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Melanin as an active layer in biosensors

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The development of pH sensors is of great interest due to its extensive application in several areas such as industrial processes, biochemistry and particularly medical diagnostics. In this study, the pH sensing properties of an extended gate field effect transistor (EGFET) based on melanin thin films as active layer are investigated and the physical mechanisms related to the device operation are discussed. Thin films were produced from different melanin precursors on indium tin oxide (ITO) and gold substrates and were investigated by Atomic Force Microscopy and Electrochemical Impedance Spectroscopy. Experiments were performed in the pH range from 2 to 12. EGFETs with melanin deposited on ITO and on gold substrates showed sensitivities ranging from 31.3 mV/pH to 48.9 mV/pH, depending on the melanin precursor and the substrate used. The pH detection is associated with specific binding sites in its structure, hydroxyl groups and quinone imine. [http://dx.doi.org/10.1063/1.4869638]

I. INTRODUCTION

Extended gate field effect transistors (EGFET) have been employed instead of traditional ion-sensitive field-effect transistor (ISFET) in pH and ion concentrations measurement in the last few years, due its flexible structure, in which a sensitive layer is deposited on an extended gate that isolates the FET from the chemical environment, promoting EGFETs a better long-term stability.[1–6] pH sensitive biosensors are extensively studied, since it has many applications, especially in the monitoring of biological systems such as blood, but also for chemical analysis and environment monitoring.[7–11]

There are many options of materials that can be used as ion sensitive layers. However there is a continuous search for layers with higher sensitivities, specially using materials like biomolecules that could present sensitivities comparable to inorganic sensors.[1,6,9] Recent studies shows that these sensors can have high sensitivities depending on the material used, like zinc oxide based sensors have sensitivity of 38 mV/pH,[1] tin oxide 56–58 mV/pH,[12] and indium tin oxide 55 mV/pH.[3] In the case of organic semiconductors, which are in general less used, nanostructured polyaniline and poly(vinylsulfonic acid) have a sensitivity of 58 mV/pH,[13] carbon nanotubes with 50.9 mV/pH,[5] and hibrid materials like vanadium oxide/hexadecylamine, 38.1 mV/pH.[14]

The Site-Binding Model can describe the working mechanism of EGFETs. In this model, specific binding-sites, can donate or accept protons from the solution, making the material surface

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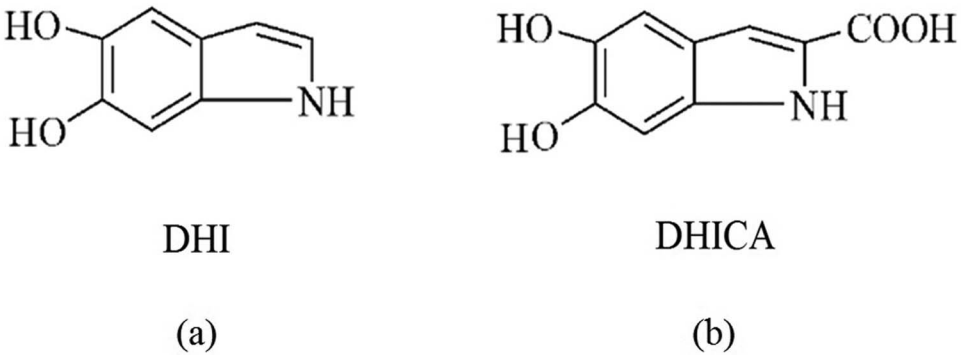

FIG. 1. Structures of Melanin main component monomers: a) 5,6 dihydroxyindol-quinone (DHI) and b) 5,6-dihydroxyindole-2-carboxylic acid (DHICA).

positively or negatively charged.[15,16] The potential (Ψ) at the interface electrolyte/active layer can be obtained from equation (1), and depends on the material of the active layer and the pH of the electrolyte:[1]

$$2,303(pH_{pzc} - pH) = \frac{q\psi}{kT} + \sinh^{-1}\left(\frac{q\psi}{kT}\frac{1}{\beta}\right) \quad (1)$$

where pH_{pzc} is the pH value at the isoelectric point, q is the elementary charge, k is the Boltzmann constant, T is the temperature and β is a parameter that reflects the sensitivity of the active layer material, and depends on the surface binding-sites density hydroxyl groups for example. The value of β can be determined from equation (2):[1]

$$\beta = \frac{2q^2 N_S (K_b/K_a)^{1/2}}{kTC_{DL}} \quad (2)$$

where N_s is the number of binding sites per unit area, K_a and K_b are constants for acid and basic dissociation, respectively, and the double layer capacitance C_{DL} is derived from the Gouy-Chapman-Stern model.[16] From equation (2) it is clear that the sensitivity depends linearly on the number of binding sites. Moreover, the larger the capacitance of the system, the lower the sensitivity.[17]

A promising biomacromolecule semiconductor for application as pH sensor is melanin. Melanins are a class of pigmentary conjugated biomacromolecule found in many biological systems, and also obtained by synthetic procedures.[18,19] Melanins do not have broad molecular regularity, and it is believed that its structure is mainly composed of macromolecules of disordered arrangements of 5,6 dihydroxyindol-quinone (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) monomers (Figure 1).[18,20–22]

The structural features of melanins make them interesting candidates for application in EGFETs, due to the large number of potential binding sites for H^+ ions; the two hydroxyl groups of the aromatic ring, the quinone imine and the hydroxyl group of carboxylic acid in the case of DHICA.[23]

Melanin films shows semiconductor behavior dependent on water content.[24] More importantly for the current application is that it is an electronic and ionic conductor.[25] In this context, our interest is to investigate the ability of melanin to act as sensitive layer in EGFETs, studying the influence of different parameters on the sensitivity of these sensors and investigate the physical mechanisms related to the operation of melanin-based EGFET biosensors.

II. MATERIAL AND METHODS

Melanin was synthesized by the oxidation of L-3-(3,4-dihydroxyphenyl)-alanine (L-Dopa) in aqueous medium. The synthesis procedure was described elsewhere.[19] The melanin powder was used to prepare three different solutions, respectively adding 700 mg (solution I),[26] 500 mg (solution II) and 300 mg (solution III) of melanin to a mixture of 5 ml of deionized water and 10 ml of

concentrated ammonium hydroxide (28%). The solutions were stirred at room temperature for 1 h and then ultrasonicated for a further hour. Subsequent centrifugations were performed for 15 min at 3500 rpm in order to remove any residual particles. The supernatants were utilized as stock solutions to produce the melanin thin films.

Thin films of melanin were produced from the three different solutions (I, II and III), deposited by spin coating (2000 rpm, 1 min) and referred as films A, B and C, respectively. Different substrates with a surface area of 1.0 cm × 2.5 cm were used: indium tin oxide coated glass (ITO – which presents a sheet resistivity of 70–100 Ω/square and 680 nm thickness – from Sigma Aldrich) and gold sputtered coated glass (100 nm thickness and resistivity of 3 Ω/square).

The thickness and homogeneity of the melanin films was analyzed through measures of Scanning Electron Microscopy (SEM) using a JEOL 6510 LV microscope. The surface morphology of the melanin films was investigated by Atomic Force Microscopy (AFM) using a SPM-9600 Scanning Probe Microscope from Shimadzu working in tapping mode. The microscope was equipped with standard silicon probes with 124 μm long cantilevers tip shaped like a polygon based pyramid with a height of 10–15 μm and typical curvature radius of less than 10 nm. The nominal spring constant of the cantilever used ranged from 34 to 51 N/m. Height and deflection signals were recorded simultaneously. All experiments were carried out in air.

Electrochemical Impedance Spectroscopy (EIS) was used to investigate the electrochemical properties of different melanin thin films. Two identical melanin films on ITO substrate were sealed parallel to each other using a thermoplastic spacer (EAF-230, Adhesive Films, Inc. USA) with a 35μm thickness, yielding symmetric 0,25 cm^2 measurement cells. Through a back filling vacuum system, the as prepared measurement cells were filled with different pH buffer solutions. Impedance measurements were carried out in the frequencies range of 100 Hz to 100 kHz, coupling a 7265 DPS Lock-In amplifier (Signal Recovery) with a 33220A power supplier (Agilent). All data were collected and analyzed using an Agilent VEE program, in order to determinate the characteristic electrochemical parameters. The obtained Nyquist plots were analyzed and the capacitance of the films were determined using an equivalent circuit model where a resistance (Re) is in series with a constant phase element (CPE) which is used instead of an ideal capacitor, in order to consider roughness and imperfections of the electrode surface.[27,28]

EGFET devices were produced by using melanin thin films (A, B and C) as the active layer attached to the gate of a commercial CD4007UB MOSFET. Melanin sensitive layers were dipped into phosphate/citrate 0.1 mol/L buffer solutions (pH range from 2 to 12), in conjunction with a silver/silver chloride (Ag^0/AgCl) reference electrode, at room temperature. The influence of total gate-source voltage (V_{GS}) and total drain-source voltage (V_{DS}) on the final drain-source current (I_{DS}) was studied as a function of pH, and the sensor responses were obtained by a computer controlled Agilent 34970A parameter analyzer.

III. RESULTS

Figure 2 shows the cross section image of a typical film of melanin using SEM. The thicknesses of the films deposited from solutions I, II and III, as determined from SEM images were respectively 400 nm, 267 nm and 233 nm.

The analysis of melanin films morphology by AFM is presented on Figure 3, where it is possible to observe the different roughness in the thin films A, B and C. For an analysis area of 5 μm × 5 μm, films A, B and C had surface roughness of 0.467 nm, 0.560 nm and 0.530 nm, respectively.

Electrochemical Impedance Spectroscopy measurements (EIS) showed that all cells made using melanin films showed a capacitive-like behavior (although not an ideal capacitor). The impedance Z of the CPE element depending on the frequency ω can be obtained from Equation (3), where Q_0 is a constant related to the effective capacitance, j is the imaginary unit and n is a real number between 0 and 1. For all analyzed films n values were close to 1, indicating that Q_0 is basically the capacitance of the systems.[29]

$$Z = \frac{1}{Q_0 (j\omega)^n} \tag{3}$$

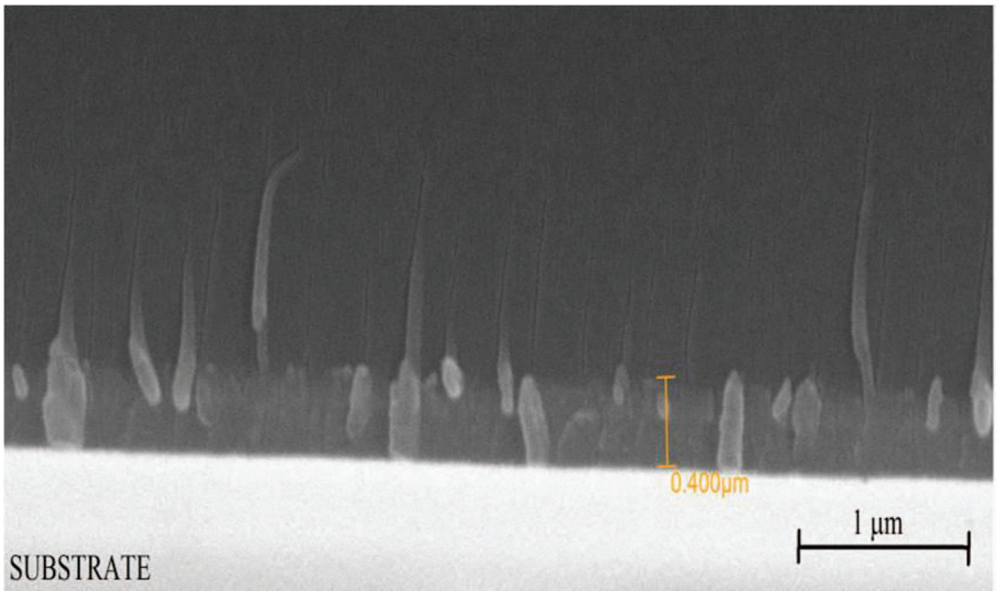

FIG. 2. SEM tilted image of melanin thin film deposited on glass from solution I.

Figure 4 shows the values of Q_0 obtained from the analysis of melanin films as a function of pH. It was not possible to measure the capacitance of the film C for pH 9 due to desorption from the substrate in such conditions.

From Figure 4 it is possible to observe that films A, B and C show similar behavior with the capacitance varying with pH. This behavior can be explained by changes in surface charge of the films according to the supply of H^+ ions in the solution, since the ions concentration influences the capacitance of the double layer.[16] Figure 4 also shows that film B have lower capacitance values than those from film A independent of pH.

Figures 5(a) and 5(b) present typical V_{DS} and V_{GS} responses, respectively, obtained for EGFETs devices with melanin thin film as active layer.

From Figure 5(a) it is possible to observe that the higher the concentration of H^+ ions in the electrolyte solution, the greater is the current in the sensor. This occurs because the EGFET devices detect the electric field created by H^+ ions, so the change in pH alters the potential between the electrolyte and thin film, modulating the intensity of the current in the MOSFET.[17]

The sensitivities of the sensors, presented in Figure 6, were obtained through fitting, following reference.[12] It can be seen that the reference electrode voltage depends linearly on pH, for I_{DS} fixed at 2 mA.

Table I presents the results of the sensitivities obtained for all sensors evaluated. In all cases, a linear response was observed across the range of pH evaluated. As can be seen the highest sensitivity was for film B. Comparing with the literature for other materials, the sensitivity found is quite satisfactory.[1,3,5,12–14] The sensitivities using gold substrates are always higher with respect to ITO. The main reason is probably due to its better conductivity.

IV. DISCUSSION

According to the Site-Binding Model and the Gouy-Chapman-Stern theory, the chemical reactions that occur on the surface of melanin films (M) can be described by equations (4) and (5), where MOH_2^+ represents the sites that received H^+ (positive sites), MOH neutral sites and MO^- the sites which have lost H^+ (negative sites).

$$MOH_2^+ \overset{K_{a1}}{\leftrightarrow} MOH + H^+ \tag{4}$$

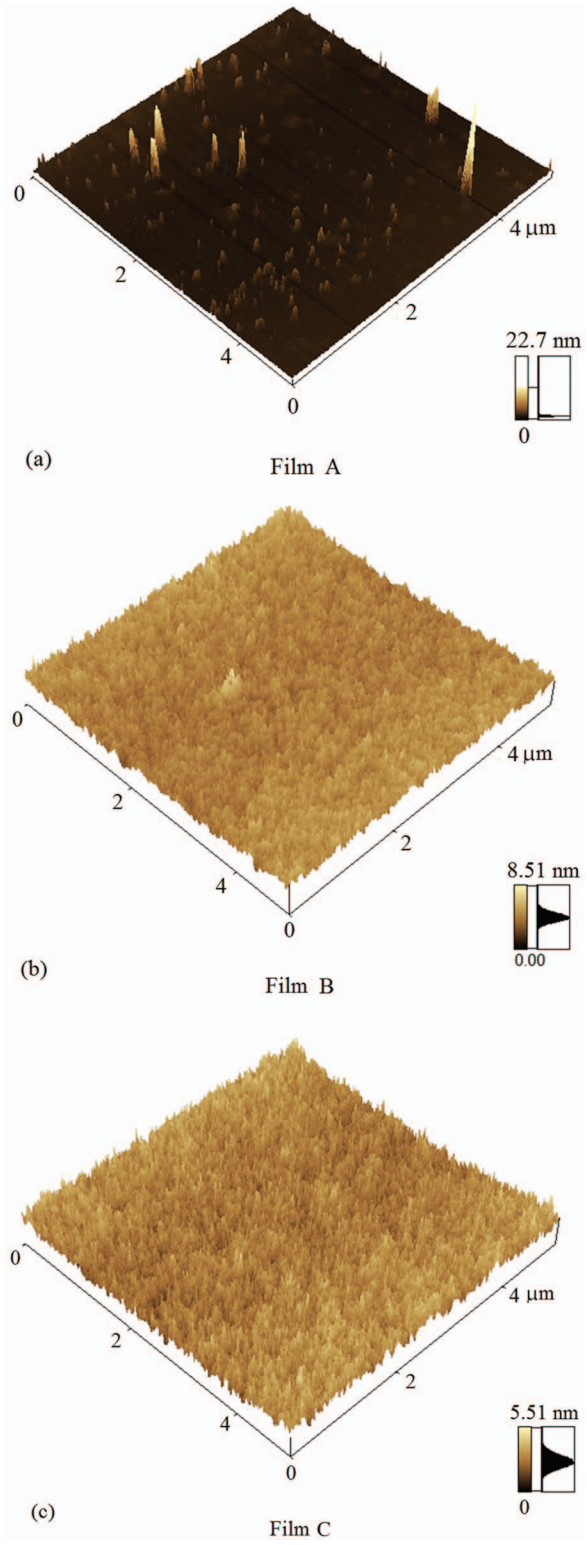

FIG. 3. Images of 5 μm $\times$ 5 μm obtained from Atomic Force Microscopy for the characterization of melanin thin films (a) A, (b) B and (c) C. The color code for depth is shown on the labels.

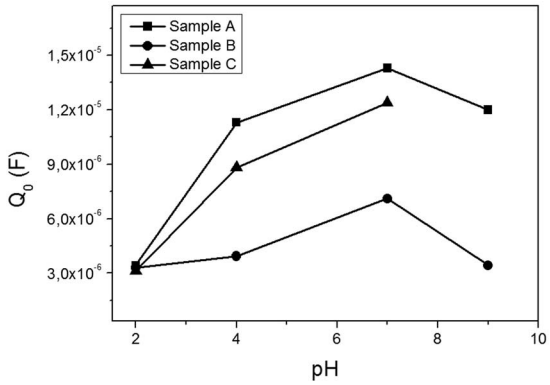

FIG. 4. Q_0 values obtained from melanin films as a function of pH.

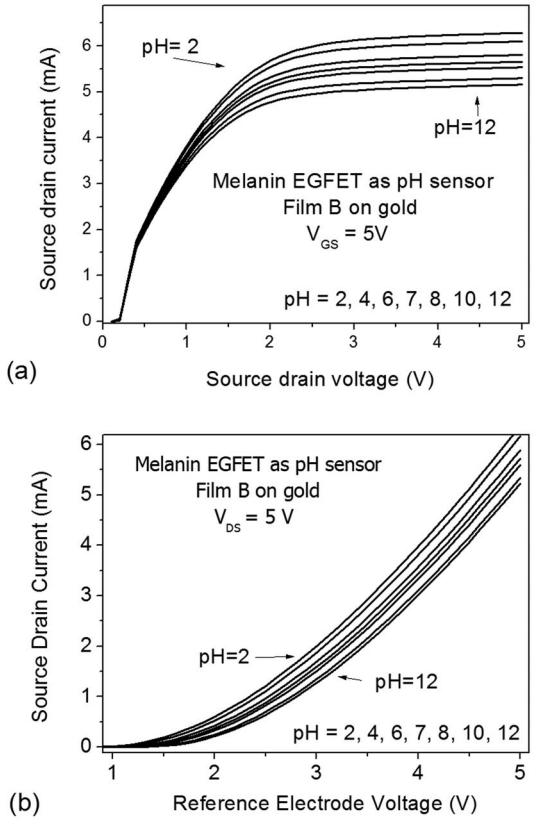

FIG. 5. Typical response of Melanin EGFET sensor in V_{DS} (a) and V_{GS} (b) measurement configurations when inserted in a solution with pH varying from 2 to 12.

$$MOH \overset{K_{a2}}{\leftrightarrow} MO^- + H^+ \tag{5}$$

The acid or basic behavior of neutral site MOH is characterized by dissociation constants Ka_1 and Ka_2, which can be calculated from equations (6) and (7). Note that the higher the values of Ka_1 and Ka_2, the higher the concentration of both conjugated pairs on the system, where MOH and MO^- species are on the film surface, and H^+ is dissociated in the solution.

$$K_{a1} = \frac{[MOH]\left[H^+\right]}{\left[MOH_2^+\right]} \tag{6}$$

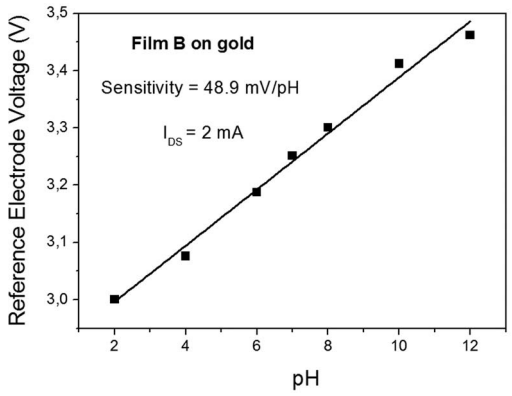

FIG. 6. Reference electrode voltage as a function of pH value. From the fit a sensitivity of 48.9 mV/pH is obtained.

TABLE I. Values obtained for the sensitivity of melanin based EGFET sensors.

Substrate	Film	Sensitivity (mV/pH)
ITO	A	31.3
ITO	B	43.9
ITO	C	39.3
Gold	A	41.2
Gold	B	48.9
Gold	C	48.0

$$K_{a1} = \frac{[MOH]\left[H^{+}\right]}{\left[MOH_2^{+}\right]} \tag{7}$$

According to equations (2) and (6) it is observed that the sensor´s response depends on the acid dissociation constant Ka, (whereas $Ka = Ka_1.Ka_2$), which in turn is related to the acid potential dissociation (pKa) through the relation $pK_a = -log\,K_a$. The pK_a values provide information about the relationship between the pH of the solution and the protonation or not of the melanin structure. For example, if the pH of the medium is lower than the pK_a for a given functional group, then it will be protonated. The pKa of the functional groups of melanin can be estimated from the monomeric units of DHI and DHICA; 4.2 to DHICA carboxylic acid, 6.0 to quinone imine group, 9.8 and 13.2 for the hydroxyl groups of DHI and DHICA, respectively.[23,30]

Based on this discussion, one can attribute two mechanisms of interaction between the electrolyte solution and the melanin films: the potential generation at this interface and the ionic flow through the film. The first mechanism occurs due to direct adsorption of ions in the Helmholtz plane, which is governed by the pKa of the binding sites of melanin. In solutions with pH values below 4.2, all hydroxyl groups from melanin are protonated, resulting in higher source drain currents (I_{DS}). As the pH is increased above the pKa of some functional groups, less binding sites are available, reducing I_{DS}. The second mechanism is the diffusion of protons from the Gouy-Chapman layer of the electrolyte into the film. In this case, the higher the pH of the electrolyte solution, the lower the supply of H^+ ions, the lower the potential generated on the film surface and, therefore, the lower the current measured in the sensor.

The total charge that can be adsorbed on the active layer of the sensor also depends on the film surface properties, and important factors may affect this behavior, like the roughness of the film. Our results indicate that compared to films A and C, film B shows greater surface roughness, or higher effective surface area, and thus more sites available for H^+ binding. Film A, in addition to a lower surface roughness, may have a more compact structure due the higher concentration of

the precursor solution that may have a negative influence on charge transport. This hypothesis was confirmed from impedance spectroscopy measurements (Figure 4), which showed that film B has a lower capacitance than film A, independent of pH. Therefore, it was found that the resistance to electron transfer as well as impedance, increased when the film thickness is increased.[28]

The use of melanin as an active layer in EGFET devices is quite promising. Melanin is easy to deposit, has a large number of hydrogen binding sites and it's amorphous nature contributes to its good sensitivity.[4]

V. CONCLUSIONS

Melanin thin films were used as the active layer in EGFET pH sensors with good sensitivity. Parameters such as the concentration of the solution used for deposition and the substrate were shown to affect the sensitivity of the sensor. Higher surface roughness provided larger effective sensing areas. A simple model was proposed with adequate description of the physical processes involved. The use of melanin in this kind of EGFET is interesting due to its low cost and easy processing.

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