An amperometric L-tryptophan sensor platform based on electrospun tricobalt tetroxide nanoparticles decorated carbon nanofibers

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Abstract:

A glassy carbon electrode (GCE) modified with electropun tricobalt tetroxide nanoparticles decorated carbon nanofibers ($\text{Co}_3\text{O}_4\text{-CNF}$) were first applied for the determination of L-tryptophan (L-Trp). The synthesized $\text{Co}_3\text{O}_4\text{-CNF}$ were characterized by scanning electron microscopy, Raman, X-ray diffraction and electrochemical impedance spectroscopy. The electrochemical detection of L-try was successfully conducted in 0.1 M phosphate solution (pH 2). The linear relationship of L-Trp was in the range of 0.005–40 μM and the detection limit reached 0.002 μM (S/N=3) with little interference from other amino acids.

Keywords: tricobalt tetroxide nanoparticles, carbon nanofibers, L-tryptophan, electrochemical determination

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1. Introduction:

L-tryptophan (L-Trp) is an essential amino acid for normal growth in infants and nitrogen balance in adults^[1]. In many biochemical process, L-Trp serves as a precursor or a vital element for forming neurotransmitter as serotonin or neurohormones as melatonin and niacin^[2]. As an essential amino acid, L-tryptophan can be only obtained through food or medical supplementation. However, the large quantity and improper metabolized of L-Trp in body can cause side effects as agitation, fever, or even diseases as hallucination, delusion etc^[3]. Therefore, the detection of L-Trp is significant in many field as food processing, bio-chemistry, pharmaceuticals, clinical analysis, etc^[4].

Various methods have been developed for the determination of L-Trp, such as high-performance liquid chromatography^[5], fluorescence spectroscopy^[6], capillary electrophoresis^[7] as well as electroanalysis^[8]. Among these methods, electrochemical method has attracted the interest of researches because of L-Trp's inherent electroactivity behavior caused by its aromatic group^[9] and the merits of electroanalysis as high sensitivity, time-saving and simple operation^[10]. Due to the sluggish electron transfer process and over-potential of L-Trp^[11], different modified electrodes have been reported for the determination of L-Trp. These modified electrodes are capable in promoting the kinetics of electron transfer process and reducing the over-potential of L-Trp in the electro-oxidation^[12]. Cobalt-based nanoparticles decorated carbon based materials stand out due to their superior catalytic performances^[13]. For instance, Ali et.al synthesized a cobalt ferrite nanohybrid on exfoliated graphene oxide compound, which shown synergetic effect towards the electro-reduction of H₂O₂ and nicotinamide adenine dinucleotide^[14]. Mujtaba et. al. decorated porous carbon material with Co₃O₄, which turned out to be a good lithium ion battery anode

materials and shown satisfied electron transfer property^[15]. Qu et. al. used one-step hydrothermal method to modify $\text{Co(OH)(CO}_3\text{)}_{0.5}$ nano-cone arrays on both sides of the thin walls of macroporous carbon nanotubes, that exhibited an improved specific capacitance and high performance as a supercapacitors^[16].

Fibrous carbon materials with one dimension on nanoscale as carbon nanofibers (CNF) are increasingly attracting the attention of scientists in recent years. One of the main advantages of CNF is its high surface area to volume ratios^[17]. In addition, carbon based materials possess properties as superior electrical conductivity, relative chemical inertness in most electrolyte solution, wide potential windows in aqueous media made them extremely suitable in constructing electrochemical sensors^[18]. Also CNF's entire surface can be easily functionalized with nanoscale materials^[19]. These synthesized CNF and CNF based multifunctional hybrids have shown potential applications in the filed as supercapacitor^[20], lithium batteries^[21], catalyst supports^[22], filter^[23] as well as chemical sensors^[24].

In this work, Co_3O_4 nanoparticles decorated carbon nanofibers (Co_3O_4 -CNF) were synthesized by electrospinning polymer precursor containing different weight percentage of metal salt, followed by successive anneal and oxidation process. The obtained Co_3O_4 -CNF were modified on a glassy carbon electrode (designate as Co_3O_4 -CNF/GCE) by electrodeposition technique and it was used in sensitive detection of L-Trp in 0.1 M phosphate solution (pH 2) with a wide linear range of 0.005 μM –40 μM .

2. Experimental

2.1 Apparatus and Chemicals

Electrochemical measurements including cyclic voltammetry (CV), differential pulse voltammetry

(DPV) and chronoamperometry were performed on a CHI 842B electrochemical workstation (Chenhua Corp., Shanghai, China). Electrochemical impedance spectroscopy (EIS) was carried on a CHI 660D electrochemical workstation (Chenhua corp., Shanghai, China). A conventional three-electrode cell with a glassy carbon electrode (GCE, $\phi=3.0$ mm) served as the working electrode, a calomel electrode as the reference electrode and a platinum electrode as the counter electrode was employed during electrochemical measurements. Scanning electron graphs were obtained by a scanning electron micrograph (JSM-6700F, 15.0 kV). Raman scattering was performed on a Raman Microscope (INVIA England) using a 545.5 nm laser source. XRD analysis was carried out on X-ray diffractometer (Haoyuan DX-2700, China).

Cobalt acetate, PAN, L-Trp, leucine, threonine alanine glucose phenylalanine, lysine, folic acid, uric acid and tyrosine were purchased from Aldrich. N,N-dimethylformamide, potassium chloride, hydrochloric acid and other chemicals were purchased from Sinopharm chemical reagent Co., Ltd. (SCRS, China). All chemicals used in this work were of analytical reagent grade and used without further purification. All experiments were carried in 0.1M phosphate solution (pH = 2). The drug sample of L-Trp (Tianshi amino acid compound tablets) was purchased from local pharmacy in Shanghai. All electrochemical experiments were carried at room temperature. The standard solutions of L-Trp with different concentration ranged in 1×10^{-5} to 1×10^{-2} M was prepared by weight and dissolved in 0.1 M HCl followed with proper dilution process by double deionized water (DDW).

2.2 Synthesis of Co_3O_4 decorated Carbon nanofibers ($\text{Co}_3\text{O}_4\text{-CNF}$)

The $\text{Co}_3\text{O}_4\text{-CNF}$ were synthesized by electrospinning technique followed with a subsequent heating process. The precursor used for electrospining were prepared by dissolving certain weight

ratio of PAN and cobalt acetate in DMF under magnetic stirring overnight at room temperature to ensure the mixture's homogeneity. Then the prepared precursor was fed into a syringe pump at the flow rate of 1ml/h. The electrospinning process was conducted by applying a voltage of 18 KV between the needle and the aluminum foil collector at a distance of 10 cm. As a control, blank PAN fiber (10 wt% PAN in DMF) and different weight ratio Co₃O₄-CNF were also synthesized under the same procedure. For carbonization, the as-prepared fibers were followed by similar procedure which reported before^[13]. For detail, the as prepared fibers were first heated up from room temperature to 280 °C at a rate of 3 °C per minute and stayed in air for 1 h for shape stabilization. Then heated up to 1000 °C at rate of 2 °C/min under nitrogen protection and stayed at the highest temperature for 2 h. After the calcination process, the synthesized material was reheated to 200°C in air and kept the temperature for 2 h to allow cobalt based nanoparticles transform to Co₃O₄ completely. For instance, the Co₃O₄-CNF obtained from the precursors with different weight percentage of Co₃O₄ loaded CNF hybrid were synthesized by adding 2.5 wt% Co(Ac)₂•4H₂O, 5 wt% Co(Ac)₂•4H₂O and 7.5 wt% Co(Ac)₂•4H₂O in to PAN polymer precursor (10 wt% PAN in DMF). For convenience, the synthesized materials were noted as 2.5 wt% Co₃O₄-CNF, 5 wt% Co₃O₄-CNF and 7.5 wt% Co₃O₄-CNF, respectively.

2.3 Preparation of modified electrode

Before the modification, a bare GCE (φ 3mm) was firstly polished by 0.05 μm alumina slurries until a mirror-like surface was acquired. Then the GCE was cleaned in 1:1(v/v) HNO₃ solution, ethanol and DDW under the help of ultrasonic bath. The synthesized Co₃O₄-CNF (1mg/mL) were dispersed in DDW with the help of ultrasonic bath as well. The Co₃O₄-CNF/GCE was gained via electrodepositing method by applying a constant potential of 1.8V for 400s in 0.1 M KCl solution

containing 200 μL 1 mg/mL Co_3O_4 -CNF.

3. Results and Discussion

3.1 Characterization of modified electrode

3.1.1 SEM, XRD and Raman spectroscopy. Fig. 1A shows the SEM image of the morphology and microstructure of the prepared Co_3O_4 -CNF. The diameter of the synthesized hybrid range from 200 to 400 nm with tens of micrometers in length. A large amount of bright contract nanoparticles about 100 nm in size randomly dispersed on the surface of CNF, which are probably derived from the decomposition of cobalt acetate. For comparison, the morphology of Co_3O_4 nanoparticles synthesized by the same electrospinning technique shown in Fig. 1B. As can be seen from Fig. 1B, the particle size of Co_3O_4 range from 100 to 500 nm and they tend to be aggregated easily. This confirms that the CNF can be served as a catalyst supporter, which avoids nanoparticles from aggregating.

XRD is a powerful tool to identify the composition of synthesized Co_3O_4 -CNF. As shown in Fig. 1C, a broad C (002) peak at about 28° indicates the amorphous behavior of the CNF. Other sharp diffraction peaks at about 18° , 32° , 38° , 40° , 46° , 57° , 61° , 67° , 79° correspond to (111), (220), (311), (222), (400), (422), (511), (440) and (533) crystalline plane of Co_3O_4 . The result shows that after the oxidation process, cobalt has been totally transformed to Co_3O_4 .

Raman spectrum is a useful tool to determinate the structure of different materials. As shown in Fig 1D, four characteristic crystalline peaks of Co_3O_4 were observed at 463, 505, 606 and 673 cm^{-1} corresponding to the E_g , F_{2g}^1 , F_{2g}^2 and A_g^1 vibration modes^[25]. In addition, two distinct peak at 1334 cm^{-1} and 1579 cm^{-1} corresponding to the defect induced D band and graphitic G band of the carbonaceous material^[26], respectively. These results indicate the complete transformation of

electrospun PAN polymeric fibers to conductive CNFs.

Fig 1 here

3.1.2 Electrochemical properties of Co₃O₄-CNF

In order to determinate the electrochemical properties of Co₃O₄-CNF, a comparison of 10 μM L-Trp's current signal on bare GCE, Co₃O₄/ GCE, CNF/GCE and Co₃O₄-CNF/ GCE was conducted in 0.1 M phosphate solution (pH 2) by CV. As shown in Fig 2A, the current signal of 10 μM L-Trp on bare GCE was very low due to the sluggish electron transfer on the surface of bare GCE. The current signal of which was only 0.3058 μA and the oxidation potential was as high as 0.9589 V. However, all of three modified electrodes showed catalytic property towards L-Trp that dramatically increased the current signal of 10 μM L-Trp. From b to d, the current signal of 10 μM L-Trp was 26.1 times, 50.1 times and 82.2 times higher than that on bare GCE. Meanwhile, the oxidation potential of L-Trp on modified electrode was negatively shifted 77.9 mV at Co₃O₄/GCE, 81.9 mV at CNF/GCE and Co₃O₄-CNF/GCE, respectively. These results suggested that both Co₃O₄/GCE, and CNF/GCE showed catalytic activity to L-Trp. Among them Co₃O₄-CNF/GCE performed the best catalytic ability towards L-Trp because of synergetic effect.

EIS was used for studying the interface characteristic of modified electrode. The typical Nyquist diagrams of bare GCE and Co₃O₄-CNF/GCE in 5.0 mM [Fe(CN)₆]^{3-/4-} solution containing 0.1 M KCl at the were shown at Fig.2 B. The Nyquist plots consist a semicircular part and a linear part corresponding to the electron transfer controlled process and the diffusion controlled process, respectively. The diameter of the semicircular part at high frequency equals to the charge transfer resistance on the electrode interface. It is shown in Fig. 2B that, the charge transfer resistance of Co₃O₄-CNF/GCE is about 50 Ω smaller than that on bare GCE (325 Ω) suggesting that the

synthesized material shown good electrical conducting property. In the meantime, the almost straight line at lower frequencies on Co₃O₄-CNF/GCE suggested a diffusion controlled process was the main controlled process during the reaction.

Fig. 2 here

3.2 Electrochemical behaviors of L-Trp on Co₃O₄-CNF/GCE

For studying electrochemical behavior of L-Trp on Co₃O₄-CNF/GCE, the effect of scan rate was investigated by CV at modified GCE in 0.1 M phosphate solution (pH 2) containing 10 μM L-Trp.

The scan rate were set in the range of 10 – 200 mV s⁻¹. As can be seen from Fig. 3, the oxidation peak currents of L-Trp rose with the increase of scan rate, at the same time the oxidation peak

current showed a good linear relationship with scan rate $v^{1/2}$. The linear regression of the oxidation

peak current and the scan rate could be expressed as $I_{pa}(\mu A) = 1.775 v^{1/2} - 4.469$ ($R = 0.997$),

indicating the electro-oxidation reactions of L-Trp is a diffusion controlled process. Furthermore,

with the increase of scan rate, E_p shifted positively. The dependence of E_p with $\log v$ could be

expressed as $E_p(V) = 0.06379 \log v + 0.7667$ ($R = 0.999$). According to Lavrion's theory, the slop

equals to $2.303RT/(1-\alpha)nF^{[27]}$. As for an irreversible electrode process, the electron transfer

coefficient α can be assumed as 0.5, the number of the electrons involved in the rate determining

step was calculated to be 1.85, which is in agreement to previous reports. So that the overall

oxidation process of L-Trp is 2 electron involved process and the mechanism of the reaction can

be deducted as scheme 1.

Fig. 3. here

Scheme1 here

3.3 Effect of operational parameters

3.3.1 Effect of Co₃O₄ loading amount

In this work different Co₃O₄ loading amount on CNF has been studied in order to understand the mechanical property and the catalytic property of the synthesized material. Fig S1 shows SEM photos of 2.5 wt% Co₃O₄-CNF, 5 wt% Co₃O₄-CNF and 7.5 wt% Co₃O₄-CNF. It can be seen from Fig. S1 A to Fig. S1 F, the more cobalt acetate added into the precursor, the more tendency that CNF became shorter. When the weight percentage of Co(Ac)₂•4H₂O reached 7.5 wt% in the precursor, the length of CNF compromised greatly. In order to study the effect of morphology, the current signal of 10 μM L-Trp was tested on these three modified electrodes. Fig. S2 is CV pattern of 10 μM L-Trp on 2.5 wt% Co₃O₄-CNF, 5 wt% Co₃O₄-CNF and 7.5 wt% Co₃O₄-CNF. L-Trp shows an irreversible oxidation on modified electrodes the current signal of L-Trp peaked at 5 wt% Co₃O₄-CNF and the current signal is 1.1 and 1.3 times higher than that on 2.5 wt% Co₃O₄-CNF and 7.5 wt% Co₃O₄-CNF which means the synthesized 5 wt% Co₃O₄-CNF shown the highest catalytic property towards L-Trp.

3.3.2 Effect of pH values.

The relationship between pH and peak current of L-Trp was illustrated in Fig. 4A to B. As it can be seen that the oxidation current peaked at pH 2 and then decrease with the increase of pH value. Besides, the oxidation potential of L-Trp at Co₃O₄-CNF shifted negatively with the increase of pH, which means the oxidation process associated with proton-transfer. A linear dependence between potentials and pH for L-Trp was as follow: $E_p(V) = 0.04614pH + 0.9716$ ($R = 0.997$).

3.3.3 Effect of electro-deposition potential

The effect of electro-deposition potential in the nature of proposed sensor is considerable important. The test was carried out in 10 mL 0.1 M KCl containing 200 μL 1mg/mL 5 wt%

Co₃O₄-CNF solution and deposition potential was implemented from 1.6–2.0 V. As shown in Fig. 4D 10 μM L-Trp current signal peaked when deposition potential reached 1.8 V and then decrease with the increase of deposition potential. As a result, 1.8 V was chosen to be the optimized deposition potential.

Fig. 4 here

3.4 Calibration curve of L-Trp

I-T was selected to explore the linear relationship between concentration of L-Trp and the oxidation current under optima condition. 0.85 V was chosen to be the applied potential. As shown in Fig. 5 the current response increased linearly with successive addition of L-Trp into the stirring phosphate solution (pH 2) at the range of 0.005 μM–40 μM. The linear regression was $I_p (\mu A) = 0.06895 + 0.1594 C (\mu M)$ ($R = 0.996$). The detection limit is estimated to be $2.0 \times 10^{-9} \text{ mol L}^{-1}$ ($S/N=3$). Table 1 lists the comparison of Co₃O₄-CNF/GCE with other carbon based sensors reported before. Our work shows a good linear working range and an attractive detection limit.

Fig 5 here

Table 1 here

3.5 Interference, reproducibility and stability of the sensor

Selectivity is a major parameter in judging the practicability of the proposed sensor. In supplementary pills, other amino acids like leucine, threonine, alanine, phenylalanine, lysine and saccharides as glucose and sucrose usually coexist with L-Trp. Table 2 shows the results of interference experiments of those coexisting component on the current intensity of 10 μM L-tryptophan on Co₃O₄-CNF/GCE. As it can be seen from the table, the sensing platform can distinguish well L-Trp from other coexisting interference. For further interferences as folic acid

and uric acid to L-Trp, those results can be found in Fig. S3.

Table 2 here

The reproducibility and stability of proposed sensor have been studied through 6 successive measurement in the 10 μ M L-Trp solution on different modified electrode. The relative standard deviation of the current signal was calculated to be 3.8%, which confirmed the reliability and reproducibility of the Co_3O_4 -CNF/GCE. The stability of the proposed sensor was studied after storing the proposed electrode 20 days in room temperature. The current signal of 10 μ M L-Trp only 4% less compared by new synthesized electrode. So that our proposed electrode shown a relatively long store life.

3.6 Real samples analysis

The practical utility of Co_3O_4 -CNF/GCE was evaluated by employing it for the determination of L-Trp in pills that purchased from local pharmacy (Tianshi muliti-amino acid pills). The concentration of L-Trp was estimated by standard addition method. The amperometric response of L-Trp was recorded at Co_3O_4 -CNF/GCE and the results were displayed in table 3. From the quantitative recovery results, the Co_3O_4 -CNF/GCE can be successfully employed for the determination of L-Trp in medicine sample.

Table 3 here

4. Conclusion

In this work, a simple and effective method for the quantification of L-Trp was investigated by electrochemical method on a Co_3O_4 -CNF/GCE. SEM and XRD result demonstrated the morphology and chemical component of synthesized materials. The synthesized Co_3O_4 -CNF/GCE shown good catalytic property towards L-Trp, at the same time it shown a wide linear range and a

good anti-interference property towards other amino acids. The detection limit of L-Trp on the proposed electrode was 2.0×10^{-9} mol L⁻¹ and it performed good accuracy in real samples' test.

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Lu Youlan and Zhao Dongsheng these two authors contributed equally to this work and should be considered as co-first authors.

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Table 1 Comparison of major characteristics at different carbon based modified electrodes for L-tryptophan's determination

Modified electrodes	Linear working range (μM)	Detection limit (μM)	Ref.
Mg–Al LDH/MWCNT/CPE ^a	3–90 90–1000	0.0068	[28]
FePt/CNT/CPE ^b	5–500	1	[29]
Co ₃ O ₄ /GR/Nafion/GCE ^c	0.05–10	0.01	[30]
TiO ₂ –MWNT/GCE ^d	1–150	0.52	[31]
SWCNH/GCE ^e	0.5–50	0.05	[32]
MWCNT/CPE ^f	0.6–9	0.03	[33]
Co ₃ O ₄ –CNF/GCE	0.005–40	0.002	This work

- Mg–Al LDH/MWCNT/CPE: multiwall carbon nanotube/Mg–Al layered double hydroxide modified carbon paste electrode.
- FePt/CNT/CPE: FePt decorated carbon nanotube nanocomposites modified carbon paste electrode.
- Co₃O₄/GR/Nafion/GCE: Co₃O₄ nanoparticles decorated graphene composite modified glassy carbon electrode.
- TiO₂–MWNT/GCE: TiO₂ nanoparticles decorated multiwall carbon nanotube modified glassy carbon electrode.
- SWCNH/GCE: single wall carbon nano-horn modified glassy carbon electrode.
- MWCNT/CPE: multiwall carbon nanotube modified carbon paste electrode.

Table 2 Results of interference experiments on the current intensity of 10 μM L- tryptophan on $\text{Co}_3\text{O}_4\text{-CNF/GCE}$.

Interferences	Concentration (μM)	Current ratio	R.S.D
Leucine	10	0.96	2.65%
Threonine	10	0.97	2.39%
Alanine	10	0.96	1.04%
Glucose	10	0.97	0.817%
Phenylalanine	10	0.96	2.21%
Lysine	10	0.97	2.55%
Sucrose	10	0.97	0.686%
Tyrosine	10	0.86	2.61%

Table3. Results of real sample analysis at $\text{Co}_3\text{O}_4\text{-CNF/GCE}$

No.	Detected (μM)	Added (μM)	Found (μM)	Recovery	R.S.D
1	1.74	2	2.09	105%	1.03%
2	1.76	4	4.05	101%	2.29%
3	1.80	6	6.10	102%	4.46%

Figure Captions:

Fig. 1 A SEM of Co_3O_4 -CNF, inset is the amplified photo, B SEM of Co_3O_4 , C XRD of Co_3O_4 -CNF and Co_3O_4 , D Raman spectrum of Co_3O_4 -CNF and Co_3O_4 , all data were acquired at 514.5 nm excitation.

Fig. 2 A CV pattern of 10 μM L-Trp on bare GCE, Co_3O_4 /GCE, CNF/GCE and Co_3O_4 -CNF/GCE in phosphate solution (pH 2), B EIS plots of bare GCE (●) and Co_3O_4 -CNF/GCE (■) in 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution containing 0.1 M KCl.

Fig. 3 CVs of Co_3O_4 -CNF/GCE in 0.1 M phosphate buffer (pH 2) with 10 μM L-Trp at scan rate ranging from 10 to 200 mV s^{-1} . Inset are the linear regression of scan rate versus oxidation potential and current signal.

Fig. 4 A CV of 10 μM L-Trp at Co_3O_4 -CNF/GCE in 0.1 M phosphate buffer pH (2 – 8) at scan rate of 100 mV s^{-1} , B the current intensity of 10 μM L-Trp at Co_3O_4 -CNF/GCE under different pH range from 2-8, C oxidation potential of 10 μM L-Trp at Co_3O_4 -CNF/GCE under different pH range from 2-8, D current intensity of 10 μM L-Trp at Co_3O_4 -CNF/GCE in pH 2 under different deposition potential from 1.6 V to 2.0 V

Fig. 5 A I-T pattern of L-Trp on Co_3O_4 -CNF/GCE in pH 2 in the range of 0.005 μM to 40 μM , the insert is the I-T pattern of L-Trp in low concentration. B the linear regression of L-Trp is in the range of 0.005 μM to 40 μM .

Scheme 1 The mechanism of electrochemical oxidation process of tryptophan

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Scheme 1

Author Biography

Prof. Yaping Ding gained her Ph.D degree in the University of Science and Technology of China and teaches in Shanghai University College of Science. She was also hired as professor in School of Materials Science and Engineering and worked as research leader in there. She is now interesting in synthesizing nano-materials and bio-functional material for electrochemical sensing.

Mr. Shengdong Zhao is a Ph.D candidate in School of Materials Science and Engineering. He is interesting in synthesizing nanomaterial and functional materials for different usage as supercapacitor, catalyst supporter and filter.

Miss Youlan Lu is a graduate student in Shanghai University, College of Science. She is now researching in the field of electrospinning carbon based materials. She has been worked in carbon nanofibers' synthesizing, characterizing and modification for three years. She is interesting in synthesizing nano-materials exploring their bio-chemical usage.

Miss Rong Fu is a first year graduate student in Shanghai University, College of Science. She is working in the field of electrospinning carbon nanofibers. She is interesting in synthesizing flexible carbon nanofiber mat and exploring their electrochemical usage.