Short Communication

Selective Determination of Uric Acid in Presence of Ascorbic Acid and Dopamine at Neutral pH Using Exfoliated Graphite Electrodes

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Abstract
Exfoliated graphite electrodes have been used to detect uric acid at neutral pHs, by following its oxidation. A linear range of 5 – 53 μM and a detection limit of 5 μM are observed at a pH of 7.0. Uric acid was found to be selectively adsorbed on exfoliated graphite surface in the presence of excess ascorbic acid. This leads to its selective determination in the presence of ascorbic acid. Simultaneous detection of uric acid, dopamine and ascorbic acid has also been demonstrated on exfoliated graphite electrodes in a pH 7 buffer.

Keywords: Exfoliated graphite, Binderless electrodes, Uric acid, Ascorbic acid, Dopamine

Uric acid (UA) is the primary end product of purine metabolism and urate is present in a concentration range of 4.1 – 8.8 mg/100 mL in serum for normal, healthy humans. The urate excretion through urine varies between 250 and 750 mg/24 h [1 – 6]. Continuous monitoring of UA in the body fluid is essential since its abnormal concentration levels lead to several diseases such as hyperuricaemia and gout [1 – 6]. Other diseases such as leukemia and pneumonia are also associated with enhanced urate levels [1]. Electrochemical oxidation of UA has widely been used for its determination [2 – 4]. However, ascorbic acid (AA) oxidation is found to interfere necessitating the selective determination of UA in presence of AA. Determination of UA using enzyme-based methods is useful due to their high selectivity [5 – 7]. Non-enzymatic methods that have been reported for the selective determination of UA include the use of polymer membranes such as osmium complex-Nafion bilayer, over-oxidized polypyrrole, poly(*o*-aminophenol), poly(4-vinylpyridine) and polyglycine [1, 10 – 14]. Low detection limit and high selectivity have been achieved on a clay-Nafion modified electrode [15]. Recently Zheng and co-workers have reported cyclodextrin modified glassy carbon (GC) electrodes for the selective determination of UA [3]. Simultaneous determination of UA, dopamine (DA) and AA has been reported on a modified gold electrode [16]. All the non-enzymatic sensors use the concept of charge-based repulsion to achieve selectivity. The drawback associated with this method is that it is restricted to a pH range of 4.2 – 5.4. Both UA and AA exist as anions beyond pH 5.4 in the alkaline range (pK_a of AA and UA are 4.17 and 5.4, respectively) [3]. Hence, it is necessary to develop a method for the selective determination of UA in the presence of AA at neutral pH. We propose the use of unmodified exfoliated graphite (EG) to selectively detect UA utilizing its adsorption properties.

It is widely known that carbon-based electrodes are suitable substrates for exploring the adsorption properties of various compounds. They are reported to be very useful for electroanalytical applications due to their favorable surface properties [1 – 2]. UA is known to adsorb on carbon-based electrodes [2, 4, 8]. However, the interference from AA could not be eliminated on an unmodified carbon electrode [2, 4, 8]. Several pretreatment procedures are reported for the activation of carbon-based electrodes for the selective determination of UA in the presence of AA [2, 4]. Improved voltammetric response of UA has been achieved using electrochemically pretreated carbon paste electrodes [4]. Anodized diamond film has been reported to selectively determine UA in the presence of 20-fold excess AA [17]. Electrochemical activation of GC electrodes in acidic medium has been recently reported [2]. However, in the case of electrochemically pretreated GC electrodes, preconcentration of UA is observed only under acidic conditions [2]. DA, a neurotransmitter, is another analytically important compound. Carbon-based electrodes have been reported for the determination of DA [18]. DA is also known to adsorb on carbon surfaces [18].

Exfoliated graphite (EG) is a low dense graphite with high temperature resistance [19]. EG is prepared by the thermal decomposition of graphite intercalation compounds (GICs) [19]. When GICs are heated past a critical temperature, the intercalated compounds vaporize and expand the graphite in the 'c' direction [19]. EG can be recompressed without any binder [20] and the mechanism of compression involves the interlocking of the layers of graphite [20]. Recompressed EG is used as seals, gaskets, electrodes and adsorption substrates [19]. We have recently reported the use of chemically functionalized EG for electrocatalysis and sensing applications [21].

Elemental analysis of EG shows that it contains 1.5% of oxygen on the surface. This is present on the surface as

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different types of functional groups. These functional groups can participate in charge-based interactions depending on the pH of the electrolyte. pH_{PZC} measurements show that the EG surface is negatively charged beyond pH 6.7 [22]. Scanning electron microscopic studies show that the polished EG surface is basal plane oriented. It is already reported that the basal plane of the graphite exhibit slow electron transfer kinetics compared to the edge planes [23]. Intentionally roughened EG electrodes has edge planes exposed to the electrolyte. The surface roughness of an intentionally roughened EG electrode using 400 emery sheet is measured to be 10 μm as observed from profilometric studies. All the electrochemical measurements have been carried out using rough EG electrodes.

Figure 1 shows the differential pulse voltammetric response of UA in a phosphate buffer of pH 7.0 on a rough EG electrode. UA undergoes irreversible oxidation and the oxidation potential is observed to be 0.26 V (vs. SCE) at pH 7.0. It is observed that the oxidation peak current of UA increases with equilibration time under open circuit conditions and reaches a constant value after 10 minutes of the addition of UA into the electrolyte. The preconcentration of UA on EG electrodes is found to be due to the favorable adsorption of UA on the EG surface. This is confirmed by checking the electrode surface for electroactivity in a fresh buffer solution after 10 min of adsorption under open circuit conditions. The adsorption of UA on carbon-based electrodes has been reported earlier [2, 4, 8] and has been explained to be due to hydrogen bonding, hydrophobic and electrostatic interactions [2]. Hydrogen bonding is highly favorable on EG based electrodes since the functional groups on the surface of EG can very well participate in the interactions. The voltammetric response of UA on EG is found to be linear with the concentration of UA. A detection limit of 5 μM of UA and a linear range of 5 – 53 μM is observed at a pH of 7.0. The experiment is carried out on the same EG surface without any surface renewal, by continuously increasing the concentration of UA in the bulk of the solution. Oxidation of potassium urate is also carried out on EG electrodes under identical conditions and the oxidation potential is observed to be 0.290 V (vs. SCE). Oxidation of UA at neutral pH on EG electrodes can be compared with other electrodes reported earlier [1 – 2]. Poly (*o*-aminophenol) modified and unmodified carbon paste electrodes show oxidation potential of 0.4 and 0.6 V (vs. Ag/AgCl) at pH 6.5, respectively [1]. Electrochemically pretreated GC electrodes show negligible preconcentration of UA at neutral pHs [2]. This may be due to the repulsion between the oxygen functional groups and the negatively charged UA at neutral pH. AA is known to be a major interference in the determination of UA.

Figure 2A shows the voltammetric response of UA and AA together in the phosphate buffer of pH 7.0 on a rough EG electrode. It is observed that the linear range of UA extends up to 34 μM in the presence of AA. The voltammetric response of UA is well separated by 0.340 V and UA can be selectively determined over AA as seen from current ratio of UA and AA. The selectivity of UA over AA arises

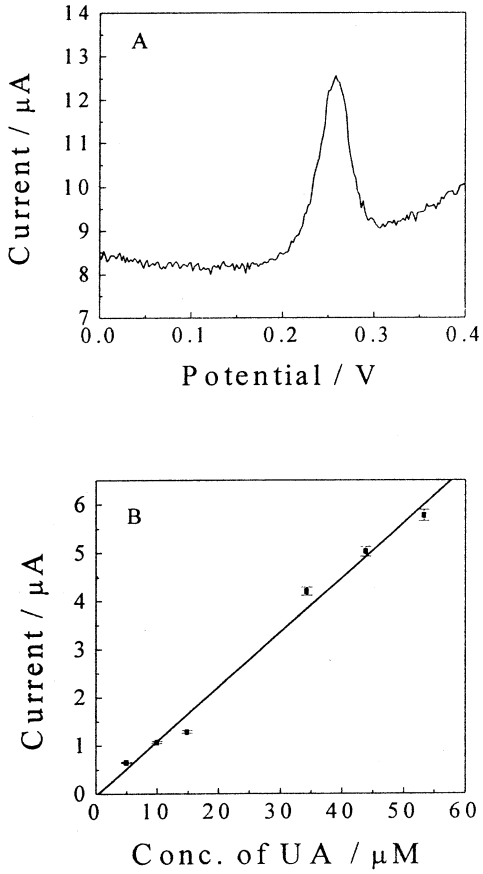

Fig. 1. A) Differential pulse voltammetric response of 34 μM UA on a rough EG electrode. Electrolyte used: 0.1 M phosphate buffer, pH 7.0, containing 0.1 M KCl. B) Calibration plot for UA ($R = 0.99$). Preconcentration time: 10 min. Geometric area: 0.07 cm^2.

from the selective adsorption of UA on EG. AA does not get adsorbed on EG surface at any pH value. This is confirmed by checking the used electrodes in fresh buffer after the analysis. Rough electrode surface exposes active edge planes that are responsible for the separation of UA and AA. However, the activation of electrode is retained only for 15 min. The oxidation potential of AA is found to shift to more positive values due to surface contamination [24]. It should be noted that the adsorption is not charge- based since at pH 7.0 both AA and UA are negatively charged as also the electrode surface. Additionally, the oxidation of UA is also observed at a pH of 4.2, where UA is neutral. The separation and selectivity of UA over AA on EG electrodes can be compared with other carbon-based electrodes and modified electrodes. The oxidation potential of AA and UA are observed to be 0.343 and 0.490 V, respectively, at a pH of 6.66 on a norepinephrine modified GC electrode [25]. Cyclodextrin modified GC electrodes show a decrease in the current response and low selectivity when pH of the electrolyte is varied from acidic to neutral [3]. This observation is explained based on the charge-based repulsion of both UA and AA at higher pHs [3]. Anodized diamond film electrodes detect UA in the presence of 20 fold excess of AA at acidic conditions [17]. In the case of EG

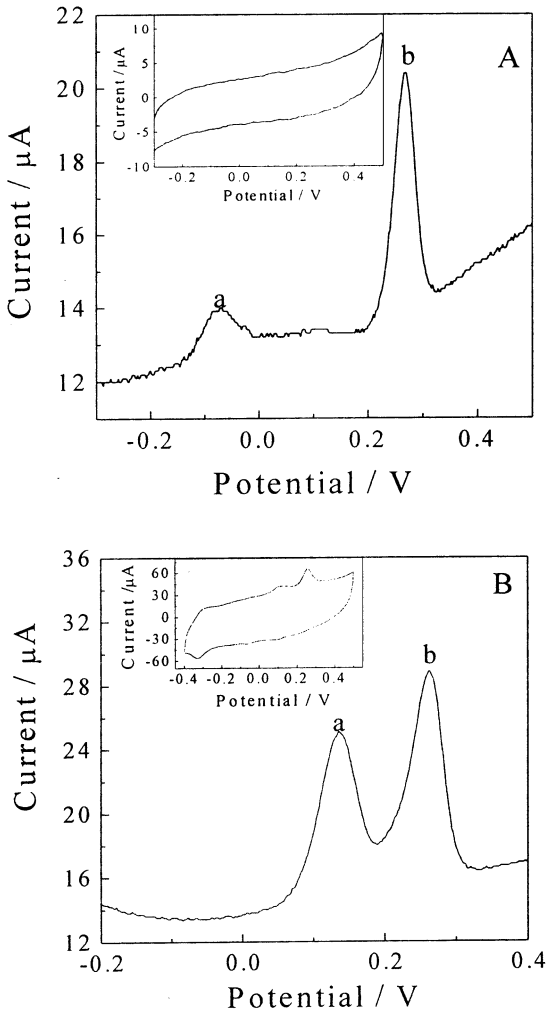

Fig. 2. A) Differential pulse voltammetric response of a) 1 mM of AA and b) 10 μM of UA on a rough EG electrode. Electrolyte used: 0.1 M phosphate buffer, pH 7.0, containing 0.1 M KCl. Preconcentration time: 10 min. Geometric area: 0.12 cm^2. Inset: Background cyclic voltammogram of the EG electrode. Scan rate: 50 mV/s. B) Differential pulse voltammetric response of a) 53.3 μM of DA and b) 53.3 μM of UA on EG electrode. Electrolyte used: 0.1 M phosphate buffer, pH 7.0, containing 0.1 M KCl. Preconcentration time: 10 min. Geometric area: 0.07 cm^2. Inset: Second cycle of the voltammogram of the EG electrode in 48 μM of UA and DA each at pH 7.0. Scan rate: 50 mV/s. Geometric area: 0.12 cm^2.

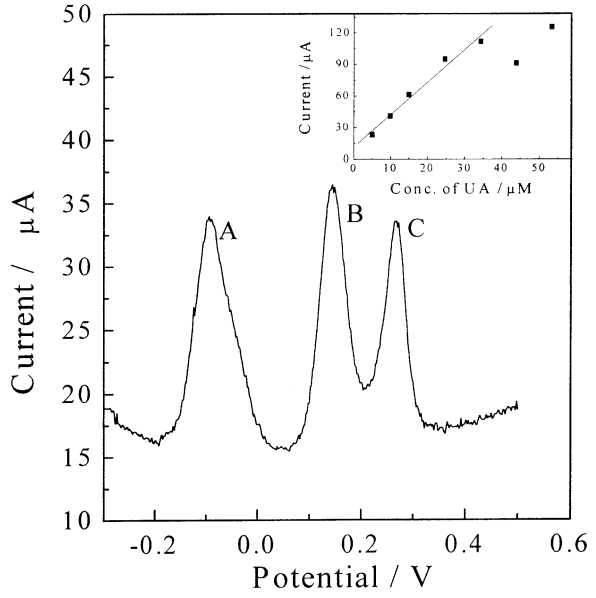

Fig. 3. Differential pulse voltammetric response of A) 2 mM of AA B) 50 μM of DA and C) 50 μM of UA on EG electrode. Electrolyte used: 0.1 M phosphate buffer, pH 7.0, containing 0.1 M KCl. Preconcentration time: 2 min. Geometric area: 0.09 cm^2. Inset: Calibration plot of UA ($R = 0.99$) in the presence of 2 mM of AA and 50 μM of DA. Preconcentration time: 10 min. Geometric area: 0.12 cm^2.

electrodes the oxidation potentials of AA and UA are observed at −0.08 and 0.26 V (Fig. 2A).

Figure 2B shows the detection of UA in the presence of DA at a pH of 7.0. Oxidation of DA is observed at 0.135 V. The signal of UA and DA are separated by 0.125 V. However, the current response for UA is found to decrease with subsequent additions of DA. This is believed to be due to the adsorption of DA and the cyclized product of DA oxidation. Oxidized form of DA is known to give an electroactive product [26]. It is confirmed that this cyclized product as well as DA get adsorbed on to EG surface very strongly. It is also known that the DA adsorbs on carbon surfaces through the catechol ring [22]. The peaks at 0.26 and 0.135 V observed on EG electrodes correspond to UA and DA, respectively, whereas the reversible peaks at −0.275 and −0.33 V correspond to the cyclized product of DA (inset: Fig. 2B). The oxidation potentials and the current values for UA and DA remain constant when a fresh, rough electrode surface is used for each measurement. The second and subsequent cycles will have the adsorbed DA, cyclized product and UA on the electrode surface. Hence, the effective area available for UA adsorption is reduced and the current response is also decreased. The competition between DA/cyclized product and UA will determine the extent of available surface for further electrochemical oxidation / adsorption of UA. DA and the cyclized product adsorb faster than UA onto EG. Moreover, preconcentration of UA takes about 10 min whereas the DA and cyclized product are adsorbed within a minute.

We have also attempted to simultaneously determine DA, UA and AA on EG electrodes. Figure 3 shows the response for the simultaneous detection of all the three components. As discussed earlier, the presence of AA has no effect on the UA signal. The DA is already known to compete for adsorption with UA. Reliable current response is obtained in the first differential pulse voltammetric measurement. Hence, a fresh surface is to be used for each measurement. The current response is found to be stable for 10 minutes after roughening the electrode surface, in the presence of all three analytes. The linear range of UA in the presence of DA and AA is observed to be up to 34 μM when a fresh surface is used for every measurement (inset Fig. 3). Analytical

utility of the EG electrodes has been examined using human urine samples. Human urine is diluted 100 times in phosphate buffer of pH 7.0 and subjected to electrochemical analysis. The amount of uric acid present in the urine is estimated to be 0.45 ± 0.07 g/L. This value is comparable to the reported values in the literature [2, 4].

Surface renewability is an important parameter that controls the reliability of the sensor. The unmodified EG electrodes are found to give stable response for all three components within the error limits. The standard deviation observed for the current values are of the order of $\pm 2\%$.

EG electrodes can be used for preconcentration of UA and DA. Selective determination of UA is achieved in the presence of excess AA based on the selective adsorption at neutral pH values. Active surfaces can be exposed by mechanical polishing of the electrode surface. Simultaneous detection of UA, DA and AA has been demonstrated and the analytical utility explored.

Experimental

Potassium urate was obtained from SD-Fine Chem, India. UA was prepared from potassium urate by hydrolysis using hydrochloric acid. DA was obtained from Aldrich, USA. AA was the product of Loba Chemicals, India. Doubly distilled water was used for all the electrochemical measurements. Buffer solutions were prepared using analytical reagent grade chemicals.

Natural graphite particles (300 – 400 μm) were obtained from Stratmin Graphite Co., NJ, USA. The natural graphite particles were intercalated with bisulfate by immersing them in $H_2SO_4 + HNO_3$ mixture (3 : 1 by volume) for 24 h. at ambient conditions. The material was then washed thoroughly with distilled water and air-dried. The dried material was introduced in a preheated furnace at a temperature of 800 °C for a min to obtain EG. EG is recompressed to give a compact pellet using the following procedure: Approximately, 150 mg of the EG powder without any binder was pressed at a pressure of 6 tons/cm^2 for about 5 h. The pellet was cut in to small pieces and mounted on a Pyrex glass tube using conducting silver epoxy and copper wire for contact. Surface of the electrodes were polished with different grades of SiC emery sheets, first with 1 500 grit and further polished with 4/0, 5/0 and 6/0 grade polishing papers to get a smooth surface. Scratching the surface against a 400-grit emery sheet in the same direction created a reproducible rough surface.

A three-electrode cell with platinum foil as the counter, calomel as the reference and rough EG as the working electrode (geometric area 0.07 – 0.12 cm^2) was used for the electrochemical measurements. Electrolyte solutions were degassed with pure nitrogen for 20 minutes prior to the experiments. Electrochemical measurements were carried out using a potentiostat/galvanostat (EG&G, PARC, USA (Versastat II)). Differential pulse voltammograms were carried out at a scan rate of 5 mV/s with a pulse height and pulse width of 50 mV and 50 ms, respectively.

Elemental analysis was carried out using a Carlo Erba (Model 1106, Italy) elemental analyzer. The EG sample was dried and degassed thoroughly before the measurements. Surface morphology of the powder and compressed pellets with different surface roughnesses was monitored using JEOL (JSM-5600 LV, Japan) scanning electron microscope, operating at 5 kV. Surface profiles of the electrodes were monitored using a stylus profilometer (from Talysurf plus, UK). Point of zero charge (pH_{PZC}) measurements was carried out using batch equilibrium method [27].

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