Int. J. Electrochem. Sci., 12 **(2017)** 6742 – 6750, doi: 10.20964/2017.07.33

International Journal of
ELECTROCHEMICAL
SCIENCE
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Preparation of a Novel N,O-carboxymethyl Chitosan/Glycine-Modified Electrode and Electrochemical Determination of Uric Acid and Ascorbic Acid

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Received: 7 December 2016 / *Accepted:* 23 April 2017 / *Published:* 12 June 2017

A N,O-carboxymethyl chitosan/glycine composite (NOCC-Gly-GC) membrane-modified electrode was prepared by coating a mixture of N,O-carboxymethyl chitosan (NOCC) and glycine (Gly) onto the surface of a glassy carbon electrode. Moreover, cyclic voltammetry was employed to characterize the electrochemical behavior of uric acid (UA) and ascorbic acid (AA) on the modified electrode. The NOCC-Gly-GC electrode was catalytically oxidized significantly, and the effective electrode area was improved with respect to that of the glassy carbon electrode. However, Gly and carboxymethyl chitosan not only increased the electrode area and active sites but also accelerated the electron transfer. In phosphate buffer solution (pH 5.59), both AA and UA were sensitive to irreversible oxidation peaks with NOCC-Gly-GC electrode. A good linear relationship between oxidation peak current and concentration was observed. After the parallel determination of mixed liquid seven times, the relative deviation ratios of AA and UA were detected to be 2.1% and 3.6%, respectively. These ratios indicated that the NOCC-Gly-GC electrode possessed electrochemical stability and reproducibility. The modified electrode exhibited well-separated oxidation peaks for UA and AA, with a potential difference of 341 mV. Thus, both simultaneous and individual measurements for UA and AA can be established. We expect the method to hold wide applications in real sample determination.

Keywords: N,O-carboxymethyl chitosan; glycine; NOCC-Gly-GC electrode; uric acid; ascorbic acid

1. INTRODUCTION

Chitosan and its derivatives have found fruitful applications in biosensors [1–2]. These materials are abundant in amino and hydroxyl groups and are hence soluble. Particularly, N,O-carboxymethyl chitosan (NOCC) is a good biocompatible material. Uric acid (UA) is a metabolite of

purine nucleotides in animals, and abnormal UA levels signify disease. Ascorbic acid (AA) is a water-soluble essential vitamin for the human body. In vivo, the vitamin can protect the body from oxidative stress. AA and UA can be detected by UV enzyme determination, high-performance liquid chromatography, electrochemical method, biosensors, fluorescence, and chemiluminescence method [3–6]. Chromatography is the most basic detection method. It is easy to operate, involves a simple mobile phase, and separates components satisfactorily. However, each sample requires a multi-sample process and high costs. UV or fluorescence methods can be influenced by other chromophores in the sample; this interference thus complicates the operation. Although the enzymatic method exhibits excellent selectivity, its high cost limits its use. By contrast, the electrochemical method holds the advantages of simple operation, low cost, high sensitivity, and low detection limit. In recent years, given the interference of AA and UA in electrode detection, detection methods with improved efficiency have been demanded for synchronous detection [7–9]. Zhang [10] prepared Au/CMK-modified glassy carbon electrode. This electrode can be used to detect dopamine (DA), AA, and UA simultaneously by cyclic voltammetry. Bagheri [11] fabricated a Fe_3O_4–SnO_2–Gr nanocomposite into a carbon paste electrode for the simultaneous detection of AA, DA, and UA. Zhang [12] developed an electrochemical biosensor (RGO–ZnO/GCE) to determine AA, DA, and UA. Lavanya [13] fabricated an electrochemical sensor for the simultaneous determination of AA, UA, and folic acid and attained good results.

To our knowledge, the fabrication of a N,O-carboxymethyl chitosan/glycine composite (NOCC-Gly-GC) membrane-modified electrode has not been reported. UA and AA can be directly determined using a glassy carbon electrode through voltammetry. However, similar oxidation potentials lead to the overlap of the volt–ampere curve. As a result, mapping the volt–ampere curve is difficult [14]. To overcome the disadvantage of the glassy carbon electrode, we coated NOCC and glycine (Gly) onto the surface of a glassy carbon electrode and prepared NOCC-Gly-GC electrodes. The overlap of the CV curve of AA and UA can be divided; thus, AA and UA can be detected simultaneously. The NOCC-Gly-GC electrodes may provide a new method for drug detection and biological analysis.

2. EXPERIMENTAL PROCEDURE

2.1 Materials and equipment

The electrochemical workstation ZENNIUM was purchased from ZAHNER Company, Germany. The instrument was equipped with a saturated calomel electrode as reference electrode (RE), platinum electrode as auxiliary electrode (CE), and NOCC-Gly-GC electrode as working electrode (WE). Chitosan (deacetylation degree 96%) was purchased from Jin Hu Hengtai County Crustacean Products Co., Ltd. N,O-carboxymethyl chitosan was prepared as described previously [15,16]. Gly was purchased from China Pharmaceutical Group Chemical Reagent Co., Ltd. AA was obtained from Tianjin Hengxing Chemical Reagent Co. Ltd., and UA was acquired from Tianjin Kaitong Chemical Reagent Co., Ltd.

2.2 Experiment method

2.2.1 Treatment of glassy carbon electrode

A glassy carbon electrode was polished, followed by the application of 0.5–0.7 μm and 30–50 nm Al_2O_3 with suede upper. Then, the electrode was cleaned with 1:1 ethanol:distilled water.

2.2.2 Electrode modification

Carboxymethyl chitosan (0.1 g) and Gly (0.1 g) were fully dissolved in 10 mL of 1% acetic acid solution. The resulting solution was then dropped, coated, and dried. A NOCC-Gly-GC-modified electrode was prepared.

3. RESULTS AND DISCUSSION

3.1 Determination of AA and UA with different electrodes

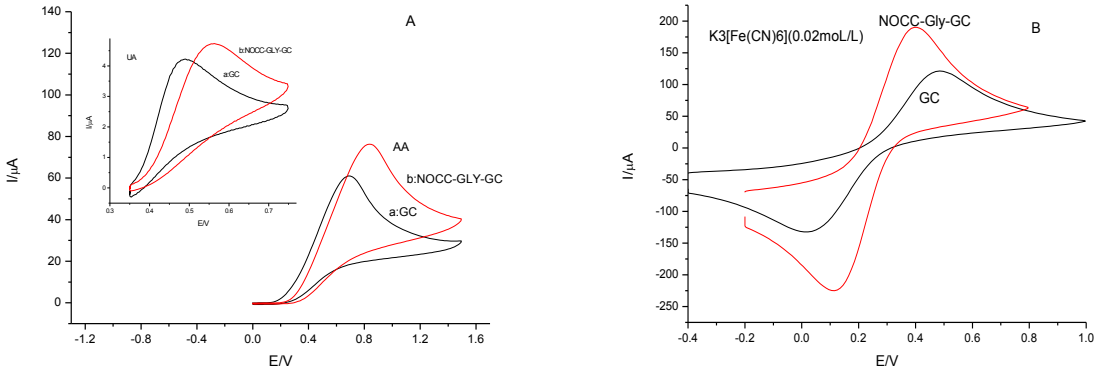

Figure 1. Cyclic voltammograms of AA, UA, $K_3[Fe(CN)_6]$ on GCE or NOCC-Gly-GC electrodes, pH = 5.59, UA (2.75×10^{-3} M), AA (0.005 M), $K_3[Fe(CN)_6]$ (0.1 M), and scan rates at 100 mV/s

AA, UA, and $K_3[Fe(CN)_6]$ were determined separately by a glassy carbon electrode, with NOCC-Gly-GC electrode as the working electrode. The measured peak current of the NOCC-Gly-GC-modified electrode was significantly higher than that of the glassy carbon electrode (Fig. 1A). The peak potential detected with the NOCC-Gly-GC-modified electrode shifted by 0.1518 V (AA) and 0.0719 V (UA) relative to those detected with the glassy carbon electrode. The $-NH_2$ and –COOH of NOCC molecules on the electrode surface can form strong hydrogen bonds between the carbonyl oxygen and the amino group of AA and UA molecules. These bonds help accelerate the electron transfer through the N···H···O and O···H···O hydrogen bond chains. Ultimately, catalytic oxidation is promoted. The modified electrode on AA and UA exerted a better catalytic effect, and the electrocatalytic reaction on the electrode was irreversible. For comparison, $K_3[Fe(CN)_6]$ was employed

as a probe to test the sensor properties of the NOCC-Gly-GC electrode as the glassy carbon electrode (Fig. 1B). Carboxymethyl chitosan and Gly improved the electrode area significantly and provided additional active sites. Furthermore, the two components accelerated the ion transfer of $K_3[Fe(CN)_6]$ and improved in electrochemical sensing properties. According to the Randles–Sevcik equation $i_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} \upsilon^{1/2} c$, larger peak currents result in larger effective electrode surface areas. The effective area of the glassy carbon electrode was calculated as 0.8121×10^{-6} cm^2, whereas that of the NOCC-Gly-GC electrode was calculated as 1.2922×10^{-6} cm^2.

3.2 Determination of AA and UA with the NOCC-Gly-GC electrode

3.2.1 Effect of different pH

The electro-oxidation of AA and UA depends on the solution pH. The peak current increased with increasing pH before pH 5.59 and then decreased for both AA and UA. This phenomenon may be due to the varying form of the compound with changes in solution pH. Thus, the interaction between modified films and the electrode substrate also changed. Both the carboxymethyl chitosan and amino groups on Gly carried positive charges at pH 5.59. These positive charges may accelerate the transmission of charge species in the film and then hasten the electrocatalytic reaction on the electrode, which was very similar to result that were already published in previous article [17]. The two dissociation stages of the AA were found with constants pK_{a1} = 4.04 and pK_{a2} = 11.34, whereas the dissociation constant of UA was determined as pK_a = 5.4. In the acidic solution of pH 5.59, AA and UA deprotonate and hence could be readily oxidized. This change consequently accelerates the electrocatalytic reaction. Therefore, in the following experiments, the pH of the buffer solution was selected to be 5.59.

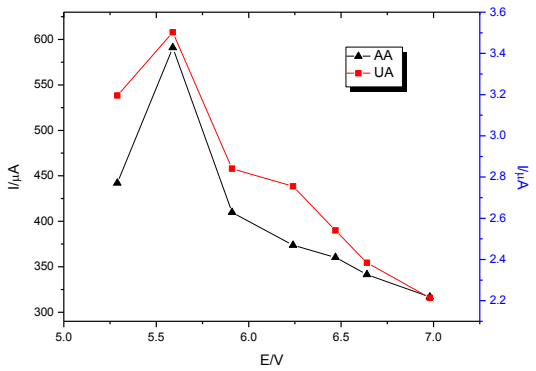

Figure 2. Influences of pH, UA (2.25×10^{-3} M), AA (0.055 M), and scan rates at 100 mV/s

3.2.2 Effect of scanning rate

The effect of scanning speed on the oxidation peak currents of AA and UA was studied using the NOCC-Gly-GC electrode as the working electrode (Figure 3). Within the range of 20–100 mV/s,

the peak current increased and the oxidation peak potential shifted with rising scanning rate. Good linear relationships of the AA and UA peak currents with v were observed. The linear equations were $I_{p(AA)}$ = 84.1009 + 2.7313*v* (*R* = 0.9984) and $I_{p(UA)}$ = 1.4414 + 0.0219*v* (*R* = 0.9993), respectively. The slopes values of the equations indicate that the oxidations of these AA and UA biomolecules on NOCC-Gly-GC are adsorption-controlled processes [18].

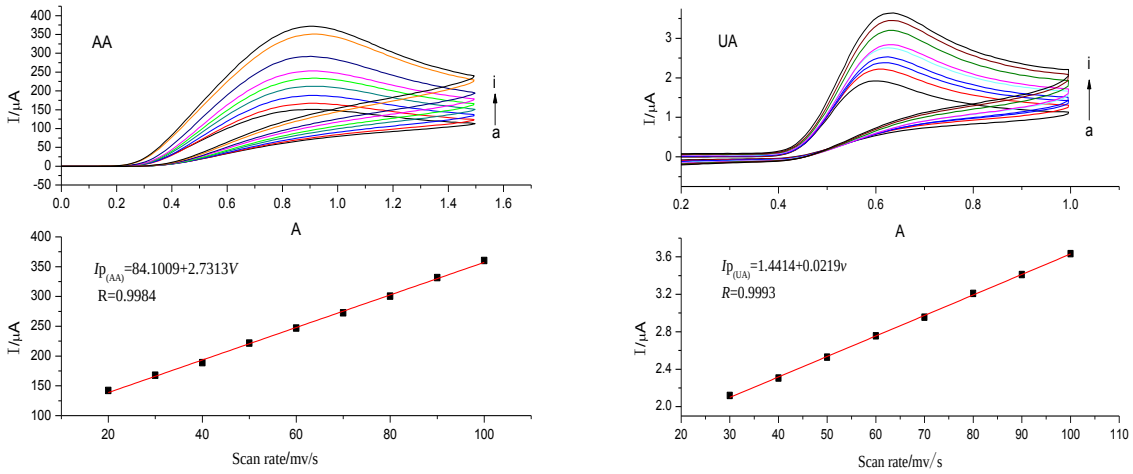

Figure 3. Cyclic voltammograms of AA and UA at different scan rates of 30, 40, 50, 60, 70, 80, 90, and 100 mV/s in PBS, pH 5.59, UA (2.0×10^{-3} M), and AA (0.035 M)

3.2.3 Effect of concentration

Table 1. Comparison of linear range and detection limit of the proposed electrode with previously reported studies

Electrode	Linear range/M		Detection limit/M		Reference
	UA	AA	UA	AA	
NOCC-Gly-GC	0.1×10^{-6} ~6.0×10^{-2}	2×10^{-5}～0.06	7.0×10^{-9}	3.0×10^{-7}	Presented method
CMK3/AuNPs/GCE	0.06×10^{-6}~16.6×10^{-5}	4.0×10^{-6}~792.0×10^{-6}	2.1×10^{-8}	7.5×10^{-7}	[10]
Fe_3O_4/SnO_2/Gr	0.015×10^{-6}~2.4×10^{-6}	0.01×10^{-6}~23.0×10^{-6}	0.005×10^{-6}	0.062×10^{-6}	[11]
Au-Graphene/GCE	1.0×10^{-6}~1.0×10^{-3}	1.99×10^{-5}~5.58×10^{-3}	4.0×10^{-7}	1.2×10^{-5}	[14]
GN/NCC/MB/GCE	4.0×10^{-6}~12.0×10^{-5}	40.0×10^{-6}~70.0×10^{-5}	3.25×10^{-6}	17.64×10^{-6}	[19]
PCA/GC	2.0×10^{-6}~1.0×10^{-4}	2.0×10^{-5}~6.0×10^{-4}	8.0×10^{-7}	3.5×10^{-7}	[20]
PABSA/CNT/GC	2.5×10^{-7}~5.0×10^{-4}	8.0×10^{-6}~ 4.0×10^{-3}	0.7×10^{-7}	5×10^{-6}	[21]
RGO–ZnO/GCE	1×10^{-6}~70×10^{-6}	50×10^{-6}~2350×10^{-6}	0.33×10-6	3.71×10-6	[22]

Cyclic voltammograms show linear relations of the oxidation peak current with the UA and AA concentrations from 3×10^{-5} M to approximately 0.05 M (AA) and from 0.1×10^{-6} M to approximately 1.0×10^{-2} M (UA) (Figure 4). The linear equation of AA was $I_{p(AA)}$ = 77.4058 + 8923.0197*c*, *R* = 0.9963, whereas that of UA was $I_{p(UA)}$ = 0.5280 + 1.4910*c*, *R* = 0.9976. The detection limits were (S/N = 3) 3.0×10^{-7} M (AA) and 7.0×10^{-9} M (UA). The results show that NOCC-Gly-GC

electrode exerts favorable electrocatalytic effects on AA and UA. The performance of NOCC-Gly-GC was compared with the other modified electrodes for the simultaneous determination of AA and UA in recent years, and the results of this comparison are shown in Table 1 [10,11,14,19–22], this method exhibited high sensitivity and lower detection limits (Table 1).

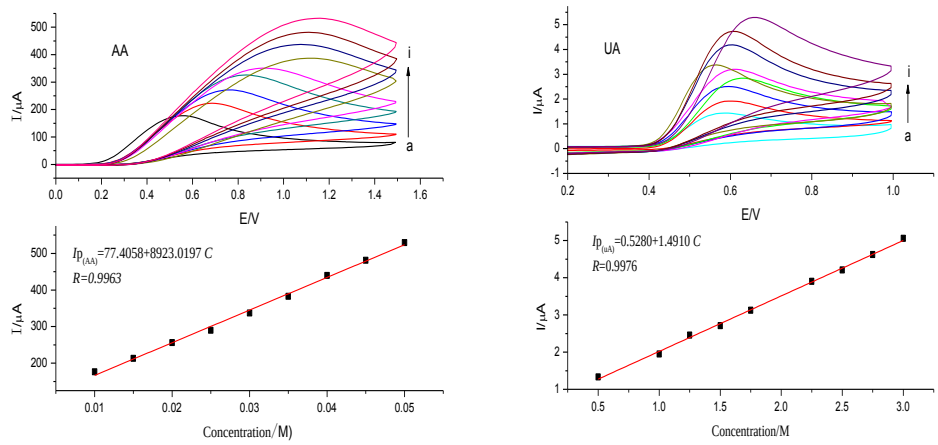

Figure 4. Cyclic voltammograms of AA and UA at various concentrations (AA: a, 0.010 M; b, 0.015 M; c, 0.020 M; d, 0.025 M; e, 0.030 M; f, 0.035 M; g, 0.04 M; h, 0.045 M; and i, 0.05 M and UA: a, 0.5×10^{-3} M; b, 1.0×10^{-3} M; c, 1.25×10^{-3} M; d, 1.5×10^{-3} M; e, 1.75×10^{-3} M; f, 2.25×10^{-3} M; g, 2.5×10^{-3} M; h, 2.75×10^{-3} M; and i, 3.0×10^{-3} M), pH = 5.59, and scan rates at 100 mV/s

3.4 Reproducibility, stability, and interference experiment

The NOCC-Gly-GC electrodes were employed in continuous scanning seven times. This continuous scanning was performed under the conditions when the relative standard deviations (RSDs) were only about 2.1% and 3.6% for UA and AA, respectively. Good reproducibility was exemplified by the NOCC-Gly-GC electrodes. The peak currents both decreased by about 11% after the electrode was exposed to air for 10 days. Thus, the NOCC-Gly-GC electrode is stable in oxygen. Figure 5 shows a pair of redox peaks at the potential of 0–1.0 V. Two obvious oxidation peaks are displayed at 0.235 and 0.576 V, which are clearly distinguished ($\Delta Ep = 0.341$ V). The two oxidation peaks were 0.235 V (UA) and 0.576 V (AA), which are consistent with the sensor results described in literature (Table 2) at 337 mV difference from the oxidation peak potential. The results show that the composites prepared from Gly and carboxymethyl chitosan can catalyze the oxidation reactions of AA and UA simultaneously. The oxidation peak potentials of these two substances are obviously distinguished. This method is expected for the simultaneous electrochemical detection of AA and UA. On NOCC-Gly-GC electrode, AA and UA can be significantly separated because the peak potential difference reached 0.341 V. When the modified electrode was used for UA determination in urine, the modified electrode could not be disturbed by high AA levels in the urine.

Table 2. Oxidation peak potentials (Ep^{ox}) of UA and AA analytes and oxidation peak potential differences (ΔEp^{ox}) of UA-AA analytes estimated on different electrodes

	Ep^{ox}/mV (UA)	Ep^{ox}/mV (AA)	ΔEp^{ox}/mV	Reference
NOCC-Gly-GC	235	576	341	Presented method
N-WCNTs/RhNPs	378	8	370	[4]
Au-Graphene/GCE	586	273	313	[14]
PABSA/CNT/GC	312	-25	337	[21]

The determination was not affected when the modified electrode was used. The electrode detected 1.2 mM AA and 0. 04 mM UA under the addition of 2.0 M H_2O_2 or 0.004 M DA. Moreover, NaCl, KCl, $ZnCl_2$, $CaCl_2$, $MgSO_4$, EDTA, and glucose did not influence the detection result. These findings demonstrated the good anti-interference property and selectivity gained by the modified electrode.

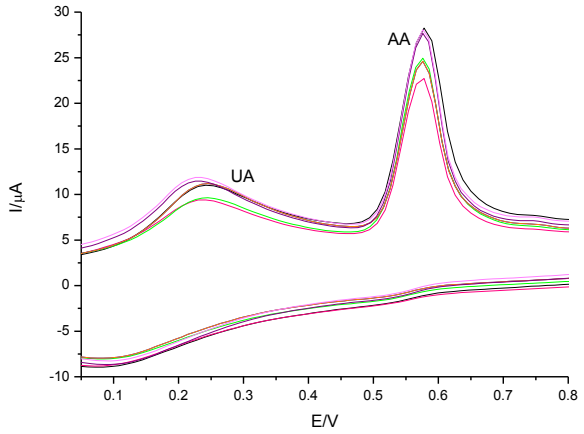

Figure 5. Reproducibility determination for AA and UA with NOCC-Gly-GC electrode; AA (4.0×10^{-4} M), UA (1.0×10^{-6} M), pH = 5.59, and scan rates at 100 mV/s

3.5 Sample determination

Recovery tests were performed using 2 mL urine sample and 5 mL 0.1 M NaOH solution in 50 mL PBS (pH 5.59) (Table 3). We obtained 10 vitamin C tablets (100 mg/tablets), which we ground finely and then accurately weighed to obtain 0.1761 g of the powder. This sample was poured into a 50 mL volumetric flask with PBS (pH 5.59). The standard addition method was used to determine the AA content in the vitamin C tablets. The results are shown in Table 4.

Table 3. Determination result for UA in urine (n = 3)

Sample (UA)	Found (μM)	Added (μM)	Total found (μM)	RSD (%, n = 3)	Recovery (%)
1	1.89	2	3.92	1.8	101.5
2	1.88	4	5.86	2.6	99.5
3	1.91	6	7.83	3.9	98.7

Table 4. Determination result of AA (n = 3)

Sample (AA)	Found (mM)	Added (mM)	Total found (mM)	RSD (%, n = 3)	Recovery (%)
1	19.06	5	24.03	2.1	99.4
2	19.06	10	29.12	1.1	100.6
3	19.06	15	33.89	3.5	98.9

4. CONCLUSION

An NOCC-Gly-GC electrode was synthesized on the surface of a glassy carbon electrode from carboxymethyl chitosan and Gly. The synthesized electrode displayed good reproducibility and stability. When the electrode was used for AA and UA determination, the peak current and concentration showed a good linear relationship. Therefore, NOCC-Gly-GC electrodes can be applied for the simultaneous detection of AA and UA.

ACKNOWLEDGMENTS
Authors are thankful to the Science and Technology Projects of Henan Province (Grant No. 132300410312) and Science and Technology Development Projects of Zhumadian City (Grant No. 13604) for the financial support.

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