Polyethylenimine Functionalized with Dopamine: Characterization and Electrocatalytic Properties

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Abstract
We report the chemical modification of polyethylenimine (PEI) by functionalization with dopamine (Do) using glutaraldehyde (Glu) as a linker (PEI-Glu-Do). The new polymer was characterized by FT-IR spectroscopy, by derivative UV-vis spectroscopy, and Cyclic Voltammetry. The PEI-Glu-Do demonstrated to have an important electrocatalytic activity allowing an important decrease in the overvoltages for the oxidation of NADH and a significant increase in its oxidation peaks currents. Different ratios of PEI:Do were assayed and the best response was obtained using PEI:Glu:Do 1:2:1. The new polymer represents an interesting and promising alternative for the electrochemical quantification of NADH and other analytes of clinical interest, as well as for the development of new electrochemical (bio)sensors.

Keywords: Polymer functionalization, Polyethylenimine, Dopamine, Electrochemical sensor, Glassy carbon, NADH, Electrocatalysis

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1. Introduction

The use of polymers for the design of electrochemical sensors has received great attention in the last years. In fact, polymers are widely used for the immobilization of biomolecules to build biosensor platforms. Their original role as electrical insulators has been progressively substituted by their electrical conductivity abilities, which offer a new and broader scope of applications. Conducting polymers have been used in different fields such as fuel cells, biosensors, electroanalysis, electrocatalysis, electrochromic displays, among others [1 – 4]. Since the first Merrifield's report in 1963 [5 – 7], functionalized polymers have become extremely useful in organic synthesis. The ability of polymers to be functionalized is closely related to the availability of their functional groups. Specifically, the new strategies have been focused on the synthesis of polymers functionalized with redox molecules to increase the number of active sites for specific bonds, and to provide a three-dimensional network that allows a more efficient electron transfer. To date, a great variety of polymers with ligands covalently bond has been synthesized [8, 9]. However, just very few have been based on the covalent immobilization of dopamine.

The development of electrochemical biosensor devices requires an efficient connection between the biomolecule involved in the biorecognition event and the electronic transducer. In this sense the conductive polymer (CPs) used as an interface between biomolecule and electrode surfaces offers several advantages [10 – 14]. The immobilization of the biorecognition element to the polymer matrix needs to be fast and stable, and most important, should ensure the biorecognition activity of the immobilized biomolecule.

Sensitive and selective amperometric biosensors based on the catalytic action of dehydrogenases mainly assisted by the soluble coenzyme nicotinamide adenine dinucleotide (NADH) have been reported [15 – 17]. In these biosensors, the analytical signal is given by the oxidation of NADH. The direct oxidation of NADH at unmodified electrodes requires large overpotential (>1 V) owing to the slow electron transfer kinetics [18]. In addition, the unmodified electrodes very often suffer from fouling by the adsorption of NADH oxidation product [19]. Considerable effort has been done to the development of new electrode materials with electrocatalytic activity towards the oxidation of NADH or mediators that allows the stable low-potential determination of NADH [20]. Several mediators have been reported in the literature, including quinones [21, 22], oxometalates [23], ruthenium complexes [24], quinonoid redox dyes such as indamines [25 – 27], phenoxazines [28, 29] and phenothiazines [30]. Although such mediator-modified electrodes significantly decrease the oxidation overpotentials and improve the stability of the NADH determination, the interference from other electroactive

molecules, the low sensitivity and the narrow linear range of the determination, restrict their analytical applications.

Several authors have described the behavior of polymers containing catechol-quinone groups as active redox centers, with interesting electrochemical activity towards the oxidation of NADH [31, 32]. There are only few reports about the catalytic activity of the oxidation product of catecholamines like dopamine (Do), epinephrine and norepinephrine on the electrooxidation of NADH [33–35]. Dopamine is an important neurotransmitter present in the central nervous system of mammalian. It plays a fundamental role not only in the functioning of central nervous system, but also in hormonal, cardiovascular and renal systems [36, 37]. The mechanism for the electrooxidation of dopamine has been proposed by Raper et al. more than 80 years ago [38]. Dopamine oxidation generate an *ortho*-quinone, that is very unstable and in turn it is converted into dopaminochrome.

The goal of this work is to synthesize and characterize a new polymeric material functionalized with dopamine, and to study the analytical applications for the development of electrochemical (bio)sensors. The proposed polymer was obtained by covalent attachment of dopamine to polyethylenimine (PEI). PEI is a polymer used in biosensors due its high biocompatibility [39, 40]. The interest in using dopamine as functionalizing agent is to take advantages of its easy oxidation in different media and electrodes. In the following sections we discuss the optimization of the synthesis conditions, and the optical and electrochemical characterization of PEI-Do. We have studied the potential application of glassy carbon electrode (GCE) modified with PEI-Glu-Do for the electrocatalytic oxidation of NADH.

2. Experimental

2.1. Reagents

Dopamine (3,4-dihydroxyphenethylamine, Do), polyethylenimine (PEI, average MW 1,200), glutaraldehyde (Glu, 25% in water), sodium cyanoborohydride, NADH and Sephadex G-10 were purchased from Sigma. Other chemicals were reagent grade and used without further purification. Ultrapure water ($\rho = 18\ M\Omega$ cm) from a Millipore-MilliQ system was used for preparing all the solutions. A 0.050 M phosphate buffer solution pH 7.40 was employed as supporting electrolyte.

2.2. Apparatus and Equipments

UV-Vis experiments were performed with a Shimadzu UV 260 spectrophotometer. IR experiments were performed with a Nicolet 5-SXC FT-IR spectrometer. The polymer samples were prepared on KBr disks.

The electrochemical measurements were performed with an EPSILON potentiostat (BAS). Glassy carbon was used as working electrode. A platinum wire and Ag/AgCl, 3 M NaCl (BAS, Model RE-5B) were used as counter and reference electrodes, respectively. All potentials are referred to the latter. The electrodes were inserted into the cell (BAS, Model MF-1084) through holes in its Teflon cover.

2.3. Synthesis of PEI-Glu-Do

Dopamine was covalently attached to PEI through the formation of imine bond between the amino groups of PEI and aldehyde groups of Glu. The pathways used for the synthesis are shown in Figure 1.

PEI functionalized with dopamine and Glu (PEI-Glu-Do) was obtained in the following way: a given amount of dopamine was added to 5 mL of a 0.11% w/v PEI solution in water. The mixture was stirred for 5 min, and then mixed with 0.050 mL of an aqueous solution of glutaraldehyde (25% P/P). The preparation was kept under constant stirring at room temperature for 4 h. The Schiff bases obtained were reduced to more stable secondary amines using sodium cyanoborohydride.

Different polymers were prepared by changing the initial concentrations of dopamine, and maintaining constant the

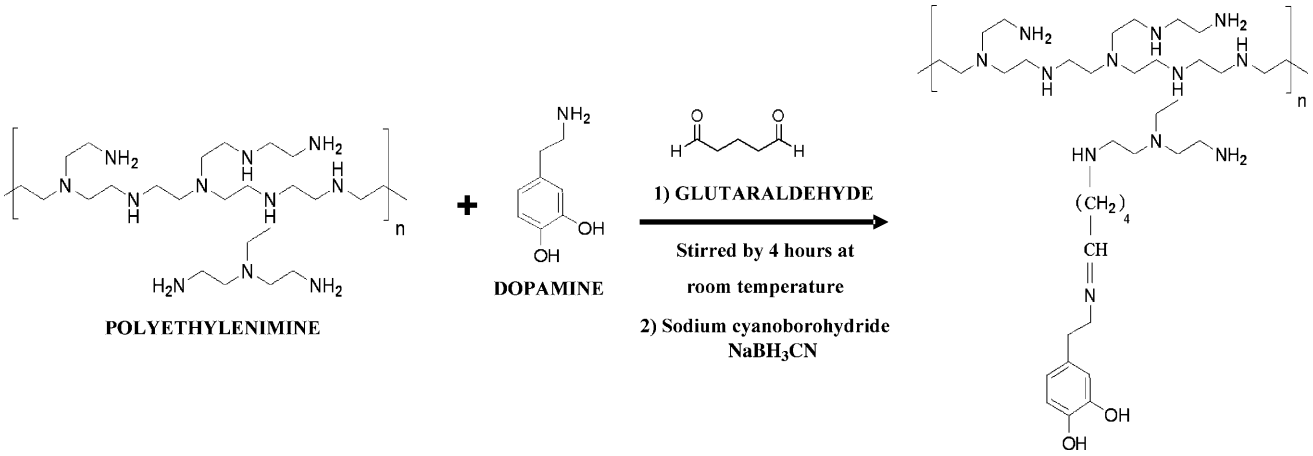

Fig. 1. Scheme of the pathways used for the synthesis of the PEI functionalization whit glutaraldehyde (Glu) and dopamine (Do).

ratio of amine groups of PEI and aldehyde groups glutaraldehyde (PEI/Glu). The final ratios of amine groups of PEI, aldehyde groups of glutaraldehyde and amine groups of dopamine in the different polymers were 1:2:1, 1:2:2, 1:2:4.

The amount of dopamine immobilized at the polymer was evaluated from UV-vis absorption.

All products were purified using Sephadex G-10 columns of 18.0 cm length and 1.3 cm width. Milli Q water was used as the elution solvent. Dextran blue was employed for the exclusion volume determination. The first fraction eluted was used for further studies because it presented the highest dopamine concentration covalently bond to PEI.

2.4. Preparation of the Glassy Carbon Electrode Modified with PEI and PEI-Glu-Do

The glassy carbon electrode (GCE) was previously polished with alumina slurries of 0.30 and 0.05 μm for 5 min and sonicated in water for 1 min. It was then modified with PEI or PEI-Glu-Do solution (GCE/PEI and GCE/PEI-Glu-Do respectively) by cycling the potential between −0.200 and +0.800 V (10 cycles) at 0.100 V s^{-1}.

2.5. Procedure

Cyclic voltammetric experiments were performed at 0.100 V s^{-1} between −0.200 and +0.800 V. All the experiments were conducted at room temperature. In all cases a 0.050 M buffer phosphate solution pH 7.40 was used as supporting electrolyte.

3. Results and Discussion

3.1. Spectroscopic Characterization of PEI-Glu-Do

3.1.1. FTIR Studies

Figure 2 illustrates FT-IR spectra of PEI (A), dopamine (B) and PEI-Glu-Do with different dopamine initial concentration: 1:2:1 (C), 1:2:2 (D) and 1:2:4 (E), before reduction reaction. The spectrum of PEI shows important peaks at 1564 and 1478 cm^{-1}, which correspond to primary and secondary amines. The bands between 970–1175 cm^{-1} (in plane deformation) and 860–900 cm^{-1} (out of plane deformation) that correspond to C–H of substitution 1:2 of dopamine benzene ring, are present in the spectrum of dopamine as well as in the spectra of the PEI-Glu-Do products (C, D and E). In addition, these bands increase with the increment of dopamine covalently bond to the polymers (B vs. C and E). The band at 1630 cm^{-1} indicates the formation of the imine (C=N) linkage, which confirms that the reaction between the NH_2 residues of both PEI and dopamine has occurred.

3.1.2. UV-Vis Studies: Characterization and Quantification of Dopamine Bonded to PEI

Figure 3 illustrates the UV-Vis absorption spectra of PEI, glutaraldehyde (Glu), dopamine (Do), and PEI-Glu-Do 1:2:1. The large overlap of the spectral bands between 190 and 350 nm makes not possible the determination of the dopamine bond to PEI (Figure 3A). In order to develop a method that allows the spectrophotometric determination of bond dopamine, we evaluate the derivative spectra. Figure 3B displays the second derivative spectra of PEI,

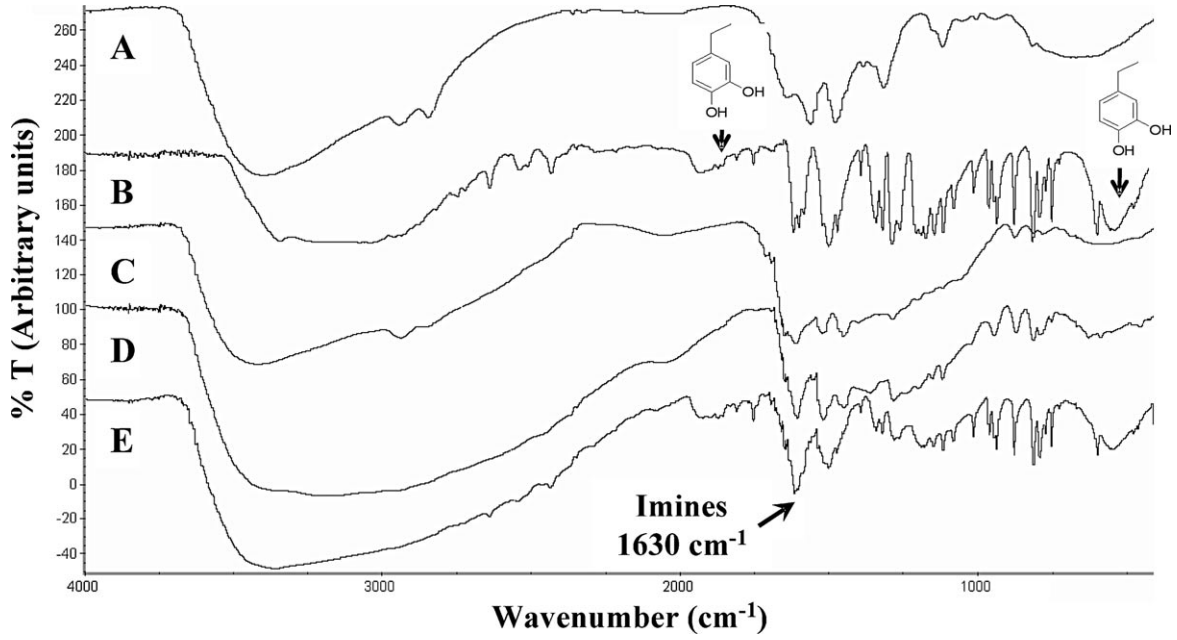

Fig. 2. FTIR spectra for unmodified PEI (A), pure dopamine (B) and functionalized PEI with glutaraldehyde (Glu) and different amount of dopamine (Do): PEI-Glu-Do 1:2:1 (C), 1:2:2 (D) and 1:2:4 (E).

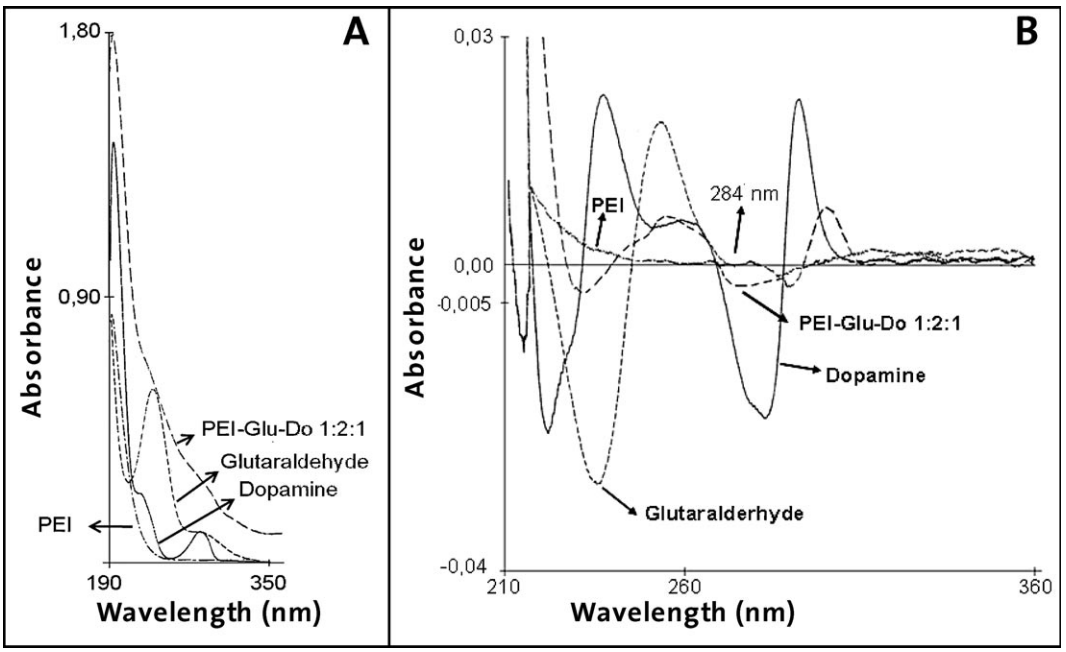

Fig. 3. UV-vis spectra of PEI, dopamine, Glu, PEI-Glu-Do 1:2:1 (A), and the second derivative spectra of the same samples (B).

glutaraldehyde (Glu) and dopamine (Do) and the polymer PEI-Glu-Do 1:2:1. The spectrum of dopamine exhibits a well-defined band with a maximum absorbance at 284 nm, while glutaraldehyde and PEI have a zero value at this wavelength. Therefore, the second derivative spectrum allows the determination of dopamine bond to the polymer at 284 nm without interference.

Table 1 shows the amount of dopamine present at the polymers synthesized with different PEI/Do ratios obtained from derivative spectrophotometrics determinations. The UV-vis spectra demonstrate that when the initial concentration of dopamine increases, the dopamine covalently attached to PEI also increases. When the concentration of dopamine in the synthesis mixture increased by a factor of 2, the amount of dopamine attached to the polymer increased about 23%. Nevertheless, an increment of 32% in the attached dopamine was reached when dopamine concentration in the synthesis solution was 4 times higher. The explanation for this nonlinear behavior has to be found on the effect of the solvent on the amount of exposed functional groups. Thus, variations in the number of dopamine fixing positions (−HC=O), have to be dependent on the coil conformation of the polymer chain, (open or closed), which in turn is depending of the quality of the solvent. Therefore an increase of the dopamine concentration in solution does not necessarily be followed by a linear increase in the number of attached dopamine. Beside of this, it also has to be considered that when dopamine covalently bonded increases, the steric hindrance problems are increased too.

Table 1. Quantification of dopamine: (a) Initial relation concentration used. (b) Dopamine concentration covalently bonded.

PEI-Glu-Do (a)	g Do/100g polymer) (b)
1:2:1	6.8 ± 0.8
1:2:2	7.5 ± 0.7
1:2:4	9 ± 1

3.2. Electrochemical Studies: Oxidation of Dopamine Bond to PEI

Figure 4 shows cyclic voltammograms (CV) obtained at 0.100 V s^{-1} for 0.500 mg/mL of PEI (·····), PEI-Glu-Do 1:2:1 (—), PEI-Glu-Do 1:2:2 (- - -), and PEI-Glu-Do 1:2:4 (- · - · -) at bare GCE. The CV for 1.0×10^{-3} M dopamine obtained in similar conditions is also showed as inset. Under these conditions, PEI does not display any faradaic process at bare GCE within the evaluated potential window. On the contrary, the CVs for the different PEI-Glu-Do show a quasireversible behavior, with an oxidation current peak at around 0.4 V, due to the two-electron conversion of dopamine to dopaminequinone [38], and the corresponding current peak due to the reduction of the electrogenerated quinone. The oxidation peak is shifted from 0.462 to 0.447 V as the amount of dopamine bond to PEI increases (compare PEI-Glu-Do 1:2:1 and 1:2:4, respectively), while the corresponding reduction peak also show a shifting from 0.063 V to 0.135 V. These results clearly demonstrate that the functionalization of PEI with dopamine turns these polymers into redox conductors, and confers to the polymer very interesting properties.

Table 2 compares cyclic voltammetric parameters for dopamine obtained from the cyclic voltammograms shown in Figure 4. The oxidation and reduction currents increase as dopamine concentration bond to the polymers increases. When the concentration of dopamine added to the synthesis

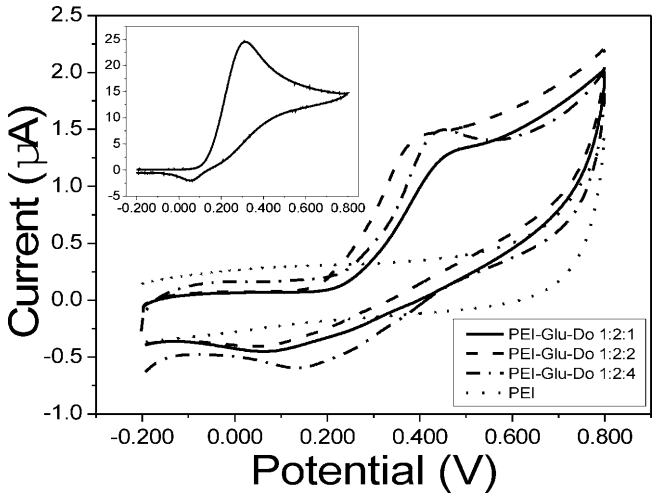

Fig. 4. Cyclic voltammograms for 0.500 mg/mL solutions of PEI (·····) and PEI-Glu-Do containing different dopamine initial concentration: 1:2:1(—); 1:2:2 (----); 1:2:4 (-·-·-) at bare GCE. Inset: Cyclic voltammograms of 1.0×10^{-3} M of dopamine at bare GCE. Scan rate: 0.100 V/s.

mixture increases by factors of 2 and 4, the oxidation current for the immobilized dopamine increases a 25 and 48%, respectively. Therefore, there is not linear relationship between the increment in the oxidation currents and the dopamine concentration present in the synthesis mixture, in good agreement with those obtained by UV-vis measurements. However, the electrochemical behavior of free dopamine is more reversible than when it is immobilized at PEI. Similarly, its behavior is more favorable when the amount of dopamine in PEI-Glu-Do increases. These results confirm that the electrochemical behavior of dopamine units bond to the polymer is not modified by the immobilization process.

Another important aspect to be evaluated is the stability of the redox signal of dopamine attached to the polymers. In this sense experiments performed one year after the preparation of the polymers (stored at 5 °C), showed the same response. These results demonstrate that the attachment of dopamine to the polymer largely improves the stability, preventing its spontaneous oxidation, in comparison with solutions of free dopamine.

Table 2. Voltamperometric parameter for bare and modified GCE with PEI-Glu- dopamine containing different dopamine initial concentration.

Electrode	ΔE_p (V)	I_{pa}/I_{pc}
GCE/PEI-Glu-Do 1:2:1	0.42	5.4
GCE/PEI-Glu-Do 1:2:2	0.35	5.3
GCE/PEI-Glu-Do 1:2:4	0.30	4.0
Bare GCE (1.0×10^{-3} M of free Do in solution)	0.27	5.2

3.3. Electrocatalytic Oxidation of NADH

One of the objectives of this study was the development of a modified GCE with capability of improving the oxidation of NADH. Figure 5 illustrates the cyclic voltammetric response obtained at bare GCE (Figure 5A) and a GCE modified with electrodeposited films of 1:2:1 (Figure 5B) and 1:2:4 (Figure 5C) PEI-Glu-Do in the absence (a) and

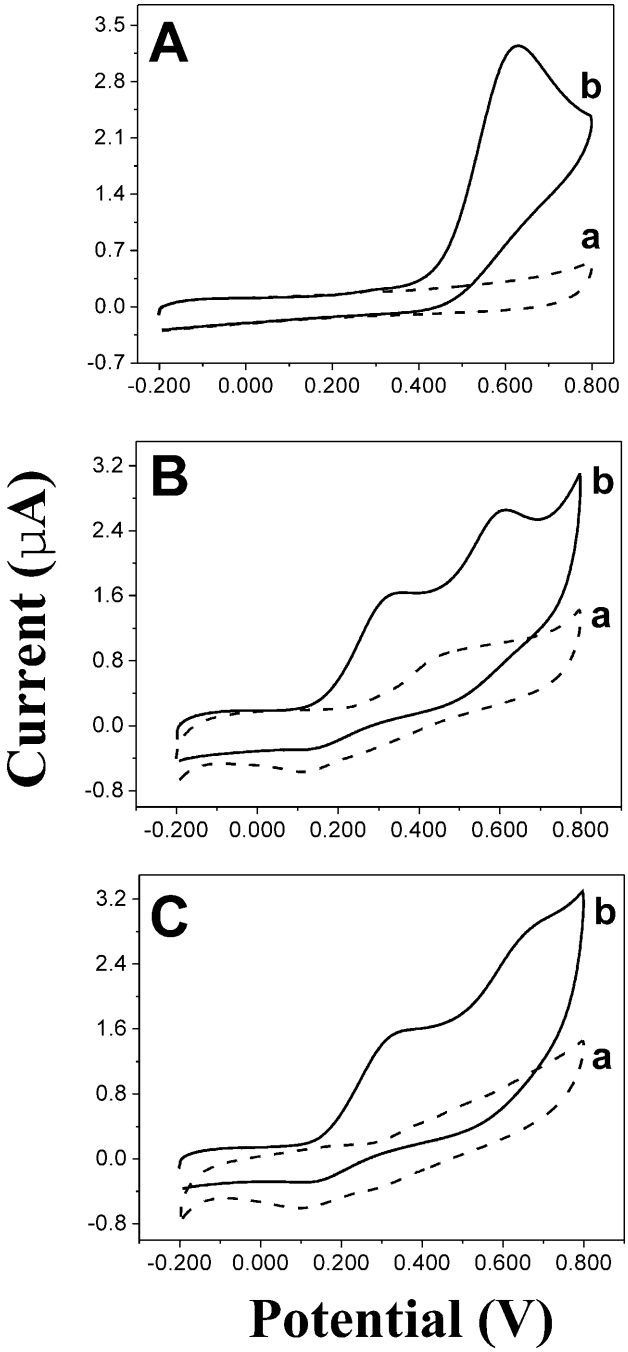

Fig. 5. Cyclic voltammograms at bare GCE (A) and modified GCE with PEI-Glu-Do containing different dopamine initial concentration: B) 1:2:1 y C) 1:2:4, in absence (a, dotted line) and in presence of 0.20 mM NADH (b, solid line). Scan rate: 0.100 V/s.

presence (b) of 0.20 mM NADH (0.050 M phosphate buffer, pH 7.4) at 0.100 V s^{-1}. At bare GCE (Figure 5A) an oxidation current peak is observed at 0.600 V due to the irreversible oxidation of NADH. Moreover, the oxidation peak at the bare electrode gradually decrease during continuous potential cycling, indicating that the electrode surface undergo fouling in the oxidation process (not shown).

At GCE/PEI-Glu-Do the oxidation signal (Figure 5B-a and 5C-a), in the absence of NADH it is observed just the redox behavior of dopamine bond to the polymer immobilized at the electrode. Upon the addition of 0.20 mM NADH it is possible to observe two processes, one at 0.300 and the other at 0.600 V. The explanation for this behavior can be obtained from the scheme shown in Figure 6. Figure 6 describes the sequences of reactions that occur at the modified GCE surface in the presence of NADH. The oxidation peak at 0.300 V is due to dopamine bond to the immovilized polymer (Figure 6A) and the process at 0.600 V is produced for the direct oxidation of the NADH that diffuse across the polymeric layer and reaches the electrode surface (Figure 6C). There is an important enhancement of the oxidation peak current at 0.300 V (Figure 5Bb and 5Cb) in the presence of NADH due to the catalytic activity of dopamine bond to the polymer on the oxidation of NADH (Figure 6B). Just a small current is observed in the cathodic scan, suggesting an electrocatalytic effect mediated by the chemical reaction between NADH and the oxidation product of dopamine (Figure 6B). The catalytic effect can be directly seen when the electrochemical response, in the presence of NADH at GCE/PEI-Glu-Do, is compared with the cyclic voltammogram of NADH oxidation at an unmodified GC electrode. Due to electrocatalytic process that ocurr between NADH and dopamine, the decrease in the overvoltage for the oxidation of NADH at GCE/PEI-Glu-Do is at least 0.300 V. The best electrocatalytical response toward NADH is obtained at GCE/PEI-Glu-Do 1:2:1. In this case the oxidation current at 0.300 V increases in a factor of 5. No improvement of the catalytic effect is obtained when the amount of dopamine in the polymer increases. It is for that reason that we conclude that is necessary only a few units of dopamine in the polymer to show efficient catalytic effect toward NADH oxidation.

One of the most important problems in the oxidation of NADH is the fouling of the electrode by the oxidation product. The cyclic voltammetric response obtained in a fresh 5.0×10^{-4} M NADH solution at GCE/PEI-Glu-Do previously cycled 10 times in another 5.0×10^{-4} M NADH solution was almost the same as the obtained in the first cycle (not shown). On the other hand, amperometric experiments performed at 0.300 V in 1.0×10^{-4} M NADH solution demonstrated that at bare GCE the signal decreases 60% after 10 min indicating that an inhibition of the electron transfer process takes place. In contrast, the response of GCE/PEI-Glu-Do (1:2:1) decreases just 30% for the same period, evidencing the advantages of the polymeric layer.

The GCE/PEI-Glu-Do has demonstrated to be very useful for the amperometric detection of NADH. Figure 7 shows the amperometric response of bare GCE (a) and GCE/PEI-Glu-Do (1:2:1) (b) at 0.300 V to successive additions of 1.0×10^{-5} M NADH. As expected, according to Figure 7, there is a significant improvement in the response

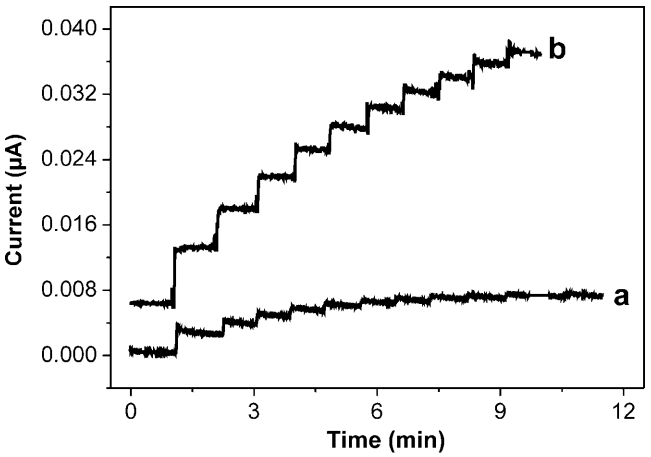

Fig. 7. Amperometric recordings for successive additions of 1.0×10^{-5} M NADH at GCE bare (a) and GCE/PEI-Do (1:2:1) (b). Working potential: 0.300 V.

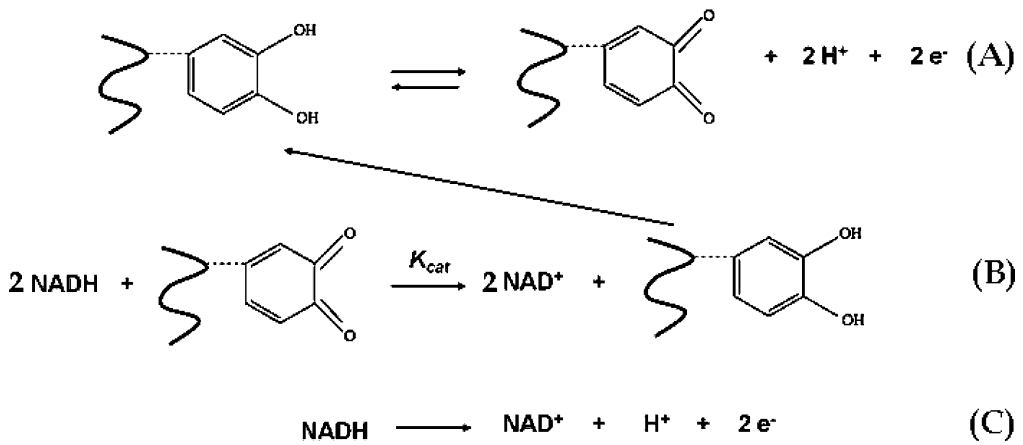

Fig. 6. Scheme showing the reaction at GCE/PEI-Glu-Do in presence of NADH.

of the GCE when polymer are present. The corresponding calibration plots show sensitivities of (20.0 ± 0.6) μAM^{-1} at bare GCE and $(49 \pm 2) \times 10^1$ for GCE/PEI-Glu-Do, indicating that PEI-Glu-Do promotes in a very efficient way the electron transfer between NADH and GCE. Therefore, the electrocatalytic activity makes posible a more sensitive (almost 25 times) and low potential amperometric determination of NADH.

In summary, the modification of GCE with PEI-Glu-Do largely improves the electrooxidation of NADH. The polymer promotes in a very efficient way the electron transfer between NADH and bonded dopamine. This behavior can be attributed to the electrocatalytic activity of quinone groups present in these new synthesized polymers.

4. Conclusions

This work proposes a new polymer obtained by the covalent attachment of dopamine to PEI using Glu as linker. The presence of dopamine in PEI-Glu-Do was demonstrated by FT-IR and UV-vis spectroscopy.

UV-vis and cyclic voltammetric studies demonstrated that the amount of dopamine bond to PEI increases when dopamine concentration in the synthesis solution increases, although in a non linear way. Electrochemical experiments demonstrate that the attachment of dopamine to PEI largely increases its stability, giving the same signal even after one year synthesis.

PEI-Glu-Do film-coated GCE exhibits remarkable electrocatalytic effects on the oxidation of NADH, by lowering the oxidation overpotentials of NADH mediated by dopamine bonded to the polymers. All these properties make PEI-Glu-Do a very interesting polymer for future developments of electrochemical (bio)sensors for different bioanalytes.

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