

Full Paper

Voltammetric Determination of the Anticancer Drug Hydroxyurea using a Carbon Paste Electrode Incorporating TiO₂ Nanoparticles

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Abstract- Hydroxyurea is an anticancer drug with high effectiveness for the treatment of different malignances. Several methods were reported for the quantification of hydroxyurea including colorimetry, high-performance liquid chromatography, and gas chromatography-mass spectrometry. Herein, a sensitive, accurate and precise nanosensor using nano TiO₂ and carbon paste for the quantification of low levels of hydroxyurea in serum samples was presented. After optimization of different experimental parameters, the proposed nanosensor showed a linear dynamic range of 8.0-180.0 mg L⁻¹ with a detection limit of 0.160 mg L⁻¹. It was found that the optimized approach could be applied to the determination of hydroxyurea in serum samples with acceptable recovery values.

Keywords- Hydroxyurea, Nanosensor, Voltammetry, nano TiO₂

1. INTRODUCTION

Hydroxyurea (HU) is an anticancer drug. Its antineoplastic properties refer to inhibition DNA synthesis via inhibition ribonucleotide reductase and reduce the level of deoxyribonucleotides [1]. HU has been used in the treatment variety malignances like melanoma, carcinoma, leukemia [2,3], thrombocytosis and polycythemia vera [4] Moreover, this drug has positive effect in the treatment sickle cell anemia and infection with human immunodeficiency virus (HIV) [5], But consumption inadequate dose of HU resulting in decrease treatment effectiveness and occurrence side effects . To ensure the accuracy of the treatment regimen, the direct measuring of HU in human serum is effective [4]. To the present time, several procedures have been used for the measuring of HU, including colorimetric [6,7], high-performance liquid chromatography [8,9] and gas chromatography–mass spectrometry (GC-MS) [10]. These procedures have some main disadvantages such as time consuming, expensive equipment and use of hazardous chemicals [11]. Due to these limitations, it is necessary to develop a sensitive, low cost and fast assay. Electrochemical techniques using chemically modified electrodes provide fast, easy, inexpensive and delicate substitutes for the quantification of different compounds in biological fluids [12,13]. Due to, biocompatibility, relatively good conductivity and high surface area, TiO_2 nanoparticles have been increasingly used as electrode modifier [14,15]. In voltammetry methods, carbon paste electrode is an common electrode and used widely as working electrode because of reproducible surface, convenient modification, ability to apply a wide range of potential and its low background contributions [5]. The purpose of this study is design and fabrication a sensitive and selective nanosensor for the determination of HU in human serum using nano- TiO_2 - modified carbon paste electrode and differential pulse voltammetry technique.

2. EXPERIMENTAL

2.1. Apparatus

All voltammetry experiments were performed with an Autolab electrochemical system from Metrohm (Herisau, Switzerland) interfaced with a personal computer for data acquisition and potential control. A three-electrode system consisted of a platinum wire as the auxiliary electrode purchased from Sarmad Teb (Tehran, Iran), $\text{Ag}/\text{AgCl}/\text{KCl}$ (saturated) as the reference electrode purchased from Sarmad Teb (Tehran, Iran) and a carbon paste electrode (CPE) as the working electrode, was used. All pH measurements were carried out using a Metrohm 827 pH meter supplied with a combined glass reference electrode.

2.2. Reagents and materials

TiO_2 nanoparticles were purchased from Acros (Geel, Belgium). Pure graphite powder was obtained from Merck (Darmstadt, Germany). HU was purchased from Sigma Aldrich

(Steinheim, Germany). and its stock solution was prepared by dissolving an appropriate amount of it in distilled water and kept in a refrigerator at 4 °C. Acetic acid, phosphoric acid, hydrochloric acid and methanol were purchased from Merck (Darmstadt, Germany).

2.3. Preparation of serum samples

During the first hour of collection, the human blood samples were centrifuged for 10 min at 1500 rpm for the separation of serum. The obtained serum samples were stored in a refrigerator at 4 °C until analysis. One mL of each serum sample was transferred into the centrifuge tubes containing different known amounts of HU and then mixed well with one mL methanol to precipitate the blood proteins. After centrifugation for 15 min at 6000 rpm, supernatants were collected and then diluted to 10 mL with 0.3 mol L⁻¹ phosphate buffer (pH 2.0).

2.4. Electrode preparation

Nano TiO₂-modified carbon paste electrode was prepared by mixing graphite powder and nano- TiO₂ (90/10 W/W%) and wetting with paraffin oil. The obtained paste was carefully hand mixed in a mortar for 20 min to ensure uniform dispersion and then packed into one end of a glass tube (3.4 mm inner diameter). Electrical contact was established via a copper wire connected to the paste. The resulting electrode was designated as nano-TiO₂/CPE. The electrode surface was gently smoothed by rubbing it on a piece of filter paper just before use. When necessary, a new surface was obtained by pushing a bit of the paste out of the tube. Bare electrode designated as bare CPE was prepared in the same way without adding Nano TiO₂. The electrodes were stored at room temperature when not in use.

2.5. Electrochemical measurement

Differential pulse voltammetry (DPV) was used for recording the electrochemical signal of HU. For this purpose, nano-TiO₂/CPE was immersed into 0.30 mol L⁻¹ phosphate buffer solution (pH 2.0) containing a given concentration of HU or into the prepared serum sample. DP voltammogram of HU was then recorded using pulse amplitude of 0.05 V and a scan rate of 50 mV s⁻¹. The current of the oxidation peak of HU at about +1.15 V was taken as analytical signal due to its correlation with the concentration of HU.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of HU at the surface of nano-TiO₂/CPE

Figure 1 represents DP voltammograms of HU at the surface of bare CPE and nano-TiO₂/CPE at the scan rate of 50 mV s⁻¹. As can be seen, at bare CPE HU shows a well-defined oxidation peak at about 1.1 V which increases noticeably at the surface of the

modified electrode. The improvement observed in the oxidation current can be attributed to the increasing of conductivity and surface area of the electrode in the presence of nano- TiO_2 .

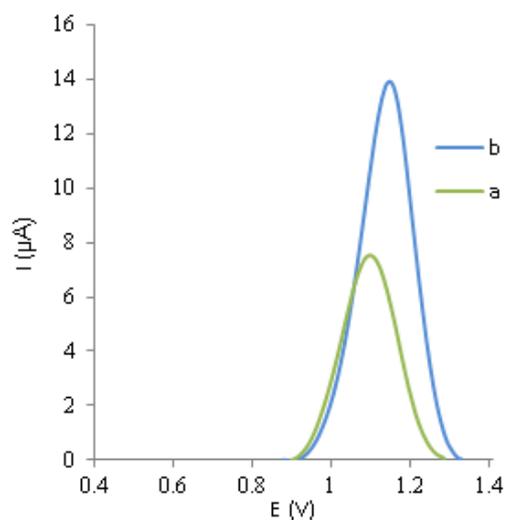


Fig. 1. DP voltammograms obtained from the oxidation of HU on the surface of bare CPE (a) and nano- TiO_2 /CPE (b). Conditions: 80.0 mg L^{-1} HU, 0.30 mol L^{-1} phosphate buffer pH 2.0, 5% nano- TiO_2 percentage

3.2. Optimization of the experimental parameters

3.2.1. Percentage of nano- TiO_2

In the present study, TiO_2 nanoparticles were used to increase the rate of electron transfer and surface area of the designed sensor.

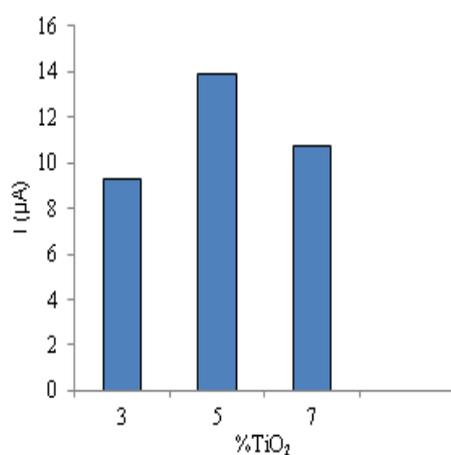


Fig. 2. Effect of nano- TiO_2 percentage on the oxidation peak current of HU. Conditions were the same as given in Fig. 1 except nano- TiO_2 percentage

So, the amount of nano- TiO_2 in the carbon paste influences on the oxidation current of HU.

To optimize the amount of TiO_2 , nano- TiO_2/CPEs containing different percentages of nano TiO_2 (3,5 and 7%) were prepared and used for the determination of 80 mg L^{-1} HU. The results obtained (Fig. 2) revealed that the highest signal was observed when using the electrode modified with 5% nano- TiO_2 . Therefore, 5% nano- TiO_2 was utilized for the construction of the nanosensor in subsequent experiments.

3.2.2. Concentration of the supporting electrolyte

Changes in the electrochemical current of HU oxidation from phosphate buffer with different concentrations ($0.050\text{--}0.40 \text{ mol L}^{-1}$) were studied. According to the results shown in Fig. 3, the oxidation signal of HU increased with increasing the concentration up to 0.2 mol L^{-1} and then remained almost stable with further concentration increasing. Thus, 0.30 mol L^{-1} was chosen as the optimal concentration of the supporting electrolyte.

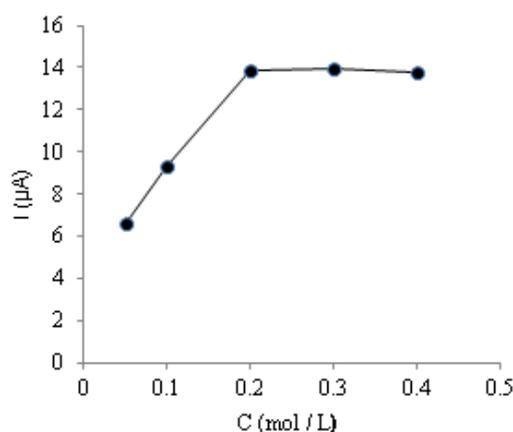


Fig. 3. Effect of concentration of the supporting electrolyte on the electrochemical current of HU. Conditions were the same as given in Fig. 1 except concentration of the supporting electrolyte

3.2.3. pH of the supporting electrode

The electrode reaction might be affected by pH of the medium in which the electrochemical signal was recorded. So, the electrooxidation of 130.0 mg L^{-1} HU in 0.30 mol L^{-1} phosphate buffer solution with different pHs in the range of 2.0–8.0 was recorded using DP voltammetry (Fig. 4A). According to the obtained results, the highest electrochemical current was observed at pH 2.0. Therefore, this pH was selected as optimum for further experiments. In addition, the anodic peak of HU shifted towards more negative potentials with increasing pH, indicating the involvement of proton in the oxidation of HU. Figure 4B illustrates the linear relationship between pH and the potential of the anodic peak with the linear regression equation of $E/V = -0.0371 \text{ pH} + 1.2387$ and the correlation coefficient (R^2) of 0.9948 suggesting an electrochemical with unequal proton-electron transfer [5].

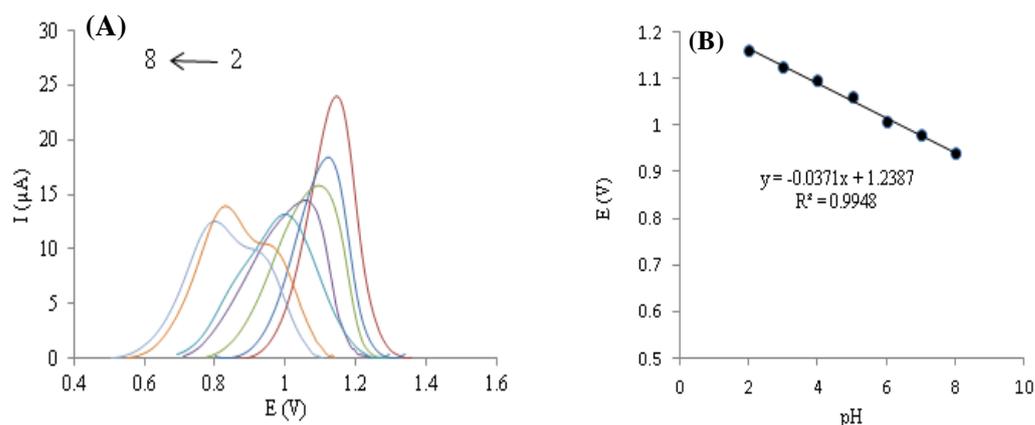


Fig. 4. (A) DP voltammograms of 130.0 mg L^{-1} HU at the surface of nano-TiO₂/CPE at different pHs of 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0. Conditions were the same as given in Fig. 1 except Ph; (B) Plot of pH versus E.

3.2.4. Nature of the supporting electrolyte

The effect of different support electrolytes on the oxidation current of HU was also evaluated. KCl/HCl mixture, phosphate buffer and acetate buffer were used for this purpose. After observing the results, phosphate buffer was chosen as the best support electrolyte.

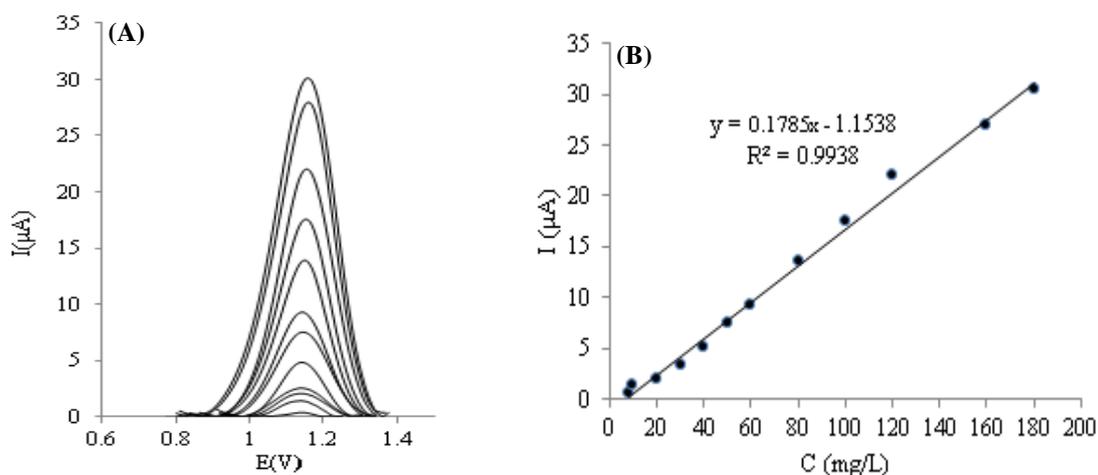


Fig. 5. (A) DP voltammograms of different concentrations of HU (from down to up: 8.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 80.0, 100.0, 120.0, 160.0 and 180.0 mg L^{-1}) obtained at the surface of nano-TiO₂/CPE; (B) Calibration curve for HU determination extracted from the voltammograms given in Fig. 5A

3.3. Calibration curve and detection limit

Under the optimized parameters, DP voltammograms of increasing concentrations of HU were recorded. It was observed that the anodic peak current increased with increasing the

concentration of HU, as depicted in Fig. 5A. The plot of peak current vs. the concentration of HU (Fig. 5B) exhibited a linear relationship between the peak current and the HU concentration in the range of 8.0–180.0 mg L⁻¹ with the linear regression equation of $I(\mu\text{A}) = 0.1785C(\text{mg L}^{-1}) - 1.1538$ and the correlation coefficient (R^2) of 0.9938. Limit of detection (LOD) calculated as $3s_b/m$ (s_b is the standard deviation of blank measurements, and m is the slope of the calibration curve) was found to be 0.160 mg L⁻¹. To ascertain the repeatability of the analyses, five replicate measurements on 30.0 mg L⁻¹ and 80.0 mg L⁻¹ HU solution were carried out using nano-TiO₂/CPE. Relative standard deviation (RSD) of the obtained peak currents was found to be 3.2% and 1.8%, respectively for 30.0 mg L⁻¹ and 80.0 mg L⁻¹ HU.

3.4. Effect of coexisting compounds on the signal of the proposed nanosensor

The effects of some possible interfering compounds such as glucose, urea, and ascorbic acid on the determination of HU with nano-TiO₂/CPE were studied. A species that cause a variation of more than 5% in the nanosensor response to HU was considered as an interfering substance.

Table 1. Effect of coexisting compounds on the determination of 40.0 mg L⁻¹ HU under the optimal conditions.

Substance	Tolerance concentration ratio
Glucose	200
Urea	100
Ascorbic acid	40

According to the results given in Table 1, no significant interference from commonly encountered matrix components was observed. It can be concluded that the proposed nanosensor is applicable to the analysis of HU in biological samples.

3.5. Detection of HU in human serum sample

In order to verify the accuracy of the approach, the standard addition method was applied for the determination of HU in a human serum sample. The serum sample prepared as mentioned previously was analyzed for HU using the nano-TiO₂/CPE and the corresponding DP voltammograms were recorded. The obtained results are reported in Table 2. As may be seen, the recoveries of the spiked samples are satisfactory indicating the acceptable accuracy of the approach. Thus, the designed nanosensor is reliable for the determination of HU in serum samples.

Table 2. Determination of HU in a serum sample using the proposed method

Sample	Added (mg L ⁻¹)	Found ^a (mg L ⁻¹)	Recovery (%)
Human serum	0	-	-
	20	20.74±0.21	103.7%
	70	70.30±0.89	100.4%

^a Mean ± standard deviation (n=3)

Table 3. Comparison of the presented method with other method reported for the determination of HU

method	Linear range(mg L ⁻¹)	LOD(mg L ⁻¹)	Ref.
Voltammetry using carbon paste electrode	15-76	0.49	[5]
Voltammetry using pencil graphite electrode	0.8-76	0.6	[3]
Gas chromatography–mass spectrometry	Up to 500	0.078	[16]
Liquid Chromatography	2-50	0.63	[8]
Nano-TiO ₂ /CPE	8-180	0.16	This work

3.6. COMPARISON

The electrochemical nanosensor presented here was compared with some other approaches previously reported for the determination of HU [3,5,16,8]. As shown in Table 3, nano-TiO₂/CPE has a wide linear range, which is greater than the other methods except one of them [16]. About LOD, a similar situation is observed so that the proposed nanosensor presents a LOD lower than the other methods except for Ref. 16. This characteristic along with the excellent repeatability of the proposed approach shows its applicability to the quantification of HU at low concentrations.

4. CONCLUSION

Since HU is an electroactive species, it can be quantified directly using voltammetry technique. Herein, an electrochemical nanosensor was fabricated by bulk modification of carbon paste electrode with TiO₂ nanoparticles. It was found that the proposed method could

be applied to determine HU in serum samples without any considerable interfering with the co-existing substances. Moreover, this technique provided high sensitivity, high-speed analysis, ease of implementation and low cost in addition to selectivity.

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