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# DNA Biosensor for the Detection of Actinomycin D

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**ABSTRACT**

The surface of a glassy carbon (GC) electrode was modified by electropolymerization with poly-lysine and citrate stabilized gold nanoparticles followed by electrostatic self-assembly of double stranded DNA. The resulted composite film modified GC electrode was used for the detection in solution or at the surface of peptide antibiotic actinomycin D (ActD), an anticancer drug. Two procedure of detection are presented and discussed.

**Keywords:** *Lysine, citrate stabilized gold nanoparticles*, *DNA biosensor, Actinomycin D*

## 1. Introduction

The interaction of DNA with drugs is among the important aspects of biological studies in drug discovery and pharmaceutical development processes. There are several types of interactions associated with ligands that bind DNA including: intercalation, noncovalent groove binding, covalent binding /cross linking, DNA cleaving, nucleoside-analog incorporation. These binding interactions determine changes to both the DNA and drug molecules in order to accommodate complex formation.[1] The integration of nanoparticles, which exhibit unique electronic, photonic, and catalytic properties, into films containing DNA or other biomaterials, which display unique recognition, catalytic, and inhibition properties, yields novel hybrid nanobiomaterials with synergetic properties and functions. [2,3] This conjugation of NPs with biomolecules could provide electronic or optical transduction of biological phenomena in the development of novel biosensors.[4,5,6] DNA can be immobilized on sensor surfaces using methods similar to those used for enzyme-based biosensors: adsorption, covalent immobilization, and avidin (or streptavidin)- biotin interaction. [7] One type of DNA immobilization is based on ionic interactions occurring between the negatively charged groups present on the DNA probe and positive charges covering the surface. For example, a chitosan film was used for the immobilization of single stranded DNA on a glassy carbon electrode. [8,9] Chitosan is a cationic polymer that can form a stable complex with the negatively charged phosphate groups of the DNA. In the present work, we have used this type of interaction for the immobilization of double stranded DNA to a positively charged surface of glassy carbon electrode. The positive charge on the glassy electrodes was obtained by electropolymerization of a mixture containing L-Lysine and low amounts of citrate stabilized gold nanoparticles. [10] The new poly-Lysine/AuNP/dsDNA film was electrochemically characterized by cyclic voltammetry and impedance spectroscopy using $[Fe(CN)_6]^{3-/4-}$ as redox probes and 0.1M $NaClO_4$ as supporting electrolyte

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whereas its topographic features were analyzed by atomic force microscopy. The poly-Lysine/AuNP/dsDNA modified electrodes were also used for the detection of an antitumor drug, namely , actinomycin D, in solution or at the surface by differential pulse voltammetry measurements in acetate buffer solution (pH=4.8). The sensing properties of the new DNA composite film are discussed.

# 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), deoxyribonucleic acid sodium salt from calf thymus, (Sigma-Aldrich, 16.7g units/mg solid, 6% sodium), acetic acid glacial (100%, Merck), sodium acetate anhydrous , (p.a.,Merck) , actinomycin D , (Sigma Aldrich, 99%) 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 (HNO3: 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 (AuNPs) were synthesized as previously presented [10].Briefly, to a mixture of 1 mL of $HAuCl_4$ ($5\times10^{-3}$ M) and 18 mL of Millipore water was added 1 mL 0.5% trisodium citrate dihydrate aqueous solution. To this solution were added drop by drop under strong stirring 10 mL of $NaBH_4$ (0.01M). The color of solution changed from yellow to purple and finally to red after the complete reduction of $HAuCl_4$ to $Au^0$ by $NaBH_4$. The resulted gold nanoparticles were 2.6 nm average diameters [10].

**2.3. Fabrication of poly-Lysine/Gold Nanomaterial/double strands DNA 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 pLys/AuNP/dsDNA films were self-assembled on the bare glassy carbon electrodes in two steps. First, the film composed of poly-Lysine and citrate stabilized gold nanoparticles was electropolymerized on the glassy carbon electrodes from aqueous phosphate buffer solution (50 mL, pH =8) in which were dissolved 0.0074g of Lysine and 500 µL of gold nanoparticles stabilized by trisodium citrate ($2.5\times10^{-4}$ M). 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, reference [10]). The resulted pLys/AuNP film modified GC electrodes were washed with Millipore water and consecutively immersed in 0.1M acetate buffer (pH= 4.8) of deoxyribonucleic acid sodium salt from calf thymus (DNA, 1.4mg/1mL) for 1 hour. The resulted pLys/AuNP/dsDNA films were gently washed with Millipore water, let to dry for 10 minutes and imaged in air by atomic force microscopy (Figure 2) 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 1). The cyclic voltammograms were recorded for various scan rates: 25, 50, 75, 100 and 125 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.

Freshly prepared pLys/AuNP/dsDNA modified electrodes were used for the electrochemical detection of actinomycin D in an aqueous acetate buffer solution (0.1M) of pH=4.8 containing 53µg/mL single stranded DNA. A pretreatment of the DNA modified electrode was performed by square wave scan at a potential of +1.4V, for 120s. Differential pulse voltammetry measurements were performed for the potential window of -0.7V to +1.4V, with amplitude of 50mV. The single strand DNA was obtained by denaturation of 5.3mg dsDNA dissolved in 100 mL (0.1M) aqueous solution of acetate buffer (pH=4.8). The dsDNA solution was boiled to 90°C, in an Erlenmeyer, for 5 minutes and cooled in an ice bath in order to stop the denaturation process.

**2.4. 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 pLys/AuNP500/dsDNA film modified glassy carbon electrodes.** In our previous work, we have presented the electrical properties of poly-Lysine/gold nanoparticle modified glassy carbon (GC) electrodes and we have demonstrated that a low amount of gold nanoparticles incorporated into the polymer network electropolymerized on the glassy carbon electrodes generates a faster electron transfer process than at the bare glassy carbon electrodes.[10] Based on this fact, we have chosen for further electrochemical studies and biosensing applications the films containing 500 µL ( the lowest amount) of citrate stabilized gold nanoparticles ($2.5\times10^{-4}$ M).[10] These films were noted by pLys/AuNP500. In order to build a DNA based biosensor, double stranded deoxyribonucleic acid sodium salt from calf thymus was electrostatically self-assembled to the pLys/AuNP500 modified GC from acetate buffer solution (pH = 4.8) for 1 h. The resulted pLys/AuNP500/dsDNA modified GC electrodes were electrochemically characterized by cyclic voltammetry and electrochemical impedance spectroscopy. Figure 1a represent the cyclic voltammograms recorded at bare glassy carbon electrode and pLys/AuNP500/dsDNA modified GC electrode in aqueous solution of 0.0005M $[Fe(CN)_6]^{3-/4-}$ and 0.1M $NaClO_4$ .The DNA layer, electrostatically self-assembled to the pLys/AuNP500 modified GC electrodes determined a decrease of peak currents in the cyclic voltammograms as compared to bare GC or pLys/AuNP modified GC (curve 2, in Figure 4a, reference [10]). This is due to a repelling effect between the outermost negatively charged DNA layer and the negatively charged redox probes from the solution. The peak to peak separation was 0.137V for a cathodic peak current of $I_c$= -5.08 µA in the CV recorded at the pLys/AuNP/dsDNA modified GC electrode as compared to 0.156V for a peak current of $I_c$= -6.05µA in the CV recorded at the bare GC electrode. The value of these parameters suggest that the diffusion of the electroactive species is slightly hindered by the outermost negatively charged DNA layer (lower peak currents in the CVs) whereas the gold nanoparticles within the poly-Lysine network assure a good electronic communication with the underlying bare GC electrode (lower peak to peak

separation than bare GC). The EIS spectra recorded at pLys/AuNP500/dsDNA modified GC electrode in aqueous solution of $[Fe(CN)_6]^{3-/4-}$ shows a high semicircle at high and intermediate frequencies and semi-infinite planar diffusion profile at low frequencies (slope unity). The fittings of the EIS spectra (table 1) were performed as previously presented [10] and show that the charge transfer resistance has increased upon the electrostatic self-assembly of DNA which is consistent to the decreased peak currents observed in the CV (red curve, Figure 1a). The heterogeneous electron transfer rate constant was estimated to be $0.27\times10^{-5}$ cm×s$^{-1}$ at the pLys/AuNP/dsDNA modified electrode which is slightly lower than at bare GC electrode ($0.32\times10^{-5}$ cm×s$^{-1}$, table 2, $n$ = 0, reference [10]).The capacitance of the pLys/AuNP500/dsDNA film, $C_f$, is slightly higher than the capacitance of the pLys/AuNP500 film (table 1, n = 2, reference [10]) suggesting an increase of the electrode area upon DNA self-assembly.

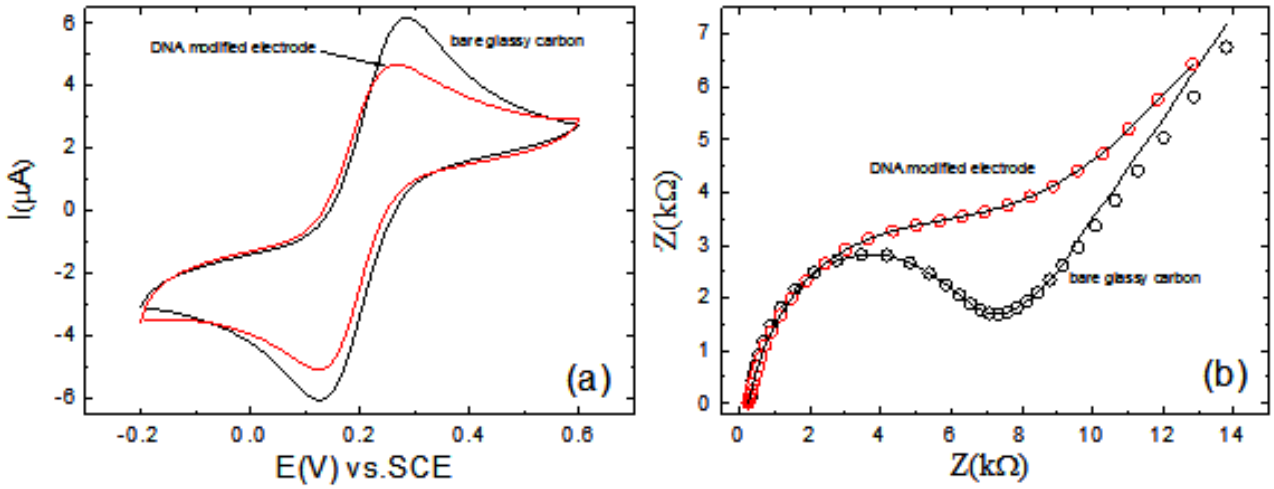


**Figure 1**.(a).Cyclic voltammograms recorded at bare glassy carbon electrode (black curve) and poly-Lysine /AuNP500/dsDNA modified GC electrode (red curve) in aqueous solution of 0.0005M $[Fe(CN)_6]^{3-/4-}$ and 0.1M $NaClO_4$.Scan rate 0.05V/s. **(b)** Nyquist diagrams recorded at bare glassy carbon electrode (experimental data, black circles), poly-Lysine /AuNP500/dsDNA modified GC electrode (experimental data, red circles) in aqueous solution of 0.0005M $[Fe(CN)_6]^{3-/4-}$ and 0.1M $NaClO_4$.The fitted data are represented by lines.

**Table1.** Parameter Values Obtained from the Fittings of the Impedance Spectra in Figure 1b Using Nonlinear Least-Square Fit[a]

| *Type of Electrode* | $R_s$ (kΩ) | $C_f$ (μF) | $R_f$ (kΩ) | $C_{dl}$ (μF) | $R_{CT}$ (kΩ) |
|---|---|---|---|---|---|
| Bare Glassy Carbon | 0.293 (0.62) | - | - | 0.31 (0.72) | 5.23 (0.80) |
| DNA film modified electrode | 0.298 (0.90) | 2.71 (0.65) | 2.33 (0.98) | 3.51 (0.85) | 6.19 (0.64) |

The change in topography upon DNA self-assembly was evaluated by tapping mode AFM (Figure 2). The roughness of the pLys/AuNP500/dsDNA film (Figure 2b) is 11.3 nm as compared to 0.65 nm for bare GC (Figure 2a). This increase of surface roughness proves the fact that the DNA layer is electrostatically attached to the poly-Lysine/gold nanoparticles film. The consecutive cyclic voltammograms (Figure 3a) recorded at the pLys/AuNP500/dsDNA modified GC electrode in aqueous solution of $[Fe(CN)_6]^{3-/4-}$ show that the pLys/AuNP500/dsDNA film is electrochemically stable due to the linear increase of peak currents with increasing scan rates (Figure 3b). This

complete electrochemical characterization suggests that the pLys/AuNP500/dsDNA film is mechanically robust and electrochemically stable. Freshly modified pLys/AuNP500/dsDNA glassy carbon electrodes were used for biosensing of an antitumor drug, namely actinomycin D as it will be presented in the following discussion.

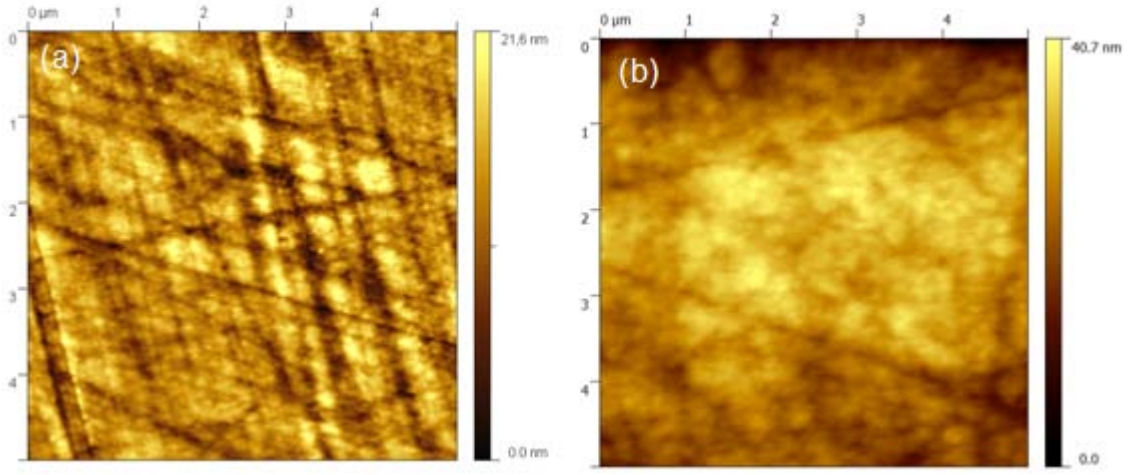


**Figure 2.** Topographic tapping AFM images of bare glassy carbon electrode (a) and Poly-Lysine/Au NP 500/dsDNA film modified glassy carbon electrode (b)

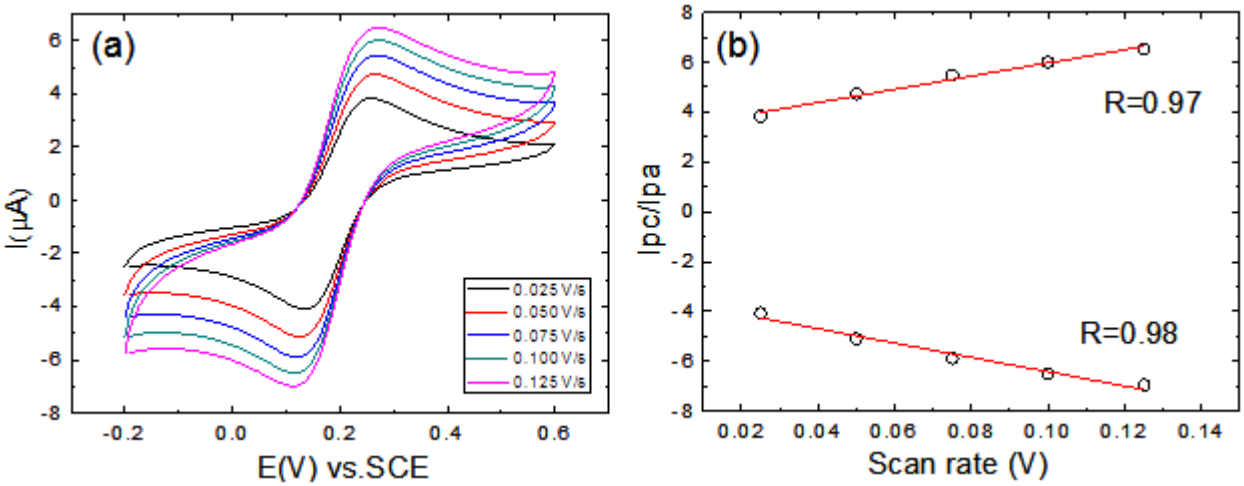


**Figure 3**.(a). Cyclic voltammograms recorded at the pLys/AuNP500/dsDNA modified electrode in aqueous solution of 0.0005M $[Fe(CN)_6]^{3-/4-}$ and 0.1M $NaClO_4$ at increasing scan rates (see inset).(b)Linear variation of anodic and cathodic peak currents with increasing scan rates.

**3.2. Biosensing properties of pLys/AuNP500/dsDNA modified GC electrode toward the detection of actinomicin D.** Actinomycin D (ActD) is an antitumor drug (Figure 4) that binds to DNA by intercalation of its phenoxazone (Pxz) moiety between base pairs via the minor groove. Once bound to the duplex, ActD sterically blocks the progression of RNA polymerase, effectively inhibiting transcription. Minor groove binding of the actinomycin D causes intimate contacts with the walls of the groove, and numerous hydrogen bonding are formed between the amino groups of the bases and the carbonyl oxygens of the ActD as well as electrostatic interactions with the phosphate backbone of the DNA duplex. The electrochemical detection of the drug at the pLys/AuNP500/dsDNA modified GC electrode was performed in acetate buffer solution (pH=4.8) containing 53μL/mL single stranded DNA. During the conditioning procedure the dsDNA film interacts with ssDNA in bulk solution and structural modification of the dsDNA immobilized on the

glassy carbon surface take place by interchain crosslinking with ssDNA in the solution. These interactions determine the formation of portions of triple helix DNA on the electrode surface.

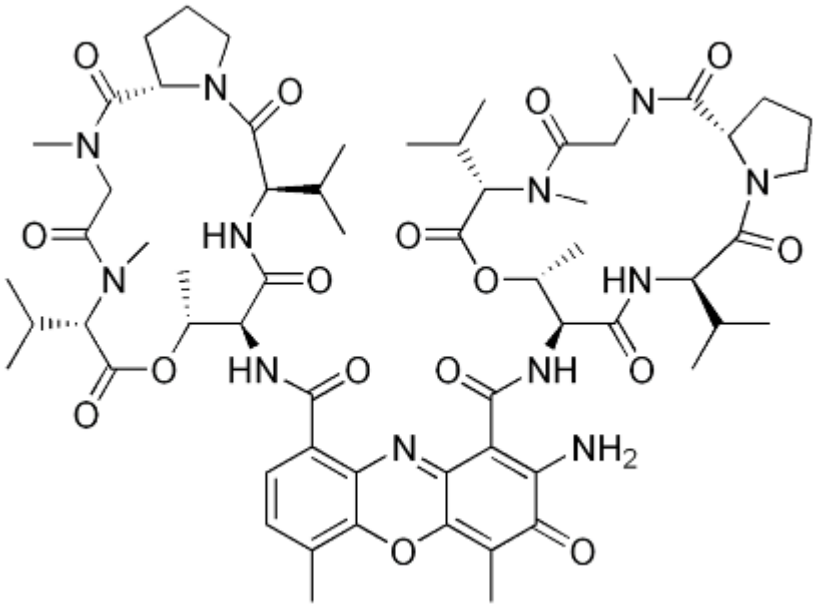

**Figure 4**.Actinomycin D

Figure 5a shows the differential pulse voltammograms (DPV) obtained when successive 25 µl aliquots of ActD 0.32m M were added to a solution of acetate buffer containing 53µL/mL ssDNA. Figure 5b clearly shows that the peaks corresponding to the ActD oxidation (in the range of -0.7V to +0.5V) decreased with increasing its concentration whereas Figure 6 shows that the peaks currents corresponding to the oxidation of guanine and adenine from ssDNA in the triple helix DNA network on the GC modified electrode also decreased. These results demonstrate the progressive intercalation of the ActD to the DNA duplex /triplex network from the GC modified electrode. The oxidation peaks of the guanine (G) and adenine (A) were observed at +0.77V and +1.06V, respectively (Figure 5a). The peak position for guanine and adenine oxidation is slightly shifted as compared to other DNA biosensors [11]. For example, Brett et al. have observed these peaks at +0.84V for guanine and +1.16V for adenine when the DNA was adsorbed directly to the GC bare surface without an intermediate film in-between the DNA layer and the underlying bare GC electrode. [11]. The less positive potentials at which the oxidation of guanine and adenine was observed in the DPVs curves at pLys/AuNP500/dsDNA/ssDNA modified GC electrodes (Figure 5) demonstrates that the oxidation of the base pairs is easier at our GC modified electrode. This is due to the electronic communication facilitated by the citrate stabilized gold nanoparticles incorporated into the poly-Lysine layer through which the duplex/triplex DNA network was connected to the bare GC surface. Also the progressive decrease of peak currents is consistent with the progressive intercalation of the drug into the DNA network from the surface of the GC modified electrode. These results are very consistent to previous published work concerning DNA based biosensor for drug detections in solution when the interaction of the drugs from solution to the DNA network at the electrode surface determined a decrease of signal in the voltammograms. [1] The limit of detection was $22.0\times10^{-7}$M ActD.

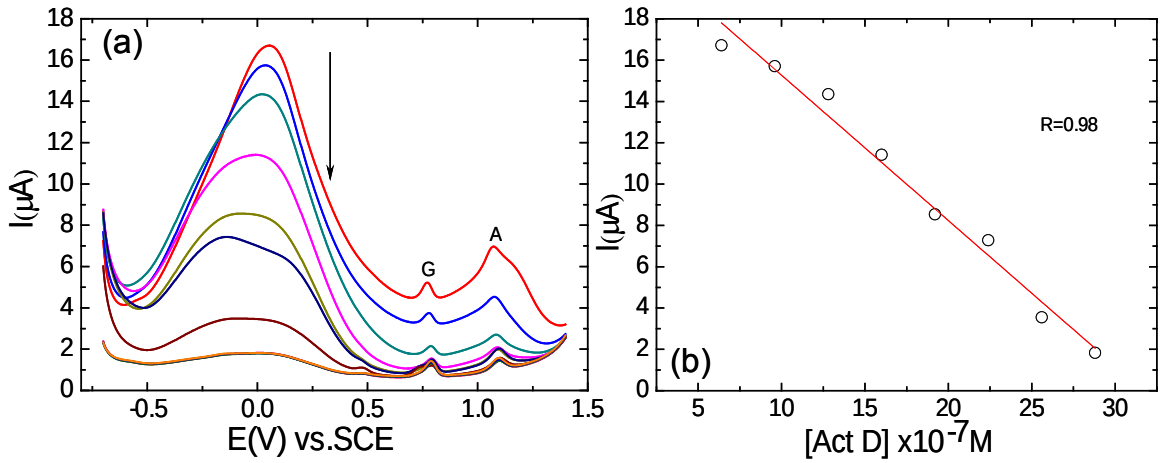


**Figure 5**.(a)Differential pulse voltammograms recorded at the pLys/AuNP500/dsDNA/ssDNA modified GC electrode in acetate buffer solution containing 53μg/mL single stranded DNA and Actinomycin D of $3.2\times10^{-7}$M to $29\times10^{-7}$M.(b)Variation of peak current at 0.05V with increasing concentration of ActD.

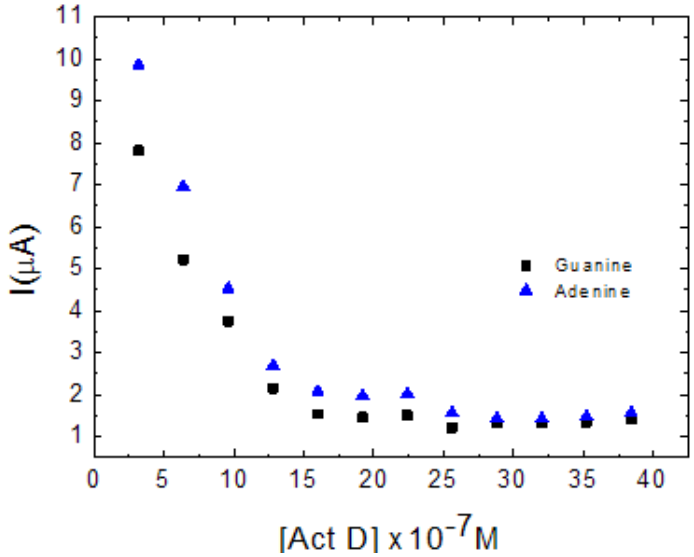


**Figure 6**.Variation of oxidation peak currents for Guanine and Adenine in the DPV from Figure 5 as a function of Actinomycin D concentration.

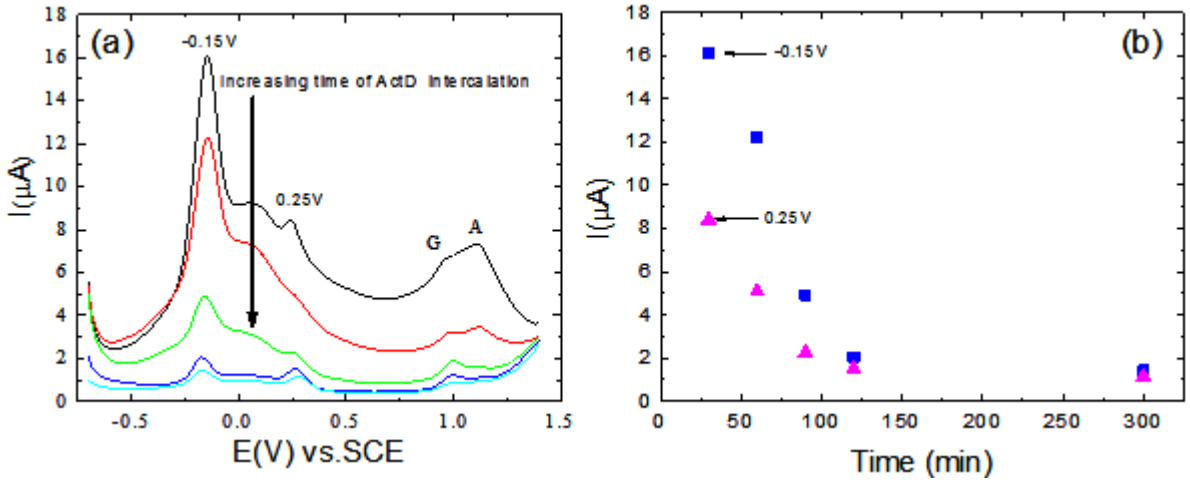


**Figure 7.** (a)Differential pulse voltammograms recorded at the pLys/AuNP500/dsDNA/ActD modified GC electrode in 0.1M acetate buffer solution at increasing times of ActD complexation :30 min (black curve), 1h (red curve), 1.5h (green curve) , 2h (blue curve) and 5 h (cyan curve).(b)Variation of oxidation peak currents of Actinomycin D for increasing duration of intercalation.

A second procedure was used for the detection of ActD. This procedure consist of placing a small aliquots of ActD solution (10µl, 0.32mM) on the surface of freshly prepared pLys/AuNP500/dsDNA modified GC electrode (without ssDNA attachment) cast in an closed box in order to avoid evaporation of the ActD solution and let to interact for 30 min, 1h, 1.5h, 2h and 5h. After each time of ActD adsorption, a differential pulse voltammogram was recorded at pLys/AuNP500/dsDNA/ActD electrode in 0.1M acetate buffer solution (pH=4.8), (Figure 7).

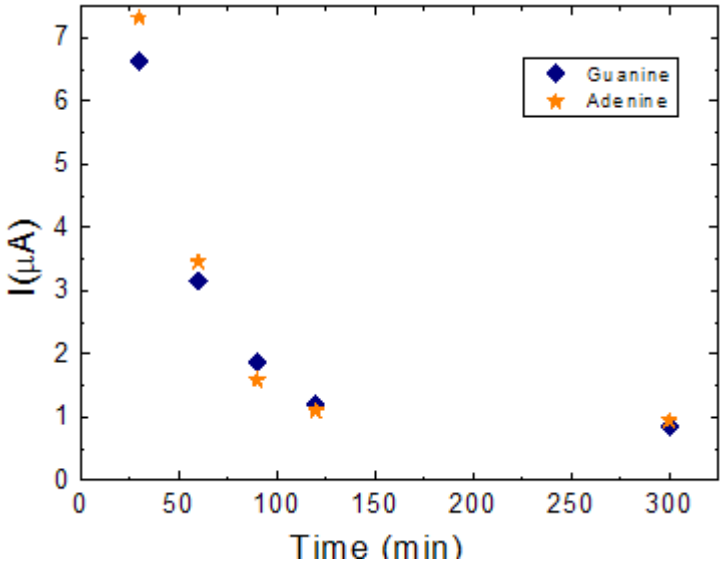


**Figure 8**.Variation of oxidation peak currents for Guanine and Adenine in the DPV from Figure 7 as a function of Actinomycin D intercalation time.

These results suggest that the interaction of DNA duplex at the surface of the electrode directly to the Act D is faster for this second type of biosensor and also the oxidation of both guanine and adenine (figure 7a and 8) takes place quit fast. Moreover, the well defined peaks of Act D suggest a higher sensitivity of the DNA duplex biosensor built following this second procedure. The peaks corresponding to the oxidation of ActD (at -0.15V and 0.25V) became more evident due to the increase of concentration near the electrode surface of ActD that intercalated with DNA duplex as compared to the previous sensing procedure when ActD is present only in solution. The gold nanoparticles from the underlying poly-Lysine network could also improve the sensitivity of this DNA biosensor toward the detection of ActD due to a better electrical conductivity at the pLys/auNP500/dsDNA modified electrode than at the pLys/AuNP500/dsDNA/ssDNA modified electrode. Overall the pLys/AuNP500/dsDNA modified glassy carbon electrodes have proven a high efficiency and selectivity for Actinomycin D detection either in solution or at the modified GC surface /solution interface.

# 4. Conclusions

We have fabricated and characterized a new biosensor composed of poly-Lysine/citrate gold nanoparticles/ dsDNA which proved to have a high efficiency for the detection of actinomycin D. Depending on the sensing procedure, either in solution or at the surface, a high selectivity was observed toward the oxidation of actinomycin D. The sensing features of this new DNA based biosensor are stable and very reproducible. This biosensor can be easily used for the detection of other antitumor drugs in solution or at interface.

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