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Sensitive and Selective Determination of Uric Acid Using Polyaniline and Iron Composite Film Modified Electrode

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We demonstrated a simple and low-cost composite composed of polyaniline and iron composite (PANI-Fe) for the determination uric acid (UA). The successful composite formation was confirmed through scanning electron microscopy, electrochemical impedance spectroscopy and electrochemical methods. The PANI-Fe composite was used to modify the surface of glassy carbon electrode (GCE) and the resulting modified electrode (PANI-Fe/GC) displayed good electrocatalytic activity to the oxidation of UA. The kinetics of the electrocatalysis and effect of scan rate were investigated. The amperometric sensor was developed based on PANI-Fe film modified electrode which delivered quick and sensitive responses to UA. The linear range is 0.05–3860 µM, detection limit is 21.5 nM, and sensitivity is 0.2981 $\mu A \mu M^{-1}$ cm^{-2}. The electrode is highly selective for UA detection. Besides, the electrode has good repeatability and reproducibility. The real-time applicability is verified in human urine samples which displayed recoveries in acceptable range.

Keywords: Conducting polymers, metals, electrocatalysis, electrochemical sensors, metabolite, Uric acid

1. INTRODUCTION

Uric acid (UA) is one of the primary end products of purine metabolism in human body [1, 2]. Generally, the average amount of UA present in urine of a healthy human is in the millimolar range (1.4–4.4 mM) and that in blood is in the micromolar (120−450 µM) range [3]. Abnormal concentrations of UA in biological fluids (blood and urine) are symptoms of several important diseases including hyperuricemia, gout, Lesch-Nyan disease etc., [4, 5] A high level of UA in the blood led to hyperuricemia and cardiovascular diseases [6]. Therefore, it is vital to develop simple and robust sensing methods for the routine analysis of UA in patients suffering from altered purine metabolism related disorders. Usually, UA, dopamine (DA) and ascorbic acid (AA) are coexisted in the extracellular body fluids, but they are biomarkers for different diseases [7, 8]. The concentration of DA (in micromolar level) is comparatively lower than that of AA (in millimolar level) in body fluids and hence the determination of UA usually encountering interference from AA rather than DA [9, 10]. Although traditional analytical methods can be used for the determination of UA, electrochemical methods are most preferable due to their simplicity, low-cost, easy-handling, rapid response time, portability and low power consuming [11]. All these three metabolites are electrochemically active and hence they are suitable for electroanalytical determinations. At conventional electrodes, all the three biomarkers are oxidized at similar oxidation potential and the voltammetric peaks usually overlap and hence difficult to determine them selectively [12]. Besides, the conventional electrodes are poor in selectivity and have low reproducibility. Therefore, rationally designed modified electrodes were developed in the past years in order to eliminate AA interference. The detection approaches are either selective detection of UA or simultaneous determination of DA, UA and AA at different voltammetric channels. So far, several modified electrodes were developed for UA determination which are includes, electrochemically pretreated carbon paste electrodes [13], well-aligned carbon nanotubes [14], preanodized clay [1], electrochemically anodized diamond film [3], methylene blue in a methyltrimethoxysilane sol-gel ceramic film [15], ordered mesoporous carbon functionalized with ferrocenecarboxylic acid-modified electrode [16], β-cyclodextrin modified copolymer of sulfanilic acid and N-acetylaniline [17], electrochemically treated pencil graphite electrodes [18], Electrochemically polymerized luminol film [19], nitrogen doped graphene [20], graphene/size-selected Pt nanocomposites [21] etc., Herein, we have prepared rod shaped iron particles decorated polyaniline composite (PANI/Fe) for the sensitive determination of UA. The composite is prepared through a straightforward solution-assisted method using cheaper precursors. The composite is suitable electrode material for the determination of UA present in human urine and serum samples.

2. EXPERIMENTAL

2.1 Chemicals and Apparatus

Aniline, and Iron (III) chloride ($FeCl_3$) were purchased from Merck. All the other reagents were purchased from Sigma-Aldrich. Double distilled water was used for all the experiments. 0.1 M phosphate buffer (pH 7.0) was prepared from sodium dihydrogen phosphate and disodium hydrogen phosphate and used as supporting electrolyte.

Electrochemical studies were performed using CHI 1205a electrochemical work station instrument (Bioanalytical Systems, Inc., USA) in a three electrode cell using modified glassy carbon electrode (GCE) as a working electrode (area 0.071 cm^2), Ag|AgCl (KCl) as reference electrode and Pt wire as counter electrode. The scanning electron microscopic (SEM) images were acquired using Hitachi S-3000 H scanning electron microscope and electrochemical impedance spectroscopy (EIS) analysis were performed using EIM6ex Zahner (Kronach, Germany).

2.2 Preparation of PANI/AC/GCE

5 mM CTAB solution was prepared in 0.5 M H_2SO_4. 40 mM aniline solution was added to the CTAB solution and the mixture was stirred using magnetic stirrer for 30 min. 50 mL of a solution 0.05M $FeCl_3$ was added drop-wise to the previous solution, continued stirring for 30 min and the temperature was maintained below 0°C. Afterwards, a pre-cooled solution of 50 mM PDS was added in dropwise to aniline solution with stirring over a period of 30 min. A dark green precipitate was formed which was filtered and washed several times with water and acetone, respectively. The purified PANI/Fe composite was dried and redispersed in ethanol (1mg mL^{-1}). Next, the surface of the GCE was polished with alumina slurry using a Buehler polishing kit. The polished GCE surface was then washed with water and dried in air-oven. 5 µl dispersion of PANI/Fe was dropped on the cleaned GCE surface and dried.

3. RESULTS AND DISCUSSIONS

3.1 Characterization of PANI-Fe/GCE

The SEM of PANI-Fe portrayed with the presence of fibers of PANI and numerous Fe metal particles (Fig. 1A and 1B). Besides, the composite featured with several cavities and catalytic sites. The morphology indicating that the composite can have high surface area which can be highly beneficial for electrochemical sensing applications.

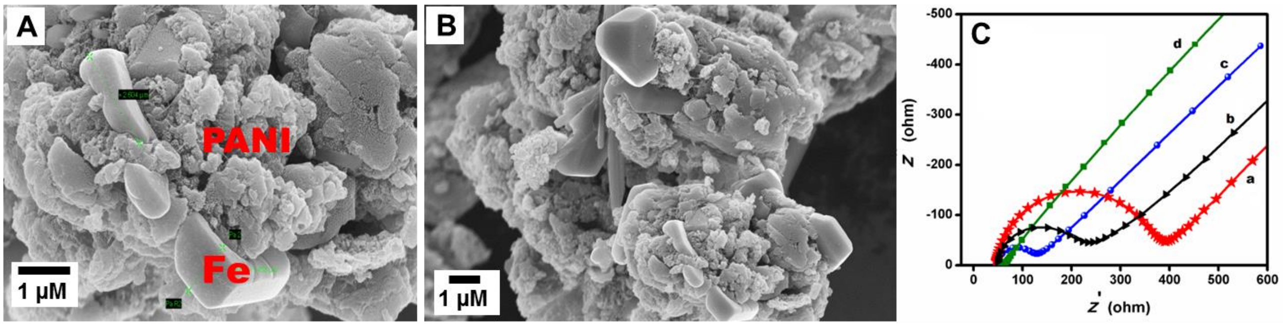

Figure 1. (A, B) FESEM images of PANI-Fe composite. (C) EIS curves of bare GCE (a), Fe/GCE (b), PANI/GCE (c) and PANI-Fe/GCE (d).

Fig. 1C displays the EIS obtained at bare GCE (a), PANI/GCE (b) and Fe/GCE (c) and PANI-Fe/GCE (d) in 0.1 M KCl containing 5 mM $Fe(CN)_6^{3-/4-}$. Randles equivalent circuit model was used to fit the experimental data and the EIS measurements were represented as Nyquist plots. The diameters of the semicircle portion of the curves were in the order of bare GCE > PANI/GCE > Fe/GCE > PANI-Fe/GCE. The R_{ct} values of bare GCE, Fe/GCE, PANI/GCE, PANI-Fe/GCE are 361 Ω, 197 Ω, 108 Ω and 22 Ω respectively. The R_{ct} value obtained at PANI-Fe/GCE is 16, 9 and 5 times smaller than the bare GCE, Fe/GCE and PANI/GCE, respectively. Thus, PANI-Fe/GCE has the lowest electrode resistance over control electrodes.

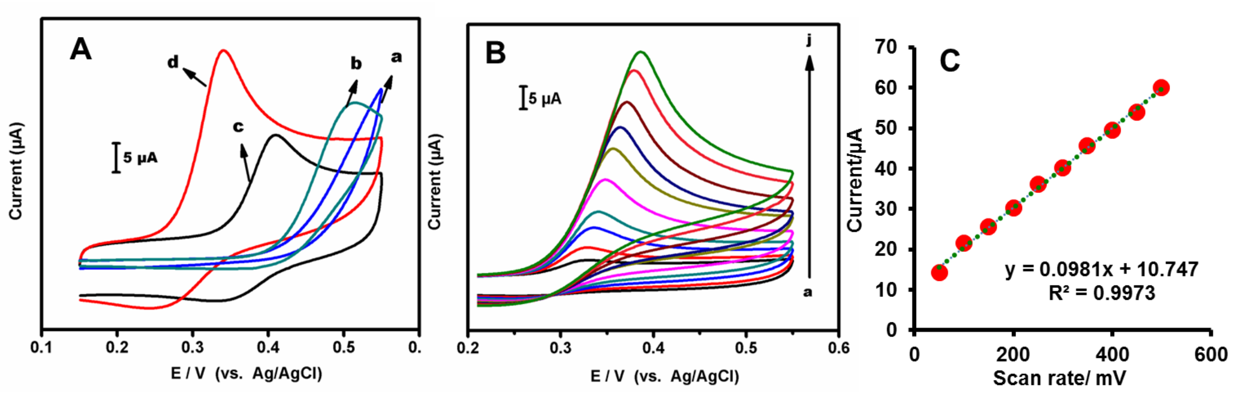

Figure 2. (A) CVs obtained at bare GCE (a), PANI/GCE (b) and Fe/GCE (c) and PANI-Fe/GCE in phosphate buffer (pH 7.0) containing 3 µM UA. Scan rate = 50 mV s^{-1}. (B) Effect of the scan rate: Cyclic voltammograms responses of PANI-Fe/GCE towards 3 µM UA at different applied scan rates from 50 to 500 mV s^{-1} (a=50, b=100, c=150, d=200, e=250, f=300, g=350, h=400, i=450 and j=500 mV s^{-1}. (C) Plot between scan rate (mV) and UA oxidation peak current (µA).

3.2 Electrocatalysis of UA

Fig. 2A shows the cyclic voltammograms (CVs) obtained at bare GCE (a), PANI/GCE (b), Fe/GCE (c) and PANI-Fe/GCE (d) in phosphate buffer (pH 7.0) containing 3 µM UA. The scan rate is 50 mV s^{-1}. The unmodified GCE displays poor electrocatalytic ability to oxidize UA. Compared with unmodified electrode, PANI/GCE and Fe/GCE have shown better electrocatalysis for UA; however the oxidation peak observed at higher overpotential. On the other hand, PANI-Fe/GCE has shown excellent electrocatalytic ability to oxidize UA at very low overpotential and sharp peak with enhanced peak current. The oxidation peak of UA is observed at PANI-Fe/GCE is 0.32 V which is about 80 mV and 180 mV lower potential than Fe/GCE and PANI/GCE, respectively. The CV results revealed that the PANI-Fe composite have synergic catalytic ability over control electrodes. Besides, the composite possess large surface area, high conductivity and abundant catalytic sites and these characteristics of the composite favored the obtained high electrocatalytic ability of the composite. The influence of different scan rates towards the electrocatalysis reaction of UA at PANI-Fe/GCE is investigated (Fig. 2B). The oxidation peak current of UA was linearly increased as the scan rate increases. The plot between oxidation peak current and scan rates displays good linearity which indicating that the oxidation process is surface confined diffusion process.

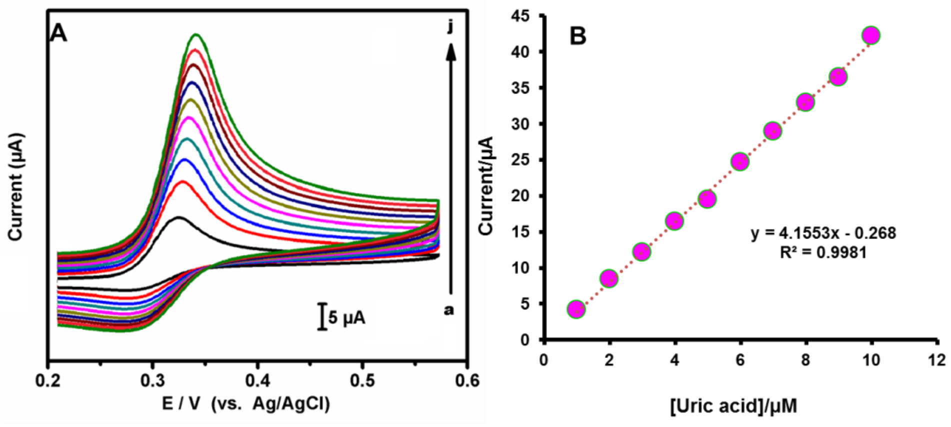

Figure 3. (A) CVs obtained at PANI–Fe/GCE in phosphate buffer (pH 7.0) containing different concentrations of UA (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 μM). (B) Plot between [UA] (μM) vs. response current (μA).

3.3 Determination of UA: voltammetry and amperometry

Table 1. Comparison of analytical parameters for the determination of UA at PANI–Fe composite film modified electrode with reported works

Electrode	Linear range/μM	Detection limit/μM	Ref.
PtAu hybrid film	20–336	0.015	[9]
f-MWCNTs/poly(neutral red) composite	0.05–30	0.015	[10]
MWCNTs	15.9-333.3	1.65	[19]
Tryptophan/gold nanoparticles/polyimidazole	6.0–486	0.5	[20]
Tryptophan/Graphene	10–1000	1.24	[21]
Pt/reduced graphene oxide	10.0-130.0	0.25	[22]
Recessed Au nanoelectrode	20–170	6.4	[23]
Au-Pd/ nanoporous stainless steel	100.0– 1200	15	[24]
hierarchical nanoporous PtTi	100-1000	5.3	[25]
PANI–Fe	0.05–3860	21.5	This work

Fig. 3A displays the CV curves obtained at PANI–Fe/GCE in phosphate buffer containing various concentrations of UA. As represented in figure, the anodic peak current corresponding to the oxidation of UA is linearly increases as the concentrations of UA increases. The plot between concentrations of UA and their corresponding response currents exhibited good linearity with slope 4.1553 μA/μM. In order to develop sensitive UA determination platform, we have adopted amperometric method. Fig. 4A displays the amperometric i-t curves obtained at PANI–Fe/GCE for sequential additions of UA into phosphate buffer (pH 7). The applied potential was + 0.32 V and electrode rotation speed was 1500 RPM. For each addition, a sharp rise in current is observed and the amperometric response current reached 95% steady-state current within 5s of UA injection. Thus, the PANI–Fe composite film showed fast and sensitive responses to each addition of UA. The concentration dependent linear plot exhibited linearity and the slope was 0.0626 µA µM^{-1} (Fig. 4B). The response was linear in wide concentration range of 0.05–3860 µM with sensitivity of 0.2981 µAµM^{-1} cm^{-2}. The limit of detection (LOD) was calculated to be 21.5 nM. The important parameters of sensor, such as LOD and linear range were comparable with previously modified electrodes along with the advantage of none-expensive electrode fabrication and rapid analysis [9, 10, 19].

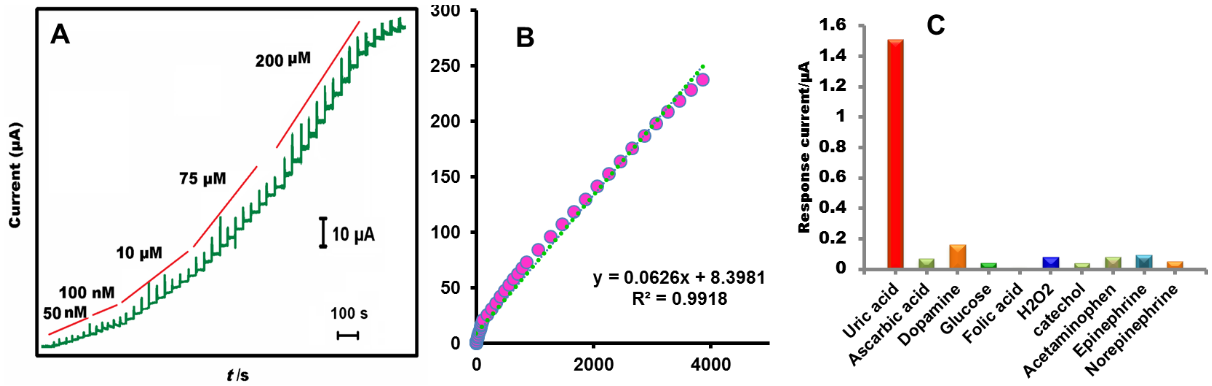

Figure 4. (A) Amperometric response obtained at PANI–Fe composite film modified electrode towards each sequential additions of UA into phosphate buffer (pH 7). The rotation speed = 1500 RPM and electrode potential = + 0.32V. (B) [UA]/µM vs. response current (µA). (C) Selectivity study: plot of response current versus UA and interferents.

3.4 Selectivity and real-time application

Selectivity of the PANI–Fe composite film modified electrode to detect UA in presence of possible interferents has been investigated. The electrocatalytic response of the electrode towards 100 µM of UA and interferents were studied and the results were given as plot in fig. 4B. As given in the plot, the PANI–Fe composite film modified electrode delivered excellent amperometric response to UA, but negligible responses to all the other analytes added. These experimental results revealed that the electrode has good selectivity to detect UA in presence of possible interferents. Therefore, the described modified electrode is a suitable candidate for the determination of UA in real samples.

Next, we have investigated the practical applicability of the PANI–Fe composite film modified electrode towards determination of UA present in spiked human urine and serum samples. The samples were collected from a healthy man. 1 mL of human urine sample was diluted with 50 mL phosphate buffer (pH 7) and known concentrations of UA were spiked into the solution. Similarly, spiked human serum sample is also prepared. Amperometric experiments were carried out using this solution and following the experimental conditions optimized for lab samples.

Table 2. Determination of UA in real samples using PANI–Fe composite

Real samples	Added/nM	Found/nM	Recovery/%	*RSD/%
Human serum	200	197.1	98.55	4.06
	500	492.3	98.46	3.79
Urine sample	200	196.5	98.25	3.19
	500	493.9	98.78	3.41

* Related standard deviation (RSD) of 3 independent experiments

For human urine sample, the PANI–Fe composite film modified electrode delivers quick and sensitive amperometric signals. The added, found and recovery values are estimated and presented in Table 2. From the table, we understood that the electrode sensitivity detects UA in real samples with satisfactory range of recoveries. Consequently, the composite is proved to be having good practical utility and it can be used for the real-time UA determination in clinical analysis.

3.5 repeatability, reproducibility and stability

Repeatability of the electrode was evaluated by performing five repeatitive measurements using indivdually prepared PANI–Fe composite film modified electrodes. The sensing ability of these electrodes were tested towards 10 µM UA. The PANI–Fe composite exhibits satisfactory repeatability with RSD of 3.85%. Similarly, reproducibility of the film was evaluated for five independent measurments (towards 10 µM UA) executed using five different modified electrodes. The electrode exhibits acceptable reproducibility with RSD of 3.92% for the determination UA. In order to determine stability of the PANI–Fe composite/GCE, its electrocatalytic response towards 10 µM UA was monitored every day. The electrode was kept stored in phosphate buffer (pH 7) at 4°C when not in use. During two weeks of storage period, 91.75% of its initial response currents towards UA was reappeared which indicates the excellent storage stability of the film.

4. CONCLUSIONS

In summary, a sensitive and selective UA detection platform was demonstrated using PANI–Fe composite. The successful formation of the composite was revealed by SEM. The EIS studies revealed that the composite has high electrical conductivity at solution-electrode interface. The electrochemical studies revealed that the composite has excellent electrocatalytic ability towards oxidation of UA at very low overpotential. The amperometric determination platform has shown sensitive signals at wide linear range of 0.05–3860 µM with high sensitivity of 0.2981 $\mu A \mu M^{-1}$ cm^{-2}. The LOD was found to be as low as 21.5 nM. Besides, the selectivity studies proved that the composite has the ability to detect UA in presence of interferents coexisted medium. The modified electrode has satisfactory stability, repeatability and reproducibility. The real sample studies revealed the promising practical applicability of the described nanocomposite in clinical diagnosis of UA.

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