Application of Fe$_3$O$_4$@SiO$_2$/GO nanocomposite for sensitive and selective electrochemical sensing of tryptophan

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Abstract

A simple strategy for determination of tryptophan (TRP) based on Fe$_3$O$_4$@SiO$_2$/GO nanocomposite modified graphite screen printed electrode (Fe$_3$O$_4$@SiO$_2$/GO/SPE) is reported. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to characterize the performance of the sensor. The Fe$_3$O$_4$@SiO$_2$/GO/SPE displayed excellent electrochemical catalytic activities. The oxidation overpotentials of tryptophan decreased significantly and its oxidation peak current increased dramatically at Fe$_3$O$_4$@SiO$_2$/GO/SPE. Under the optimized experimental conditions tryptophan showed linear response over the range of 1.0-400.0 μM. The lower detection limit was found to be 0.2 μM for tryptophan. The practical application of the modified electrode was demonstrated by measuring the concentration of tryptophan in real samples.

Keywords

Tryptophan; Fe3O4@SiO2/GO nanocomposite; graphite screen printed electrodes; voltammetry

Introduction

Tryptophan (Trp) is a vital amino acid for humans and herbivores as the precursor of hormones, neurotransmitters and other relevant biomolecules. Tryptophan is also a precursor of the neurotransmitter serotonin [1-4]. It has been implicated as a possible cause of schizophrenia in people who cannot metabolize it properly. When improperly metabolized, it creates a waste product in the brain that is toxic, causing hallucination, delusions depression, Alzheimer’s and Parkinson’s diseases. Therefore, determination of tryptophan is very important in blood and urine sample in these disease. This compound is sometimes added to dietary, food products, pharmaceutical formulas due
to the scarcely presence in vegetables [5-8]. Hence, a simple, fast, selective, sensitive and accurate method for the determination of this analyte is very important. Methods for the determination of tryptophan are mainly based on HPLC [9] and spectrophotometric procedures [10]. Most of these methods involve laborious and slow procedures with the modification of tryptophan by numerous reagents.

The electroanalytical methods, with respect to their sensitivity, accuracy, less expensive and simplicity have been more intentioned in recent years for analytes determination [11-14].

Screen printed electrodes (SPes) allowing the mass production of reproducible, economical and disposable devices. Other substantial features of these SPes which make them suitable for on-site analysis are related to their capability to be connected to portable instrumentation and its miniaturized size [15-18]. In order to improve the efficiency of usual electrodes for sensor applications, a conventional modification method is applied. Modified electrodes not only render better electrocatalytic activity, higher sensitivity and selectivity, but also lower detection limit in comparison with traditional electrodes [19-26].

Nano materials are recognized as engineered particles with considerable surface to volume ratio and dimension less than 100 nm [27-30]. Application of nanoparticles in the construction of electrochemical sensors has increased recently [31-35].

Graphene is a two dimensional (2-D) sheet of carbon atoms in a hexagonal configuration with atoms bonded by sp² bonds. These bonds and this electron configuration provide this arterial with extraordinary properties, such as large surface area, theoretically 2630 m²/g for a single layer, and double that of single walled carbon nanotubes (SWCNTs). It also shows excellent thermal and electrical conductivity. Due to its unique electronic properties, large surface area, rich edge defects, a tunable band gap, room temperature Hall effect, strong mechanical strength, high elasticity and thermal conductivity; it exhibits remarkable conductivity, and sensing capability [36-38]. Magnetic iron oxide nanoparticles such as maghemite (γ-Fe₂O₃) or magnetite (Fe₃O₄), are used in decoration of graphene to obtain improved magnetic, optical, and electrochemical properties in graphene. This combination which leads to improve the properties of graphene, make it a great option for the modification of electrodes [39].

By showing advantageous properties of superparamagnetism, low toxicity and reusability, Fe₃O₄ nanoparticles (Fe₃O₄NPs) have played a major role among the most widely used magnetic sorbents. Application of bare Fe₃O₄ NPs, however, is limited owing to its oxidation characteristics and lack of target selectivity. Thus, the bare Fe₃O₄NPs is required to be functionalized in order to protect them from oxidation and raise selectivity and adsorption efficiency of a target analyte. In addition, the silica (SiO₂) has been widely used as catalysis, electronic device and amorphous materials. It should be noted that SiO₂ has attracted much attention as sensing material in the design of biosensors [40-42].

The most promising and favourable coating material proved to be silica, as it not only protects magnetic nanoparticles against oxidation and agglomeration at wide pH range, but also improves their chemical stability. Moreover, the surface of silica is often finished with a silanol group, which can react with various chemicals and silane coupling agents to conjugate with a variety of biomolecules and specific ligands. Thus, SiO₂ layers have good compatibility and hydrophilicity and indispensable properties for the use of these materials in biomedical applications [43].

The present work aims to employ the electrochemical method for determination of tryptophan at the newly synthesized Fe₃O₄@SiO₂/GO nanocomposite modified screen printed electrode. The modified electrode was used successfully as an electrochemical sensor for determination of tryptophan in the real sample as a sensitive, selective, simple and rapid method.
Experimental

Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with the General Purpose Electrochemical System (GPES) software. The screen-printed electrode (DropSens, DRP-110, Spain) consists of three main parts which are a graphite counter electrode, a silver pseudo-reference electrode and a graphite working electrode (diameter: 1 mm). A Metrohm 710 pH meter was used for pH measurements.

Tryptophan and all other reagents were of the analytical grade, and they were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts over the pH range of 2.0-9.0.

Synthesis of Fe$_3$O$_4$@SiO$_2$/GO nanocomposite

Graphene oxide nano sheets were synthesized from natural graphite flakes based on the modified Hummers and Offeman’s method. The reduced graphene oxide (0.096 g) was dispersed in 40 ml of water, and the solution was kept in ultrasonic bath for 1 h. The mixture was further stirred vigorously for 30 min at 60 °C. Next, 177 mg of FeCl$_3$·6H$_2$O was added under the stirring. After the mixture was stirred vigorously for 30 min under the N$_2$ atmosphere, 95 mg of FeSO$_4$·7H$_2$O was added and stirred under the N$_2$ atmosphere for 30 min. Finally, 30 mL of the 6 % NH$_4$OH aqueous solution was added into the mixture, drop by drop, at 60 °C for 1 h and then reacted for another 2 h. The N$_2$ atmosphere was used during the reaction to prevent critical oxidation. The reaction mixture was then centrifuged, washed with double distilled water, and then dried. The obtained black precipitate was Fe$_3$O$_4$/GO nanoparticles and was ready for use. Core–shell Fe$_3$O$_4$@SiO$_2$/GO nanocomposites were prepared by growing silica layers onto the surface of Fe$_3$O$_4$/GO, as described by Lu et al. [43]. Twenty-five millilitres of ethanol, 1 mL of water, 1 mL of ammonium hydroxide, and 150 μL of tetraethyl orthosilicate (TEOS) were added in a 250 ml three-neck flask in a 40 °C water bath. Fe$_3$O$_4$/GO was added to the above-mentioned solution under mechanical stirring. Aliquots of the mixture were taken out after 12 h by centrifugation, and then washed with water and vacuum-dried at 60 °C overnight. The morphology of the product was examined by SEM. Fig. 1 depicts the SEM pictures of Fe$_3$O$_4$@SiO$_2$/GO nanocomposites.

Preparation of the electrode

The bare screen printed electrode was coated with Fe$_3$O$_4$@SiO$_2$/GO, as shown in the following. A stock solution of Fe$_3$O$_4$@SiO$_2$/GO in 1 mL of the aqueous solution was prepared by dispersing 1 mg of Fe$_3$O$_4$@SiO$_2$/GO with ultrasonication for 1 h, while 2 μl of aliquots of the Fe$_3$O$_4$@SiO$_2$/GO/H$_2$O suspension solution was cast on the carbon working electrodes, followed by waiting until the solvent was evaporated at room temperature.
Preparation of real samples

Urine samples were stored in a refrigerator immediately after collection. Ten millilitres of the samples were centrifuged for 15 min at 2,000 rpm. The supernatant was filtered out by using a 0.45 µm filter. Next, different volumes of the solution was transferred into a 25 mL volumetric flask and diluted to the mark with phosphate buffer saline (PBS) (pH 7.0). The diluted urine samples were spiked with different amounts of tryptophan. The tryptophan contents was analysed by the proposed method by using the standard addition method.

The serum sample was centrifuged, and after filtering, diluted with PBS (pH 7.0) without any further treatment. The diluted serum sample was spiked with different amounts of tryptophan. The tryptophan contents was analysed by the proposed method by using the standard addition method.

Discussion

Electrochemical behaviour of tryptophan at the surface of various electrodes

The electrochemical behaviour of tryptophan depends on the pH value of the aqueous solution. Therefore, the pH optimization of the solution seems to be necessary in order to obtain the best results for the electrooxidation of tryptophan. Thus, the electrochemical behaviour of tryptophan was studied in 0.1 M PBS at different pH values (2.0-9.0) at the surface of Fe₃O₄@SiO₂/GO/SPE by voltammetry. The peak current of tryptophan is increased with the increase of pH and reached the highest value when the pH of PBS is 7.0. Thus, the subsequent determination experiment was performed in 0.1 M pH 7.0 PBS (Fig. 2).

![Fig. 2. Plot of I_p vs. various pH (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0)](image)

Fig. 2 depicts the CV responses for electrooxidation of 100.0 µM tryptophan at the and unmodified SPE (Curve a), GO/SPE (Curve b), SiO₂/GO/SPE (Curve c) and Fe₃O₄@SiO₂/GO/SPE (Curve d) The peak potential occurs at 725 mV due to the oxidation of tryptophan, which is about 145 mV more negative than the unmodified SPE. Also, Fe₃O₄@SiO₂/GO/SPE shows much higher anodic peak current for the oxidation of tryptophan compared to the other electrodes, indicating that the modification of the unmodified SPE with Fe₃O₄@SiO₂/GO nanocomposite has significantly improved the performance of the electrode towards tryptophan oxidation.
The effect of potential scan rates on the oxidation current of tryptophan has been studied (Fig. 4). The results showed that increasing the potential scan rate induced an increase in the peak current. In addition, the oxidation processes are diffusion controlled, as deduced from the linear dependence of the anodic peak current ($I_p$) on the square root of the potential scan rate ($\nu^{1/2}$) for tryptophan.

Tafel plot was drawn from the data of the rising part of the current–voltage curves recorded at a scan rate of 10 mV s$^{-1}$ for tryptophan. This part of voltammogram, known as the Tafel region is affected by electron transfer kinetics between substrate (tryptophan) and Fe$_3$O$_4$@SiO$_2$/GO/SPE. Tafel slope of 0.1528 V/decade was obtained, which agree well with the involvement of one electron at the rate determining step of the electrode process [44], assuming charge transfer coefficients $\alpha = 0.61$ for tryptophan.

**Chronoamperometric measurements**

Chronoamperometric measurements of tryptophan at Fe$_3$O$_4$@SiO$_2$/GO/SPE was carried out by setting the working electrode potential at 0.75 V vs. Ag/AgCl/KCl (3.0 M) for various concentrations of tryptophan (Fig. 5) in PBS (pH 7.0). For electroactive materials (tryptophan in this case) with a diffusion coefficient of $D$, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [44]:

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2}$$
where $D$ and $C_b$ are the diffusion coefficient (cm$^2$ s$^{-1}$) and the bulk concentration (mol cm$^{-3}$), respectively. Experimental plot of $I$ vs. $t^{-1/2}$ was employed with the best fits for different concentrations of tryptophan (Fig. 5A). The slopes of the resultant straight lines were then plotted against tryptophan concentrations (Fig. 5B). From the resultant slope and the Cottrell equation, the mean value of $D$ was found to be $2.5\times10^{-5}$ cm$^2$/s for tryptophan.

**Fig. 5.** Chronoamperograms obtained at Fe$_3$O$_4$@SiO$_2$/GO/SPE in 0.1 M PBS (pH 7.0) for different concentrations of tryptophan. The numbers 1–5 correspond to 0.1, 0.5, 1.0, 1.5 and 2.0 mM of tryptophan. Insets: (A) Plots of $I$ vs. $t^{-1/2}$ obtained from chronoamperograms 1–5. (B) Plot of the slope of the straight lines against tryptophan concentrations.

**Calibration plots and limits of detection**

The electrooxidation peak currents of tryptophan at the surface of Fe$_3$O$_4$@SiO$_2$/GO/SPE can be used to determine tryptophan in the solution. Since differential pulse voltammetry (DPV) has the advantage of having an increase in sensitivity and better characteristics for analytical applications, DPV experiments were performed by using Fe$_3$O$_4$@SiO$_2$/GO/SPE in 0.1 M PBS containing various concentrations of tryptophan (Fig. 6). The results show that the electrocatalytic peak currents of tryptophan oxidation at the surface of Fe$_3$O$_4$@SiO$_2$/GO/SPE were linearly dependent on tryptophan concentrations over the range of 1.0–400.0 µM (with a correlation coefficient of 0.9981) while the detection limit ($3\sigma$) was obtained as 0.2 µM. These values are comparable with values reported by other research groups for determination of tryptophan (Table 1).
Table 1. Comparison of the efficiency of some modified electrodes used in the electro-oxidation of tryptophan

| Electrode              | Modifier                                                                 | LOD, µM  | LDR, µM        | Ref. |
|------------------------|--------------------------------------------------------------------------|----------|----------------|------|
| Glassy carbon          | Silver nanoparticles-decorated reduced graphene oxide                     | 7.5      | 10.0-800.0     | [45] |
| Glassy carbon          | Cu-nanoparticles incorporated overoxidized-poly (3-amino-5-mercapto-1, 2, 4-triazole) | 0.16     | 4.0-144.0      | [46] |
| Carbon paste            | Carbon nanoparticles and reduced graphene oxide                          | 65.0     | 80.0-1000.0    | [47] |
| Glassy carbon          | Poly (9-aminoacridine) functionalized multi-walled carbon nanotubes      | 0.8      | 1.0-500.0      | [48] |
| Screen printed         | Fe₃O₄@SiO₂/GR Nanocomposite                                              | 0.2      | 1.0-400.0      | This work |

Real sample analysis

The new Fe₃O₄@SiO₂/GO nanocomposite modified screen printed electrode was also applied for the determination of tryptophan in human blood serum and urine samples by using the standard addition method. Differential pulse voltammetry (DPV) experiments were done for different real samples. The results for the determination of the tryptophan in real samples are given in Table 2. Satisfactory recoveries of the experimental results were found for tryptophan. The reproducibility of the method was demonstrated by the mean relative standard deviation (RSD).

Table 2. Determination of tryptophan in human blood serum and urine samples.

| Sample            | c / µM (n=5)           | Recovery, % | RSD, % |
|-------------------|------------------------|-------------|--------|
|                   | Spiked | Found |               |         |
| Human blood serum |         |       |               |         |
| 0                 | -      | -     | -             | -       |
| 7.5               | 7.6    | 101.3 | 3.2            |
| 12.5              | 12.2   | 97.6  | 2.8            |
| 17.5              | 17.9   | 102.3 | 1.8            |
| 27.5              | 27.3   | 99.3  | 2.4            |
| Urine             |         |       |               |         |
| 0                 | -      | -     | -             | -       |
| 10.0              | 9.7    | 97.0  | 1.9            |
| 20.0              | 20.5   | 102.5 | 2.8            |
| 30.0              | 30.4   | 101.3 | 2.2            |
| 40.0              | 40.3   | 100.7 | 3.3            |

The repeatability and stability of Fe₃O₄@SiO₂/GO/SPE

The long-term stability of the Fe₃O₄@SiO₂/GO/SPE was tested over a 3-week period. When CVs were recorded after the modified electrode was stored in atmosphere at room temperature, the peak potential for tryptophan oxidation was unchanged and the current signals showed less than 2.6 % decrease relative to the initial response. The antifouling properties of the modified electrode toward tryptophan oxidation and its oxidation products were investigated by recording the cyclic voltammograms of the modified electrode before and after use in the presence of tryptophan. Cyclic voltammograms were recorded in the presence of tryptophan after having cycled the potential 20 times at a scan rate of 50 mV s⁻¹. The peak potentials were unchanged and the currents decreased by less than 2.4 %. Therefore, at the surface of Fe₃O₄@SiO₂/GO/SPE, not only the sensitivity increase, but the fouling effect of the analyte and its oxidation product also decreases.
Conclusion

The present study demonstrates the construction of Fe$_3$O$_4$@SiO$_2$/GO/SPE and its application for the determination of tryptophan. The tryptophan oxidation was catalyzed at pH 7.0 and its peak potential were shifted to a less positive potential at the tryptophan surface. This modified electrode offers a considerable improvement in voltammetric sensitivity toward tryptophan, compared to the bare electrode. Differential pulse voltammetry (DPV) exhibits a linear dynamic range from 1.0-400.0 μM and a detection limit of 0.2 μM for tryptophan. The modified electrode showed high stability, good reproducibility and fast response for the detection of tryptophan concentrations. Moreover, the proposed method was applied to determination of tryptophan in real sample with satisfactory results.

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