Electrooxidation of DNA at Glassy Carbon Electrodes Modified with Multiwall Carbon Nanotubes Dispersed in Chitosan

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Abstract
We report on the analytical performance of glassy carbon (GCE) electrodes modified with a dispersion of multiwall carbon nanotubes (CNT) in chitosan (CHIT) for the quantification of DNA. The electroanalytical response of the resulting electrodes was evaluated using differential pulse voltammetry, while the electrochemical reactivity of the film surface was characterized using scanning electrochemical microscopy. Different treatments of the modified GCE were evaluated to improve the stability of the film and the accumulation of DNA. The guanine oxidation signal of double stranded calf-thymus DNA after 3-min accumulation was 20 times higher at GCE/CHIT-CNT cross-linked with glutaraldehyde (GTA) than at bare GCE, while the peak potential was around 45 mV less positive. The guanine oxidation signal demonstrated to be highly reproducible, with 3.4% *RSD* for 5 different electrodes. The treatment with sodium hydroxide demonstrated to be not effective since the resulting films were less stable and the guanine oxidation signal was ten times smaller compared to electrodes prepared with the GTA treated films. The effect of chitosan molecular weight used to prepare the dispersion and the amount of carbon nanotubes dispersed were evaluated. The response of single stranded DNA and oligo$(dG)_{15}$ is also discussed.

Keywords: Carbon nanotubes, Chitosan, Glassy carbon electrode, DNA, Glutaraldehyde, Scanning electrochemical microscopy

1. Introduction

In the last years different approaches have been conducted to improve the performance of electrochemical biosensors. In this sense, carbon nanotubes (CNTs) have largely contributed to the success of new electrochemical biosensors due to their exceptional electronic properties, electric conductivity and catalytic effects [1 – 3]. Different strategies to modify electrode surfaces with these nanostructures have been proposed and the usefulness to detect several analytes has been widely demonstrated [1 – 3].

One of the problems to prepare biosensors based on CNTs is their low solubility in usual solvents. Functionalization of CNTs surface is one of the alternatives to allow their dispersion, derivatization and further adsorption or attachment of different biomolecules [4]. The dispersion of CNTs in different media followed by the immobilization at solid surfaces is another interesting approach to prepare electrochemical sensors. In this sense, dispersions in acidic solutions [5, 6], *N*,*N*′-dimethylformamide [7] as well as within composite matrices using different binders like Teflon [8], bromoform [9], mineral oil [10 – 16] and inks [17] have been successfully used. Cai et al. [18] have reported a sensitive electrochemical DNA biosensor based on the covalent attachment of 5′-aminooligonucleotides at oxidized multiwalled carbon nanotubes dispersed in *N*, *N*′-dimethylformamide and deposited on glassy carbon surfaces. Erdem et al. [19] have proposed the use of oxidized multiwall carbon nanotubes dispersed in *N*,*N*′-dimethylformamide and deposited on glassy carbon and pencil graphite electrodes for the direct detection of nucleic acids and DNA hybridization.

The resulting electrodes modified with CNTs have been employed for the detection of numerous bioanalytes including glucose [8, 10], DNA [11], homocystein [16], neurotransmitters and related compounds [9, 10, 13], ethanol [8, 12], aminoacids [20] among others. Polymers like Nafion [21, 22], polyethylenimine [23] and chitosan [24] have been also used to disperse CNTs.

Chitosan (CHIT) is a copolymer of β-(1 – 4)-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose with good biocompatibility and film-forming properties. Acidic solutions of chitosan have been used to electrostatically immobilize single-stranded DNA (ssDNA) for sequence-specific DNA detection [25]. Electrostatically self-assembled multilayers of chitosan and dsDNA were also built up on gold surfaces [26]. The dispersions of CNTs in CHIT (CNT-CHIT) have been

prepared using different procedures and its structure, chemical and mechanical properties have been characterized by several techniques [27 – 31]. They have been used as chemical sensors for nitrite [32] bromide [33], insulin [34] and NADH [35], and also for the preparation of enzymatic biosensors by the incorporation of enzymatic systems as glucose dehydrogenase [36] cholesterol oxidase [37] horseradish peroxidase [38] glucose oxidase [39] and laccase [40]. Yao et al. [41] have developed a biosensor based on chitosan doped with carbon nanotubes to detect DNA using methylene blue (MB) as DNA-redox indicator. They demonstrated that chitosan film doped with CNT not only increases the electroactive surface of the electrode, but also allow the improvement in the charge transfer between the electrode and methylene blue.

In this paper we study the electrooxidation of DNA at glassy carbon electrodes modified with multiwall carbon nanotubes (CNT) dispersed in chitosan. This work is focused on the determination of the DNA accumulated at glassy carbon electrodes modified with the CNT-CHIT film from the electroactivity inherent to the nucleic acid itself, without any redox indicator using differential pulse voltammetry. The presence of CNT dispersed in chitosan on the electrode surface was characterized by linear sweep voltammetry, while the electrochemical reactivity of the film was characterized by Scanning Electrochemical Microscopy.

In the following sections we discuss the advantages of using glutaraldehyde as cross-linking agent as well as the effect of the molecular weight of chitosan and the amount of carbon nanotubes in the dispersions on the response of the resulting electrode for the quantification of nucleic acids.

2. Experimental

2.1. Reagents

Hydrogen peroxide (30% V/V aqueous solution) was purchased from Merck. Glutaraldehyde (GTA) (25% V/V aqueous solution) was purchased from Baker. Ferrocene methanol (FcOH), Chitosan (CHIT) of low (Cat N° 44886 – 9), medium (Cat. N° 44887 – 7) and high molecular weight (Cat. N° 41941 – 9) were obtained from Aldrich.

Multiwall carbon nanotubes (MWCNT) 1 – 5 μm long and (30 ± 15) nm diameter were obtained from NanoLab (USA). CNTs were oxidized by chemical treatment with a mixture of concentrated sulfuric and nitric acids 1:1 by refluxing for 3 hours. After that, the suspension was filtered and washed with water until neutral pH.

Double stranded calf thymus DNA (dsDNA) (activated and lyophilized, Cat. N° 4522) was from Sigma. Oligo$(dG)_{15}$ was obtained from Integrated DNA Technology. Stock solutions of dsDNA and oligo$(dG)_{15}$ (1000 and 250 ppm, respectively) were prepared with tris-EDTA (TE) buffer (1× concentrate, 20 mM Tris-HCl, 1 mM EDTA, pH 8.0). Single stranded DNA (ssDNA) was obtained by dsDNA heating in boiling water bath for 5 min followed by fast cool in ice bath.

All solutions were prepared with ultrapure water ($\rho =$ 18 MΩ) from a Millipore-MilliQ system (MQ water). Buffer solutions of 0.20 M formate pH 5.00 or 0.10 M phosphate pH 7.40 were employed as supporting electrolytes. All chemicals were used as received.

2.2. Apparatus

Differential pulse voltammetry (DPV), scanning electrochemical microscopy (SECM) and linear sweep voltammetry (LSV) measurements were performed with a CHI 900 or CHI 440 setup (CH Instruments Inc., USA). For SECM measurements a ca. 10 μm diameter home made carbon fiber electrode served as SECM tip while glassy carbon electrodes (GCE) of 3 mm diameter (Model CHI104, CH Instruments) were used as SECM substrate. A platinum wire and Ag/AgCl, 3 M NaCl (BAS, Model RE-5B) were used as counter and reference electrodes, respectively. All potentials are referred to that reference electrode. A magnetic stirrer provided the convective transport when necessary.

2.3. Preparation of the Working Electrode

Prior to surface modification, the GCE was cleaned by polishing with 0.05 μm alumina slurries for 1 min and sonicated in water for 20 s. The oxidized CNTs were dispersed in a 1.0% W/V chitosan solution prepared in 1.0% V/V acetic acid solution by sonication for 30 min. The immobilization of CNTs was performed by casting the GCE with 10 μL of the CHI-CNT dispersion. The resulting electrodes were called GCE/CHIT-CNT. The optimum conditions were drying of the dispersion dropped onto the GCE for 45 min at room temperature followed by the reaction with 3.0% V/V GTA for 2 s (GCE/CHIT-CNT/GTA). After that, the electrode was washed by immersion in MQ water for 10 s.

For comparison, GCE/CHIT-CNT was also treated in different ways: by immersion in 1.00 M NaOH solution for 30 min or in 0.3% V/V GTA for 2 s and washed with MQ water 10 s.

2.4. Procedure

2.4.1. DNA Detection

It consisted of DNA adsorption followed by transference to supporting electrolyte for the voltammetric transduction. Some experiments were performed without medium exchange.

DNA adsorption: The given electrode was immersed in a stirred supporting electrolyte solution containing DNA and the accumulation was performed at open circuit potential for a given time. The electrode containing the adsorbed DNA layer was washed for 10 s with the buffer solution where the transduction will be performed.

Voltammetric transduction was performed by DPV under the following conditions: potential increment 0.04 V, pulse amplitude 0.05 V, pulse width 0.017 s, and pulse period 0.2 s. The anodic current at around 1.0 V, corresponding to the guanine oxidation, was used as analytical signal.

2.4.2. Linear Sweep Voltammetry Experiments

They were carried out in 20 mM H_2O_2 prepared in phosphate buffer solution (0.10 M, pH 7.40) between -0.250 V and 1.000 V at 0.100 Vs^{-1}.

2.4.3. SECM Experiments

Principles of SECM: The feedback mode is the main quantitative operation mode of SECM and it is essentially based on the measurement of the current produced when the tip is brought close to the substrate in the presence of a redox mediator [42, 43]. The potentials of the tip and substrate electrodes are controlled with a bipotentiostat to ensure that the reactions at both, the tip and substrate, occur at diffusion-controlled rates. The oxidation or reduction of the redox mediator is produced at the tip, while at the substrate, only when the electrodes are very close, the applied potential permits the regeneration of the redox mediator, i.e., a feedback between the electrodes is taking place. Thus, when the tip is far from the substrate and an adequate potential is applied, the steady-state current ($i_{T,\infty}$), is given by $i_{T,\infty} = 4nFDCa$ (where F is the Faraday constant, n is the number of electrons transferred in the tip reaction, D is the diffusion coefficient of electroactive species, C is the bulk concentration of the species and "a" is the tip radius). In the positive feedback case, a current higher than $i_{T,\infty}$ is observed ($i_T > i_{T,\infty}$) when the tip is brought close to the substrate, meaning that the substrate acts as a conductive surface producing an additional flux of the redox mediator at the tip surface. On the contrary, in the negative feedback case, a current lower than $i_{T,\infty}$ is observed ($i_T < i_{T,\infty}$) when the tip is close to the substrate, meaning that the substrate acts as an electrical insulator hindering the flux of the redox mediator at the tip surface.

SECM Experimental Procedure: The experiments were carried out in 0.10 M phosphate buffer solution pH 7.40, using FcOH as redox mediator. The tip potential was held at 0.500 V to produce the oxidation of FcOH, while the substrate potential was held at 0.000 V to permit the feedback between the electrodes, since $FcOH_{ox}$ generated at the tip is reduced at this potential regenerating the parent FcOH.

Part of the CHIT-CNT film (no more than 1/3 of the surface) was removed from the glassy carbon modified electrode. Then, an approach curve was conducted on the exposed glassy carbon surface at a tip scan rate of 0.5 μm/s. The tip was stopped when i_T reaches 1.25 times the value of $i_{T,\infty}$. According to the theoretical curve that describes the dependence of the i_T with the distance between the tip and the substrate (d), 1.25 times of $i_{T,\infty}$ corresponds to a $d \approx$ 10 μm, when a 5 μm tip radius is used [44].

After the approach curve, the tip was moved in the x-direction to make sure that the tip is over the film and a series of constant height 100 μm × 100 μm area SECM images were recorded at a tip scan rate of 1 μm/s.

The results are presented in the dimensionless form of I_T, by normalizing the experimental feedback current (i_T) by the steady-state current obtained when the tip was far from the substrate ($i_{T,\infty}$), i.e., $I_T = i_T/i_{T,\infty}$

3. Results and Discussion

3.1. Characterization of the GCE Modified with CHIT-CNT Films

Since CNTs have important catalytic effects on the oxidation of hydrogen peroxide [10, 17, 18], the presence of CNTs on the glassy carbon surface was evaluated from linear sweep voltammetry experiments in 20 mM hydrogen peroxide solution. Figure 1 shows the voltammetric response of H_2O_2 obtained at GCE (dotted line), GCE/CHIT (dashed line) and GCE modified with 0.50 mg/mL CNT dispersed in CHIT (solid line). While the oxidation of hydrogen peroxide starts at 0.520 V at GCE, a drastic shifting to more positive potentials (0.655 V) was observed at GCE/CHIT electrode, indicating that the chitosan film blocks in some degree the redox response of H_2O_2 on the GCE surface. This behavior has been previously observed for other redox systems [40].

In the presence of CNT a catalytic effect is observed for both the electrooxidation and the electroreduction of H_2O_2. The oxidation starts at 0.470 V while the reduction starts at -0.040 V. For GCE modified with CNT-CHIT dispersions containing different amounts of carbon nanotubes (0.25, 0.50, 1.00, 1.50 and 2.00 mg/mL), the oxidation and reduction signals for hydrogen peroxide increase with the increment in the amount of CNTs, although the corresponding overpotentials remain almost constant for dispersions containing more than 0.25 mg/mL CNTs (not shown). This fact

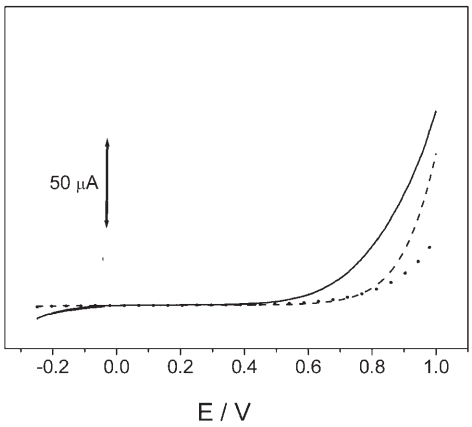

Fig. 1. Linear sweep voltammograms of GCE (dotted line), GCE/CHIT (dashed line), and GCE/CHIT-CNT (0.50 mg/mL CNT) (solid line) in 20 mM H_2O_2 prepared in 0.10 M phosphate buffer solution pH 7.40. Scan rate: 0.100 Vs^{-1}. Treatment with GTA: 3.0% V/V GTA for 2 s.

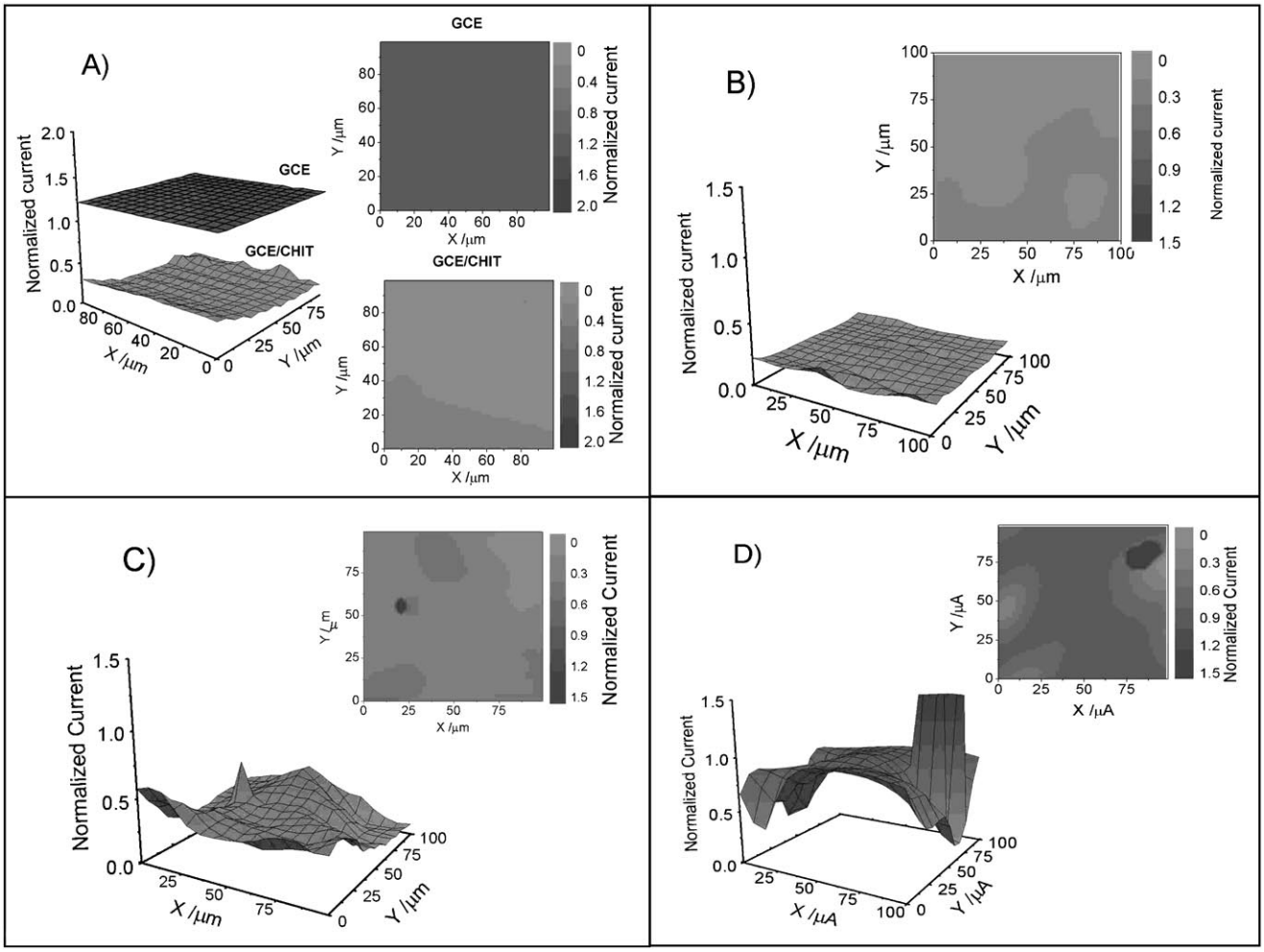

Fig. 2. SECM surface-plot images recorded using the feedback mode in solution of 1.0 mM ferrocene methanol of A) GCE and GCE/CHIT/GTA electrodes; B) GCE/CHIT-CNT/GTA (0.25 mg/mL CNT); C) GCE/CHIT-CNT/GTA (0.50 mg/mL CNT); D) GCE/CHIT-CNT/GTA (1.00 mg/mL CNT). Experimental conditions: supporting electrolyte 0.050 M phosphate buffer pH 7.40; tip carbon fiber of 5 μm radius; Image parameters: 100 × 100 μm at 1 μm/s tip scan. Insets show the corresponding contour images. Treatment with GTA: 3.0% V/V GTA for 2 s.

demonstrates that the polymeric layer does not impair the electrocatalytic activity of the dispersed CNTs.

The glassy carbon surface modified with different CNTs dispersions was also studied by SECM. Figure 2 displays the images obtained when the tip is scanned in close proximity (ca. 10 μm) to the substrate electrode: GCE (A), GCE/CHIT (A) or GCE/CHIT-CNT with different content of CNT (B, C and D). From Figure 2A it is possible to observe that GCE and GCE/CHIT present a homogeneous electrochemical activity. In fact, the bare GCE image presents a typical substrate conductive behavior, with current values of 1.25 times the steady-state current, i.e., the $I_T = 1.25$. When chitosan is present at the GCE surface the normalized current decreases from 1.25 to 0.30. This behavior is compatible with a negative feedback between the tip and the substrate indicating that CHIT partially blocks the electrochemical response of FcOH. When CNTs are present on the surface of GCE the images show that the normalized currents increase with the amount of CNT, although they never reach the values obtained for GCE (Fig. 2B, C and D). In all cases, regions of different electroactivity can be found according to the distributions of CNTs on the GC surface.

3.2. Electrochemical Behavior of dsDNA at GCE Modified with CHIT-CNT Films Treated with GTA and NaOH

The exposure of CGE/CHIT-CNT to DNA solutions prepared in acidic media produces the dissolution of chitosan and consequently the loss of the film [41]. For this reason, different chemical treatments were performed to improve the film stability before the adsorption of DNA. Two strategies were used to stabilize the CHIT-CNT film, through chitosan hydrogels formation by the addition of glutaraldehyde as covalent cross-linker [45] or by immersion in a 1.0 M NaOH solution [46].

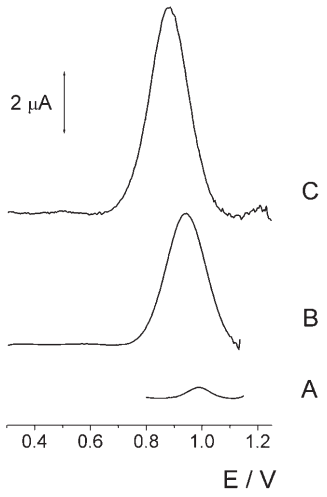

Fig. 3. Differential pulse voltammograms obtained at A) glassy carbon electrode (GCE), B) GCE/CHIT/GTA, and C) GCE/CHIT-CNT/GTA (0.50 mg/mL) after 3 min accumulation in 30.0 ppm dsDNA at open circuit potential with transference to 0.20 M sodium formate pH 5.00. Treatment with GTA: 3.0% V/V GTA for 2 s.

The presence of DNA adsorbed at GCE/CHIT-CNT/GTA was evaluated by DPV from the oxidation of guanine residues. Figure 3 shows differential pulse voltammograms for calf-thymus double stranded DNA obtained after 3 minutes accumulation at open circuit potential at different electrodes: (A) GCE, (B) GCE modified with 10 μL chitosan (1.0% W/V in a 1.0% V/V acetic acid) and treated with 3.0% V/V GTA (2 sec) and (C) GCE modified with 10 μL of the CNT-CHIT dispersion (0.50 mg/mL) and treated with 3.0% V/V GTA (2 sec). As expected [47], at bare GCE the guanine residues are oxidized at 0.988 V with a peak current (i_p) of (0.33 ± 0.01) μA. When the chitosan film is adsorbed and treated with GTA, the oxidation current of dsDNA increases almost 10 times compared to that obtained at GCE ($i_p = (4.1 \pm 0.1)$ μA) and the peak is wider. This increase in current indicates that in the presence of CHIT treated with GTA the amount of adsorbed DNA also increases.

Several processes could be responsible for the interaction of DNA with CHIT after treatment of CNT-CHIT film with GTA. The most important would be the covalent binding between amine residues of dsDNA bases and the groups obtained after the activation of the amine CHIT residues with GTA. The presence of free unreacted GTA within the hydrogel can not be completely excluded, and these molecules of GTA could be accessible for the interaction with DNA bases allowing the accumulation of more DNA molecules. Zeiger et al. [48] have reported the reaction of glutaraldehyde with deoxyribonucleosides at pH 6.5 in solution at 37 °C to form reaction products with deoxyadenosine, deoxycytidine, and deoxyguanosine. Finally, the electrostatic interaction between unreacted amine residues (positive) of CHIT and DNA, could be another mechanism, although this effect would be less important due to the massive reaction with GTA.

In the presence of CNTs (C) the current due to guanine oxidation is almost 20 times higher than that at GCE (6.519 ± 0.004) μA vs. (0.33 ± 0.01) μA. In addition, a negative shifting in the peak potential of around 45 mV (average) is observed when CNTs are present at the electrode surface, suggesting that CNTs not only facilitates the adsorption of DNA but also the electron transfer to the electroactive residues.

Figure 4 displays DPVs for 60 ppm dsDNA adsorbed for 5 min at CGE/CHIT-CNT after treatment with 1.0 M NaOH for 30 min or GTA (0.3 or 3.0% V/V) for 2 s. It is clear that the treatment of the GCE/CNT-CHIT has a strong influence on the adsorption of DNA and further electrochemical response. The electrodes immersed in NaOH show a signal for guanine oxidation remarkable small and a shifting in the oxidation peak potential of 140 mV in the negative direction compared to the electrodes treated with GTA. Moreover, the voltammetric response for dsDNA accumulated at GC/CHIT and GC/CHIT-CNT electrodes treated with 1 M NaOH for 30 min present the same peak current value ($i_p =$ 1.2 μA for dsDNA 30 ppm, not shown). The increment of the pH of the film produces some neutralization of the positive charges of CHIT, and as a consequence of that, there is a decrease of the electrostatic adsorption of DNA. This fact is an indication that in this case the DNA adsorption step is limiting the electroanalytical response [46]. Smaller DNA oxidation signals are obtained when using more diluted GTA solution (0.3% V/V), although even after treatment with 0.3% V/V GTA it is possible to enhance the DNA signal by increasing the interaction time with GTA (not shown). Another interesting result is that the DNA oxidation peak potential obtained for GTE/CNT-CHIT treated with GTA is higher than that obtained for

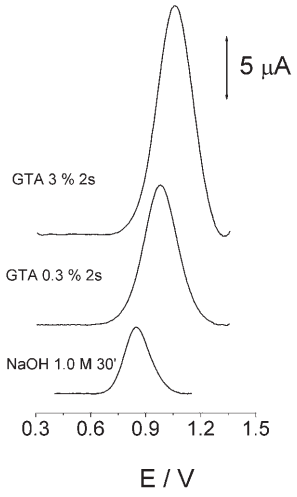

Fig. 4. Differential pulse voltammograms obtained at GCE/CHIT-CNT (0.50 mg/mL) electrodes treated with NaOH 1.0 M for 30 min; 0.3% V/V GTA for 2 s and 3.0% V/V GTA for 2 s after the adsorption from 60 ppm dsDNA solution for 5 min at open circuit potential.

electrodes treated with NaOH, suggesting an important compromise of the bases in the interaction with GTA. Therefore, the treatment with 3.0% V/V GTA for 2 sec was selected as optimum.

The dsDNA-accumulation time at GCE/CHIT-CNT/GTA plays an important role on the guanine oxidation signal. Figure 5 shows that the voltammetric signal increases up to 5 min, leveling off for longer times. Three minutes were selected as optimum considering further analytical applications.

The effect of the medium exchange was also evaluated. The dsDNA was accumulated for 3 min at open circuit potential and then it was transferred to the formate buffer solution (0.20 M pH 5.00). Under these conditions, the current increased 15% (not shown), suggesting a rearrangement of the adsorbed DNA layer in a conformation more accessible for electrooxidation. Consequently, subsequent work was done with medium exchange.

3.3. Effect of CNT Amount and the Chitosan Molecular Weight on the dsDNA Response

The influence of the amount of CNTs in the dispersion on the adsorption and electrooxidation of dsDNA was also studied (Fig. 6A). At variance with the behavior observed for hydrogen peroxide (Figure 1), no significant differences were observed for electrodes prepared with diverse amounts of CNTs (0.25, 0.50, 1.00, 1.50 and 2.00 mg/mL). Hydrogen peroxide is a small molecule that can easily diffuses through the structure of the film. However, in the case of dsDNA, the main event is the adsorption at the electrode surface facilitated by the interaction with GTA.

In addition, the influence of the CHIT molecular weight used to disperse the CNTs on the oxidation of dsDNA was investigated. Figure 6B shows the average of the DPV response obtained with 0.50 mg/mL CNT-CHIT dispersions using CHIT of different molecular weight (MW), high, medium and low. An increase of 40% in the oxidation current of dsDNA was found when using GCE modified with dispersions prepared with medium MW CHIT in comparison with low MW CHIT. A small increment was observed using GCE modified with CNTs dispersions prepared with high MW chitosan. These results suggest that the presence of a larger number of amine residues facilitates the adsorption of DNA. The selected CHIT was the one of medium molecular weight.

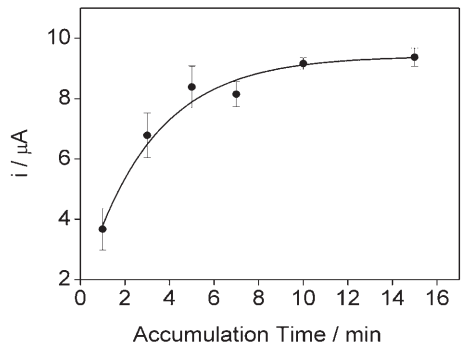

Fig. 5. Dependence of the oxidation currents with the accumulation time from a 30 ppm dsDNA solution at open circuit potential on GCE/CHIT-CNT/GTA electrode without medium exchange. Other experimental conditions as in Figure 3.

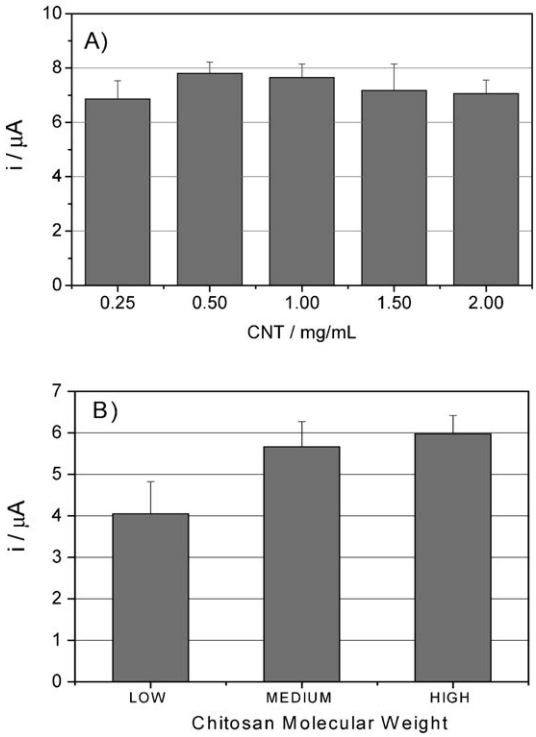

Fig. 6. DPV oxidation currents at GCE/CHIT-CNT after the adsorption of 30 ppm dsDNA and transference to 0.20 M formate buffer pH 5.00 as function of A) CNT concentration in the dispersion and B) chitosan molecular weight employed in the dispersion. Other experimental conditions as in Figure 3.

3.4. Analytical Applications

Figure 7 shows a calibration plot for dsDNA performed using a GCE modified with 0.50 mg/mL CNT-CHIT dispersion treated with GTA after 3 min accumulation. A linear relationship was obtained up to 90.0 ppm dsDNA with a sensitivity of (0.2376 ± 0.0008) μA L mg^{-1}, $r = 0.99997$.

The stability of the dispersion was evaluated during 30 days from the oxidation signal of dsDNA. The oxidation current of dsDNA decreased 33% after three days, to remain almost constant thereafter (not shown). The inter-electrode reproducibility was evaluated using 5 different GCE/CHIT-CNT/GTA. The average of the currents obtained after 3 min of dsDNA (30.0 ppm) accumulation was (5.8 ± 0.2) μA with a *RSD* of 3.4%, indicating that the whole protocol for preparing the DNA-modified-GCE/CHIT-CNT is highly reproducible. The reproducibility in the preparation of the CNT-CHIT dispersions was analyzed by using GCE modified with three fresh dispersions from the

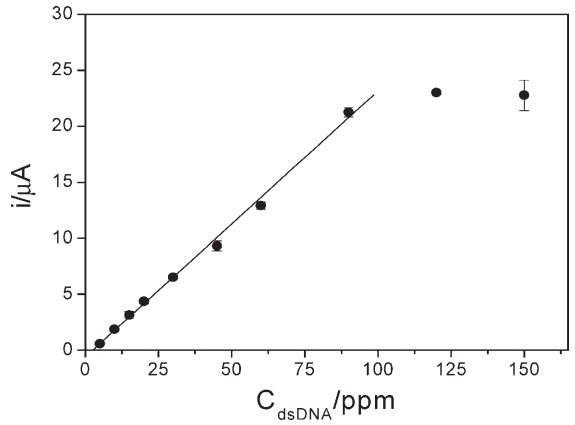

Fig. 7. Calibration plot obtained at GCE/CHIT-CNT electrode for dsDNA after medium exchange to a 0.20 M formate buffer solution pH 5.00. Other experimental conditions as in Figure 3.

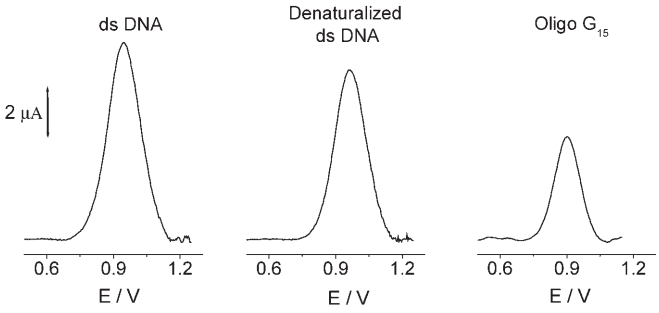

Fig. 8. Differential pulse voltammograms of GCE/CHIT-CNT (0.50 mg/mL) electrodes after adsorption of 30 ppm native (A) and denatured (B) dsDNA and 10 ppm oligo (dG_{15}) (C) and transference to 0.20 M formate buffer pH 5.00. Other experimental conditions as in Figure 3.

signal of dsDNA after the adsorption for 3 min at open circuit potential and medium exchange. The i_p values for the electrodes prepared with the different dispersions were: (8.6 ± 0.2) μA, (7.8 ± 0.4) μA and (7.5 ± 1.5) μA indicating an adequate reproducibility (*RSD* of 7%) in the methodology employed.

The behavior of different nucleic acids was also evaluated. Figure 8 shows the response of GCE/CHIT-CNT/GTA to different nucleic acids: dsDNA, denatured dsDNA and oligo$(dG)_{15}$. Very sensitive signals were obtained in all cases, although, at variance with the direct adsorption at GCE, the signal for single stranded DNA is slightly smaller than that for dsDNA. At this stage we have not a conclusive explanation for this behavior. It could be due to the rigidity inherent to dsDNA compared to ssDNA that would make more important the compromise of its bases in the covalent bond with CHIT-GTA.

4. Conclusions

In summary, we are reporting a new alternative for the successful quantification of nucleic acids. The performance of the glassy carbon electrode modified with CNT/CHIT as a new platform for the immobilization of DNA has been demonstrated, in this case in connection with dsDNA, ssDNA and oligo$(dG)_{15}$. The treatment of the CNT-CHIT has demonstrated to be a very important variable, being the cross-linking with 3.0% V/V glutaraldehyde, the best one. The DNA immobilized at the glassy carbon electrodes modified with CNT-CHIT dispersion and treated with GTA was evaluated from the direct oxidation signal of the guanine residues without needing of redox markers. This new platform opens the doors to new strategies for the development of biosensors based on the use of dsDNA as analytical tool.

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