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Electrochemical Determination of Dopamine Based on Self-Assembled Peptide Nanostructure

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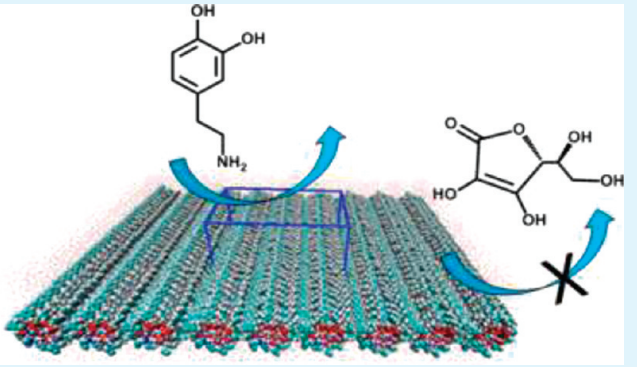
ABSTRACT: Self-assembled peptide nanostructures are electronically insulating as are most biomaterials derived from natural amino acids. To obtain additional properties and increase the applicability of peptide nanomaterials, some chemical modifications can be performed and materials can be functionalized to form hybrid compounds. In this work, we described the formation of *L*-diphenylalanine nanotubes (PNTs) with cyclic-tetrameric copper(II) species containing the ligand (4-imidazolyl)-ethylene-2-amino-1-ethylpyridine $[Cu_4(apyhist)_4]^{4+}$ in the Nafion membrane on a vitreous carbon electrode surface. This copper complex has been studied as structural and functional models for the active centers of copper containing redox enzymes. Scanning electron microscopy was used to confirm the formation of the nanostructures. The electrochemical properties of the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion film on a glassy carbon electrode were characterized using cyclic voltammetry and square-wave voltammetry and showed high electrocatalytic activity toward the oxidation of dopamine (DA). The detection sensitivity was found to be enhanced by the use of copper(II) complex in the PNTs/Nafion films. Under the optimum conditions, the square-wave voltammetry peak height was linearly related to the DA concentration over two concentration intervals, viz., 5.0−40 μmol L^{-1} and 40−1000 μmol L^{-1}. The detection limit was 2.80 μmol L^{-1} (S/N = 3), and ascorbic acid did not interfere with the DA detection. These results suggested that this hybrid bioinorganic system provides an attractive advantage for a new type of electrochemical sensors. The detection sensitivity was found to be enhanced by use of PNTs.



KEYWORDS: *L*-diphenilalanine nanostructures, copper(II) complexes, biomimetic sensors, dopamine

1. INTRODUCTION

The self-assembly of organic nanostructures and hybrid materials relies on noncovalent and reversible interactions, such as hydrogen bonding, metal coordination, hydrophobic effects, van der Waals forces, $\pi-\pi$ interactions, and electrostatic interactions. When combined, these forces form organized, complex entities based on the association of the self-assembling components. The specificity and the selectivity provided by both natural and artificial biomolecules makes them well-suited for this task and offer a way to design complex nanoscale assemblies.[1]

The nanostructures obtained from biomolecules are attractive because of their biocompatibility, ability for molecular recognition, and ease of chemical modification, which are important factors for various applications.[2] The functionalization of these materials has greatly facilitated the study of biological systems, which can be utilized in biosensor devices, catalytic activity and molecular recognition.[3−6] Thus, the challenge faced by synthetic chemistry in the area of molecular electronics is to prepare molecules with specific and well-defined functions (i.e., molecules that can be used at a molecular level as wires, switches, diodes, etc.). The controlled assemblies of selected components of supramolecular species will allow the preparation of nanoscale materials with sophisticated electronic properties.[7,8]

Peptide nanotubes are superior building blocks for biosensors because their robust nature and directed self-assembly enables the fabrication of miniaturized sensor chip platforms in a bottom-up process without the need for sophisticated lithographic methodologies.[9] When peptide nanotubes are coupled with adequate transducers, the resulting hybrid bioinorganic devices can improve the signal-to-noise ratio and specificity of the sensor.[10]

Recently, a novel biosensor for hydrogen peroxide was developed by combining the known properties of microperoxidase-11 (MP11) as an oxidation catalyst and the interesting properties of *L*-diphenylalanine peptide nanotubes (PNTs) as a supporting matrix to create an effective bioelectrochemical interface.[11] In this case, the synthesized MP11/PNTs were immobilized onto the ITO electrode surface via layer-by-layer deposition using poly(allylamine hydrochloride) in positively charged polyelectrolyte layers. The PNTs provided a favorable microenvironment for MP11 to perform direct electron transfer to the electrode surface.

In more recent work,[12] we reported an effective approach to the construction of a biomimetic sensor of multicopper oxidases

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by immobilizing a cyclic-tetrameric copper(II) species containing the ligand (4-imidazolyl)ethylene-2-amino-1-ethylpyridine (apyhist) in the Nafion membrane on a glassy carbon electrode surface. This modified electrode showed excellent electrocatalytic activity toward the reduction of oxygen. We also provided evidence that the cyclic tetranuclear imidazolate-bridged complex $[Cu_4(apyhist)_4]^{4+}$ acts as a catalyst for the intramolecular, four-electron reduction of O_2 and on the kinetics of this mediated reaction. Besides, we have reported that the tetranuclear copper(II) complex act as active species for the homogeneous catalysis of phenol or catechol oxidation.[13−15] The construction of a biomimetic sensor using copper(II) complexes is a formidable challenge in the development of analytical procedures for the determination of various analytes.[16]

In this work, we describe the characteristics of the hybrid film of PNTs modified with a tetranuclear copper(II) complex $[Cu_4(apyhist)_4]^{4+}$ in a Nafion membrane on a glassy carbon electrode surface for determination of dopamine (DA) with square wave voltammetry. The electrochemical behavior of the modified electrode has been analyzed, and the role of the copper(II) complex as an electron mediator has been discussed. Electrochemical detection of DA has received much interest because of its importance in central nervous system and easy oxidation property.[17] A major interference is created by ascorbic acid (AA) which presents by 10^3 times at higher level than DA in human brain.[18] For selective detection of DA, many different strategies have been used to modify the electrode surface.[19] The present study is also concerned with the simultaneous detection of dopamine in the presence of ascorbic acid by using PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion film-coated electrode.

2. EXPERIMENTAL SECTION

2.1. Materials and Instrumentation. Sodium hydroxide, hydrochloric acid, potassium chloride, hydrogen peroxide (29%), absolute ethyl alcohol, and methyl alcohol. were purchased from Synth. Histamine dihydrochloride, 1,1,1,3,3,3-hexafluoride-2-propanol (HFP), diphenylalanine peptide (*L*-Phe···*L*-Phe), 2-acetylpyridine, potassium phosphate monobasic, DA and AA were purchased from Sigma-Aldrich. Potassium phosphate dibasic trihydrate was purchased from Carlo Erba Reagents. Perchlorate copper(II) was purchased from Acros Organics. Nafion (5% solution) was purchased from DuPont, and 0.5 and 0.03 μm alumina suspensions were purchased from Panambra Zwick.

Cyclic and square wave voltammetry were performed using Potentiostat/Galvanostat μAutolab Fra 2, Type III. Measurements of pH were performed with a Metrohm-Pensalab Model 827 pH Lab pH meter with combined glass electrodes. The cleaning of the electrodes was performed in an UltraSonic Cleaner, model SW2000F, purchased from Sanders bath heating. All solutions were prepared using pure water with resistivity of 18.2 MΩ cm at 25 °C, which was produced using a Direct-Q System, Millipore.

2.2. Functionalization of Peptide Nanotubes with a Copper(II) Complex. The copper(II) complex $[Cu_4(apyhist)_4]^{4+}$ was prepared following the methodology described in the literature.[13] The *L*-diphenylalanine nanotubes (PNTs) were prepared by dissolving the lyophilized form of the peptide *L*-Phe···*L*-Phe compound in HFP at a concentration of 100 mg mL^{-1} and diluting to 5 mg mL^{-1} in ultrapure water.[11] The diluted mixture was centrifuged for 15 min in a MPW centrifuge at 1200 x rpm. The supernatant was removed and the precipitate was placed in a solution of $[Cu_4(apyhist)_4]^{4+}$ (8 mg) previously dissolved in 1 mL of ultrapure water followed by the addition of a 5% Nafion solution (200 μL). This solution was ultrasonicated for at least 15 min at room temperature to obtain a homogeneous blue solution.

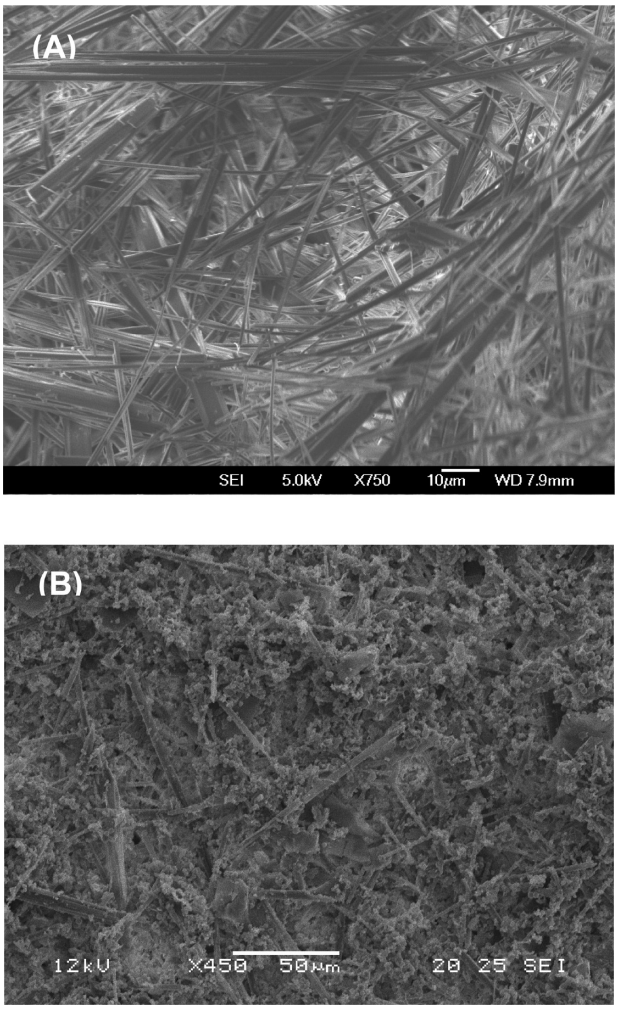


Figure 1. SEM images of (A) PNT-$[Cu_4(apyhist)_4]^{4+}$ hybrid material and (B) Nafion/PNT-$[Cu_4(apyhist)_4]^{4+}$ hybrid film on a GC electrode.

2.3. Fabrication of the Modified Electrode. A glassy carbon electrode (0.071 cm^2) was carefully polished with 0.5-μm and 0.03-μm alumina and was ultrasonicated in ethanol and twice-distilled water. The modified GC electrode was prepared by adding a drop (4.5 μL) of the mixture solution described previously directly to the top of the electrode. The solvent was allowed to evaporate at room temperature.

2.4. Electrochemical Measurements. Square-wave voltammetry (SWV) measurements for DA oxidation were performed in an unstirred, nondeaerated 0.1 mol L^{-1} phosphate buffer solution (pH 7.0) by applying a potential between −1.0 and +1.0 V vs SCE at a frequency of 80 Hz and a pulse amplitude of 50 mV. A 10 mL aliquot of the supporting electrolyte was transferred into the cell, and 5 or 40 μL of the DA solution (0.01 mol L^{-1}) was added using a micropipet.

3. RESULTS AND DISCUSSION

3.1. SEM Characterization of PNT-$[Cu_4(apyhist)_4]^{4+}$ Hybrid Material and a PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion/GC electrode. Figure 1 shows the SEM images of the PNT-$[Cu_4(apyhist)_4]^{4+}$ hybrid material and PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion film on a GC electrode. In both cases, the obtained nanotubes were long and interacted with each other to generate fibrils that were

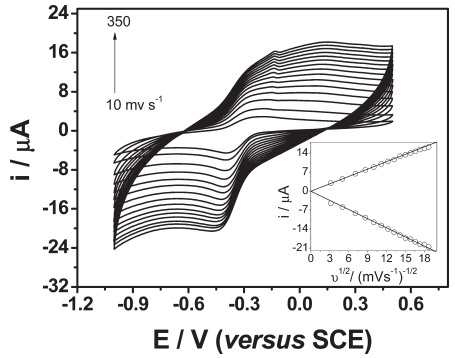


Figure 2. Cyclic voltammograms of PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion at different scan rates (from inner to outer: 10, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, and 350 mVs^{-1}) in pH 7.0 phosphate buffer solution (0.1 mol L^{-1}). All potentials are given vs SCE. The inset shows the dependence of redox peak currents on the potential sweep rates.

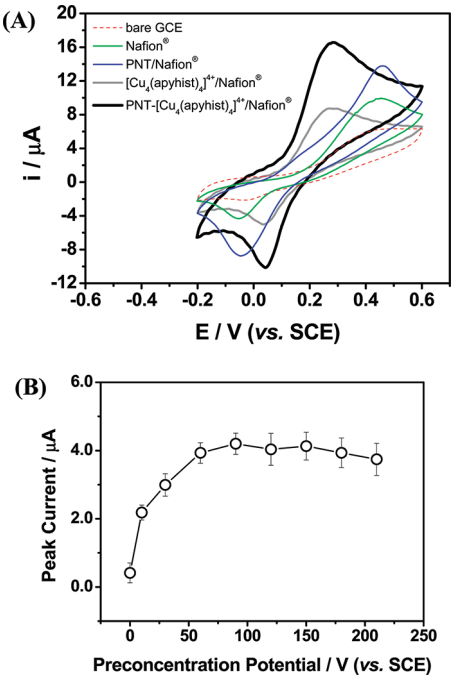


Figure 3. (A) Cyclic voltammograms on bare GCE, Nafion, PNT/Nafion, $[Cu_4(apyhist)_4]^{4+}$/Nafion and PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion-modified carbon electrodes in 0.1 mol L^{-1} phosphate buffer solution (pH 7.0) in the presence of 1×10^{-3} mol L^{-1} DA solution (50 mVs^{-1}); (B) Influence of the preconcentration time on the square-wave voltammetry peak current of 10 μmol L^{-1} DA obtained at the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion/GCE. The preconcentration step was followed by transfer to a blank solution. Potential pulse amplitude = 50 mV, frequency =80 Hz and ΔEs= 4 mV.

350−500 nm thick, as shown in Figure 1A. The membrane of the Nafion was uniformly distributed onto the glassy carbon electrode surface (Figure 1B). The ease of dispersion of the PNTs and copper(II) complex in the polymer matrix may be attributed to the hydrophobic interaction between PNTs and the Nafion polymer as well as the electrostatic interaction between the sulfonate groups from Nafion and $[Cu_4(apyhist)_4]^{4+}$.

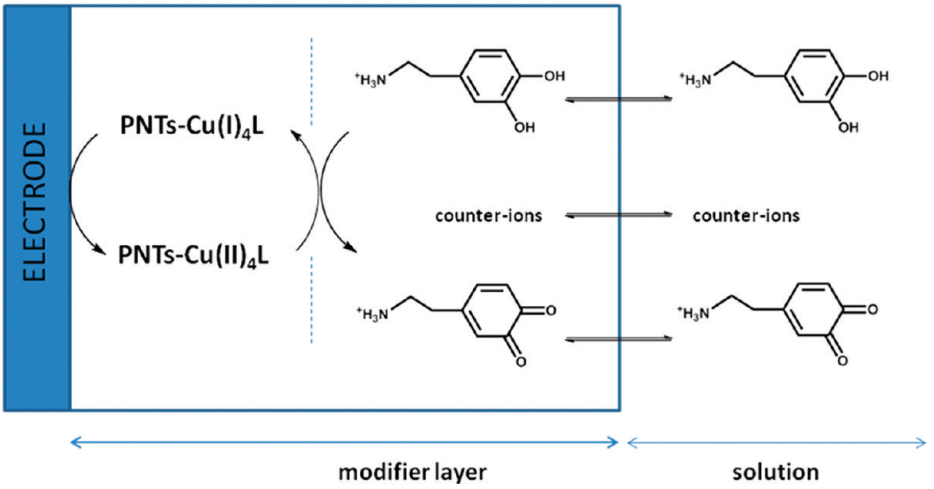
Figure 2 shows the cyclic voltammograms of PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion modified electrodes in a phosphate buffer solution (pH 7.0) at various scan rates. This voltammetric behavior could be attributed to the Cu^{II}/Cu^{I} redox couple at approximately −0.30 V (vs SCE), based on spectroelectrochemical data obtained in phosphate buffer solution pH 7.0 (0.1 mol L^{-1}), in the range 0.40 to −0.70 V versus SCE (data not shown). By scanning to negative potential, it was possible to see a strong decrease in the $\pi \rightarrow \pi^*$ ligand bands at 258 and 320 nm, with the simultaneous increasing of a broad band at 470 nm assigned as a charge transfer Cu(I)→ imine band, also observed in others macroacyclic copper(I) complexes.[20] This experiment confirms the assignment of the voltammetric waves and the bands in electronic spectra performed previously for compound of copper(II) studied.

Both the cathodic and anodic peak currents increased linearly with increasing square roots of the scan rates, which indicated that the process was driven by a diffusion-controlled redox rather than the electrochemical interaction of $[Cu_4(apyhist)_4]^{4+}$/Nafion film with the surface of the glassy carbon electrode.[12] In this case, the limiting factor at relatively high scan rates for PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion/GC electrode was the diffusion of ions at the coating layer of modified electrode, probably because of an increase in the hydrophobic properties of surfaces treated with peptide nanostructures; a similar behavior has been observed for carbon nanotubes.[21,22] Furthermore, the magnitude of the peak current of the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion/GC electrode was much larger than the same electrode in the absence of PNTs,[12] which indicated that the presence of the peptide nanostructure enhanced the electrochemical response toward the redox reactions between the copper ion and the modified electrode. This same behavior has been recently demonstrated by Gazit et al. and Matsui et al., in which the peptide nanostructure-modified electrodes that significantly improved the surface area of the electrode and induced electron transfer in the chemical reaction.[2,23]

The adsorbed fraction of copper(II) on the surface of the PNT/Nafion/GC electrode was calculated using the following equation: $\Gamma = Q/nFA$, where Q is the charge involved in the reaction, n is the number of electrons transferred, F is the Faraday constant, and A is the electrode area. The value of Γ = 12.6 nmol cm^{-2} of copper(II) complex was calculated and was corrected for capacitive current using digital background subtraction. The effect of the PNTs on the capacitive background current is also shown in the voltammograms in Figure 2. Depending on the amount of deposit, the ratio of capacitive to analytical current can be adjusted.[24] However, with voltammetric techniques, such as square wave voltammetry, capacitive background currents can be effectively suppressed to develop electrochemical sensors on the basis of peptide nanostructures.[25]

3.2. Electrochemical Behavior of DA. The electrochemical behavior of DA in pH 7.0 phosphate buffer solution was examined using cyclic voltammetry. On the bare and Nafion-modified carbon electrodes, a large peak-to-peak separation have been observed for 1.0×10^{-3} mol L^{-1} DA, as shown in Figure 3A. However, when the bare GCE surface was coated with Nafion film, well-defined cathodic and anodic peaks have been obtained, due to high cation-exchange capacity of Nafion attracts the cation dopamine from bulk solution to the electrode surface. On the $[Cu_4(apyhist)_4]^{4+}$/Nafion/GCE the anodic and cathodic peak separation are smaller, probably because of the $[Cu_4(apyhist)_4]^{4+}$ complex, which improves the absorption efficiency and electrochemical reactivity of DA. The copper(II) complex serves to generate active sites of the Cu(II)-peroxide adduct inside the electrode for the transfer of the charge through the interface to catalyze the oxidation of dopamine in vitro.[26]

Scheme 1. Idealized structure of the $[Cu_4(apyhist)_4]^{4+}$ catalyst immobilized in the PNT/Nafion®-modified carbon electrode surface and proposed mechanism of DA reaction



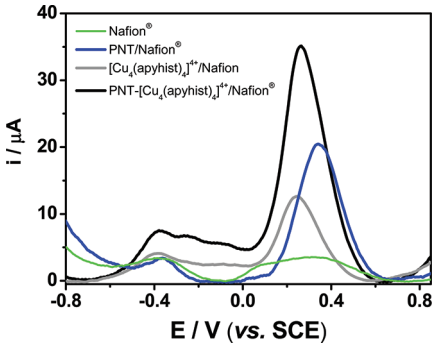


Figure 4. Square-wave voltammograms obtained for 400 μmol L^{-1} DA solution in 0.1 mol L^{-1} phosphate buffer (pH 7.0) for Nafion, PNT/Nafion, $[Cu_4(apyhist)_4]^{4+}$/Nafion and PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion-modified carbon electrodes. Potential pulse amplitude = 50 mV, frequency =80 Hz and ΔEs= 4 mV.

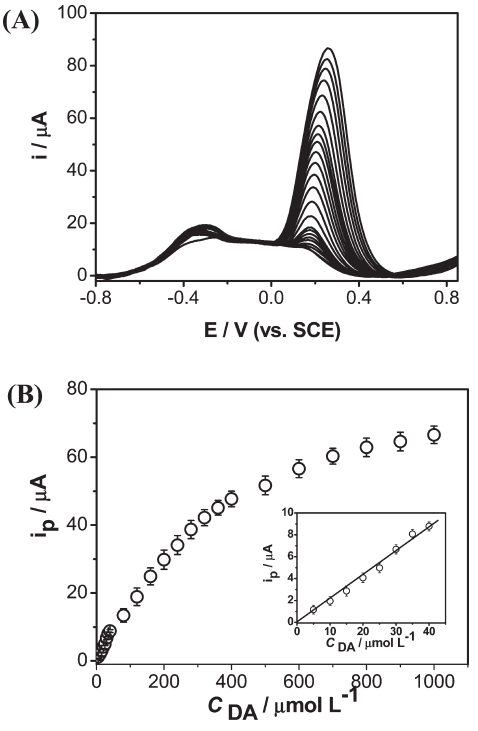


Figure 5. (A) Square-wave voltammograms for DA in PBS (pH 7.0) for the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion carbon electrode. Each addition of DA was 5 or 40 μmol L^{-1}; (B) Linear relation observed between peak current (i_p) vs concentration of DA over two concentration intervals, viz., 5.0−40 μmol L^{-1} and 40−1000 μmol L^{-1}. Potential pulse amplitude =50 mV, frequency =80 Hz and ΔEs= 4 mV.

Comparing with the bare and Nafion/GC electrodes, the voltammetric responses of DA at the PNT/Nafion and PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion-modified carbon electrodes showed increased peak current intensity, which may be due to the incorporation of dopamine into the lattice-peptide, can be used as an effective preconcentration step prior to the voltammetric measurement. In fact, the peak current increases as the preconcentration time increases and starts to level off around 90 s, as shown in Figure 3B. Therefore, to increase the sensitivity of detection, a longer preconcentration time is needed for the lower concentration of DA.

Moreover, it can be seen that the redox current of DA at the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion/GC electrode is higher than that at the $[Cu_4(apyhist)_4]^{4+}$/Nafion-modified carbon electrode. It may be due to the synergic effect of copper complex and *L*-difenilalanine nanotubes, because the surface area of the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion/GC electrode increased, the edge plan sites presented on the PNT surface can improve the electron transfer between the modified electrode and DA. A similar behavior has been observed with biocompatible conducting polymer poly(*N*-methylpyrrole)/Au nanoparticles deposited on a glassy carbon electrodes.[27] It was found that the redox peak currents for the PNMPy/AuNP-modified GCE were much higher than that PNMPy-modified GCE without AuNPs. Thus, the AuNPs generate many active sites inside the electrode for the transfer of the charge through the interface, inducing good contact with the PNPMPy matrix.[27]

According to the above discussions, the course of DA oxidation at the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion-modified GC

Table 1. Performance Comparison of the DA Sensors Based on Different Electrode Materials[a]

electrode type =	linear range (μmol L^{-1})	detection limit (μmol L^{-1})	ref
PNMPy/AuNP-modified GCE	up to 20×10^3	1.5	27
PNCPy/AuNP-modified GCE	$100-10 \times 10^3$	100	31
Au nanowire electrodes	0.4−250	0.4	32
biomimetic chitosan film	0.5−19.2	0.36	33
EPPGE-SWCNT-Fe_2O_3	3−32	0.36	34
MWNTs-IL-Gel	1−100	0.10	35
MWNTs-CoPc	3.11−93.2	0.26	36
MWNTs	0.5−400	0.20	37
dopamine polymer film	1−600	0.20	38
penicillamine self-assembled monolayer	8.06−1060	0.46	39
N-acetylcysteine self-assembled monolayer	1−200	0.80	40
DL-homocysteine self-assembled monolayers	5−500	0.50	41
$[Cu(bpy)_3]Cl_2 \cdot 6H_2O$-Nafion	9−230	4.80	42
Pt/Nafion	3−60	0.01	43
graphene/Nafion	4−100	2.64	44
Nafion/Pt@Au/CNT	up to 120	0.08	45
CNT/titania/Nafion	0.5−500	0.1	46
PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion	5−40	2.80	in this work

[a] PNMPy-poly(*N*-methylpyrrole); PNCPy-poly[*N*-(2-cyanoethyl)pyrrole]; AuNP-gold nanoparticles; MWNTs-multi-walled carxcbon nanotubes; SWNTs-single-walled carbon nanotubes; EPPGEs-edge-plane pyrolytic graphite electrode; CoPc-cobalt phthalocyanine; IL-ionic liquid; bpy-(2,2...-bipyridine).

electrode could be described as in Scheme 1. The voltammetric response of the modified electrode for dopamine was based in two redox steps, which can be described by following electrocatalytic mechanism: the first involves the electrochemical oxidation of copper(I) in the hybrid film producing copper(II) complex on the electrode surface, followed by the electron transfer of the dopamine and consequently regeneration of the copper(I) in the complex. The anodic peak current obtained in ca. +0.25 V (vs SCE) was proportional to the dopamine concentration in solution. Therefore, the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion/GC electrode exhibited high electrocatalytic activity toward DA oxidation, leading to improve the reversibility and enhance the electron transfer kinetics. The incorporation of copper(II) complex into the net-like PNT film was effective to improve the electrocatalytic activity of the modified electrode.

3.3. Voltammetric determination of DA. Figure 4 shows the square wave voltammograms for the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion, $[Cu_4(apyhist)_4]^{4+}$/Nafion, and PNT/Nafion electrodes in the presence of 400 μmol L^{-1} DA solution in 0.1 mol L^{-1} phosphate buffer (pH 7.0). For the Nafion and PNT/Nafion electrodes, the electrooxidation of DA occurred at ca. 380 mV, and the peak obtained was rather broad, suggesting slow electron transfer kinetics. However, a well-defined oxidation peak was obtained in the presence of $[Cu_4(apyhist)_4]^{4+}$ on the modified electrode surface, respectively. For PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion composite film, the oxidation peak potential shifted to *ca.* 255 mV, with an increase in the response to DA compared to the $[Cu_4(apyhist)_4]^{4+}$/Nafion and PNT/Nafion electrodes. This may be the result of the larger surface area of the PNT nanostructures on the electrode surface, which may enhance the catalytic activities of the electrode and adsorb DA more easily than electrodes with less surface area. It appears that DA is capable of interacting with the tubule-free amide sites via hydrogen bonds that promote more efficient absorption and electron transfer reactions on the structure electrode−solution interface. Moreover, the immobilization of the copper(II) complex $[Cu_4(apyhist)_4]^{4+}$ in the PNT/Nafion membrane appears to act as a promoter by lowering the potential of DA oxidation with a negative shift in its anodic peak with an increase in peak current. The presence of the copper(II) complex significantly improves the current response of the sensor by inducing electron transfer in the chemical reaction. This is a result of the formation of a hybrid system between the copper(II) complex and the PNT/Nafion structure.

The two anodic peaks at −350 and −160 mV were assigned to the existence of two different copper(II) complexes in aqueous solution because this system shows a strong dependence on the pH for equilibrium involving a mononuclear complex $[Cu(apyhist)(H_2O)_2]^{2+}$.[12]

The square wave voltammograms of various concentrations of DA for the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion modified carbon electrode are shown in Figure 5. Under the optimized conditions, the SWV peak height was linearly related to the DA concentration over two concentration intervals, viz., 5−40 μmol L^{-1} and 40−1000 μmol L^{-1}, the linear dependence obeyed the following equations:

$$i_p = 0.2158c_{DA} \ (R^2 = 0.9932)$$

$$i_p = 0.1143c_{DA} + 5.718 \ (R^2 = 0.9872)$$

The slopes of the both equations were 0.2158 and 0.1143 μA/μmol L^{-1}, respectively. The detection limit of 2.80 μmol L^{-1} could be estimated (S/N = 3). The decreased slopes with increasing the DA concentration may be explained as follows: with the increasing of DA concentration, the main contributions to the peak currents of DA were changed gradually from the adsorption of DA at the electrode surface into the diffusion of DA to the electrode surface, which results in the decreased different slopes of the two concentration intervals.[28]

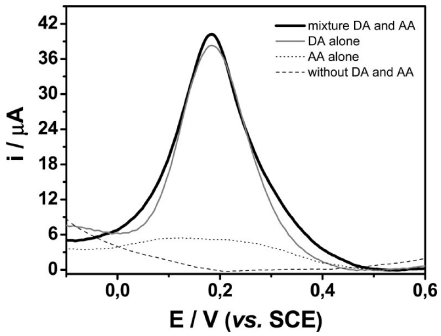


Figure 6. Square-wave voltammograms of the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion/GC electrode in the absence of DA, in the presence of 400 μmol L^{-1} AA alone, and in a mixture of 400 μmol L^{-1} AA/400 μmol L^{-1} DA. Potential pulse amplitude = 50 mV, frequency = 80 Hz, and ΔE_s = 4 mV.

The effect of square wave frequency on the peak current and peak potential of DA was investigated in the range 10−150 Hz. The oxidation peak current of DA shows a linear relationship with the scan rate in the range (10−150 Hz) and the dependence of i_p vs frequency can be represented by the equation: $i_p = 0.088f - 22.31$ ($R^2 = 0.9680$). This behavior suggests that the electrode process is adsorption-controlled,[29] since the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion film shows hydrophobicity by incorporating uncharged hydroquinone molecule within the film. The adsorbed compound was removed from the electrode surface by applying the potential of +25 mV for 100 s. This process is repeated at least thrice after each run in order to ensure full desorption of the compound.

The sensitivity of the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion hybrid film was two times greater than that of the $[Cu_4(apyhist)_4]^{4+}$/Nafion electrode. The detection limit (3σ/slope) of DA was estimated to be 2.80 μmol L^{-1}, which is relatively low as compared to other DA sensors reported in the literature based on different electrode architectures (see Table 1). Thus, it is clear that modified carbon electrode PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion can be promising for the determination of DA.

To verify the selectivity of the PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion/GC electrode for DA detection, SWVs were obtained in the presence of *L*-ascorbic acid (AA). As shown in Figure 6, the anodic peak potentials of DA appeared at +204 mV, whereas the AA anodic current was almost negligible because the negative charged PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion hybrid film repels the negatively charged ascorbate anion. This feature allows positively charged DA to reach the electrode surface because at pH 7.0 PBS, DA (pK_a = 8.87) exits as cations, whereas AA (pK_a = 4.17) exits as anions.[30]

The relative standard deviation (RSD) of the same sensor in ten successive measurements was 1.5% for 10 μmol L^{-1}, and ten different electrodes were fabricated for determining 10, and the RSD was 2.6%, indicating that PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion film had excellent reproducibility. Furthermore, the stability of the modified electrode was investigated. The peak current retained 96% of its initial response after its storage in air for 2 weeks. The results revealed the good reproducibility and stability of the sensor.

4. CONCLUSIONS

This work demonstrates that PNTs are easily and homogeneously dispersed into a Nafion membrane solution. A uniform and stable PNT/Nafion composite film was achieved as a result of electrostatic interactions between the sulfonate groups of Nafion and the copper(II) complex, thus developing a novel chemically modified electrode. A Nafion membrane was used to block interferences, such as ascorbate, which exhibits an oxidation potential close to that of DA. The PNT-$[Cu_4(apyhist)_4]^{4+}$/Nafion/GC electrode has provided good electrocatalytic properties and a satisfactory detection limit for DA. The modification of PNTs with metallic complexes not only enhanced the peak current but decreased the overpotential toward the anodic oxidation of DA. Therefore, this work illustrates a simple and novel approach for the development of a voltammetric sensor based on peptide-nanostructured modified electrodes. Because of its stable response, this sensor may find future application as a disposable dopamine microsensor at physiological concentrations in microvolume samples.

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