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Electrochemical determination of uric acid in reptilian excreta and human urine using gold modified pencil graphite electrode.

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Abstract: This article reports the determination of uric acid (UA) present in reptilian excreta (from common house lizard Hemidactylus flaviviridis) and human urine using gold modified pencil graphite electrode (GPGE) prepared through electrodeposition method. The modification of the electrode was confirmed by scanning electron microscopic (SEM) and electrochemical impedance spectroscopic (EIS) studies. Electrochemical detection of UA was carried out by cyclic voltammetric (CV) and differential pulse voltammetric (DPV) techniques in the potential range of –200 to +700 mV versus saturated Ag/ AgCl/ saturated KCL reference electrode. The results demonstrated that the GPGE exhibited high electro-catalytic activity and good analytical performance towards the oxidation of UA. In addition GPGE displayed good reproducibility and good selectivity towards the determination of UA present in the biological samples.
Key words: Cyclic Voltammetry; Differential pulse voltammetry; GPGE; Uric acid.

1. Introduction

Uric acid (2,6,8,-trihydroxy purine) is an important evolutionary significant biomolecule and it plays a vital role in adaptation of animals to completely dry terrestrial habitat. Animals like reptiles and birds are successfully adopted by conserving water by excreting its nitrogenous metabolic waste products in the form of UA [1]. Because UA and its salts are insoluble in water (6 mgL^{-1}), the withdrawal of water from the urine causes UA and its salts to precipitates, due to this bird excrete in semisolid and lizards excrete in fully dry white mass as excretory product (contains nearly 90 % of UA) [2-4]. UA of reptiles and birds differ from that of mammals as both are not of the same origin. In reptiles, UA represents the end product of protein metabolism. On the other hand, UA in mammals (human beings, apes and dalmatian dogs) represents the end product of purine metabolism whereas in ruminants like cow and buffalo, the principal final product of purine metabolism is water soluble allantoin [5-6]. Enzyme uricase present in ruminants animals responsible for the formation of allantoin from uric acid. But in case of human beings, apes and dalmatian dogs uricase enzyme is absent and hence the body fluids contains UA. The evolution has taken place in such a way that the presence of UA in the body fluids of these animals acts as antioxidant, responsible for long life and scavenger of singlet oxygen [7-9]. Normal human beings excrete UA about 400 mg to 700 mg through urine per day. Abnormal levels of UA present in urine or body fluids of human beings leads to the symptoms of several diseases such as Gout, Hyperuricemia and Lesch-Nyan disease [10]. So the determination of UA in human blood or urine is

a powerful indicator in diagnosing diseases and the determination of UA in reptiles and other animals had evolutionary significance. Hence it is important to develop techniques to detect UA in body fluids as well as in urine. The development of a simple and rapid methodology for the determination of UA has attracted attention in recent years. Among various methods, electrochemical method of detection of UA shows a higher selectivity and sensitivity. Electrochemical methods received much more interest because they are more sensitive, less expensive, less time consuming and can potentially be applied for real-time analysis.

In order to resolve Ascorbic acid (AA), Dopamine (DA) and UA, which are oxidized almost at similar potential on conventional electrodes, chemically modified electrodes are frequently being used. Carbon based electrodes are widely used in electroanalytical research because of their chemical inertness, relatively wide potential window, low background current, and suitable for different types of analysis. There are several types of carbon materials available that are suitable for electrochemical applications, among them glassy carbon [11-15], modified carbon nanotubes [16], chitosan-graphene modified glassy carbon [17], poly(sulfonazo III) modified glassy carbon [18], poly(vinyl alcohol) covalently modified glassy carbon [19], pyrolytic graphite [20], zinc-nickel nanoalloy coated composite graphite [21], gold nanoparticles modified glassy carbon [22], exfoliated graphite [23], pre-anodized nafion-coated glassy carbon [24], poly(p-toluene sulfonic acid) modified electrode [25] are some example.

In the present work, the GPGE electrode was used for the determination of UA present in human urine, common house lizard excreta and in buffalo urine. The low cost of GPGE and its easy fabrication could be an alternative method in the electrochemical detection of UA in routine clinical and pharmaceutical analysis.

2. Experimental

2.1. Apparatus and Reagents

All the voltammetric measurements were performed using electrochemical workstation (CHI 660D, USA). All the experiments were conducted in a standard three-electrode assembly incorporating pencil graphite electrode (PGE) or gold modified pencil graphite electrode (GPGE), platinum wire and Ag/AgCl (KClsat) as working, auxiliary and reference electrodes respectively. The surface morphology of the modified electrode was performed by scanning electron microscope (FEI-Quanta 200).

All the chemicals used were of analytical grade. *Uric acid* and chloroauric acid were procured from Loba Chemicals, (Mumbai, India). Potassium dihydrogen phosphates, di-potassium hydrogen phosphate were procured from Merck (Mumbai, India). Potassium hexacyanoferrate(III) was procured from Merck (Darmstadt, Germany) Double distilled water was used for the preparation of all the reagents.

2.2. Preparation of pencil graphite electrode (PGE) and gold modified pencil graphite electrode (GPGE).

A working electrode was prepared with 2B pencil graphite leads (0.5 mm diameter) procured from Camlin Ltd (Mumbai, India).The posterior end of the pencil lead was connected to a copper wire for electrical contact, and then the pencil lead was inserted in the plastic tube and filled with epoxy resin. After 24 hours (time required for the setting of epoxy resin), the anterior end of electrode was scrapped using a sharp knife and polished using alumina slurry down to 0.05 μm on a polishing cloth.

The polished PGE was sonicated and finally washed with double distilled water. The prepared PGE was subjected to electrodeposition using chronoamperometry in 1mM chloroauric acid at the applied potential of -1.0 V versus saturated Ag/AgCl (KCl_{sat}) electrode with a pulse width of 300 seconds. After electrodeposition the electrode was washed with double distilled water and used for the electrochemical experiments.

2.3. Collection of samples

Common house lizard (Hemidactylus flaviviridis) excreta was collected from lizards dwell in chemistry building, Kuvempu University, Buffalo urine collected from farmer house near Kuvempu University, Shankaragatta and Human urine sample was collected from Health Center, Kuvempu University, Shankaragatta, India.

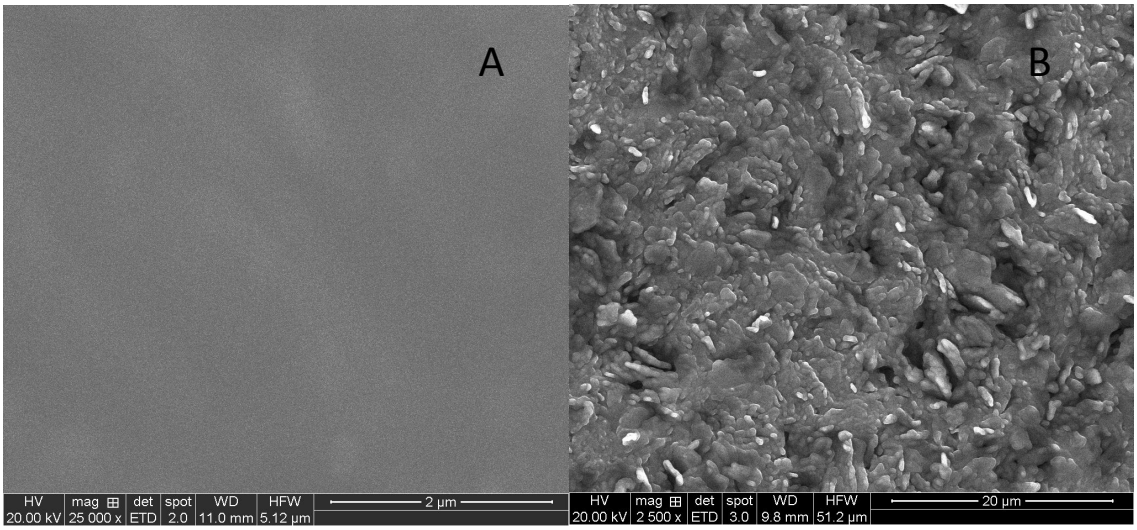


Fig. 1. Scanning electron micrographs of (A) PGE and (B) GPGE

3. Results and Discussions

3.1. Morphological characterization of PGE and GPGE

Scanning electron microscopy can directly represent the information of the electrode surface. Fig.1A and 1B shows the surface morphology of PGE and GPGE. As shown in the Fig.1A the surface of PGE is very smooth and uniform. The SEM pictures of PGE and GPGE showed significant difference in the surface morphology, which suggest that the gold nanoparticles were embedded onto the surface of PGE (**Fig.1B).** The presence of gold nanoparticles on the surface of the electrode increases the effective surface area and hence increased the electron transfer rate. The stability and sensitivity of the modified electrode has been confirmed by the performance of GPGE in the electrochemical investigation of $[Fe(CN)_6]^{3-/4-}$ system.

3.2. Electrochemical behavior of $[Fe(CN)_6]^{3-/4-}$ at PGE and GPGE.

The redox response of $[Fe(CN)_6]^{3-/4-}$ couple is a valuable and convenient probe to characterize the electrochemical performance of the modified electrode. The performance of GPGE can be monitored via the redox behavior of $[Fe(CN)_6]^{3-/4-}$ couple. Fig. 2A represents the cyclic voltammogram obtained at PGE and GPGE in 0.1 M KCl containing 10 mM $K_3[Fe(CN)_6]$. The PGE shows a reversible voltammogram with a peak to peak separation of 96 mV for the $[Fe(CN)_6]^{3-/4-}$ couple (curve b). The redox response of $[Fe(CN)_6]^{3-/4-}$ couple at GPGE was observed with a peak separation of 78 mV. The reduction in peak to peak separation potential at GPGE clearly indicated the better performance of GPGE than that of PGE. From Fig. 2A it can be seen that the magnitude of peak currents for $[Fe(CN)_6]^{3-/4-}$ couple at GPGE was higher than that of the unmodified PGE. The increase in peak currents is attributed to the high specific surface area of GPGE and good catalytic properties of gold nanoparticles.

Electrochemical impedance spectroscopy (EIS) was used to investigate the features of GPGE surface. The electrochemical impedance spectra at PGE and GPGE for $[Fe(CN)_6]^{3-/4-}$ couple were measured at the formal redox potential (Fig. 2B). It can be seen that at the unmodified PGE, a semicircle with an almost straight tail line is observed (curve b). The value of charge transfer resistance were determined from the Randle's equivalent circuit. The GPGE shows a straight line (curve a), implying that a diffusion controlled electrochemical process of the redox couple occurs at the electrode surface. However, the diameter of the high frequency semicircle was obviously absent due to the surface modification of the gold, suggesting that a significant acceleration of $[Fe(CN)6]^{3-/4-}$ redox reaction occurred in presence of gold nanostructures. The resistance of GPGE was much smaller than that of PGE and this may be attributed to the better conductivity of the modified electrode [26]. The impedance plots obtained were in good agreement with the CV behavior.

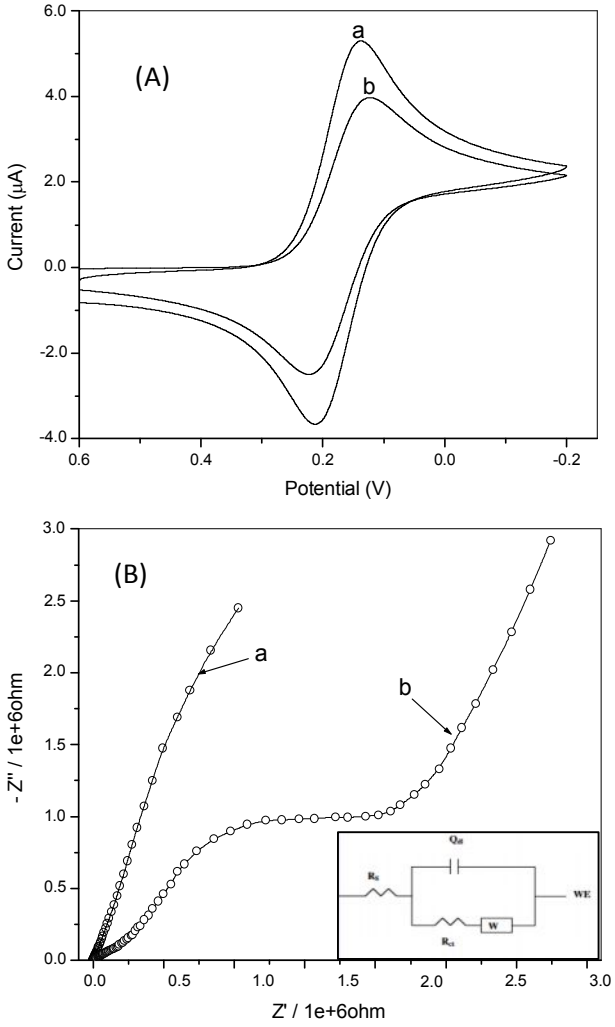
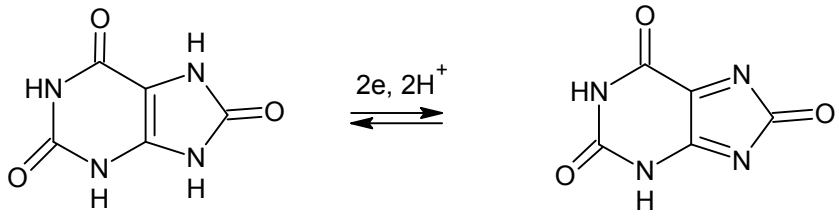


Fig. 2. (A) Cyclic voltammogram for 10 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl solution at a scan rate of 50 mVs^{-1} : GPGE (a) and PGE (b). (B) Nyquist plots for 10 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl solution at GPGE (a) and PGE (b), frequency range: 10 kHz-0.1Hz. Inset of 2B is the equivalent circuit.

3.3-Electrochemical determination of UA at PGE and GPGE

Fig. 3 shows the electrochemical response of 0.2 mM UA at PGE and GPGE in phosphate buffer of pH 7.0. Cyclic voltammetric study shows a quasi-reversible reaction process for UA. UA is first converted to the diimine through a 2*e*, $2H^+$ process (scheme 1). Under identical conditions, the GPGE produces significantly increased peak current compared to that of PGE which provides clear evidence for the catalytic effect of gold modified PGE. GPGE shows 2.4 times enhanced current sensitivity than that of PGE for 0.2 mM UA. The reaction mechanism for the redox process of UA is as shown in scheme 1.



Scheme 1.

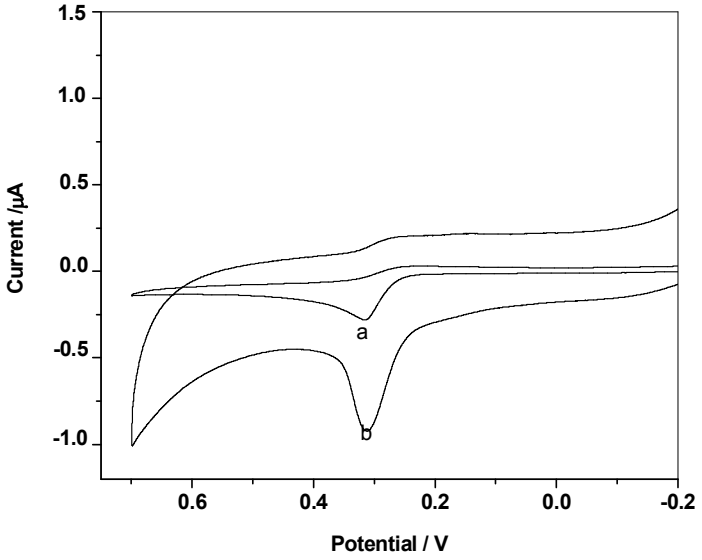


Fig. 3. Cyclic voltammograms for 0.2 mM UA in 0.1 M phosphate buffer solution of pH 7.0 at PGE (a) and GPGE (b) with a scan rate of 50 mVs^{-1}.

3.3. Electrochemical determination of Ascorbic acid (AA), Dopamine (DA) and UA at PGE and GPGE.

The major problem associated in the determination of UA present in biological sample is due to the interference caused by AA and DA, which co-exists in biological fluids at considerable amount. Usually these species oxidizes simultaneously, incapacitating selective detection of AA, DA and UA. The main objective of the present study was to utilize modified GPGE for the individual and simultaneous determination of AA, DA and UA. Before examining the simultaneous determination, the individual oxidation of AA, DA and UA has been studied using PGE and GPGE. DPV technique was used for the determination of AA (0.5 mM), DA (0.2 mM) and UA (0.2 mM) in 0.1 M phosphate buffer of pH 7.0. At PGE, oxidation peaks for AA, DA and UA were observed at 228 mV, 153 mV and 344 mV respectively (data not shown). Fig 4 shows the oxidation peaks at -44 mV, 136 mV and 200 mV for AA, DA and UA respectively at GPGE, which indicated the good electrocatalytic activity of GPGE.

The simultaneous determination of AA, DA and UA has been carried out at PGE and GPGE. As shown in Fig 5, (curve a) PGE was unable to separate the oxidation peaks of AA, DA and UA. However at GPGE three well resolved oxidation peaks were observed (Fig. 5, curve b). These results clearly indicated that the modified GPGE has got better selectivity and sensitivity towards the simultaneous determination UA in presence of AA and DA.

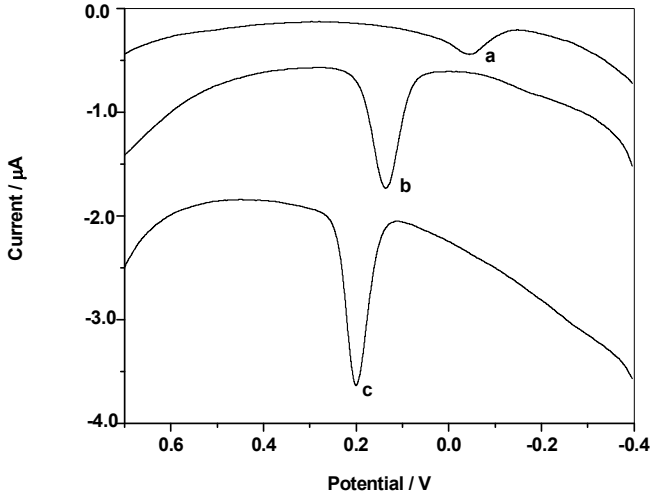


Fig.4. Differential pulse voltammograms of 0.5 mM AA (a), 0.2 mM DA (b) and 0.2 mM UA in 0.1 M phosphate buffer solution of pH 7.0 at the GPGE.

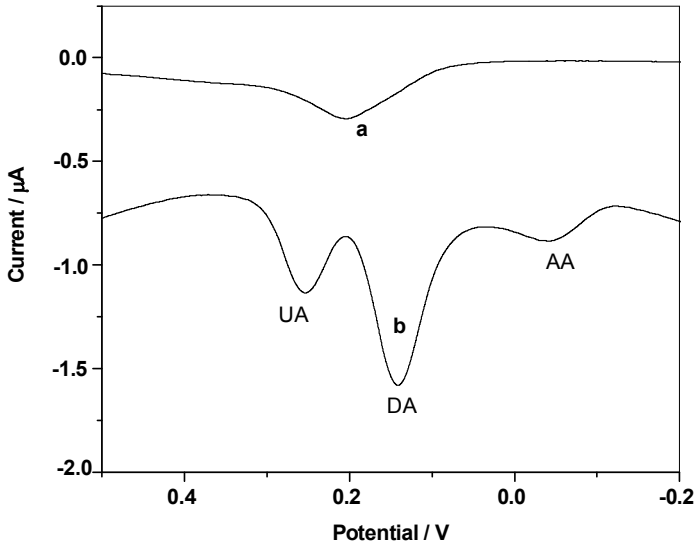


Fig.5. Differential pulse voltammogram obtained for the mixture of 0.5 mM AA, 0.2 mM DA and 0.2 mM UA in 0.1 M phosphate buffer of pH 7.0 at PGE (a) and GPGE (b).

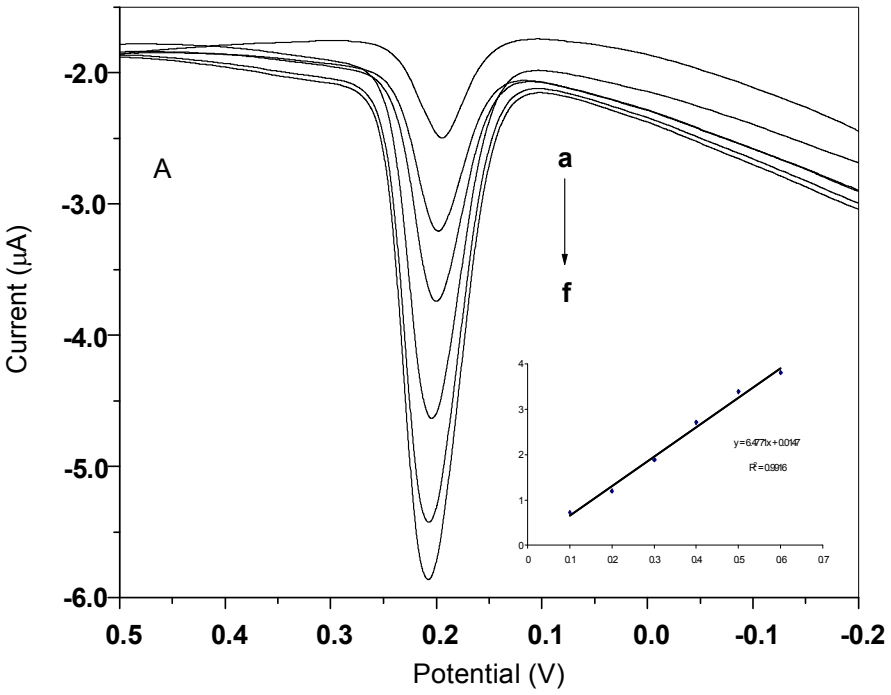


Fig.6. (A) DPV of 0.1 M phosphate buffer solution (pH 7.0) containing different concentration of UA (a→f: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mM); (B) corresponding calibration curve based on the voltammogram.

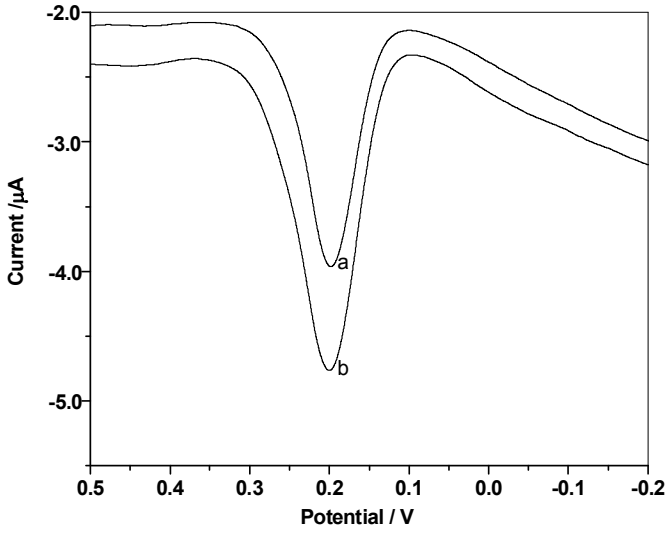


Fig.7. Differential pulse voltammogarms obtained at GPGE in 0.1 M phosphate buffer solution pH 7.0 (a) human urine and (b) human urine + 2 ml of 0.08 mM commercial UA.

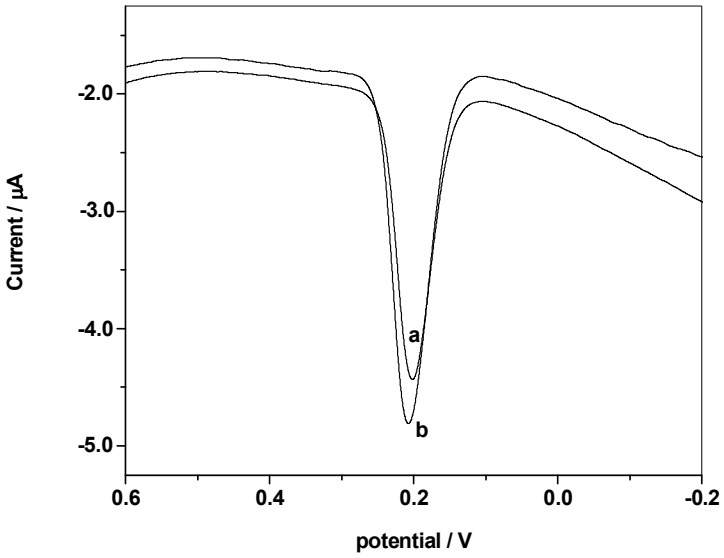


Fig.8. Differential pulse voltammograms obtained at GPGE in 0.1 M phosphate buffer solution pH 7.0. (a) Reptile excreta and (b) Reptile excreta + 2 ml of 0.08 mM commercial UA.

3.4-Differential pulse voltammetric studies of UA at GPGE

The determination of UA at GPGE was performed using DPV technique. Fig. 6 shows the DPV curves for different concentrations of UA in 0.1 M phosphate buffer of pH 7.0. The peak current corresponding to the oxidation of UA increases with increase in concentration. The voltammetric calibration curve for UA as shown inset of Fig. 6 is linear in the studied concentration range of 0.1 to 0.6 mM with regression co-efficient, R^2= 0.9983.

3.5-Determination of UA in real samples

The practical utilization of GPGE for the detection of UA in the real samples was tested by determining the concentration of UA in reptile excreta, human urine and buffalo urine. A known weight (1.75 mg) of common house lizard excreta (white solid portion) was dissolved in 25 mL phosphate buffer solution of pH 7.0 and the solution was used for DPV studies as such. The dilution process can help to reduce the matrix effect in the real samples. An anodic peak at 200 mV was observed and is attributed to the oxidation of UA (Fig. 7, curve a). To confirm the observed anodic peak was due to UA, the sample was spiked with 0.08 mM commercial UA and the resulting DPV is shown in Fig. 7 curve b. An increase in the peak current confirmed that the peak observed at 200 mV was due to the oxidation of UA. The recovery rate of the spiked sample was 99.9 % [2]. The application of the electrode was extended for the measurement of UA in Human urine. Fig. 8**,** curve a shows the DPV curve obtained for 1 mL of urine in 25 mL phosphate buffer of pH 7.0. An anodic peak at 200 mV was observed and is attributed to the oxidation of UA. To confirm the observed anodic peak was due to UA, the sample was spiked with 0.08 mM commercial UA and the resulting DPV is shown in Fig. 8, curve b. An increase in the peak current confirmed that the peak observed is due to the oxidation of UA. The recovery rate of the spiked sample was 102 %.

DPV studies were also carried out for the detection of UA in buffalo urine sample. A known volume (1mL) of the sample was diluted to 25 ml with phosphate buffer of pH 7.0. No oxidation peak was observed corresponding to UA in the applied potential range. This result indicated that the urine of buffalo does not contain UA, but contains the major purine metabolic product allantoin, which is an electroinactive compound in the applied potential range. The percentage of UA present in buffalo urine is very minute [5-6].

4. Conclusion

The current work demonstrates that the GPGE can be used to detect the presence of UA in biological fluids**.** The modified GPGE shows good selectivity, sensitivity and stability for the electrocatalytic oxidation of UA. Unlike PGE, GPGE could separate the oxidation peak potential of AA, UA and DA effectively. The proposed method has been applied to describe the qualitative and quantitative estimation of UA in reptile excreta and human urine with satisfactory results. These results are of great significance from the viewpoint of practical applications.

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