A Novel Glucose Biosensor based on Poly(azulene-co-3-thiophene acetic acid) Conducting Films

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Catalytic properties of poly(azulene-co-3-thiophene acetic acid) films were tested as mediator-carrying enzyme for glucose detection. Poly(azulene-co-3-thiophene acetic acid) films were characterized using Cyclic Voltammetry (CV), Electrochemical Impedance Spectroscopy (EIS) and Scanning Electron Microscopy (SEM) methods. The cross-linking method for glucose oxidase (Gox) immobilization on the electrode surface was employed. The calibration curves for glucose amperometric detection were obtained in the range between 40-200μM. The detection limit was 27.65 μM.

Keywords: conducting polymers, glucose biosensor, poly(azulene-co-3-thiophene acetic acid), cyclic voltammetry, electrochemical impedance spectroscopy

The biosensor development based on immobilization of biological compounds such as proteins, enzymes, antibodies to reactive functional group of conducting polymer have attracted considerable attention due to their applications in areas like bioanalytical chemistry and clinical diagnosis [1-2]. Conducting polymers have been employed as tools in order to amplify signal response of the glucose oxidase-glucose interaction. Polyazulene is reported to exhibit good magnetic, optical and electrochemical properties and high conductivity (10^{-2} – 1 S/cm^{-1}) in its doped form [3-7]. Polythiophene derivates due to their high conductivity (about 10^{-2} S/cm^{-1}) in doped form, electrochemical stability, excelent processing possibilities and biocompatibility are attractive materials for practical devices such as sensors and bioelectronics (neural prothesis) [8-9]. The properties of conducting polymers could be modified over a wide range of conductivity, processability, stability and solubility by copolymerization [10-15]. Due to the fact AZ and 3-thiophene acetic acid (3-TAA) shows relatively close oxidation potentials the electrosynthesis of the copolymer can be accomplished. The resulted copolymer films contain functional carboxyl groups. Suitable functional groups, such as –COOH or –NH_2, are generally needed for biosensors development. Thiophene derivates containing such functional groups are difficult to electropolymerize due to the substantial nucleofilic character exhibited by the reactive group which attacks the radical cation (polaron) intermediates formed during electropolymerization [16]. A key point in obtaining a conducting film of 3TAA is to maintain a sufficient growth rate by using relatively high monomer concentration (above 0.2M) and positive enough polymerization potential (1.6 V) [17]. In turn, it has been proved earlier in [18] in the study concerning electrochemical copolymerization of 50 mM 3-thiophene acetic acid with 50 mM 3-methyl-thyophene that: (i) COOH group is incorporated into copolymer structure and it is not taking part to the electrochemical polymerization reaction; (ii) the copolymerization process is potential dependent, so that it is possible to obtain a copolymer film at a potential at which only one monomer species can be oxidized; (iii) the copolymerization mechanism involves the radical cation attacks on a neutral monomer in order to form the copolymer.

The immobilization of biological molecules to the conducting polymer can be performed using three methods: (i) entrappment of biomolecules in a conducting polymer film [19]; (ii) electrostatic binding of biomolecules (proteines) directly to specific charged groups generated on the polymer surface; (iii) covalent attachment of the biomolecules to a conducting polymer film containing reactive functional groups (–NH_2, –COOH). The latter method received much attention due to its advantages [prevents biomolecule denaturation, allows immobilization biomolecules in different experimental conditions (*p*H, ionic ionic strength), reduces the leaching of the captured biomolecules)] on the other two methods [20]. It is imperious needed that covalent linkage between biological compounds and the polymeric matrix to maintain its biomolecule activity, to increase its electrode stability and ensure biological event between analyte and immobilized biomolecule.

Nowadays the development of non-enzymatic glucose biosensor has been subject of extensive research comparing with enzymatic glucose sensors [21]. However, the superior selectivity and relative non-toxicity of enzymatic glucose sensors makes it the suitable the choice in glucose biosensor industry for blood sugar monitoring of people with diabetes.

In this study we report the development and characterization of new biosensors for glucose detection. The Au conventional electrode is modified by electrochemical deposition of copolymer film based on azulene and 3-thiophene acetic acid, followed by glucose oxidase immobilization via carboxyl groups of the copolymer. Up to our knowledge it is the first time when it is reported the use of poly(azulene-co-3-thiophene acetic acid) films as the housing matrix for the GOx immobilization.

Experimental part

Chemicals and materials

All chemicals were used without additional purification. Azulene (Fluka), 3-thiophene acetic acid (Fluka), tetra-n-butylammonium hexaflorophosphate (**$TBAPF_6$**) (Fluka)

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and acetonitrile (Sigma-Aldrich) were used as received for film depositions. Glucose oxidase from *Aspergillus niger* (Sigma) 200 U/mg, N-(3-dimethyl aminopropyl)-N'-ethyl carbodiimide hydrocloride (EDC) (Fluka), N-Hydroxysuccinimide (NHS)(Fluka) were used for enzyme coupling.

Electrochemical measurements

Cyclic voltametry (CV) and electrochemical impedance spectroscopy (EIS) experiments were performed with an Autolab PGSTAT 302N (Ecochemie) containing a FRA module, with a three-electrode cell, at room temperature. Gold disk electrodes (Metrohm) with a 2-mm diameter were used as working electrodes, while a platinum spiral wire was used as a counter electrode. As reference it was employed a wire of Ag/AgCl electrode. Before using the Pt spiral wire electrode was annealed in a Bunsen flame for the removal of organic residues. EIS experiments were carried out by scanning in the 100 KHz to 100 mHz frequency range with applied AC signal amplitude of 10 mV. The impedance spectra were analyzed using ZView software (Scribner Associates Inc.) All the electrochemical measurements were performed inside a Faraday cage.

The gold working electrodes were successive mechanically polished with 1, 0.3, 0.05 μm alumina powders and subsequently electrochemically cleaned in 0.1 M H_2SO_4 by cycling the potential between 0 V and 1 V (vs. Ag/AgCl) at 0.05 Vs^{-1}.

Films depositions procedure

The copolymerization of 5 mM azulene(AZ) and 5 mM 3-thiophene acetic acid (3TAA) was performed in an acetonitrile solution containing 0.1 M $TBAPF_6$ as support electrolyte. The copolymer films of poly(AZ-co-3TAA) were deposited on Au electrodes by sweeping the working electrode potential both in the potential range from –0.6 to 1.2 V (denoted as narrow copolymer film **NCF**) and –0.6 to 1.8 V (called as wide copolymer film **WCF**), at a 0.05 V/s scan rate at room temperature for 5 consecutive potential cycles. The thickness of the coating was established by integration of the charge passed through cell during electrochemical copolymerization.

Morphological analysis

The morphology of poly(AZ-co-3TAA) films deposited onto a Pt plate was evaluated by scanning electron microscopy using a Quanta™ 3D FEG (Fei Company) equipment.

GOx biosensor assembling

The Au/poly(AZ-co-3TAA) modified electrodes were rinsed with acetonitrile and then with bidistilated water. After that each electrode was placed for 1 h in a solution of 0.1M PBS (phosphate buffer with 0.9%NaCl; *p*H=6.3) containing 0.015M EDC and 0.03M NHS in order to activate the COOH functional group. Further, the activated copolymer modified electrodes were rinsed with PBS and they were immersed in a solution of PBS containing 400 U/mL GOx for 1 h. The Au/poly(AZ-co-3TAA)/GOx electrodes were stored in 0.1M PBS at 4°C. The Au/poly(AZ-co-3TAA)/Gox electrode was used for glucose detection. Prior to use, the 0.25M glucose stock solution was allowed to mutarotate in order to be reached a thermodynamic equilibrium between α- β- and γ- glucose isomers. So that, the glucose stock solution was kept at 4°C for 24 h before use.

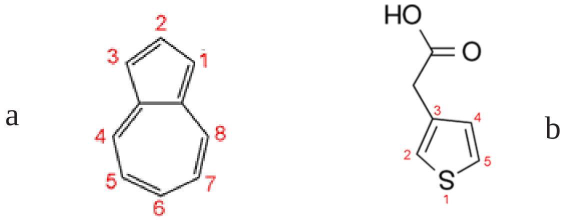

Fig. 1 The molecular structure of the monomers a) azulene (AZ) and b) 3-thiophene acetic acid (3TAA)

Results and discussions

Electrochemical copolymerization

The chemical structure of the starting monomers is shown in figure 1.

Polyazulene and poly(3-thiophene acetic acid) electro deposition onto Au electrodes was investigated as a parallel study in two potential ranges, one between –0.6 to 1.2 V and the other one between –0.6 to 1.8 V, both at 0.05 V/s scan rate, for 5 consecutive potential scans. The conducting coating obtained in the former potential domain is denoted as NCF (copolymer film obtained in narrow potential range), and the one achieved in the latter potential range is labeled WCF (copolymer film obtained in wide potential range). Copolymer films with distinct structures could be obtained by switching the potential over two different ranges.

CV during separate polymerization of 5 mM AZ and 5 mM 3TAA onto Au electrodes were performed (data not shown). The oxidation of the 3-thiophene acetic acid single monomer onto Au substrate occurs at 1.07V and 0.94V vs. Ag/AgCl for narrow potential range and wide potential range, respectively. In the case of azulene polymerization, the oxidation of the monomer takes place at 0.90V and 0.89V vs. Ag/AgCl for narrow potential range and wide potential range, respectively. These oxidation potentials are in good accordance with the data previously reported onto Pt substrate [17, 22-24].

According to [4], the highest spin density occurs at 1- and 3- positions in the case of azulene structure and 2- and 5- positions for thiophene structure, respectively. So, these are the positions of the monomers where the random copolymerization most likely occurs (fig. 1).

In figure 2, in the first scan the oxidation peak was sharp and it appeared at 0.86 V and 0.88V for NCF and WCF, respectively. The peak corresponds mainly to the oxidation of azulene monomers to an unstable radical cation which undergoes to dimerization reaction and subsequently the film is electrodeposited on electrode surface. The oxidation of the 3-thiophene acetic acid monomer onto Au substrate occurs at 1.07V vs. Ag/AgCl for NCF, at lower current density (aprox 2 magnitude orders) than that corresponding to the oxidation of azulene monomers. Thus, the oxidation peak appeared in the first anodic scan could be assign to the contribution of azulene and 3-thiophene acetic acid monomers. By sweeping the potential to cathodic direction, two peaks appeared at 0.32V/-0.18V for NCF and 0.36 V/ -0.10V vs. Ag/AgCl for WCF, respectively. In the second scan there are two oxidation peaks at 0.43 V /0.79 V (NCF) and 0.44 V /0.80 V vs. Ag/AgCl (WCF), respectively. The first one is associated with the oxidation of the copolymer formed in the first potential cycle and the last is attributed to the contribution of oxidation of the azulene and 3-thiophene acetic acid monomers. During the subsequent potential scans, the peak potentials corresponding both to the copolymer and monomers oxidation does not change significantly. The constant increasing of the anodic and cathodic peak currents with successive scans is consisted with the growth of an

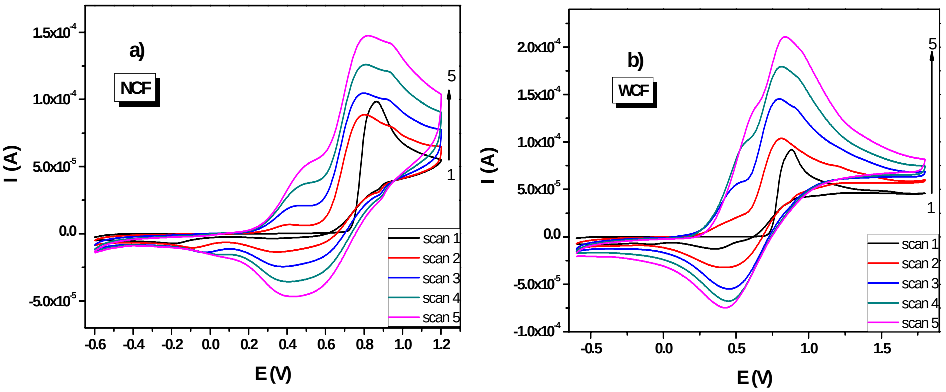

Fig.2. Cyclic voltammograms of azulene and 3-thiophene acetic acid copolymerization onto Au-disc electrode at 50 mV/s when potential range was scaned **a)** between -0.6 and 1.2V and **b)** between -0.6 and 1.8 V vs. Ag/AgCl, respectively.

adherent, conducting and insoluble copolymer film onto Au electrode.

The thickness of the copolymer film was controlled by the potential cycle number applied in the electrochemical polymerization process and it was calculated from the integrated charge densities involved in the reaction process, using the following equation:

$$g = \frac{q_{copol} M_{monomers}}{nFA_{Au}\rho}$$

where q is the total charge consumed during copolymerization, $M_{monomers}$ is the molar mass of azulene and respectively 3-thiophene acetic acid monomers, n represents the number of electrons taking part in oxidation of one monomer unit, F the Faraday's constant ρ, copolymer films with distinct structures could be obtained. is the film density approximated to 1 g/cm^3 and A_{Au} the area of the Au disk electrode surface coated with copolymer film, expressed in square centimeters. For a 2-mm Au disk electrode the surface area was calculated to 0.0314 cm^2. In the calculation of copolymer thickness we considered total current efficiency for monomers deposition, not taking into consideration the charge consumed by soluble oligomers. The charge consumed in copolymerization reaction was 0.192 C/cm^2 (for NCF) and 0.454 C/cm^2 (in the case of WCF). In addition, the thickness of the copolymer films were computed from CV recorded in copolymerization process and it exhibited 5.37 μm and 12.74 μm, for NCF and WCF, respectively.

Welzel et al. [18] demonstrate that the radical cation (polaron) or dication (bipolaron) of 3-methyl thiophene attacks the thiophene ring of 3TAA and forms the copolymer when the applied potentials exceed the oxidation potential of 3-methyl thiophene. By copolymerization of azulene and 3TAA occures the azulenium radical cations (polarons) or bications (bipolaron) attacks on the 2- and 5- position of thiophene 3-acetic acid. The contribution of the AZ in the copolymer film is expected to be major in the case of NCF due to the fact that the smallest oxidation potential was exhibited in the case of AZ monomer. In the case of WCF, due to the extended applied potential, the 3TAA content in the copolymer structure is expected to increase significantly compared with NCF.

Electrochemical characterization of poly(AZ-co-3TAA) films

The redox properties of the Au/poly(Az-co-3TAA) films were studied in the transfer solution of 0.1M TBAPF$_6$ in ACN. The cyclic voltammograms were recorded during electrochemically oxidation (p-doping) of copolymer films. The degree of doping y (charge per mole of monomers unit) and the surface concentration of the electroactive sites Γ were calculated [25-26] according to the following equations $y = \frac{2Q_a}{(q_{copol} - Q_a)}$; $\Gamma = \frac{Q_a}{yFA_{Au}}$ where Q$_a$ is the anodic charge of the copolymer film in the p-doping processes and the other symbols are defined previous in the paper having their usual electrochemical meaning. The Q charge consumed in the redox doping processes is 88.53 10^{-3} C/cm^2 and 146.17 10^{-3} C/cm^2 for NCF and WCF, respectively The doping level calculated in the case of NCF (1.73) is higher than the one of WCF (0.95). These values of y demonstrated that poly(AZ-co-3TAA) synthesized in narrow potential range is more conducting comparing with WCF.

In CV figures (fig. 3a and 3b) the three consecutive scans are shown for the potential window -0.4 V to 0.9V, where the two films are electroactive. Both films proved very good electrochemical activity and reversibility during the doping-undoping process. In the case of NCF, the broad anodic and cathodic peaks could be associated with the variety of redox–active anchored sites and their interaction within the copolymer layer [18]. In fact, the calculated concentration of electroactive sites in the film,Γ, is higher for WCF (1.60 x 10^{-5} mol cm^{-2}) than NCF (5.33 x 10^{-6} mol

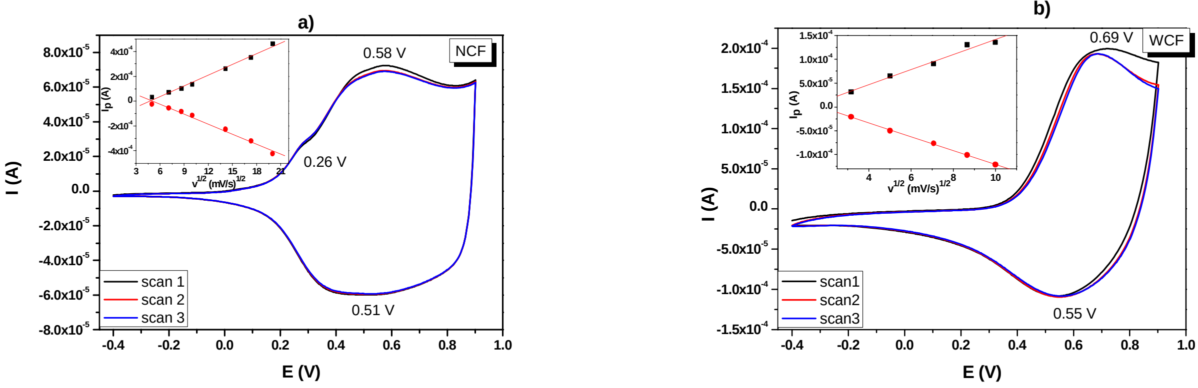

Fig. 3. Cyclic voltammograms of copolymer films a) NCF and b) WCF onto Au electrode recorded in a 0.1M TBAPF$_6$/ACN monomer free solution at 50 $mV \cdot s^{-1}$. Inset in panel a) and b): Dependence of peak current on the square root of scan rate for a) NCF b) WCF in a 0.1 M TBAP acetonitrile solution, recorded in the potential window -0.4 – 0.9 V. The scan rates increase from a) 25, 75, 50, 100, 200, 300, 400 mV/s and b) 10, 25, 75, 50, 100 mV/s.

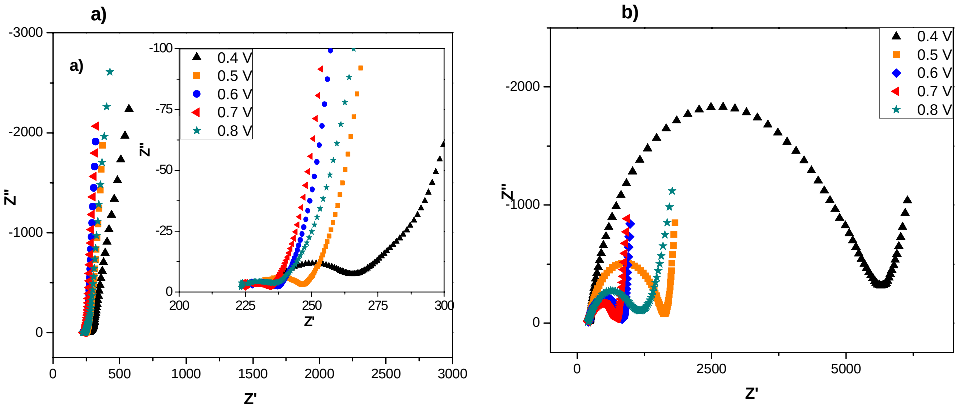

Fig. 4. Nyquist plots for the two copolymer films **a) NCF** and **b) WCF**, deposited on Au (2 mm) disc electrode in 0.1M TBAPF$_6$ / acetonitrile solution, at various applied potentials 0.4, 0.5, 0.6, 0.7, 0.8 V vs Ag/AgCl. Frequency was in the range of 100 kHz to 100 mHz. Amplitude of alternative voltage was 10 mV.. *Inset* Nyquist plots in high frequency range

cm^{-2}). The shape of the consecutive voltammograms recorded for both copolymers did not change substantially during potential scans showing a stable copolymer film formation. An anodic peak at 0.26 V appears as a shoulder incorporated in a broad peak at about 0.58 V vs. AgAgCl in the case of NCF films, this could be associated with the coexistence of two different macromolecular species [18, 27].

When the thickness of the copolymer film is increased (from 5.37 μm to 12.74 μm) the onset of poly(AZ-co-A3TAA) oxidation peak shifts 0.2V in positive direction. This means that the oxidation of the film occurs not only at the electrode surface but also in the bulk of the copolymer film [17-18, 28].

During the scan rate changes from 10 to 400 mV . s^{-1} the anodic and cathodic peaks current increased with the increasing of scan rate. This confirms the good adherence of the electroactive copolymers to electrode surface. In the insets of figure 3 the linear dependences of the peaks currents vs. square roots of the scan rate emerged for both copolymer films, indicating diffusion controlled processes. The difusion coeficients provided by Randles-Sevcik Equation [29] involving the transfer of one electron for both NCF and WCF are 1.38·10^{-5} cm^{2}s^{-1} and 2.29·10^{-5} cm^{2}s^{-1}, respectively. These values indicated that PF_6^- diffuses in and out the copolymer film during doping/dedoping processes, faster for the WCF than for the NCF.

The electrochemical behaviour of the investigated coatings is strongly affected by the thickness and history of the copolymer films. The changes in the morphology of copolymer conducting films containing AZ and 3TAA could be associated with increasing of film thickness and subsequently to different conjugation length. In the case of NCF the effective conjugation length is extended due to the relatively long copolymer chain, the surface is homogenous and the incorporation of anions of the supporting electrolyte into the coating is slower. In the case of WCF the efective conjugation length is a mixture of long and short chain, the surface is homogenous and porous and the incorporation of into the coating is faster. According to literature [30] a similar type of behaviour is reported for film of different thicknesses.

Electrochemical impedance spectroscopy (EIS) of poly(AZ-co-3TAA) films

The impedance data of the investigated copolymer coatings were obtained in a monomer free solution at various dc applied potentials ranging between -0.5V and 1.4V(vs. Ag/AgCl), covering the domain in which the film is transformed from insulating to conducting and after that to overoxidizied state.

In the potential range between -0.5 and 0.3 V vs. Ag/AgCl the copolymer film obtained on both potential domains is an electronic insulator. In this region a purely resistive behaviour of the films is observed and no ionic transfer occurs.

In the potential range between 0.4V and 0.9V vs. Ag/AgCl, both films (NCF and WCF) are in conducting state (fig. 4). In this region the copolymer film undergoes a transition to the best conducting state. The increase in the conductivity of NCF may be attributed to the increase of the effective conjugation length. The extension of effective conjugation length facilitates the easy flow of charge between coating and Au electrode surface. The best conducting potential was found to be 0.7 V for both NCF and WCF proved by the fact that the coatings exhibit the smallest diameter of semicircle in Nyquist plot, and in consequence the low charge transfer resistance. The R$_{ct}$ values for WCF are higher than the values obtained for NCF. The Rct high value obtained for WCF compared with NCF is due to the increasing of the thickness of the film.

In the potential range between 1V and 1.4V vs. Ag/AgCl, the copolymer films are overoxidized and became electrochemically inactive [17]. In this region a purely resistive behaviour of the coatings is displayed and no ionic transfer is evidenced.

Several equivalent circuits were used for model electrode-conducting polymer-electrolyte interfaces. The best model that described the electrode-poly(Az-co-3TAA) coating-electrolyte interface is illustrated in the figure 5. The chi-squared (χ^2) obtained from the experimental data and fitting response was within the range 10^{-4} - 10^{-3}.

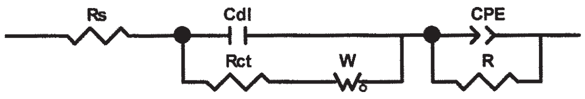

Fig.5 Equivalent electrical circuit used for EIS simulation.

The electrochemical parameters of copolymer films were evaluated using ZView software. In Nyquist plots three zones were evidenced: the semi-circle appeared at high frequencies is related to the charge transfer resistance at the copolymer/solution interface (Rct), the Warburg impedance, due to the diffusion of supporting electrolyte counter ions from solution to copolymer film during charge discharge process and a linear capacitive behavior related to the doping mechanism at low frequencies. The equivalent circuit was built by series components: first is the bulk solution resistance (Rs); the second component is a parallel combination between the double layer capacitance (Cdl) and a series consisting in charge transfer resistance of the copolymer film (Rct) and open Warburg element (W$_0$); the third series is a parallel connection between a constant phase element (CPE) and a charge transfer resistor (R) both elements associated with the electrode/coating interface.

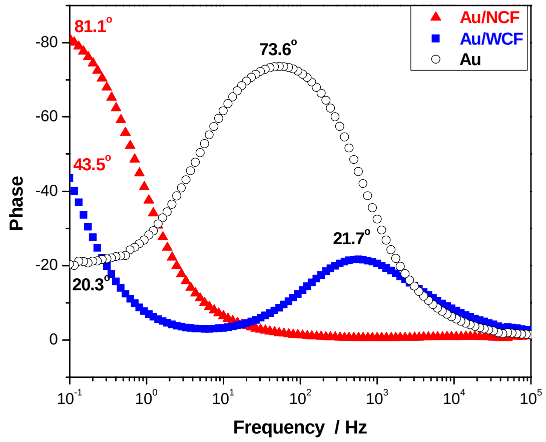

Fig. 6. Bode phase angle diagram for Au naked electrode and of Au/poly(AZ-co-3TAA) obtained both in narrow potential range (NCF) and wide potential range (WPR) at 0.7V vs Ag/agCl in monomer free solution containing 0.1M $TBAPF_6$ in ACN. Spectra were obtained between 0.1 Hz and 100 KHz. Amplitude of the alternative voltage was 10 mV.

Table 1
PARAMETERS OBTAINED FOR THE Au/POLY(AZ-co-3TAA) FILM AS FUNCTION OF THE APPLIED POTENTIAL IN THE POTENTIAL REGION WHERE THE FILM IS IN CONDUCTING STATE.

Abbreviation of copolymer films	E_{appl} (V)	R_{sol}(Ω)	R_{ct} (Ω)	C_{dl} (F)	σ (S/cm)
NCF	0.4	237	22.99	$5.67\cdot10^{-7}$	$7.44\cdot10^{-3}$
	0.5	230.8	11.45	$8.17\cdot10^{-7}$	$1.49\cdot10^{-2}$
	0.6	227	8.622	$9.78\cdot10^{-7}$	$1.98\cdot10^{-2}$
	0.7	224.4	7.957	$1.23\cdot10^{-6}$	$2.15\cdot10^{-2}$
	0.8	223.8	9.61	$1.43\cdot10^{-6}$	$1.78\cdot10^{-2}$
WCF	0.4	230.9	4697	$1.23\cdot10^{-6}$	$8.64\cdot10^{-5}$
	0.5	219.3	1351	$1.92\cdot10^{-6}$	$3.00\cdot10^{-4}$
	0.6	209.6	612.7	$3.30\cdot10^{-6}$	$6.62\cdot10^{-4}$
	0.7	204.4	548.9	$9.93\cdot10^{-6}$	$7.39\cdot10^{-4}$
	0.8	206.2	978.4	$1.52\cdot10^{-5}$	$4.15\cdot10^{-4}$

Bode phase angle plots (fig 6) exhibited phase angle changes with frequency for the Au, Au/NCF and Au/WCF electrodes. Bode plots showed changes in the electronics of Au, Au/NCF and Au/WCF electrodes. In the medium frequency domain, the maximum phase angle was noticed for Au naked electrode at -73.6°, while Au/WCF exhibited -21.7° phase. This observation points out the transition from semi-metallic behaviour for Au to semiconductor one for WCF in the mentioned frequency range. In the low frequency domain (0.1 Hz), in the region where electronic of the electrode system is minimally perturbed, the Au/NCF exhibited semi-metallic behaviour (phase angle -81.1°) while Au/WCF (phase angle -43.5°) and Au electrode (phase angle -20.3°) presented semiconductor behaviour. In Bode-phase plots of poly(AZ-co-3TAA) the maximum phase angle of -81° was achieved for NCF at 0.1 Hz frequency. These values are in good agreement with literature data for similar copolymer systems [31].

The charge transfer conductivity of the copolymer films (σ) was estimated from impedance data using $\sigma = \frac{g}{R_{ct}A_{Au}}$ [32-33], where the symbols are previously defined in the paper with their usual electrochemical meaning. The charge transfer conductivity for copolymer films with different doping levels is showed in table 1. The values of charge transfer conductivity for NCF (2.15 10^{-2} S/cm) and WCF (7.39 10^{-4} S/cm) at the optimum potential applied for doping processes 0.7V were compared. The best conductivity was found to be for NCFs. These findings supports the data obtained in electrochemical characterization of the film on both potential investigated domains.

Obtaining of conducting polymer films containing electroactive azulene and 3-thiophene acetic acid in the copolymer skeleton was also confirmed by the conductivity study. According to literature [34-35] the conductivity of copolymer film is found to increase with the decrease of 3TAA units. This means that the content of 3TAA units is higher in the case of the poly(AZ-co-3TAA) film synthesized in wide potential domain comparing with NCF; it is also worth noting that both copolymer films investigated exhibiting good redox behaviour when subject to a doping process.

Morphological characterization of the copolymer matrices

The morphology of the copolymer films deposited onto Pt plate was evidenced by scanning electron microscopy (SEM). SEM images of the two modified electrodes CFN and CFW respectively, were investigated.

The morphological aspects (fig.7) of the azulene and 3-thiophene acetic acid copolymer films are different, depending on the potential domain used for electro-deposition. Both copolymer films showed caulifower-like structure. The NCF (fig. 7a) revealed to include small islands consisting of globular cauliflower-like units similar to those observed for P3TAA [36], while WCF (fig. 7a) exhibits a granular structure. The granular structure of the WCF was developed by increasing potential range so that, the surface area of the copolymer film increased corresponding to the growth of the coating. According to SEM images the NCF is more compact on the electrode surface in comparison with WCF, which has rather a rough structure. NCF structure possesses a better mechanical strength comparing with WCF, so that SEM results corroborate with cyclic voltammetry data recorded during redox characterizations. WCF exhibits better homogenous morphology than that of NCF [16, 37].

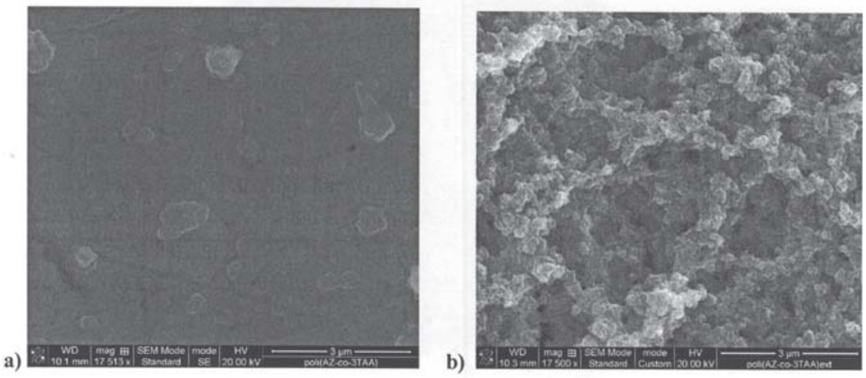

Fig.7. SEM images of poly(AZ-co-3TAA) films: a) NCF; b) WCF. Potentiodynamic coatings were obtained from 5 mM AZ and 5 mM A3TAA in 0.1M $TBAPF_6$/ACN, during 5 consecutive cycles at a scan rate of $0.05Vs^{-1}$

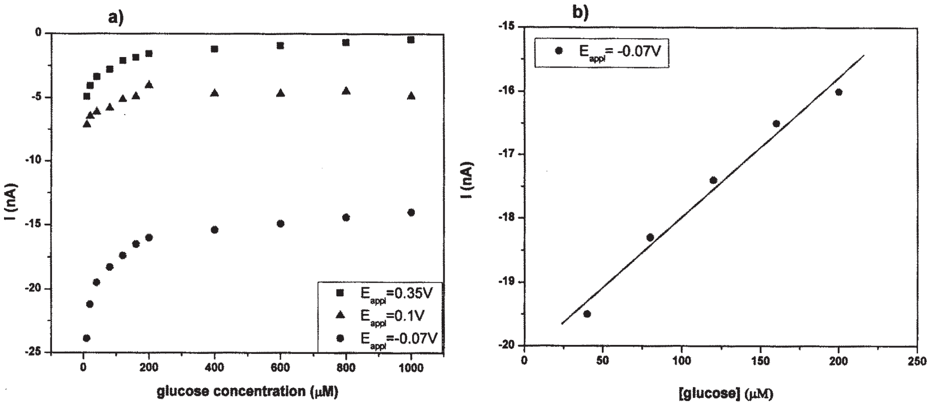

Fig. 8. a) Typical amperometric response of GOx-immobilized flms of poly (AZ-co-3TAA)to glucose in 0.1 M PBS (pH=6.3). The applied potential was: 0.35 V, 0.1 V and -0.07V. b) Calibration curve for the amperometric response of the enzime modified electrode at -0.07V applied potential, in the concentration range 40-200 μM.

Amperometric detection of glucose with Au/poly(AZ-co-3TAA)/Gox

GOx was immobilized covalently through carboxyl group of the copolymer films. The amperometric response towards glucose detection of Au/WCF/Gox at various applied potential (0.35, 0.10 and -0.07 V) is exhibited in fig. 8. Optimal conditions for the enzyme immobilization were investigated and the biosensor was tested for glucose sensing within the concentration range of 10-1000 μM D-glucose. A linear response of the modified enzyme electrode to glucose in the range 40-200 μM was obtained. The best response was achieved when the applied potential was -0.07V vs. Ag/AgCl and a sensitivity of 0.7 nA cm^{-1} μM^{-1} was obtained. The detection limit was found to be 27.65 μM. The response of the Au/WCF/Gox biosensor to such cathodic potential is a great achievement due to the fact that the effect of interference such as ascorbic acid, uric acid, dopamine etc is avoided. In literature the enzymatic biosensors containing conducting polymer as housing matrix for glucose detection are reported at more positive applied potentials, generally in the domain 0.2-0.65 V [38-40]. Details of the matter regarding interference effects and obtaining a better sensitivity are still under investigation.

Conclusions

The formation and properties of novel redox–active films based on azulene and 3-thiophene acetic acid were investigated. The obtained electroactive coatings exhibited good doping-undoping redox behaviour. The electrocatalytic properties of Au/poly(Az-co-3TAA)/Gox towards glucose detection were demonstrated. The obtained electrochemical sensors showed a linear response to glucose over concentrations range from 40 to 200 μM with a sensitivity of 0.7 nA $cm^{-1}\mu M^{-1}$ at the –0.07 V vs. Ag/AgCl applied potential. The detection limit was 27.65 μM.

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