Original Research Article

Square wave voltammetric quantification of folic acid, uric acid and ascorbic acid in biological matrix

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A B S T R A C T

Nowadays, modified electrodes with metal nanoparticles have appeared as an alternative for the electroanalysis of various compounds. In this study, gold nanoparticles (GNPs) were chosen as interesting metal nanoparticles for modifying carbon paste electrode (CPE). GNPs and the gold nanoparticles-modified carbon paste electrode (GNPs/CPE) were characterized by UV–Vis spectroscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). GNPs/CPE as a simple and sensitive electrode was used to study three important biological molecules: folic acid (FA), uric acid (UA) and ascorbic acid (AA). Square wave voltammetry (SWV) was used as an accurate technique for quantitative measurements. A good linear relation was observed between anodic peak current (i\text{pa}) and FA (5.2 × 10⁻⁶ – 2.5 × 10⁻³ M), UA (1.2 × 10⁻⁶ – 2.1 × 10⁻³ M) and AA (1.2 × 10⁻⁶ – 2.5 × 10⁻³ M) concentrations in simultaneous determination of these molecules.

1. Introduction

Nanomaterials have shown novel and unique properties, which are dependent on their nano scale dimension, size and shape [1–4]. These properties change nanomaterials from their bulk and make them interesting in a variety of scientific fields, especially in electrochemical studies [5–7]. Nanomaterials provide at least three important functions for electroanalysis, i.e., the roughening of the conductive sensing interface, catalytic features and conductivity properties [8,9]. In this study, gold nanoparticles (GNPs) were chosen as the metal nanoparticles for modifying the carbon paste electrode (CPE). Due to the unique optical, electronic, and molecular-recognition properties of GNPs, they are the subject of substantial researches, applied in a wide variety of areas, including electron microscopy, electronics, nanotechnology, and materials science. The size and shape of colloidal GNPs have a great effect on their properties and applications. Electrochemical studies have revealed some properties of GNPs like improving the electrode conductivity and enhancing surface area, facilitating the electron transfer and improving detection limit, which make it a promising modifier candidate [10,11]. The methods such as spectroscopy and chromatography are used for analytical experiments, but due to their complexity and high cost, electrochemical methods using modified electrodes with nanoparticles, nanofibers, carbon nanotubes and other nanomaterials are used in several new works [12,13].

CPEs are a good choice for electrochemical analysis, because of their wide potential window, lower residual currents than glassy carbon electrodes and easy preparation [14]. Gold nanoparticles-modified carbon paste electrode (GNPs/CPE) is used in many studies to determine a variety of analytes including vitamins, drugs, dyes, ions, heavy metals and so on in biological or non-biological matrixes [15–17]. Vitamins are organic molecules; that their adequate amounts are necessary for normal activity and regular metabolism of the body and its functions [18].

Folic acid (FA), (2S)-2-{(2-amino-4-hydroxypteridin-6-yl)methyl]amino}pheny]formamido]pentanedioic acid, also known as folate (the form naturally occurring in the body) or vitamin B9, exists naturally in a wide variety of foods such as broccoli, cabbage, fruits and nuts. FA is essential to numerous bodily functions, and its functions [18].
high-performance liquid chromatography [24] and electrochemical techniques have been used [25,26].

Uric acid (UA), 7,9-Dihydro-1H-purine-2,6,8(3H)-trione, is a water soluble molecule. UA, an end product of purine metabolism in the body, is defecated by urine. Aberrant levels of UA are symptoms of some diseases like hyperpiesia, gout and Lesch-Nyhan syndrome [27]. Numerous analytical methods including colorimetry (optical detection) [28], enzymatic [29] and electrochemical techniques have been applied to determination of UA [30,31].

Ascorbic acid (AA), (5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-furan-2(5H)-one, also known as l-ascorbic acid or vitamin C, is a water soluble vitamin, which is widely present widely in biological systems. AA is a natural antioxidant and is an essential substance for prevention or treatment of cold, mental diseases and infertility [32]. Because of the importance of AA, it is used in supplemental dietary intakes. Liquid chromatography [33], enzymatic techniques [34] and electrochemical techniques are most common methods to determination of AA [35,36].

Among all of the mentioned techniques in detection and determination of these molecules, electrochemical methods have attracted more interest because of their simplicity, low cost and high accuracy and precision.

FA, UA and AA usually co-exist in human biological fluids such as blood and urine. Because of the significant effect of these molecules on healthy activity of the body, a simple, sensitive and simultaneous determination method of FA, UA and AA is needed to be developed in all fields of biomedical chemistry, diagnostic researches and analytical chemistry, especially in electrochemical studies. Since these molecules exhibit responses at close potentials in voltammetric studies, some materials such as carbon based compounds and noble metals have been used to determine FA, UA and AA in presence of each other [37]. But up to now, no other work in the literature has been reported on the application of GNP/CPE for electrochemical studies of UA, AA and FA simultaneously.

In this work, we propose a GNP/CPE. Electrochemical behaviors of FA, UA and AA were studied individually and simultaneously, and their electrochemical parameters were calculated. At optimum conditions, which mean pH of 6 and scan rate of 0.1 V/s, the peak separations of FA–UA and UA–AA were 0.372 and 0.252 V, respectively, which are large enough for simultaneous determination of FA, UA and AA. The analytical applicability of the proposed modified electrode was evaluated by standard addition method for determination of these molecules in human urine without any further treatment.
2. Experimental

2.1. Apparatus and reagents

A three-electrode system including an unmodified and modified carbon paste electrode as working electrode, an Ag/AgCl electrode as reference electrode and a rod of Pt as auxiliary electrode was applied to obtain the electrochemical data. Electrochemical measurements were conducted using the µAutolab PGSTAT 30 electrochemical analyzer (Ecochemie BV, Utrecht, the Netherlands) connected to a computer with general purpose electrochemical system software package (NOVA) and the PalmSens LITE (version 1.8.0.0) for amperometric studies. HAuCl₄·3H₂O and tri-sodium citrate dihydrate were purchased from Merck (Darmstadt, Germany). Other reagents and chemicals were of analytical grade and used without further purification. All solutions were prepared with distilled water.

2.2. GNP synthesis

According to the published papers [38–40], all glasswares were cleaned and then rinsed several times with distilled water. 0.5 mL of 1% (m/v) sodium citrate solution was added to 50 mL of 0.01% (m/v) boiling HAuCl₄ solution. The mixture was boiled for 15 min and stirred for 15 min to produce colloidal GNP s after removing the heating source. Finally the GNP s solution was transferred to a dark–colored bottle and was stored in a refrigerator.

![Fig. 3. Cyclic voltammograms of CPE (a) and GNP/CPE (b) of (A) FA, (B) UA and (C) AA at a concentration of 1×10⁻⁴ M, respectively; (D) Square wave voltammograms of CPE (a) and GNP/CPE (b) of a mixture of FA, UA and AA at a concentration of 1×10⁻⁴ M for each analyte.](image)

![Fig. 4. Ep-pH for FA, UA and AA at GNP/CPE.](image)
2.3. Electrode construction

We made the working electrode by Ertalon and used a copper wire (2 mm in diameter) for its electrical contact. The unmodified CPE was prepared by mixing graphite powder with a suitable amount of mineral oil and thorough hand mixing (about 60:30, m/m). The modified electrode was prepared by mixing above composite with GNPs colloidal solution. According to Fig. 1, after adding 40 µL of colloidal GNPs solution (about 10%, m/m), anodic peak current (i_{pa}) is nearly constant, so 40 µL was chosen as optimum amount of GNPs. Finally a portion of the composite mixture (carbon paste and modified carbon paste) was packed into a fitted ertalonic tube in the end of electrode (2 mm in interior diameter).

2.4. Preparation of real sample

Urine sample was collected from a volunteer. To determine the FA, UA and AA contents after homogenization, the urine was filtered by a filter paper. A 10 µL of filtered urine sample was transferred into a 10 mL volumetric flask containing phosphate buffer solution (PBS) (pH 6) and made up to the volume.

3. Results and discussion

3.1. Characterization of GNPs and modified electrode

As can be seen in Fig. 2A, the maximum absorbance of colloidal GNPs solution in UV–Vis increased at around 530 nm, which means the approximate size of synthesized GNPs was lower than 20 nm. In the visible range, the optical spectrum of spherical gold particles with an average size of 3.4 nm or higher was generally dominated by the plasmon band, and a peak at around 520 nm was caused by the excitation of surface plasmons [41,42]. To confirm the formation of GNPs and its diameter, typical transmission electron microscopy (TEM) image of synthesized colloidal GNPs solution is shown in Fig. 2B. It can be seen that GNPs with a mean diameter around 10 nm were synthesized.

To study the surface morphology and the effect of GNPs as a modifier on the CPE, scanning electron microscopy (SEM) images before and after adding GNPs are shown in Figs. 2 C and D, respectively. These images illustrate with the optimum amount of GNPs solution, the surface area and the porosity of CPE were increased extensively, which will improve the performance of modified electrode and facilitate the electron transferring between solution and electrode surface.

3.2. Electrochemical behavior of modified electrode

The electrochemical behavior of FA, UA and AA on GNPs/CPE was investigated by cyclic voltammetric (CV) and square wave voltammetric (SWV) techniques. The CV profiles for oxidation of FA, UA and AA, separately and simultaneously are shown in Figs. 3A, B, C. CVs exhibit anodic oxidation peaks at about 0.620 V, 0.405 V and 0.126 V, respectively for FA, UA and AA. Fig. 3D shows the SW-voltammograms of a solution containing a mixture of 1×10^{-4} M of FA, UA and AA. Presence of a good peak separation between three analytes demonstrates the possibility of simultaneous determination of FA, UA and AA by GNPs/CPE.

According to the Randles-Sevcik equation [43]:

\[ i_{pa} = (2.69 \times 10^{5}) n^{1/2} A D^{1/2} v^{1/2} C, \]

where \( i_{pa} \) is the anodic peak current (A), \( n \) is the number of transferred electrons, \( A \) is the electroactive surface area (cm²), \( v \) is the scan rate (V/s) and \( C \) is the concentration of \( K_{3}[Fe(CN)_{6}] \) as the probe (mol/cm³), the electroactive surface area of both CPE and GNPs/CPE was calculated. The area of CPE (0.0634 cm²) is less than GNPs/CPE area (0.0979 cm²) which showed that GNPs as an effective modifier provide a large surface and facilitate the electron transfer between the electrode and the solution [38].
Scheme 1. Proposed oxidation mechanisms of FA, UA and AA on the surface of GNP/CPE.

Fig. 6. (A) SW-voltammograms of FA in a concentration range from $1 \times 10^{-6}$ (a) to $1 \times 10^{-4}$ (e) M in presence of constant concentration of UA and AA at $2 \times 10^{-7}$ M in 0.1 M PBS (pH=6); (B) SW-voltammograms of UA in a concentration range from $1 \times 10^{-6}$ (a) to $1 \times 10^{-4}$ (e) M in presence of constant concentration of FA and AA at $2 \times 10^{-7}$ M in 0.1 M PBS (pH=6); (C) SW-voltammograms of AA in a concentration range from $1 \times 10^{-6}$ (a) to $1 \times 10^{-4}$ (e) M in presence of constant concentration of FA and UA at $2 \times 10^{-7}$ M in 0.1 M PBS (pH=6); (D) Simultaneous determination SW-voltammograms of FA, UA and AA over the concentration range from $1.2 \times 10^{-6}$ (a) to $2.5 \times 10^{-5}$ (g) M in 0.1 M PBS (pH=6).
at about pH 6.5 and then decreases. In Fig. S2B, maximum current of graphs. According to Fig. S2A, the current of FA achieves a maximum with increased solution pH, the peak current increases in all three examples. To do so, the CV for $3.8\times10^{-4}$ M FA, UA, and AA were examined in 0.1 M phosphate buffer solutions over a pH range of 5.5–7.5, 4–7.5 and 5–7.5, respectively. The $i_{pa}$ and the $E_{pa}$ were found to be markedly dependent on the pH. Figs. S1A, B and C illustrate the effect of pH on FA, UA, and AA, respectively. As can be seen in Fig. S1, with increased solution pH, the peak current increases in all three graphs. According to Fig. S2A, the current of FA achieves a maximum at about pH 6.5 and then decreases. In Fig. S2B, maximum current of UA appears at about pH 5.5 and in Fig. S2C, the maximum current of AA achieves at about pH 6. Thus, the optimum pH of FA, UA and AA were chosen as 6.5, 5.5 and 6, respectively.

To find the electrochemical behavior and the number of participating electrons and protons in oxidation mechanisms of these molecules, the equations of $E_{pa}=$pH +1.156, $r^2=0.997$, $E_{pa}=-0.0645$ pH +0.7802, $r^2=0.9913$ and $E_{pa}=-0.0531$ pH +0.5676, $r^2=0.9945$, respectively, have a nearly Nernst equation slopes, which means the presence of equal electrons and protons in the oxidation processes of FA, UA and AA.

### 3.3.2. Effect of potential scan rate

To find information about electrochemical reactions and oxidation mechanisms of FA, UA and AA at the GNPs/CPE, the effects of scan rate on the $i_{pa}$ and the $E_{pa}$ of these molecules were examined in 0.1 M PBS containing $3.8\times10^{-4}$ M of analytes at the pH of 6.5, 5.5 and 6, respectively. The voltammograms in Figs. 5A, B and C represent the oxidation processes of FA, UA and AA.

As shown in Fig. 5A (inset a), over the scan rate ranges of 0.02–0.26 V/s, a linear equation between $i_{pa}$ and $v$ ($r^2=0.9921$) and $E_{pa}$ and $v$ ($r^2=0.9950$), which demonstrates an adsorption-controlled process for the electrochemical reaction of folic acid at the GNPs/CPE. Accumulation time ($t_{acc}$) and accumulation potential ($E_{acc}$) of FA were calculated to be 60 s and 0.2 V, respectively [38].

The insets a of Figs. 5B and C both indicate a linear relation between $i_{pa}$ (A) and $v^{1/2}$ ($r^2=0.9936$) over the scan rate ranges of 0.02–1 V/s for $3.8\times10^{-4}$ M UA and AA, respectively. The equations are $i_{pa}=5\times10^{-5}v^{1/2} - 5\times10^{-7}$ ($r^2=0.9986$) and $i_{pa}=1\times10^{-5}v^{1/2} +5\times10^{-8}$ ($r^2=0.9936$) for UA and AA, accordingly. It means that the reactions

| Parameter | Folic acid | Simultaneous determination | Uric acid | Simultaneous determination | Ascorbic acid | Simultaneous determination |
|-----------|------------|-----------------------------|-----------|-----------------------------|---------------|-----------------------------|
| In presence of FA and AA | $I_{pa}$ ($\mu A$) | 0.741 | 0.733 | 0.356 | 0.361 | 0.121 | 0.109 |
| In presence of FA and AA | $L_{DW}$ | $1\times10^{-6}$–$1\times10^{-4}$ | $5.2\times10^{-6}$–$2.5\times10^{-5}$ | $1\times10^{-5}$–$1\times10^{-4}$ | $2.1\times10^{-5}$–$2.5\times10^{-5}$ | $1\times10^{-7}$–$1\times10^{-4}$ | $2.5\times10^{-5}$–$2.5\times10^{-5}$ |
| Sensitivity $a$ | | 0.0219 | 0.2793 | 0.037 | 0.3959 | 0.0106 | 0.3121 |
| Intercept $b$ | | 2.7×$10^{-8}$ | 3.2×$10^{-7}$ | 3.9×$10^{-8}$ | 1.2×$10^{-7}$ | 7.0×$10^{-8}$ | 3.5×$10^{-7}$ |
| $r^2$ | | 0.9907 | 0.9902 | 0.9910 | 0.9917 | 0.9925 | 0.9906 |

* In presence means as a constant concentration of two analytes and increasing concentration of third analyte, and simultaneous means increasing concentration of all three analytes.

* Average anodic peak potentials (V) extracted from SW-voltammograms.

* Linear dynamic range (M).

* Sensitivity is the same slope of linear regression equation (A/M).

* Detection limit (M) (n=3).

* Intercept of calibration curves (A).

* Correlation coefficient.
of UA and AA are diffusion-controlled through the modified electrode.

According to the slopes of insets b of Figs. 5A, B and C and using the Laviron’s equation (Eq. (1)) [44], the number of involved electrons in the oxidation processes of FA, UA and AA was determined.

\[
E = E^0 - \frac{R T}{n F} \log \left( \frac{I}{nF \alpha} \right) + \frac{R T}{n F} \log \left( \frac{I}{nF \alpha} \right)
\]

(1)

Where \(E\) and \(E^0\) are redox and formal potentials (V), respectively. \(R\) is the gas constant (J/K mol), \(T\) is the system temperature (K), \(n\) is the charge transfer coefficient, \(I\) is the number of involving electrons, \(F\) is the Faraday number (C/mol), \(k_i\) is electron rate coefficient, and \(v\) is the potential scan rate (V/s). The equations of the plots are \(E_{pa}=0.0862 \log u+0.812 \quad (r^2=0.9945)\), \(E_{pa}=0.0639 \log v+0.5113 \quad (r^2=0.9961)\) and \(E_{pa}=0.0583 \log u+0.2844 \quad (r^2=0.9806)\) for FA, UA and AA, respectively. The \(u\) and \(v\) values of these molecules were calculated as X.0, 1.06 and 1.10, respectively. If we assume \(u=0.5\), which is used for irreversible redox reactions, the number of electrons transferred in oxidation processes of FA, UA and AA was found to be 2.60, 2.12 and 2.20, respectively, that assuming \(n=2\). Consequently, according to the obtained results in Section 3.3.1, all three oxidation mechanisms involve two electrons-two protons (Scheme 1) [45,46].

As can be seen in Scheme 1, the oxidation process of FA is performed by losing the \(H^+\) of C(9) and the \(H^+\) of N(10), and folic acid turn into the stable form called dehydroascorbic acid [47-49].

3.4. Repeatability of GNPs/CPE

To demonstrate the precision of applied method, the experiments were repeatedly performed in an identical solution containing \(2 \times 10^{-4}\) M of analytes in 0.1 M PBS (optimum pH) with the same GNPs/CPE. It was found that for 10 repetitive measurements, the relative standard deviation (RSD) was 5%, which indicates a reproducible maintainability for the GNPs/CPE.

3.5. Simultaneous determination of FA, UA and AA

SWV technique was used for simultaneous determination of FA, UA and AA at pH 6 in 0.1 M PBS and potential scan rate of 0.1 V/s. In order to illustrate the possibility of the proposed method for simultaneous determination of these molecules at different concentrations, four SW-voltammograms were recorded separately, as can be seen in Fig. 6. First, the SWV curves were recorded by increasing the concentration of one molecule from \(1 \times 10^{-6}\) M to \(1 \times 10^{-4}\) M and holding the concentration of other two compounds constant at \(2 \times 10^{-7}\) M (Figs. 6A–C). This procedure was done for all three molecules and their analytical parameters are listed in Table 1.

Fig. 6D shows the simultaneous increasing concentration of FA, UA and AA in 0.1 M PBS (pH 6) from \(1 \times 10^{-6}\) to \(2.5 \times 10^{-5}\) M. The obtained SW-voltammograms represented a good linearity in the concentration ranges (Fig. S3).

3.6. Interference study of modified electrode

It is an important factor for a modified electrode to discriminate the similar interfering species to the target analytes. The selectivity of GNPs/CPE was investigated by determining different foreign species in a \(1 \times 10^{-6}\) M solution of FA, UA and AA in human urine. As can be seen in Fig. 7, some common substances were added to a stirring buffered solution (pH 6) at 0.8 V. When 30 µL of \(1 \times 10^{-2}\) M of each FA, UA and AA solution was added to PBS, the current increased significantly, while interferences at the same condition showed no important signals. Therefore, the GNPs/CPE exhibits good selectivity for determination of FA, UA and AA.

3.7. Application of GNPs/CPE

To represent the applicability of GNPs/CPE for analysis of FA, UA and AA in real samples, human urine as a biological fluid was selected. First, the SW-voltammograms were recorded without addition of the analytes to prepared urine sample (explained in Section 2.4). Then, the real sample was spiked by known concentration of FA, UA and AA. The results are given in Table 2, which confirmed that the GNPs/CPE illustrated a good efficiency for the determination of FA, UA and AA simultaneously.

Table 3 shows the comparison of some parameters of different sensors with GNPs/CPE. As can be seen, in comparison to the mentioned methods [37,46,50-52], the sensitivity, detection limit and pH obtained by GNPs/CPE for FA, UA and AA determination are comparable to the electrochemical techniques using other modified electrodes.

4. Conclusions

This study demonstrates a simple and sensitive voltammetric technique for determining FA, UA and AA in biological medium. GNPs/CPE showed an obvious increase in surface area and porosity after adding gold nanoparticles to carbon paste. Electrochemical parameters such as pH were optimized and oxidation mechanisms of FA, UA and AA were proposed by finding the number of involved electrons and protons. The linear dynamic ranges of GNPs/
CPE in determination of three molecules, separately and simultaneously, were calculated by square wave voltammetry. Amperometric curve was applied to study the interferences and square wave voltammetry was used to illustrate the applicability of the proposed method to analysis of human urine as a biological fluid.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jphspa.2017.01.002.

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