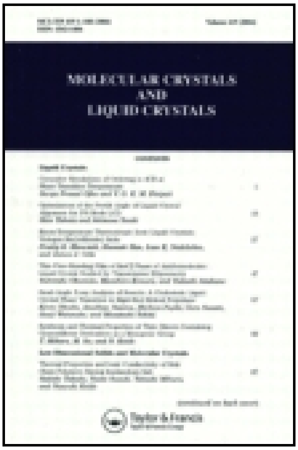

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Electropolymerization Compounds Used for the Obtainment of Modified Electrodes and Electrochemical Biosensors

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Electropolymerization Compounds Used for the Obtainment of Modified Electrodes and Electrochemical Biosensors

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Modified electrodes were obtained from pyrrole and aniline on different substrates like: platinum, titanium, zinc or aluminium substrates by electropolymerization or by co-electropolymerization from synthesis solutions containing different ratio of monomers. Cyclic voltammetry and electrochemical impedance spectroscopy were used to investigate the electrochemical properties of the resulting modified electrodes. Further, an amperometric biosensor for glucose was prepared by immobilization of glucose-oxidase into a poly(o-phenylenediamine) film by simple one-step electropolymerization on platinum substrate. The rejection of interference species may be additionally increased by placing additional layers of Prussian Blue that ensured electrode operation at low potential values or of polypyrrole, respectively.

Keywords: amperometric biosensor; coatings; electropolymerization; polyaniline; polypyrrole

INTRODUCTION

In recent years, the search for organic materials exhibiting electronic conductivity has become an active subject of study by both chemists and physicists. Various polymers were synthesized exhibiting electronic conductivities in their oxidized or reduced states. All the polymers in these systems possess π-electron systems, otherwise they could not be conductive. In their pure state they are usually not conductive, but some of them may have semiconductive properties. They become conductive upon doping by suitable chemical or

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electrochemical oxidations. Conductive organic polymers may be useful for a large number of practical applications.

In this article, the polypyrrole films electrodeposited on pure titanium, aluminium and zinc substrates using galvanostatic or potentiodynamic methods were studied related to the electrochemical properties and protective corrosion properties.

Polymers as polypyrrole and polyaniline have been studied and continue to be studied very actively, especially since these polymers can be very conveniently prepared by simple electrochemical method. Because the oxidation potential of aniline (0.8 V vs. SCE) and pyrrole (1.2 V vs. SCE) are near, we believe that simultaneous oxidation and co-deposition of polyaniline and polypyrrole is possible by changing the applied potential. In this article, we have studied also the formation of polyaniline–polypyrrole composite coatings on platinum and titanium substrates using galvanostatic and potentiodynamic methods. The electrochemical properties of these composite coatings were studied by cyclic voltammetry.

Also the immobilization of biomolecules in or on electropolymerized films is gaining importance [1–3]. The electrochemical entrapment of biomolecules in organic polymers involves the application of an appropriate potential to the working electrode soaked in aqueous solution containing both biomolecule and electropolymerizable monomer. The most widely-held opinion is that the immobilization of biomolecules in electrogenerated polymers is partly due to electrostatic interactions between negatively or positively charged proteins and polymer films, the proteins being incorporated within the polymer as counter-ions. However, most often the electropolymerization process requires very high concentrations of a supporting electrolyte or a buffer. As a result the incorporation of protein molecules in the growing polymer film is more likely due to the presence of protein molecules in the immediate vicinity of the electrode surface and does not result from specific electrostatic interactions. Moreover, the biomolecule immobilization by electrochemical entrapment in conducting or non conducting polymer films requires also high concentrations of biomolecule during the electropolymerization process.

EXPERIMENTAL

Polypyrrole films doped with different anions were deposited on different substrates accordingly with the data specified in Table 1.

The electrosynthesis was carried out in a one compartment cell generally using an aqueous (bidistilled water) solution. As cycling solution we used KCl or Na_2SO_4 aqueous solutions of different concentrations.

TABLE 1 Obtainment Conditions of Polypyrrole Films on Different Substrates

Substrate	Treatment	$C_{pyrrole}$	Dopant anions	Electrosynthesis method	Characteristics of the method	Supporting electrolyte
Platinum	Mechanical polishing	0,05 M	Cl^-, dodecylsulphate (DS)	Galvanostatic	$i = 1\,mA\,cm^{-2}$	0.1 M KCl
				Potentiodynamic	(−200, 800)mV 10 mV/s; 10–50 cycles	
Titanium	Mechanical polishing	0,05 M	Cl^-, dodecylsulphate (DS)	Galvanostatic	$i = 1\,mA\,cm^{-2}$ $t = 10$ minutes	0.1 M KCl
Aluminium	1. Mechanical polishing 2. immersion in 0.1 M KOH, $t = 10$minutes	0,05 M	Dodecylsulphate (DS), 1,4-bis(2-ethylhexyl) sulphosuccinate (AOT)	Potentiodynamic	(0, 800) mV; 5 mV/s; 50–100 cycles	0.1 M Na_2SO_4
Zinc	1. Mechanical polishing	0,5 M	Oxalate	Galvanostatic	$i = 15\,mA\,cm^{-2}$, $i = 25\,mA\,cm^{-2}$ $t = 10$ minutes	3.5% NaCl

Also, polyaniline, polypyrrole and poly(aniline-co-polypyrrole) film were electropolymerized onto a platinum wire substrate by applying galvanostatic impulses. The polymerization was carried out in a 0.05 M monomer + 0.5 M H_2SO_4 electrolytic solution. In the case of the synthesis of copolymers, the concentration of one monomer was maintained constant at 0.05 M and the proportion in moles of the other one was varied from 0.05 M to 0.01 M. It can say that, the initial concentration of the co-monomers was varied from 1:1 to 5:1. The electrochemical characteristics of the coatings were studied by cyclic voltammetry. As cycling solutions were used 0.25 M Na_2SO_4 and 0.25 M NaCl aqueous solutions.

In the case of glucose biosensor obtainment, Prussian Blue was electrodeposited using cycling voltammetry method for 5 cycles in the potential range $-800 \div 500$ mV, at a scan rate of 50 mV s^{-1} from a solution containing $1.5 \cdot 10^{-3}$M $K_3[Fe(CN)_6]$; $2 \cdot 10^{-3}$ M $FeCl_3$; 0.1 M KCl; 10^{-3} M HCl. Polypyrrole films were deposited onto the substrate covered with Prussian Blue film by potential cycling within the limits of $-150 \div 700$ mV, using a scan rate of 50 mV s^{-1} for 30 cycles. The electrosynthesis was carried out using an aqueous solution containing 0.05 M pyrrole (that was freshly distilled) and 0.1 M KCl. Glucose oxidase (GOx) (from Aspergillus niger, 10000 U/g) was immobilized in the poly(o-phenylenediamine) layer using the cyclic voltammetry method ($0 \div 600$ mV, 50 mV s^{-1}, 50 cycles). The synthesis solution contained $5 \cdot 10^{-3}$ M o-phenylenediamine, 0,1 M KCl and 50 mg/L glucose oxidase in a buffer solution of $pH = 5.2$ (1.5726 g/L acetic acid; 10.0457 g/L sodium acetate). For the amperometric measurement of glucose sensing, the current was measured at potential of 0 V in an oxygen saturated phosphate buffer solution of $pH = 7.4$.

RESULTS AND DISCUSSION

The synthesis and properties of polypyrrole (PPY) establishing itself as a novel material in the field of conducting polymers, have been extensively studied [4–7]. It has been shown that along with a number of other parameters, the anions incorporated in the positively charged polymer for charge compensation influence the growth rate, the morphology of the synthesized polypyrrole films and the speed and extent of the electrochemical response. The electrochemical redox process of PPY is affected by the anions because of the motion of anions and protons in the polymer accompanying the redox of PPY. Because the available explanations to the findings about the anion effects are not yet satisfactory, in the present work we investigated further the anion effects on electrosynthesis and electrochemical activity of PPY on pure titanium, aluminium and zinc substrates in different aqueous solutions.

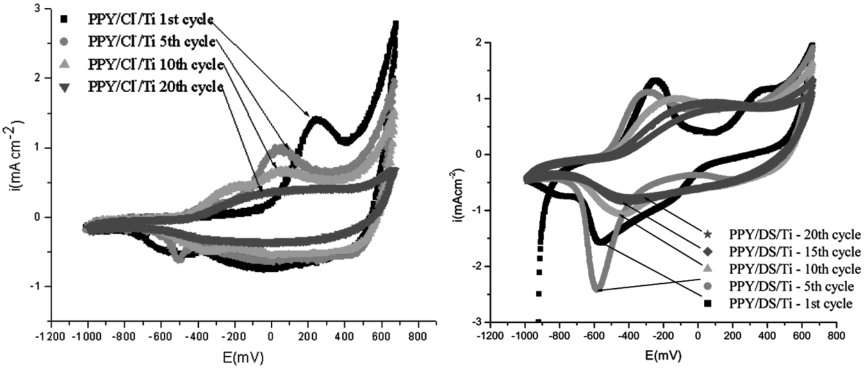


FIGURE 1 The cyclic voltammograms of PPY(Cl^-)/Ti and of PPY(DS)/Ti films for different cycles in aqueous solution of 0.1 M KCl (the cycling electrolyte).

In Figure 1 are presented the cyclic voltammograms of PPY(Cl^-)/Ti and PPY(DS)/Ti modified electrodes, respectively, in an aqueous solution of 0.1 M KCl. The broad peaks observed in the voltammograms suggest a complicated redox process for the obtained modified electrodes. These peak systems were explained in a previous paper [8].

The comparison between the behaviour of the two types of electrodes shows some major differences. The PPY(DS)/Ti modified electrodes shows much sharper peaks and lower peak separation than PPY(Cl^-)/Ti modified electrodes, this indicates that the first type of the electrode is characterized by faster and more reversible electrochemical redox processes. The higher electroactivity of the PPY(DS)/substrate modified electrode was expected on the basis of the fact that the large amphiphilic DS (dodecylsulphate anion) surfactant anion is not easily released after its incorporation into the polymer film. This fact is partially assigned to its size and partially to the compatibility of the polar end with the charged matrix and of the unpolar end with the neutral (reduced) matrix.

We reported in a previous paper that in the case of platinum substrate the redox processes which take place during of the potential scan are much more active than those which take place on the modified electrodes with titanium substrate [8].

This fact we can explain thus: titanium is a valve metal and its corrosion resistance is due to the formation of a TiO_2 passive film. When the working electrode of titanium is anodic polarized, it is easily oxidized giving a non-conducting dielectric film (TiO_2) on the electrode surface. The TiO_2 film acts as an excellent barrier, inhibiting the charge transfer reaction and thus inhibiting the redox processes which take place on it. Our experimental results are in concordance with these characteristics of TiO_2 passive film. Because the PPY polymeric film was galvanostaticaly electrodeposited on titanium substrate results that the PPY doped film forms simultaneously with TiO_2 passive film. This fact explains why the PPY(Cl^-)/Ti and PPY(DS)/Ti modified electrodes have an electroactivity much lower than the PPY(Cl^-)/Pt and PPY(DS)/Pt modified electrodes.

The electrochemical impedance spectroscopy (EIS) was used to investigate the behaviour of the PPY(Cl^-)/Ti and PPY(DS)/Ti modified electrodes. One of the most important applications of EIS is the evaluation of corrosion by polymer coatings. Figure 2a gives the impedance spectra in Nyquist coordinates for PPY(Cl^-)/Ti modified electrode after obtainment at a potential of 0 V in an aqueous 0.1 M KCl solution and the range frequencies of 10^{-2}–10^5 Hz.

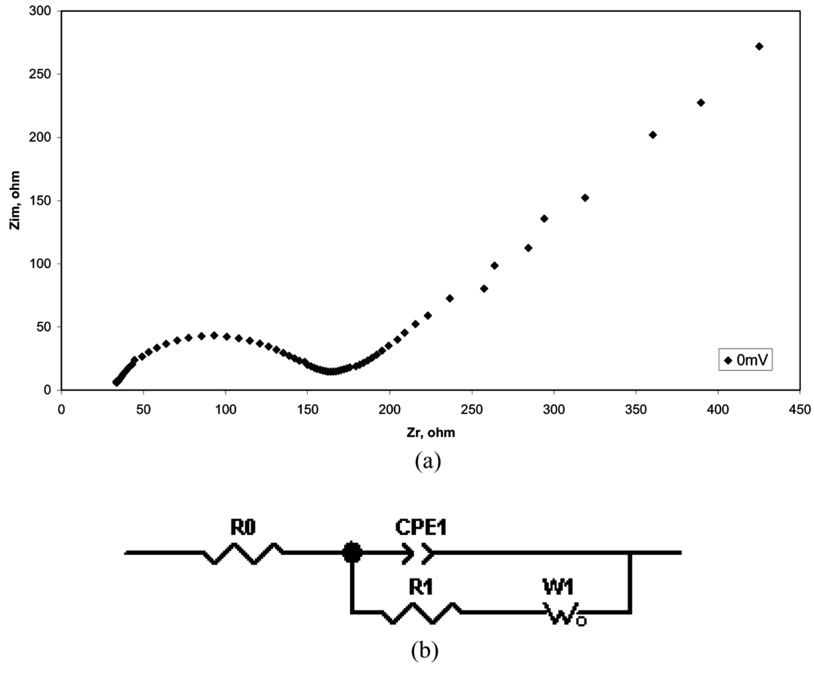


FIGURE 2 The impedance spectra in Nyquist coordinates for PPY(Cl^-)/Ti modified electrode after obtainment at the potential of 0 V in an aqueous 0.1 M KCl solution and the range frequencies of 10^{-2}–10^5 Hz (a) and the equivalent circuit used for the interpretation of the data (b).

Here a single semicircle for charge transfer process and a diffusion tail at low frequency region are present. The semicircle of charge transfer process is severely depressed and the slope of diffusion tail is less than 45°. These could result from the porous polymer film formed on the metal surface. The diffusion impedance element suggests that the electrochemical processes might be controlled by active species diffusing to the metal surface or corrosion products diffusing away from the metal surface through the polymer film.

The equivalent circuit used for the interpretation of the data is shown in Figure 2b.

Diffusion is represented by a Warburg element and the non-homogenous nature of the electrode material was represented by a CPE with impedance given by: $Z_{\mathrm{CPE}} = (1/\sigma)(j\omega)^{-\alpha}$, where σ and α are positive constants. A CPE describes a capacitor when $\alpha = 1$, in which case $\sigma = C$ and tends to a resistor as $\alpha \rightarrow 0$.

A similar behaviour we obtained for PPY(DS)/Ti modified electrodes and hence, impedance spectra may be interpreted with the same equivalent circuit (see Fig. 2b). In Table 2 are given the values of circuit elements obtained fitting the experimental data to the equivalent circuit proposed. From this table it can be observed that the charge transfer resistance has higher values in the reduced state ($-500\,\mathrm{mV}$) which decrease once with the oxidation states ($0\,\mathrm{mV}$). Also, comparative analysis of the impedance data have been shown that, the modified PPY(DS)/Ti electrode has a lower charge transfer resistance than PPY(Cl^-)/Ti modified electrode at the same potential. This fact we can explain thus: the polymer films doped with large anions (amphiphilic anions) have a higher electroactivity and the redox processes are more reversible and take place with higher rate.

It was observed that the electrodeposition of PPY on aluminium substrates is more difficult than in the case of platinum or titanium

TABLE 2 Values of Circuit Elements Obtained Fitting the Experimental Data Obtained for PPY(Cl^-)/Ti; PPY(DS)/Ti Films to the Proposed Equivalent Circuit

Dopant anion	Circuit element E. mV	R0, Ω	R1, Ω	CPE1, $\Omega^{-1}s^{\varphi}$	W1-R, Ω	W1-T, s^{φ}	χ
Cl^-	0	31.2	115	$1.36 \cdot 10^{-5}$, $\varphi = 0.77$	63.62	0.89, $\varphi = 0.28$	0.004
DS	−500	30.5	124.6	$2.94 \cdot 10^{-5}$, $\varphi = 0.90$	44.6	0.31, $\varphi = 0.31$	0.0015
DS	0	31.1	61.12	$2.47 \cdot 10^{-5}$, $\varphi = 0.90$	51.8	0.96, $\varphi = 0.42$	0.0021
	Maximum error	0.5%	1.2%	7.6%, respectively 1%	5.6%	6.4%, respectively 0.6%	

substrates. The Al_2O_3 film acts as an excellent barrier, inhibiting an electron transfer and this inhibiting the electrochemical formation of polypyrrole (PPY) on it. We obtained the electrodeposited PPY film on aluminium by removal the barrier oxide film (Al_2O_3) from the aluminium surface. For that, prior to anodic polarization the electrode surface was etched in 0.1 M KOH for a period of 10 minutes.

The polymeric film was electrodeposited using potentiodynamic method thus, the electrode potential was scanned on the range of ($0 \div 800\,mV$) with a sweep rate of $5\,mV \cdot s^{-1}$ for a period of 50 cycles in aqueous solutions: 0.1 M pyrrole + 0.1 M SDS and 0.1 M pyrrole + 0.02 M AOT. After this anodic polarization a successful formation of both films have been taken, the porous Al_2O_3 film and the polymeric PPY film in their pores at the same time.

The two films interact between them and we can consider that, the bilayer – film was obtained which consisted of a barrier – type Al_2O_3 and an electronically – conducting PPY film. The formation of Al_2O_3 proceeds two interfaces, namely the aluminium/Al_2O_3 and the Al_2O_3/PPY interfaces. We consider that, the species are continuously generated at metal/oxide interface. Then they migrate through growing Al_2O_3 layer toward the solution under high electric field, while oxide ions (O^{2-}) migrate toward the aluminium electrode [9]. At the interface of Al_2O_3/PPY, the Al^{3+} encounter water species to form Al_2O_3 film.

The polarization behaviour of the PPY(DS)/Al and PPY(AOT)/Al modified electrodes was studied by cyclic voltammetry in 0.1 M N_2SO_4 aqueous solutions. In Figure 3 are shown the cyclic voltammograms of PPY(DS)/Al modified electrode at the different cycles in 0.1 M N_2SO_4.

From analysis of Figure 3, one can see that, at the anodic potential scan two anodic current peaks (oxidation peaks) are obtained, while at the reverse potential scan, two catodic current peaks (reduction peaks) are obtained. The appearance of these anodic and cathodic peaks was explicated in a previous paper [10]. The shape of the voltammetric curve also corroborates the reported mechanism. The same behaviour is obtained for the PPY(AOT)/Al modified electrode. In both cases it was observed that the increasing of the number of cycles leads in all the cases to increasing of the height of anodic and catodic peaks and hence, to increasing of the anodic and catodic current peaks respectively; the PPY(DS)/Al modified electrode shows much sharper peaks and lower peak separation than the PPY(AOT)/Al modified electrode, this indicates that the former is characterized by faster and more reversible electrochemical redox processes. This is confirmed by Table 3, where the voltammetric data of the two electrodes are compared and summarized.

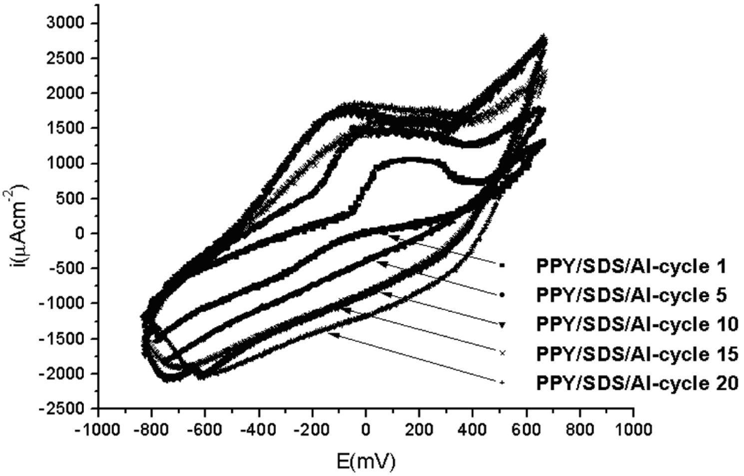


FIGURE 3 Cyclic voltammograms of PPY(DS)/Al modified electrodes at different cycles in 0.1 M Na_2SO_4 at a scan rate of $10\,mV\,s^{-1}$ and 25°C.

In Figure 4 are given the impedance diagrams for PPY (DS)/Al modified electrode at the open circuit potential. The uncoated aluminium electrode shows a single capacitive semicircle on Nyquist diagrams traducing the influence of Al_2O_3 film/pure aluminium interface. It can be seen that the PPY(DS)/Al electrode has a higher polarization resistance then aluminium electrode uncoated. The two time constants that are present in Bode diagrams suggest the two interfaces at the electrode surface, which are: Al_2O_3 film/Al substrate and PPY(DS) film/Al_2O_3 film.

TABLE 3 The Kinetic Parameters of PPY(AOT)/Al and PPY(DS)/Al Modified Electrodes for Different Cycles in Aqueous Solution of 0.1 M Na_2SO_4 (the Cycling Electrolyte)

Doping anion	Cycle number	ipa_1 ($mA \cdot cm^{-2}$)	Epa_1 (mV)	ipa_2 ($mA \cdot cm^{-2}$)	Epa_2 (mV)	ipc_1 ($mA \cdot cm^{-2}$)	Epc_1 (mV)
AOT	1	4.87	170	–	–	–	–
AOT	5	7.54	−50	–	–	–	–
AOT	10	7.81	−20	–	–	9.66	−690
AOT	15	8.80	−125	–	–	10.03	−730
AOT	20	9.18	−100	–	–	10.1	−600
DS	10	8.73	−190	12.30	200	–	–
DS	15	9.16	−156	13.45	270	–	–
DS	20	9.22	−120	14.73	340	–	–

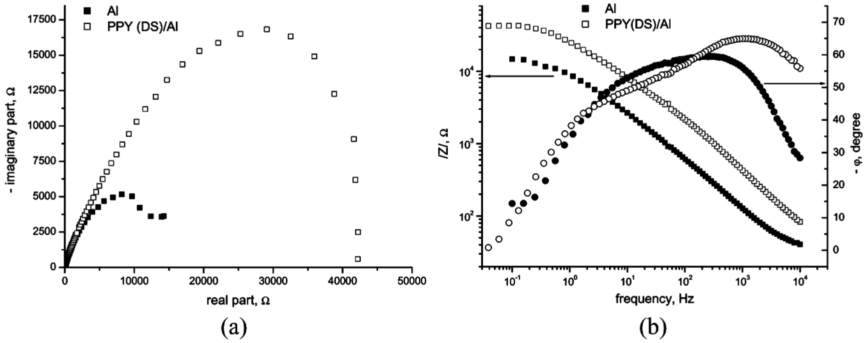


FIGURE 4 Nyquist (a) and Bode (b) diagrams for Al and PPY(DS)/Al electrodes in 0.1 M Na_2SO_4 at the rest potential.

In the case of the electropolymerization of pyrrole on zinc electrodes we must take into account the fact that a zinc electrode is characterized by a very negative oxidation potential that induces its dissolution before the oxidation potential of pyrrole is reached. Therefore, the monomer oxidation is inhibited and consequently the electropolymerization reaction is prevented. To avoid this situation, it is necessary to perform a passivation on the metallic surface by a pre-treatment or by using a suitable supporting electrolyte during the electropolymerization. Homogeneous and adherent polypyrrole films have been obtained in sodium oxalate aqueous medium on zinc electrodes previously treated by immersion in sodium sulfide solution for 24 h.

We have obtained the polypyrrole layer onto zinc electrode by galvanostatic method. The electrosynthesis process of polypyrrole has been performed in an aqueous solution of 0.2 M $Na_2C_4H_4O_6$ and 0.5 M Py at different current densities. This medium has proved to be particularly favorable for slowing down the corrosion rate of zinc electrode without any preliminary passivation treatment.

In Figure 5 are shown the cyclovoltammograms of PPY/Zn electrode in aqueous solution of 3.5% NaCl at a potential sweep of 50 mV s^{-1}. Analyzing Figure 5, one can see that at the potential sweep, on the cyclovoltammogram two anodic peaks of oxidation appear, respectively two cathodic peaks of reduction at reverse sweep. The broad peaks observed in the voltammogram suggest a complex redox process for PPY/Zn modified electrode.

It can be seen that the redox couple are very stable and that, the current peaks increase with increasing of the cycles number. Also it was observed that the obtainment of the PPY layer on the zinc electrode surface by galvanostatic method and the use of a current

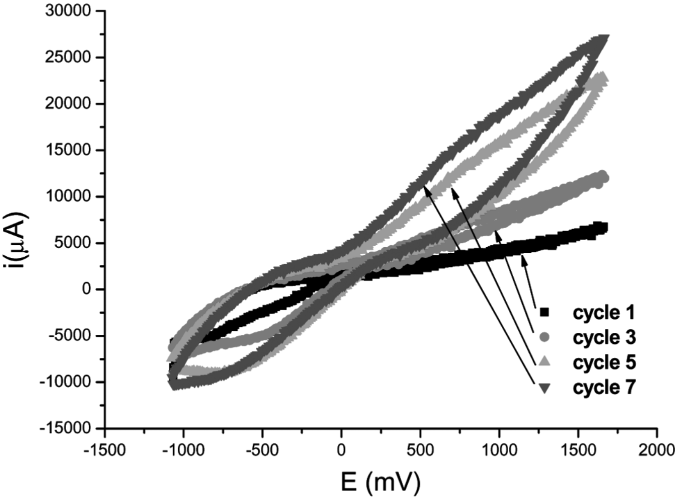
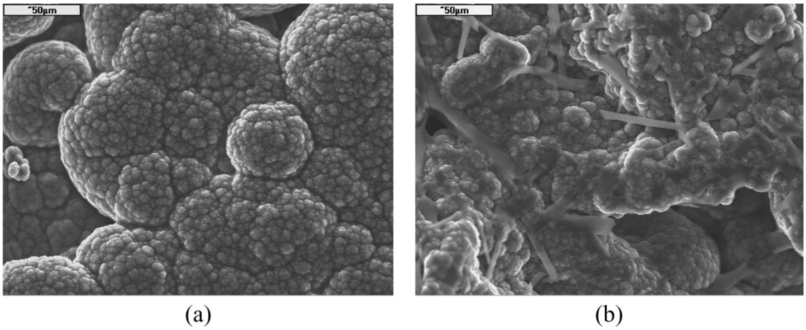


FIGURE 5 Cyclic voltammograms of PPY/Zn electrode in 3.5% NaCl, $50\,mV\,s^{-1}$ (PPY galvanostatic polymerization at $25\,mAcm^{-2}$).

density higher than $15\,mA\,cm^{-2}$ led to a more conductive and porous polypyrrole film.

Studies were also devoted to PANI deposition on zinc electrodes. PPY electrosynthesis was used as a pretreatment for the zinc substrate in order to deposit PANI on these substrates with almost no metal dissolution [11]. In a two-step electropolymerization, the first monomer is electrochemically polymerized on the electrode surface and it is used as an electrode for the polymerization of second monomer. Electrochemically formed copolymers are multilayered systems, which are synthesized by subsequent electropolymerization of two or more layers of different polymers on the same electrode surface. The second layer, electrochemically formed from a different polymer, is most probably located within the network of the first one thus forming a complex structure instead of a separate layer.

The PANI/PPY/Zn modified electrodes were obtained by galvanostatic electrodeposition of polyaniline from a solution of 0.5 M An + 0.2 M $Na_2C_4H_4O_6$ with H_2SO_4 addition on the PPY/Zn modified electrode at a current density of $1\,mA\,cm^{-2}$. The most significant quality test of the generated system lies in the stability of the electrochemical response in monomer free 3.5% NaCl solution, which can be taken as a measure of the efficiency of the PPY coating in protecting the underlying zinc electrode. It was found that the PANI/PPY/Zn modified electrode is more stable and more conductive than the systems



(a) (b)

FIGURE 6 SEM micrographs of a) PPY film obtained $15\,mA\,cm^{-2}$ and b) PANI/PPY film obtained by PPY deposition $15\,mA\,cm^{-2}$ followed by PANI deposition $1\,mA\,cm^{-2}$.

obtained in another conditions. As it can be seen, the redox couples are more clearly defined and much more active. We concluded that the polyaniline film is much better obtained when the PPY substrate is deposited at a current density of $15\,mA\,cm^{-2}$.

SEM micrographs point out the cauliflower shape of the polypyrrole layer and the polyaniline deposition on top the cauliflowers in a strip shape of approximately 4 μm width (see Fig. 6).

In this article we report also the formation of polyaniline – polypyrrole composite coatings on platinum substrates using galvanostatic and potentiodynamic methods. The electrochemical properties of these composite coatings were studied by cyclic voltammetry.

Electrochemical synthesis of poly(aniline-co-pyrrole) from different precursor solutions produces homogeneous and adherent black films. They show great electroactivity in the cycling solutions and the voltammetric profiles are very similar to the potential values of the current peaks being located at intermediate values of the typical electrochemical responses of the homopolymers.

First we obtained the PANI films on the platinum substrates in a synthesis solution of 0.05 M aniline + 0.5 M H_2SO_4 by galvanostatic method. Characterization of the PANI obtained films was made by cyclic voltammetry in a solution free of monomer, in this case we used an aqueous solution of 0.25 M Na_2SO_4. Figure 7 shows the cyclic voltammogram of PANI/Pt modified electrode in 0.25 M Na_2SO_4 at the first cycle and a scan rate of $20\,mV\,s^{-1}$.

From Figure 7, we can see that at the increasing anodic potential sweep, on the anodic branch appear three anodic oxidation peaks while, at the reverse potential sweep on the cathodic branch appears

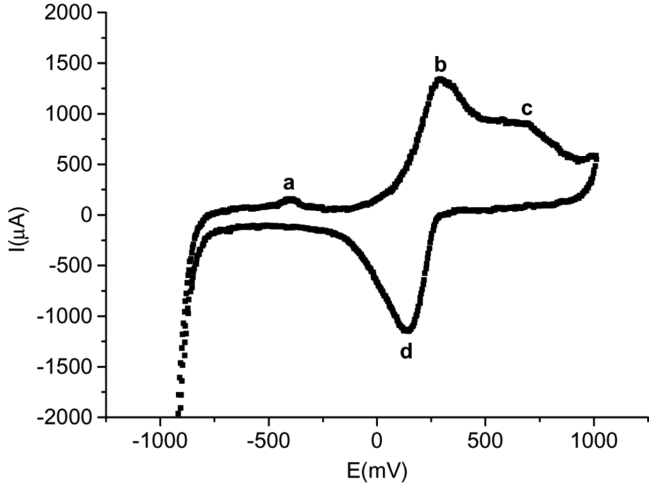


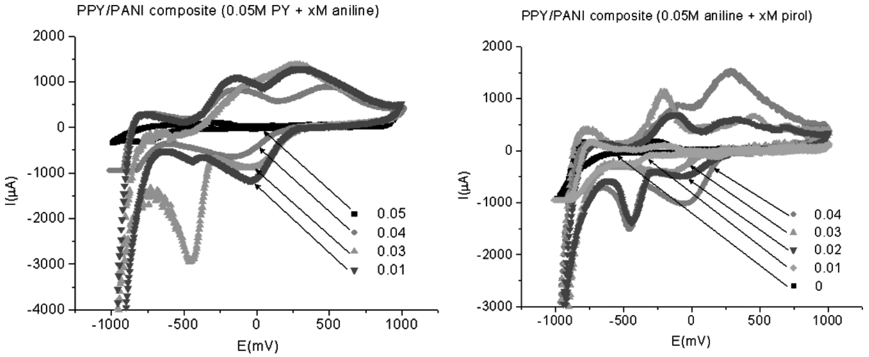
FIGURE 7 The cyclic voltammogram of PANI/Pt modified electrode in 0.25 M Na_2SO_4 at the first cycle and a scan rate of $20\,mV\,s^{-1}$.

only one reduction peak (d). It is well known that polyaniline can exist in three different oxidation states such as leucoemeraldine (fully reduced form), emeraldine form (partially oxidized form) and pernigraniline (fully oxidized form). These forms of polyaniline are dependent on the applied potential.

Further, we have studied the formation of polyaniline – polypyrrole composite coatings on platinum substrate using constant current electrolysis (galvanostatic method) in aqueous media (see experimental section). It was shown that the structure, yield and the morphology of the coatings were dependent on the feed ratio of monomers (aniline and pyrrole) and the applied potential. The resulting composites also showed significant changes in electrochemical behaviour when compared with the homopolymers. As the oxidation potential of aniline (0.8 V vs. SCE) and pyrrole (1.2 V vs. SCE) are near, we believe that simultaneously oxidation and co-deposition of polyaniline and polypyrrole is possible by changing the applied potential.

Figure 8 illustrates the cyclic voltammetric behaviour of the PANI/PPY composite film that was prepared on platinum substrate in manner described in experimental section.

It was observed that the electrochemical response of the copolymers is not the simple addition of those related to PANI and PPY. This fact would be an indication of the formation of a new material with differentiated properties. In general, it can be shown that the behaviour of



(a) (b)

FIGURE 8 The cyclic voltammograms of the PANI/PPY composite film obtained at the cycle number 3 in 0.25 M Na_2SO_4 at a scan rate of $20\,mV\,s^{-1}$.

the current – potential curves is very much influenced by the molar feed ratio of monomers – see for comparison Figure 8 and Table 4.

The immobilization of biomolecules in electropolymerized films for biosensors obtainment is also reported. Amperometric enzyme biosensors form the majority of commercial biosensor devices available on the market today. Amperometric biosensors operate at a fixed potential with respect to a reference electrode and the current generated by the oxidation or reduction of the substrate at the surface of the working electrode is measured. Amperometric biosensors are based on redox enzymes and these enzymes use molecular oxygen as an electron acceptor and produce hydrogen peroxide in the reaction with their substrates. Biosensors based on these enzymes work by following

TABLE 4 The Kinetic Parameters of the PANI/PPY Composite Film Obtained at the Cycle Number 3 in 0.25 M Na_2SO_4 at a Scan Rate of $20\,mV\,s^{-1}$

xM pyrrole	xM aniline	ipa_1 ($mA\,cm^{-2}$)	Epa_1 (mV)	ipa_2 ($mA\,cm^{-2}$)	Epa_2 (mV)	ipc_1 ($mA\,cm^{-2}$)	Epc_1 (mV)	ipc_2 ($mA\,cm^{-2}$)	Epc_2 (mV)
0.05	0.01	1.10	−150	1.30	300	0.73	−440	1.17	−30
0.05	0.02	1.00	−120	1.35	280	1.60	−460	0.96	−50
0.05	0.03	0.90	−95	1.40	250	2.95	−445	0.87	−40
0.05	0.04	0.83	−140	0.90	490	0.65	−160	0.03	450
0.05	0.05	0.18	−260	0.07	800	0.04	−280	0.03	100
0.01	0.05	0.11	−215	0.09	806	0.27	−480	0.11	190
0.02	0.05	0.70	−130	0.60	305	1.38	−440	0.50	−40
0.03	0.05	1.10	−200	0.56	446	0.39	−250	–	–
0.04	0.05	0.87	−100	1.50	280	1.50	−460	1.02	−50
0.05	0.05	0.18	−260	0.07	800	0.04	−280	0.03	100

either the oxygen consumption or the hydrogen peroxide production as a measure of the substrate (analyte) concentration. For instance, the glucose sensor amperometrically detects the hydrogen peroxide, which is produced during the oxidation of glucose catalyzed by the enzyme glucose oxidase (GOx):

$$\text{Glucose} + O_2 \rightarrow \text{gluconolactone} + H_2O_2$$

It is very important to use some selective electrocatalysts which are able to lower the overvoltage of H_2O_2 electrooxidation or electroreduction, thus enabling to provide the detection at much lower potentials, where interferents do not discharge [12,13]. From such electrocatalysts, Prussian Blue seems to be very promising for the use in biosensors.

In the present article, we report a combination of both of these approaches – the obtaining of a glucose sensor based on glucose oxidase immobilized in a poly(o-phenylenediamine) layer placed on a platinum electrode modified by PB and PPY layers.

In Figure 9 are presented the cyclic voltammograms corresponding to Prussian Blue film electrodeposition.

As we mentioned above PB layer functions as electrocatalyst for hydrogen peroxide reduction or oxidation. Thus, an operating potential of around 0 V was chosen, because interferents such as ascorbic acid supposedly not to be discharged at this potential. Biosensor response was obtained by H_2O_2 detection based on its reduction at PB/Pt electrode at the potential of 0 V: $H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$.

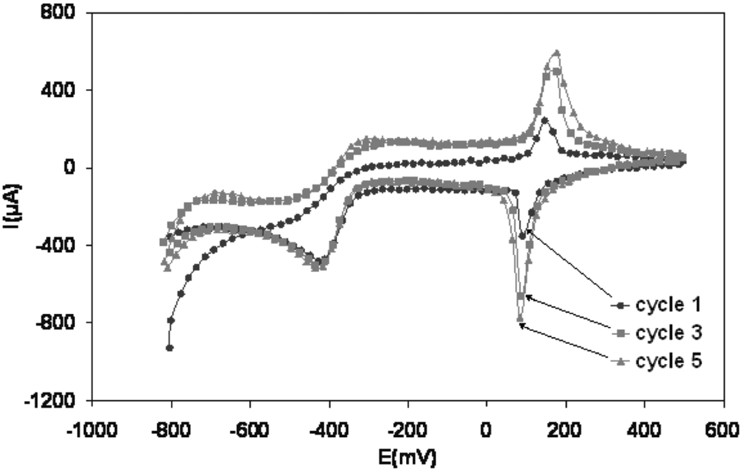


FIGURE 9 Cycling voltammograms corresponding Prussian Blue film electrodeposition.

The biosensor prepared by electropolymerization of pyrrole in presence of GOx over the PB layer didn't show sensitivity to glucose. At the chosen operating potential the currents corresponding to the redox processes in polypyrrole are higher then the ones corresponding to H_2O_2 reduction which results from the enzyme reaction. An interesting alternative is the use of non-conducting polymer films such as poly(o-phenylenediamine) (p(oPD)) [14–16]. The resulting films are thin, hydrophobic and insulating and have therefore obvious advantages in terms of stability and applicability towards miniaturized devices. In addition to immobilizing the enzyme, these films allow rapid diffusion of substrate and product but also act as permselective membranes to improve selectivity and provide a barrier against electrode fouling.

Thus, better results were obtained when glucose oxidase was immobilized in a poly(o-phenylenediamine) film. This polymer presents low redox currents at potentials around 0 V. But we observed a low stability of poly(o-phenylenediamine) film deposited onto PB film. In order to increase the stability of the enzymatic layer we used an intermediate layer of polypyrrole. The electrode covered with these layers was not only more stable, but the diffusion resistance to interferents at the electrode surface was high.

In Figure 10 are presented the cyclic voltammograms reflecting the growth of the polypyrrole layer on PB/Pt modified electrode. It can be seen that after 30 cycles the peaks corresponding to PB redox processes diminished in height, and only current characteristic of polypyrrole was observed.

The poly(o-phenylenediamine) film was synthesised in presence of GOx on the PPY/PB/Pt modified electrode. Because poly(o-phenylenediamine) is a non-conducting polymer, the thickness of its films is self-limited: as successive layers are formed onto previous ones, the electrical resistance increases and diffusion across existing layers is hindered. The increasing the monomer electrolysis time over 50 cycles do not improve the exclusion properties of the poly (o-phenylenediamine).

Before the glucose sensing measurements, the p(oPD)-GOx/PPY/PB/Pt electrode was polarized at an appropriate potential value for 5–10 min. The responses to glucose reached their steady state values within 20 s after glucose injection. Figure 11 shows calibration plots for p(oPD)-GOx/PPY/PB/Pt electrode for the freshly prepared biosensor and after storing in a phosphate buffer solution of $pH = 7.4$ for 30 hours, respectively.

The kinetic parameters, Michaelis Menten constant (K_M) and maximum response current (i_{max}) were obtained, respectively, from

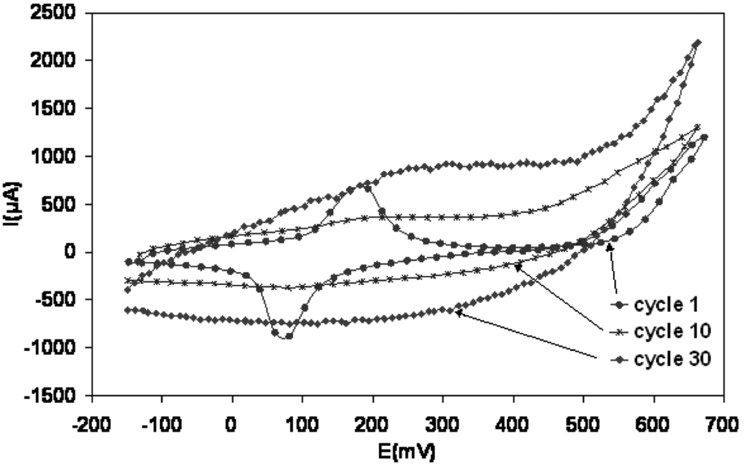


FIGURE 10 Cycling voltammograms corresponding polypyrrole film formation on the PB/Pt modified electrode from aqueous solution containing 0.05 M pyrrole and 0.1 M KCl.

the slope and intercept of the Lineweaver-Burk type-plot (1/I vs. 1/[S]) based on the equation:

$$1/i = 1/i_{\max} + 1/i_{\max} \cdot K_M/[S],$$

where i is the measured current and $[S]$ is the glucose concentration.

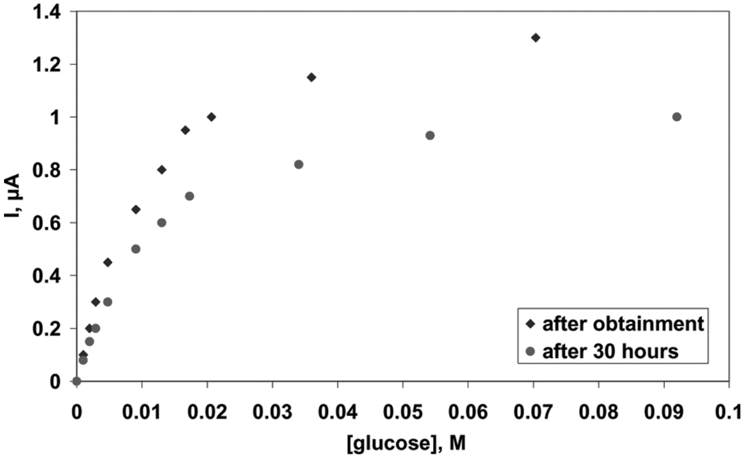


FIGURE 11 Calibration plot of the response current of biosensor with the glucose concentration after biosensor obtainment and after 30 hours keeping in a phosphate buffer solution of pH = 7.4.

The apparent Michaelis Menten constant was 19 mM after biosensor obtainment and 14 mM after 30 hours storing in a phosphate buffer solution of $pH = 7.4$.

CONCLUSIONS

The results indicate that the mechanism of the redox process for doped polypyrrole films is complex and may be governed by the diffusion of the anions and cations of the electrolyte.

In the case of platinum substrate the redox processes which take place during of the potential scan are much more active than those which take place on the modified electrodes with titanium substrate.

Comparative analysis of the impedance data have been shown that, the modified PPY(DS)/Ti electrode has a lower charge transfer resistance than PPY(Cl^-)/Ti modified electrode at the same potential.

The obtainment of PPY film by electropolymerization on aluminium substrate it is possible after remove the oxide barrier film (Al_2O_3) from the electrode surface. The formation process of PPY film and the porous film of Al_2O_3 takes place at the electrode surface simultaneously. The two films interact between them and form a composite material at the electrode surface.

The stability of this composite material increase with the number of cycles and with anodic polarization.

The electrochemical impedance spectra of polypyrrole films in aqueous media respond to the changes occurring during the insulator-conductor transition. These include charge carrier generation, conformational changes, a higher porosity and increased ionic transport capabilities.

The polymeric coatings obtained in sodium tartrate aqueous solutions at the zinc electrode surface are homogeneous, compact and strongly adherent to the working electrode surface. Generally, the PPY film formed at $15\,mA\,cm^{-2}$ current density is uniform, thick and its surface is characterized by a cauliflower-like structure consisting of microspherical grains with different sizes.

Electrochemical polymerization of aniline can be easily performed by galvanostatic method onto a PPY/Zn modified electrode. A bilayer system can be generated and it exhibits the usual PANI electrochemical response and is stable upon cycling in NaCl solution.

The anticorrosion properties of conductive polymers is due to the fact that such materials, when they are in the conductive states, are capable of displacing the electroactive interface from the metal/solution to the polymer/solution one.

A multilayer glucose biosensor was constructed by depositing an p(oPD)-GOx film on the previously formed PPy and PB films. Sensors based on p(oPD)-GOx film show a diminished sensitivity to ascorbate interference. The combination of Prussian Blue layer ensuring electrode operation at low potential values and of an interference – rejecting PPY layer enables to decrease drastically the influence of ascorbate interference.

The linear range, sensitivity and kinetic parameters of K_M and i_{max} of the biosensor were obtained from the experimental data and they are comparable with the values cited in literature.

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