Determination of Uric Acid Using TiO\textsubscript{2} Nanoparticles Modified Glassy Carbon Electrode

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Abstract: In the present study, the electrochemical behavior of uric acid (UA) was evaluated using a titania nanoparticle (TiO\textsubscript{2}) coated glassy carbon electrode (GCE). TiO\textsubscript{2} nanoparticles are synthesized and characterized using Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Analysis (EDX) techniques. The electrochemical behavior of uric acid on both bare GCE and Titania coated GCE electrodes were studied through differential pulse voltammetry. Titania-coated GCE showed a higher current at the lower potential for the oxidation of uric acid when compared to bare GCE. The sensor's improved electrocatalytic activity was observed to detect uric acid in a 0.1 M phosphate buffer saline (PBS) solution at pH 7.0. A good linear relationship was observed between electrical response and the concentration of uric acid in the range of 1 to 9 μM. Under optimized experimental conditions, the limit of detection (LOD) was found as 0.764 μM. The sensor has expressed considerable sensitivity towards UA detection without interference and is successfully used to determine UA in human urine samples.

Keywords: uric acid; electrocatalytic activity; TiO\textsubscript{2} nanoparticles; electrochemical sensor.

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1. Introduction

Uric acid (UA) is an important nitrogen-containing compound present in both animals and plants. It comprises 2,6,8- trihydroxy purine, which exhibits keto-enol tautomerism and converts to the corresponding urate. The presence of more uric acid and urate leads to accumulation as calculi in the joints, and connective tissues cause arthritis and rheumatic pain, and excess deposition of uric acid and urate causes kidney failure. Uric acid is a biomolecule in urine and blood and is generated by breaking the purine in the body’s metabolic process [1]. The normal level of uric acid in the blood is 0.12 mM – 0.45 mM, and in urine is 2 mM [2]. Increased uric acid levels in body fluids can cause diseases like toxemia of pregnancy, hyperuricemia, etc. [3-4]. Several studies suggested that the unusual UA level in blood serum is the risk factor, can cause cardiovascular disease, and influence the circulatory system [5]. In this context, UA sensing with commercial monitoring, anticipated sensitivity, and accuracy in human body fluids is crucial to disease sense. Different techniques, including HPLC, fluorescence, calorimetry, and direct electrochemical detection, have been developed to quantify uric acid in biological fluids. The electrochemical technique is widely used and has
been proven to be an excellent method for UA determination due to its rapid response, simple handling, specificity, low cost, and high sensitivity [6-7].

Usage of transition metal oxide nanoparticles is increased because of their potential applications. Recently modification of magnetic nanoparticles was done by coating with either metal oxides or noble metals [8-10]. Among several transition metal oxides, TiO₂ became promising material due to its unique characteristics like high chemical stability, better conductivity, biocompatibility, harmfulness, and low cost. Titania nanoparticles of various shapes were used as electrode material for different electrochemical-sensing applications because of their better catalytic and electrical properties [11]. In our previous work, we fabricated TiO₂/rGO/GCE for the quantification of Rifampicin. [12]. PtCu@rGO-TiO₂ and TiO₂/rGO were used for oxygen reduction reactions [13, 14]. The other amino acid tryptophan was detected using a glassy carbon electrode modified with silver doped TiO₂ nanoparticles (Ag-TiO₂ NPs/GCE) [15]. Recently several papers have been published in the literature to detect uric acid using modified nanotubes, nanoparticles, and nanofibres. [16-22]

This work reports the electrochemical behavior of UA on titania nanoparticle-modified glassy carbon electrodes (GCE). The developed electrochemical sensor exhibits an improved electrocatalytic activity towards detecting uric acid in 0.1 M PBS of pH 7.00. The sensor has expressed considerable sensitivity and selectivity towards UA detection without any interference and successfully determined UA in human urine samples.

2. Materials and Methods

CH-Instrument model No. CH1610D (USA) was adopted for the voltammetric studies. It comprises a conventional three-electrode system consisting of modified and/or unmodified glassy carbon electrodes as the working electrode. Silver/silver chloride with saturated KCl and platinum electrodes are used as reference electrodes and counter electrodes. With the help of a pH meter Elico model (LI-120), the solutions' pH values are determined.

Uric acid, Sodium dihydrogen phosphate (NaH₂PO₄), HNO₃, and titanium n-butoxide are acquired from HI-media (Mumbai). Disodium hydrogen phosphate (Na₂HPO₄) was procured from Sigma-Aldrich and used as provided. The uric acid stock solution was prepared by dissolving the required amount in 0.1 M NaOH solution. Phosphate buffer solution (PBS) is a supporting electrolyte, and it was prepared from 0.1 M disodium hydrogen phosphate and 0.1 M sodium dihydrogen phosphate. All the reagents are AR grade and utilized as supplied. The ultrapure water supplied by Millipore milli-Q system was used throughout the experimental work for the dissolution purpose.

The sol-gel method was adopted to prepare Titania nanoparticles [23]. In this process, first solution A was prepared by dissolving 5 mL of titanium n-butoxide in 15 mL of ethanol followed by ultrasonic dispersion. Solution B was prepared by mixing 5 mL of water, 1 mL of HNO₃, and 20 mL of absolute ethanol. Then solution A was slowly added dropwise to solution B under vigorous stirring. Subsequently, the temperature increased up to 80°C under stirring. The resulting gel was dried at 84°C for 4 hours, and then the resulting mixture was calcined in a furnace at 450°C for 2 hours to obtain a crystalline powder of TiO₂ nanoparticles.

The prepared TiO₂ nanoparticles (3.0 mg) were dispersed in ethanol (1.0 mL) to form a homogenous suspension. To obtain the TiO₂ NPs modified GC electrode, 10 µL of TiO₂ NPs solution was deposited on a bare GCE surface and dried at room temperature. The prepared modified electrode was thrice washed with double distilled water, dried, and used for further studies.
3. Results and Discussion

3.1. SEM, TEM, and EDX analysis.

The surface and morphological studies of titania nanoparticles were investigated by scanning electron microscopy. Pictures of SEM provided in Figure 1a give information about the surface structure, such as nanoparticles' shape and size. TiO$_2$ nanoparticles size is in the range of 70-150 nm and spherical and uniformly distributed. The energy-dispersive x-ray spectroscopy determines the composition of a sample. EDX of TiO$_2$ is presented in Figure 1b, which shows the presence of Ti and O elements and evidence for the formation of pure TiO$_2$ nanoparticles.

![Figure 1. (a) SEM image of TiO$_2$ nanoparticles; (b) Representing the presence of Ti and O thorough EDX.](https://biointerfaceresearch.com/)

3.2. Electrochemical oxidation of UA at the TiO$_2$.

The pH values of electrolytes have a significant influence on the UA electrochemical sensors. The effect of pH on uric acid oxidation was studied between 5.5 to 8.0. The maximum current was observed at pH 7.0, so the pH 7.0 solution was selected further throughout the experiment. The anodic peak potential of uric acid shifted gradually to a negative value representing the participation of protons in the electrochemical oxidation process.

![Figure 2. Cyclic voltammogram (CV) of blank (a) at bare GCE and CV of 25 mM UA on bare GCE (b) and TiO$_2$ NPs/GCE (c) electrodes in the presence of 0.1 M PBS (pH 7.0) at a scan rate of 50 mVs$^{-1}$.](https://biointerfaceresearch.com/)
Figure 2 describes the cyclic voltammograms (CVs) bare GCE in the absence of UA (a), in the presence of 25 mM UA (b), and CV of TiO$_2$ NPs/GCE in the presence of 25 mM UA (c) in 0.1 M PBS at pH 7.0 at scan rate 50 mVs$^{-1}$. The CV curve for bare GCE showed a relatively oxidized peak at 336 mV with a peak current of 25.6 µA; Moreover, no cathodic peak was observed in the backward reaction. On the other hand, an oxidation potential at 292 mV, peak current (Ipa) of 40.6 µA was observed for the modified TiO$_2$ modified GC electrode. Introduction of TiO$_2$ nanoparticles onto the surface of GCE enhances the anodic peak current relative to the bare GCE. It is clear from these results that the presence of TiO$_2$ NPs improves UA oxidation.

3.3. Effect of scan rate.

Variable scan rates study describes the impact of scan rate upon electrocatalytic-oxidation of 25 mM UA on TiO$_2$ NPs/GCE ranged from 50 – 400 mVs$^{-1}$ and is depicted in Figure 3 a. It is observed that UA oxidation peak current (Ipa) also increased on increasing the scan rate. As shown in Figure 3 b, the excellent relationship of current vs. square root of the scan rate (R$^2$ = 0.9955) is obtained over the scan rate range.

Figure 3. (a) CV measurements of modified TiO$_2$ NPs/GCE electrode at the potential scanning range from 50 to 400 mV s$^{-1}$ in 0.1M PBS solution at pH 7.0 condition; (b) Calibration plots for the anodic peak current vs. square root of scan rate.

Figure 4. (a) Differential pulse voltammetry (DPV) recorded for TiO$_2$ NPs/GCE in 0.1M PBS pH 7.0 UA with the range of concentrations of 1-9 µM (b) calibration plots of I$_{pa}$ vs. UA concentrations.
3.4. Analytical performance evaluation analysis of real samples.

Figure 4a shows the differential pulse voltammogram (DPV) curves for UA oxidation on TiO$_2$ NPs/GCE in 0.1 M PBS at pH 7.0 at various concentrations (1-9 M). The investigations were conducted using DPV parameters of a pulse height of 50 mV and a pulse time of 200 ms. The different oxidation peaks observed at 0.44 V, along with a higher current response as UA content increases, show that TiO$_2$ NPs/GCE can be used to quantify UA (Figure 4b).

Linear regression equation is given as: $\text{I}_{\text{pA}}$ (μA) = 1.7312 $x$ + 8.1937 (μA) ($R^2 = 0.9965$). The limit of detection (LOD) and limit of quantification (LOQ) can be calculated using the formulae given below [24]:

$$\text{LOD} = \frac{3\sigma}{m}$$  \hspace{1cm} (1)

$$\text{LOQ} = \frac{10\sigma}{m}$$  \hspace{1cm} (2)

Here, $\sigma$ is the standard deviation (SD), $m$ is the slope of the calibration curve. On substituting the values in equations 1 and 2, LOD is found to be 0.764 μM, and LOQ value is 2.54 μM. These values are comparable to those achieved by other electrochemical sensors as per the data provided in Table 1 [25-34]. The current electrode is identical to, if not superior to, the previous electrodes. The current electrode has a noticeable advantage over the composites with several previously reported components but failed to functionalize graphene nanosheets with zinc tetraaminophthalocyanine, methylcellulose/graphene oxide/iron oxide nanohydrogels.

Table 1. Comparison of linear dynamic range and low limit of detection of Uric acid using TiO$_2$ NPs/GCE and other electrochemical sensors.

<table>
<thead>
<tr>
<th>Electrochemical sensor</th>
<th>Linear dynamic range (mol L$^{-1}$)</th>
<th>Limit of Detection (mol L$^{-1}$)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SnO$_2$-NP/MWCNTs/CPE</td>
<td>3.0×10$^{-6}$ – 2.0×10$^{-4}$</td>
<td>1.0 × 10$^{-6}$</td>
<td>[25]</td>
</tr>
<tr>
<td>nano-Au/p-TA/GCE</td>
<td>1.0×10$^{-3}$ – 2.8×10$^{-4}$</td>
<td>0.8 × 10$^{-6}$</td>
<td>[26]</td>
</tr>
<tr>
<td>N-DHPB/MWCNT/PE</td>
<td>5.0×10$^{-8}$ – 4.2×10$^{-4}$</td>
<td>2.0 × 10$^{-6}$</td>
<td>[27]</td>
</tr>
<tr>
<td>HCNTs/GCE</td>
<td>6.7×10$^{-6}$ – 6.5×10$^{-5}$</td>
<td>1.5 × 10$^{-6}$</td>
<td>[28]</td>
</tr>
<tr>
<td>CNT&amp;NF</td>
<td>4.0×10$^{-6}$ – 4.0×10$^{-4}$</td>
<td>1.0 × 10$^{-6}$</td>
<td>[29]</td>
</tr>
<tr>
<td>NgB/CPE</td>
<td>12.5×10$^{-6}$ – 7.5×10$^{-4}$</td>
<td>5.0 × 10$^{-6}$</td>
<td>[30]</td>
</tr>
<tr>
<td>ZIF-11/GCE</td>
<td>50×10$^{-6}$ – 540×10$^{-6}$</td>
<td>0.48 × 10$^{-6}$</td>
<td>[31]</td>
</tr>
<tr>
<td>Uricase/carbon nanotube/carboxymethylcellulose electrode</td>
<td>20×10$^{-6}$ – 50000×10$^{-6}$</td>
<td>2.8 × 10$^{-6}$</td>
<td>[33]</td>
</tr>
<tr>
<td>Methylcellulose/graphene oxide/iron oxide nanohydrogel/GCE</td>
<td>0.5×10$^{-6}$ – 140×10$^{-6}$</td>
<td>0.17×10$^{-6}$</td>
<td>[33]</td>
</tr>
<tr>
<td>Zinc Tetraaminophthalocyanine functionalized graphene nanosheets/GCE</td>
<td>0.5×10$^{-6}$ – 100×10$^{-6}$</td>
<td>0.15×10$^{-6}$</td>
<td>[34]</td>
</tr>
<tr>
<td>TiO$_2$ NPs/GCE</td>
<td>1.0 × 10$^{-6}$ – 9.0 × 10$^{-6}$</td>
<td>0.76×10$^{-6}$</td>
<td>Present work</td>
</tr>
</tbody>
</table>

3.5. Analysis of real samples.

Performance of the TiO$_2$ NPs/GCE checked by applying it to detect uric acid in actual urine samples. The recovery method was adopted to quantify uric acid in the actual urine sample. Here 2 mL of a human urine sample was taken and diluted with 0.1 M PBS (pH 7.0) till the disappearance of uric acid DPV peak present in it, and then it is spiked with uric acid with the concentration of 0.01 0.02 and 0.03 mM. These samples were analyzed by DPV using TiO$_2$ NPs/GCE, and the corresponding results are presented in Table 2. The values presented in Table 2 showed satisfactory results with a recovery of 97.0 to 99.6%.

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Table 2. Application of the method to actual urine samples

<table>
<thead>
<tr>
<th>Real sample</th>
<th>Spiked (mM)</th>
<th>Found (mM)</th>
<th>Recovery %</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine sample</td>
<td>0.01</td>
<td>0.097</td>
<td>97.0</td>
<td>2.0</td>
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<tr>
<td></td>
<td>0.02</td>
<td>0.019</td>
<td>95.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>0.0299</td>
<td>99.6</td>
<td>2.9</td>
</tr>
</tbody>
</table>

4. Conclusions

In this work, TiO$_2$ nanoparticles were synthesized through the sol-gel method and used to fabricate TiO$_2$NPs/GCE by coating on a glassy carbon electrode to determine Uric acid. The TiO$_2$NPs/GCE exhibited significant electrocatalytic activity, better sensitivity, and selectivity towards uric acid oxidation at pH 7.00. TiO$_2$ NPs/GCE exhibited satisfactory results for the determination of uric acid with LoD of 0.764 μM and LoQ of 2.54 μM. The value obtained is lower and comparable with the other reported modified electrodes. Hence TiO$_2$ NPs/GCE may act as an excellent sensor for determining uric acid, and the method has been successfully applied to determine uric acid in urine samples.

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Conflicts of Interest

The authors declare no conflict of interest

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