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Synthesis of poly-Lysine/Gold nanoparticle films and their electrocatalytic properties

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ABSTRACT

Films composed of citrate stabilized gold nanoparticles (AuNPs) and Poly-Lysine (pLys) were fabricated by electropolymerization on glassy carbon (GC) electrodes from 0.1M phosphate buffer solution at pH = 8. The films were topographically analyzed by atomic force microcopy whereas their electrocatalytic properties were studied by cyclic voltammetry and electrochemical impedance spectroscopy in aqueous solution using 0.0005M $[Fe(CN)_6]^{3-/4-}$ as redox probes and 0.1M $NaClO_4$ as supporting electrolyte. The electron transfer process proved to be more efficient at the pLys-AuNP modified electrodes containing low amounts of gold nanoparticles (500 µL, 2.5×10^{-4}M) than at the bare glassy carbon electrode. The heterogeneous electron transfer constant increased 2 orders of magnitude at the pLys-AuNP modified electrodes proving a more efficient electron transfer than at bare GC. These new films have a great potential for DNA biosensor applications.

Keywords: *citrate stabilized gold nanoparticles, Lysine, electropolymerization, electrocatalytic properties*

1. Introduction

The functionalization of various types of electrodes with nanomaterials represents an intense research subject due to the wide range of applications in analytical sensing. The large variety of nanomaterials and methods used to fabricate composite films on various types of surfaces offers great diversity of sensing applications for chemical, electrochemical or biochemical detection of analytes in solution. [1,2] The techniques employed to fabricate multilayers of nanoparticles include: (i) ligand exchanges combined with dithiol linkers, (ii) electrostatic or coordinative interactions with the functional groups present on the nanoparticles surface or (iii) covalent linking using amide coupling [1,2,3]. Nanomaterials exhibit unique electronic, optical, and catalytic properties due to their size, morphology, large surface area, and possible quantum confinement. [4,5].Gold nanoparticles (Au NPs) are widely used for different studies due to their chemical stability. The incorporation of these nanomaterials into thin films will improve the electrocatalytic properties of the modified surfaces depending on the size, shape and density of nanoparticles. [6,7,8]. In this paper we present a new method for gold nanomaterial/polymer film fabrication based on simultaneous electropolymerization of L-Lysine (Lys) and citrate stabilized gold nanoparticles (AuNPs) from 0.1M phosphate buffer solution at pH =8. This procedure has allowed the direct fabrication of a polymer matrix filled with nanoparticles and chemically bonded to the surface of

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glassy carbon (GC) electrodes. The modified electrodes were topographically analyzed by atomic force microscopy whereas their electrochemical properties and stability were evaluated by cyclic voltammetry and electrochemical impedance spectroscopy.

2. Experimental section

2.1.Chemicals. Hydrogen tetrachloroaurate trihydrate (III) ,($HAuCl_4$, 99.99%, Sigma Aldrich), lysine, ($C_6H_{14}N_2O_2$,≥ 98%, Aldrich), sodium borohydride ($NaBH_4$, 96%, Sigma Aldrich), citrate trisodium dihydrate, ($(HOC(COONa)CH_2COONa)_2 \cdot 2H_2O$, Fluka, %), potassium hexacyanoferrate (III), potassium hexacyanoferrate (II) (pa quality) and $NaClO_4$ (pa quality), H_2SO_4 (pure, Pronalab), H_2O_2 30% (Fluka) were used as received. Millipore filtered water (resistivity > 18 MΩ·cm) was used to prepare all aqueous solutions and for rinsing. Prior use, all the glassware were cleaned with freshly prepared *aqua regia* (HNO_3: HCl =1:3, % v/v), rinsed abundantly with Millipore water and dried.

2.2. Synthesis of spherical gold nanoparticles stabilized by trisodium citrate. The gold nanoparticles stabilized by trisodium citrate were synthesized as follows: 1 mL of $HAuCl_4$ (5×10^{-3} M) was added to 18 mL of Millipore water and stirred. To this solution was added 1 mL 0.5% trisodium citrate dihydrate aqueous solution and consecutively were added drop by drop under strong stirring 10 mL of $NaBH_4$ (0.01M). The solution mixture was stirred for additional 30 minutes. The resulted gold nanoparticles were characterized by UV-Vis spectroscopy and transmission electron microscopy.

2.3. UV-Vis spectroscopy measurements. The UV-Vis spectra of freshly prepared gold nanoparticles solution was recorded on a UV-Vis spectrophotometer from ScanSci in the range of 200 nm to 900 nm, using quartz cuvettes with 1cm light path.

2.4. Fabrication of poly-lysine and gold nanomaterials films on glassy carbon electrodes. All the electrochemical measurements were performed on a PGSTAT 302N potentiostat (EcoChemie B.V., The Netherlands) using a conventional three-electrode cell equipped with a working electrode of glassy carbon (0.0314 cm^2 area), a platinum electrode as the counter electrode and a saturated calomel electrode as the reference electrode. The poly-Lysine and gold nanoparticle films were fabricated by electropolymerization on glassy carbon electrodes. First, 0.0074g of Lysine was dissolved in 50mL of phosphate buffer (0.1M, pH=8).To this solution were added 500µL or 1500µL of gold nanoparticles stabilized by trisodium citrate (2.5×10^{-4} M) and stirred for homogenization. The resulted mixture was poured into the electrochemical cell and deaerated with nitrogen for 10 minutes. Consecutive cyclic voltammograms were recorded for a potential window of -0.2V to 1.5V, until stabilization (no increase of peak currents was observed (Figure 2). The resulted poly-Lysine/AuNP films were characterized by atomic force microscopy (Figure 3) whereas their electrochemical properties were evaluated by cyclic voltammetry and electrochemical impedance spectroscopy using 0.0005M $[Fe(CN)_6]^{3-/4-}$ as redox probes and 0.1M $NaClO_4$ as supporting electrolyte (Figure 4). Cyclic voltammograms were recorded for various scan rates: 25, 50, 75, 100 and 150 mV/s, within the potential window of -0.2 V to 0.6 V, at a step potential of 6mV. Electrochemical impedance spectroscopy (EIS) measurements were performed for a frequency range of 10000Hz to 1Hz, with amplitude of 10 mV at the midpeak potentials determined from the CVs. For deaeration, gaseous nitrogen was purged in the 0.1M $NaClO_4$ /0.0005M $[Fe(CN)_6]^{3-/4-}$ aqueous solution before each measurement for 15 minutes.

2.5. Atomic Force Microscopy measurements. The film modified glassy carbon electrodes were imaged in air using a Molecular Imaging, PicoLe AFM in tapping mode. Silicon cantilevers (Nanosensors) with a resonance frequency of 200-400 kHz were used for the surface topography measurements. Topographic, amplitude, and phase images were recorded for each layer with a resolution of 512 pixels × 512 pixels. AFM measurements were carried out several times and reproducible results were obtained.

3. Results section

3.1. Characterization of Poly-Lysine/Au citrate nanoparticle films. The synthesized gold nanoparticles stabilized by citrate showed a surface plasmon band at 515 nm (Figure 1) which corresponds to an average diameter of 2.6 nm. [9]

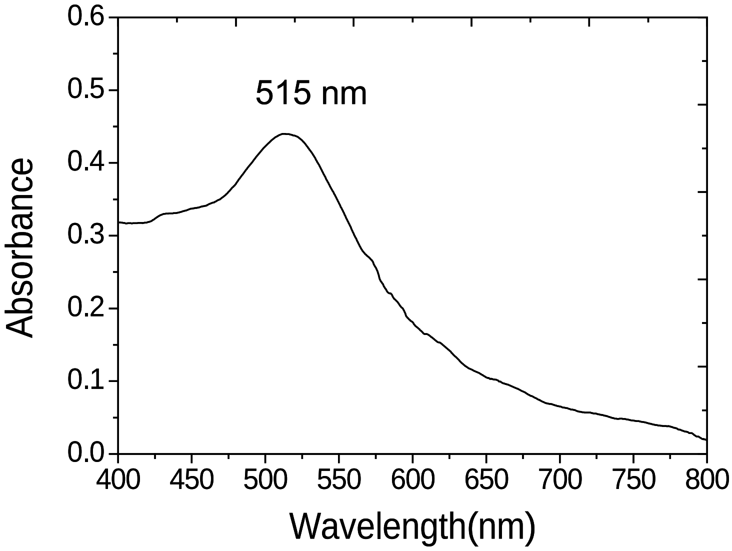

Figure 1. UV-Vis spectra of gold nanoparticles stabilized by trisodium citrate

Figure 2 presents the consecutive cyclic voltammograms recorded at the glassy carbon electrode during the electropolymerization of 0.0074 g Lysine (pKa = 10.5) and 500µL gold nanoparticles stabilized by citrate dissolved in 50 mL of PBS aqueous solution (pH=8). At this pH the resulted poly-Lysine (pLys) matrix is positively charged whereas the incorporated citrate stabilized gold nanoparticles are negatively charged. These opposite charges facilitate the up-take of gold nanoparticles into the polymer matrix during the electropolymerization process. The overall charge of the pLys/AuNPs citrate films proved to be dependent on the low (500µL, pLys/AuNP-500) or high amount (1500µL, pLys/AuNP1500) of gold nanoparticles incorporated into the polymer matrix, as it will be further demonstrated. The film formation on the glassy carbon electrode stabilized after 25 consecutive scans. The surface topography of the resulted films was analysed by atomic force microscopy measurements. Figure 3 shows the topographic tapping mode (left side) and amplitude AFM images (right side) of bare glassy carbon electrode (a,b), pLys/AuNP 500 film (b,c) and pLys/AuNP 1500 film(e,f). The surface roughness (RMS) increased from 0.65 nm for bare glassy carbon electrode (Figure 2a) to 9.28 nm for pLys/AuNP-500 (Figure 2c) and decreased to 6.77 nm for the film pLys/AuNP-1500 (Figure 3e). A higher amount of gold nanoparticles stabilized by citrate incorporated into the poly-L-Lysine matrix tends to soften the film (lower RMS) implying a more compact structure of the pLys/AuNP-1500 film than the pLys/AuNP-500 film which could be expected due to the stronger electrostatic attraction between the higher number of negatively charged AuNPs and Poly-Lysine matrix positively charged.

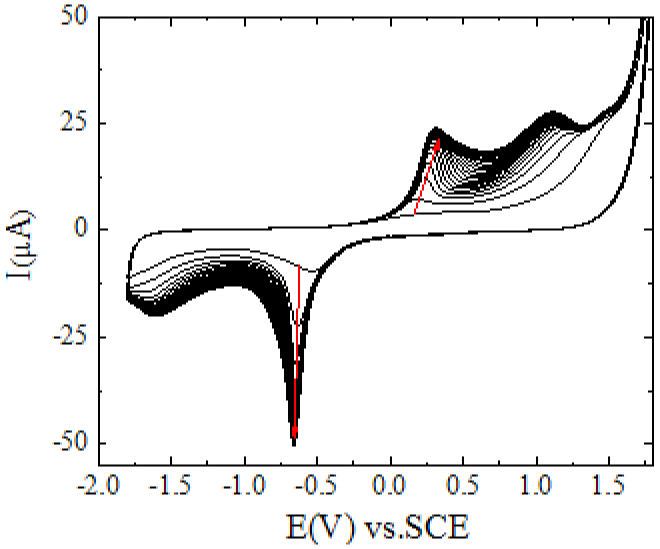

Figure 2. Consecutive cyclic voltammograms recorded during electropolymerization process at the glassy carbon electrode in 0.1M phosphate buffer solution, 50 mL (pH=8) of 0.0074g Lysine and 500μL gold nanoparticles stabilized by citrate (2.5×10^{-4} M). Scan rate 50mV/s.

These results are consistent to previous published work in which poly-Lysine was used to electrostatically self-assembly gold nanoparticles stabilized by mercaptosuccinic acid (MSA) on gold electrodes. [10] The poly-Lysine layer has determined a higher increase of roughness whereas the gold nanoparticles stabilized by mercaptosuccinic acid determined a decrease of film's surface roughness. [10] The higher content of AuNPs incorporated into the poly-Lysine matrix is proven by a higher number of aggregates observed in the topographic tapping AFM images (Figure 3e and f) of pLys-Aucit1500 modified GC electrode. The sizes of the spherical features observed in the AFM images suggest that the gold nanoparticles are forming aggregates within the polymer matrix which could be expected due to the strong electrostatic attraction between these oppositely charged materials. The electrocatalytic properties of the pLys/AuNP films were studied as a function of AuNPs content by cyclic voltammetry and electrochemical impedance spectroscopy measurements. Figure 4a shows the cyclic voltammograms recorded at the pLys/AuNPs films containing two different volumes of AuNPs: 500 μL (2.5×10^{-4} M), curve n =2 or 1500 μL (2.5×10^{-4} M), curve n = 3 dissolved in 100 mL aqueous solution of 0.0005M $[Fe(CN)_6]^{3-/4}$ and 0.1M $NaClO_4$. For comparison, the cyclic voltammograms recorded at bare glassy carbon electrode (curve n = 0) and poly-Lysine modified electrode (without AuNP, curve n =1) in aqueous solution 0.0005M $[Fe(CN)_6]^{3-/4-}$and 0.1M $NaClO_4$ are presented in Figure 4a. The cyclic voltammograms (Figure 4a) show a very interesting behaviour of the Poly-Lysine and poly-Lysine/AuNP modified carbon electrodes toward negatively charged probes in solution. The highest peak to peak separation in the CVs was recorded at the bare glassy carbon electrode being 0.156V for a cathodic peak current of I_c= -6.05μA. For the pLys and pLys/AuNP modified GC electrodes the peak to peak separation decreased and the peak currents varied as follows: 0.134V, I_c= -4.64μA (n = 1), 0.120V, I_c= -6.19μA, (n = 2), 0.127V, I_c= -5.11μA (n = 3). These parameters values demonstrate that the electron transfer process is more efficient at the pLys/AuNP500 modified electrode (lowest peak to peak separation and highest increase of peak currents) than at the bare glassy carbon electrode and the other two modified electrodes.

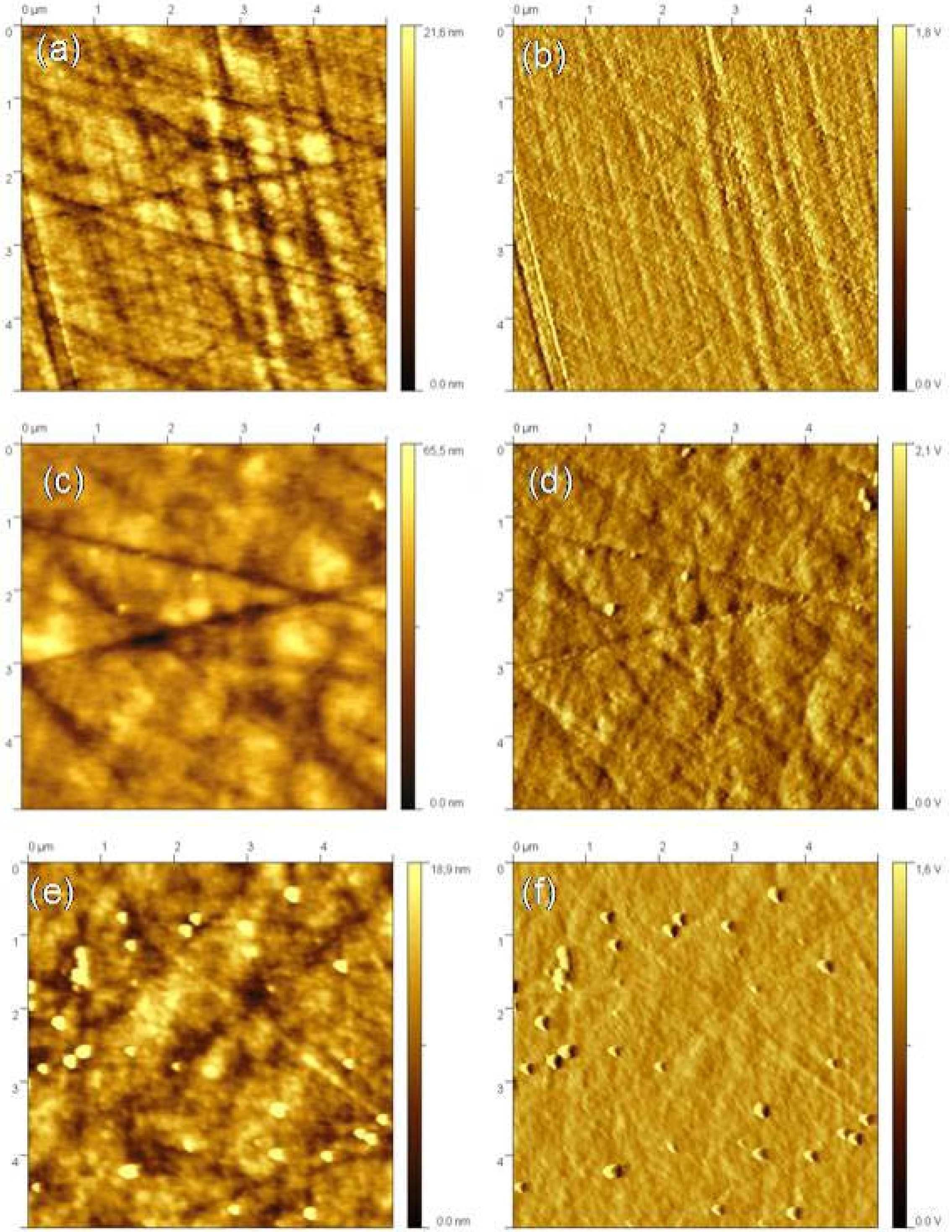

Figure 3. Topographic tapping (left) and amplitude (right) AFM images of bare glassy carbon electrode (a,b), Poly-Lysine/Au NP 500 film (b,c) and poly-Lysine/AuNP 1500 film(e,f).

In consequence, a low amount of citrate stabilized gold nanoparticles assures an efficient electron transfer process and facilitates the diffusion of the $[Fe(CN)_6]^{3-/4-}$ within the film toward the underlying bare glassy carbon electrode.

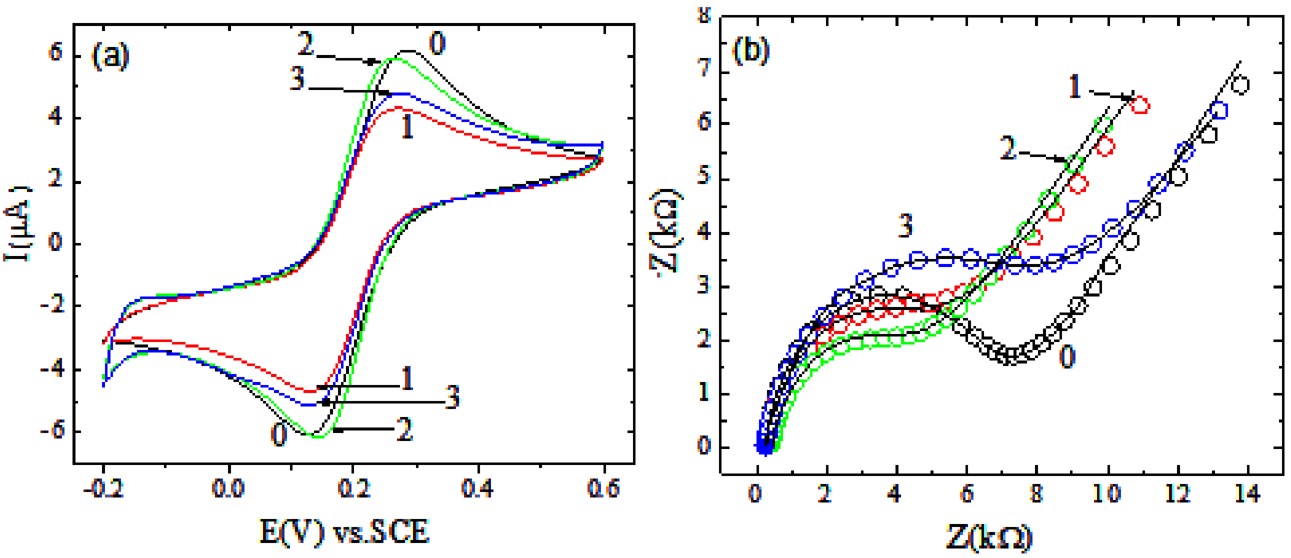

Figure 4.(a).Cyclic voltammograms recorded in aqueous solution of 0.0005M $[Fe(CN)_6]^{3-/4-}$ and 0.1M $NaClO_4$ at bare glassy carbon electrode (n =0), poly-Lysine modified GC electrode (n =1), poly-Lysine/AuNP 500 modified GC electrode (n =2) and poly-Lysine/Au NP 1500 modified GC electrode (n=3). Scan rate 0.05V/s.**(b)** Nyquist diagrams of poly-Lysine/AuNP films modified electrodes recorded in aqueous solution 0.0005M $[Fe(CN)_6]^{3-/4-}$ and 0.1M $NaClO_4$ .The type of layer, n , is indicated close to the curves and correspond to the same notation as for CVs. The experimental data are represented by circles whereas the fitted data are represented by lines.

These results are consistent to our previous published work in which the electrocatalytic properties of multilayered poly-Lysine/Au-MSA NPs films have shown a strong repelling effect toward negatively charged probes in solution when the outermost layer was the Au-MSA NPs layer.[10] The difference here is that the citrate stabilized gold nanoparticles are incorporated into the film by electropolymerization whereas in the previous work small negatively charged gold nanoparticles stabilized by mercaptosuccinic acid were self-assembled electrostatically to the polymer in a layer-by-layer architecture. Additionally, at low concentration the AuNPs formed less aggregates (compare Figure 3c and 3e) and in consequence they maintain their catalytic activity within the film due to their high active surface area. A more detailed evaluation of the electrochemical properties of these new films was performed by EIS measurements. Figure 4b shows the Nyquist diagrams recorded at the bare glassy carbon and the modified electrodes in aqueous solution 0.0005M $[Fe(CN)_6]^{3-/4-}$and 0.1M $NaClO_4$. The EIS spectra were fitted using Randles circuit or the equivalent electrical circuit presented in Figure 8 of reference [6], and the nonlinear least-square fitting procedure. The parameters values obtained from the fittings, given in table 1, agree very well with the experimental data. The EIS spectra feature variable semicircles at high and intermediated frequencies depending on the type of film and semi-infinite planar diffusion at low frequencies (slope unity), (Figure 4b). The double layer capacitance, C_{dl}, has increased at film modified GC electrodes but it has decreased with increasing the content of the AuNPs incorporated into the film suggesting an electronic communication between the AuNPs within the film and the underlying bare GC carbon electrode. The capacitance of the film, C_f, has decreased with increasing the number of AuNPs incorporated into the pLys matrix suggesting a transition from large and bulky structure of the poly-Lysine film (pLys, n = 1, high area of the electrode) to a more compact and organized film, the pLys/AuNP1500 (n= 3) with a smaller area, which is consistent with the AFM images (Figure 3).

The apparent charge transfer resistance, R_{CT}, has decreased several orders of magnitude upon electropolymerization of Lysine and AuNP on the GC bare electrode. The lowest R_{CT} value was obtained from the fittings of the Nyquist spectrum recorded at the pLys/AuNP500 modified GC electrode suggesting that the process of electron transfer is the fastest at this modified electrode.

Table1. Parameter Values Obtained from the Fittings of the Impedance Spectra in Figure 4b Using Nonlinear Least-Square Fit[a]

n	R_s (kΩ)	C_f (μF)	R_f (kΩ)	C_{dl} (μF)	R_{CT} (kΩ)
0	0.293	-	-	0.31	5.23
	(0.62)			(0.72)	(0.80)
1	0.292	2.54	2.00	3.21	3.12
	(0.23)	(0.74)	(0.98)	(0.85)	(0.64)
2	0.335	2.15	1.59	2.74	2.50
	(0.87)	(0.56)	(0.70)	(0.93)	(0.89)
3	0.298	1.95	2.50	2.63	4.34
	(0.53)	(0.78)	(1.10)	(0.75)	(0.73)

[a] The percent errors from the fit for each element are given in parentheses.

These results demonstrate that the charge transport mechanism at the pLys/AuNP modified GC electrodes in the presence of the redox probe $[Fe(CN)_6]^{3-/4-}$ is electron transport mediated by the AuNPs. Moreover, the ionic diffusion is highly favored by the electrostatic attraction between the negatively charged probes in solution and the polymer matrix which is positively charged. The experimental heterogeneous electron-transfer rate constants, $k_{\exp}$, (table 2) can be evaluated based on EIS fitting parameters and equation (1):

$$K_{\exp} = \frac{RT}{n^2 F^2 A R_{CT} [c]} \qquad (1)$$

where R is the gas constant, T is the temperature (K), A is the electrode area (0.0314 cm^2), $[c]$ corresponds to the bulk concentration of the redox probe (0.005 molcm^{-3}), and n is the number of transferred electrons per molecule of the redox probe (n = 1) for $[Fe(CN)_6]^{3-/4-}$ probes. [6] The heterogeneous electron-transfer rate constant has increased 2 orders of magnitude at the pLys/AuNP500 film proving a faster electron transfer at this modified electrode than at bare glassy carbon electrode which is consistent with the decreased peak to peak separation and slightly increased peak currents in the cyclic voltammograms (Figure 4a, n = 2). The improved electrocatalytic properties of the pLys/AuNP modified GC electrodes represent a great potential for DNA biosensor applications. [11] Further studies are performed in our laboratory in order to evaluate the biosensing properties of these films.

Table2. Heterogeneous Electron-Transfer Rate Constants Calculated for Each Film Electropolymerized on GC electrodes Using the R_{CT} Parameters Obtained from the Fittings of the EIS Spectra and Based on eq.1.

n	k_{et} (cm×s^{-1})
0	0.32×10^{-5}
1	0.54×10^{-5}
2	0.68×10^{-5}
3	0.39×10^{-5}

4. Conclusions

New composite polymer/AuNP films were fabricated by electropolymerization on glassy carbon electrodes. Based of the EIS and voltammetry studies, we conclude that the main charge transport mechanism at the pLys/AuNP modified GC electrodes in the presence of the redox probe $[Fe(CN)_6]^{3-/4-}$ is electron transport mediated by the Au NPs incorporated into the polymer matrix. Additionally, the ionic diffusion of the redox probes is highly favorable due to the electrostatic attractive interaction between the negatively charged probes in solution and positively charged polymer within the pLys/AuNP composite film.

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