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A Highly Selective Electrochemical Sensor for L-Tryptophan Based on a Screen-Printed Carbon Electrode Modified with Poly-*p*-Phenylenediamine and CdS Quantum Dots

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ABSTRACT: A new highly selective electrochemical sensor for the determination of L-tryptophan was proposed by modifying the surface of screen-printed carbon electrodes (SPCEs). The surface of SPCE was firstly modified by electropolymerization of *p*-phenylenediamine (PPD). The polymer film was then covalently linked with cysteamine capped cadmium sulfide quantum dots (Cys-CdS QDs) by using glutaraldehyde (GA) as a cross-linker resulted in an organic–inorganic hybrid composite film (QDs/GA/PPD/SPCE). The modified electrode was applied as a working electrode for detecting various amino acids. It was found that the modified electrode gave an electrochemical response selectively to L-tryptophan over other amino acids. The experimental parameters, including pH of solution, buffer types, electropolymerization cycles, scan rate, and accumulation time, were studied and optimized. The proposed sensor can be used to detect L-tryptophan with a low detection limit of 14.74 μmol L^{-1} with good precision and the relative standard deviation less than 3.7%. The modified electrode was used to detect L-tryptophan in beverage samples and gave satisfactory recoveries from 91.9 to 104.9%. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40356.

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INTRODUCTION

L-Tryptophan (Trp) is one of the essential amino acids functioning as a normal growth control in infants and as a nitrogen balance control in adults for humans and herbivorous animals.[1] In many biochemical processes, L-tryptophan is an essential precursor of a neurotransmitter such as serotonin and neurohormones such as melatonin and niacin. L-Tryptophan cannot be synthesized directly in human body. Therefore, it can only be obtained through food products, dietary, or added to supplement typical diets.[2,3] However, L-tryptophan can cause schizophrenia in people who cannot metabolize it properly.[4] Moreover, a large amount or the improper metabolized of L-tryptophan can cause a side effect to humans, including hallucinations, delusions, agitation, confusion, and fever.[1,2] Therefore, determination of low concentration of L-tryptophan is important. Simple and less expensive detection techniques are of great interest.

Many analytical methods such as spectrophotometry,[5–7] liquid chromatography,[8,9] fluorescence,[10] chemiluminescence,[11] and capillary electrophoresis[12–14] have been used for detection of amino acids. Although these methods have advantages of sensitivity, accuracy, and low detection limit, they often suffer from complicated operations, cumbersome pretreatment steps, time consuming, and matrix interferences.

Recently, electroanalytical techniques have been widely applied in the analysis of various biological species. Such techniques have many advantages such as high selectivity, high accuracy, high sensitivity, simplicity, and low cost. Various electrode surface modifications on carbon,[1,2,15,16] graphite,[17,18] platinum,[19,20] and gold[4] electrodes were used. Carbon electrodes have been widely used because of the ease of construction, compatibility with various types of modifiers, and low cost. Moreover, carbon electrodes give a low background current compared with graphite and metal electrodes.[21] Nevertheless, the response of L-tryptophan at bare carbon electrodes is not satisfactory because of slow heterogeneous electron transfer processes and the direct electrochemical oxidation of L-tryptophan takes place at high potential.[2,16] Therefore, to overcome these drawbacks, the electrodes must be chemically modified with various compounds such as nanoparticles,[2,16,17,22] poly(9-aminoacridine) functionalized multiwall carbon nanotube,[15] hemin,[23] butyrylcholine,[24] glutamic acid,[25] cerium hexacyanoferrate,[26] and 4-aminobenzoic

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acid.[27] In addition, there have been reports on the combination of polymer films and inorganic materials to fabricate the electrochemical sensors for L-tryptophan such as gold nanoparticles/over-oxidized-polyimidazole (GNPs/PImox),[28] iron ion-doped natrolite zeolite-multiwalled carbon nanotube (FeNAZ/MWCNTs),[29] and nano-TiO_2/ferrocene carboxylic acid (FCCa/TiO_2).[30] Modified electrodes using these hybrid organic–inorganic materials can improve the response characteristics toward the L-tryptophan oxidation compared with unmodified electrodes.

Recently, the interest of conducting polymer from aromatic diamines has increased because of its versatile functionality, high permselectivity to various electroactive species, and easy preparation. Among diamines that are suitable to the oxidative polymerization, phenylenediamine is usually used.[31,32] Polymerization of phenylenediamine can be performed via electrochemical oxidation on a conductive surface. Poly-*p*-phenylenediamine (PPD) film can improve the electron transfer from the redox species to the electrode surface. In addition, a nanocrystalline semiconductor or quantum dots (QDs) have emerged as the attractive materials used in chemical sensors. Quantum dots (QDs) can be used in several aspects such as fluorescence probes,[33–35] electrochemical markers,[36–38] sensitized solar cells,[39,40] and cell imaging[41,42] depending on the type, capping molecule, and size of nanoparticles. Interactions of quantum dots to several chemical species resulting in fluorescence changes have been reported.[43] However, quantum dots may be also useful in electrochemical sensor as a selective recognition part analog to its function in the optical sensor. The combination of PPD and quantum dots may provide an excellent type of electrochemical sensors. It is expected that the hybrid materials of the conducting polymer films and nanoparticles would significantly improve the electrocatalytic properties, stability, and reproducibility of the electrode, decrease drawbacks in overpotential and increase the reaction rate.[44]

In this work, we proposed a new electrochemical sensor for selective detection of L-tryptophan. A screen-printed carbon electrode (SPCE) was modified with PPD by electropolymerization method and then linked with cysteamine capped CdS quantum dots (Cys-CdS QDs) via glutaraldehyde (GA). Parameters affecting the detection sensitivity by the modified electrode (QDs/GA/PPD/SPCE) were studied and optimized. The feasibility of the proposed sensor in real applications was demonstrated by the detection of L-tryptophan in beverage samples.

EXPERIMENTAL

Reagents and Materials

L-Alanine, L-arginine, L-histidine, L-leucine, L-lysine, L-phenylanaline, and L-tryptophan were purchased from Acros Organics (Belgium). L-Asparagine, L-glutamic acid, L-proline, L-tyrosine, and L-valine were purchased from Fluka (Switzerland). L-Aspartic acid and DL-glutamine were purchased from Biochemicals. L-Cysteine, L-glycine, D-phenylalanine, L-serine, and L-threonine were purchased from Sigma-Aldrich (USA). L-Isoleucine was purchased from Fisher Scientific (India). DL-Methionine was obtained from Himedia (India). GA (25% solution) was obtained from LobaChemie (India). An SPCE with diameter of 4 mm was purchased from Metrohm (Switzerland). Cysteamine capped CdS quantum dots were synthesized by procedures described previously.[45] PPD was purchased from Fluka (Switzerland). Hydrochloric acid was purchased from Lab Scan Asia (Thailand). Sodium hydroxide, sodium sulfate and di-sodium hydrogen phosphate were purchased from Carlo Erba (Italy). All reagents used were of analytical grade. All aqueous solutions were prepared in deionized water with the specific resistivity of 18.2 MΩ cm from RiO_s^{TM} Type I Simplicity 185 (Millipore water).

Instrumentation

Electrochemical measurements, including cyclic voltammetry (CV), linear sweep voltammetry (LSV), and squarewave voltammetry (SWV), were performed with an Autolab PGSTAT101 potentiostat/galvanostat. The Nova 1.7 software was used to control the system and process data. A conventional three electrodes system was used. An SPCE and a modified electrode (QDs/GA/PPD/SPCE) were used as working electrode, whereas the platinum sheet was used as counter electrode and Ag/AgCl as reference electrode. Experiments were performed at ambient temperature. A pH meter, Ultra Basic DenVER was applied for the pH adjustment.

Preparation of the Modified SPCE

The solution of 10 mmol L^{-1} PPD containing 25 mmol L^{-1} hydrochloric acid and 20 mmol L^{-1} sodium sulfate was polymerized on a bare carbon electrode by cyclic voltammetry using potential cycling in the range of −0.4 to 1.5 V at a scan rate of 10 mV s^{-1} for 20 cycles. After electropolymerization, of sodium hydroxide solution (5 mmol L^{-1}) was dropped on the electrode surface to neutralize excess HCl and rinsed with water. Then, electrode was further modified with 0.5% GA by dropping the solution on the electrode and kept in a close container for 1 h to allow the reaction completed. Then, the electrode was rinsed with water and 5 mmol L^{-1} of cysteamine capped CdS quantum dots (Cys-CdS QDs) was dropped on the electrode. The electrode was allowed to stand for 1 h and rinsed with water prior to use. Finally, the obtained modified electrode, QDs/GA/PPD/SPCE, was used as a working electrode for the sensing of L-tryptophan.

Electrochemical Measurement of L-Tryptophan by QDs/GA/PPD/SPCE

The electrocatalytic properties of L-tryptophan at QDs/GA/PPD/SPCE were investigated by using cyclic voltammetry (CV) and linear sweep voltammetry (LSV), and potentials were scanned from +0.7 to +1.05 V versus Ag/AgCl. Determination of L-tryptophan was performed by squarewave voltammetry (SWV) obtained by scanning potentials from +0.6 to +1.4 V versus Ag/AgCl with a step and amplitude of 0.005 V. Typically, 20 mmol L^{-1} phosphate buffer (pH 2.0) containing 20 mmol L^{-1} KCl was used as a supporting electrolyte. After the background current declined to a steady value, L-tryptophan solution was added, and the resulted current from electrocatalytic oxidation of L-tryptophan was recorded. L-Tryptophan concentration was obtained by measuring the heights of the oxidative peaks against the standard curve.

RESULTS AND DISCUSSION

In this work, we proposed the use of SPCE as an electrode substrate. This sensor platform may be suitable for constructing a

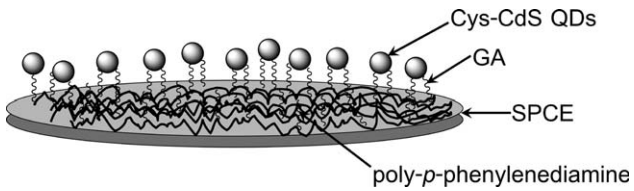

Figure 1. Schematic representation of the modified electrode (QDs/GA/PPD/SPCE).

portable sensor. However, it is not easy to modify a recognition unit directly on the SPCE surface. Therefore, electrochemical polymerization of PPD was chosen for surface modification. The resulting polymer film can further react easily with GA via the imine formation. Because the capping molecule of the CdS QDs is cysteamine, it can, therefore, directly react with the aldehyde moiety. We expect that SPCE modified with the hybrid organic–inorganic materials would give a better electrochemical response toward L-tryptophan. The possible chemical structure of the modified electrode is demonstrated in Figure 1.

Electropolymerization of PPD on SPCE

The surface of SPCE was firstly covered with the polymer film of PPD by electropolymerization.[19] The thickness of the polymer film can be controlled by the number of scan cycles. The reproducibility of the electrode surface using this approach is impressive. Figure 2 shows the cyclic voltammogram recorded during the electropolymerization of a solution containing 10 mmol L^{-1} PPD in 25 mmol L^{-1} hydrochloric acid and using 20 mmol L^{-1} sodium sulfate as a supporting electrolyte. The cyclic voltammetry was applied for the electropolymerization by scanning the potential between +1.5 and −0.4 V (vs Ag/AgCl) with a scan rate of 10 mV s^{-1}. The obtained CV profiles of PPD thin film exhibited the obvious oxidation peak at +0.40 V (vs Ag/AgCl) with a small reduction peak around +0.00 V (vs Ag/AgCl). In addition, the peak current increased with increasing the number of scan cycles. The results confirm the continuous build up of PPD thin film on the surface of a bare SPCE. Increasing the number of electropolymerization cycles gave higher peak currents. However, the oxidation peak increased until stabilized around 20 cycles (see the inset in Figure 2). This behavior was attributed to the decrease of PPD conductivity upon increasing the thickness of the film. The broader peaks were observed during the ongoing scanning, indicating the permanent growth of the polymer film. These results revealed that PPD films were formed on the surface of SPCE by electropolymerization.[15]

Electrochemical Behavior of L-Tryptophan on Modified Electrodes

Aims of SPCE surface modification focus on the sensor sensitivity and selectivity. We investigated the response of the sensor toward L-tryptophan after each modification stage. The experiment was performed by using 0.5 mmol L^{-1} L-tryptophan in 20 mmol L^{-1} phosphate buffer (pH 2.0) containing 20 mmol L^{-1} KCl. SPCEs at each modification stage were used as working electrode and the cyclic voltammograms were recorded at the potential range from +1.05 to +0.7 V (vs Ag/AgCl). The results are showed in Figure 3 and the peak position and peak current

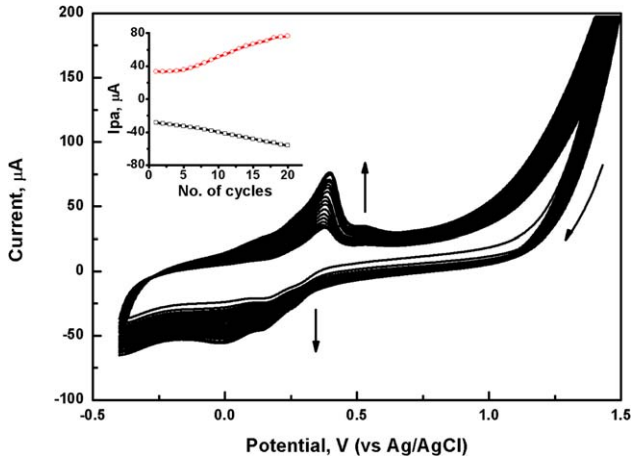

Figure 2. CVs during electropolymerization of 10 mmol L^{-1} *p*-phenylenediamine in 25 mmol L^{-1} HCl and 20 mmol L^{-1} Na_2SO_4 at scan rate of 10 mV s^{-1} on bare SPCE. Inset: oxidation and reduction peak current on CV response. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

are summarized in Table I. From the cyclic voltammogram, it clearly seen that L-tryptophan has no response on a bare SPCE (curve a) and a SPCE modified with QDs (curve b) whereas the cyclic voltammograms of L-tryptophan shows a broad oxidation peak current around 0.88 V (vs Ag/AgCl) on the PPD/SPCE (curve c). This may indicate that the PPD plays an important role in the enhancement of L-tryptophan oxidation of the SPCE electrode. However, when adding GA into the PPD film, the peak current was reduced which signified the imine formation of GA on PPD film. Then, Cys-CdS QDs having amine groups on the surface were added. It can be seen that the peak position did not change, but the peak current increased significantly. This result suggested that Cys-CdS QDs were adsorbed on the layer of PPD/GA. In addition, the result from curve b supported that we cannot directly put Cys-CdS QDs onto the SPCE without PPD and GA.

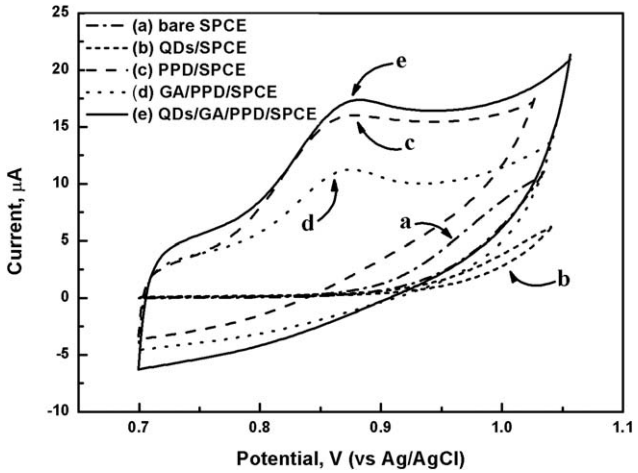

Figure 3. The CVs of 0.5 mmol L^{-1} L-tryptophan in 20 mmol L^{-1} phosphate buffer (pH 2.0) containing 20 mmol L^{-1} KCl on modified electrodes with scan rate of 10 mV s^{-1}.

Table I. Peak Currents (I_{pa}) and Peak Potentials (E_{pa}) of L-Tryptophan Obtained From CVs Using the Modified Electrodes for the Electrochemical Oxidation of 0.5 mmol L^{-1} L-Tryptophan

Electrode	I_{pa} (μA)	E_{pa} (V)
Bare SPCE	n.d.	n.d.
QDs/SPCE	n.d.	n.d.
PPD/SPCE	6.38	0.87
GA/PPD/SPCE	3.89	0.87
QDs/GA/PPD/SPCE	6.25	0.88

n.d.; not detectable

From these investigations, the improvement of electrochemical responses of the modified electrode may be due to the intensive properties of PPD and Cys-CdS QDs. The conductive polymer PPD can obviously enhance electron transfer from the oxidation of L-tryptophan to the SPCE surface. Cys-CdS QDs on the PPD layer act as a recognition unit that can interact with L-tryptophan in the solution due to hydrogen bonding interactions. Actually, the determination of adsorb species on the electrode surface can provide higher current response because no mass transport is needed. Therefore, it can be concluded that the hybrid material between PPD and QDs is suitable for improving the detection sensitivity of L-tryptophan.

Moreover, to show the importance of the modification materials, further experiments were performed by using a bare SPCE and a modified SPCE to measure the solution in the absence and presence of 0.5 mmol L^{-1} L-tryptophan. The comparison results are shown in Figure 4. No significant electrochemical response of L-tryptophan on a bare electrode was observed. On the other hand, the oxidation peak current of L-tryptophan was detected on the modified electrode. This result indicated the modified electrode (QDs/GA/PPD/SPCE) can be used for the selective and sensitive detection of L-tryptophan.

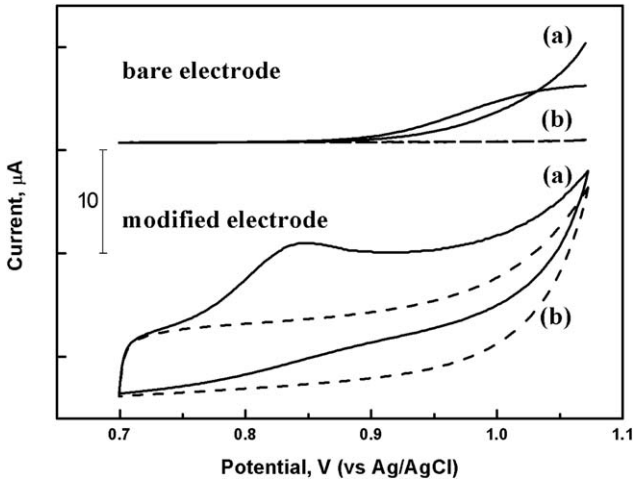

Figure 4. CVs of bare and modified SPCE (a) in the presence of 0.5 mmol L^{-1} L-tryptophan in 20 mmol L^{-1} phosphate buffer (pH 2.0) containing 20 mmol L^{-1} KCl, (b) in the absence of L-tryptophan.

Optimization of Parameters

Effect of pH and Buffer. The pH is an important factor that may affect the electrochemical reaction of L-tryptophan. Moreover, the solution pH can affect the charge on the QDs surface that may result in different interactions between QDs and L-tryptophan. Preliminary experiments with three different pH buffer solutions, acetic–acetate (pH 4.5), Tris-HCl (pH 7.5) and boric–borate (pH 10.0) buffer showed that in the acidic solution gave the better response. However, the type of acidic buffer solution at pH 4.5, including phosphate, phthalate and acetate did not affect the peak current significantly. Thus, the studied pH ranged from 2.0 to 4.5 in phosphate buffer solution was investigated in details. Figure 5(a) shows the effect of pH on the peak current response of 0.5 mmol L^{-1} L-tryptophan on the modified electrode with 20 mmol L^{-1} phosphate buffer solution containing 20 mmol L^{-1} KCl as the supporting electrolyte. It revealed that the peak current of L-tryptophan increased significantly from pH 4.5 to 2.5 and then became stable at pH lower than 2.5. From the studied pH, the maximum current response was obtained at pH 2.0 to 2.5. Thus, phosphate buffer solution at pH 2.0 was chosen for further experiments. Moreover, the oxidation peak potential of L-tryptophan showed the positive shift with decreasing of pH. In Figure 5(b), a plot of

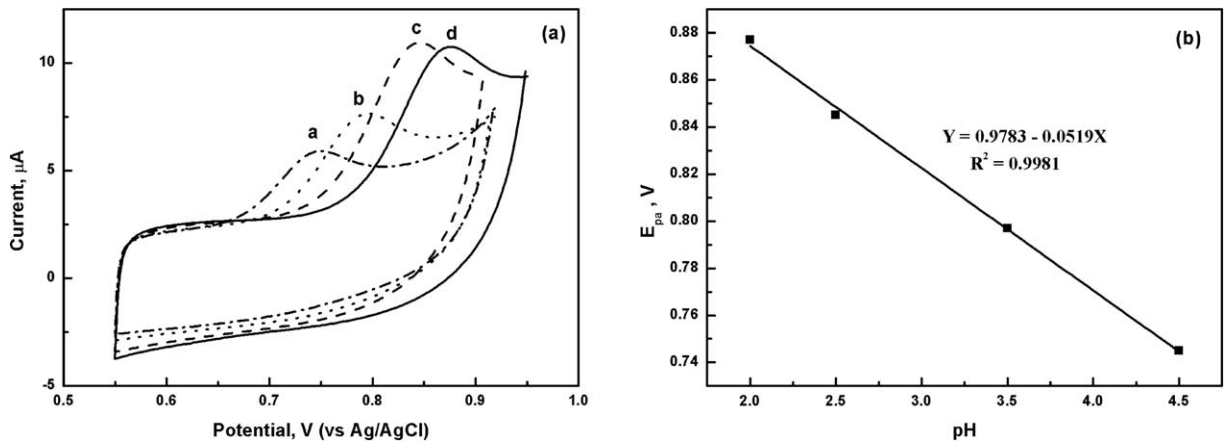

Figure 5. The effect of pH (a) CVs response of modified electrode in the presence of 0.5 mmol L^{-1} L-tryptophan in 20 mmol L^{-1} phosphate buffer containing 20 mmol L^{-1} KCl in pHs: (a) 4.5, (b) 3.5, (c) 2.5, and (d) 2.0. (b) Plot of peak potential versus pH.

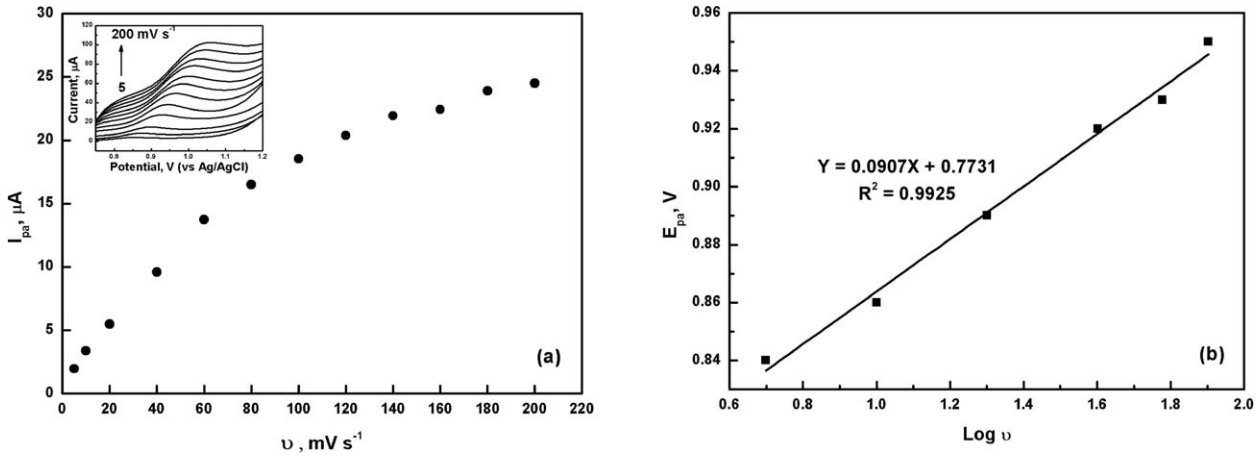

Figure 6. Effect of scan rate (a) LSVs response of 0.5 mmol L^{-1} L-tryptophan in 20 mmol L^{-1} phosphate buffer (pH 2.0) containing 20 mmol L^{-1} KCl at scan rate 5–200 mV s^{-1}. (b) Plot of peak potential versus logarithm of scan rate.

peak potential versus pH was linear with a linear regression equation, E_{pa} (V) = 0.9783 – 0.0519pH with a correlation coefficient of $R^2 = 0.9981$. This result suggested that protons may take part in the oxidation process[15,46] by protonating the N-indole unit and reducing the oxidizing ability of L-tryptophan.

Effect of Scan Rate. The influence of scan rate on peak current and peak potential of L-tryptophan by using the modified SPCE was investigated by linear sweep voltammetry (LSV) mode in the solution composed of 0.5 mmol L^{-1} L-tryptophan, 20 mmol L^{-1} phosphate buffer solution pH 2.0 and 20 mmol L^{-1} KCl as the supporting electrolyte. The results show in Figure 6(a), the oxidation peak current increased dramatically upon increasing the scan rate. A good linear relationship between I_{pa} and scan rate was obtained over the range of 5 – 80 mV s^{-1}. The linear regression equation was I_{pa} (µA) = 0.1959 ν (mV s^{-1}) + 1.4198 with a correlation coefficient of 0.9942. These phenomena suggest that the reaction of L-tryptophan at the modified SPCE is controlled by adsorption.[15,46] However, at higher scan rates, the peak current slightly increased. Moreover, the peak potential slightly shifted to more positive potentials upon increasing the scan rate. The results show that at higher scan rates a kinetic limitation between L-tryptophan and modified electrode surface can occur. The scan rate of 100 mV s^{-1} was chosen for further investigation.

To determine the number of the electron transfer associated with the oxidation of L-tryptophan at the modified SPCE, the peak potential was plotted against the logarithm of the scan rate (log ν) in the range of 5–80 mV s^{-1}. Figure 6(b) shows the linear correlation between the peak potential and the logarithm of the scan rate expressed as $E_{pa} = 0.0907 \log \nu + 0.7731$ with $R^2 = 0.9925$. This relationship can be explained by Laviron's theory.[15,47] The slope of this plot is equal to $2.303RT/\alpha n_\alpha F$ and α value was assumed to be 0.5 due to a totally irreversible electrode reaction. Therefore, based on this relationship, the n_α can be calculated to be 1.30. This result indicated that transfer of two electrons and two protons involved in the electrooxidation of L-tryptophan.

Effect of Accumulation Time. The role of the QDs on the modified electrode is to interact with and adsorb L-tryptophan on the modified surface. To verify this hypothesis, the accumulation time of the L-tryptophan on the electrode surface was demonstrated. The experiment was performed by using the same L-tryptophan concentration, the increasing of accumulation time should increase the response current if the L-tryptophan actually adsorb on the electrode surface. As shown in Figure 7, the peak current of L-tryptophan increased proportionally with increasing of accumulation time (the inset shows the voltammograms at the accumulation time between 0 and 300 s.). The result suggested that L-tryptophan was firstly adsorbed on the QDs surface before the electrooxidation reaction occurred. Although the higher accumulation time can provide higher current, in order to decrease the analysis time, the accumulation time at 180 s was chosen.

Interference Study

To evaluate the influences of some potential interferences on the determination of L-tryptophan, 20 L-amino acids (5 mmol L^{-1}), alanine, arginine, asparagines, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, (D-, L-) phenylalanine, proline, serine, threonine, tyrosine, and valine were individually mixed with 0.5 mmol L^{-1}

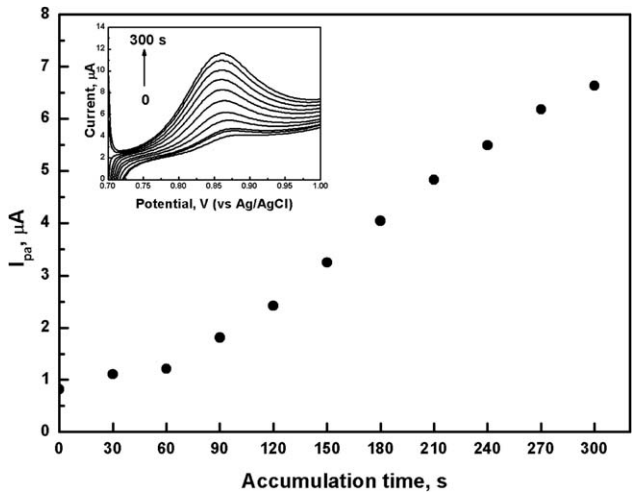

Figure 7. Effect of the accumulation time on peak current of 0.5 mmol L^{-1} L-tryptophan in 20 mmol L^{-1} phosphate buffer (pH 2.0) containing 20 mmol L^{-1} KCl. Inset: LSVs at scan rate of 100 mV s^{-1}.

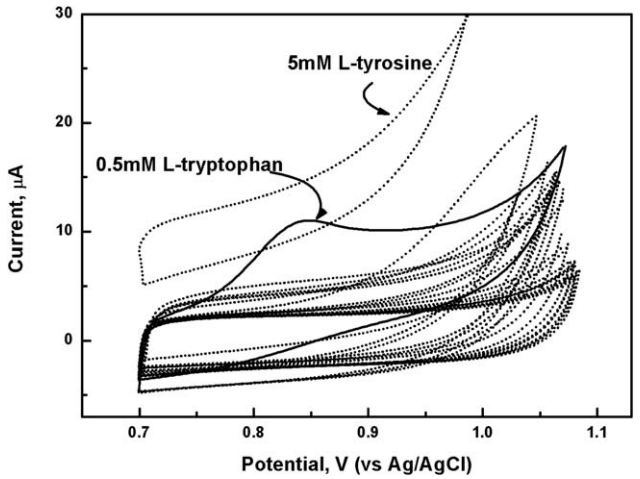

Figure 8. Effect of interferences on CVs response of 0.5 mmol L^{-1} L-tryptophan and 20 interferences (5 mmol L^{-1} each) on QDs/GA/PPD/SPCE.

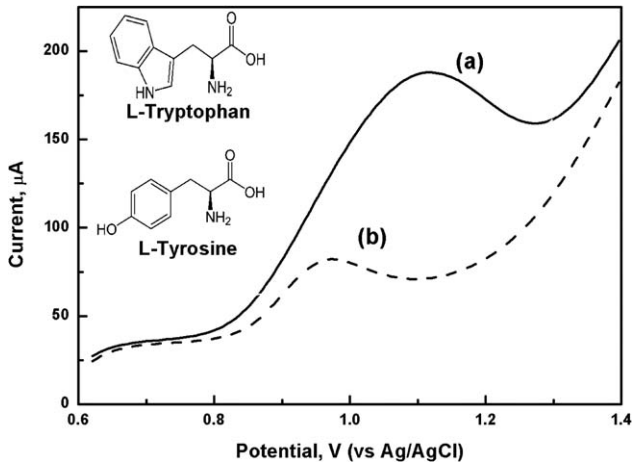

Figure 9. SWVs in the presence of 0.5 mmol L^{-1} (a) L-tryptophan and (b) L-tyrosine in 20 mmol L^{-1} phosphate buffer (pH 2.0) containing 20 mmol L^{-1} KCl.

L-tryptophan and measured by the modified SPCE under the optimized conditions. The results in Figure 8 show that the responses current of the mixed solution are not different from than that of L-tryptophan only for most studied amino acids except L-tyrosine. L-Tyrosine can also undergo the oxidation reaction on the modified SPCE when applying squarewave potential waveform. Figure 9 shows the comparison of the resulted voltammogram between the measurements of 0.5 mmol L^{-1} L-tryptophan and L-tyrosine. It can be seen that L-tyrosine gave a smaller peak current (27.8 μA) than L-tryptophan (72.2 μA). In addition, oxidation peak potentials of L-tyrosine and L-tryptophan were different: +0.97 V for L-tyrosine and +1.12 V for L-tryptophan. Therefore, use of the proposed sensor to detect L-tryptophan may not be interfered from L-tyrosine because the electrooxidation peak potential of L-tyrosine was far away and the peak current was much smaller than that of L-tryptophan. In addition, the interfering from other tested amino acids was negligible. Therefore, it can be concluded that the modified electrode provides a high selectivity toward L-tryptophan.

The selectivity of this sensor toward L-tryptophan may be due to many reasons. The main reason is due to the L-tryptophan contain the indole moiety that can be oxidized more easily than other oxidizable amino acids.

The QDs may be classified as an efficient absorbent. The nanometer size of QDs can provide larger surface area for accommodating more numbers of amino groups on the surface of the modified electrode. Therefore, QDs increase the number of adsorbed L-tryptophan on the surface of modified SPCE via hydrogen bonding interactions.

Basically, all amino acids can possibly form hydrogen bonding interactions with the amino groups on the surface of QDs. However, the degree of hydrogen bonding interactions may be different due to different amino acid side chains. Among the studied amino acids, only L-tryptophan contains the indole moiety which can form hydrogen bonds with the amino groups of the QDs. Moreover, the hydrophobicity of the side chain group of amino acids can also increase the adsorption ability on the modified surface as seen from the results of both aromatic amino acids (L-tryptophan and L-tyrosine). From these reasons, the modified SPCE shows higher detection selectivity toward L-tryptophan than other studied amino acids.

Analytical Performances

To apply the proposed sensor as the L-tryptophan probe, it is important to evaluate the analytical performance of the new sensor. A squarewave waveform was applied in the quantitative analysis due to the better detection sensitivity. The calibration curve was first evaluated under optimal condition by increasing the L-tryptophan concentrations as a function of peak current by using the modified SPCE. The resulted calibration curve shows in Figure 10. It can be seen that the peak currents increased linearly upon increasing L-tryptophan concentration. The regression equation was found as peak current (μA) = 80.554[trp, mmol L^{-1}] – 4.5668 ($R^2 = 0.9941$) within the working concentration range of 0.1–0.5 mmol L^{-1}. The detection limit was calculated using the equation $Y_{LOD} = \bar{X}_B + 3S_B$, where Y_{LOD} is the signal for detection limit,

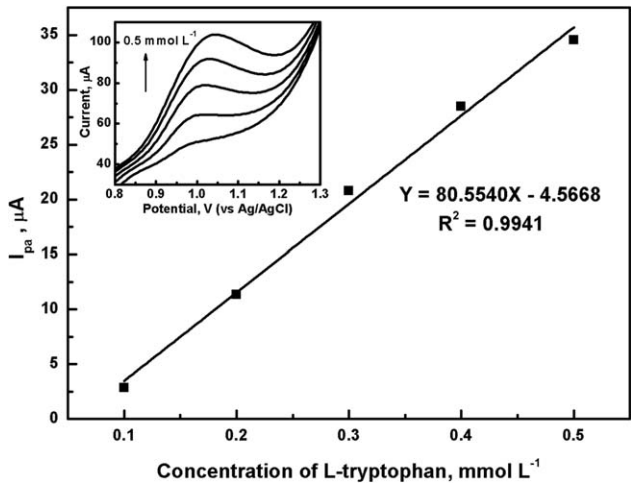

Figure 10. Calibration curve of L-tryptophan. Inset: SWVs obtained for L-tryptophan in 20 mmol L^{-1} phosphate buffer (pH 2.0) containing 20 mmol L^{-1} KCl.

Table II. The Content of L-Tryptophan and Recoveries in Samples Using Standard Addition Method

Sample	Content of L-tryptophan (μmol L^{-1})	Content of L-tryptophan (g/100 g)	Added (μmol L^{-1})	Found (μmol L^{-1})	RSD (%), $n = 3$	Recovery (%)
S1	42.4	0.3	150.0	179.9	3.1	91.9
			200.0	236.3	4.3	96.6
S2	n.d.	n.d.	150.0	142.8	2.2	95.2
			200.0	193.1	2.1	96.6
S3	n.d.	n.d.	150.0	152.6	1.4	101.7
			200.0	198.3	0.8	99.1
S4	n.d.	n.d.	150.0	148.9	2.9	99.3
			200.0	209.9	4.4	104.9

n.d., not detectable.

$\bar{X}_B$ the mean of blank signal and S_B the standard deviation of the blank signal. The theoretical detection limit of L-tryptophan was 14.74×10^{-6} mol L^{-1}. The repeatability of the sensor was studied by using the same modified electrode for 10 repetitive measurements of 0.5 mmol L^{-1} L-tryptophan. The relative standard deviation (RSD) was 3.61%, indicating that the repeatability of the detection of L-tryptophan on the modified electrode was satisfactory. In addition, the modified electrode can be used for at least 200 times.

Determination of L-Tryptophan in Beverage Samples

To evaluate the applicability of the proposed sensor, the modified SPCE was applied to detect L-tryptophan in beverage samples. The content of L-tryptophan was determined with the standard addition method in order to prevent the matrix effect. Four beverage samples were analyzed following; the sample beverage (2.5 mL) was diluted with 20 mmol L^{-1} phosphate buffer pH 2.0 containing 20 mmol L^{-1} potassium chloride and analyzed using squarewave voltammetry (SWV) with a step and amplitude of 0.005 V. The results are shown in Table II. L-Tryptophan was found only in sample S1 at 42.4 μmol L^{-1}. The results indicated that the modified electrode could be efficiently used for the direct determination of L-tryptophan in real samples. The recovery tests were also performed. The recoveries were obtained in the range from 91.9 to 104.9% (see Table II).

Comparison of the Proposed Sensor with Other Related Electrochemical Sensors

Determination of L-tryptophan by using the electrochemical sensor approach is attractive to analytical chemists. Several materials and strategies have been proposed to obtain better selectivity and sensitivity as summarized in Table III. It can be seen from Table III that most of the detection limits obtained by electrochemical sensors were lower than micromolar range. Although the detection limit of the proposed sensor is not significantly better than that reported in the previous literatures, selectivity and simplicity of the proposed sensor are remarkable. On the other hand, the proposed SPCE can be used as a disposal sensor, especially in a clinical analysis.

CONCLUSION

A new selective electrochemical L-tryptophan sensor based on the modification of SPCE was demonstrated. The SPCE was modified by conducting polymer–nanocrystalline semiconductor hybrid materials (QDs/GA/PPD/SPCE). The combination of

Table III. Comparison of the Efficiency of Some Modified Electrodes in the Electrochemical Determination of L-Tryptophan

Modified material	Based electrode	Possible interference	Detection limit (μmol L^{-1})	[Ref.]
Poly(9-aminoacridine) functionalized multiwalled carbon nanotubes	Glassy carbon electrode	Tyrosine	0.81	[15]
4-Aminobenzoic acid polymer film	Glassy carbon electrode	Tyrosine	0.2	[27]
Butyrylcholine	Glassy carbon electrode	Tyrosine	0.6	[24]
Hemin	Glassy carbon electrode	Tryptophan derivatives	0.025	[23]
Gold nanoparticle	Carbon ionic liquid electrode	Cysteine, Tyrosine	4.0	[22]
Gold nanoparticle	Glassy carbon electrode	Tyramine	0.08	[16]
Gold nanoparticles/carbon nanotube film	Glassy carbon electrode	—	0.01	[2]
Carbon nanotube, carbon black, and copper nanoparticle	Screen-printed carbon electrode	Cysteine, Tyrosine	3.8	[48]
Poly(p-phenylenediamine) polymer film/cysteamine capped cadmium sulfide quantum dots	Screen-printed carbon electrode	Tyrosine	14.74	This work

these materials provided the significant improvement of the electrochemical response and selectivity toward L-tryptophan comparing with a bare SPCE. This sensor can be used to detect the concentration of L-tryptophan at micromolar level. The proposed sensor was applied to detect the L-tryptophan in real beverage samples with satisfactory results. In addition, this sensor platform is potentially suitable for fabrication of a portable sensor for point of care.

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REFERENCES

1. Deng, K. Q.; Zhou, J. H.; Li, X. F. *Colloid. Surface. B.* **2013**, *101*, 183.
2. Guo, Y.; Guo, S.; Fand, Y.; Dong, S. *Electrochim. Acta.* **2010**, *55*, 3927.
3. Prabhu, P.; Babu, R. S.; Narayanan, S. S. *Colloid. Surface. B.* **2011**, *87*, 103.
4. Gholivand, M. B.; Pashabadi, A.; Azadbakht, A.; Menati, S. *Electrochim. Acta.* **2011**, *56*, 4022.
5. Nagaraja, P.; Yathirajan, H. S.; Vasantha, R. A. *Anal. Biochem.* **2003**, *312*, 157.
6. Yu, W.; Zhang, H.; Chen, G.; Tu, C.; Ouyang, P. *Microchim. Acta.* **2004**, *146*, 285.
7. Reynolds, D. M. *Water Res.* **2003**, *37*, 3055.
8. Yamada, K.; Miyazaki, T.; Shibata, T.; Hara, N.; Tsuchiya, M. *J. Chromatogr. B.* **2008**, *867*, 57.
9. Kutlán, D.; Molnár-Perl, I. *J. Chromatogr. A.* **2003**, *987*, 311.
10. Sikorska, E.; Gliszczyńska-Świglo, A.; Insińska-Rak, M.; Khmelinskii, I.; Keukeleire, D. D.; Sikorski, M. *Anal. Chim. Acta.* **2008**, *613*, 207.
11. Lin, Z.; Chen, X.; Cai, Z.; Li, P.; Chen, X.; Wang, X. *Talanta* **2008**, *75*, 544.
12. Ilisz, I.; Fodor, G.; Berkecz, R.; Iványi, R.; Szente, L.; Péter, A. *J. Chromatogr. A.* **2009**, *1216*, 3360.
13. Underberg, W. J. M.; Waterval, J. C. M. *Electrophoresis* **2002**, *23*, 3922.
14. Simionato, A. V. C.; Moraes, E. P.; Carriho, E.; Franco, M.; Tavares, M.; Kenndler, E. *Electrophoresis* **2008**, *29*, 2051.
15. Güney, S.; Yıldız, G. *Electrochim. Acta.* **2011**, *57*, 290.
16. Li, C.; Ya, Y.; Zhan, G. *Colloid. Surface. B.* **2010**, *76*, 340.
17. Shahrokhian, S.; Bayat, M. *Microchim. Acta.* **2011**, *174*, 361.
18. Liu, Y.; Xu, L. *Sensors* **2007**, *7*, 2446.
19. Sayyah, S. M.; El-Rehim, S. S. A.; El-Deeb, M. M.; Kamal, S. M.; Azooz, R. E. *J. Appl. Polym. Sci.* **2010**, *117*, 943.
20. Lakard, B.; Herlem, G.; Lakard, S.; Fahys, B. *J. Mol. Struc-Theochem.* **2003**, *638*, 177.
21. Shahrokhian, S.; Fotouhi, L. *Sensor. Actuat. B-Chem.* **2007**, *123*, 942.
22. Safavi, A.; Moneni, S. *Electroanal.* **2010**, *22*, 2848.
23. Nan, C. G.; Feng, Z. Z.; Li, W. X.; Ping, D. J.; Qin, C. H. *Anal. Chim. Acta.* **2002**, *452*, 245.
24. Jin, G. P.; Lin, X. Q. *Electrochem. Commun.* **2004**, *6*, 454.
25. Liu, X.; Luo, L.; Ding, Y.; Ye, D. *Bioelectrochemistry* **2011**, *82*, 38.
26. Fang, B.; Wei, Y.; Li, M.; Wang, G.; Zhang, W. *Talanta* **2007**, *72*, 1302.
27. Huang, K. J.; Xu, C. X.; Xie, W. Z.; Wang, W. *Colloid. Surface. B.* **2009**, *74*, 167.
28. Wang, C.; Yuan, R.; Chai, Y.; Chen, S.; Hu, F.; Zhang, M. *Anal. Chim. Acta.* **2012**, *741*, 15.
29. Noroozifar, M.; Khorasani-Motlagh, M.; Akbari, R.; Parizi, M. B. *Biosens. Bioelectron.* **2011**, *28*, 56.
30. Raoof, J. B.; Ojani, R.; Baghayeri, M. *Sensor. Actuat. B-Chem.* **2009**, *143*, 261.
31. Li, X. G.; Huang, M. R.; Duan, W. *Chem. Rev.* **2002**, *102*, 2925.
32. Li, X. G.; Huang, M. R.; Chen, R. F.; Jin, Y.; Yang, Y. L. *J. Appl. Polym. Sci.* **2001**, *81*, 3107.
33. Thesana, W.; Tuntulani, T.; Ngeontae, W. *Anal. Chim. Acta.* **2013**, *783*, 65.
34. Noipa, T.; Martwiset, S.; Butwong, N.; Tuntulani, T.; Ngeontae, W. *J. Fluoresc.* **2011**, *21*, 1941.
35. Li, J.; Zhu, J. *J. Analyst* **2013**, *138*, 2506.
36. Tang, D.; Hou, L.; Niessner, R.; Xu, M.; Gao, Z.; Knopp, D. *Biosens. Bioelectron.* **2013**, *46*, 37.
37. Martín-Yerga, D.; González-García, M. B.; Costa-García, A. *Sensor. Actuat. B-Chem.* **2013**, *182*, 184.
38. Li, Y.; Han, M.; Bai, H.; Wu, Y.; Dai, Z.; Bao, J. *Electrochim. Acta.* **2011**, *56*, 7058.
39. de la Fuente, M. S.; Sańchez, R. S.; Gonzaĺez-Pedro, V.; Boix, P. P.; Mhaisalkar, S. G.; Rincoń, M.E.; Bisquert, J.; Mora-Seró, I. *J. Phys. Chem. Lett.* **2013**, *4*, 1519.
40. Zhao, J.; Wu, J.; Yu, F.; Zhang, X.; Lan, Z.; Lin, J. *Electrochim. Acta.* **2013**, *96*, 110.
41. Chang, J. C.; Rosenthal, S. *J. Meth. Mol. Biol.* **2013**, *991*, 149.
42. Zhang, Q. *Meth. Mol. Biol.* **2013**, *995*, 179.
43. Wang, X.; Ruedas-Rama, M. J.; Hall, E. A. H. *Anal. Lett.* **2007**, *40*, 1497.
44. Li, Y. X.; Wang, P.; Wang, L.; Lin, X. Q. *Biosens. Bioelectron.* **2007**, *22*, 3120.
45. Noipa, T.; Tuntulani, T.; Ngeontae, W. *Talanta* **2013**, *105*, 320.
46. Fang, B.; Liu, H.; wang, G.; Zhou, Y.; Jiao, S.; Gao, X. *J. Appl. Polym. Sci.* **2007**, *104*, 3864.
47. Laviron, E. *J. Electroanal. Chem.* **1974**, *52*, 355.
48. Carvalho, R. C.; Mandil, A.; Prathish, K. P.; Amine, A.; Brett, C. M. A. *Electroanal.* **2013**, *25*, 903.