and also the electrocatalytic activity of the sensor for the electrooxidation of diclofenac molecules. The influence of the adsorption of the oxidation product of diclofenac molecules at the surface of the electrode on the electrochemical response of DS was also investigated. For this purpose, two continuous scan cycles for Au–PtNPs/f-MWCNTs/AuE were recorded. In the first cycle (curve c), a well-defined anodic peak is observed at about 0.57 V which gives rise to a cathodic peak at around 0.3 V in the reverse scan mode. In the subsequent cycle (curve d), a new anodic peak is appeared at 0.34 V which forms a reversible couple with the cathodic peak at 0.3 V. The reversible couple is formed at less positive potentials because of the redox property of the oxidation product of diclofenac. Also, a decrease in the oxidation peak current of diclofenac is observed in curve d due to the strong adsorption of the oxidation products of diclofenac at the surface of the electrode which blocks the effective reaction sites at the electrode surface. Therefore, based on the results which are reported by the other research groups, when the potential sweep is initiated in the positive direction at the surface of Au–PtNPs/f-MWCNTs modified electrode, diclofenac is irreversibly oxidized giving rise to an oxidation peak at 0.57 V [36,37].

3.3. Effect of scan rate

In order to investigate the effect of potential sweep rate on the oxidation peak current (Ip) of diclofenac, CVs were recorded at different scan rates from 10 to 400 mV s⁻¹ and (B) plot of the peak current versus scan rate for the oxidation of DS at Au–PtNPs/f-MWCNTs/AuE.
scan rates (0.01–0.4 V/s, Fig. 4A). As shown in Fig. 4A, the anodic peak current increases with increasing the scan rate and a slight shift in the oxidation peak potential (E\text{p}) towards more positive values is observed which indicates that the electron transfer reaction is irreversible. A linear relationship can be observed between the anodic peak current and the scan rate which confirms the adsorption controlled of the electrochemical process on the surface of the modified electrode.

3.4. Effect of pH

The influence of pH on the electrochemical behavior of diclofenac was studied in the pH range of 5 to 10 using PBS solutions. It was found that the peak potential is shifted towards less positive values with increasing the pH of the solutions which indicates the involvement of the proton in the electrochemical reaction. As can be seen in Fig. 5B, there is a linear relationship between the peak potential (E\text{p}) and pH with a regression equation:

\[ E_p(V) = -0.0459 \text{pH} + 0.9222 \quad (R^2 = 0.9912) \]

The slope of the equation is close to the Nernstian value of −59 mV which indicates that the equal number of protons and electrons are involved in the oxidation of diclofenac. This result is consistent with the diclofenac oxidation mechanism which is shown in Scheme 1 [36]. As is evident in Fig. 5C, the oxidation peak potential of DS, increases up to pH 7 and then decreases. Therefore, pH 7 was selected as an optimum pH value for the electrochemical experiments.

3.5. Analytical performance

The differential pulse voltammetry method was used for determination of different concentrations of DS in solutions using the proposed sensor under the optimized experimental conditions. The obtained results are shown in Fig. 6. As is evident in Fig. 6A, the peak current increases with increasing the concentration of DS in solution. The calibration curve is linear in the concentration range of 0.5 to 1000 μM with the regression equation of \( I_p = 0.0126C + 0.417 \), with a correlation coefficient \( R^2 \) of 0.9947. The detection limit (based on 3Sb/m) of the fabricated sensor was investigated using diclofenac solutions with different concentrations (50 and 500 μM) and the relative standard deviation (RSD) was found to be 1.7%. Also, the repeatability of the fabricated sensor was investigated using diclofenac solutions with different concentrations (50 and 500 μM) and the relative standard deviation was obtained 2.5%. The reproducibility of the fabricated sensor, was also checked through determining the concentration of 50 μM DS solution by five independent electrodes and the RSD value was estimated 2.6%. The sensor stability was investigated by storing the modified electrode in a refrigerator at 4 °C for two weeks and detecting the same concentration of DS every day. The obtained results exhibit no obvious decrease in the current response after two weeks. The practical application of the proposed sensor was investigated for determination of DS in tablet and urine samples by using standard

![Scheme 1. Oxidation mechanism of diclofenac sodium.](image)

**Fig. 6.** (A) Differential pulse voltammograms of DS in the concentration range of 0.5–1000 μM and (B) calibration curve of the sensor in different concentrations of DS.

<table>
<thead>
<tr>
<th>Method</th>
<th>Linear range (μmol L(^{-1}))</th>
<th>Detection limit (μmol L(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCPE</td>
<td>10–140</td>
<td>3.28</td>
<td>[38]</td>
</tr>
<tr>
<td>NHMN</td>
<td>196–2650</td>
<td>31.7</td>
<td>[39]</td>
</tr>
<tr>
<td>APTase sensor</td>
<td>0–1000</td>
<td>0.27</td>
<td>[40]</td>
</tr>
<tr>
<td>VFMCNTPE</td>
<td>5–600</td>
<td>2</td>
<td>[41]</td>
</tr>
<tr>
<td>GO-COOH-GCE</td>
<td>1.2–400</td>
<td>0.09</td>
<td>[42]</td>
</tr>
<tr>
<td>BDD</td>
<td>5–50</td>
<td>0.14</td>
<td>[43]</td>
</tr>
<tr>
<td>Au–PtNPs/F–MWCNTs/AuE</td>
<td>0.5–1000</td>
<td>0.3</td>
<td>This work</td>
</tr>
</tbody>
</table>

The influence of some of the possible interferences such as K\(^+\), citric acid, ascorbic acid, uric acid, glucose, sucrose and fructose on determination of DS was investigated by addition of interfering species into a 50 μM DS solution under the optimized experimental conditions. The change in the peak current of DS molecules in the presence of interfering species (500 μM) is shown in Fig. 7. The change of DS peak current (ΔI\text{p}) was calculated by subtracting the currents which recorded in the presence and absence of interfering species in solution. As is evident in Fig. 7, the presence of interfering species in solution has no significant effect on the DS current response and the peak current changes are < 5%.

3.6. Determination of DS in real samples

The practical application of the proposed sensor was investigated for determination of DS in tablet and urine samples by using standard...
addition method. The DS tablet sample (Hakim Pharmaceutical Co., Tehran, Iran, 25 mg and 100 mg) was finely ground in a mortar and dissolved in 100 mL deionized water by ultrasonication. Then, the prepared solution was filtered and diluted with 0.1 M phosphate buffer (pH 7) and it was transferred to a voltammetric cell for electrochemical measurements.

Drug-free human urine sample was centrifuged for 10 min at 3500 rpm and the supernatant of the solution, was collected and diluted 50-fold with PBS (pH 7) and then spiked with different concentrations of DS. The differential pulse voltamograms of urine sample are shown in Fig. 8. A well-defined oxidation peak of diclofenac molecules at the surface of Au–PtNPs/_CNTs modified gold electrode is observed at 0.58 V. The detection limit was calculated as 0.9 µM. The obtained results are given in Table 2. The results show that the values of recoveries and relative standard deviations (RSDs) are acceptable and the proposed sensor is a promising tool for determination of DS in both pharmaceutical and human urine samples.

4. Conclusions

In this research work, a gold–platinum (Au–Pt) bimetallic nanoparticle decorated multi walled carbon nanotubes (f-MWCNTs) modified gold electrode was developed for determination of diclofenac sodium in pharmaceutical and biological samples. The proposed electrochemical sensor showed a good electrocatalytic activity towards the DS oxidation due to the synergetic effects of gold–platinum (Au–Pt) bimetallic nanoparticles and multi walled carbon nanotubes which are presented at the surface of the electrode. Furthermore, the modified electrode was applied successfully for determination of DS in tablet and urine samples.

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References


