Possibilities and limitations in miniaturized sensor design for uric acid

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Uric acid (UA) has been under intensive investigation by electrochemists owing to its important role as a metabolite in biological fluids. One of the major problems in biological determinations of uric acid comes from electrochemical interferences such as ascorbic acid (AA), which has a similar oxidation potential, $E_{1/2} = 200$ mV versus SCE, at graphite electrodes, and is present at high concentrations in biological systems. UA undergoes a fast electron transfer reaction, $k_e = 54$ s$^{-1}$, at carbon fiber electrodes. These characteristics make UA an excellent candidate for fast scan voltammetric (FSV) determinations. This paper presents the results of FSV at bare carbon fiber electrodes. The results show good selectivity and sensitivity in the determination of low concentrations of UA in the presence of high concentrations of AA. By increasing the scan rate above 500 V s$^{-1}$, voltammograms of UA in the presence of AA can be resolved because of the kinetic differences in the response of the two anions, without the need for a permselective film on the electrode. Results are also presented that demonstrate an effective way to reach a stable background current at bare carbon fiber electrodes, which is required in FSV because the signal from the analyte is smaller than the electrochemical signal from the background current. Signal-to-noise ratios at bare carbon fiber electrodes in FSV are improved because the high temporal resolution in fast scan methods allows the acquisition of a large number of scans that can be signal averaged in a short period of time. In addition, large signals can be measured because the voltammetric peak current increases with increase in scan rate.

Keywords: Uric acid; fast scan voltammetry; bare carbon fiber electrodes

Uric acid (UA) [7,9-dihydro-1H-purine-2,6,8(3H)-trione] is the principal end product of purine metabolism; therefore, its determination serves as a marker for the detection of disorders associated with purine metabolism such as gout and Lesch–Nyhan syndrome.2,3 Gout occurs when sodium urate crystals are deposited in the joints, soft tissue, bursae and tendons and Lesch–Nyhan syndrome is an x-linked chromosome disorder that results in the absence of the enzyme hypoxanthine–guanine phosphoribosyl transferase (HGPRT). Hyperuricemia (elevated concentrations of UA) may indicate other medical conditions such as kidney injury,4 leukemia5 and pneumonia.6 Diagnosis is confirmed by monitoring UA serum or urinary levels. The normal UA serum levels range from 4.1 to 8.8 mg dL$^{-1}$ and urinary excretion is typically 250–750 mg d$^{-1}$.7 UA has been determined in the clinical laboratory by colorimetric, enzymatic and electrochemical methods. The most popular colorimetric method involves the oxidation of UA to allantoin and CO$_2$ by phosphotungstic acid, which is reduced to a tungsten blue chromophoric compound. The absorbance between 660 and 720 nm is proportional to UA concentration.8,9 Numerous modifications of this colorimetric procedure have been developed10–12 but the tendency of UA to coprecipitate with plasma proteins, the possible formation of turbidity in the final colored solution, the non-linearity of the relationship between the color yield and concentration of UA over the range of UA concentrations commonly encountered and interferences from compounds such as ascorbic acid (AA) and other reducing agents can restrict the analytical applications of this method.13

Enzymatic methods are more selective than colorimetric methods. One of the most popular enzymatic methods monitors the decrease in absorbance (292 nm) which results from the oxidation of UA to allantoin:

$$\text{uric acid} + \text{O}_2 + 2 \text{H}_2\text{O} \xrightarrow{\text{uricase}} \text{allantoin} + \text{H}_2\text{O}_2 + \text{CO}_2 \quad (1)$$

The reaction has a high specificity but the high background absorbance of the matrix may produce poor color development, and possible enzyme inhibition by purines present in the sample, such as xanthine, may lead to poor reproducibility.14

Other enzymatic procedures have been developed based on amperometric measurements of H$_2$O$_2$ produced in this reaction [see eqn. (1)].15 These procedures use the anodic electroactivity of peroxide but its oxidation can require relatively high applied potentials ($> 0.4$ V) and consequently this system is susceptible to interferences from readily oxidizable interferents. Different approaches have been used to eliminate the interferences16,17 but the complexity of the procedure and the lack of selectivity still need to be overcome.

New methods based on surface-modified electrodes have been used.18–21 One method uses a cobalt phthalocyanine base (CoPc) tailored for the electrocatalytic oxidation of H$_2$O$_2$ in combination with a cellulose acetate–uricase bilayer. The amperometric calibrations were linear over the range 1.3 $\times$ 10$^{-6}$–1 $\times$ 10$^{-3}$ M but the response time required to reach 95% of the steady-state current was long (15 min).19

Levels of serum UA are frequently elevated in patients with hypertension or ischemic heart disease.22–23 although the biological mechanism that links hyperuricemia to ischemic heart disease is uncertain.24 Rarely, the heart is the site of deposition of urate crystals but high levels of UA may be involved in platelet adhesiveness25 and aggregation.26

Ischemia, local deficiency of blood supply and reperfusion are fundamental concepts in transplant surgery. To remove a donor organ and place it in a recipient requires the cessation of natural circulatory flow for a variable length of time. Oxidative stress takes place when reactive free radical oxidants are formed when oxygen is reintroduced to ischemic tissue.27,28 Because reactive free radicals are difficult to measure directly, changes in antioxidants such as UA are often used as an indication of oxidative stress. UA was found to increase by over 300% after ischemia and 600% during the first 30 min of reperfusion in an isolated liver model of ischemia/reperfusion.29 These new
important areas for UA determinations require selective, rapid determinations with low detection limits. Since biological matrices are complex, the methods should be selective to avoid interferences. Fast determinations are also required because real-time monitoring is necessary to determine fast concentration changes; for example, in the myocardial interstitial space, adenosine and its metabolites such as UA are important markers of ischemia and regulators of blood flow, and may produce cardioprotection against ischemia. A fast method to assess the concentration of adenosine and its metabolites is necessary to determine their involvement in mediating these effects.30

Finally, low detection limits are required because of the low concentration of UA in biological systems such as heart microdialyzates. The concentration of UA in samples taken 60 min after implantation of a microdialysis probe in an isolated rat heart was $1.8 \times 10^{-6}$ M.31 and in a sample taken 60 min after implantation of a microdialysis probe in a rat liver it was $5.4 \pm 2.0 \times 10^{-6}$ M.29

The use of ultramicroelectrodes (UMEs) has grown rapidly in the last decade22–34 because of the advantages of their small dimensions. Several advantages arise from the reduction in electrode size.35–37 The small physical dimensions of UMEs make them suitable for in vivo measurements.38 The small ohmic drop and cell time constant associated with UMEs allow fast scan voltammetry (FSV) to be performed.39

One of the major problems with the in vivo determinations of UA comes from electrochemical interferences such as AA.40 Electrodes coated with anion-excluding films such as Nafion41 or a thick film of overoxidized polypyrrole42 are not practical for selective determinations of UA (anion) in the presence of AA because the response of UA and AA is suppressed by the anion-excluding films attenuate anion diffusion. In this work, we investigated the use of FSV at scan rates above 500 V s$^{-1}$ to improve the selectivity and sensitivity of UA in the presence of AA at bare carbon fibers. By increasing the scan rate above 500 V s$^{-1}$, voltammograms of UA in the presence of AA can be resolved because of the kinetic differences in the response of the two anions and also, simultaneously, an improvement in the S/N ratio is achieved because of the high temporal resolution which allows the acquisition of a large number of scans that can be signal averaged in a short period of time.43

FSV determinations are limited by the interference from a large background current. The background current has at least two components: double-layer capacitance associated with the layer of ions which forms at the solution/electrode interface and the oxidation and reduction of surface-bound species. The sensitivity of FSV analysis depends on the stability of the background current.44 We established experimentally that a stable background current can be obtained at carbon fiber electrodes after 30 min of continuous cycling of the electrode in the experimental potential window. The possible effects of the surface electrochemical pre-treatment which results from cycling on the electrode response (peak separation and peak current in voltammetry) to UA and AA are discussed.

**Experimental**

**Chemicals**

All chemicals were used as received. Ascorbic acid (Mallinckrodt, St. Louis, MO, USA), potassium hexacyanoferrate(III) (Sigma, St. Louis, MO, USA) and uric acid (Sigma) were prepared in $7 \times 10^{-2}$ M potassium phosphate buffer (pH 7.4). All determinations were performed at room temperature.

**Electrodes**

A saturated calomel electrode (SCE) was used as a reference electrode. Carbon fiber (7 μm diameter; Textron Specialty Materials, Lowell, MA, USA) was used as the working electrode. The fiber was first connected to a copper wire with silver epoxy (EPO-TEK 410 E; Epoxy Technology, Billerica, MA, USA). When the silver epoxy had dried, the copper wire was inserted and sealed into a micropipet tip and epoxy was used to insulate the carbon electrode. The epoxy was made by mixing the shell (Shell Epon 828; Miller-Stephenson Chemical, Danbury, CT, USA) and hardener (m-phenylenediamine; Miller-Stephenson Chemical, Danbury, CT, USA). The mixture was heated in a water-bath until the epoxy became transparent and water-like. The micropipet tip was filled with the liquid epoxy and the electrode was left overnight at room temperature and then cured in an oven for 1 h at 150 °C. After curing, the tip of the electrode was sanded off on a polishing wheel using 600-grit silicon carbide paper, and finally the surface was polished gently on a polishing wheel (Ecomet I; Buehler, Evanston, IL, USA) with an Alpha A polishing cloth (Mark V Laboratory, East Granby, CT, USA), with a α-alumina suspension of 0.1 μm particle size (Gamal; Fisher Scientific, Pittsburgh, PA, USA). Immediately after polishing, the electrodes were dipped in propan-2-ol45 for 10–15 min and then sonicated in water for 5 min. The electrodes were electrochemically pre-treated in $7 \times 10^{-2}$ M potassium phosphate (pH 7.4) for 30 min by continuous cycling46 at 10 V s$^{-1}$ in a potential window from $-1.0$ to $1.5$ V versus SCE. The limiting current of hexacyanoferrate(III) was measured at 0.0 V versus SCE before and after electrochemical pre-treatment (ECP).

**Instrumentation**

The instrumental set-up for fast scan voltammetry has been described previously.46 A function generator (Universal Programmer, Model 175, EG&G Princeton Applied Research, Princeton, NJ, USA) was used to apply a triangular waveform to an SCE in a two-electrode configuration potentiostat. The current at the working electrode was converted to voltage, amplified and recorded by a digital oscilloscope (LeCroy Model 9310; Chestnut Ridge, NY, USA). The stored waveform was transferred from the oscilloscope to a computer for plotting of the data.

With this set-up, 250 background scans in phosphate buffer at the experimental scan rate, in a potential window from $-1.0$ to $1.5$ V versus SCE, were recorded, stored, averaged and used for background subtraction, and 250 scans in the analyte solution, in the same potential window, were recorded, stored, averaged and used for digital processing of the data. The average background was subtracted from the average analyte signal. For convenience of performing background subtraction, analyte and buffer solutions were injected into the electrochemical cell with a syringe which allowed the solutions to be pumped into the cell without moving the electrodes.45 A copper mesh Faraday cage was used to minimize the environmental noise.

**Results and discussion**

**Electrochemical pre-treatment**

The background current observed in FSV in a blank solution is due to several processes, including double-layer charging, redox reactions of the surface functional groups, such as quinones, and redox reactions of impurities in the electrolyte solution. The amplitude of the background current may be more than two orders of magnitude greater than that of the oxidation current of an analyte. Consequently, FSV requires a stable background current to produce a reliable background that can be used in background subtraction. The formation of oxides47 and the roughness of the electrode surface can change during a voltammetric determination, and these changes can produce significant modifications of the background current. In previous work,45 we established that a stable background current can be obtained at a carbon fiber electrode after 30 min of continuous
cycling of the electrode in the potential window from −0.8 to 1.2 V versus SCE in 7 × 10⁻² M potassium phosphate buffer (pH 7.4) at a scan rate of 100 V s⁻¹. In addition, we found that it is possible to enhance the sensitivity of the carbon fiber electrode by this form of ECP. ECP may produce an oxide layer at the surface of the carbon fiber; the layer arises from the oxidation of the surface with a formation of carboxyl and/or quinone groups. The formation of the oxide film at the surface of the carbon fiber UME may increase the sensitivity of the UME to cations such as dopamine. In this work, we modified our original ECP procedure to increase the sensitivity in UA determinations and the selectivity in the presence of AA. Here, electrodes were electrochemically pre-treated, using the same fast scan procedure that was used previously, but the scan rate and the potential window were modified. The scan rate was 10 V s⁻¹ and the potential window was from −1.0 to 1.5 V versus SCE. The lower scan rate and the more positive potential of this ECP can produce a larger current density for a longer time at the UME than the ECP that was used previously. A comparison of voltammograms (1) and (2) in Fig. 1(a) shows a significant change in the overall background current after the first 30 min of continuous cycling in the potential window from −1.0 to 1.5 V versus SCE. The charging current increases in the entire potential window. The absence of significant changes in the overall background current after additional 30 and 60 min of continuous cycling [curves (3) and (4)] indicates that a reasonably stable background can be reached after 30 min of continuous cycling in this potential window.

Voltammograms (1) and (2) in Fig. 1(b) show the background current after the original and the new ECP, respectively. With the new ECP [voltammogram (2)] a higher charging current in the entire potential window is observed. The larger current density for a longer time at a UME in the new ECP may produce nanocracking of the UME in addition to surface cleaning. The nanocracked surface can exhibit fast electron transfer activity to electroactive anions and cations. Determination of the apparent radius of the graphite fiber electrodes before and after ECP Hexacyanoferrate(III), Fe(CN)₆³⁻, was used to evaluate the apparent radius of the electrodes before and after ECP, because of its absence of adsorptive behavior at carbon fiber electrodes and its well known redox properties. Cyclic voltammetry at a low scan rate, 50 mV s⁻¹, was used to find the apparent radius of the UME before and after the ECP by considering a disk equivalent area of the electrode. The apparent radius of the UMEs before and after ECP is 2.3 ± 0.1 and 3.2 ± 0.1 µm, respectively. These values were calculated from the voltammograms in Fig. 2(a) by using the diffusion coefficient of Fe(CN)₆³⁻, D₀ = 7.7 × 10⁻⁶ cm² s⁻¹.

To study possible changes in the kinetics of Fe(CN)₆³⁻ before and after ECP, plots of the potential versus log ([Iₚ/ I] were obtained. A plot before ECP gave a slope of 132 ± 5 mV, indicative of an irreversible wave for a one-electron process. The same plot after ECP gave a slope of 74 ± 3 mV, indicative of a quasi-reversible behavior. After ECP, the half-wave potential for the oxidation of Fe(CN)₆³⁻ shifted from 168 ± 10 to 161 ± 5 mV versus SCE as expected for this probe. Polished surfaces, before ECP, are characterized by polishing debris and different levels of impurities and surface oxides. ECP not only removes all these possible impurities, but also produces a more active surface.

Voltammetry of ascorbic acid and uric acid at low scan rates AA (anion) is an electroactive interferent in the determination of UA (anion). Voltamograms of AA at the physiological pH of 7.4 are illustrated in Fig. 2(b). After ECP, the half-wave potential for the oxidation of AA is shifted from 195 ± 19 to 182 ± 15 mV versus SCE as expected for this probe. In order to verify changes in the kinetics of AA before and after ECP, plots of the potential versus log ([Iₚ/ I] were obtained. The slope of the plot before and after ECP was 183 ± 9 and 139 ± 8 mV, respectively. Even though AA kinetics are faster after the ECP, both plots are indicative of an irreversible wave for a two-electron process. The wave height after ECP is lower than that before ECP and implies a lower sensitivity for AA. Voltamgrams of UA at physiological pH are illustrated in Fig. 2(c). After ECP, the half-wave oxidation potential for UA is shifted from 366 ± 19 to 211 ± 7 mV versus SCE. The position of the oxidation wave after ECP is close to that observed by Vavrin, E°C = −80 mV versus SCE, in a polarographic determination of AA at pH 7.4 at 25 °C. In order to verify changes in the kinetics of AA before and after ECP, plots of the potential versus log ([Iₚ/ I] were obtained. The slope of the plot before and after ECP was 183 ± 9 and 139 ± 8 mV, respectively. Even though AA kinetics are faster after the ECP, both plots are indicative of an irreversible wave for a two-electron process. The wave height after ECP is lower than that before ECP and implies a lower sensitivity for AA.
shape of the wave after ECP suggested possible adsorption at the surface of the carbon fiber electrode, in agreement with previous results.39

The possible influence of adsorption on the UA oxidation current was investigated at high scan rates. The slope of the logarithm of the oxidation peak current as a function of the logarithm of the scan rate was determined. The slope of the plot in Fig. 3 shows that UA oxidation is predominantly diffusion controlled. The slope in the scan rate range 100–2000 V s\(^{-1}\) was 0.50, which is the theoretical value for a diffusion-controlled process.58

ECP not only can be used to reach a stable background current as demonstrated above but can also improve the selectivity and sensitivity in FSV. Gonon et al.39 developed the first ECP procedure for carbon fiber UMEs which offered an important improvement in sensitivity and selectivity for dopamine determinations in vivo. In their ECP, the electrodes were immersed in pH 7.4 phosphate buffer solution and the ECP was achieved by applying an alternating triangular waveform potential to the working electrode. The parameters of this ECP were as follows: frequency 70 Hz, lower potential limit 0 V, upper potential limit +5 V, duration 20 s, followed by continuous application of a potential of +1.5 V for 20 s. After ECP, the differential pulse voltammograms showed sharper, better resolved peaks for AA and 3,4-dihydroxyphenylacetic acid (DOPAC).

Detection of UA without interference from AA

Previous studies by fast scan methods (100–400 V s\(^{-1}\) range) have shown that UA has faster kinetics than AA at carbon fiber electrodes.39 Therefore, selective determinations of UA in the presence of AA should be possible by FSV at high scan rates, even though UA and AA are both anions at physiological pH and have similar redox potentials.

Fig. 4 shows the cyclic voltammograms of 1.13 \(\times\) 10\(^{-4}\) M UA (dashed line) and 1.13 \(\times\) 10\(^{-3}\) M AA (solid line) at a scan rate of 100 V s\(^{-1}\) in 7 \(\times\) 10\(^{-3}\) M potassium phosphate buffer (pH 7.4) at a carbon fiber electrode after ECP. It is clear that after ECP: (a) 1.13 \(\times\) 10\(^{-4}\) M UA and 1.13 \(\times\) 10\(^{-3}\) M AA at scan rate of 500 V s\(^{-1}\) in 7 \(\times\) 10\(^{-2}\) M potassium phosphate buffer (pH 7.4) at a carbon fiber electrode after ECP. Both voltammograms present only one oxidation peak around 0.5 V versus SCE. The current-to-UA concentration ratios (i/uC) for UA and the UA–AA mixture are 125 and 130 nA mm\(^{-1}\), respectively. The similar values of i/uC and the similar peak potentials values, \(E_p\), in both voltammograms are a clear indication that the response of AA becomes insignificant relative to that of UA at 500 V s\(^{-1}\).43 Therefore, the peaks that are observed are a result of the oxidation of UA in the presence of slowly reacting AA. Although at 500 V s\(^{-1}\) AA does not show a clear oxidation peak, which is expected at more positive potentials, it may be the principal factor behind the poor background subtraction that is observed in the solution containing UA, shown in the voltammogram in Fig. 5(b).

Fig. 6 shows voltammograms of (a) 5.0 \(\times\) 10\(^{-5}\) M UA and (b) a mixture of 5 \(\times\) 10\(^{-5}\) M UA and 1 \(\times\) 10\(^{-4}\) M AA at a scan rate of 1000 V s\(^{-1}\) in 7 \(\times\) 10\(^{-2}\) M potassium phosphate buffer (pH 7.4) at a carbon fiber electrode after ECP. The voltammograms are virtually the same, verifying that a very well defined response of UA can be obtained at low concentrations of UA in the presence of high concentrations of an interfering anion such as AA at a fast scan rate.

The potential used in the determinations of UA at 500 and 1000 V s\(^{-1}\) was 0.5 V versus SCE. All UA signals were background subtracted. UA currents used to calculate the

![Fig. 2](image-url) Cyclic voltammetry at a carbon fiber disk electrode (approximately 7 \(\mu\)m diameter). The dashed line is the response before ECP and the solid line that after ECP: (a) 1 \(\times\) 10\(^{-4}\) M Fe(CN)\(_3\)\(^{3-}\); (b) 2.02 \(\times\) 10\(^{-4}\) M AA; and (c) 4.0 \(\times\) 10\(^{-4}\) M UA. Scan rate, 50 mV s\(^{-1}\); 5 \(\times\) 10\(^{-2}\) M phosphate buffer (pH 7.4) as supporting electrolyte.
sensitivity values that are listed in Table 1 were based on averaged results of at least three determinations. The limits of detection (LODs) were calculated based on three times the S/N. No additional S/N optimization such as smoothing was used. The results show improved sensitivity at faster scan rates but the higher LOD at higher scan rates is a result of the increased background current at the high scan rates.

An additional advantage of FSV as used here is the shorter time required to complete a voltammogram, which facilitates signal averaging. Other approaches to optimizing the S/N were used by Baur et al..60 The S/N ratios as a function of the number of cycles are summarized in Table 2. The high frequency noise was rapidly reduced by signal averaging as the number of scans increased. The results illustrate an improvement in S/N with increase in the number of scans in spite of the simultaneous increase in the background current, which may account, together with non-random noise, for the lower than theoretical (S/N \(\approx n^{1/2} \)) increase in S/N seen in Table 2 with increase in the number of cycles. Higher noise may also account for higher peak current signals that are measured with a smaller number of cycles; a decrease in the noise with increase in the number of cycles contributes to some decrease in the measured peak signal and a decrease in the standard deviation of current measurements in Table 2.

**Conclusions**

We have demonstrated the analytical utility of FSV at electrochemically pre-treated carbon fiber electrodes in the determination of UA. High selectivity and sensitivity at 1000 V s\(^{-1}\) allow the determinations of low concentrations of UA in the presence of high concentrations of AA at a carbon fiber electrode without a permselective film.

The voltammetric properties of carbon fiber electrodes are drastically modified by the ECP used here. Faster kinetics,
increased background current and a shift of peak potentials for all the systems studied [UA, AA and Fe(CN)₃⁴⁻], after ECP, can be caused by the high current density at the fiber during the ECP at positive potentials. This high current density may not only modify the electrode surface but may also produce nanocracks in the surface. The increase in the background current density may not be caused by the high current density at the fiber during the ECP process. A stable background current can be reached after ECP, after 30 min of ECP.

High scan rates allow the acquisition of many signals for signal averaging, improving the S/N and the sensitivity in the determination of UA.

References


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