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# A superior sensor for the electrochemical detection of Tryptophan in food samples using Ag-doped TiO<sub>2</sub> nanoparticles modified glassy carbon electrode

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This work presented preparation and characterization of silver doped TiO<sub>2</sub> nanoparticles modified glassy carbon electrode (Ag-TiO<sub>2</sub> NPs/GCE) and application to electrochemical detection of tryptophan as amino acids in food. The TiO<sub>2</sub> NPs and Ag-TiO<sub>2</sub> NPs were electrodeposited on GCE and their structural characterization with SEM and XRD exhibited that TiO<sub>2</sub> NPs was synthesized in rutile phase on GCE in sponge-like structure and Ag-TiO<sub>2</sub> NPs/GCE had the higher porosity and roughness than undoped electrode which indicated the higher effective surface area. The electrochemical measurements with cyclic voltammetry (CV) and amperometry showed that the nanostructured surface of electrode behaved as mediator and shuttled the electron between electrolyte and electrode surface and decreased the kinetic barrier in transfer of electron. Therefore, the comparable liner range (10 to 220  $\mu$ M), the higher sensitivity (0.9150  $\mu$ A/ $\mu$ M) and lower detection limit (0.003  $\mu$ M) were obtained for Ag-TiO<sub>2</sub> NPs/GCE to determine tryptophan compared to other reported tryptophan sensors. The stability, repeatability and selectivity of response for Ag-TiO<sub>2</sub> NPs/GCE was evaluated towards interference effect on determination of tryptophan which showed high anti-interfering capability and selective response of the Ag-TiO<sub>2</sub> NPs/GCE as tryptophan sensor. The practicability of the proposed sensors was used for the determination of tryptophan in egg white as the real sample that presented the tryptophan content in the prepared real sample is 0.10 µM. Furthermore, the acceptable values of obtained recoveries (102.50% to 90.00%) and RSDs (1.44% to 3.25%) indicated that the proposed sensors could satisfactorily be applied for determination of tryptophan in food samples.

**Keywords:** Electrochemical sensor; Amino acids; Tryptophan; Silver doped TiO<sub>2</sub> nanoparticles; Cycle voltammetry

## **1. INTRODUCTION**

Amino acids are organic molecules which contain both an amino group and a carboxyl group. These are components of proteins and metabolized in resting muscle [1, 2]. Amino acids have an important role to the regulation of digestion and appetite via hormones, neurons and neurotransmitters [3, 4]. Sensory proteins are another function of amino acids that it causes to transmit the nutrient state of the environment to the mechanistic target of rapamycin complex and thus regulate microautophagy. Moreover, biological functions are the most important role of amino acids in the human body [5-7]. For example, glutamic acid and gamma-amino acid as neurotransmitters in brain, hydroxyproline as collagen component, glycine as biosynthetic precursor to porphyrins in red blood cells, carnitine in lipid transport, glucogenic amino acids in glucose formation reaction, and tryptophan as precursor of the neurotransmitter serotonin and hormone melatonin [8]. Therefore, biological and nutritional characterizations of amino acids lead to extensive use as supplements in nutrient solutions in food products, pharmaceutical products, animal feed, cosmetics and fertilizers [9, 10].

Amino acids like tryptophan are called essential amino acids because they cannot synthesize in the human body and they must be supplied with a diet. Tryptophan ((2S)-2-amino-3-(1H-indol-3-yl) propanoic acid) as an aromatic amino acids is extendedly contributed in the biosynthesis of proteins. It is composed of  $\alpha$ -amino group,  $\alpha$ -carboxyl groups and indole rings which join to a methylene group. This amino acid trough synthesis neurotransmitter serotonin plays a major role in sleep, emotional moods, pain control, regulating appetite, inflammation, and intestinal peristalsis [11, 12]. Studies showed that consumption- of tryptophan-rich foods can improve sleep quality, anxiety and depression symptoms by enhancing the serotonin level in the brain [13-15]. Therefore, it is necessary to use tryptophan-rich foods and dietary supplements to provide the required tryptophan. Furthermore, potential side effects of tryptophan supplementation can be tremor, dry mouth, mild nausea and dizziness. Food sources of tryptophan are dairy products, turkey, beef, quinoa, poultry, barley, bananas, brown rice, and fish, baking chocolate, soybeans, potatoes, russet and peanuts. Thus, many studies have been conducted for electrochemical characterization and determination of amino acids and tryptophan [16-18]. Studies showed that use of doped or nanostructured sensors presented influence the chemical and physical properties [19-22], and promote the sensing properties [23-26]. Therefore, this study was carried out for electrodeposition, characterization of Ag-TiO<sub>2</sub> NPs/GCE and application to electrochemical determination of tryptophan in egg white samples.

#### **2. MATERIALS and METHOD**

Prior to the synthesis, the glassy carbon electrode (GCE) was polished with alumina powder (96%, 2-3  $\mu$ m, Lianyungang ZhongAo Aluminum Co., Ltd., China) on a polishing cloth, and ultrasonically washed with ethanol and deionized water, respectively. TiO<sub>2</sub> nanoparticles were electrodeposited on GCE in electrolyte which consisted 3 M KCl (99%, Lianyungang KANDS Chemical Co., Ltd., China), 0.01 M H<sub>2</sub>O<sub>2</sub> (50%, Qingdao HiseaChem Co., Ltd., China), and 0.01 M titanium butoxide (Ti(C<sub>4</sub>H<sub>9</sub>O)<sub>4</sub>, 97%, Sigma-Aldrich) under linear sweep applied potential –1.5 V to 1 V at 10 mV/s for 35 minutes. For synthesis the Ag-TiO<sub>2</sub> NPs/GCE, 0.05 mM AgNO<sub>3</sub> (99%, Anson Bio-Technology Co., Ltd., China) was added to electrolyte. Finally, the electrodeposited electrode was taken out from the solution and rinsed with deionized water.

All electrochemical studies and electrodeposition process were conducted on potentiostat/galvanostat (Autolab, Netherlands) in electrochemical cell which contained Pt wire as

counter electrode, Ag/AgCl (3 M KCl) as reference electrode and bare and modified GCE as working electrode. The electrochemical measurements were performed in 0.1 M KOH (90%, Qingdao HiseaChem Co., Ltd., China) solution and 0.1M phosphate buffer solution (PBS) which prepared from Na<sub>2</sub>HPO<sub>4</sub> (99.0%, Ningxia Baiyun Carbon Co., Ltd., China).In order to preparation real sample, the eggs were purchased from local market, the egg whites were separated from egg yolks. The separated egg whites were dried in room temperature, and 1 g powder of dried egg whites was added to 11 of 0.1 M PBS.

The morphology of electrodeposited TiO<sub>2</sub> NPs/GCE and Ag-TiO<sub>2</sub> NPs/GCE was studied via scanning electron microscopy (SEM). The structural property of samples was done by X-ray diffraction (XRD) at 35 kV, 10 mA and wavelength radiation of CuK $\alpha$  ( $\lambda$ =1.5418 Å), USA).

#### **3. RESULTS AND DISCUSSION**

The SEM images of the TiO<sub>2</sub> NPs/GCE and Ag-TiO<sub>2</sub> NPs/GCE are presented in Figure 1. As observed, the synthesized TiO<sub>2</sub> NPs/GCE is covered by a sponge-like structure which contains the spherical shape TiO<sub>2</sub> NPs with good dispersion. The average diameter of TiO<sub>2</sub> NPs is 42 nm. For Ag-TiO<sub>2</sub> NPs/GCE, there is higher porosity and roughness than undoped electrodes which indicate the higher surface area. Moreover, the distribution of Ag NPs on the TiO<sub>2</sub> surface is not uniform, and doped electrodes are covered by irregular shaped particles. The average diameter of Ag-TiO<sub>2</sub> NPs is 55 nm. There are more aggregates of tiny crystals of Ag-TiO<sub>2</sub> NPs that are useful for their catalytic properties in electrochemical activity.



Figure 1. FESEM images of the (a) TiO<sub>2</sub> NPs/GCE and (b) Ag-TiO<sub>2</sub> NPs/GCE electrodes.

Figure 2 reveals the XRD pattern of TiO<sub>2</sub> NPs and Ag-TiO<sub>2</sub> NPs powders. There are diffraction peak at  $2\theta = 27.33^{\circ}$ ,  $35.92^{\circ}$ ,  $41.09^{\circ}$ ,  $54.18^{\circ}$ ,  $56.49^{\circ}$  and  $68.78^{\circ}$  which attributed to formation of the (110), (101), (111), (211), (220) and (301) planes of rutile phase of TiO<sub>2</sub>, respectively according to JCPDS card no.77-0441. XRD pattern of Ag-TiO<sub>2</sub> NPs shows the XRD pattern of the same as that of TiO<sub>2</sub> NPs with diffraction peaks at  $2\theta=38.09^{\circ}$ ,  $44.29^{\circ}$  and  $64.42^{\circ}$  which associated with (111), (200)

and (220) planes of fcc phase of Ag that these are well matched with JCPDS card no.65-2871. The results are evidence to form mixed phases of Ag doped  $TiO_2$ .



Figure 2. XRD patterns of the (a) of TiO<sub>2</sub> NPs and (b) Ag-TiO<sub>2</sub> NPs powders.

Figure 3 shows the recorded CVs of GCE, TiO<sub>2</sub> NPs/GCE and Ag-TiO<sub>2</sub> NPs/GCE in 0.1 M KOHpH 9.5 at a scan rate of 10 mV/s. It has not observed any recorded peak for GCE. For TiO<sub>2</sub> NPs/GCE, the cathodic and anodic peaks are appear at -0.01 V and -0.16V that is associated with Ti ions reduction and oxidation of reduced Ti ions, respectively [27-29]. For Ag-TiO<sub>2</sub> NPs/GCE, the recorded CV shows two pairs of redox peaks which contained a pair of cathodic and anodic peak at 0.07V and 0.03V, respectively, and another pair of cathodic and anodic peak at 0.37V and 0.33 V, respectively. Both redox peaks are correlated with oxidation Ag to Ag<sub>2</sub>O and reduction Ag<sub>2</sub>O to Ag[30, 31] that confirms to introduction and anchoring the Ag NPs inside the TiO<sub>2</sub> network.

Figure 4 exhibits the recorded CVs of GCE, TiO<sub>2</sub> NPs/GCE and Ag-TiO<sub>2</sub> NPs/GCE in 0.1 M PBS pH 7 at a 10 mV/s scan rate with addition of tryptophan. As observed from Figure 4, there are irreversible oxidation peaks at potential of 0.87, 0.80 and 0.72 V for GCE, TiO<sub>2</sub> NPs/GCE and Ag-TiO<sub>2</sub> NPs/GCE, respectively. The recorded peak currents for GCE, TiO<sub>2</sub> NPs/GCE and Ag-TiO<sub>2</sub> NPs/GCE are -1.03, 3.70 and 4.67  $\mu$ A, respectively. Accordingly, the oxidation potential and current are decreased and increased with modification of the GCE by nanoparticles, respectively. These are related to the nanostructured surface of electrode that it behaves as a mediator and shuttles the electron between electrolyte and electrode surface and decreases the kinetic barrier in transfer of electron [32]. Moreover, the nanostructured surface of electrodes causes enhancement of effective surface area. Ag-TiO<sub>2</sub> NPs/GCE shows the lower peak potential and higher peak current that it is due to higher

conductivity of Ag NPs [33]. Therefore, the well electrocatalytic behavior is obtained for the determination of tryptophan, as consequence the following measurements were conducted on Ag-TiO<sub>2</sub> NPs/GCE.



**Figure 3.** The recorded CVs of (a) GCE, (b) TiO<sub>2</sub> NPs/GCE and (c) Ag-TiO<sub>2</sub> NPs/GCE in 0.1 M KOH pH 9.5 at a scan rate of 10 mV/s and potential range of -0.8 V to 0.6 V.

Figure 5a shows the stability effect of electrocatalytic response of Ag-TiO<sub>2</sub> NPs/GCE in presence of 1.5  $\mu$ M tryptophan solutions in 0.1M PBS (pH 7) at a 10 mV/s scan rate. As seen, the difference of the first and 100<sup>th</sup> recorded electrocatalytic current of tryptophan is ignorable (less than 7 %) which referred to the solution. Figure 5b shows the recorded CVs of Ag-TiO<sub>2</sub> NPs/GCE in 0.1M PBS (pH 7) at a 10 mV/s scan rate with successive additions of 1.5, 3, 4.5, 6 and 7.5  $\mu$ M tryptophan solutions. As seen, the tryptophan oxidation peak is linearly increases with increasing the tryptophan concentrations. With increase in the concentration of tryptophan, the anodic peak current increases, indicating that phenyl ring in tryptophan has been oxidized by the active Ag-TiO<sub>2</sub> NPs/GCE. Ag is oxidized to Ag<sup>+</sup> on the electrode surface and the tryptophan acts as a reducing agent [34].

Further electrochemical studies were performed using amperometry technique for obtaining the sensitivity, linear range and detection limit of Ag-TiO<sub>2</sub> NPs/GCE as tryptophan sensor in 0.1M PBS at 0.70 V with successive additions of tryptophan. Figures 6 shows the recorded DPV and calibration plot which demonstrated to the fast response of electrode to addition tryptophan, and result in a limit of detection of  $0.003\mu$ M, sensitivity of 0.9150  $\mu$ A/  $\mu$ M and liner range from 10 to 220  $\mu$ M.



Figure 4. The CV curves of (a) GCE, (b)  $TiO_2$  NPs/GCE and (c) Ag-TiO\_2 NPs/GCE in 0.1 M PBS (pH 7.0) at a scan rate of 10 mV/s in presence 1.5  $\mu$ M tryptophan solution and potential range of 0.22 V to 1.0 V.



**Figure 5.** The CV curves of Ag-TiO<sub>2</sub> NPs/GCE in 0.1 M PBS (pH 7.0) at a scan rate of 10 mV/s toward addition of tryptophan and potential range of 0.22 V to 1.0 V for study of (a)the stability effect and (b) concentration effect.

Table 1 exhibits the comparison between the obtained linear range, detection limit and sensitivity values of Ag-TiO<sub>2</sub> NPs/GCE and other reported tryptophan sensors which revealed to higher sensitivity and lower detection limit values of Ag-TiO<sub>2</sub> NPs/GCE than other reported

tryptophan sensors due to excellent structural features like large electroactive surface area of Ag-TiO<sub>2</sub> NPs, faster electron transfer rates and good conductivity of Ag NPs [35, 36].



**Figures 6.** (a) The amperometric response and (b) calibration curve of Ag-TiO<sub>2</sub> NPs/GCE in 0.1 M PBS (pH 7.0) at 0.70 V rotating speed of 1000 rpm to successive additions of tryptophan.

Table 1. Comparison betw	veen the performances	s of Ag-TiO2 NPs/GCE	and different reported sensor
for tryptophan deter	mination		

Electrode	Method	Linear Range	Detection	Sensitivity	Ref.
		( <b>µM</b> )	limit (µM)	(μ <b>Α/μ</b> Μ)	
Ag-TiO2 NPs/GCE	AMP <sup>a</sup>	10 - 220	0.003	0.9150	This
					work
AuNPs/ carbon paste	DPV <sup>b</sup>	6.0–200.0	0.65	0.2563	[37]
electrode				0.0704	
Carbon black	SWV <sup>c</sup>	0.025–4.8	0.011	0.2039	[38]
nanoballs/CNTs/GCE					
pencil graphite leads	DPV	0.154–200.0	0.046	0.3119	[39]
MWCNTs-	DPV	4.9 -64.1	1.6	0.4085	[40]
cetyltrimethylammonium					
bromide/GCE					
graphene oxide-	SWV	5-700	1.0	0.0346	[41]
NiOnanocomposite					
/carbon paste electrode					
hydroquinone derivative	DPV	20.0-	5.5	0.0257	[42]
/CNTs/carbon paste		800.0			

electrode						
benzofuran /CNTs/carbon electrode	derivative paste	DPV	100.0-400.0	1.16	0.044	[43]

<sup>a</sup>AMP: amperometry<sup>b</sup> DPV: differential pulse voltammetry <sup>c</sup> SWV: Squarewave voltammetry

In order to investigate the interference effect of Ag-TiO<sub>2</sub> NPs/GCE to determination of tryptophan, the recorded electrocatalytic current using amperometry technique in 0.1M PBS (pH 7) at 0.70 V to first addition 10  $\mu$ M of Tryptophan and 20  $\mu$ M of dopamine, tartaric acid, ascorbic acid, uric acid, folic acid, 1-histidine, 1-asparagine, 1-lysine, nitrite and epinephrine, respectively, and second addition 10  $\mu$ M of tryptophan. Figure 7 displays the recorded electrocatalytic currents for first and second additions of 10  $\mu$ M tryptophan are 9.09  $\mu$ A and 9.12  $\mu$ A, respectively, which indicated to stability and repeatability response of Ag-TiO2 NPs/GCE towards determination of tryptophan. In addition, recorded electrocatalytic currents of substances additions are insignificant which in agreement with other reports of tryptophan sensors [44, 45]. Therefore, the results corroborated the anti-interfering capability of the Ag-TiO<sub>2</sub> NPs/GCE for determination of tryptophan.



**Figures 7.** (a) The electrochatalytic current using amperometry technique on Ag-TiO<sub>2</sub> NPs/GCE to in 0.1M PBS (pH 7.0) at 0.70 V to additions 10  $\mu$ M of tryptophan and 20  $\mu$ M of different substances at 0.70 V rotating speed of 1000 rpm.

The practicability of the proposed sensors was used for the determination of tryptophan in egg white by amperometry technique in prepared real sample pH 7.0 at 0.70 V. Figure 8 shows the recorded amperometry of Ag-TiO<sub>2</sub> NPs/GCE in successive additions of 0.1  $\mu$ M tryptophan solution

and its calibration plot. Results present the tryptophan content in the prepared real sample is 0.10  $\mu$ M which is closed to reported value by ion exchange liquid chromatography [46]. Moreover, Table 2 shows the analytical results of determination tryptophan in egg white prepared samples with the Ag-TiO<sub>2</sub> NPs/GCE by standard addition. As observed, the acceptable values of obtained recoveries (102.50% to 90.00%) and RSDs (1.44% to 3.25%) indicate that the proposed sensors could satisfactorily be applied for determination of tryptophan in food specimens.



**Figure 8.** (a) The amperometric response of Ag-TiO<sub>2</sub> NPs/GCE in real sample of egg white (pH 7.0) at 0.70 V at 0.70 V rotating speed of 1000 rpm to successive addition of 0.1  $\mu$ M tryptophan solution and (b) its calibration plot.

**Table 2.** The analytical results of tryptophan in egg white samples (n = 4)

Sample	Spiked (µM)	Found	Recovery (%)	<b>RSD</b> (%)
		(µM)		
Egg	0.10	0.09	90.0	1.44
white	0.20	0.18	90.0	2.11
	0.30	0.29	96.6	2.72
	0.40	0.41	102.5	3.25
	0.50	0.48	96.0	3.21

#### **4. CONCLUSION**

This study was performed for preparation and application of silver doped TiO<sub>2</sub> nanoparticles modified glassy carbon electrode (Ag-TiO<sub>2</sub> NPs/GCE) as electrochemical sensor of tryptophan in food.

The TiO<sub>2</sub> NPs and Ag-TiO<sub>2</sub> NPs were electrodeposited on GCE. The morphology and of crystalline structures of electrodeposited samples were studied with SEM and XRD and their results exhibited that the synthesized TiO<sub>2</sub> NPs/GCE in rutile phase was covered by sponge-like structure which contained the spherical shape TiO<sub>2</sub> NPs with good dispersion and Ag-TiO<sub>2</sub> NPs/GCE had the higher porosity and roughness than undoped electrode. Electrochemical characterizations with CV and amperometry showed that the nanostructured surface of electrode behaved as mediator and shuttled the electron between electrolyte and electrode surface and decreased the kinetic barrier in transfer of electron. Moreover, nanostructured surface of electrode caused to enhancement of effective surface area and led to higher sensitivity (0.9150  $\mu$ A/ $\mu$ M) and lower detection limit (0.003  $\mu$ M) of Ag-TiO<sub>2</sub> NPs/GCE to determination of tryptophan compared to other reported tryptophan sensors. The liner range was obtained from 10 to 220 µM. The stability, repeatability, interference effect and selectivity of response of Ag-TiO2 NPs/GCE was evaluated towards determination of tryptophan. Results showed high antiinterfering capability and selective response of the Ag-TiO<sub>2</sub> NPs/GCE as tryptophan sensor. The practicability of the proposed sensors was used for the determination of tryptophan in egg white as a real sample that its result presented the tryptophan content in the prepared real sample is 0.10 µM. Moreover, the acceptable values of obtained recoveries (102.50% to 90.00%) and RSDs (1.44% to 3.25%) indicated that the proposed sensors could satisfactorily be applied for determination of tryptophan in food samples.

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