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Preparation of voltammetric biosensor for tryptophan using multi-walled carbon nanotubes

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Abstract−Multi-walled carbon nanotubes (MWCNTs) were grown by chemical vapor deposition. The effect of the composition of carbon paste electrode on its voltammograms was evaluated in basic solution with 5.0×10^{-5} M tryptophan (Trp). It was found that addition of MWCNTs to the carbon paste would generate the peak current of Trp because of its catalytic effect on the redox process. The pH strongly affects the peak potential of Trp. The best analytical response was obtained at pH 13.0. The anodic peak currents were proportional to Trp concentrations in the range of 1.0×10^{-9}-1.0×10^{-4} M under the optimized experimental conditions. The detection limit was 2.2×10^{-10} M. The effect of potential scan rate on the peak potential and peak current of tryptophan was investigated. The correlation of the peak currents against $v^{1/2}$ (v is the scan rate) is linear, which is very similar to a diffusion-controlled process. The proposed biosensor was applied to the determination of Trp in pharmaceuticals formulations successfully.

Key words: Biosensor, Cyclic Voltammetry, Multi-walled Carbon Nanotubes, Chemical Vapor Deposition, Electrocatalytic Effect

INTRODUCTION

Tryptophan is an essential amino acid with diverse physiological roles, functioning both independently or via incorporation into the structure of larger molecules or polymers, such as proteins. It is a precursor for biologically important molecules, such as the neurotransmitter serotonin and the neurohormone melatonin. Abnormal levels of serotonin and melatonin have been shown to be associated with depression and Alzheimer's and Parkinson's diseases, respectively. It has been shown that the control of dietary intake of tryptophan (through food or supplements) and the resulting physiological concentrations in the human body has had a positive effect on the regulation of the synthesis of serotonin [1-4]. Tryptophan, found as a component of dietary protein, is particularly plentiful in chocolate, oats, bananas, dried dates, milk, yogurt, cottage cheese, red meat etc [5,6].

The analysis of amino acids is commonly based on liquid chromatography and capillary electrophoresis with pre- or post-column labeling and spectroscopic detection of the corresponding derivatives [7-9]. So far, several analytical methods for the determination of pharmaceutical mixtures including voltammetry [2,10], HPLC [11-14], spectrofluorimetry [15], spectrophotometry [16], capillary electrophoresis [17,18], flow-injection analysis [19-21], gas chromatography/mass spectrometry [22], thin-layer chromatography/fluorescence detection [23], ion exchange chromatography [24], amperometric determination [25], far-IR absorption and reflection spectra [26], ion chromatography [27], cyclic voltammetry [28] and liquid chromatography with fluorescence detection [29] have been reported. Concentration of amino acids in biological samples is low; therefore, it is necessary to use a highly sensitive method that provides determination of these analytes at subordinate concentrations. Electrochemical analytic technique is an attractive method due to simplicity, low expense, high sensitivity and possibility of miniaturization. However, there are the following problems for the direct electrochemical oxidation of Trp at common electrodes: (1) the electron transfer reaction of Trp is slow and high potential is required for determination, and (2) strong adsorption of Trp at the surface of metallic electrode makes Trp detection unsatisfactory. These problems have been solved using modified electrodes [30]. Construction of electrochemical sensors using modified electrodes continues to be an area of great interest, and a relatively large amount of electrochemical research has been devoted to the development and application of these sensors for the analysis of biologically active compounds. Modification of electrodes with suitable materials facilitates the electrochemistry of the redox biological compounds, which generally results in increased selectivity and sensitivity of the determinations. Among these methods, chemically modified electrodes (CMEs) have been applied for the determination of Trp in real samples [31, 32]. Zeolite-modified electrodes (ZMEs), the important type of the CMEs, have been largely developed during the past two decades because they enable to combine the intrinsic properties of the zeolites (mainly their ion-exchange capacity and their size selectivity at the molecular level) with electron transfer reactions. Zeolite-modified carbon paste electrodes (ZMCPEs) have been largely and successfully applied for electroanalytical purposes [33].

Due to the unique properties of carbon nanotubes, including enhanced electronic properties, high chemical stability and mechanical strength, and rapid electrode kinetics, they have received considerable attention as modifiers in the field of electrochemical sensing. Therefore, CNT-based electrochemical sensors generally offer higher sensitivities, lower limits of detection, and faster electron transfer

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kinetics than traditional carbon electrodes [34].

We assess in this work the benefit of the immobilization of zeolite beta particles and MWCNTs at the carbon paste electrodes for improving the performance of CMEs in electroanalysis, via analysis of the pharmaceutical samples of the Trp.

EXPERIMENTAL PROCEDURE

1. Chemicals and Reagents

All reagents were of analytical grade. All solutions were prepared with double-distilled water. Stock solutions of tryptophan were freshly prepared as required at the desired pH (7.0-14.0) and purged with pure nitrogen gas (99.999%) for 5 min before the voltammetric measurements. During the experiments, nitrogen gas was passed over the surface of test solutions in order to avoid entrance of oxygen into the solution.

2. Synthesis of Multi-walled Carbon Nanotubes

The nanotubes were grown by chemical vapor deposition. Several transition metal catalysts have been shown to be active for generation of carbon nanotubes [35].

In this work MWCNTs were synthesized from acetylene on a Fe : Co : $CaCO_3$ catalyst at 720 °C [36]. For the production of carbon nanotubes, approximately 100 mg of catalyst containing 5 wt% Fe-Co with a mole ratio of 1 : 1 was weighed and spread into a thin layer onto a quartz boat positioned horizontally inside of a resistive tube furnace under nitrogen flow. The furnace temperature was then set at the reaction temperature, while accurately controlled. When temperature reached 720 °C, acetylene was introduced at 3.0 ml/min, while the flow of nitrogen maintained at 200 ml/min. After the system was rinsed with nitrogen, reaction product was collected from the quartz boat. For purification, raw MWCNT samples were sonicated (40 kHz) in diluted nitric acid (30% HNO_3) for 30 min, filtered, washed with distilled water to remove acid and finally dried at 120 °C overnight. The residue of as-prepared MWCNTs was placed inside a Pyrex tube and oxidized in a furnace at 350 °C in air for different time periods to remove carbon impurities. Fig. 1 shows the SEM image of synthesized MWCNT. The diameter, length, purity and other characters of synthesized MWCNTs are summarized in Table 1.

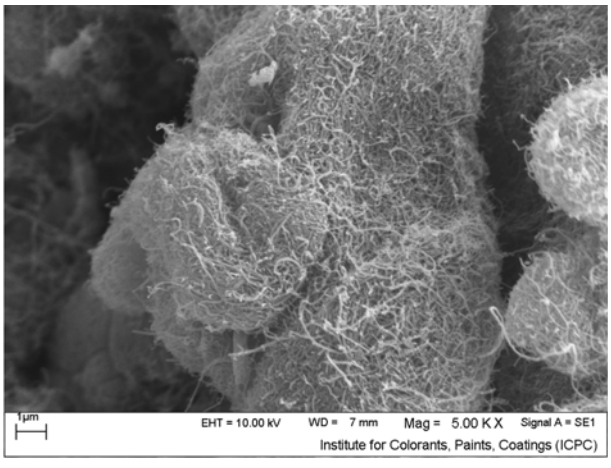

Fig. 1. SEM image of MWCNT.

Table 1. Properties of synthesized MWCNTs

Catalyst	Co : Fe
Color	Black
Purity	>95%
Outside diameter (OD)	8-15 nm
Inside diameter (ID)	3-5 nm
Length	10-50 μm
Special surface area (SSA)	235 m^2/g
Bulk density	0.07 g/cm^3
True density	~2.1 g/cm^3

3. Preparation of the MWCNT-modified Carbon Paste Electrodes (MWCNT-CPEs)

The unmodified carbon-paste electrode was prepared by mixing graphite powder with an appropriate amount of paraffin and thorough hand mixing in a mortar and pestle, and a portion of the composite mixture was packed into the end of a Teflon tube. Electrical contact was made by forcing a glassy carbon electrode down the Teflon tube and into the back of the paste. The modified electrode was prepared by mixing unmodified paste with MWCNT and then mechanically homogenized.

4. Apparatus

The voltammograms were recorded with μAutolab FRA2 Potentiostat-Galvanostat. A three-electrode system was used with MWCNT-*CPEs* as working electrode, a platinum rod electrode as the counter electrode and an Ag/AgCl as the reference electrode. All tests were at room temperature. A digital pH meter (Jenway 370) was applied for pH adjustment.

RESULTS AND DISCUSSION

1. Voltammetric Studies on Trp

1-1. Type and Amount of Modifiers

Mixing of the carbon paste with two types of modifiers (beta zeolite and MWCNT) remarkably increases the sensitivity of the car-

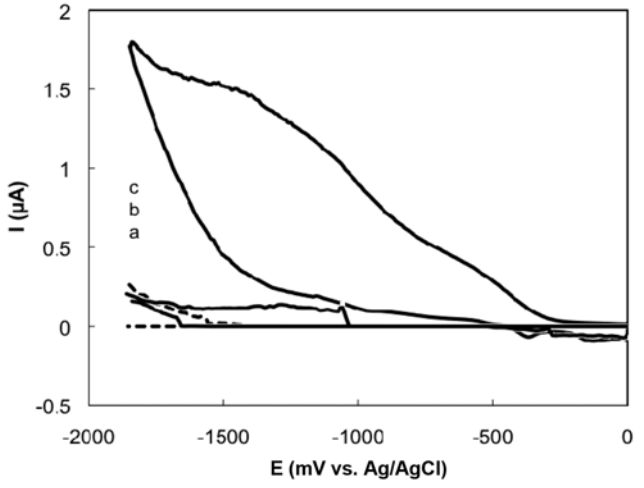

Fig. 2. Effect of various modifiers on CPE response at scan rate of 300 mVs^{-1} (a) Blank (only CP), (b) 5 wt% Beta zeolite, (c) 5 wt% MWCNT.

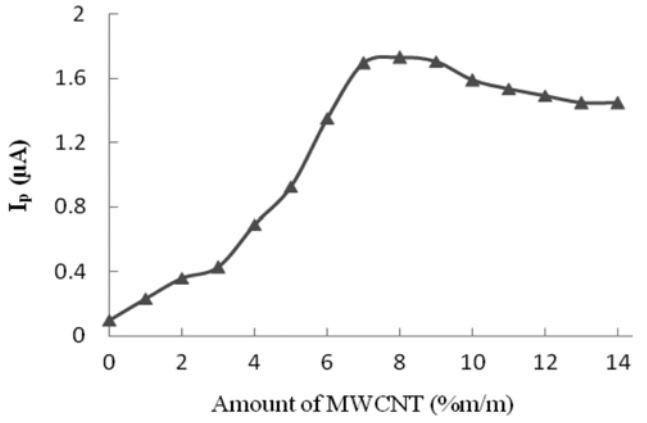

Fig. 3. Effect of MWCNT content of MWCNT-CPEs on anodic peak current at scan rate of 300 mVs^{-1}.

bon-paste electrodes. In this work, beta zeolite and MWCNT were tested as modifier. The effect of the composition of carbon paste electrode on its voltammetric profiles was evaluated in basic solution (pH 13) with 5.0×10^{-5} mol l^{-1} Trp, as presented by Fig. 2. The best modifier should give the highest Ip value and resolution and the longest distance from the discharging current of the background. As it can be observed, the 5 wt% MWCNT-modified carbon paste electrode gives the highest peak intensity values; in contrast, other electrodes present useless or weak signals. Therefore, MWCNT was selected as the best modifier to determine the Trp in later experiments, while other electrodes were rejected.

The anodic peak currents of Trp were strongly related to the content of MWCNT in carbon paste. Fig. 3 shows the influence of MWCNT amount varying from 0% to 14% (m/m) in the carbon paste on the anodic peak current (I_{pa}). It was found that the increase of MWCNT content in the carbon paste would increase the peak current of Trp because of its catalytic effect on the redox process. The carbon paste electrode with modifier amounts higher than 10% (m/m) presented resistive voltammetric signals. This probably occurs due to the decrease of the graphite content in the paste and consequently the reduction of conductive material in the electrode. The optimum content of modifier was 8% for the Trp determination.

1-2. Effect of Solution pH

The effect of pH of the Trp solution (5.0×10^{-5} mol l^{-1}) on the electrode potential was investigated. The electrochemical behavior of the carbon paste electrode modified with MWCNT (8% m/m) was studied in a large pH range between 9.0 and 14.0. The pH value was increased gradually using NaOH (0.1 or 1.0 mol l^{-1}). Fig. 4 illustrates the dependence of the peak potential and peak current on the pH of the solution. As can be seen, the highest sensitivity was obtained at pH 13.0 (Fig. 4(a)). The anodic peak potential corresponding to the tryptophan oxidation shows a linear variation with pH with a slope of −50.31 mV/pH as shown in Fig. 4(b). This suggests that the total number of electrons and the protons taking part in the charge transfer is the same. When the pH value was lower than 12.0, the anodic peak current of Trp would decrease and peak potential shifted toward the negative direction. Therefore, the results indicate that a solution at pH 13.0 could be chosen for further studies.

1-3. Influence of Scan Rate

The effect of potential scan rate between 25 and 500 mVs^{-1} on

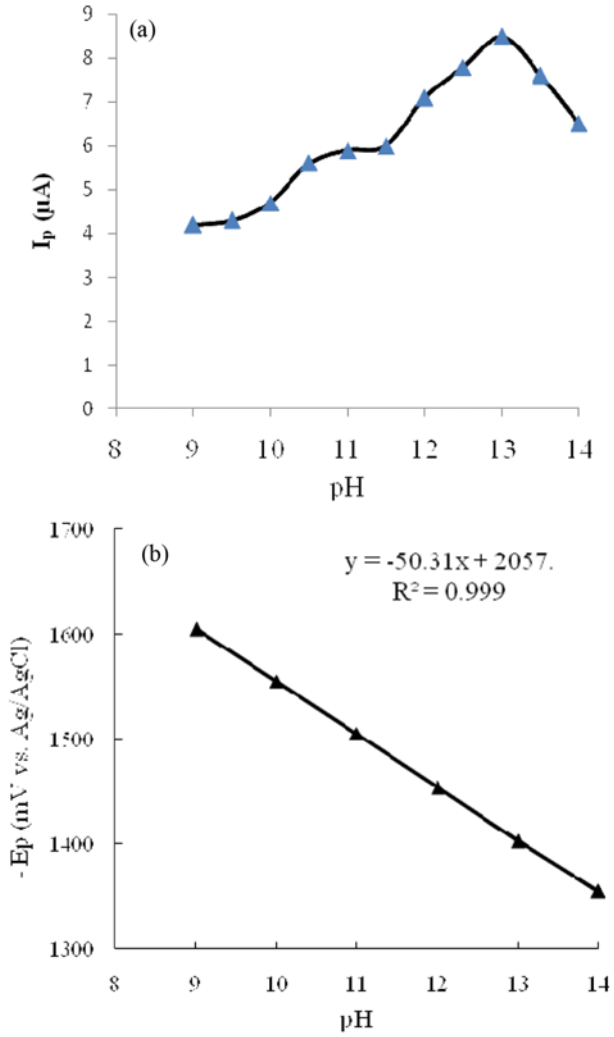

Fig. 4. Influence of pH on the (a) peak current and (b) peak potential of the modified carbon paste electrode with 8% (m/m) MWCNT in solution containing 5.0×10^{-5} mol l^{-1} Trp at scan rate 300 mVs^{-1}.

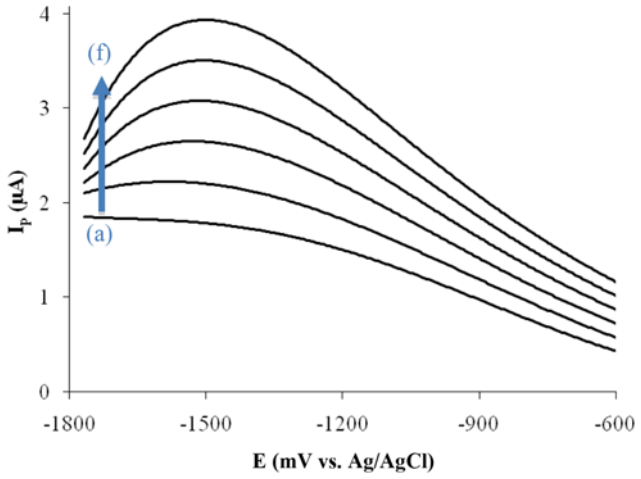

Fig. 5. Variation of peak current with potential scan rates (a) 25, (b) 50, (c) 100, (d) 200, (e) 300 and (f) 400 mVs^{-1} in pH 13.0 and 5.0×10^{-5} mol l^{-1} Trp.

the peak current of Trp was also evaluated. A linear relationship (I_p=0.47 $\nu^{1/2}$+1.82) was found between I_p and the square root of the scan rate ($\nu^{1/2}$) in the part of the range (R^2=0.9895) as shown in Fig. 5. The peak currents for Trp increase by increasing scan rate to 400 mVs^{-1}. This is evidence that the kinetic of electron transfer on the electrode surface is not sufficiently fast as consequence of the nature of the matrix whose internal resistance is considerable. For scan rates higher than 400 mVs^{-1}, the voltammetric profiles become distorted, which can be associated with a slow electron transfer rate between the graphite and MWCNT in the paste. From these results, a scan rate of 300 mVs^{-1} was chosen for further studies since it results in voltammograms with better peak definition.

1-4. Calibration Curve, Repeatability and Lifetime of ZMCPE

After optimizing the operating conditions for the carbon paste electrode modified with MWCNT, we made cyclic voltammetric measurements in solutions containing different Trp concentrations in the range of 1.0×10^{-10}-1.0×10^{-4} mol l^{-1}. Fig. 6 shows that by addition of Trp in solution up to 1.0×10^{-3} mol l^{-1}, the anodic peak current increased significantly and then it leveled off. The electrode has a linear dynamic range 1.0×10^{-9}-1.0×10^{-4} mol l^{-1}, with R^2=0.9840 and a detection limit of 2.2×10^{-10} mol l^{-1} (three times the standard deviation of the blank/slope). The repeatability of the voltammetric measurements was evaluated making ten successive cyclic voltammograms of the MWCNT modified carbon paste electrode at optimized conditions. A Trp concentration level of 2.5×10^{-6} mol l^{-1} was selected to examine the repeatability of the voltammetric sensor. For this study, relative standard deviation was 3.4%, indicating that the modified electrode presents good stability and repeatability, within a confidence level of 95%. The lifetime of the proposed electrode was at least 2 months and more than 25 determinations were performed using the same electrode surface.

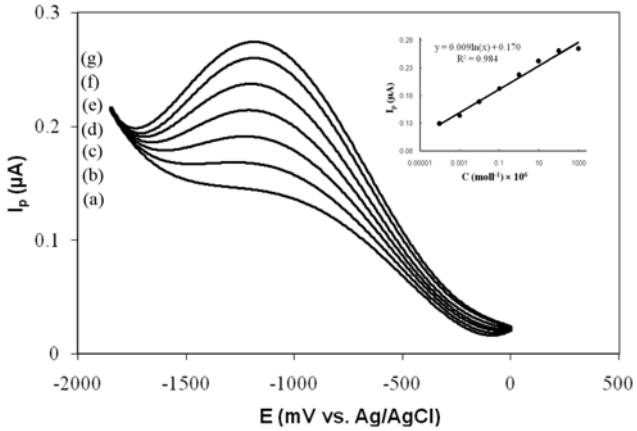

Fig. 6. Calibration curve of Trp. Variation of peak current with concentration of Trp (a) 1.0×10^{-10}, (b) 1.0×10^{-9}, (c) 1.0×10^{-8}, (d) 1.0×10^{-7}, (e) 1.0×10^{-6}, (f) 1.0×10^{-5}, and (g) 1.0×10^{-4} mol l^{-1} Trp from down to up concentrations at pH 13.0 and scan rate of 300 mVs^{-1}.

2. Voltammetric Analysis of Pharmaceutical Synthetic Samples by the MWCNT-CPE

The analytical utility of the method was assessed by applying it to the determination of tryptophan in some synthetic samples. A foreign compound was considered to interfere seriously when it gave a determination error of more than 5%. The effect of all examined compounds over tryptophan on the measured analytical signal is given in Table 2. The recovery and relative standard deviation values given in Table 2 were calculated from six determinations. From the results, we concluded that the presence of excess of all concomitant compounds did not significantly influence the determination of Trp upon the experimental conditions, and the method is relatively selective for determination of the Trp in the pharmaceutical samples. On the other hand, this table indicates the high accuracy and precision of the present work as compared with those previously reported by other methods [12,15,16], which require more complicated instrumentations or time-consuming pretreatment steps.

Table 2. Experimental results for Trp determination in the presence of interfering species

Sample	Composition (μM) respectively	Trp (μM)/ Found	Recovery (% of nominal value)	R.S.D. (%)
Trp, Vitamin E	3, 45	3.15	105.0	2.7
Trp, Vitamins A and E	4, 35, 30	4.14	103.5	3.9
Trp, Vitamin B_{12} and AA[a]	5, 45, 45	4.77	95.4	4.0
Trp, Cysteine and Starch	5, 40, 45	4.81	96.2	4.6

[a]Ascorbic acid

Table 3. Summary of the dynamic ranges and detection limits for voltammetric determination of Trp at the various modified electrodes

Electrode type	Modifier	Dynamic range (μM)	Detection limit (μM)	References
Carbon paste	β zeolite	0.5-5000	0.1	[37]
Boron-doped diamond nanowires	-----	-----	0.5	[38]
Carbon paste	Cobalt salophen	0.5-50	0.1	[31]
Carbon paste	Y zeolite	0.2-150	0.06	[39]
Glassy carbon	Poly(4-aminobenzoic acid) film	1-100	0.2	[40]
Glassy carbon	Gold nanoparticles	0.09-50	0.08	[41]
Glassy carbon	Gold nanoparticles-CNT	0.030-2.5	0.010	[42]
Carbon nanofibers	------	0.1-119	0.1	[43]
Carbon paste	MWCNT	0.001-100	0.00022	This work

The combination of sensitivity, selectivity and simplicity of this method makes it an excellent and versatile technique.

Table 3 summarizes the dynamic ranges and detection limits for voltammetric determination of Trp at the various modified electrodes which were reported previously [31,37-43].

CONCLUSIONS

The carbon paste electrode modified with MWCNT was prepared and applied for the voltammetric determination of Trp in pharmaceutical preparations with good sensitivity, excellent detection limit and wide dynamic range. The proposed sensor provides a simple low-cost method which offers a direct selective determination of Trp in pure solutions and in its pharmaceutical formulation without prior separation or derivatization steps with high selectivity, accuracy, precision and sensitivity. The reliability and stability of the CPE modified by MWCNT offer a good possibility for extending the technique in pharmaceutical analysis. The working electrode surfaces can be prepared and renewed easily by mechanical polishing. The ease of preparation of the electrode in combination with the excellent detection limit and high selectivity represent important factors for use of the electrode in routine quantitative analysis.

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