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Ethylendiamine-functionalized multi-walled carbon nanotubes prevent cationic dispersant use in the electrochemical detection of dsDNA

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ABSTRACT

In this paper, for the first time, an electro-analytical method based on the direct adsorption of double-stranded deoxyribonucleic acid (dsDNA) at glassy carbon electrode modified with ethylendiamine (eda) functionalized multi-walled carbon nanotubes (eda-CNT) is reported. The carbon nanotubes were functionalized with ethylendiamine via chemical modification of the carboxyl groups, and the eda-CNTs were characterized by different techniques, including Fourier transform infrared (FT-IR) spectroscopy, thermo gravimetric analysis (TGA), elemental analysis and cyclic voltammetry, and compared with pristine (CNT) and oxidized (ox-CNT) MWCNTs.

The presence of eda on the CNT surface allows them to disperse in a phosphate buffer solution at pH 7.0 and provides an environment that promotes the electrostatic adsorption of dsDNA. At eda-CNTs, the adsorption of dsDNA is improved and a linear correlation between the oxidation current of guanine bases and the accumulation time is observed. This result indicates that the presence of positive charges on the surface of the nanotube plays an important role in the attraction of the dsDNA molecule.

A more sensitive detection of DNA was obtained compared with CNT and ox-CNT when eda-CNT where used with a linear range from 5 to 60 ppm, a sensitivity of $0.0315 \pm 0.0003\ \mu A\ mg^{-1}\ L$ and a LOD of 0.971 ppm after a 10-min accumulation which is lower than that obtained previously using cationic dispersing agents. The analytical performance reported is comparable with that reported previously for cationic polymers in terms of linear range and LOD values.

1. Introduction

Carbon nanotubes (CNTs) are widely used in the electrochemical field due to their favorable properties, such as enhanced detection sensitivity, electrocatalytic effects and reduced fouling [1–3]. Moreover, CNTs have proven to be good modifiers as electrode materials, mainly because of their chemical stability, wide electrochemical potential window, and biocompatibility [1,4,5]. Currently, the poor dispersibility of these nanostructures in both organic and inorganic solutions is one of the biggest obstacles to their use in biosensors. To use CNTs in the electrochemical evaluation of DNA, it was necessary to improve the dispersibility of the nanotubes in aqueous solution and to introduce positive charges to promote the adsorption of biological molecules onto the resulting surface. Different research lines have been used to overcome this difficult problem. For example, non-covalent surface modification of the CNT with surfactants, polymers, etc., was used [6–9]. In the development of DNA biosensors, these systems were tested to both improve the dispersibility of the nanotubes and to allow the adsorption of DNA molecules [8–11]. Bollo et al. [8] studied the electro-oxidation of dsDNA onto a MWCNT-chitosan/glassy-carbon electrode by direct adsorption of the macromolecule. The presence of a positively charged film, due to the charges in the polymer, favored the adsorption of the dsDNA onto the modified-electrode.

Although non-covalent surface modification is readily achieved, covalent CNT functionalization can be better controlled to produce more stable nanomaterials [12]. Commonly, the first step in this method is to incorporate carboxylic acid groups (CNT-COOH) that can be, in a second step, derivatized by others molecules [6,12–14]. An example of such functionalization is the attachment of an amide bond. This can be accomplished, for example, by activating the carboxylic acid group with thionyl chloride ($SOCl_2$) and then covalently attaching any (bio)molecule that contains a free-amine group [13,15]. Vucović et al. [16] attached amines onto MWCNTs, thereby improving their dispersibility in aqueous media. Singh et al. [17] reported that SWCNTs functionalized with ammonium are capable of penetrating human cells and facilitating DNA delivery. Previous reports [17–19] have shown that for positively charged

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CNTs (CNT-NH_3^+) the electrostatic interactions prevail over the hydrophobic ones. Yang et al. [20] compared the adsorption of DNA, in solution, onto CNTs with and without charged amine groups. The results demonstrated that the amount of adsorbed DNA is four times higher for CNT-NH_3^+ compared to CNT, thus demonstrating the importance of electrostatic interactions. In 2006 Yang et al. [21] reported the electrooxidation of dsDNA by a CNT-based electrode modified with ethylenediamine via electrochemical synthesis. Although a well-defined oxidation peak corresponding to dsDNA oxidation was observed, no analytical performance was reported for the electrode, and the linear range was restricted up to 40 ppm. The remainder of the paper describes the study of the interaction between promethazine hydrochloride and immobilized dsDNA.

Therefore, covalent surface modification of CNTs with positively charged functional groups, e.g., amines, allows us to improve the dispersibility of CNTs in aqueous media and to avoid the use of a dispersant agent that could block the electroactive surface of the modified-electrode [3]. Additionally, amine-functionalized CNTs are suitable for the immobilization of molecules by electrostatic interaction [22,23], thus favoring the adsorption of DNA molecules (either single or double stranded DNA) to generate biosensors for future applications in studies of DNA damage or hybridization.

In this paper, the preparation of ethylendiamine-functionalized multi-walled carbon nanotubes (eda-CNT) is described. The first step was to introduce oxidative functional groups by chemical oxidation, and then, ethylendiamine was directly coupled through an amide bond. The presence of different functional groups in the MWCNTs was chemically and electrochemically characterized and compared with pristine CNTs. These functionalized-CNTs were dispersed in aqueous media and used to generate modified-glassy carbon electrodes for the immobilization of calf-thymus double-stranded deoxyribonucleic acid (dsDNA). Electrooxidation of the adsorbed dsDNA was studied by differential-pulse voltammetry. The effect of the concentration of CNTs onto the electrode and the accumulation time of the dsDNA onto the modified-electrodes was investigated.

2. Experimental

2.1. Apparatus

Fourier-transform infrared (FTIR) spectra were obtained using a Bruker Vertex 70 spectrometer. All samples were prepared as pellets using spectroscopic grade KBr. Elemental analysis was performed with a Flash 1112 analyzer from Thermo Fisher Scientific. Thermo gravimetric analysis (TGA) was performed using a Setaram SETSYS Evolution 16/18 analyzer. The samples were scanned within the temperature range of 0–1500 °C at a heating rate of 5 °C min^{-1}. Cyclic voltammograms (CV) and differential pulse voltammograms (DPV) were recorded using a CHI 900 setup (CH Instruments Inc., USA). A three-electrode cell was used with an Ag/AgCl, 3 M NaCl (BAS) and a platinum wire as a reference and auxiliary electrodes, respectively. The working electrode was a glassy carbon electrode (GCE, CH. Instrument) modified with MWCNTs. A magnetic stirrer provided the convective transport when necessary.

2.2. Reagents

MWCNT (1–5 μm long and 30 ± 15 nm diameter) was provided by NanoLab (USA). Ethylenediamine (eda) and thionyl chloride ($SOCl_2$) were obtained from Sigma–Aldrich. Calf-thymus double-stranded DNA (dsDNA) (activated and lyophilized, Cat. N° 4522) was from Sigma. Stock solutions of dsDNA (1000 ppm) were prepared with Tris–EDTA buffer solution (20 mM Tris–HCl, 1 mM EDTA, pH 8.0). A 0.2 M phosphate buffer solution (PBS), pH 7.4 was used as a supporting electrolyte for the DNA studies. All the other chemicals, such as H_2SO_4 and HNO_3, were of analytical grade and used as received. All solutions were prepared with ultrapure water (ρ = 18 MΩ cm) from a Millipore-Milli-Q system.

2.3. Surface modification of MWCNTs

2.3.1. Oxidation of MWCNTs (ox-CNTs)

A small fraction of the MWCNTs (<100 mg) were immersed in a 50 mL H_2SO_4/HNO_3 (3:1) solution in a round-bottomed glass flask. The mixture was heated to ~110 °C for 3 h, and the experiment was considered to have started at the moment the first reflux drops appeared. Oxidized MWCNTs (ox-CNTs) were filtered and washed thoroughly with deionized water until a neutral pH was reached, after which they were dried at 50 °C for 24 h.

2.3.2. Amidation of MWCNTs (eda-CNTs)

ox-CNT were modified with eda in a two-step reaction (Scheme 1). First, 100 mg of ox-CNTs were refluxed with 20 mL of $SOCl_2$ for 24 h. The temperature was controlled between 65 and 70 °C. After the reaction, the remaining $SOCl_2$ was evaporated and MWCNT-COCl was obtained. Second, 20 mL of eda was added to MWCNT-COCl and the mixture was heated between 30 and 40 °C for 12 h. The unreacted eda was eliminated by evaporation to dryness. The eda-CNTs were washed with 0.1 M HCl, Milli-Q water and acetone and dried under vacuum at 50 °C for 24 h.

2.4. Characterization of functionalized MWCNTs

Pristine and functionalized MWCNTs samples were characterized by several techniques. FTIR and elemental analysis were used to estimate the surface groups on the CNTs before and after chemical treatment. The Kaiser Test was used to estimate the free amine groups on the ethylendiamine-functionalized CNTs. Thermogravimetric analysis (TGA) provided information on the changes in the thermal stability of the samples. Finally, cyclic voltammetry was used to determine the electrochemical behavior of the MWCNTs.

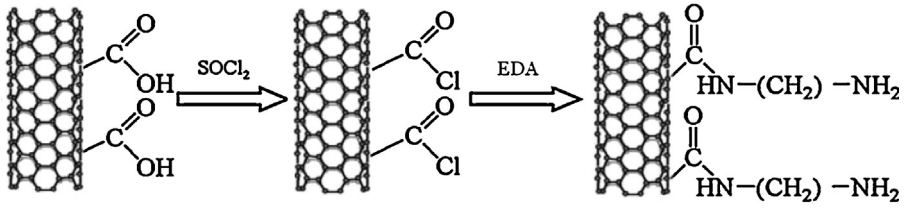
2.5. Interaction between dsDNA and MWCNTs in solution

Pristine CNTs, ox-CNTs and eda-CNTs were dispersed in 0.2 M formiate buffer solution (FBS), pH 5.0 by sonicating for 45 min with a concentration of 1 mg mL^{-1}. The dispersions were then mixed with a dsDNA solution (45 ppm). Three samples of 2 mL were prepared: (1) 45-ppm dsDNA solution in 0.2 M FBS, pH 5.0; (2) CNTs in 0.2 M FBS, pH 5.0 (1 mg mL^{-1}); and (3) 45-ppm dsDNA solution + CNTs (1 mg mL^{-1}) in 0.2 M FBS, pH 5.0. The mixture of CNT and dsDNA was achieved by using a vortex system for 5 min to obtain a homogeneous dispersion. The samples were incubated for 1 h and 24 h in darkness at room temperature and under controlled stirring. Then, the samples were ultracentrifuged at 13500 rpm for 150 min. The supernatant was collected, and the absorbance of the samples at 260 nm was measured. Comparing the results between samples (1) and (3), we calculated the amount of dsDNA that does not interact with the CNTs.

2.6. Electrochemical measurements

2.6.1. Modification of GCEs with pristine CNTs, ox-CNTs and eda-CNTs

1 mg of Pristine CNTs, ox-CNTs and eda-CNTs were dispersed in 1 mL of 0.1 M PBS (pH 7.4) by sonication for 15 min. The sonication procedure was repeated thrice. Prior to surface modification, each GCE were polished with 0.3 and 0.05-μm alumina slurries for 1 min. The immobilization of CNT was performed by casting the GCE with 8 μL of the CNT dispersion. The optimum conditions



Scheme 1. Schematic illustration showing the preparation of ethylendiamine-functionalized (eda-*CNT) MWCNTs from oxidized carbon nanotubes (ox-CNT).

involved drying the dispersion onto the GCE for 15 min at 50 °C. The resulting modified electrodes were called GCE/CNT, GCE/ox-CNT and GCE/eda-CNT.

2.6.2. Electrochemical detection of dsDNA onto CNTs-modified electrodes

DNA adsorption: The given electrode was immersed in a stirred supporting electrolyte solution containing 45 ppm of dsDNA, and accumulation was allowed at open circuit potential for a given time. The electrode containing the adsorbed DNA was washed for 10 s using buffer solution and transferred into a buffer solution cell and a differential pulse voltammogram (DPV) was performed. The experiments were conducted in quadruplicate.

DPV operating conditions: potential increment of 0.004 V, pulse amplitude of 0.05 V, and pulse period of 0.2 s. The anodic current was approximately 1.0 V, which corresponds to the guanine oxidation that was used as an analytical signal.

3. Results

3.1. Chemical characterization of CNTs

To corroborate the incorporation of ethylendiamine onto the MWCNTs surface, FT-IR was performed for the pristine carbon nanotubes (MWCNT) and their derivates, ox-CNT and eda-CNT; the corresponding results are shown in Fig. 1.

In our previous work [2], we performed a complete characterization of both MWCNT and ox-CNT samples to determine the optimum conditions for incorporation of carboxylic acids into the nanotube structure. According to those results, and as observed in Fig. 1, CNTs present a few bands, indicating the presence of small fractions

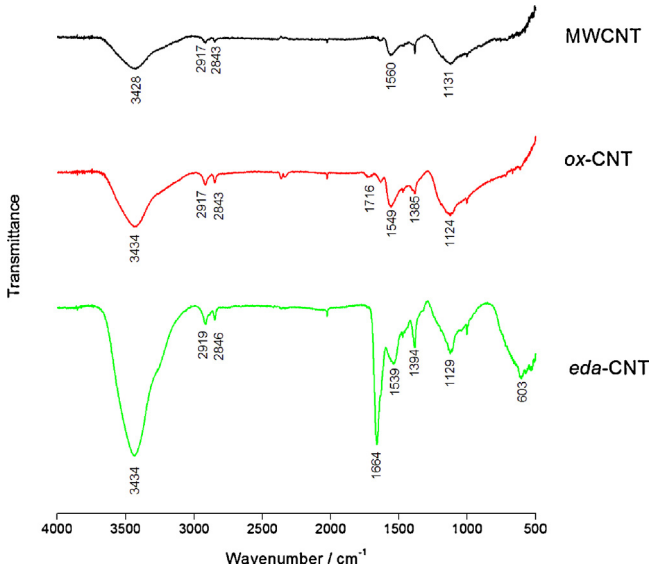


Fig. 1. FTIR spectra of pristine MWCNT, ox-CNT and eda-CNT.

of functional groups most likely incorporated during their synthesis process. After chemical oxidation, there was an increase in the bands at approximately 3400 cm^{-1} and 1100 cm^{-1}, and a new band developed at approximately 1700 cm^{-1} that corresponds to carboxylic acid groups. The band observed at 3434 cm^{-1} was assigned to the O—H stretching increase for ox-CNT, which indicates the presence of different oxygenated functional groups (e.g., carboxylic acids, hydroxyl and phenol groups). After chemical oxidation, all the bands between 1300 and 1050 cm^{-1} that were associated with ether, alcohol and phenol groups increased their intensity.

The FT-IR spectrum of the eda-CNTs exhibited a broad peak at 3434 cm^{-1} that is associated with the N—H bond stretching, which overlapped with the O—H stretching present in CNTs and ox-CNTs. The N—H bond also presents a band at 1539 cm^{-1} that is assigned to the N—H in-plane deformation for primary amines. The functionalization of ox-CNTs with ethylendiamine is confirmed with the appearance of a new band at a lower frequency (1664 cm^{-1}) corresponding to the stretching of the amide carbonyl group (N—C═O) and the absence of the band at 1716 cm^{-1} assigned to C═O in the —COOH groups. The presence of the amide bond is also verified by two bands, one at 603 cm^{-1} corresponding to the deformation of the N—H bond of secondary amines and another band at 1539 cm^{-1} that is associated with the deformation of the C—N bond. Finally, we observed a band at 1124 cm^{-1} that could be assigned to the C—N stretching of the carbon atom bonded to the amino-terminal group [24].

Elemental analysis performed on the MWCNT, ox-CNT and eda-CNT samples (Table 1) indicate that MWCNTs have small quantities of oxygenated groups, which increased by three times when they were treated with a sulfo-nitric mixture (also shown in Fig. 1). When ox-CNTs were functionalized with ethylendiamine, an increase in the percentage of nitrogen and hydrogen was observed, which indicates that the incorporation of the eda chain to the sample was successful.

Those results were also observed by Montesa et al. [25] with single-walled carbon nanotubes (SWCNTs) functionalized with amines. Furthermore, the decrease observed in the oxygen content of the eda-CNTs compared to that of the ox-CNTs may also provide evidence for the covalent attachment of the amines [26].

The surface content of the amino-terminal functions present on the eda-CNTs was determined with the aid of the quantitative Kaiser test [27]. The amount of free amino groups was found to be $(1.9 \pm 0.2) \times 10^2$ μmol g^{-1}, which is smaller than that expected according to the N% obtained with elemental analysis. This result indicates that not all of the amino groups of ethylendiamine that are incorporated to MWCNT are in a free-form and that some of the EDA chains are most likely covalently attached between two

Table 1
Elemental analysis of MWCNTs and their derivates of ox-CNTs and eda-CNTs.

	C (%)	H (%)	O (%)	N (%)
MWCNTs	98.08	0.13	1.02	0.07
ox-CNTs	96.32	0.16	3.49	0.17
eda-CNTs	90.91	1.70	1.34	6.15

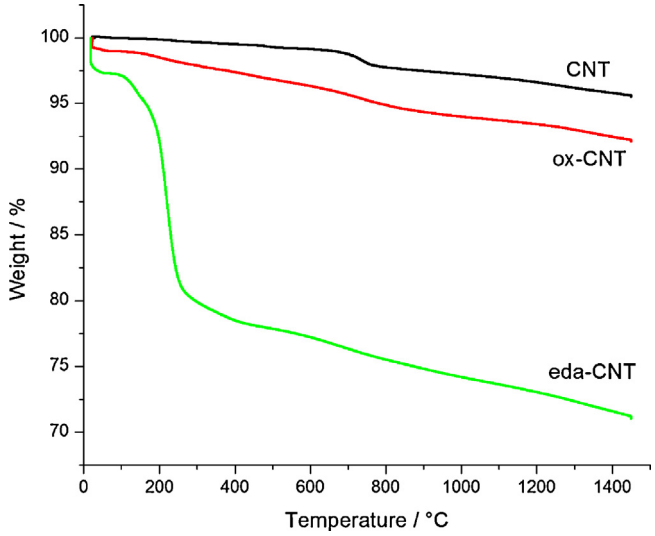


Fig. 2. Thermogravimetric curves of pristine MWCNT, ox-CNT and eda-CNT.

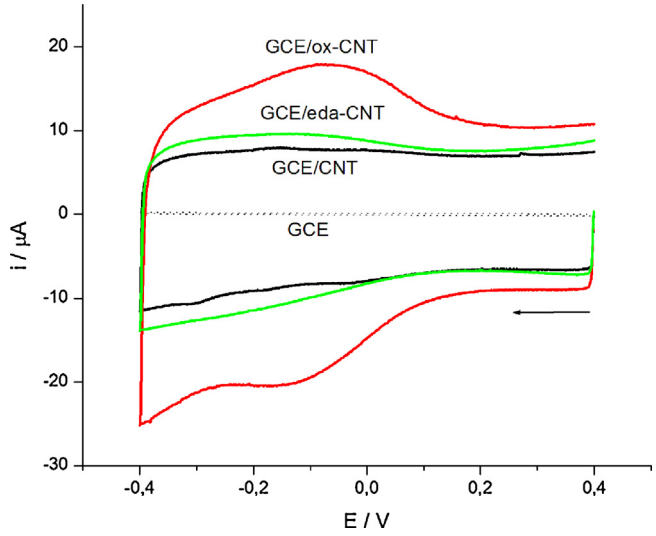


Fig. 3. Cyclic voltammograms of bare GCE and modified-GCE with CNTs (1 mg mL^{-1}): MWCNT (GCE/CNT), oxidized (GCE/ox-CNT) and eda-functionalized CNT (GCE/eda-CNT), recorded in 10 mM PBS, 0.1 M NaCl and pH 7.0. Sweep rate: 50 mV s^{-1}. The arrow indicates the direction of the scan.

nanotubes. The latter is in agreement with previously produced SWCNTs functionalized with ethylendiamine [28].

Fig. 2 shows that the thermal degradation of the ethylendiamine-functionalized CNTs is greater than that of MWCNT and ox-CNT. The pristine MWCNTs were stable and hardly decomposed under 1500 °C. Below 150 °C, a lower weight loss was observed for all samples, which corresponds to evaporation of the adsorbed water [2,29]. Between 150 °C and 350 °C, the weight loss for ox-CNTs (~1%) is primarily attributed to the CO_2 evolution of the carboxylic groups. For eda-CNTs, the weight loss in the same interval of temperature can be associated to the loss of the ethylendiamine covalently attached to the nanotube through an amide bond (18%). This result is in agreement with the FT-IR and elemental analysis results. Thermal degradation from 350 °C to 500 °C may be attributed to the elimination of hydroxyl/ether functionalities attached to the CNT walls. Finally, at temperatures between 500 °C and 600 °C, the observed degradation corresponds to the decarboxylation of lactone and anhydrides groups; while above 600 °C, the weight loss could originate from phenols, ethers and carbonyl/quinones [2]. The sample with the greatest weight loss at all temperatures was eda-CNT (>20%). Another finding is that none of the samples were completely decomposed.

3.2. Electrochemical characterization of CNTs

Cyclic voltammetry of the CNT samples was performed in 10 mM PBS, pH 7.0 and 0.1 M NaCl (Fig. 3).

The modified glassy carbon electrode that was generated with pristine CNTs (GCE/CNT) revealed no electrochemical signal in the range of −0.4 to 0.4 V, indicating that the small amounts of oxygenated functional groups observed with FT-IR are not electrochemically active. The CV profile is rectangular with good symmetry, indicating a typical electric double-layer behavior [30]. With oxidized-CNT, (GCE/ox-CNT) a redox couple was observed at approximately −0.1 V, which is similar to previous reports [2,31]. We suggest that the redox couple corresponds to the carboxylic acid groups incorporated in the CNT structure that are reduced in the first step and then oxidized, even though other oxygenated groups such as quinone could contribute because they are also electroactive [32]. However, according to Fuente et al. [33], the reduction of small pyrones is thermodynamically much easier than the reduction of large pyrone structures, such as pyrones in carbon nanotubes, which is mainly due to geometric effects. Thus, using representative quinonoid structures, the theoretical values of the potential reduction for pyrone-like groups on carbon surfaces were calculated by Fuente. These potentials exhibited different values, which were all far from the experimental data shown in Fig. 3, supporting our assumption that the redox process observed correspond to —COOH groups instead of other oxygenated groups. Additionally, Andreas et al. [34] demonstrated that the pH value of the solution affects the electrochemical behavior of carbon-based electrodes; they demonstrated that the redox peak associated to quinones is drastically reduced if the pH of the electrolyte is increased up to ca. 3. The loss of these peaks is most likely due to the lack of available protons for the oxidation/reduction of the quinone groups. In Fig. 3, a pH of 7.0 was used in the experimental conditions; therefore, the redox peaks observed cannot be related to quinones.

After modification of ox-CNTs by ethylendiamine (GCE/eda-CNT), the redox couple observed in the vicinity of −0.1 V disappears completely, indicating a voltammetric profile similar to that obtained for GCE/CNT. As the redox peaks in the vicinity of −0.1 V are associated with carboxylic acids, its disappearance as a result of the amidation reaction indicates that CNTs were covalently functionalized with ethylendiamine.

The CNT-modified electrodes were also characterized by cyclic voltammetry in PBS (0.2 M and pH 7.4) (figure not shown), with the resulting capacitance values being related to the electroactive area of each electrode. When pristine MWCNTs are used to modify the GCE, the resulting capacitance value was 310 μF. This capacitance value rises to 394 μF when the CNTs are oxidized. This result indicates an increase in both the double-layer capacitance and the pseudocapacitance due to the newly incorporated electroactive groups. The increase in the double-layer capacitance due to oxygenated functional groups is attributed to an enhanced accessibility of hydrophilic carbon coverage in the aqueous electrolyte [2]. The capacitance of the ox-CNTs is decreased when it is functionalized with ethylendiamine (250 μF). This result indicates that the access of the electrolyte to the surface is lower, which is most likely due to an increase in the packing of the nanotubes before the functionalization. A similar capacitance reduction was observed for carbon fibers after electrochemical modification by eda [35].

To determine the electron-transfer properties of the modified electrodes, a cyclic voltammetry study was conducted for the $Fe(CN)_6^{-3}/Fe(CN)_6^{-4}$ couple (figure not shown). For the GCE, the

Table 2
Percentage of the decrease in the signal DNA absorbance, at 260 nm, after interaction with each type of MWCNTs.

Incubation time	1 h	24 h
MWCNTs	25%	79%
ox-CNTs	10%	77%
eda-CNTs	33%	81%

typical cyclic voltammogram was observed. When the GCE/CNT was used, the electrochemical behavior of $Fe(CN)_6^{-3}/Fe(CN)_6^{-4}$ remained unchanged, but an increment in the current values was observed. This behavior could be explained by the increase in the electroactive area of the electrode when it is modified with carbon nanotubes, as represented in the capacitance values discussed earlier. With the GCE/ox-CNT, the voltammetric profile exhibits higher current values than GCE/CNT, due to the increase in the electroactive area. For GCE/eda-CNT, the intensities of the redox peaks of $Fe(CN)_6^{-3}/Fe(CN)_6^{-4}$ are higher than those of GCE/CNT. Although GCE/eda-CNT has a smaller electroactive surface according to the capacitances and compared with GCE/CNT, their positive charges apparently played an important role. The presence of positive charges on the surface of the nanotube, due to the amine groups, could electrostatically attract the negative charges of the $Fe(CN)_6^{-3}/Fe(CN)_6^{-4}$ couple. Thus, the redox mediator is attracted to the surface in quantities greater than for GCE/CNT, which is uncharged. The loss of the electroactive area with the ethylendiamine incorporation does not block the approach of $Fe(CN)_6^{-3}/Fe(CN)_6^{-4}$. The positive charges on the nanotube improve the response of the modified-electrode compared with the bare GCE and the GCE/CNT.

3.3. dsDNA/CNTs interaction in solution

The pristine CNTs exhibit fewer side defects compared to the functionalized CNTs; theoretical studies demonstrated that the interaction between pristine MWCNTs and dsDNA occurs mainly by Van der Waals interaction [36]. In this sense, the presence of functional groups in the CNT structure could disfavor the interaction between the bases of the dsDNA and the surface of the nanotube. In the specific case of ox-CNTs, the interaction between negative dsDNA (due the phosphate backbone) and negative ox-CNTs should diminish primarily by electrostatic repulsion. However, eda-CNT has positively charged functional groups, which could attract the dsDNA to the eda-CNT [19]. According to these assumptions, the order of interactions of the CNTs with dsDNA is expected to be ox-CNTs < pristine CNTs < eda-CNTs.

Studies regarding the interaction between dsDNA and different CNTs in solution were conducted according to a modified version of a protocol from Xiao et al. [20]. The experimental pH value of 5.0 ensures the presence of charged functional groups on both ox-CNTs and eda-CNTs (—COO^- and NH_3^+, respectively). Three samples were prepared in 0.2 M FBS as described in experimental section, and the decreases in the maximum absorbance observed for dsDNA after incubation with CNTs for 1 h and 24 h are summarized in Table 2.

From the data shown in Table 2, the interaction of dsDNA with the different CNTs is clearly dependent on the incubation time. For 1 h of incubation time, it is possible to distinguish between the effects of the functional groups on the interaction of the nanotube with dsDNA. The eda-CNT has the highest percentage of signal loss (33%), followed by pristine CNT (25%) and ox-CNT (10%). These results allowed us to infer that with 1 h of incubation time, there is a predominance of electrostatic interactions between the amine groups of eda-CNT and the phosphate backbone of dsDNA. This would allow a greater amount of DNA to be adsorbed compared to the amounts adsorbed onto pristine CNTs or ox-CNTs. The negative charges of the ox-CNTs diminished the interaction with dsDNA, as we expected.

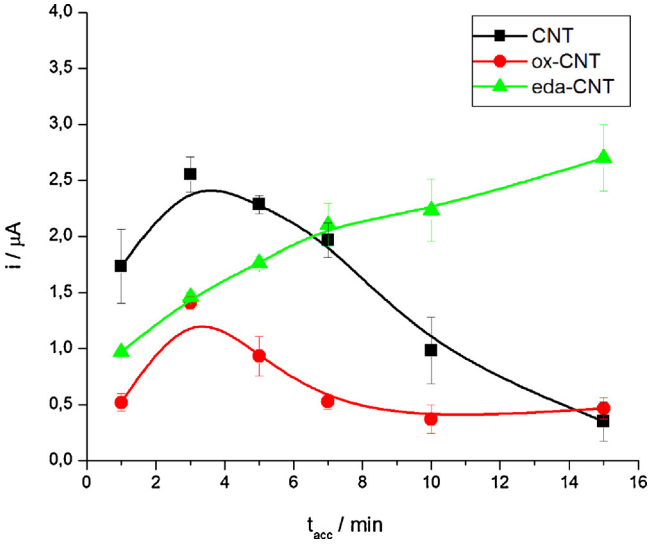


Fig. 4. Current values for the electrooxidation of 45 ppm of dsDNA at different accumulation times for GCE/CNT, GCE/ox-CNT and GCE/eda-CNT. Dispersion of 0.5 mg mL^{-1} CNTs in 0.2 M PBS, pH 7.4.

With 24 h of incubation time, the percentages of signal loss are similar between the different CNTs. In this case, the electrostatic interaction that initially facilitates the approach of the macromolecule is no longer important, and now the dsDNA adopts a spatial conformation that would allow the interaction of nucleobases with the sidewall of the nanotube through π-type interactions. In contrast, with 1 h of incubation time, the electrostatic interactions would predominate, as described above.

3.4. Electrooxidation of dsDNA at GC-modified electrodes

The presence of DNA adsorbed at the GC-modified electrodes was evaluated by differential pulse voltammetry (DPV) from the oxidation of the guanine residues. The influence of the amount of pristine CNTs in the dispersion on the adsorption of 45-ppm dsDNA after a 5-min accumulation onto GCE/CNT was studied (not shown). The guanine residues were oxidized at ~1.0 V. The highest current values were obtained when the concentration of CNTs was 1 mg mL^{-1}. However, with a concentration of 0.5 mg mL^{-1}, the voltammogram is more well-defined and has a less capacitive current. This capacitive current makes it more difficult to evaluate the oxidation signal of the dsDNA at a higher CNT concentration, e.g., for 4 mg mL^{-1}, thereby making it difficult to quantify the amount of oxidized DNA. Therefore, 0.5 mg mL^{-1} was selected as the CNT dispersion concentration for comparative purposes. Fig. 4 shows the average current values obtained for the oxidation of dsDNA at different accumulation times for GCE/CNT, GCE/ox-CNT and GCE/am-CNT, where it is clear that the dsDNA-accumulation time plays an important role in the guanine oxidation signal, which is dependent on the nanotube used.

At the GCE/CNT, the electrooxidation of dsDNA reaches a maximum value at 3 min. Subsequently, the current value slightly decreases, indicating that the maximum adsorption of DNA is reached. When the nanotubes are oxidized (GCE/ox-CNT), the current values obtained are smaller than those of GCE/CNT at any accumulation time used. The presence of oxygenated functional groups directly affects the adsorption of dsDNA, which is most likely due to a repulsive effect between the negative charged phosphate backbone of dsDNA and the carboxylic acid groups of the ox-CNT. This result is in agreement with the UV–vis studies

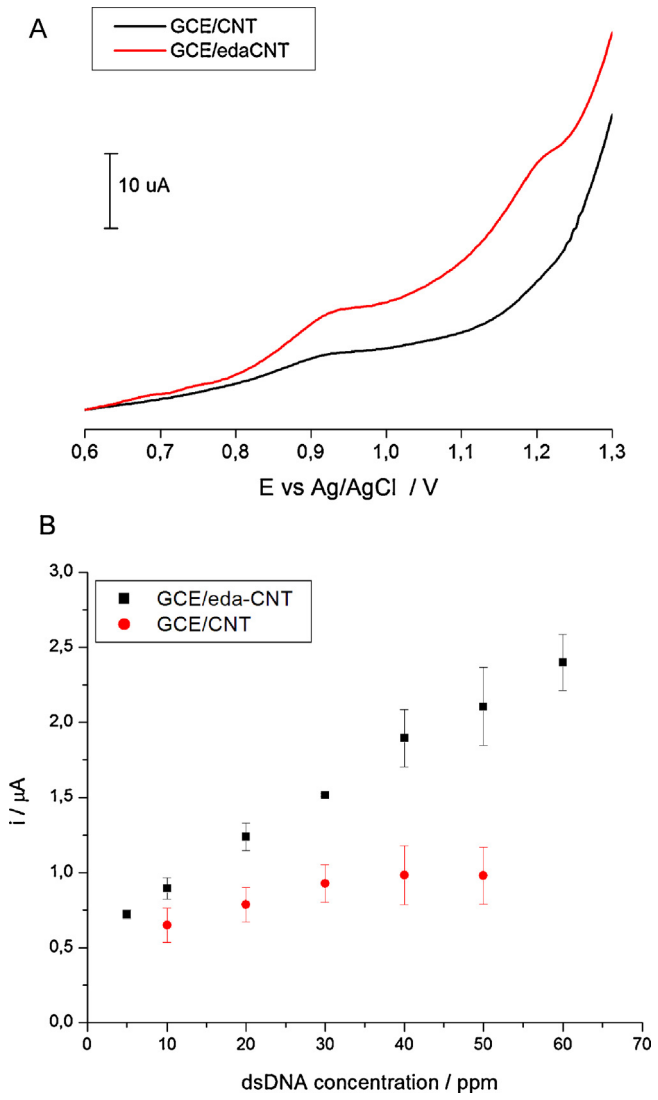


Fig. 5. (A) DP Voltammograms for 45 ppm dsDNA solutions at GCE/CNT and GCE/eda-CNT electrodes after 10-min accumulation. (B) Dependence of dsDNA peak current of GCE/CNT and GCE/eda-CNT electrodes on dsDNA concentration. Dispersion of 0.5 mg mL^{-1} CNTs in 0.2 M PBS, pH 7.4. Accumulation time of 10 min.

discussed above. The current values obtained with GCE/ox-CNT are less than the 18% of the current values obtained with GCE/CNT.

Using the GCE/eda-CNT modified electrode, the current values increased with the accumulation time. At lower accumulation times, the response is similar to the other electrodes, indicating that the presence of functional groups on the surface of the carbon nanotubes it is not relevant for dsDNA adsorption. Although the values obtained with GCE/eda-CNT are not the highest, it clearly shows that the response of the electrode is improved when the nanotubes has functional groups with a positive charge instead of a negative charge. Indeed, the current values increase linearly with the accumulation time used without reaching a maximum value at 15 min, unlike the trend that was observed for the other modified electrodes. This result corroborates the results observed by UV–vis spectroscopy, where the adsorption process of dsDNA is initially favored by electrostatic interactions.

3.5. Analytical performance of modified-electrodes

Fig. 5A shows the DP voltammograms of dsDNA oxidation obtained using GCE/CNT and GCE/eda-CNT after 10 min of accumulation time. A better signal resolution was observed when eda-CNT are modifying the GCE electrode, and also it was possible to observe the oxidation signal of adenine residues at 1.200 V.

On the other hand, Fig. 5B shows the dsDNA peak current dependence with the dsDNA concentration; GCE/ox-CNT was not considered for this study due the low adsorption capacity of dsDNA. The linear range, reproducibility and limit of detection were studied using DPV under the optimized conditions in PBS (0.2 M, pH 7.4) and following the guanine oxidation peak closed to 0.900 V. The reproducibility of the CNT-modified electrodes was estimated by comparing the oxidation peak currents of 45 ppm dsDNA solution at 10 min of accumulation time. The relative standard deviation (RSD) of four CNT-modified GCEs was calculated to be 13% for GCE/CNT and 0.6% for GCE/eda-CNT, indicating an improvement in the reproducibility when eda-CNT was used.

A linear range between 10 and 30 ppm with a sensitivity of 0.0139 ± 0.0002 µA mg^{-1} L (correlation coefficient = 0.999) were determined for GCE/CNT. Under this condition, the calculated LOD was 0.82 ppm, (obtained as $3s/S$, where s is the standard deviation of the blank and S the slope of the calibration plot). For GCE/eda-CNT, the linear range was from 5 to 60 ppm with a sensitivity of 0.0315 ± 0.0003 µA mg^{-1} L (correlation coefficient = 0.999). Based on that result the LOD was 0.97 ppm after a 10-min accumulation. According to these results, GCE/eda-CNT has a better sensitivity than GCE/CNT (almost 3 times higher) and a large linear range of dsDNA concentration.

Comparing the analytical performance obtained with GCE/eda-CNT, where no cationic dispersing agent was used, we were able to obtain a similar linear range than that reported previously by us using chitosan as a dispersing agent [8]. Other work, using the cationic polymer polyethylenimine as a dispersing agent, report a sensitivity of 1.09 ± 0.06 µA mg^{-1} L but have a very short linear range (2.5 and 25.0 ppm) and a higher LOD than our system (1.65 vs. 0.97 ppm) [21]. Moreover, Rivas et al. [37], who used a more complex system based on carbon nanotubes (CNT) dispersed in glucose oxidase (GOx) (GCE/CNT-GOx) reported a similar linear range (5.0–70 ppm) but with an almost 2 times lower sensitivity (0.016 ± 0.001 µA mg^{-1} L) and a LOD of 1.7 ppm.

4. Conclusions

A simple, sensitive and rapid dsDNA quantification method is reported for the first time using covalent functionalized CNT which permits to avoid the use of cationic polymers to disperse CNT. Electrochemical characterization of the modified-electrodes revealed that eda-functionalization decreased the capacitance of the electrode compared to ox-CNT in buffer solution, and did not affect the electron transfer of the surface when a redox mediator was studied.

The functionalization of CNTs with ethylendiamine largely facilitates the adsorption of dsDNA using an aqueous media as a dispersant agent, and the GCE/eda-CNT analytical performance was comparable with that reported previously for cationic polymers in terms of linear range and LOD values.

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