Selective voltammetric method for uric acid detection using pre-anodized Nafion-coated glassy carbon electrodes

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Electrochemically pre-anodized Nafion-coated glassy carbon electrodes (NCGCEs) were used for the selective determination of uric acid (UA) by square-wave voltammetry. The major interference from ascorbic acid (AA) was overcome owing to the distinct ability of the pre-anodized NCGCE to yield its best performance at around pH 5. At pH 5, AA exists in the anionic form whereas UA is in the cationic form, since their pK_a values are 4.1 and 5.4, respectively, at 25 °C. Consequently, the Nafion film repels the negatively charged AA and selective sensing of UA can be achieved. Moreover, the anodized surface has a high affinity towards UA through hydrogen bonding. Hence the combination of pre-anodization and the Nafion film modification works excellently in the determination of UA. Under optimized conditions, the detection limit for UA has been improved to 10 nM (S/N = 3). The practical analytical utility of the method is demonstrated by the measurement of UA in human urine without any preliminary treatment.

Keywords: *Nafion; uric acid; ascorbic acid; voltammetry; glassy carbon electrode*

Uric acid (UA) is the primary product of purine metabolism and its inconsistency in human body causes many diseases, such as gout, hyperuricaemia and Lesch–Nyan disease.[1] Hence monitoring the concentration of UA in biological fluids is very important. The major obstacle in monitoring UA is the interference from other compounds such as ascorbic acid (AA) and dopamine which co-exist in the biological fluids[2–4] and have almost the same redox potential as UA. Various methods, such as the adsorption/medium exchange approach,[5,6] enzyme-based techniques,[7–11] the use of polymer-modified electrodes with[12] and without[11,13,14] catalyst and the use of electrochemically pre-treated carbon paste electrodes[15] or clay-modified electrodes[16] have been developed for the selective determination of UA. We describe here a relatively simple voltammetric method for the sensitive and selective detection of UA using pre-anodized Nafion-coated glassy carbon electrodes (NCGCEs).

It is generally found with GCEs that a particular pre-treatment method, which activates the electrode surface with respect to a specific electrochemical reaction in solution, can deactivate other reactions. Moreover, even for a given redox system, variations in the pre-treatment history can cause wide alterations in the heterogeneous electron transfer rate constant often by many orders of magnitude.[17] Hence, in any electrochemical studies with a GCE, its surface pre-treatment method must first be formulated. Various surface pre-treatment methods have been reported to improve the electrochemical performance of carbon surfaces, particularly from GCE substrates. These include electrochemical methods[18–25] and non-electrochemical methods,[26–29] such as polishing, vacuum heat treatment, laser irradiation, fracturing and high-intensity ultrasonic treatment. Among these procedures for electrode activation, electrochemical methods of pre-treatment have been used extensively since they are convenient, inexpensive and rapid and have the additional advantage that they can be performed *in situ* within the electrolyte. Both cathodization[30] and anodization[18–25] have been reported to activate the electrode surface with respect to specific redox systems, but the latter method has predominantly been employed.

In this paper, we report the effect of surface pre-anodization on the determination of UA on a GCE and an NCGCE. At pH 5, AA exists in the anionic form whereas UA is in the cationic form, since their pK_a values are 4.1 and 5.4, respectively, at 25 °C.[31] Consequently, the Nafion film repels the negatively charged AA and the selective sensing of UA can be achieved. Such a pre-treatment method yields the best performance for UA oxidation around pH 5, hence the interference of AA can be completely overcome on the NCGCE. Moreover, unlike the case of the carbon paste electrode reported earlier,[15] where the electrode material was found to become swollen after prolonged use, the GCE shows good stability even after prolonged use.

Experimental

Chemicals and reagents

Nafion perfluorinated ion-exchange powder as a 5% m/v solution in a mixture of lower aliphatic alcohols and 10% water was obtained from Aldrich (Milwaukee, WI, USA). UA (Sigma, St. Louis, MO, USA), AA (Wako, Osaka, Japan) and all other compounds (ACS certified reagent grade) were used without further purification. Aqueous solutions were prepared with doubly distilled, de-ionized water.

Apparatus and procedure

All the electrochemical experiments were carried out with a Bioanalytical Systems (West Lafayette, IN, USA) BAS 100B electrochemical analyzer. A BAS Model VC-2 electrochemical cell was employed. The three-electrode system consisted of GCE as working electrode, an Ag/AgCl reference electrode (Model RE-5, BAS), and a platinum wire auxiliary electrode. Since dissolved oxygen did not interfere with the anodic voltammetry, no deaeration was performed. The GCE working electrode was first hand polished using a polishing kit (BAS), then a uniform Nafion coating was achieved by covering the GCE surface of 0.07 cm^2 geometric area with 6 μl of Nafion at the desired concentration and spin-coated at 3000 rpm. The pre-anodization of both the GCE and NCGCE were performed in the respective supporting electrolyte. FTIR measurements were carried out with a Bruker (Karlsruhe, Germany) Equinox 55 spectrometer.

The general procedure for the UA detection is as follows. After modifying the GCE with Nafion of the desired composition, the NCGCE is pre-anodized at the desired pre-anodization potential for a given time. Then preconcentration is carried out at a specific potential just before detection. The NCGCE can be easily renewed by cleaning at +2.0 V for 30 s in 0.05 M citrate buffer solution (pH 5). The pre-anodization procedure itself can result in a renewed electrode.

Results and discussion

Electrochemical behavior

The square-wave (SW) voltammogram of 10 μM UA on the GCE in citrate buffer (pH 5) is illustrated in Fig. 1. The SW voltammetric method was chosen because it has been proved to be very sensitive in the determination of micromolar amounts of these analytes on carbon surfaces.[12] The electrochemical response of UA oxidation on a polished but non-pre-treated GCE is very weak and almost no peak is observed. On the other hand, the polishing and subsequent pre-treatment of the GCE by anodizing at +2.0 V gives a well defined peak at +0.47 V corresponding to UA oxidation. Note that the background current is also found to increase after pre-anodization. Similarly, the non-pre-treated NCGCE does not yield any peak but the pre-anodized electrode yields a very large peak, larger than that with the pre-anodized bare GCE. Even with the pre-anodized NCGCE the background current increases considerably. As the peak magnitude for NCGCE is higher than that for the bare electrode, the further work described here was restricted to this electrode alone. Of course, the modification of the Nafion film is mainly for the selective sensing of UA.

Linear sweep voltammetry was carried out to study whether adsorption or diffusion controls the electrode process. Linear plots were observed for peak current *versus* square root of scan rate, indicating that the charge transfer is a diffusion controlled process. However, the plots, instead of passing through the origin, yielded a negative intercept. Therefore, to infer more about the nature of the process, versatile chronocoulometric experiments were employed. The total charge obtained with this technique can be accounted for by three additive terms, which consist of the charge due to diffusing species, the double layer charging and the faradaic component due to the reduction of adsorbed component. If the process is a perfectly diffusion controlled process, the plot of Q *versus* $t^{1/2}$ is expected to pass through the origin without any intercept. On the other hand, if there is adsorption from the electroactive component there will be an intercept. Only an approximate value of the surface excess can be achieved by comparing the intercept of the plot obtained for a solution containing the analyte with the intercept for the same experiment performed with supporting electrolyte only. Reversed chronocoulometry was preferred to obtain the exact double layer charging component even in the presence of the adsorbed state.

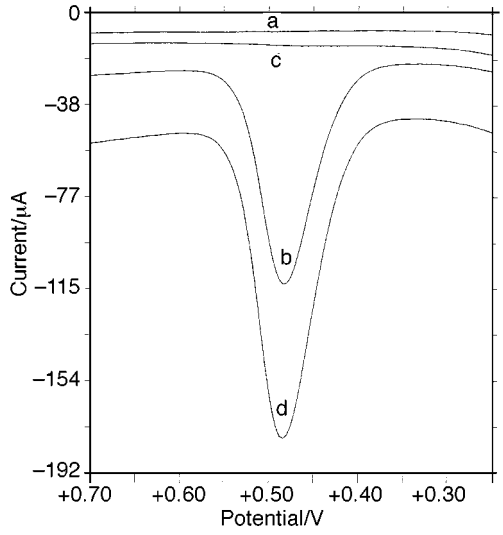

Fig. 1 SW voltammograms for 10 μM UA in 0.05 M citrate buffer (pH 5) at the GCE (a and b) and NCGCE (c and d) without (a and c) and with (b and d) electrochemical pre-treatment. Conditions: P_a = +2.0 V; t_a = 90 s; P_p = +0.3 V; t_p = 10 s. SW parameters: modulation amplitude, 40 mV; modulation frequency, 150 Hz; modulation step, 4 mV.

The chronocoulometric data for UA oxidation are given in Table 1 for NCGCE with and without pre-treatment. As can be seen, for the NCGCE without pre-treatment the forward and reverse intercepts are almost equal (0.57 μC in the forward step and 0.50 μC in the reverse step). This allows the important conclusion that the charge observed in the forward scan is simply due to the charging of the double layer and no adsorption of UA occurs on the non-pre-anodized NCGCE. For the pre-anodized electrode, the amounts of charge for these two plots are different. The double layer charging component is found to be 2.75 μC whereas the charge in the forward step is 4.01 μC. After subtracting the double layer charging component, we obtain an absolute charge of 1.26 μC due to the adsorbed component. From this charge, the surface coverage is calculated to be about 9.3×10^{-11} mol cm^{-2}, which is reminiscent of monolayer coverage.[17] These results indicate that, on the pre-anodized NCGCE, oxidation occurs in the adsorbed state in the initial stage of the charge transfer and diffusion sets in on top of that. It is very important to note that adsorption becomes possible only on the pre-anodized surface.

It is well known that the pre-anodization of a GCE has great influence on electron transfer reactions.[16] It is generally believed that the pre-anodization followed by cathodization for a very short time introduces >C=O functional group on the surface,[21,26,30] which can mediate electron transfer reactions at the interface.[32] There are also other models that suggest porous electrode surface formation even to the extent of multi-layers, apart from >C=O formation on the surface, with the amount and the size of the pores depending on the pre-anodization potential.[33] Hence there is a considerable increase in electroactive area and also the bare electrode behaves as a polymer-modified electrode with diffusion complications because of these multi-layers. Recent X-ray photoelectron spectroscopic studies on exclusively pre-anodized electrodes also confirm this.[30] Of the total amount of surface functional groups generated on pre-anodization, 35% is occupied by >C=O groups. Most important, this study indicates that the surface formed >C=O groups will be reduced and oxidized, slowly giving rise to large background current. Overall, there are two possible ways to induce such phenomenon. First, the slow oxidation and reduction of the >C=O functional group can induce a large background current. Second, the introduction of polar functional groups at the surface and accompanying ion incorporation in the inner Helmholtz plane would contribute to the increase in the double-layer capacitance of the GCE and hence the increase in background current.[21] Note that the increased background current in the present investigation has a phenomenological analogue to these reports.

Nagaoka and co-workers[22,23] studied the redox reaction of adsorbed catechol on a pre-anodized surface and found three redox pairs, contrary to the observation of a single peak on a highly ordered pyrolytic graphite electrode. They explained that, apart from the normal adsorption peak, other peaks arise

Table 1 Chronocoulometry at the NCGCE with and without electrochemical pre-treatment. Experimental conditions: E_i = +0.2 V; E_f = +0.9 V; pulse, 250 ms; 10 s of preconcentration at open circuit. Intercepts were obtained from Anson plots

Electrolyte	Intercept*/μC	Intercept†/μC
Citrate buffer (pH 5)	0.48	−0.46
Citrate buffer (pH 5)‡	2.76	−2.58
10 μM UA in citrate buffer (pH 5)	0.57	−0.50
10 μM UA in citrate buffer (pH 5)‡	4.01	−2.75

* Anodic response. † Cathodic response. ‡ Electrochemical pre-treatment conditions: P_a = +2.0 V; t_a = 90 s.

due to the redox process of catechol adsorbed on the micropore surface. The peak potential differences may arise from the structural variations of the adsorption sites. In the present investigation, however, no additional peak was observed. It is therefore believed that the increased current is not due to an increased surface area with a porous structure but to some chemical interaction between the >C=O groups and the UA. It is also important to note that the peak potential is not shifted for UA but only a peak current increase is observed, showing that the electron transfer rate is not altered on pre-anodization. Instead, the availability of UA near or on the electrode surface has been increased by the presence of >C=O groups on the surface.

One possible reason for the preferential adsorption of UA on the pre-anodized electrode could be the positive chemical interaction between the UA and the surface produced >C=O. We strongly emphasize chemical interaction because although the oxidized surface has energetically different sites on the surface, UA yielded only one peak, unlike the multiple peaks observed for catechol oxidation where physical interaction was postulated.[22,23] One possible chemical interaction could be the hydrogen bonding between the more acidic –H of the UA and the >C=O groups present on the surface, as shown in Fig. 2. Note that we have already observed hydrogen bonding of UA in poly-4-vinylpyridine film in a neutral medium and reported that such bonding can lead to loading of UA into the neutral PVP.[14]

The existence of hydrogen bonding was confirmed by FTIR spectroscopy. The reflection mode FTIR spectrum of UA in KBr shows absorption bands for N–H stretching at 3139, 3011 and 2819 cm^{-1}, whereas for UA adsorbed on the pre-anodized bare GCE there are absorption bands for N–H stretching at 2967, 2933 and 2863 cm^{-1}. It should be noted that the values of the two high energy absorption bands are considerably lowered for UA adsorbed on the pre-anodized GCE owing to hydrogen bonding. Unfortunately, it is very difficult to probe the hydrogen bonding at the pre-anodized NCGCE by reflective FTIR owing to the presence of the Nafion film. We attribute the peak enhancement in this case also to the increase in UA accumulation by hydrogen bonding, although an increase in permselectivity by the pre-anodized NCGCE cannot be ruled out, as discussed below.

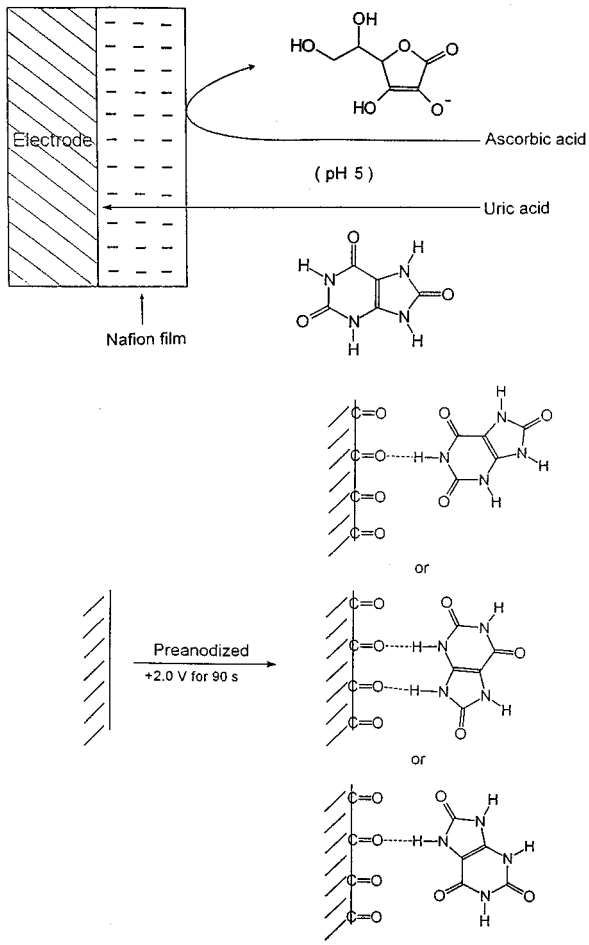

Fig. 2 Reaction scheme for the pre-anodized NCGCE in UA detection.

Effect of pH *on the oxidation of UA*

The pH range is very critical for the voltammetric characteristics of UA and also as Nafion film, so the effect of pH was studied in detail. The solution pH has a great influence on the UA oxidation on the pre-anodized NCGCE, by altering both the peak current and the shift in peak potential. The peak potential for UA oxidation shows a linear variation with pH and is shifted to more negative potentials with a slope of −60 mV pH^{-1}, suggesting that the total number of electrons and the protons taking part in the charge transfer is the same. As UA oxidation is known to occur by a two electron transfer, the number of protons involved is also predicted to be two. The peak current shows very interesting non-linear characteristics with pH, as illustrated in Fig. 3. The peak current remains almost constant in the pH range 1–4 (region I), increases very steeply between pH 4 and 5 (region II) and decreases at higher pH (region III). In the pH range 1–4, UA exists as a neutral molecule since its pK_a is around 5.4. In region I, since the dissociation constant is far from the pH values, the –H ionization (*i.e.*, the acidic character of UA) will be negligible. Hence the hydrogen bonding between the –H of UA and the >C=O groups on the electrode surface will be very weak, which leads to a very small amount of UA on the surface and hence a very small peak current is observed in this region. In the same way, the large current spike at pH values very close to the dissociation constant can be understood. In region II, the –H is almost on the brink of ionization at these pH

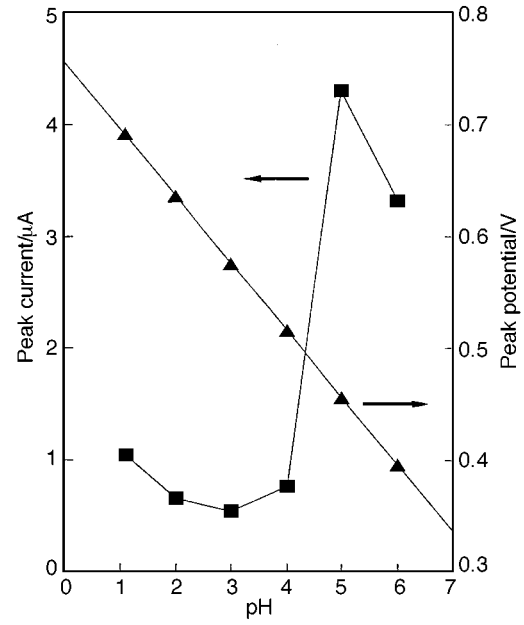

Fig. 3 Dependence of the peak current and peak potential on pH in SWV for 20 μM UA at the NCGCE with electrochemical pre-anodization treatment. Conditions: P_a = +2.0 V; t_a = 30 s; P_p = +0.2 V; t_p = 10 s. SW parameters: modulation amplitude, 25 mV; modulation frequency, 15 Hz; modulation step, 4 mV.

values, hence the acidic character will be the highest for UA. Hydrogen bonding will be more feasible between UA and the >C=O groups, leading to a large amount of UA on the surface and a large peak current is observed. Similarly, the decrease in the peak current in solutions of pH higher than the dissociation constant is accountable in the same way. Because UA is ionized at these pH values and exists in anionic form, almost no UA can be exchanged into the Nafion film.

The observed trend can also be explained as follows. Following anodization the film has been depleted of cationic species and especially of protons. The measurements at different pHs are indicative of a permselective membrane. Below pH 4, protons predominate over UA in solution. Between pH 4 and 5, UA becomes the predominant source of protons for the film and therefore a large increase in the current signal is observed as UA permeates the film. Above pH 6, UA is essentially ionized and therefore unable to enter a predominantly anionic film and therefore the current begins to fall. However, note that the effect of the increase in permselectivity by Nafion on the sensitivity is minor, as indicated in Figs. 1(b) and (d).

Optimization of parameters for detection

To arrive at the optimum conditions for UA detection on the pre-treated NCGCE, both the electrode conditions and the SW parameters must be optimized. As far as the electrode conditioning is concerned, the pre-anodization potential (P_a) and the Nafion composition were considered. The effect of P_a on the NCGCE is illustrated in Fig. 4(A). The peak current is constant up to +1.6 V and increases further very steeply to +2.2 V. The literature shows that the irreversible oxidation of GCE starts around +1.2 V, introducing the >C=O functional groups proportionately on the surface. The steep increase in the peak current can thus be understood by a substantial chemical interaction between the UA and the large numbers of >C=O groups present. The saturation of the peak current beyond +2.2 V could be due to saturation of >C=O production on the surface during the pre-treatment.[32] The pre-anodization time (t_a) also has a great influence on the peak current, as illustrated in the Fig. 4(B). The peak current increases linearly with pre-anodization time and shows saturation at longer times. From the above results, P_a = +2.0 V is fixed for the sensitive detection of UA on the pre-anodized NCGCE. The Nafion concentration was varied as another electrode parameter while the maximum peak current was observed for a 4% m/v solution as shown in Fig. 5. An increase in Nafion concentration obviously increases the film thickness, and hence the ion-exchange capacity. However, a too thick film may render the mass transfer difficult and result in a decrease in the current response.

The other major factors that should be considered are the preconcentration potential (P_p), the preconcentration time (t_p) and the SW parameters. The effect of P_p is illustrated in Fig. 6(A), where P_p was varied from +0.8 to −0.8 V. The peak current remains almost constant in the potential range from +0.8 to +0.5 V. Obviously in this potential range any UA that arrives at the electrode surface during the preconcentration process will be oxidized and there will be no accumulation. There is a sudden increase in the peak current at +0.4 V and a decrease on further increase in P_p. The decrease in the peak current probably occurs because at these negative potentials the >C=O groups are electrochemically reduced and their abundance on the surface declines substantially. Hence the optimum P_p should be +0.3 V for the sensitive determination of UA. A plot of t_p *versus* peak current is illustrated in Fig. 6(B), where the peak current initially increases and at about 30 s it attains saturation. For convenience, a t_p of 10 s was chosen for all subsequent electrochemical measurements.

Using the pre-anodization conditions mentioned above, the effect of the SW response on the pre-anodization was studied since the peak current for UA obtained in SW voltammetry is dependent on various parameters, such as SW amplitude, SW frequency and step height. The peak current initially increases with increase in SW amplitude and reaches a maximum around 40 mV. Hence this value was fixed as the optimum limit in the detection of UA. Similarly, the effect of SW frequency was studied and 150 Hz was found to be the optimum. We studied the effect of step width and interestingly it showed little influence on the peak current. Overall, the best signal-to-background current characteristics can be obtained with the following instrumental settings: modulation amplitude, 40 mV; modulation frequency, 150 Hz; and modulation step, 4 mV.

Analytical characterization

First the interference effect was studied. Various possible interfering substances, such as urea, purine, glucose, sucrose and AA, were examined for their effect on the determination of

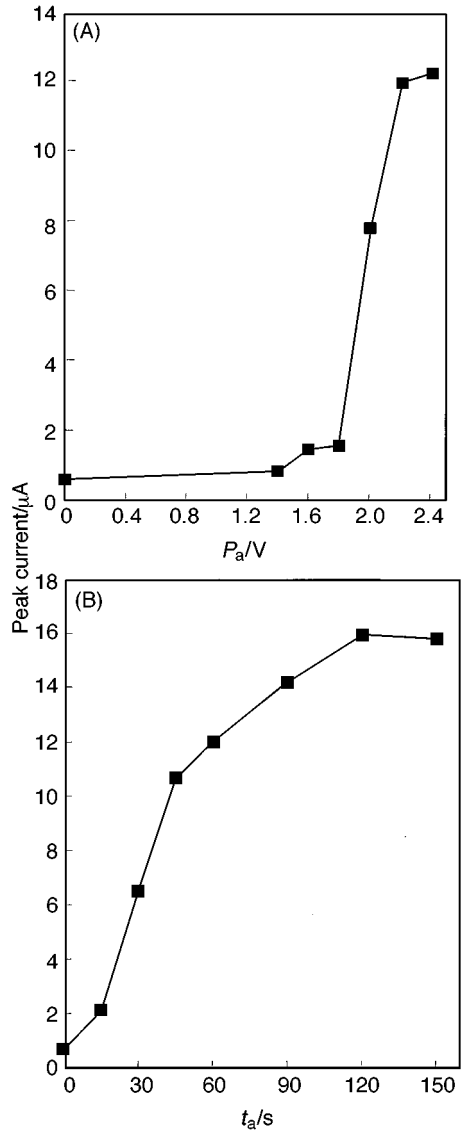

Fig. 4 Effects of (A) P_a and (B) t_a at the NCGCE on the SW voltammetric response of 20 μM UA. Conditions: (A) P_p = +0.2 V; t_p = 10 s; t_a = 30 s; and (B) P_p = +0.2 V; t_p = 10 s; P_a = +2.0 V. SW parameters as in Fig. 3.

1 μM UA under the optimum conditions. There was no substantial change in the peak current. The data are summarized in Table 2. It is well known that the UA and AA co-exist in many biological samples. As AA is a serious interferent, its effect was investigated in detail. Under the optimum conditions, the SW voltammograms obtained for 10 μM UA with and without the 300 μM of AA indicated almost no change in peak current. In fact, the tolerance limit was around 0.5 mM. Although the oxidation potentials of UA and AA are close, AA is not detected because of the tuning of the electrode surface to work at its best selectivity at pH 5. As the pK_a value of AA is 4.1, it exists as an anion at pH 5 and is unable to enter Nafion film. Therefore, it is very reasonable to expect no interference for UA in the presence of AA at this pH.

Linear calibration curves were obtained over the ranges 0.1–3 and 5–10 μM in citrate buffer solution (pH 5) (Fig. 7). The slope (μA μM^{-1}) and correlation coefficient are 23.71 and 0.996 over the range 0.1–3 μM and the detection limit (S/N = 3) is as low as 10 nM. Note that the detection limit is at least two orders of magnitude lower than those for most of the other methods. To characterize the reproducibility of the method, repetitive measurement–regeneration cycles were carried out in 10 μM UA. To characterize the reproducibility of the Nafion-coated GCE, repetitive measurement–regeneration cycles were carried out in 10 μM UA. The results of 15 successive measurements showed an RSD of $< 2\%$.

Since the acceptable tolerance of AA concentration for the determination of UA is as high as 0.5 mM, the method is applicable to the direct analysis of urine samples. As shown in Table 3, three human urine samples from laboratory personnel were analyzed for UA content with the method presented above. In order to fit into the linear range, all the samples used for detection were diluted 5000-fold. The dilution process can actually help in reducing the matrix effect of real samples. In order to ascertain the correctness of the results, the samples were spiked with certain amounts of UA at about the same concentrations as found in the samples themselves. The recoveries for the spiked urine samples were good, between 102.0 and 99.5%.

Conclusions

The combination of pre-anodization to increase the sensitivity and Nafion film modification to improve the selectivity was found to work excellently in UA detection. The functional groups generated on the GCE surface during pre-anodization undergo chemical interaction with the UA, probably through hydrogen bonding, and adsorption is facilitated. The electrochemically pre-anodized NCGCEs can be applied to the

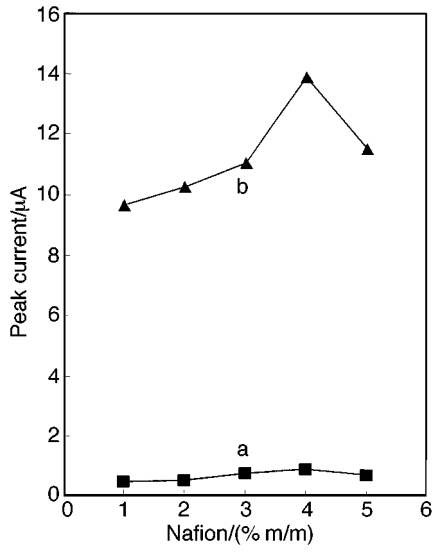

Fig. 5 Effect of film thickness at the NCGCE without (a) and with (b) electrochemical pre-treatment on the SW voltammetric response of 50 μM UA. Conditions as in Fig. 3.

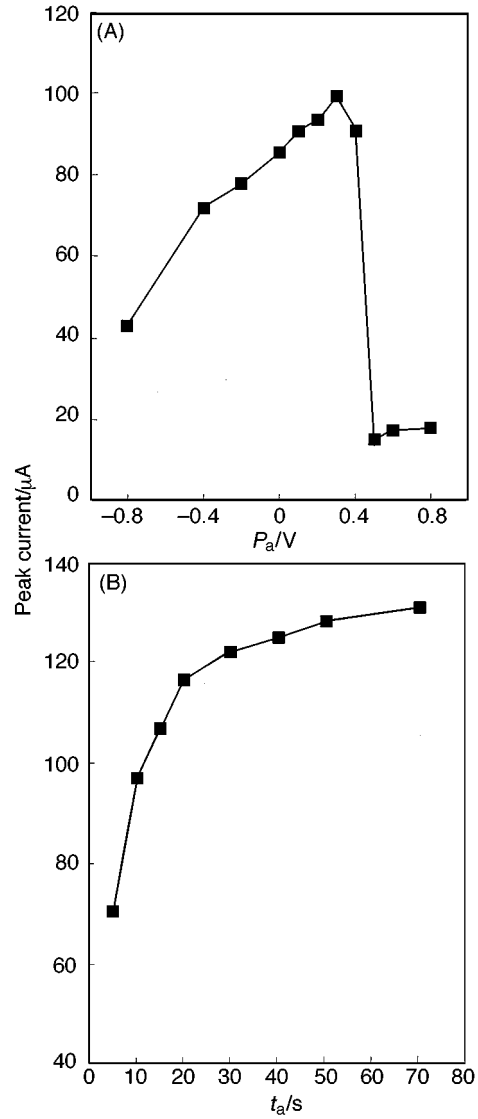

Fig. 6 Effects of (A) P_p and (B) t_p on the peak current in SWV for 10 μM UA at the NCGCE. Conditions: (A) P_a = +2.0 V; t_a = 90 s; t_p = 10 s; and (B) P_a = +2.0 V; t_a = 90 s; P_p = +0.3 V. SW parameters: modulation amplitude, 40 mV; modulation frequency, 150 Hz; modulation step, 4 mV.

Table 2 Influence of potential interferents on the response of UA. UA concentration = 1 μM

Interferent	Concentration/ μM	Signal change (i_{UA} = 100%)
AA	10	+0.93
	50	−1.12
Urea	50	−1.83
Purine	10	−2.83
Cytosine	10	−2.42
Cysteine	10	−3.07
Glucose	1	−5.42
Oxalate	1	−2.63
Adenine	1	−2.18

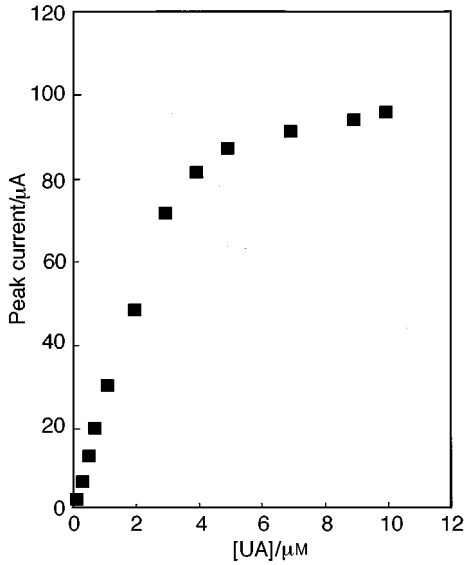

Fig. 7 Dependence of the SWV peak current on the concentration of UA. Conditions P_a = +2.0 V; t_a = 90 s; P_p = +0.3 V; t_p = 10 s. SW parameters as in Fig. 6.

Table 3 Determination of UA in three urine samples with the NCGCE. Number of samples assayed was five

Parameter	Urine 1	Urine 2	Urine 3
Original value (ppm)	0.122 ± 0.003	0.077 ± 0.002	0.115 ± 0.002
Spike (ppm)	0.034	0.034	0.034
Recovery (%)	100.7 ± 0.8	102.0 ± 0.5	99.5 ± 0.7
Total value (ppm)*	610.2 ± 14.5	383.7 ± 10.9	575.2 ± 11.4

* Total value obtained by multiplying the detected value with the dilution factor of 5000.

detection of UA in urine samples. The recovery of spiked UA in urine samples was good. More importantly, the pre-anodized electrodes can be easily regenerated and the interference of AA can be easily avoided.

The authors gratefully acknowledge financial support from the National Science Council of the Republic of China under Grant NSC 87-2113-M-005-020.

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Paper 8/01532E
Received February 23, 1998
Accepted April 16, 1998