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A novel electrochemical biosensor for the detection of uric acid and adenine

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1. ABSTRACT

A novel electrochemical biosensor for the detection of uric acid and adenine was prepared based on a gel containing multi-walled carbon nanotubes and room-temperature ionic liquid of 1-octyl-3-methylimidazolium hexafluorophosphate. The electrochemistry of uric acid and adenine was studied in this gel modified electrode. There was a significant two-way electrocatalytic activity upon both oxidation and reduction of uric acid. Similar to a bare glassy carbon electrode, uric acid undergoes a $2e,2H^+$ oxidation in phosphate buffer in the modified electrode. A diimine, the oxidation product of uric acid, was found to be an unstable intermediate, which was converted by a follow-up hydration reaction to an imine alcohol, with the reaction rate constant of 8.5 +/- 0.3 $M^{-1}\cdot s^{-1}$ according to Nicholson's theory. Under optimum conditions, linear calibration graphs were obtained over the concentration range of 1.0×10^{-7} M ~ 1.0×10^{-5} M (uric acid) and 1.0×10^{-5} M ~ 6.0×10^{-4} M (adenine). Based on the signal-to-noise ratio of 3, the detection limits of the current technique was found to be as low as 9.0×10^{-8} M (uric acid) and 2.0×10^{-6} M (adenine), respectively. This novel biosensor was successfully applied for the assay of uric acid in human urine. Because of its good stability and long-term durability, such a gel modified electrode can provide a simple and easy approach for sensitive detection of uric acid and adenine.

2. INTRODUCTION

Uric acid arises within physiological fluids as a result of various biochemical processes involving purine degradation. It has long been acknowledged as a key interferent in the application of electrochemical techniques to the analysis of physiological fluids (1,2). Therefore, the analytical value of detecting uric acid has often been overlooked but more recent clinical investigations have revealed that the purine is a key player in a number of metabolic processes that are of considerable diagnostic significance (3-7). Uric acid is present in human serum or in urine only in extremely small amounts. The typical concentrations of uric acid within serum and urine normally reside in the 0.1-0.4 mM and 1.2-2.4 mM range, respectively (8). Substantially increased uric acid levels have been recognized as a symptom of many diseases. While commonly regarded as an indicator of gout, current interest in metabolic syndrome has identified urate (the salt of uric acid) as a versatile handle through which the progress of cardiovascular diseases (6), kidney diseases (9-11) and a number of diabetic complications can be gauged (12). On the contrary, in February of 2005, the researchers in Thomas Jefferson University found that increasing levels of uric acid might help cut some of the potentially devastating "secondary" cellular damage that occurs following a spinal cord injury. This new finding may lead to new treatments for such injuries (13). Consequently, the

significance of developing a method for the assay of uric acid in human urine or blood with high precision and accuracy is highly topical.

The mechanistic and kinetic intricacies of various substitutions upon the purine base have been extensively explored and reviewed (14). Recently, Davis et al reviewed the strategies for improving the detection of uric acid (15), the technologies used for its detection and also those previously employed for its removal are reviewed with the aim of highlighting how the seemingly contrasting approaches are evolving to aid the development of new sensing devices for clinical analysis. New attentions have been focused on all kinds of modified electrode with excellent electrochemical properties (16-21). The typical values of detection limit are 0.1 to 1.0 μM by modifying electrode, it even can get as low as 1 nM by means of sol-gel or polymer film method.

Adenine, a purine base (nitrogenous base), is one of the most important organic molecules for life as we know it today. It is an integral part of DNA, RNA, and ATP. Its primary end-product is uric acid, from the catabolism of dietary and endogenous nucleic acid. Direct electrochemical detection of adenine is studied at copper (22), mercury (23,24) and other kinds of modified carbon (25-27) electrodes. The detection limit of adenosine is about micromole level by means of fast scan voltammetry (26,27).

Carbon nanotubes (CNs) can form gels when mixing them with imidazolium ion-based room-temperature ionic liquid (RTILs) by grinding (28). Several scientists have developed the excellent electrocatalytic properties of such a gel in the redox behavior of different biomolecules (29). Our group has been involving in the development of chemically modified electrode based on CNs and RTILs. For example, the multi-walled carbon nanotubes (MWNTs) gel of 1-butyl-3-ethylimidazolium hexafluorophosphate was coated on a glassy carbon electrode, where the direct electrochemistry of proteins was studied. The preliminary investigation has demonstrated that such a gel electrode is thermal stable with high conductivity, and that the proteins adsorbed on the electrode can still retain their activities (30). We have also reported the selective detection of dopamine in the presence of ascorbic acid and uric acid at another multi-walled carbon nanotubes gel modified electrode of 1-octyl-3-methylimidazolium hexafluorophosphate (31). The oxidation peaks of dopamine, ascorbic acid and uric acid in their mixture can be well separated since the peak potential of ascorbic acid is shifted to more negative values, while that of uric acid is shifted to more positive values due to the modified electrode. As a result, dopamine can be determined in the presence of uric acid and more than 100 times excess of ascorbic acid. The modified electrode has been successfully applied for the assay of dopamine in human blood serum as well. This kind of modified electrode provides a platform for fabrication of biosensors, which shows promising application to detect various biomolecules.

Here, we describe a cyclic voltammetric and differential pulse voltammetric studies of uric acid and adenine at different modified electrodes. Comparing with bare glassy carbon electrode and MWNTs modified glassy carbon electrode, the MWNTs-ionic liquid gel modified electrode shows more excellent electrocatalytic properties. Hence both uric acid and adenine are highly sensitively detected.

3. MATERIALS AND METHODS

3.1. Materials

MWNTs were produced by catalytic chemical vapor deposition (CCVD) method, and provided by the Department of Chemical Engineering of Tsinghua University of China as gifts. The details of synthesis were reported elsewhere (32,33). The purity of the MWNTs is about 99%. The ionic liquid of 1-octyl-3-methylimidazolium hexafluorophosphate ($OMIMPF_6$) was synthesized according to the procedures described in the references (34,35). The $OMIMPF_6$ has been characterized by 1H NMR and IR, and its purity was proven to be very high. Uric acid was purchased from Merck. Adenine sulfate was purchased from Acros. Water was triply distilled with a quartz apparatus. Highly purified nitrogen was used for deaeration. All other reagents were of analytical grade. The human urine samples were obtained from healthy people and were diluted 10 times with 0.1 M phosphate buffer (pH 7.08) before using.

3.2. Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a CHI 660 electrochemical workstation (Shanghai, China). The working electrode was a glassy carbon electrode or a modified glassy carbon electrode, the auxiliary and reference electrodes were platinum wire and saturated calomel electrode (SCE), respectively.

3.3. Preparation of gel modified electrode

The gel was got by grinding 12 mg MWNTs and 0.2 mL $OMIMPF_6$ with an agate mortar for about 20 min, and it would be available for at least three months. The multi-walled carbon nanotubes-ionic liquid gel modified glassy carbon electrode (denominated as MWNTs-IL-Gel/GCE in this paper) was fabricated as described before (31). As the thickness of the modified layer has great effect on the electrochemical properties of MWNTs-IL-Gel/GCE, it was carefully controlled to be consistent during each explore. All voltammograms of MWNTs-IL-Gel/GCE were recorded after reaching equilibrium within the tested aqueous solution.

3.4. Electrochemical measurements

The buffer and sample solutions were purged with highly purified nitrogen for at least 5 minutes prior to the experiments. Nitrogen atmosphere was maintained over the solutions during the experiments. All experiments were carried out at room temperature (18 ± 2°C).

4. RESULTS AND DISCUSSION

4.1. Electrochemical characteristics of uric acid at MWNTs-IL-Gel/GCE

As shown in Figure 1a, in the 0.1 M phosphate buffer (pH 7.08), no redox peak appears at the bare glassy

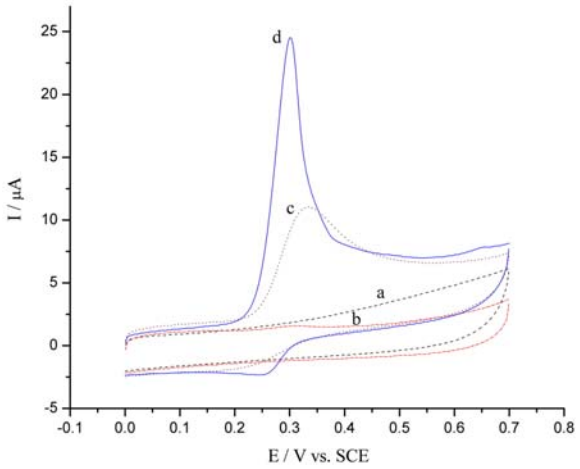

Figure 1. Cyclic voltammograms of 0.1 M phosphate buffer solution (pH 7.08) in the absence (a,b) or presence (c,d) of 0.2 mM uric acid at bare glassy carbon electrode (a,c) or MWNTs-IL-Gel/GCE (b,d). Scan rate: 0.05 V/s.

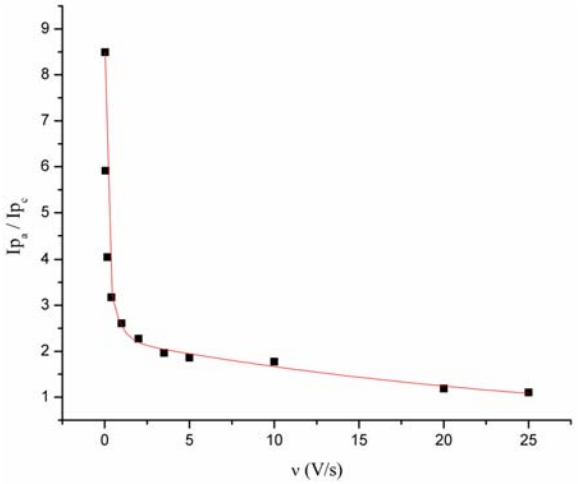

Figure 2. Relationship between Ip_a/Ip_c and scan rate ν. Other conditions as in Figure 1d.

carbon electrode. When 0.2 mM uric acid was added into the solution, there is a quasi-reversible electrochemical reaction process of uric acid, and the reduction peak is not very obvious compared with the oxidation peak (Figure 1c). Based on Dryhurst's result (14), it should be due to a following-up reaction occurring with the electrochemical process. When using the method of drop-coating 1.5 mg of MWNTs dispersed in 1 ml triply distilled water on the glassy carbon electrode, it shows a strong adsorptive activity towards uric acid, which is consistent with the previous report (36). The charging currents are enhanced greatly and the peak current is also depended on the preconcentration time. Thus, it shows almost the same electrochemical behaviors of uric acid at this MWNTs electrode as at the bare glassy carbon electrode.

However, at MWNTs-IL-Gel/GCE, the charging currents trend to be flat in the phosphate buffer (Figure 1b). In the presence of uric acid, the peak currents are much larger than those at the bare glassy carbon electrode, and an obvious reduction peak appears at the potential of 0.26 V (Figure 1d). The oxidation peak potential shifts more negatively. Meanwhile, the difference of peak potentials (ΔEp) is ca. 40 mV, which has been decreased about 100 mV compared with the bare glassy carbon electrode. It can be concluded that such a gel electrode may have a significant two-way electrocatalytic activity upon both oxidation and reduction of uric acid.

The redox peak currents increase linearly with the square root of the potential scan rate in the range from 0.01 to 0.6 V/s. After washing the electrode with a large amount of triply distilled water and afterwards putting it in a blank solution (0.1 M phosphate buffer), the peak current disappeared. This result shows that uric acid is hardly adsorbed at the surface of this gel modified electrode and that the electrode reaction is controlled by the diffusion of uric acid in the solution.

The oxidation peak of uric acid is well behaved in 0.1 M phosphate buffer solution in the range of pH 2.60-10.60. The relationship between the oxidation peak potential and the pH was directly investigated and a linear regression equation for $Ep_a = 0.74 - 0.061$ pH (Ep_a, V; correlation coefficient, $r = 0.9986$) was obtained, which showed that in the process of electrode reaction the uptake of electrons should be accompanied by an equal number of protons.

In order to get a better resolution among the voltammograms, differential pulse voltammetry (DPV) was employed. A $W_{1/2}$, width of the peak at half height in DPV curve, was measured as 52 mV with the parameters setting of increasing $E = 4$ mV and amplitude = 20 mV, respectively. Based on the equation $W_{1/2}=3.52RT/nF$ (37), n was calculated as 1.7, which indicates that uric acid is undergoing two-electron oxidation during the process occurring at the modified electrode. As shown in Figure 2, the ratio of oxidation peak current (Ip_a) to reduction peak current (Ip_c) first decreased greatly, then gradually approaches to 1, with the increasing of scan rate. It shows the electrode reaction is sill accompanied by a similar following-up reaction to the case at the bare glassy carbon electrode.

In summary, uric acid is first converted to the reactive dimine species through a $2e,2H^+$ process. Increasing scan rate results in the re-reduction of the dimine before nucleophilic attack from water and the production of the imine alcohol. After the addition of a second water molecule, there is the final intra-molecular degradation to allantoin (15). The basic scheme is outlined within Figure 3.

Based on Nicholson's theory (38), for the case of charge transfer followed by an irreversible chemical reaction, the ratio of oxidation to reduction peak currents was constant for a constant value of the parameter $k_f\tau$, where k_f and τ stand for the chemical reaction rate constant and the time in seconds from $E_{1/2}$ to the switching potential, respectively. They constructed a working curve for the ratio

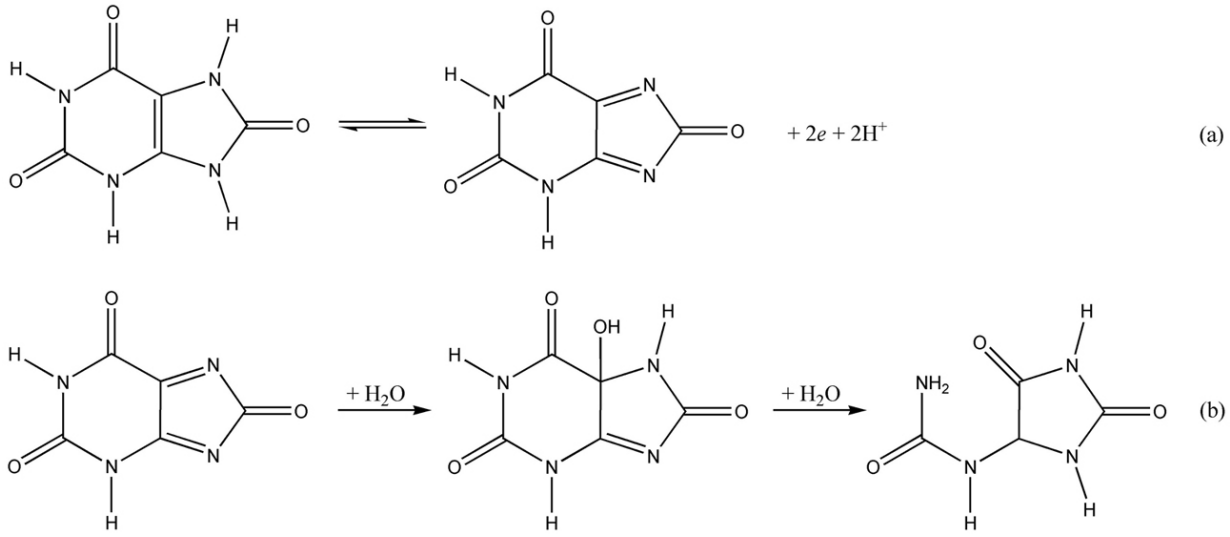

Figure 3. The reaction mechanism for the redox process of uric acid.

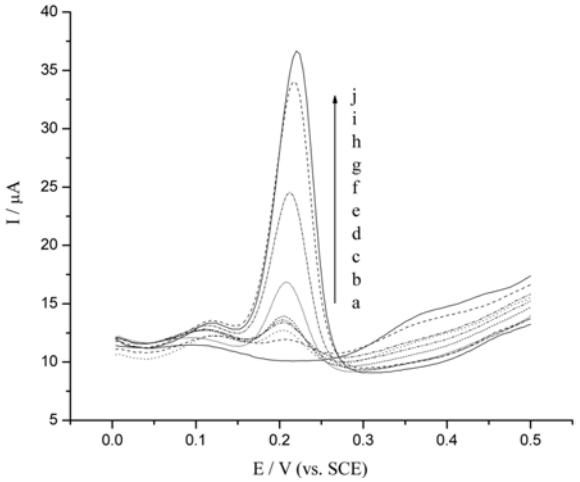

Figure 4. Differential pulse voltammograms with correction of background currents for different concentrations of uric acid at MWNTs-IL-Gel/GCE. The concentration of uric acid (μM): (a)0; (b) 0.1; (c) 0.3; (d) 0.5; (e) 0.7; (f) 1.0; (g) 3.0; (h) 5.0; (i) 7.0; (j) 10.0. Scan rate 0.02 V/s.

of peak currents Ip_a/Ip_c as a function of $k_f\tau$. If $E_{1/2}$ is known, a rate constant can be calculated from a single cyclic voltammogram. It should be noted that in Nicholson's system the reactant of follow-up chemical reaction is the product of reduction reaction, which is different from this system. As the $E_{1/2}$ value can be estimated from the mean of oxidation and reduction potential, the rate constant k_f for the follow-up hydration reaction was calculated as 8.5±0.3 $M^{-1}\cdot s^{-1}$.

4.2. Sensitive detection of uric acid at MWNTs-IL-Gel/GCE

An enhanced peak current can be used to improve the detecting sensitivity for uric acid. As shown in Figure 4, a series of well-defined DPV peaks for uric acid are obtained. After the correction of background currents, the detection limit of uric acid is ca. 9.0×10^{-8} M in neutral solution, and linear calibration graphs were obtained over the uric acid concentration range 1.0×10^{-7} M to 1.0×10^{-5} M. The linear equation is $Ip_a = 1.41 + 2.56$ C (Ip_a, μA; C, μM; correlation coefficient, $r = 0.9977$). All measurements were carried out at least three times with good reproducibility (R.S.D. ≤3%, $n = 5$). Compared with the detection limit of uric acid at the bare glassy carbon electrode, which is 1.0×10^{-4} M, the one at MWNTs-IL-Gel/GCE has been improved over 1000 times.

4.3. Sensitive detection of adenine at MWNTs-IL-Gel/GCE

As shown in Figure 5a, there is an oxidation peak of adenine at about 1.08 V at the bare glassy carbon electrode. At MWNTs-IL-Gel/GCE, the peak current is much larger (see Figure 5b) and the peak potential is shifted to more negative value (from 1.08 V to 0.99 V). The oxidation peak currents increase linearly with the scan rate in the range from 0.01 to 0.6 V/s, which shows that the electrode reaction is controlled by adsorption. In the range of pH 3.80-10.00, the relationship between the oxidation peak potential and pH was investigated and a linear regression equation for $Ep_a = 1.28 - 0.052$ pH (Ep_a, V; correlation coefficient, $r = 0.9867$) was obtained, which indicated that the uptake of electrons should be accompanied by an equal number of protons.

The detection limit of adenine is ca. 2.0×10^{-6} M in neutral solution at MWNTs-IL-Gel/GCE by means of DPV, which is about 20 times lower than the one at bare glassy carbon electrode. The linear calibration graphs were obtained over the adenine concentration range 1.0×10^{-5} M to 1.0×10^{-4} M and 1.0×10^{-4} M to 6.0×10^{-4} M. The linear equations are $Ip_a = 0.21 + 9.59$ C (Ip_a, μA; C, μM; correlation coefficient $r = 0.9974$) and $Ip_a = 0.57 + 5.85$ C (Ip_a, μA; C, μM; correlation coefficient $r = 0.9994$),

Table 1. Experimental results for the determination of uric acid in human urine.

Sample No.	UA Spiking (10^5 M)	UA found (10^5 M)	Recovery
1	—	2.22	—
2	2.0	1.97	98.6%
3	2.0	1.98	99.2%
4	2.0	2.07	103.7%
Mean			100.5%

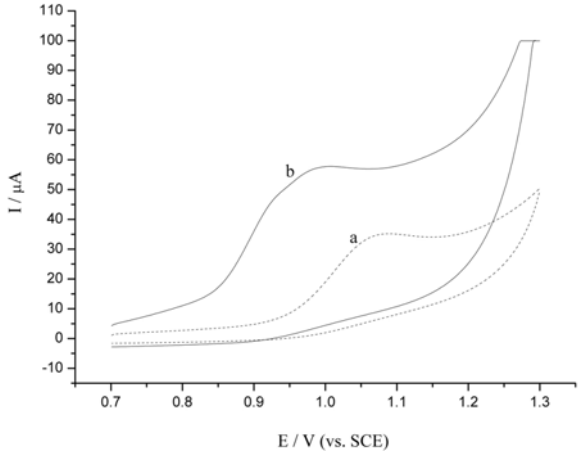

Figure 5. Cyclic voltammograms of 0.2 mM adenine in 0.1 M phosphate buffer solution (pH 7.08) at (a) bare glassy carbon electrode and (b) MWNTs-IL-Gel/GCE. Scan rate: 0.05 V/s.

respectively. All measurements were carried out at least three times with good reproducibility (R.S.D. ≤3%, n = 5).

When using MWNTs modified electrode instead of MWNTs-IL-Gel/GCE, the peak potential of adenine is 0.87 V, which shows better electrocatalytic oxidative property to adenine than at MWNTs-IL-Gel/GCE. However, as to the other electrochemical characteristics, such as the stability of the modified electrode and the detecting sensitivity, MWNTs-IL-Gel/GCE shows more advantages.

4.4. A comparison between MWNTs/GCE and MWNTs-IL-Gel/GCE

As discussed above, when using MWNTs/GCE, the background currents are enhanced remarkably and it shows electrocatalytic oxidation characteristic to adenine. On the other hand, it shows little electrocatalytic oxidation characteristic to uric acid.

Comparing with MWNTs/GCE, the developed method of using MWNTs-IL-Gel/GCE for the sensitive detection of uric acid and adenine has more advantages. First, MWNTs-IL-Gel/GCE has prominent two-way electrocatalytic redox property to uric acid. The detection limit can be improved over three orders of magnitude, which can be used in sensitive detection applications such as the highly accurate elevating of uric acid levels variation. Meanwhile, it has electrocatalytic oxidation property to adenine and improves the detection limits as well. Second, the MWNTs-IL-Gel can be attached at the glassy carbon electrode directly by rubbing, which can form a well-proportioned modification. Such a fabrication method can be manipulated more easily and can save more operation time. On the contrary, the MWNTs cannot be attached directly at the surface of glassy carbon electrode by rubbing, and the drop-coating procedure may cause the injector being blocked easily. Third, it might be the most important. Due to the low vapor tension of the ionic liquid, the MWNTs-IL-Gel has good stability and long-term durability, it can be available for at least 3 months.

In a word, as a novel electrode-modifying material, the MWNTs-IL-Gel integrates the advantages of both MWNTs and ionic liquid, which is a new, simple and convenient method to detect uric acid and adenine with higher sensitivity. The combination of function-designable ionic liquid and carbon nanotubes having excellent electrochemical characteristics should be a very good platform for developing excellent and cheap biosensors for some biomolecules.

4.5. Analytical application

In human urine, uric acid was detected and its concentration was measured. The other substances in the urine sample, such as proteins, do not interfere with the determination of uric acid. In the 100 times diluted urine sample, the concentration of uric acid is measured as 2.22 mM, which is consistent with the normal result. The satisfactory results are shown in Table 1. Unfortunately, the detection of adenine in a real sample using this gel modified electrode could not get good results, which needs further investigation.

5. CONCLUSIONS

A simple, quick and sensitive electrochemical technique has been developed for the uric acid detection, which is based on the application of MWNTs-IL-Gel/GCE. Comparing with previous electrodes and measurements, the detection limit is lower, the electrode stability is better and the experiment procedure is more convenient and easier to be controlled. This technique has been used in the determination of uric acid in human urine with satisfactory result. The simplicity, stability and durability make MWNTs-IL-Gel/GCE a very good platform for biosensors. Moreover, combining the environment-friendly and designable ionic liquid with carbon nanotubes having excellent electrochemical characteristics may be a new idea for developing some novel and powerful biosensors.

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