Adsorption of Guanine, Guanosine, and Adenine at Electrodes Studied by Differential Pulse Voltammetry and Electrochemical Impedance

Ana Maria Oliveira-Brett,* Luís Antônio da Silva, and Christopher M. A. Brett*

Departamento de Química, Universidade de Coimbra, 3004-535 Coimbra, Portugal

Received June 27, 2001. In Final Form: October 18, 2001

Guanine, guanosine, and adenine have been electrochemically oxidized at a glassy carbon electrode in aqueous solution. Electrochemical impedance is shown to be a valuable probe of adsorption of the oxidation products on the electrode surface. Voltammetric and impedance studies at different concentrations have been carried out. Correlation between the results for the different compounds helps in understanding the adsorption process and the preconcentration properties of purine bases. Irradiation with ultrasound during recording of the impedance spectrum enabled adsorption to be avoided and the electrode process of the solution-soluble species to be investigated, and oxidation rate constants were calculated.

Introduction

It is of fundamental importance for understanding the function of DNA purine bases to understand their spontaneous adsorption on surfaces. For this reason, the electrochemical study of nucleic acids and their adsorption on different types of electrode materials has recently been of great interest.[1–3] Most of the work has investigated either the reduction of the pyrimidine and purine bases on the mercury electrode[4–6] or their oxidation at glassy carbon electrodes.[7,8] Electrochemically induced adsorption has usually been observed. Adsorption of guanine and guanosine on pyrolytic graphite electrodes has been fully characterized, and the implications for the determination of guanine in the presence of guanosine have been discussed.[9] A similar behavior was found at glassy carbon electrodes.[10]

The electrochemical oxidation mechanism of guanine and adenine was investigated and follows a very similar mechanism to that for other purines.[11–17] It is pH dependent, and the electron transfer process occurs in consecutive steps with the formation of very strongly adsorbed oxidation products and dimers on the electrode

surface for both compounds. However, although the adsorbates were identified,[14,15] their adsorption on the electrode surface has never been investigated.

The objective of this work was to study the adsorption of guanine, of its corresponding nucleoside, guanosine, of adenine, and of their oxidation products on a glassy carbon electrode. For this purpose, differential pulse voltammetry and electrochemical impedance were used. Understanding the adsorption process of these components of DNA is necessary to get insight into the behavior of DNA-modified electrodes previously investigated using electrochemical impedance.[18] This paper also demonstrates that sonoelectrochemical impedance is an effective technique for avoiding complications due to adsorbed material.

Experimental Section

Guanine, adenine, and guanosine (Sigma, >99% purity) were used as received without further purification. All other reagents were of analytical grade, and all solutions were prepared using Millipore Milli-Q ultrapure water (conductivity < 0.1 μS cm^{-1}). Stock solutions of the bases were prepared in 0.2 M acetate buffer at pH 4.5. The stock solution of guanosine (1.5×10^{-2} M) was made by slow heating of the base in electrolyte until close to 100 °C. Experiments were carried out in pH 4.5, 0.2 M acetate buffer at 25 ± 1 °C.

A glassy carbon working electrode (Tokai GC 20) of diameter 3 mm, a Pt wire counter electrode, and a Ag/AgCl (3.0 M KCl) reference electrode were placed in a one-compartment microcell using 200 μL of solution.

Voltammograms were obtained using a PC-controlled PG-STAT10 Autolab (EcoChemie) potentiostat/galvanostat with GPES 4.5 software. Differential pulse (DP) voltammograms were registered in the potential interval 0 to +1.4 V versus Ag/AgCl, and the DP conditions were as follows: pulse amplitude, 50 mV; pulse width, 70 ms; scan rate, 5 mV s^{-1}.

Electrochemical impedance measurements were carried out using a Solartron 1250 frequency response analyzer, coupled to a Solartron 1286 electrochemical interface controlled by Zplot software; fitting of the spectra was done by ZSIM CNLS software. A rms perturbation of 5 mV was applied over the frequency range 65 kHz to 0.01 Hz with five frequencies per decade at chosen potentials in the range 0.0–1.4 V versus Ag/AgCl.

Sonoelectrochemical impedance experiments were carried out by irradiating the glassy carbon electrode with ultrasound during the running of impedance experiments. A Vibra cell model 501

* To whom correspondence should be addressed. Telephone: +351-239-835295. Fax: +351-239-835295. E-mail: brett@ci.uc.pt.

(1) Palecek, E. In *Topics in Bioelectrochemistry and Bioenergetics*; Milazzo, G., Ed.; Wiley: London, 1983; Vol. 5, p 65.

(2) Oliveira-Brett, A. M.; Matysik, F.-M. *Bioelectrochem. Bioenerg.* **1997**, *42*, 111.

(3) Oliveira-Brett, A. M.; Matysik, F.-M. *J. Electroanal. Chem.* **1997**, *429*, 95.

(4) Dryhurst, G.; Elving, P. J. *Talanta* **1969**, *16*, 855.

(5) Elving, P. J.; Pace, S. J.; O'Reilly, J. E. *J. Am. Chem. Soc.* **1973**, *95*, 647.

(6) Palecek, E. *Electroanalysis* **1996**, *8*, 1.

(7) Yao, T.; Musha, S. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 2307.

(8) Dryhurst, G. *Talanta* **1972**, *19*, 769.

(9) Dryhurst, G. *Anal. Chim. Acta* **1971**, *57*, 137.

(10) Yao, T.; Taniguchi, Y.; Wasa, T.; Musha, S. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2937.

(11) Dryhurst, G.; Face, G. F. *J. Electrochem. Soc.* **1970**, *117*, 1259.

(12) Brett, C. M. A.; Oliveira-Brett, A. M.; Serrano, S. H. P. *J. Electroanal. Chem.* **1994**, *366*, 225.

(13) Subramanian, P.; Dryhurst, G. *J. Electroanal. Chem.* **1987**, *244*, 137.

(14) Dryhurst, G. *J. Electrochem. Soc.* **1969**, *116*, 1411.

(15) Dryhurst, G.; Elving, P. J. *J. Electrochem. Soc.* **1968**, *115*, 1014.

(16) Goya, R. N.; Dryhurst, G. *J. Electroanal. Chem.* **1982**, *135*, 75.

(17) Oliveira-Brett, A. M.; Piedade, J. A. P.; Serrano, S. H. P. *Electroanalysis* **2000**, *12*, 969.

(18) Brett, C. M. A.; Oliveira-Brett, A. M.; Serrano, S. H. P. *Electrochim. Acta* **1999**, *44*, 4233.
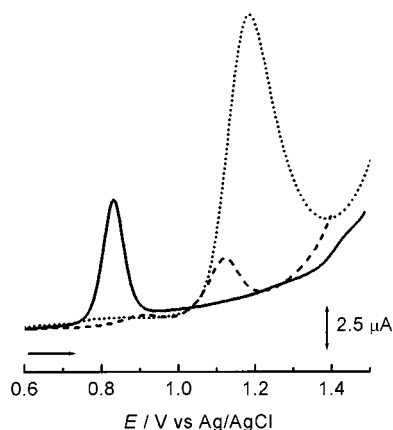
Figure 1. DP voltammograms for (—) 3×10^{-5} M guanine, (- - -) 5×10^{-5} M adenine, and ($\cdot \cdot \cdot$) 5×10^{-5} M guanosine, in pH 4.5, 0.2 M acetate buffer: scan rate, 5 mV s^{-1}; amplitude, 50 mV.

20 kHz sonic horn (Sonics & Materials Inc.) with a 3 mm diameter Ti-tipped probe was employed, placed at a distance of 6 mm from the glassy carbon electrode surface. The electrodes were immersed in a thermostated cell of 20 cm^3 capacity.

Results and Discussion

Oxidation of Guanine. The oxidation of guanine occurs at ~0.85 V, as shown in the differential pulse voltammogram in Figure 1. A saturated solution of concentration 1 mM, well above the solubility limit for guanine, was prepared; the filtered supernatant was used for the voltammetric experiments in Figure 1, the actual concentration in the electrolyte solution being 3×10^{-5} M.[2] The peak width at half-height of ~70 mV shows that the oxidation follows quasi-reversible electrode kinetics under the conditions employed. Since the first, rate-determining, step of the oxidation involves two electrons,[7,8] a peak width of 45 mV would be expected for reversible kinetics. In fact, guanine oxidation occurs in two consecutive steps. The first step is oxidation to 8-oxoguanine (8-oxoG) followed by the one-electron oxidation of guanine dimers.[13,17] The oxidation of 8-oxoG, corresponding to a reversible two-electron/two-proton process, occurs at +0.55 V, and oxidized 8-oxoG undergoes rapid hydrolysis.[16,17] So it does not interfere with the measurements made here. The guanine dimers formed during the oxidation of guanine are strongly adsorbed on the electrode surface and are reversibly oxidized.

Electrochemical impedance experiments offer an excellent way to further probe the adsorption phenomenon and the influence of base concentration and of potential. Thus, spectra were recorded at different applied potentials in the interval 0.0–1.0 V and for different concentrations. Figure 2a shows complex plane impedance plots registered at different potentials for 3×10^{-5} M guanine in acetate buffer electrolyte, and Figure 2b shows consecutive spectra recorded at an applied potential of +0.85 V versus Ag/AgCl, just before the current peak reaches its maximum value.

The various factors which influence the shape of the impedance plots are now considered. Figure 2a shows that, at values of potential below that necessary for guanine oxidation, the impedance behavior is essentially capacitive modified by surface roughness effects, leading to a straight line of angle less than 90°. At +0.80 V—the beginning of the oxidation peak (as shown in Figure 1)—the charge-transfer reaction begins to have an influence on the shape of the spectrum. At +0.85 V a high-frequency semicircle appears because of the charge-transfer reaction, and at
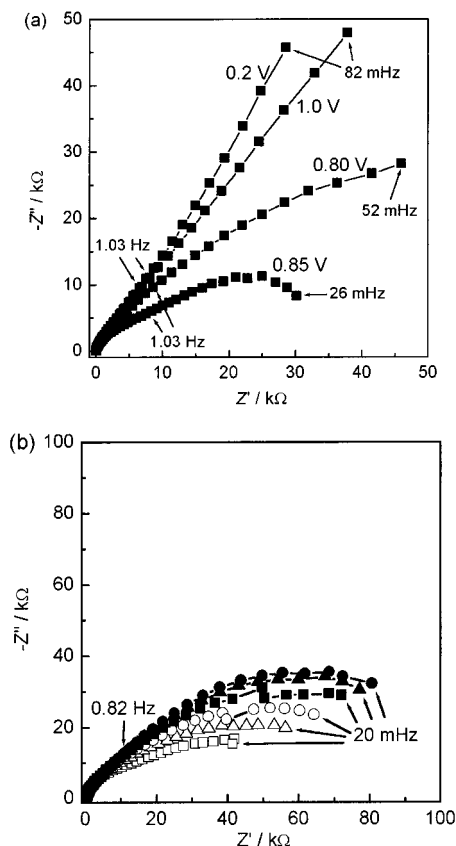

Figure 2. Complex plane impedance spectra for 3×10^{-5} M guanine in pH 4.5, 0.2 M acetate buffer: (a) different applied potentials; (b) consecutive spectra at +0.85 V, from scan 1 (□) to scan 6 (●).

low frequency the behavior can be attributed to relaxation of the double layer due to reaction products adsorbed on the electrode surface. Diffusion-limited oxidation at +1.0 V leads to a straight line in the impedance plot. These phenomena cannot be observed by differential pulse voltammetry. From Figure 2b it can be seen that there is an increase in the size of the semicircle with successive scans, showing the effect of surface blocking on the measured charge-transfer resistance.

The spectra were fitted to the electrical equivalent circuit shown in Figure 3a. The charge-transfer reaction is represented by a charge-transfer resistance, R_{ct}, in parallel with a constant phase element CPE$_1$ with characteristics of a capacitance, to take into account the frequency dispersion often related directly to electrode roughness,[19] which is normally found for solid electrodes. The low frequency double layer relaxation is described by the R_2/CPE$_2$ parallel combination in series with R_{ct}. The CPE exponents found here had values between 0.90 and 0.80. R_Ω represents the ohmic resistance of the cell. At potentials less than necessary for guanine oxidation, R_{ct} and R_2/CPE$_2$ were removed from the electrical equivalent circuit. An example of the fitting procedure is shown in the Bode plot of Figure 3b.

Taking surface roughness into account, as described above, values of interfacial capacitance were found between 5 and 12 μF, the lowest values being at potentials close to that for oxidation of guanine, as would be predicted. Values of CPE$_2$ were around 0.5 mF cm^{-2}, an order of magnitude expected for adsorbed species.

Fitted values of R_{ct} are given in Table 1, together with the corresponding values of oxidation peak current, I_p,

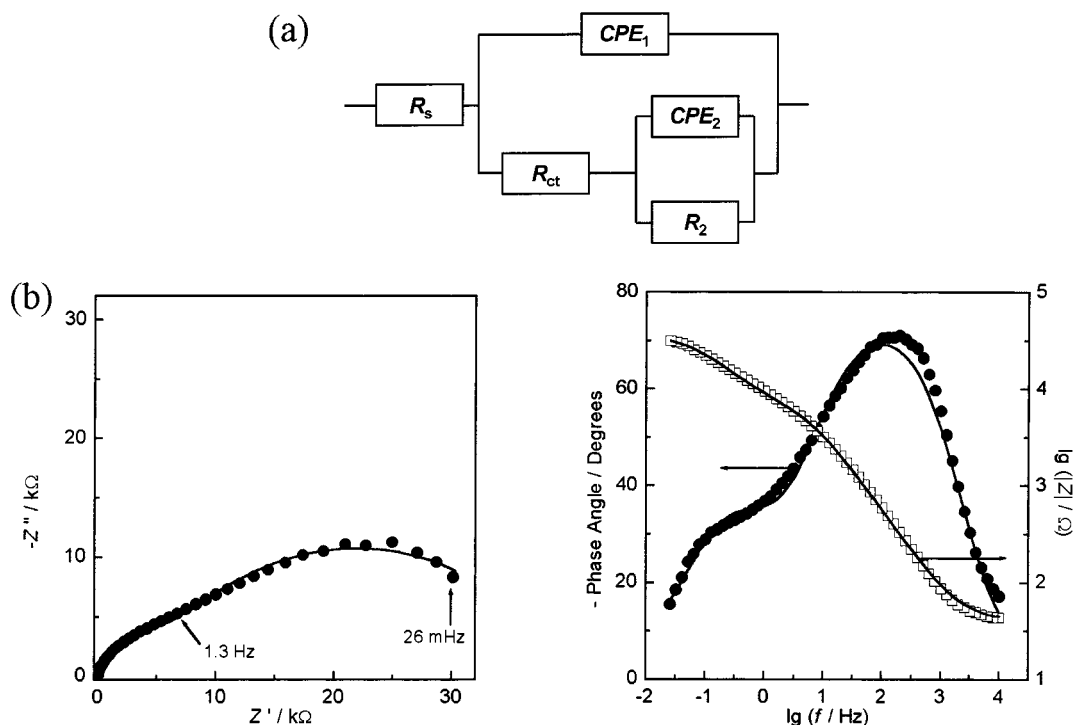(19) De Levie, R. *Electrochim. Acta* **1964**, *9*, 1231.

(a)

(b)

Figure 3. Fitting of impedance spectra: (a) Equivalent circuit. (b) Fitting at 0.85 V for 3×10^{-5} M guanine; data from Figure 2a. The solid line shows the result of fitting to the equivalent circuit.

Table 1. Guanine Oxidation Peak Current, I_p, from DPV and R_{ct} from Impedance Spectra at +0.85 V for Consecutive Scans, in pH 4.5, 0.2 M Acetate Buffer

scan no.	$I_p/\mu A$ (differential pulse)	$R_{ct}/k\Omega$ (impedance)
1	6.39	24.2
2	6.42	32.1
3	6.39	38.0
4	6.51	41.8
5	6.66	45.2
6	6.85	47.8

from differential pulse voltammetry. Comparison shows that both R_{ct} and I_p values increase with scan number. In differential pulse voltammetry, the peak current increases by ~3% and R_{ct} increases by ~90% after six scans. In fact, the value of I_p is more or less constant over the first three scans and then begins to increase slightly. This can be attributed to the contribution from strongly adsorbed guanine dimers,[14] formed on the electrode surface between guanine and the adsorbed guanine oxidation products which slowly cover the surface. It was verified that after 20 scans the peak current begins to drop. However, each impedance experiment takes approximately 10 min and is conducted at a fixed value of applied potential, where an electrode reaction is occurring all the time. Therefore, the influence of adsorption of oxidation products blocking the surface is seen already in the second frequency scan.

To remove the effect of blocking by adsorption products, impedance spectra were recorded while irradiating the electrode surface with ultrasound, the purpose being to exploit the cleaning effect of ultrasound as well as prevent blocking of the electrode surface. Several different ultrasound power levels were tried, to ascertain what was the minimum power level necessary for the cleaning effect to operate while minimizing the temperature perturbation. Second, experiments were done under irradiation after recording of spectra under silent conditions several times consecutively to test reproducibility. An example is shown in Figure 4. The diameter of the high-frequency semicircle is R_{ct} = 5.1 kΩ, significantly lower than the values
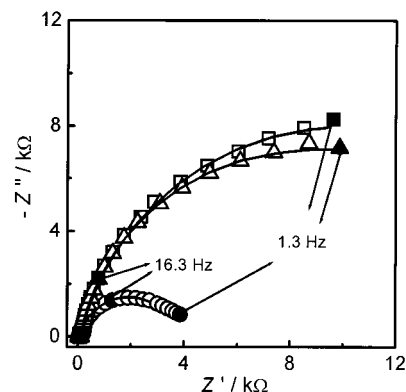

Figure 4. Impedance spectra of 3×10^{-5} M guanine in pH 4.5, 0.2 M acetate buffer at 0.85 V: (□) before application of ultrasound; (○) with ultrasound power = 135 W cm^{-2}; (△) after ultrasound.

calculated in the absence of ultrasound. Assuming a simple electrode process, the equation

$$R_{ct} = \frac{RT}{(nF)^2 A k_0 c_{\infty}}$$

can be employed in order to calculate the standard rate constant, k_0, from the high-frequency part of the spectra, where A is the electrode area, $n = 2$, and all other symbols have their usual meanings. A value for the standard rate constant of 6.21×10^{-3} cm s^{-1} is obtained.

Oxidation of Adenine. A differential pulse voltammogram for adenine is in Figure 1 and shows the oxidation occurring at +1.1 V versus Ag/AgCl, and the greater peak width at half-height than that for guanine demonstrates that the oxidation reaction is slower. It occurs in three steps, and one of the oxidation products is adenine dimers.[14,15] Adenine adsorbs very strongly on the electrode surface, compared to the adsorption of the oxidation products and the dimers. The more positive oxidation
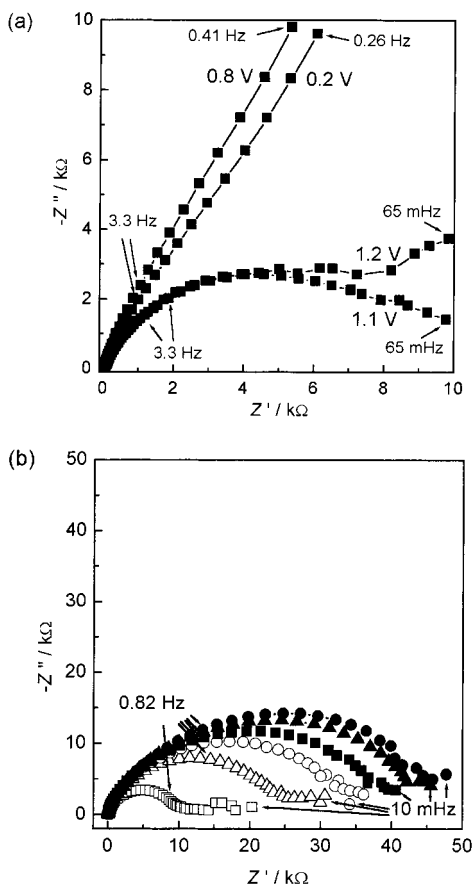
Figure 5. Complex plane impedance spectra for 1×10^{-3} M adenine in pH 4.5, 0.2 M acetate buffer: (a) different applied potentials; (b) consecutive spectra at 1.1 V, from scan 1 (□) to scan 6 (●).

Figure 6. Complex plane impedance spectra for different concentrations of adenine at an applied potential of +1.2 V: (□) 1×10^{-3}; (■) 5×10^{-3}; (●) 10×10^{-3}; (○) 15×10^{-3} M. The inset is a plot of R_{ct}^{-1} versus adenine concentration.

Table 2. Adenine Oxidation Peak Current, I_p, from DPV and R_{ct} from Impedance Spectra at +1.1 V for Consecutive Scans, in pH 4.5, 0.2 M Acetate Buffer

scan no.	$I_p/\mu A$ (differential pulse)	$R_{ct}/k\Omega$ (impedance)
1	78.0	9.8
2	12.0	22.2
3	11.7	31.6
4	10.0	37.5
5	10.2	43.0
6	10.9	46.7

potential than that of guanine is due to the difference in chemical structure. Additionally, the more polar structure also means that, unlike the case of guanine, there is no problem of solubility in acetate buffer. At much higher concentrations of 1 mM, after the first scan the values of current diminish drastically, although the way in which blocking of the electrode surface occurs is qualitatively similar to that of guanine.

Complex plane impedance spectra recorded at different applied potentials are shown in Figure 5a, and the results of consecutive scans for the same concentration are shown in Figure 5b. Once again, as for guanine, there is a capacitive behavior before the oxidation reaction begins and then a charge-transfer semicircle appears. However, the second semicircle due to relaxation of the double layer, which was pronounced for guanine, is not so for adenine. Successive measurements at +1.1 V show a similar variation with scan number as that for guanine. The variations of current and of R_{ct} are shown in Table 2 and can be compared with the values in Table 1 for guanine. Comparatively speaking, the surface is blocked by adenine much faster, which can be ascribed to its greater polarity. The variation of impedance spectra for different adenine concentrations is not large (Figure 6 and inset), which demonstrates that saturation adsorption occurs readily and on a time scale less than that of the impedance experiment.

Once again, the cleaning effect of ultrasonic radiation leads to a reduction in the high-frequency semicircle. Values of 8 kΩ are obtained, and this corresponds to a standard rate constant of 1.19×10^{-4} cm s^{-1} for a two-electron process.
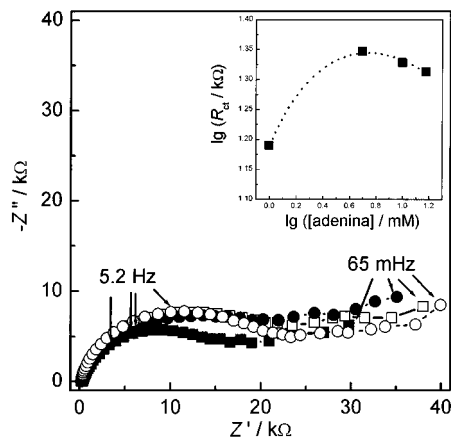
Oxidation of Guanosine. Although guanine and guanosine have similar structures, the potentials necessary for their electro-oxidation are different. As shown by the DPV of guanosine in Figure 1, guanosine oxidizes at ~1.1 V in acetate buffer compared to ~0.85 V for guanine. This difference can be attributed to the influence of the ribose group in making the removal of protons and electrons from the nitrogen base more difficult via inductive effects. However, the values of current in subsequent scans decrease, as in the case of adenine, owing to adsorption of oxidized guanosine on the surface of the electrode. The variation of peak current with concentration for the first and second DPV scans, at high concentrations, shows a pronounced reduction in current due to adsorption. There is a saturation effect on increasing the concentration, which can be ascribed to blocking the electrode surface.

The various factors which influence the shape of the impedance plots are shown in Figure 7. This demonstrates that, as for guanine, at values of potential below those necessary for guanosine oxidation, the impedance behavior is essentially capacitive modified by surface roughness effects. At +1.0 V—the foot of the oxidation peak in Figure 1—the charge-transfer reaction begins to have an influence on the shape of the spectrum. From Figure 8 it can be seen that there is a decrease in the size of the semicircle with increasing concentration, the excess of reagent at the surface facilitating electron transfer.

In further experiments, impedance spectra were recorded sequentially at +1.1 V for a guanosine concentration of 1.0×10^{-3} M, and these demonstrate, more conclusively, how adsorption products block the electrode surface For the first two scans, two time constants are evident, as discussed above. They then become fused together and the semicircles become more depressed. Values of R_{ct} were calculated and are illustrated in Table 3. It was verified that the increase in R_{ct} was much greater for the highest concentrations of guanosine tested.
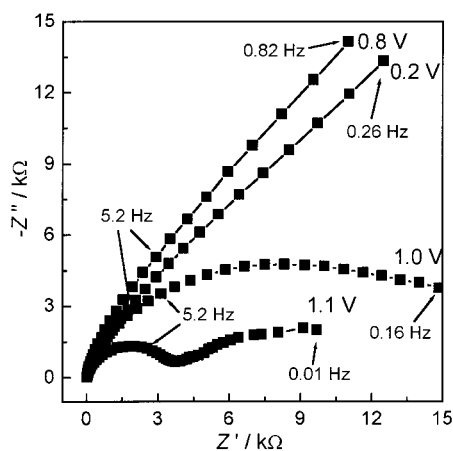
Figure 7. Complex plane impedance spectra for 1×10^{-3} M guanosine in pH 4.5, 0.2 M acetate buffer at different applied potentials.
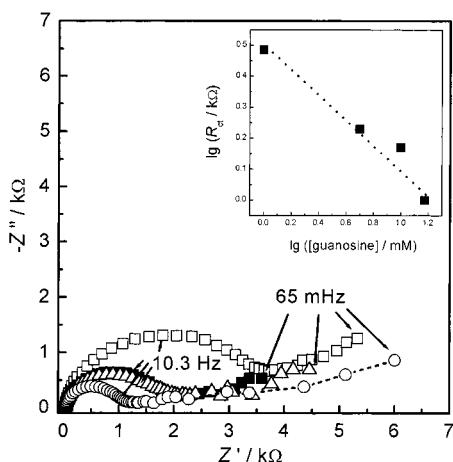
Figure 8. Complex plane impedance spectra for different concentrations of guanosine at an applied potential of 1.1 V: (□) 1×10^{-3}; (■) 5×10^{-3}; (△) 10×10^{-3}; (○) 15×10^{-3} M. The inset is a plot of R_{ct}^{-1} versus guanosine concentration.

Table 3. Values of R_{ct} from Impedance Spectra of Guanosine at +1.1 V for Consecutive Scans, in pH 4.5, 0.2 M Acetate Buffer

| | R_{ct} for the following values of [guanosine]/mM | | | |
scan no.	1	5	10	15
1	3.0	2.4	2.2	1.6
2	6.2	8.2	12.0	16.0
3	8.1	15.2	22.4	29.1
4	9.8	27.9	31.1	42.2
5	12.0	33.8	34.2	56.8
6	13.2	37.0	37.0	65.5

and must be due to the presence of the sugar group. Adenine and guanine show similar adsorption characteristics. In all cases, the application of ultrasound leads to removal of the adsorbed species and the values of oxidation rate constant are similar. It suggests that the strengths of adsorption of the DNA bases on the glassy carbon surface are similar. This can have important implications for the preparation of electrochemical DNA biosensors.

Conclusions

Differential pulse voltammetry and electrochemical impedance have demonstrated that guanine, adenine, guanosine, and their oxidation products are electrochemically adsorbed on glassy carbon electrode surfaces, and these behaviors show a concentration dependence. The dependence of guanine adsorption on concentration is least evident in these data, owing to its low solubility in aqueous acetate buffer solution. Adsorption causes an increase in R_{ct} from electrochemical impedance and a decrease in oxidation current. The time scale of adsorption is brought into evidence by comparison of differential pulse voltammetric and impedance results.

It is shown that irradiation by ultrasound during the recording of impedance spectra does not interfere with the measurements and removes adsorbed products, thus being a promising avenue for the measurement of rate constants without the influence of adsorption.

Taking surface roughness into account, as described previously, values of capacitance were found between 5 and 12 μF, the lowest values being at potentials close to that for oxidation of guanosine, as would be predicted.

Application of ultrasound, as before, leads to a smaller and constant value of the charge-transfer resistance and is successful in removing adsorbed species from the electrode surface. Standard rate constants from sonoelectrochemical impedance spectra are again on the order of 1.0×10^{-4} cm s^{-1}.

Comparative Remarks. Comparison of the three bases shows that adsorption is least for guanosine, as reflected by the lower values of charge-transfer resistance,