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ADSORPTION AND ELECTROOXIDATION OF DNA AT GLASSY CARBON PASTE ELECTRODES

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This work reports the study of the adsorption and electrooxidation of nucleic acids on glassy carbon paste electrodes (GCPE) by using chronopotentiometric stripping analysis. The influence of electrochemical pretreatments, binder content, supporting electrolyte, accumulation potential, and time on the adsorption and further electrooxidation of oligo$(dG)_{21}$ is discussed. The adsorption behavior of double stranded calf thymus DNA (dsDNA) on GCPE is also evaluated. Trace (ppb) levels of oligo$(dG)_{21}$ and (ppm) levels of dsDNA can be readily detected following short accumulation periods, with detection limits of 21 ppb and 200 ppb for oligo$(dG)_{21}$ and dsDNA, respectively. The confined DNA layer demonstrated to be stable in air, in 0.200 M acetate buffer pH 5.00, and in 0.020 M phosphate buffer pH 7.40 + 0.50 M NaCl.

Keywords: DNA adsorption; DNA-biosensors; DNA electrooxidation; DNA-modified electrode; Glassy carbon paste electrode; Potentiometric stripping analysis

INTRODUCTION

Since the first report on the electrochemical behavior of nucleic acids by Palecek (1960), several electrochemical methodologies have been proposed for the direct quantification of nucleic acids, as well as for the evaluation of the DNA damage (J. Wang 1995; J. Wang et al. 2000; Mikkelsen 1996; Palecek and Fojta 2001; Rivas and Pedano 2006; Rivas, Pedano, and Ferreyra 2005). In the last decade, the advantages of using DNA as a biorecognition element in affinity biosensors have been largely demonstrated (Palecek 2009; Qiang, Hongying, and Bin 2009; Kerman, Vestergaard, and Tamiya 2009).

Carbon materials have been widely used for studying the adsorption and oxidation of nucleic acids. Graphite paste (CPE), graphite (Cai et al. 1996), highly oriented pyrolitic graphite (Cai et al. 1996; Gorodetsky and Barton 2006), and screen

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printed (SPE) (J. Wang et al. 1998) have been successfully used. Carbon nanotube paste (CNTPE) (Pedano and Rivas 2004), glassy carbon modified with nanotubes (Bollo, Ferreyra, and Rivas 2007), and boron doped diamond (Prado et al. 2002) have demonstrated to be very useful for this task.

Pang et al. (1996) have reported the immobilization of nucleic acids on oxidized surfaces of glassy carbon electrodes (GCE) by deposition of the DNA solution and further evaporation of the solvent. Several studies (Oliveira Brett, Serrano, Gutz, and La-Scalea 1997a, 1997b; Oliveira Brett, Serrano, Gutz, La-Scalea, and Cruz 1997; Oliveira Brett et al. 1999) have demonstrated the successful use of glassy carbon as substrate for preparing DNA modified electrodes to evaluate the voltammetric behavior of nitroimidazoles. Oliveira Brett et al. (2004) have also proposed the detection of all deoxyribonucleotides at adequately conditioned GCE. Z. Wang et al. (2000) have reported the adsorption and oxidation of denatured and double stranded DNA at glassy carbon electrodes by using differential pulse voltammetry and in situ FTIR spectroelectrochemistry. We have studied the DNA layer adsorbed on GC electrodes by using AFM, SEM, Ellipsometry, Raman spectroscopy, Cyclic voltammetry, and Potentiometric Stripping Analysis (Pedano and Rivas 2003, 2005; Pedano et al. 2008). We have demonstrated that the oxidative electrochemical pretreatments were not favorable for the adsorption and further oxidation of DNA and that the interaction of DNA with the surface is driven mainly by hydrophobic interactions.

In this work, we are reporting, for the first time, the use of a glassy carbon composite obtained by dispersion of glassy carbon microspheres with mineral oil, called glassy carbon paste electrode (GCPE), to study the adsorption and electrooxidation of DNA. J. Wang et al. (2001) have proposed for the first time the glassy carbon paste electrode as electrochemical sensor. Since then, several authors used this composite electrode for electroanalytical applications. Anik (2007) have reported the use of GCPE modified with xanthine oxidase for the quantification of xanthine and hypoxanthine. Barek et al. (2004) have presented the voltammetric adsorptive stripping of 1-nitropyrene at GCPE. Ricci et al. (2003) have investigated the analytical application of a GCPE modified with Prussian Blue. Our group (Rodríguez and Rivas 2002) have studied the electrochemical behavior of glassy carbon paste electrode under different conditions and the analytical performance of biosensors prepared by dispersion of polyphenol oxidase within this composite material. However, to the best of our knowledge, there is no report about the adsorption and electrooxidation of DNA at this surface.

In the following sections we discuss the influence of different experimental conditions like electrochemical pretreatments, binder content, supporting electrolyte, accumulation potential and time on the adsorption and further electrooxidation of oligo$(dG)_{21}$. The analytical performance for the determination of oligo and polynucleotides is also discussed.

EXPERIMENTAL

Materials

Oligo$(dG)_{21}$ was purchased from Life Technologies (Grand Island, New York, USA) as the ammonium salts. Double stranded calf thymus DNA (dsDNA) (activated and lyophilized, catalog number 4522) was purchased from SIGMA

(St. Louis, MO). Glassy carbon spherical powder 0.4–12 μm, type 2, was obtained from Alfa AEsar. All other reagents were of analytical grade.

The DNA stock solutions (nominally 1000 mg/L) were prepared with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8,0).

Ultrapure water ($\rho = 18$ MΩcm) from a Millipore-MilliQ system was used for preparing all the solutions.

Apparatus

All constant-current chronopotentiometric experiments were performed with a TraceLab Potentiometric Stripping Unit PSU 22 (Radiometer, France) connected to a PC. According to the Trace Lab protocol, the potentials were sampled at a frequency of 30 kHz and the derivative signal (dT/dE) vs. potential (E) were recorded following baseline fitting.

The glassy carbon paste electrode was prepared by mixing in an agata mortar glassy carbon microparticles with mineral oil. A portion of the resulting paste was packed firmly into the cavity (3.0 mm diameter) of a Teflon tube. The electric contact was established through a stainless steel screw. A new surface was obtained by smoothing the electrode onto a weighing paper. The three-electrode system consisted of a 3 mm diameter glassy carbon paste working electrode, an Ag/AgCl reference electrode (BAS Model RE-5B) and a platinum wire auxiliary electrode. The electrodes joined the cell through holes in its Teflon cover. All potentials are referred to that reference electrode. A magnetic stirrer was used during the DNA accumulation step.

Procedure

The sequence detection consisted of the DNA immobilization and chronopotentiometric transduction.

Nucleic acid immobilization. A freshly polished glassy carbon paste electrode was immersed in a stirred 0.200 M acetate buffer pH 5.00 containing DNA and it was held at a constant potential for a given time.

The DNA-modified electrode was washed before the medium exchange with 0.200 M acetate buffer pH 5.0 for 5 seconds.

Chronopotentiometric transduction. The experiment was performed in 0.200 M acetate buffer solution pH 5.00 applying a constant current of 8.0 μA with an initial potential of 0.500 V. The anodic signal at around 1.0 V, corresponding to the guanine oxidation, was used as the analytical signal.

Repetitive measurements were carried out by polishing the surface of GCPE and repeating the aforementioned assay. All experiments were performed at room temperature.

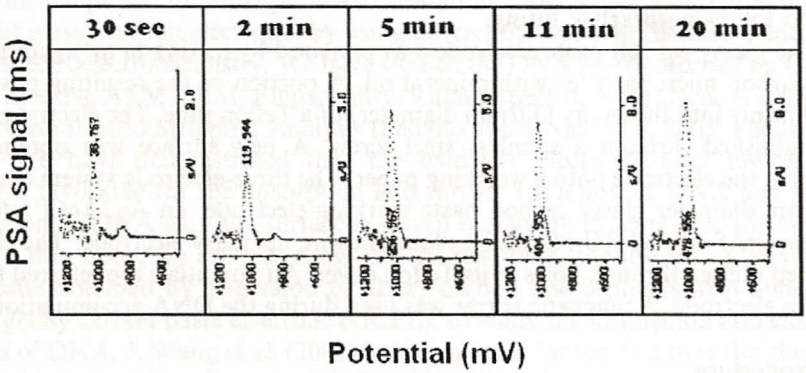
RESULTS AND DISCUSSION

Study of the Experimental Conditions for the Adsorption and Electrooxidation of DNA

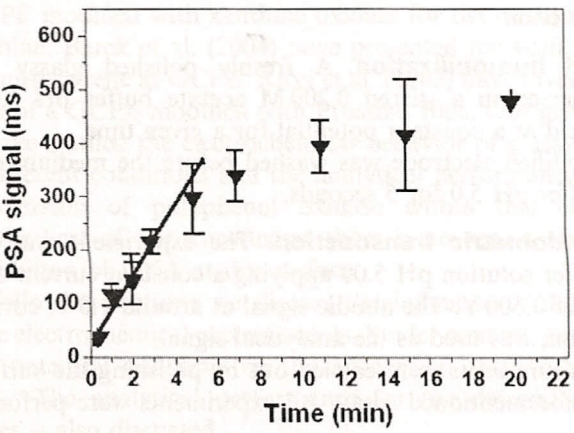
Effect of adsorption time and potential. Figure 1a displays the chronopotentiograms obtained in 0.200 M acetate buffer solution pH 5.00 after accumulation

at 0.200 V in a 0.50 mg/L oligo$(dG)_{21}$ solution for 30 s, 5.0 min, 11.0 min, and 20.0 min using a GCPE containing 15.0% of mineral oil. The adsorption and electrooxidation of DNA was evaluated from the guanine oxidation signal at around 1.0 V. Well defined signals are obtained at this small concentration even after short accumulation times, demonstrating the excellent capability of the PSA unit to detect trace levels. Figure 1b shows the effect of the accumulation time upon the PSA response. The PSA signal increases with the accumulation time up to 4.0 min, to remain almost constant thereafter. The slope for the linear portion is $(63 \pm 7) \times 10^2\,\mathrm{ms \cdot min^{-1}}$.

The influence of the accumulation potential upon the chronopotentiometric response for oligo$(dG)_{21}$ is shown in Figure 2. The measurements were performed



(a)



(b)

Figure 1. (a) Chronopotentiograms obtained in 0.200 M acetate buffer solution pH 5.00 at untreated glassy carbon paste electrode after accumulation periods of 30 sec, 5 min, 11 min, and 20 min at 0.500 V in a 0.200 M acetate buffer solution pH 5.00 containing 0.50 mg/L oligo$(dG)_{21}$. Stripping current: 8.0 μA; Initial potential: 0.500 V. (b) Effect of the adsorption time on the guanine electrooxidation signal obtained in 0.200 M acetate buffer solution pH 5.00 at GCPE after different accumulation periods in a 0.200 M acetate buffer solution pH 5.00 containing 0.50 mg/L oligo$(dG)_{21}$. Stripping current: 8.0 μA; Initial potential: 0.500 V.

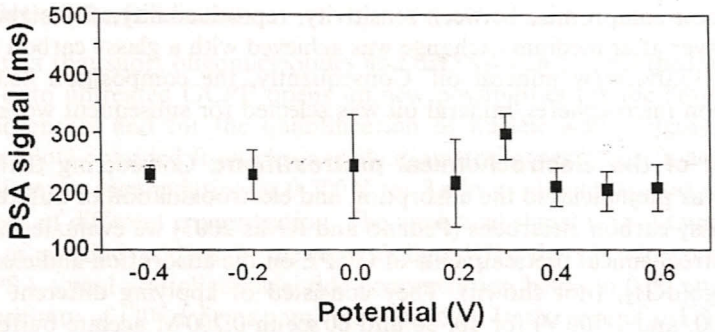


Figure 2. Effect of the accumulation potential on the chronopotentiometric response of oligo$(dG)_{21}$ at GCPE. Adsorption conditions: 3 min at different potentials in 0.200 M acetate buffer solution pH 5.00 + 0.50 mg/L oligo$(dG)_{21}$. Stripping conditions: constant current: 8.0 μA, initial potential: 0.500 V, electrolyte: 0.200 M acetate buffer solution pH 5.00.

in 0.200 M acetate buffer solution pH 5.00 following 3.0 min accumulation from 1.00 mg/L oligo$(dG)_{21}$ solution at GCPE containing 85.0% w/w glassy carbon microspheres and 15.0% mineral oil. In contrast to the potential dependence observed for the accumulation of DNA at carbon (graphite) paste electrodes, where the adsorption was favored at positive potentials (Wang et al. 1997), the guanine oxidation signal remains essentially the same when the accumulation potentials changes from 0.60 to −0.40 V. This behavior suggests that the main interaction mode between the bases and glassy carbon is not electrostatic.

Effect of the electrode composition. The effect of the amount of binder in the composite on the adsorption and electrooxidation of DNA was evaluated from the chronopotentiometric guanine oxidation signal obtained in a 0.200 M acetate buffer solution pH 5.00 after accumulation for 3.0 min at 0.500 V in a 1.00 ppm oligo$(dG)_{21}$ solution. We evaluated three compositions of the composite: 90:10, 85:15, and 80:20% w/w glassy carbon microspheres powder/mineral oil, respectively (not shown). Even when the composite containing 10.0% oil allowed to obtain the highest sensitivity, it also presented the highest background signals, poor reproducibility, and it was difficult to handle. The poor reproducibility could be associated to some inhomogeneity originated as a consequence of the small amount of binder. The increment of mineral oil up to 20.0% w/w gave smaller choronopotentiometric signals, demonstrating that the adsorption of DNA largely depends on the amount of mineral oil in the composite, probably due to a more difficult adsorption of nucleic acids at glassy carbon microparticles in the presence of an excessive amount of oil. At variance with graphite paste electrode, GCPE prepared with 30.0% w/w mineral oil was difficult to handle.

The influence of the medium exchange on the DNA layer adsorbed at GCPE of different composition was evaluated from the guanine electrooxidation signal after accumulation for 3.0 min at 0.500 V from 1.00 ppm oligo$(dG)_{21}$ solution. In the case of the paste containing just 10.0% mineral oil, the loose of signal was really negligible. For GCPE prepared with 15.0% w/w oil, the signal decreased in a 15.0%. These results demonstrate once more the importance of the oil content in the composite on the adsorption of the nucleic acid.

The best compromise between sensitivity, reproducibility, and stability of the adsorbed layer after medium exchange was achieved with a glassy carbon composite containing 15.0% w/w mineral oil. Consequently, the composition 85.0%/15.0% glassy carbon microspheres/mineral oil was selected for subsequent work.

Effect of the electrochemical pretreatment. Considering that any pretreatment was prejudicial to the adsorption and electrooxidation of different nucleic acids at glassy carbon electrodes (Pedano and Rivas 2003) we evaluate the effect of diverse electrochemical pretreatments of GCPE on the adsorption and electrooxidation of oligo$(dG)_{21}$ (not shown). They consisted of applying different potentials (1.500, 1.600, and 1.700 V) for 10, 30 and 60 sec in 0.200 M acetate buffer solution pH 5.00. Except for the treatment at 1.500 V for 30 sec, any other pretreatment was not effective, mainly due to the facilitated solvent oxidation at the activated electrode and the consequent more difficult definition of the guanine oxidation signal.

Effect of the background electrolyte. The influence of the acetate concentration (pH 5.00) upon the adsorption of 0.50 ppm of oligo$(dG)_{21}$ at GCPE after 3.0 min accumulation at 0.200 V is illustrated in Figure 3. It is important to consider that the adsorption of the nucleic acid is highly determined by its conformation in solution previous to the adsorption step. In this sense, the screening of the negative charges of the phosphate backbone has a crucial role, especially when the electrostatic interaction is important. The guanine oxidation signal decreases for acetate concentrations lower than 0.200 M due to a poor screening of the negative charges that result in a conformation where the accessibility of guanine residues for electrooxidation is not favored. The fact that the guanine oxidation signals remain constant for higher acetate concentrations suggests a poor electrostatic interaction of DNA with GCPE, in agreement with the effect of the adsorption potential previously described.

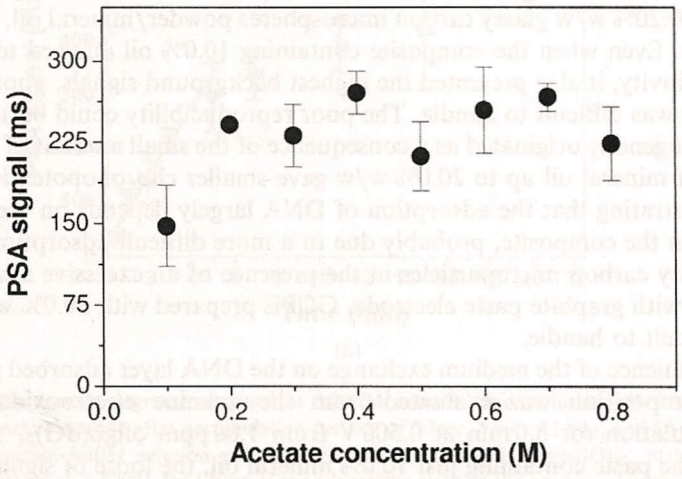
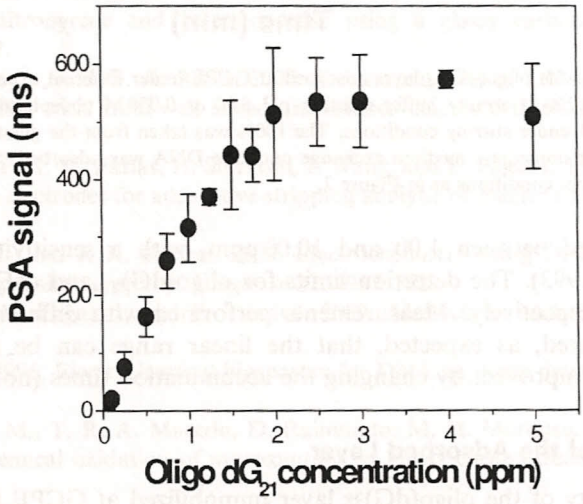


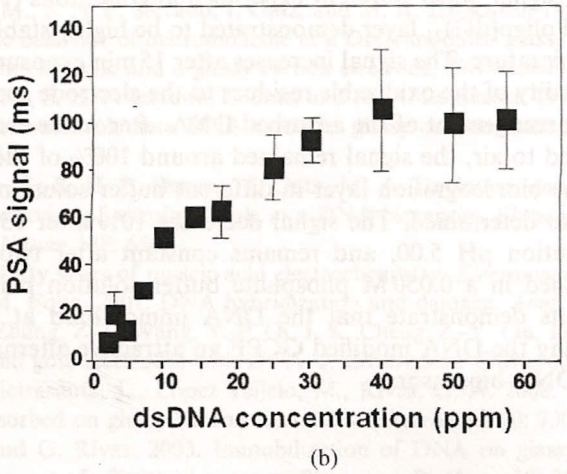
Figure 3. Effect of the concentration of the acetate buffer solution upon the adsorption of 0.50 mg/L oligo$(dG)_{21}$. Adsorption conditions: 3.0 min at 0.200 V in acetate solutions of different concentration containing 0.5 mg/L oligo$(dG)_{21}$. Other conditions as in Figure 2.

Analytical Performance

The fact that short oligonucleotides and dsDNA can be adsorbed and preconcentrated onto untreated GCPE opens up new possibilities for the development of affinity biosensors and for the quantification of nucleic acids. Figure 4 displays calibration plots obtained from chronopotentiometric experiments in acetate buffer solution after the accumulation at 0.200 V for 3 min in oligo$(dG)_{21}$ (a) and dsDNA (b) solutions of different concentration. The analytical signal was obtained from the oxidation of guanine residues. In the case of oligo$(dG)_{21}$ there is a linear relationship between PSA signal and oligonucleotide concentration between 0.05 and 1.50 ppm, with a sensitivity of (307 ± 5) ms ppm^{-1} ($r = 0.9991$). In the case of dsDNA, a linear



(a)



(b)

Figure 4. Calibration plots for oligo$(dG)_{21}$ (b) obtained after 3 min accumulation at 0.200 V in a 0.200 M acetate buffer solution pH 5.00. Other conditions as in Figure 2.

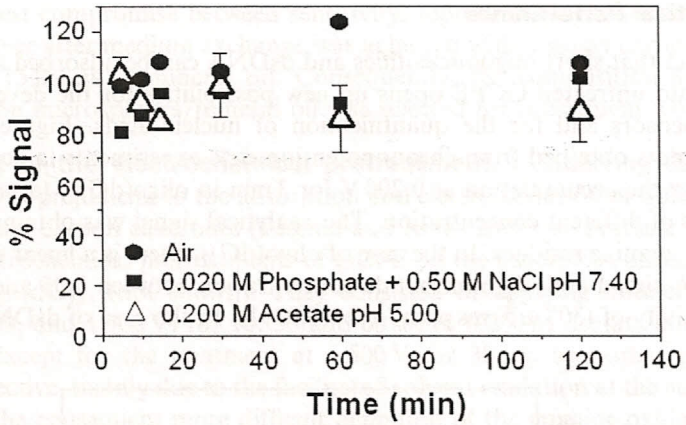


Figure 5. Stability of the oligo$(dG)_{21}$ layer adsorbed at GCPE under different experimental conditions: exposed to air, in 0.200 M acetate buffer solution pH 5.00 or 0.050 M phosphate buffer solution pH 7.40 + 0.500 M NaCl under stirring conditions. The 100% was taken from the guanine electrooxidation signal obtained after immediate medium exchange once the DNA was adsorbed. Oligo$(dG)_{21}$ concentration: 3.0 ppm. Other conditions as in Figure 2.

range is obtained between 1.00 and 10.00 ppm, with a sensitivity of (6.0 ± 0.3) ms ppm^{-1} ($r = 0.993$). The detection limits for oligo$(dG)_{21}$ and dsDNA are 21 ppb and 200 ppb, respectively. Measurements performed with different accumulation times demonstrated, as expected, that the linear range can be shifted and the detection limits improved, by changing the accumulation times (not shown).

Stability of the Adsorbed Layer

The stability of the oligo$(dG)_{21}$ layer immobilized at GCPE from 3.00 mg/L oligo$(dG)_{21}$ for 3 min at 0.200 V was evaluated from the chronopotentiometric guanine oxidation signal under different experimental conditions (Fig. 5).

The confined oligo$(dG)_{21}$ layer demonstrated to be highly stable when exposed to air at room temperature. The signal increases after 15 min exposure, probably due to a better accessibility of the oxidizable residues to the electrode surface as a consequence of some rearrangement of the adsorbed DNA after water evaporation. After three hours exposed to air, the signal remained around 100% of the original value. The stability of the biorecognition layer in different buffer solutions under stirring conditions was also determined. The signal decreases 10% after 15 min in 0.200 M acetate buffer solution pH 5.00, and remains constant after two hours. Similar results were obtained in a 0.050 M phosphate buffer solution pH 7.40 + 0.500 M NaCl. These results demonstrate that the DNA immobilized at the electrode is highly stable making the DNA modified GCPE an attractive alternative for further developments of DNA biosensors.

CONCLUSIONS

The results presented here demonstrated the effective immobilization of DNAs on glassy carbon paste surfaces. The independence of the guanine oxidation signal

with the accumulation potential, and the behavior at high ionic strengths indicate that, as in the case of glassy carbon electrode, the interaction between DNA and the electrode surface is mainly hydrophobic. The high stability of the adsorbed layer either exposed to air or under stirring conditions in acetate or phosphate buffer solutions, makes the DNA-modified-glassy carbon paste electrode a useful tool for future developments of genosensors.

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