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Electrochemical Determination of Tyrosine and Nitrite Using CS/CMWNTs/GCE-modified Electrode

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A novel electrode was successfully made by modifying a glassy carbon electrode (GCE) with a chitosan (CS)/carboxylated multi-walled carbon nanotube (CMWNT) composite. The electrochemical behavior of L-tyrosine (L-Tyr) and nitrite (NO_2^-) was investigated using cyclic voltammetry, and the detection conditions of L-Tyr and NO_2^- were explored. The effective area of the CS/CMWNTs/GCE was calculated to be 7.2534×10^{-6} cm^2. The surface area of the electrode membrane surface increased significantly. Accelerating the electron transfer of $K_3[Fe(CN)_6]$ is beneficial for the adsorption and enrichment of the measured substance. The results showed that the amperometric response of the sensor reached a maximum when the pH of phosphate-buffered saline (PBS) was 3.6 and 4.7 for L-Tyr and NO_2^-, respectively. The modified electrode could detect L-Tyr and NO_2^- in the linear concentration ranges of 1.0×10^{-8}–2.0 M and 1.5×10^{-8}–0.3 M, respectively. The detection limits were 1.6×10^{-9} M and 2.1×10^{-9} for L-Tyr and NO_2^-, respectively (S/N = 3). These results demonstrate the high stability and reproducibility of detection, obtained using the CS/CMWNTs/GCE. The modified electrode was applied to determine the L-Tyr content of amino acid injections and the NO_2^- content of salted radish.

Keywords: Carboxylated multi-walled carbon nanotubes, chitosan, CS/CMWNTs/GCE, tyrosine, NO_2^-

1. INTRODUCTION

Pickling salt contains a large amount of nitrite. Nitrite is commonly misused, and doses as low as 0.3–0.5 g can be toxic; more than 3 g can be enough to form a protein with hemoglobin, leading to hypoxia that could result in respiratory failure and death[1,2]. Tyrosine (L-Tyr) is a biochemical reagent that can be used to treat hyperthyroidism[3]. Many methods for the determination of NO2- and L-Tyr have been reported internationally.

NO_2^- and L-Tyr can be detected using high-performance liquid chromatography, spectrophotometry, atomic absorption spectrometry, ion chromatography, fluorescence, catalytic spectrophotometry, and chemiluminescence methods. However, these methods are subject to a number of shortcomings, such as poor sensitivity and stability, duration, interference by ions, toxicity, secondary pollution, and complex or time-consuming measurement processes. In contrast, electrochemical methods have the advantages of low cost, simple operation, low detection limits, high sensitivity, and an extensive range of applications[4-9]. Carboxylated multi-walled carbon nanotubes (CMWNTs) exhibit promising properties for electrode conduction, including high conductivity and specific surface area. They have superior ability to promote electron transfer reactions because electrode materials can accelerate the exchange of electrons and significantly improve the signal strength[10]. When used alone, chitosan (CS) has poor conductivity. It can be used as a multi-walled carbon nanotube (MWCNT) dispersant in the preparation of chemically modified electrodes[11]. In recent years, given the interference of L-Tyr and NO_2^- in electrode detection, research interest in detection methods with improved efficiency has increased[12-20]. Li-prepared ERGO-NiHCF/GCE modified glassy carbon electrodes14, showed a very high electrocatalytic activity toward nitrite oxidation, and the current of the oxidation peak had a linear relationship with the concentration of nitrite. Qiao[15] and coworkers prepared a glassy carbon electrode (GCE) modified with a composite film composed of copper oxide, reduced graphene oxide (rGO)， and MWCNTs in a two-step procedure. The electrochemical behavior of L-Tyr was also investigated on the resulting CuO- rGO-MWCNTs/GCE-modified electrode, with a detection limit of 5.0 × 10-9 M. Kanchana[17] prepared a Ce-HA-modified GCE which showed an excellent electrocatalytic activity for resolving the overlapping voltammetric responses of NE, UA, and Tyr into three well-defined voltammetric peaks. Yu[21] prepared glassy carbon electrode modified with graphene and multi-walled carbon nanotubes composite film (GR/MWCNT/GCE), which successfully detected nitrite with a detection limit of 5.0×10^{-8} M.

The combination of carboxylated MWCNTs and chitosan created a high-performance composite (CS/CMWNTs/GCE) membrane-modified glassy carbon. Compared to most of the materials used to modify the electrode, CMWNTs can provide better analytical performance, better electrical properties, and larger active surface area, which can enhance electron transfer, electrode conductivity, and analytical sensitivity of the electrode. However, due to the poor dispersibility of CMWNTs, currently existing methods mostly involve the use of surfactants for dispersion, or various modifications to the CMWNTs to achieve its dispersion in the monomer solution. We utilized the synergistic effect of CS-dispersed MWCNTs and good biocompatibility. The CS/CMWNTs composite films were modified on glassy carbon electrode by the synergistic effect of CS-dispersed MWCNTs and the electrocatalytic performance. Thus, a novel modified electrode was obtained. The resulting electrode had a strong enrichment and synergistic catalytic performance, leading to higher redox activity, which in turn increases the electrochemical response. To our knowledge, this technology is uncommon and has been less reported in the literature. L-Tyr and NO2- can be directly determined using the CS/CMWNTs/GCE electrode through voltammetry. This method has the advantages of simple preparation, low a cost, high sensitivity, and fast and convenient operation. For these reasons, CS/CMWNTs/GCE electrodes are promising for the detection of amino acid injections and analysis of nitrites in salted radishes.

2. EXPERIMENTAL

2.1 Materials and Equipment

Cyclic voltammetry (CV) measurements were performed on a Zennium electrochemical workstation (Zahner, Germany). The following chemicals were used: chitosan (degree of deacetylation 96%) from Hengtai Jinhu Carapace Products Co. Ltd.; sodium nitrite from Tianjin Kay Tong Chemical Reagent Co. Ltd.; L-tyrosine from Sinopharm Group Chemical Reagent Co. Ltd.; carboxylated multi-walled carbon nanotubes (d $\leqslant$ 50 nm) from Chengdu Institute of Organic Chemistry; and compound amino acid injections from Guangdong Litai Pharmaceutical Co. Ltd.

2.2 Method

A GCE was polished in an ultrasonic bath for 5 min, first in a 1:1 solution of nitric acid: ethanol, then in distilled water.

Chitosan (0.1 g) was placed in a 0.5% acetate solution. CMWNTs (0.01 g) were then added before ultrasonication. The resulting black suspension (5 μL) was applied to the GCE to prepare a CS/CMWNTs modified GCE.

A three-electrode system was used, including a platinum electrode as the counter (auxiliary) electrode (CE), a saturated calomel electrode as the reference electrode (RE), and CS/CMWNTs/GCE as the working electrode (WE).

3. RESULTS AND DISCUSSION

3.1 Effect of pH and electrochemical behavior of $K_3[Fe(CN)_6]$

A K_2HPO_4-KH_2PO_4 buffer was used as the bottom liquid. $K_3[Fe(CN)_6]$ (1.5 × 10-3 M) was employed as a probe to test the sensor properties of the CS/CMWNTs/GCE and the GCE (Fig. 1B). The peak current of K3[Fe(CN)6] was clearly larger with the modified electrode. The composite membrane has a strong enrichment ability and synergistic catalytic performance, which is beneficial to the redox reaction of more active substances, thereby enhancing the voltammetric response. At the same time, the redox peak is symmetrical on the modified electrode, indicating that the electrochemical reaction of the probe ion on the modified electrode is a quasi-reversible process[17]. For reversible processes, the Randles–Sevcik equation is used:

$$i_p = 2.69\times10^5 n^{3/2} A D^{1/2} \upsilon^{1/2} c$$

The effective area of the GCE was calculated as 1.6302×10^{-6} cm^2, whereas that of the CS/CMWNTs/GCE electrode was calculated as 1.7186×10^{-5} cm^2, indicating that CMWNTs can increase the rate of electron transfer. The addition of CMWCNTs also promotes the formation of a network- meshed structure, allowing more active substances to be adsorbed and thereby enriching the electrode surface. The surface area of the membrane increased significantly, promotinged the electrochemical reaction of the measured material, and acceleratinged the electron transfer of Fe^{2+} to Fe^{3+}.

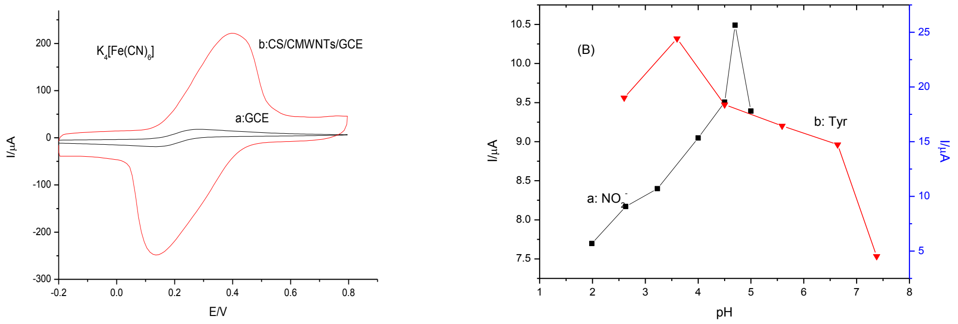

Figure 1. $K_3[Fe(CN)_6]$ (1.5×10^{-3} M) (**A**) on different electrodes (a: GCE, b: CS/CMWNTs/GCE), Influences of pH **(B)** (NO_2^- : 5×10^{-4} M), L-Tyr: 3.75×10^{-4} M)**;** scan rates at 100 mV/s

As shown in Fig. 1B a, the NO_2^- content of PBS buffer solutions containing NO2- (5 × 10-4 M), and with pH = 1.99, 2.63, 3.23, 4.00, 4.50, 4.70, 5.00, and 5.8, was verified using the CS/CMWNTs/GCE as the WE. The pH of the solution affects the catalytic oxidation of NO_2^- because the reaction mechanism can be expressed as follows [22,23]:

$$H^+ + NO_2^- \longleftrightarrow HNO_2;$$

$$NO_2^- - e^- \rightarrow NO_2;$$

$$2NO_2 + H_2O \rightarrow 2H^+ + NO_3^- + NO_2^-.$$

The NO_2^- oxidation peak current reaches a maximum when pH = 4.7. Therefore, a buffer solution of pH 4.7 was selected as the supporting electrolyte.

Curve b in Fig. 1B represents PBS buffer solutions containing L-Tyr (3.75×10^{-4} M), and with pH = 2.6, 3.6, 4.5, 5.59, 6.64, and 7.38, at a sweep speed of 100 $mV \cdot s^{-1}$. The oxidation peak current of tyrosine in the modified electrode decreases with increasing pH. At pH 7.38, the peak current almost disappears. Furthermore, the oxidation peak potential of L-Tyr shifts negatively as pH increases. This may be attributable to the oxidation of tyrosine during proton transfer and adsorption. Protonated tyrosine was adsorbed on the electrode surface. The stronger the acidity of the solution, the greater the degree of protonation and the higher the adsorption rate, so the peak current increased. Therefore, the PBS buffer solution with pH = 3.6 was selected for the determination of L-Tyr content.

3.2 Effect of pH and scanning rate

Fig. 2A shows the CV curves of NO_2^- (2×10^{-3} M, pH 4.7) with the CS/CMWNTs/GCE. The results showed that the oxidation peak current of NO_2^- increased as the sweep velocity was increased;

the peak current and the scanning rate had a linear relationship. This result indicates that the reaction with the electrode was irreversible. The linear equation is:

$I_{p(NO2-)}$ = 17.0540 + 1.0428υ， R^2= 0.9970. The redox reactions of NO_2^- are attributed to the adsorption control process, which differs from some previous findings[5,14] while agreeing with others[21].

The effect of scanning speed on the oxidation peak currents of L-Tyr (0.70 mM) was studied using the CMWNT/bent/GCE as the WE in a pH 3.6 acetic acid-sodium acetate buffer solution with various sweep velocities (Fig. 2 B). The oxidation peak current *I*p has a linear relationship with the square root of the sweep velocity υ:

Ip(Tyr)= 5.8095 + 1.6522$\upsilon^{1/2}$, R^2=0.9979,

indicating that the electrochemical oxidation of L-Tyr on the CS/CMWNTs/GCE was controlled by diffusion. As the scanning rate increased, the peak potential shifted positively, which is typical of irreversible reactions. This finding is consistent with those in the literature[3, 16].

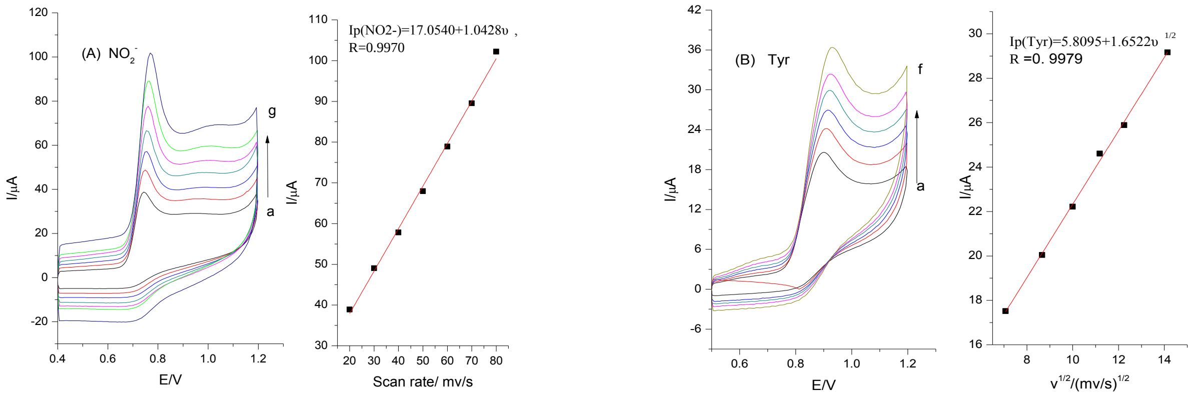

Figure 2. Cyclic voltammograms of NO_2^-(2 × 10^{-3} M, pH 4.7; A a-g: 20, 30, 40, 50, 60, 70, 80 mV·s^{-1}), and L-Tyr (0.70 × 10^{-3} M pH 3.6; B a-g: 10, 50, 75, 100, 125, 150, 200 mV·s^{-1}) at various scan rates.

3.3 Detection limit, reproducibility, stability, and interference experiments

The relationship between NO_2^- concentration and the peak current was studied using CV under the optimized experimental conditions (Fig. 4A). The results show that the NO_2^- oxidation peak current increased with concentration. The linear relationship of the oxidation peak current with the NO_2^- concentrations was in the range of 1.5 × 10^{-8}–0.3 M (Fig. 4A). The linear equation representing NO_2^- concentration was:

I_p (NO_2^-) = 1.2156 + 2.8047*c*， R^2= 0.9985，

and the detection limits were (S/N = 3) 2.1 × 10^{-9} M. These results demonstrate that the modified electrode exerts favorable electrocatalytic effects on NO_2^-. Compared with previously reported systems, the electrode presented a wider linear range and a lower detection limit[19-21].

The CS/CMWNTs/GCE was used as the working electrode to perform CV, in pH ＝ 3.6 PBS solution. As seen in Fig. 4B, the oxidation peak current had a linear relationship with L-Tyr concentration in the range of 1.0×10^{-8}～2.0 M, and the linear equation was:

I_p(Tyr) = 6.7016 + 3950.3878 c (R^2= 0. 9961).

The detection limit was $1.6{\times}10^{-9}$ M S/N = 3).

Seven CS/CMWNTs/GCE were prepared in the same manner. The relative standard deviations of the Tyr and NO_2^-peak currents were 1.1% and 1.8%. The prepared electrode was used for two days, and the NO_2^- response signal was kept above 94%. These results demonstrate the high reproducibility and stability of the prepared electrode. The effects of some common ions on the determination of NO_2^- (4.0×10^{-4} M) and L-Tyr (0.012 mM) were investigated under the same experimental conditions. The results show that while Na^+, Zn^{2+}, Ca^{2+}, Co^{2+}, Ni^+, Cd^{2+} Fe^{3+}, NO_3^-， and PO_4^{3-} have no effect on NO_2^- determination, H_2O_2 produces significant interference. No interference was observed by taurine, curcumin, tryptophan, uracil, phenylalanine, serine, glutamic acid, or proline for L-Tyr detection.

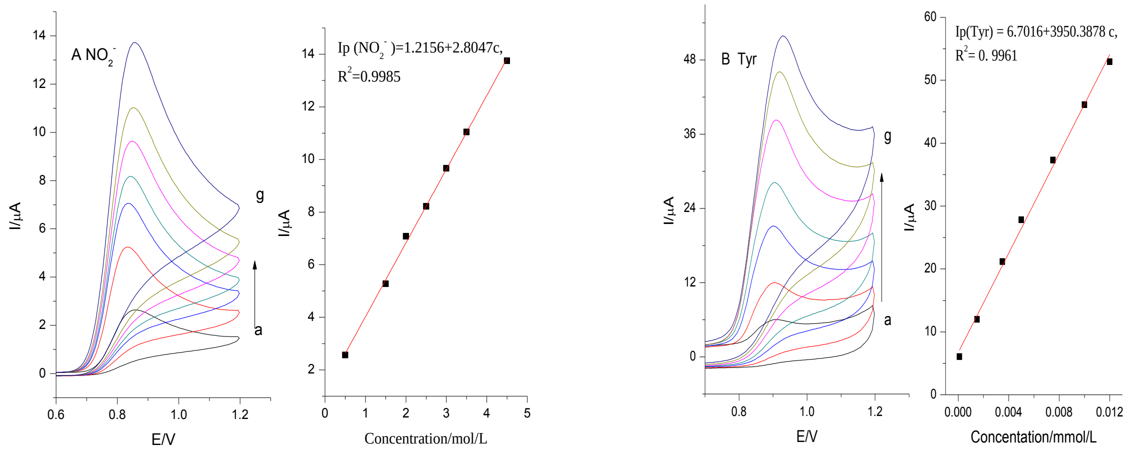

Figure 3. Cyclic voltammograms of NO_2^- and L-Tyr at various concentrations(NO_2^-， a~g: 0.0005, 0.0015, 0.002, 0.0025, 0.003, 0.0035, 0.0045 M, pH = 4.7)and（L-Tyr , a~g : 0.0001, 0.0015, 0.0035, 0.005, 0.0075, 0.01,0.012 mM, pH = 3.6）, scanning rate = 100 mV·s^{-1}.

Table 1. Comparison of the linear ranges and detection limits of Tyr and NO_2^- obtained with the present technique and with previously reported methods

Electrode	Linear range (M)		Detection limit(M)		Reference
	Tyr	NO_2^-	Tyr	NO_2^-	
CS/CMWNTs/GCE	$1.0{\times}10^{-8}$～2.0	$1.5{\times}10^{-8}$～0.3	$1.6{\times}10^{-9}$	$2.1{\times}10^{-9}$	Present method
ERGO/NiHCF /GCE		$0.8{\times}10^{-6}$～$25.0{\times}10^{-5}$		$2.0{\times}10^{-6}$	[14]
Cu-Ag /GO/GCE		$8.0{\times}10^{-9}$～$2.0{\times}10^{-6}$		$8.0{\times}10^{-9}$	[18]
CS-CoO/GCE		$8.0{\times}10^{-7}$～$6.0{\times}10^{-3}$		$1.0{\times}10^{-7}$	[20]
G R/MWCNT/GCE		$1.0{\times}10^{-7}$～$1.7{\times}10^{-3}$		$5.0{\times}10^{-8}$	[21]
P5-SA/MWNT	$9.0{\times}10^{-6}$～$2.0{\times}10^{-4}$		$6.0{\times}10^{-6}$		[3]
CuO-rGO-MW CNTs /GCE	$2.0{\times}10^{-8}$～$1.8{\times}10^{-4}$		$5.0{\times}10^{-9}$		[15]
MWNTs /CPE	$3.5{\times}10^{-6}$～$2.0{\times}10^{-3}$		$3.2{\times}10^{-7}$		[16]

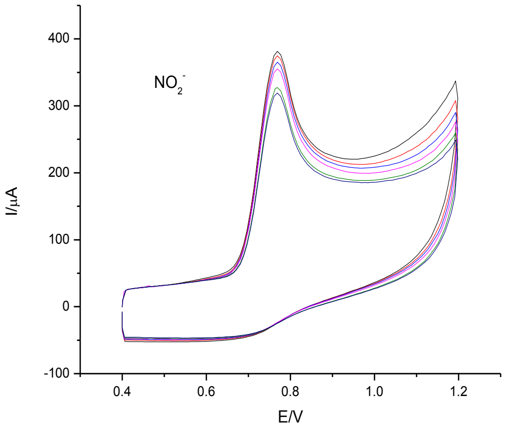

Figure 4. Reproducibility of CS/CMWNTs/GCE electrode: NO_2^- (0.09 M), pH = 4.7 and scan rates = 100 mV/s

3.4 Sample detection

Commercially available salted radishes (30 g) were soaked in 50 mL of distilled water for two days and then mashed, filtered, and washed three times with a constant volume of distilled water (500 mL). Samples (1 mL) were then added to PBS (pH = 4.70) in a buffer solution. Varying amounts of NaNO2 were used for spiked recovery experiments run in triplicate and in parallel; three copies of the amino acid injection sample, L-Tyr, were analyzed using the CS/CMWNTs/GCE under the optimized experimental conditions. The results, shown in Table 2, show that the nitrite content of the salad was 70.1 mM, which is much higher than the national standard. Furthermore, the L-Tyr amounts were consistent with the compound amino acid injection.

Table 2. Determination of NO_2^- in salted radish and L-Tyr in amino acid injections (n = 3)

Sample	Found (mmoL/L)		Added (mmoL/L)		Total found (mmoL/L)		RSD (%, n=3)		Recovery (%)	
	L-Tyr	NO_2^-	L-Tyr	NO_2^-	L-Tyr	NO_2^-	L-Tyr	NO_2^-	L-Tyr	NO_2^-
1	1.37	1.41	1	2	2.39	3.43	2	2.5	102.0	101.0
2	1.37	1.41	2	4	3.43	5.38	2.3	0.8	103.0	99.3
3	1.37	1.41	3	6	4.34	7.51	1.6	1.9	99.0	101.6

4. CONCLUSIONS

A novel modified GCE was prepared, modified using chitosan and carboxylated multi-walled carbon nanotube film. The modified electrode has excellent electrochemical properties and is a highly sensitive electrocatalyst for L-Tyr and NO_2^- detection with low detection limits (The detection limits for L-Tyr and NO_2^- were 1.6×10^{-9} M and 2.1 × 10-9 , respectively), wider linear ranges, good reproducibility and stability, and high sensitivity. The method presented herein provides a promising way to determine NO_2^- content in salted radishes and L-Tyr content in amino acid injections.

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