A Gold Nanoparticles-Modified Carbon Paste Electrode for Uric Acid Determination in Human Urine without Pretreatment Steps

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Received: 30 April 2013 / Received in revised form: 31 July 2013 / Accepted: 5 August 2013 / Published online: 30 August 2013

Abstract- Uric acid (UA) is a water soluble compound which is produced from purine metabolism in human body. High or low levels of UA are symptoms of some deficiencies in human body, so finding a simple and sensitive method for determination of UA is important. A new chemically modified electrode was fabricated by gold nanoparticles (GNPs) and carbon paste electrode (CPE) for determination of UA in human urine. Gold nanoparticles-modified carbon paste electrode (GNPs/CPE) was characterized by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Some important experimental parameters such as amount of modifier, pH and scan rate (ν) effects were studied by cyclic voltammetry and the best values for analysis were chosen. To propose a mechanism for oxidation of UA on the surface of modified electrode, the number of involved electrons and protons and diffusion coefficient of UA (DUA) was calculated. Square wave voltammetry (SWV) was used as an accurate technique for quantitative measurements. A good linear relation was observed between anodic peak current (ipa) and UA concentration (CUA) in the range of 2×10⁻⁷ to 2×10⁻⁴ mol L⁻¹, and the detection limit (DL) was obtained to be 3.9×10⁻⁸ mol L⁻¹, that is comparable to recent studies. This paper demonstrates a novel, simple, selective and rapid sensor for determination of UA in the analytical real samples.

Keywords- Uric Acid, Gold Nanoparticle, Modified Carbon Paste Electrode, Square Wave Voltammetry, Human Urine
1. INTRODUCTION

In humans and higher primates, UA is the final oxidation product of purine metabolism and is excreted in urine. The enzyme xanthine oxidase makes uric acid from xanthine and hypoxanthine, which in turn are produced from other purines [1]. Scheme 1 illustrates the heterocyclic structure of UA. In humans, purines are excreted as uric acid. High amounts of purines are found in animal food products, such as liver and sardines. A moderate amount of purine is also contained in beef, pork, poultry, fish and seafood, asparagus, cauliflower, spinach, mushrooms and so on [2].

High levels of uric acid is called hyperuricemia, in many instances, people have elevated uric acid levels for hereditary reasons, although diet or reduced excretion by the kidneys may be the other factors [3,4]. Low concentrations of uric acid (hypouricemia) can have numerous causes such as low dietary zinc intakes or Fe deficiency [5]. Improper levels of UA are symptoms of some diseases like hyperpiesia, gout and Lesch-Nyhan syndrome [6,7].

Numerous analytical methods including colorimetry (optical methods), enzymatic and electrochemical techniques have been applied to determination of UA [8–11]. Low cost, simplicity, high reproducibility, rapid response and low detection limit are some advantages that cause extensive interest to electrochemical methods.

Carbon paste electrode (CPE) because of its specific properties namely easy preparation and lower residual currents [12,13] has been used for determination of a vast verity of species like drugs, vitamins and so on. To promote the efficiency of CPE, different modifiers can be used. These modifiers have important effects on parameters of carbon paste electrode in electrochemical measurements.

For modification of electrodes, metal nanoparticles have emerged as a reliable alternative in electroanalysis of various compounds [14,15]. Nanometals roughening the conductive sensing interface provide catalytic features and represent conductivity properties [16].

In this study, GNPs as specific and important metal nanoparticles were chosen for the modification of CPE. There are some methods for synthesis of GNPs including Turkevich method [17], Brust method [18] and Perrault method [19]. Turkevich method was utilized for GNPs synthesis in the present work. Electrochemical studies have shown some properties for GNPs like improving the electrode conductivity and surface area enhancement, facilitating the electron transfer and detection limit improvement, which makes it a promising candidate for electrode modification [20,21]. In this work, UA was diffused toward the surface of the gold nanoparticles-modified carbon paste electrode (GNPs/CPE) and its oxidation peak current (ipa) was used for determining of UA in human urine sample without any more steps prior to sample analysis. The present study describes a valid, simple, rapid, selective and sensitive procedure for determining of UA by employing the square wave voltammetry (SWV) on GNPs/CPE.
2. EXPERIMENTAL

2.1. Apparatus and reagents

Electrochemical measurements were conducted using the Autolab PGSTAT 30 electrochemical analyzer (Ecochemie BV, Utrecht, the Netherlands) connected to a computer with General Purpose Electrochemical System software package. A three-electrode system including a carbon paste electrode (unmodified and modified), an Ag/AgCl electrode and a Pt rod, as working electrode, reference electrode and auxiliary electrode, respectively, was used to obtain the electrochemical data. HAuCl₄·3H₂O and tri-sodium citrate dihydrate were purchased from Merck (Darmstadt, Germany). Other reagents and chemicals were of analytical grade and were used without further purification except graphite powder. All solutions were prepared with distilled water.

2.2. Graphite powder purification

100 g of graphite powder was washed with concentrated HCl about 2 h at room temperature, then filtered and dried. The acid washed sample was mixed with a 20% (w/v) NaOH solution and rinsed with hot distilled water to reach the pH of 7.0. Then it was washed and dried in laboratory environment [22].

2.3. GNPs synthesis

GNPs were prepared according to the prior published papers [23,24]. First, glasswares were cleaned in a HNO₃: HCl (1:3) solution and then rinsed with distilled water thoroughly. 0.5 mL of 1% (w/v) of sodium citrate solution was added to 50 mL of 0.01% (w/v) of HAuCl₄ boiling solution. The mixture was boiled for 15 min and then to produce colloidal GNPs stirred for 15 min after removing the heating source. The synthesized solution was stored in a dark-colored bottle in refrigerator.

2.4. Construction of the electrode

The electrode was made by Ertalon and a copper wire with 2 mm diameter was used for its electrical contact. The carbon paste electrode was prepared by mixing graphite powder (60% w/w) with appropriate amount of paraffin oil (about 30% w/w) and thorough hand mixing. The modified electrode was prepared by mixing unmodified paste with GNPs (about 10%, w/w). In the long run, a portion of the mixture (unmodified and modified) was packed into a tube in the end of electrode.

2.5. Preparation of sample

Urine sample was collected from a volunteer and to determine the UA content after homogenization, the urine was filtered by a filter paper. The collected solution was used without any more treatments. A 10 μL of filtered urine sample was transferred into a 10 mL volumetric flask containing phosphate buffer (pH=5.5) and made up to the volume.
3. RESULTS AND DISCUSSION

3.1. Structure characterization of modified electrode

First, we used the UV-Vis spectra to confirm the synthesis of GNPs. As can be seen in Fig. 1A, maximum absorbance appears at around 530 nm, which means the size of synthesized GNPs is lower than 20 nm approximately [25]. This result was confirmed by TEM image of synthesized colloidal gold nanoparticles solution (Fig. 1B). According to Fig. 1B, the average diameter of synthesized GNPs is about 10 nm.

The surface morphology of CPE and modifying effect of GNPs on it was find out by SEM images before and after adding of GNPs. Fig. 1C is showing the carbon paste surface and Fig. 1D is showing the surface of carbon paste with optimum amount of GNPs. SEM images show the porosity and surface increment of the electrode after presence of nano-sized gold particles. Consequently, it will increase the efficiency of modified carbon paste electrode and facilitate the electron transfer between solution and surface of electrode.

Fig. 1. (A) UV–Vis spectrum of colloidal gold solution; (B) TEM image of colloidal gold nanoparticles with a mean diameter of 10 nm; (C) SEM image of carbon paste electrode before adding the modifier; (D) SEM image of GNPs/CPE with optimum amount of gold nanoparticles
3.2. Electrochemical behavior of modified electrode

To evaluate the electrochemical behavior of GNPs/CPE, potassium ferrocyanide solution was used. Fig. 2A displays the cyclic voltammograms of bare CPE (a) and GNPs/CPE (b) in K₃[Fe(CN)₆]₃⁻ 5×10⁻³ mol L⁻¹ containing 0.1 mol L⁻¹ KCl solution. The cyclic voltammograms corresponded to the GNPs/CPE exhibited a redox peak smaller than that of bare CPE. This CVs proved the presence of negative charges on GNPs/CPE that repels the negatively charged K₃[Fe(CN)₆]₃⁻/₄⁻ ions, and causing a lower current signal [26].

Fig. 2. (A) Cyclic voltammograms of bare CPE (a) and GNPs/CPE (b) in the solution of K₃[Fe(CN)₆]₃⁻ 5×10⁻³ mol L⁻¹ containing KCl 0.1 mol L⁻¹. (B) Cyclic voltammograms of (a) CPE and (b) GNPs/CPE of UA 4×10⁻⁵ mol L⁻¹
Moreover, to study the electrochemical behavior of UA, cyclic voltammetry was used too. Fig. 2B shows that there was no redox peak at unmodified CPE (a), while in the presence of GNPs in the paste matrix; a significant oxidation peak can be observed (b). The effective influence of GNPs for determining of the UA can be established by these observations.

3.3. Optimization of experimental conditions

3.3.1. Optimum amount of GNPs

The concentration of GNPs in the carbon paste electrode as an effective parameter on the voltammetric responses was investigated by CV technique at different amount of colloidal gold solution. To finding the optimum amount of GNPs, some electrodes containing 25, 30, 35, 40, 45 and 50 μL of GNPs were prepared and under identical conditions examined for their voltammetric signals. As can be seen in Fig. 3, after adding 40 μL of colloidal GNPs solution, with increasing the modifier amount, anodic peak current is nearly constant, consequently 40 μL (about 0.006 mol L\(^{-1}\)) was chosen as optimum amount of GNPs.

Fig. 3. Anodic peak currents (A) against different volume of colloidal gold nanoparticles (μL)

3.3.2. Effect of solution pH

PH is one of the variables which mainly and strongly influence the current and shape of voltammograms, so finding the optimum pH of electrochemical system is important. The CVs for a series of 0.01 mol L\(^{-1}\) UA solutions in 0.1 mol L\(^{-1}\) phosphate buffers were examined (pH 4–7.5). The anodic peak currents \(i_{pa}\) and the anodic peak potentials \(E_{pa}\) were found to be dependent on the pH. Fig. 4A shows that with solution pH rising, the
highest peak current and finest voltammogram appears at about pH 5.5. Thus, pH 5.5 was chosen as the optimum pH and other measurements were done at this pH.

According to Fig. 4B the peak potential decreases with solution pH increasing by the equation of $E_{pa}=-0.0645pH+0.7802$, $r^2=0.9913$. The slope, -0.0645, is close to 0.059 at the Nernst equation slope. Therefore, the number of protons and electrons that are involved at the oxidation process of UA are equal.

**Fig. 4.** (A) CVs of $4\times10^{-4}$ mol L$^{-1}$ UA at GNPs/CPE in different phosphate buffer solutions. pH 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, respectively from right to left. (B) Anodic peak potential (V) against pH; scan rate 0.1 V s$^{-1}$
3.3.3. Effect of potential scan rate

As another important factor in electrochemical study, the potential scan rate (ν) effect on the anodic peak current (i<sub>pa</sub>) and the anodic peak potential (E<sub>pa</sub>) of UA was examined at the buffered solution (pH 5.5) containing 3.8×10<sup>−4</sup> mol L<sup>−1</sup> UA Fig. 5A. It can be seen that by increasing the scan rates, i<sub>pa</sub> increased and E<sub>pa</sub> shifted to more positive potentials. As shown in Fig. 5(B), a linear equation between i<sub>pa</sub> (A) and ν<sup>1/2</sup> (V<sup>1/2</sup> s<sup>−1/2</sup>) over the scan rate ranges of 0.02 to 0.9 V s<sup>−1</sup> for 3.8×10<sup>−4</sup> mol L<sup>−1</sup> UA was found; i<sub>pa</sub>=5×10<sup>−5</sup>ν<sup>1/2</sup>+5×10<sup>−7</sup> (r<sup>2</sup>=0.9986), that indicates the electrochemical reaction of UA at the GNPs modified electrode is a diffusion-controlled process.

![Graph A](image1)

![Graph B](image2)

**Fig. 5.** (A) Cyclic voltammograms using the GNPs/CPE in the pH of 5.5 at scan rates 0.02 to 0.9 V s<sup>−1</sup> (as shown by (a) to (s)). (B) Linear relation between anodic peak current, i<sub>pa</sub>, and square root of scan rates, ν<sup>1/2</sup>, at the 0.1 mol L<sup>−1</sup> buffer solution of pH 5.5
According to Fig. 5A, the anodic peak potential ($E_{pa}$) shifted to positive potentials as scan rate was increased. The plot of $E_{pa}$–log $\nu$, showed a linear relation between $E_{pa}$ and log $\nu$ with the equation of $E_{pa}=0.0639\log\nu+0.5113$ ($r^2=0.9961$), that indicates an irreversible mechanism for the UA electrocatalytic oxidation on the surface of GNPs/CPE.

According to Laviron's model, for an irreversible process [27], plotting the $E_{pa}$ vs. log $\nu$ (Fig. 6A) gives a straight line with the slope of $2.3RT/(1-\alpha)nF$, where, $\alpha$ is the electron transfer coefficient and for an irreversible reaction, it considered equal to 0.5. So by replacing the slope of plotted line and other constants, the number of involved electrons, $n$, is calculated about 2. Consequently, according to the result of section 3.3.2, two electrons and two protons are involved at the oxidation process of UA (shown in scheme 1).

In addition, the Tafel curves [28] for a solution of $3.8\times10^{-4}$ mol L$^{-1}$ UA at different scan rates in PBS (pH 5.5) were plotted (Fig. 6B) and the slopes used to find the number of involved electrons and the protons, and explanation of oxidation mechanism. The slopes acquired from plots of log $i_{pa}$ against $E$, show the values of $\alpha n$ in the range of 0.6 to 0.9 in scan rate ranges of 0.02 to 0.1 V s$^{-1}$ on the modified carbon paste electrode. According to the slope of Tafel’s plot, $\alpha nF/2.3RT$, and supposing the $\alpha=0.5$, a two electron-two proton mechanism can be attributed to oxidation of UA, similar to the Laviron’s model (mentioned above).

Scheme 1. Chemical structure of uric acid and its oxidation mechanism on the surface of GNPs/CPE.
Fig. 6. (A) Anodic peak potential, $E_{pa}$, against log $ν$ for a 3.8×10^{-4} mol L^{-1} UA solution at PBS (pH 5.5). (B) Tafel curves of 3.8×10^{-4} mol L^{-1} UA at different scan rates in 0.1 mol L^{-1} PBS (pH 5.5).

Basically, uric acid is oxidized in a primary 2e⁻-2H⁺ oxidation of the C₄=C₅ bond to give a readily reducible and highly reactive bis-imine. Complete hydration of the bis-imine gives rise to uric acid-4,5-diol, that at intermediate pH, breaks down to alloxan, allantoin, urea, and occasionally traces of parabanic acid [29].

3.4. Chronoamperometric study

The diffusion coefficient of UA ($D_{UA}$) was investigated by chronoamperometry and using the Cottrell equation [30].

$$i=nFAD^{1/2}C/\pi^{1/2}t^{1/2}$$  \hspace{1cm} (1)

Where, $n$ is the number of involved electrons, $F$ is Faraday number (C mol L⁻¹), $A$ is the effective electrode surface area (cm²) (calculation not mentioned), $C$ is the UA concentration.
(mol L⁻¹) and \( t \) is the time (s). In Fig. 7, the chronoamperograms for (a) \( 2 \times 10^{-5} \), (b) \( 4 \times 10^{-5} \) and (c) \( 8 \times 10^{-5} \) mol L⁻¹ of UA are shown. First the plot of \( I-t^{-1/2} \) was drawn (Fig. 7, inset A) using the data obtained from these curves. The equations of \( i=0.001t^{-1/2}+0.0002 \), \( i=0.002t^{-1/2}+0.0005 \) and \( i=0.004t^{-1/2}+0.0007 \) are corresponded to above concentrations, respectively. Then, the slopes of these straight lines were plotted against UA concentration (Fig. 7, inset B). The diffusion coefficient of UA was calculated from the slope of the last plot and it was equal to \( 1.976 \times 10^{-5} \) cm² s⁻¹.

![Fig. 7. Chronoamperograms of GNPs/CPE in the optimum condition of UA solutions (a) \( 2 \times 10^{-5} \), (b) \( 4 \times 10^{-5} \) and (c) \( 8 \times 10^{-5} \) mol L⁻¹. Inset A: Plot of \( I-t^{-1/2} \) obtained from chronoamperograms. Inset B: Plot of the slopes of the lines obtained from \( I-t^{-1/2} \) diagrams against UA concentration (mol L⁻¹)]
3.5. Linear range and detection limit of UA

To obtain the linear range and then calculation of detection limit, under optimized conditions acquired in previous sections, square wave voltammograms for oxidation of UA in different concentrations were carried out (Fig. 8). As shown in the inset of Fig. 8, around the concentration of 2×10^{-4} mol L^{-1}, the calibration curve has leveled off. The two linear ranges, between \( i_{pa} \) and \( C_{UA} \) are shown in the inset of Fig. 8 with the calibration equation of \( i_{pa}=0.115C_{UA}+2\times10^{-7} \) \( (r^2=0.9905) \) and \( i_{pa} = 0.0376C_{UA}+2\times10^{-5} \) \( (r^2=0.9902) \), where \( i_{pa} \) is the anodic peak current (A) and \( C_{UA} \) is the concentration of UA (mol L^{-1}). GNPs/CPE as a modified sensor showed good linear ranges from 2×10^{-7} to 2×10^{-4} and 2×10^{-4} to 1×10^{-3} mol L^{-1} for UA. The DL of UA was determined using the 3\( \sigma_{bl}/m \) ratio equal to 3.9×10^{-8} mol L^{-1}, where \( \sigma_{bl} \) is the standard deviation of the mean value for 8 SWVs of the blank solution (phosphate buffer solutions of pH 5.5) and \( m \) is the sensitivity of UA that is equal to 0.115 A mol^{-1} L and acquired from calibration equation. The resultant linear range and DL of the proposed electrode is comparable to values reported previously, which their comparison is represented in Table 1.

Table 1. Comparison of different sensors for the determination of UA

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Modifier</th>
<th>Linear range (mol L^{-1})</th>
<th>pH</th>
<th>Technique</th>
<th>Sample</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCE(^a)</td>
<td>Chitosan-MWNT(^b)</td>
<td>2×10^{-6} to 8×10^{-5}</td>
<td>5.5</td>
<td>DPV</td>
<td>Urine</td>
<td>[31]</td>
</tr>
<tr>
<td>GCE</td>
<td>MWNT film</td>
<td>1×10^{-7} to 1×10^{-4}</td>
<td>5.5</td>
<td>DPV</td>
<td>Human serum</td>
<td>[32]</td>
</tr>
<tr>
<td>Gold</td>
<td>SWNT(^c)</td>
<td>1×10^{-7} to 2.5×10^{-5}</td>
<td>5.0</td>
<td>CV</td>
<td>–</td>
<td>[33]</td>
</tr>
<tr>
<td>GCE</td>
<td>PEDOT(^d)</td>
<td>1×10^{-5} to 2×10^{-5}</td>
<td>7.4</td>
<td>DPV</td>
<td>–</td>
<td>[34]</td>
</tr>
<tr>
<td>CPE(^e)</td>
<td>GNPs(^f)</td>
<td>2×10^{-7} to 2×10^{-4}</td>
<td>6.5</td>
<td>SWV</td>
<td>Human urine</td>
<td>This work</td>
</tr>
</tbody>
</table>

\(^a\) Glassy carbon electrode
\(^b\) Chitosan-Multi walled carbon nanotubes
\(^c\) Single-walled carbon nanotube
\(^d\) Poly(3,4-ethylenedioxythiophene)
\(^e\) Carbon paste electrode
\(^f\) Gold nanoparticles
3.6. Interference study of modified electrode

Probable interferences of some compounds and ions, which commonly existed in biological samples, in a solution containing $1.0 \times 10^{-5}$ mol L$^{-1}$ UA, in phosphate buffer solution (pH=5.5) was investigated by SWV. The results showed that most species such as glucose, Na$^+$, Cu$^{2+}$, Zn$^{2+}$, NO$_3^-$ and Cl$^-$ did not interfere in a 1000-fold concentration. It was found no interference in a 200-fold amount of Fe$^{2+}$, K$^+$, SO$_4^{2-}$, Br$^-$ and the same concentration of ascorbic acid (AA). These results illustrated a good selectivity of GNPs/CPE for UA determination in complicated samples.

![SWVs in the buffer solution of pH 5.5 and scan rate of 0.1 V s$^{-1}$ of UA solutions](image)

**Fig. 8.** SWVs in the buffer solution of pH 5.5 and scan rate of 0.1 V s$^{-1}$ of UA solutions: (a) $2 \times 10^{-7}$, (b) $6 \times 10^{-7}$, (c) $8 \times 10^{-7}$, (d) $2 \times 10^{-6}$, (e) $4 \times 10^{-6}$, (f) $8 \times 10^{-6}$, (g) $2 \times 10^{-5}$, (h) $4 \times 10^{-5}$, (i) $6 \times 10^{-5}$, (j) $8 \times 10^{-5}$, (k) $2 \times 10^{-4}$, (l) $4 \times 10^{-4}$, (m) $6 \times 10^{-4}$, (n) $8 \times 10^{-4}$ and (o) $1 \times 10^{-3}$ mol L$^{-1}$.

Inset: Two linear ranges of UA taken from SWVs in the optimum condition

3.7. Real sample analysis

In order to demonstrate the applicability of this method for real samples analysis, human urine as a biological fluid was analyzed. The content of UA in prepared human urine (section 2.5) in an ordinary condition was determined by the GNPs/CPE by adding the specific
concentration of standard solution of UA as a standard addition method that is a precious analytical method to overcome the probable matrix interferences. Table 2 shows the recovery of UA by spiking the analyte solution into diluted urine samples. An average recovery of 103.2% for the UA added to analytical samples was obtained (Table 2).

Table 2. Results of recovery analysis for UA spiked in urine samples using the GNPs/CPE

<table>
<thead>
<tr>
<th>No.</th>
<th>Spiked (µmol L(^{-1}))</th>
<th>Found(^{a}) (µmol L(^{-1}))</th>
<th>RSD</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>4.92 ± 0.07</td>
<td>1.42</td>
<td>98.40</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>10.79 ± 0.04</td>
<td>0.37</td>
<td>107.90</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>33.99 ± 0.60</td>
<td>1.76</td>
<td>99.97</td>
</tr>
<tr>
<td>5</td>
<td>74</td>
<td>78.19 ± 1.50</td>
<td>1.92</td>
<td>105.66</td>
</tr>
</tbody>
</table>

\(^{a}\)\(x = \bar{x} \pm s_x\) for \(n=3\) (\(s_x\) means standard deviation)

4. CONCLUSION

This study demonstrates a simple, rapid and sensitive voltammetric method for determination of UA in human urine sample. Characterization of the electrode demonstrated an obvious increase in surface area and porosity after presence of gold nanoparticles as an efficient modifier of carbon paste electrode. Electrochemical parameters such as pH and scan rate were optimized and in the achieved optimum conditions, diffusion coefficient of UA (\(D_{UA}\)) was calculated. The modified carbon paste electrode by gold nanoparticles (GNPs/CPE) at this study showed a low detection limit and great concentration linear range. Results achieved by the proposed electrode, were shown the comparable sensitivity, linear range and DL of the GNPs/CPE for the determination of UA.

Acknowledgement

We gratefully acknowledge the post-graduate office of Guilan University for supporting this work.

REFERENCES