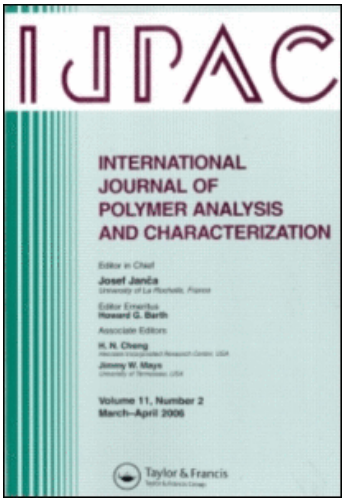

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Amperometric Biosensor for Dopamine Determination Based on Over-Oxidized Polypyrrole-Plant Tissue Composite

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Amperometric Biosensor for Dopamine Determination Based on Over-Oxidized Polypyrrole-Plant Tissue Composite

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Abstract: An amperometric biosensor based on over-oxidized polypyrrole (OPPy) coated on a sol-gel bioelectrode was developed by entrapment of plant tissue into sol-gel matrix. Polymer film was electrochemically synthesized by electro-oxidation of 0.1 M pyrrole in aqueous solution onto the sol-gel biosensor. PPY was then over-oxidized by scanning potential from −0.2 to 1.1 V versus SCE at a scan rate of $20\,mVs^{-1}$ in 0.5 M NaOH. The OPPy-modified sol-gel bioelectrode showed a high catalytic activity toward the electro-oxidation of dopamine (DA). The effects of pH, pyrrole concentration, and potential range of polypyrrole over-oxidation on the response of the electrode were investigated. The modified bioelectrode presented a linear response range for DA from 9.99×10^{-6} M up to 1.1×10^{-3} M by amperometry. Amperometric measurements were performed at a constant potential of 0.4 V versus SCE. The diffusion coefficient of $6.86 \times 10^{-8}\,cm^2\,s^{-1}$ for DA was also obtained using chronoamperometry study. The responses of the over-oxidized polypyrrole-coated sol-gel electrode and uncoated sol-gel electrode were compared. The sensitivity and the stability of the biosensor were also determined. The biosensor was applied for the determination of dopamine in pharmaceutical formulations.

Keywords: Amperometry; Biosensor; Dopamine; Over-oxidized poly pyrrole; Plant tissue

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INTRODUCTION

Conducting polymers have attracted much attention, largely because of their many applications in solar cells, light-weight batteries, electroanalytical applications, and chemical sensors and biosensors.[1–5] In the past decade, an elegant strategy of biosensor construction based on the entrapment of biomolecules in polymer films during their electrogeneration on electrode surface appeared.[6] Early works described the entrapment of glucose oxidase within the growing network of conducting polypyrroles,[7,8] thus, the enzymes are incorporated in the growing polymer. The electrically conducting polymers act as excellent materials for immobilization of biomolecules for the preparation of biosensors.

Among the conducting polymers, polypyrrole and its derivatives play an important role due to their versatile applicability. Degradation of polypyrrole conductivity can be obtained by electrochemical oxidation at high potentials; the doping ions are expelled from the polymer film, and oxygen-containing groups such as carbonyl and carboxyl are introduced to the pyrrole unit, resulting in a porous structure film and remarkable permselective properties.[9] Conducting polymers are used to enhance speed, sensitivity, and versatility of biosensors to measure different analytes. They are also known to be compatible with the biological molecules in neutral aqueous solutions.

Several methods for the immobilization of biomolecules into the polymer matrix have been successfully applied. Tian et al.[10] developed a novel amperometric glucose biosensor by an electrochemical formation of polypyrrole membrane in the presence of glucose oxidase on the ceramic carbon electrode. Over-oxidation polypyrrole/poly (O-phenylenediamine) bilayer biosensors have been developed with the enzymes glucose oxidase or cholesterol oxidase entrapped within an inner polypyrrole (PPy) layer.[11] An electrochemical biosensor was developed by electrodeposition of gold nanoclusters on insulating over-oxidized polypyrrole (OPPy) film modified glassy carbon electrode.[12]

A straightforward method for constructing a cholesterol biosensor by entrapment of cholesterol oxidase within a polypyrrole film electropolymerized in a flow system is proposed by Vidal et al.[13]

The sol-gel technique has attracted much attention recently.[14] Several reports on the use of sol-gel chemistry in the development of biosensors were published in the past few years.[14–19]

The enzymes can be immobilized within sol-gel matrices still maintaining their native properties; this makes the technique a potential tool for the development of new biosensors. The excellent properties of sol-gel materials such as chemical inertness, optical transparency,

negligible swelling in aqueous solution, low temperature encapsulation, and mechanical stability endued these materials with an extensive role in the preparation of chemical sensors and biosensors.[17]

The entrapped species such as chemical materials and biomolecules preserved their chemical properties or bioactivities well. The most interesting thing is that the leaching of these entrapped species did not occur or occurred very slowly. The reason for this is that the sol-gel materials are usually formed by hydrolysis of an alkoxide precursor followed by condensation to yield a polymeric oxo-bridged SiO_2 network, and the immobilized species are encapsulated within the physically rigid network. Plant tissue containing the enzymes and these plant-based biosensors offer a good alternative compared with biosensors based on isolated enzymes. These biosensors have some advantages, such as low cost, simplicity of construction, no need for a co-factor for enzyme regeneration, biocatalytic activity, and improved stability.[20]

In recent years plant tissue biosensors based on materials such as banana,[20] potato,[21] spinach,[22] mushroom,[23] coconut,[24] palm tree,[25] and asparagus[26] have been reported.

In this work, we propose a novel system for construction of the OPPy-modified sol-gel derived biosensor based on electrodeposition of polypyrrole on the sol-gel matrix-containing plant tissue. It describes a bioelectrochemical system for the determination of dopamine (DA), which is used as a model system involving redox couple for assay of electrode function.

EXPERIMENTAL SECTION

Reagents and Instrumentation

Dopamine and other reagents were of analytical grade supplied by Merck. Pyrrole, analytical grade, was from Merck and purified by double distillation, stored in a refrigerator, and kept from light. Methyltrimethoxysilane (MTMOS) and graphite powder were purchased from Merck. The fresh apple used in the biosensor construction was purchased at a local market. It was stored at 4°C until use. All the chemicals were obtained from commercial sources and used without further purification. A sol-gel derived bioelectrode was used as working electrode. A platinum wire was employed as counter electrode and a saturated calomel electrode (SCE) served as the reference electrode. Voltammograms were obtained with a PGSTAT 20 Autolab potentiostat from Eco Chemie (The Netherlands).

Preparation of Sol-Gel Bioelectrode

The silica sol was prepared by mixing 0.6 mL MTMOS, 0.1 mL HCl (0.1 M), and 0.2 mL water. A thick section of the apple pulp was obtained by cutting with a spatula. A 0.032 g portion of the pulp was mixed with 0.3 g graphite powder and was allowed to dry in free air. The resulting mixture was mixed with the homogeneous silica solution and the paste was packed into one end of a 2 mm i.d. tube to a length of 3 mm, subsequently allowed to dry and gel at least two days at 4°C. The surface of all electrodes was removed by mechanical polishing with 1200-grit polishing paper. The electrodes were rinsed thoroughly with water to yield shiny surfaces. Copper wire contacted to the other end, providing the electric contact.

Preparation of OPPy-Modified Sol-Gel Derived Bioelectrode

The sol-gel bioelectrode was placed in a solution containing 0.05 M pyrrole and 0.1 M $LiClO_4$, and polypyrrole formed electrochemically on the sol-gel derived bioelectrode by the cyclic voltammetry method. In this procedure, the potential was scanned from -0.2 to 0.7 V at the scan rate of 20 mVs^{-1} three times. Then PPy was over-oxidized by scanning potential from -0.2 to 1.1 V versus SCE at the scan rate of 20 mVs^{-1} seven times in 0.5 M NaOH.

RESULTS AND DISCUSSION

The PPy and OPPy films were prepared by the cyclic voltammetry method as mentioned above. An obvious peak appeared during the first voltammetry cycle. During the second and third cycles, this peak disappeared. It indicates that an irreversible electrochemical process of the polymer film has been achieved during the first cycle. In this process, the electrode surface was activated and the polymer lost its conductivity. Figure 1 shows the voltammograms recorded during the over-oxidation process of PPy.

Cyclic Voltammetric Characterization

The cyclic voltammograms of sol-gel electrodes were recorded between -0.4 and 0.8 V using a scan rate of 20 mVs^{-1}. Figure 2 shows the cyclic voltammograms obtained with the sol-gel derived electrode (a) and the OPPy modified sol-gel derived electrode (b) in the presence of 10^{-4} M

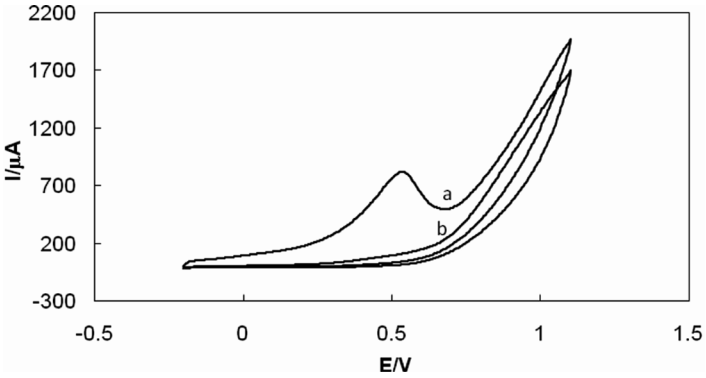

Figure 1. Repetitive CVs for the over-oxidation process of PPy doped with $LiClO_4$ in 0.5 M NaOH: (a) first cycle; (b) second cycle. Scan rate: 100 mVs^{-1}.

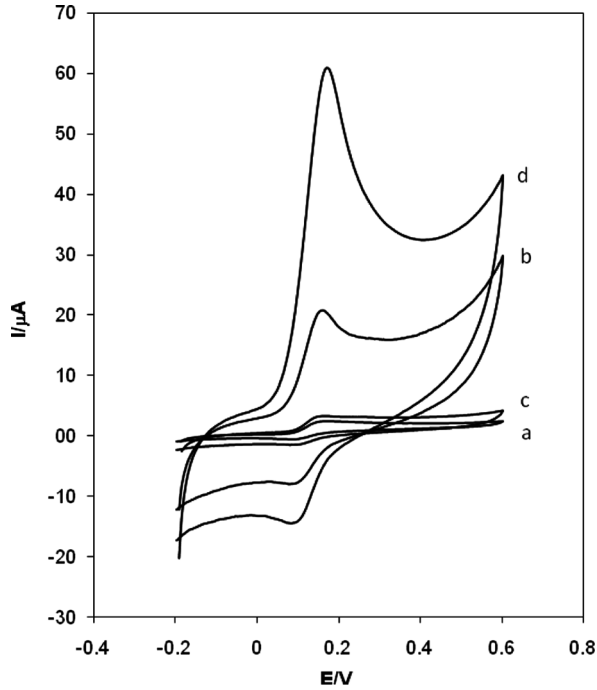

Figure 2. Cyclic voltammograms obtained with the sol-gel derived electrode (a) and the OPPy-modified sol-gel derived electrode (b) in the presence of 10^{-4} M dopamine, the sol-gel derived bioelectrode (c), and the OPPy-modified sol-gel bioelectrode (d) in 0.1 M phosphate buffer, pH = 7.0.

DA in 0.1 M phosphate buffer solution (pH 7.0). The cathodic and anodic peaks appearing at this electrode were ascribed to the electrochemical redox reaction of DA (Figure 2(a)). The formation of the over-oxidized polypyrrole film on the sol-gel electrode leads to increase the peak currents and the kinetics of electrode reactions (Figure 2(b)). This indicates an enhanced diffusion flux. The electrocatalytical reaction of DA was performed by adding the apple tissue in the sol-gel bulk because of the existence of polyphenol oxidase enzyme (Figure 2(c)). A remarkable increase in the OPPy-modified sol-gel derived bioelectrode was observed by modifying the bioelectrode surface in the presence of OPPy (Figure 2(d)). We assume that the OPPy plays the role of modifier and mediator.

Optimization of Parameters of Electropolymerization/ Over-oxidation Step

In order to investigate the potential range of the electropolymerization process, different potential ranges were applied and in each range, the sol-gel electrodes were used in a phosphate buffer solution with 0.05 M pyrrole and 0.1 M $LiClO_4$. The experiment was carried out in this way: the initial potential was fixed and the final potential varied from 0.7 to 1.2 V. Optimum range was found from -0.2 to 0.7 V. In this range, dopamine peak exhibited higher current.

Potential range of over-oxidation stage was optimized as mentioned previously; the amount of potential range was from -0.2 to 1.1 V.

In the over-oxidation stage, scan rate and number of cycles were studied. In order to optimize scan rate, 10, 20, 40, and 60 mVs^{-1} rates were tested. By considering a higher current of dopamine peak, 20 mVs^{-1} was selected for subsequent studies, and the optimized number of cycles was seven.

Optimization of Experimental Conditions

To investigate the effect of pH of the DA solution, buffer solutions (0.1 M) were prepared ranging from pH 4.6 to 8.0 and the OPPy-modified bioelectrodes were settled in the cell with different buffer solutions containing 10^{-4} M DA. Figure 3(a) shows the cyclic voltammograms recorded for pH 4.6–8.0 at the scan rate of 30 mVs^{-1}.

The effect of pH on the peak potential and current of DA was studied for the OPPy-modified bioelectrode. The peak potential values of DA were dependent on pH and shifted toward a less positive potential with an increase of the pH of the buffer solution. It means that the redox couple of DA includes proton transfer in the reduction and oxidation

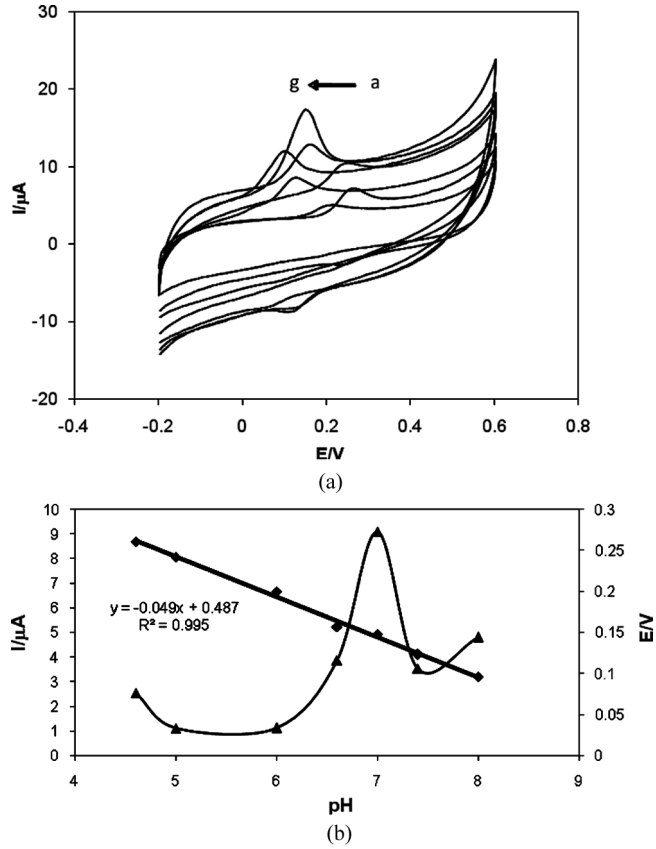

Figure 3. (a) Cyclic voltammograms of 10^{-4} M dopamine obtained with the OPPy-modified sol-gel derived bioelectrode in 0.1 M phosphate buffer at different pH values: a, 4.6; b, 5.0; c, 6.0; d, 6.6; e, 7.0; f, 7.4; g, 8.0. (b) Dependence of peak currents and peak potentials on pH for 10^{-4} M dopamine at the OPPy-modified sol-gel derived biosensor with scan rate of 30 mV/s.

processes. As can be seen from Figure 3(b), the current response increases with increasing pH and then reaches the maximum value at pH 7.0. From these results, pH 7.0 was selected for subsequent studies.

The effect of the amount of apple tissue on the OPPy-modified bioelectrode response was studied in our previous work.[19] An increase of the tissue percentage of the bioelectrode results in an increase in the bioelectrode response. Increasing the tissue percentage beyond 10.6% caused a lower response. Based on these results, the bioelectrode with a percentage of 10.6% tissue was used.

The effect of the pyrrole concentration on the voltammetric signal of the OPPy-modified bioelectrode in a solution containing 10^{-4} M

DA was studied in the range from 0.05 to 0.3 M. From the results, 0.05 M pyrrole concentration was selected as optimum value for further studies. Perchlorate concentration was 0.1 M in the polymerization step.

The influence of the scan rate varying from 10 to 300 mVs^{-1} on the voltammetric response of the OPPy-modified sol-gel derived bioelectrode was studied. Figure 4 shows the cyclic voltammograms of the OPPy-modified bioelectrode in the presence of 10^{-4} M DA at 0.1 M PBS. The anodic currents increase and the peak potentials shift as the scan rate increases. When the peak current values were plotted against $v^{1/2}$, a linear relationship with $R^2 = 0.9949$ was obtained. This behavior suggests that the oxidation process is controlled by the diffusion.

Under the optimum conditions, the cyclic voltammograms at different concentrations of DA were recorded and peak currents were linearly dependent on DA concentration.

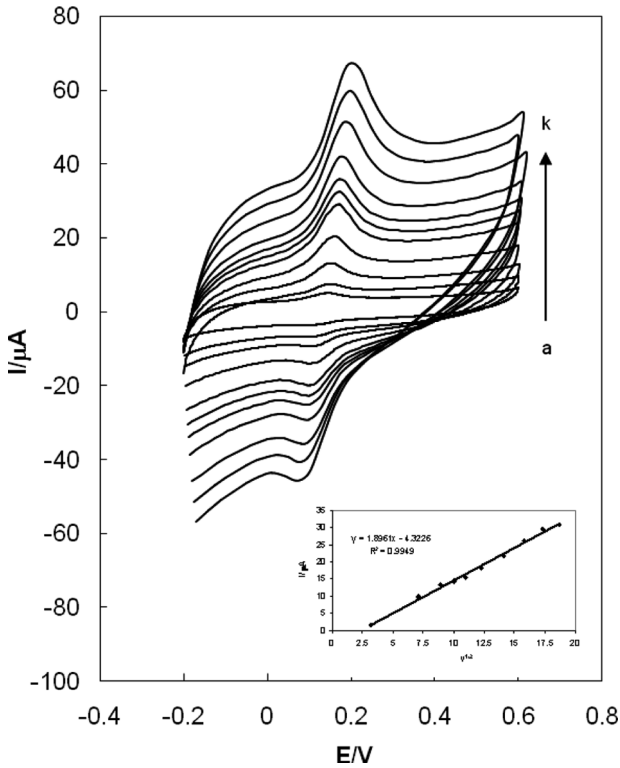

Figure 4. Cyclic voltammograms of OPPy-modified sol-gel bioelectrode in 0.1 M buffer solution (pH 7.0) containing 10^{-4} M dopamine at scan rates: a, 10; b, 20; c, 30; d, 50; e, 80; f, 100; g, 120; h, 150; i, 200; j, 250; k, 300. Inset: Dependence of the peak current with square root of the scan rate.

Chronoamperometry

Oxidation of DA at the OPPy-modified sol-gel derived bioelectrode was studied by the chronoamperometry technique. The obtained chronoamperograms for different concentrations of DA are given in Figure 5. As Figure 5 shows, an increase in concentration of DA was accompanied by an increase in anodic currents obtained for a potential step of 400 mV versus SCE. From the chronoamperometric studies, the diffusion coefficient of DA for the OPPy-modified bioelectrode can be determined. The relationship between diffusion coefficient and bulk concentration can be described by the Cottrell equation[27]:

$$I = nFAD^{1/2}C/\pi^{1/2}t^{1/2}$$

where D and C are the diffusion coefficient ($cm^2\,s^{-1}$) and the bulk concentration (M), and n, F, and A are electron number, Faraday number, and the electrode area, respectively. The plot of I versus $t^{-1/2}$ constructed from the Cottrell equation is linear, and from the slope, the value of D can be obtained. The slope of the resulting straight line was then plotted

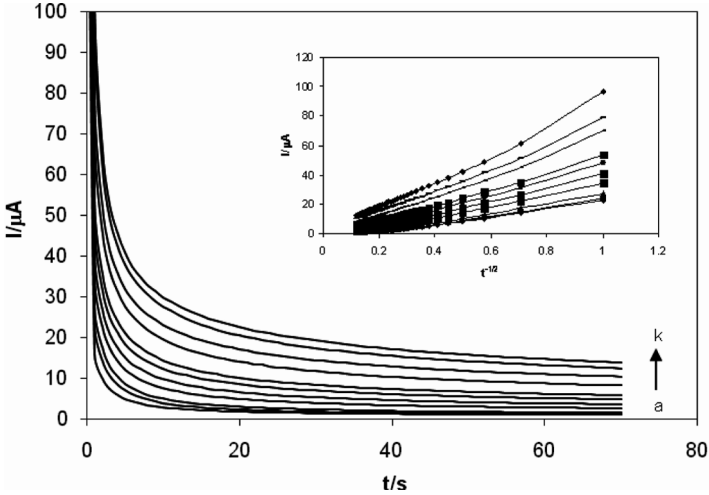

Figure 5. Chronoamperometric response of the OPPy-modified sol-gel derived bioelectrode in 0.1 M phosphate buffer solution (pH 7.0) containing different concentrations of dopamine potential 400 mV vs. SCE. Inset: Plot of I vs. $t^{-1/2}$ obtained from chronoamperometric experiments for the OPPy-modified sol-gel derived bioelectrode in 0.1 M buffer solution (pH 7.0) containing DA concentrations: a, 1.99×10^{-5}; b, 5.96×10^{-5}; c, 9.9×10^{-5}; d, 1.48×10^{-5}; e, 1.96×10^{-4}; f, 3.1×10^{-4}; g, 4.39×10^{-4}; h, 5.84×10^{-4}; i, 7.41×10^{-4}; j, 9.09×10^{-4}; k, 1.1×10^{-3} M.

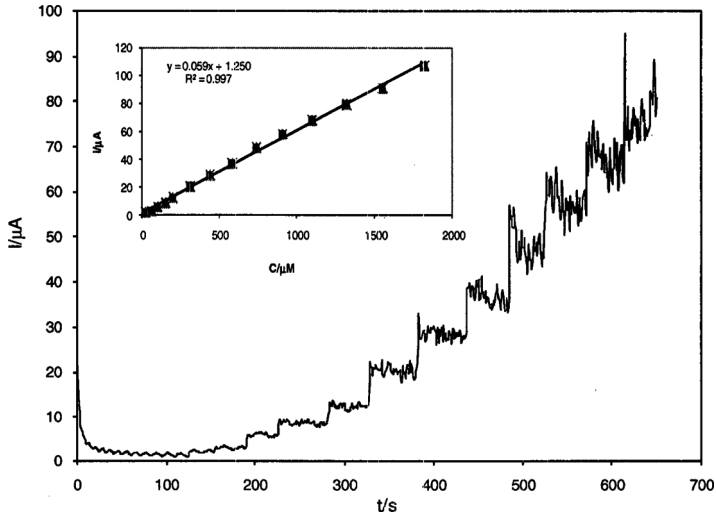

Figure 6. Amperometric response of the OPPy-modified sol-gel bioelectrode kept in 400 mV in 0.1 M phosphate buffer solution, pH 7.0, containing different concentrations of dopamine from 9.99×10^{-6} M to 1.1×10^{-3} M. Inset: calibration plot for concentrations of dopamine.

versus the concentration of DA, which allowed calculating the diffusion coefficient of 6.86×10^{-8} cm^2 s^{-1} for DA.

Amperometry

Figure 6 shows the effect of concentration of the DA on the amperograms of the OPPy-modified sol-gel derived bioelectrode when the potential was kept at 400 mV during the successive addition of DA. The modified bioelectrode presented a linear response range for DA from 9.99×10^{-6} M up to 1.1×10^{-3} M.

Limit of Detection and Stability

A calibration plot constructed from the data in Figure 6 gives a limit of detection (LOD) of 4.1×10^{-6} M.

The repeatability of the response current of the OPPy-modified sol-gel bioelectrode was investigated at various DA concentrations. The relative standard deviation (R.S.D.) was 3.5% for 13 successive assays. The stability and lifetime of the sensor were investigated by measuring

the biosensor response over a four-month period. The response current of the sensor decreased to 20% after 70 days.

The proposed method was used for the determination of DA in dopamine hydrochloride injection (DHI) solution. Constant concentration of the DHI solution and aliquots of standard DA solution were added into the electrochemical cell, and then the concentration of DA was determined by standard addition method, resulting in mean values of $208 \pm 4\%$ mg of DA per injection.

CONCLUSION

A novel bioelectrode was developed for the determination of DA by using plant tissue in the sol-gel network coated with over-oxidized polypyrrole. The OPPy-modified sol-gel biosensor exhibits good electrocatalytic properties towards DA. The determination of DA can be performed in the range of 9.99×10^{-6}–1.1×10^{-3} M with a detection limit of 4.1×10^{-6} M.

Good stability, reusability, and lifetime were obtained. The low cost, simple, and fast fabrication of the sensor makes it superior to other techniques. The bioelectrode was applied for the determination of DA in pharmaceutical formulations.

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