# Voltammetric Behavior of Nitroimidazoles at a DNA-Biosensor

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**Abstract**
The newly developed DNA-biosensor is a very promising tool for the investigation and study of the action of drugs specifically designed to interact with DNA. In this work the electrochemical reduction of nitroimidazole drugs was studied in the presence of DNA immobilized onto the surface of a glassy carbon electrode. This enabled preconcentration of the drug onto the electrode surface, which was then electrochemically reduced to the corresponding hydroxylamine which followed by reoxidation give the nitroso compound and subsequently an azoxycompound. Moreover, as the target of the nitroimidazole action was the DNA, the damage caused to DNA on the electrode surface by a reduction product of this drug could be detected in situ.

**Keywords:** DNA damage, Bioelectrochemistry, Nitroimidazoles, DNA targeted drugs

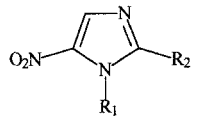
## 1. Introduction

Some drugs act directly on nucleic acids to disrupt replication, transcription and translation. They can be divided into three groups: intercalating cytostatic agents, alkylating agents and chain 'cutters'. It has been shown that reduced nitroimidazoles bind to DNA [1], the damage to DNA resulting in strand breaking and helix destabilization. Unwinding occurs without any detectable binding to DNA of a nitroimidazole.

Nitroimidazoles are drugs selectively toxic to anaerobic bacteria and protozoa [2]. The most important derivatives have substituents in the N1 position of the heterocyclic ring of 5-nitroimidazole (Scheme 1).

The electrochemical reduction of nitroimidazoles follows a complex mechanism and in theory the nitro group is able to receive up to six electrons to form the corresponding amine [3]. Under anaerobic and low oxygen pressure conditions metronidazole (2-methyl-5-nitroimidazole-1-ethanol) follows a reduction mechanism similar to that of nitrobenzene [3]. Biologically, the mechanism of action of this class of compounds is dependent on the reduction of the nitro group. In fact, the biochemical lesion of DNA is caused by the intermediate radicals during subsequent oxidation of the products of reduction of the nitro group [2, 4].

Electrochemical methods have been used to study the reduction mechanism of nitroimidazoles as antimicrobial agents [5], and in pharmaceutical [6] and clinical [7] analysis. Both mercury [8] and solid electrodes [9], modified with DNA, have been developed with the aim of detecting the interaction and damage of species with DNA [10–12] as well as for studying the voltammetric behavior of several drugs [13–15]. Some authors [16, 17] proposed that for 2-nitroimidazoles the hydroxylamine formed by reduction of the nitro group would be responsible for the biological activity of these compounds, forming an adduct with guanine residues by a phosphate ester bond, but others [1, 3, 4] suggested that for the 5-nitroimidazoles this mechanism occurs to a lesser extent, the nitro radical being responsible for the biochemical lesion of DNA.



METRONIDAZOLE: $R_1$ = $-CH_2CH_2OH$ ; $R_2$ = $-CH_3$

TINIDAZOLE: $R_1$ = $-CH_2CH_2SO_2Et$ ; $R_2$ = $-CH_3$

SECNIDAZOLE: $R_1$ = $-CH_2CH_2(OH)Me$ ; $R_2$ = $-CH_3$

NIMORAZOLE: $R_1$ = $-CH_2CH_2N$⟨O⟩ ; $R_2$ = $-H$

Scheme 1.

Electrochemical reduction of nitroimidazole derivatives [4, 18–20] shows two reduction waves in aqueous acid media, the first involving four electrons and corresponding to the reduction of the nitro group to form the intermediate hydroxylamine (–NHOH) and the second involving two electrons and corresponding to the reduction of the hydroxylamine to amine ($-NH_2$). However, for ornidazole [21] the second wave disappears at higher pH values and for other nitroimidazoles, only one wave occurs in alkaline media corresponding to a six electron transfer.

We have verified [14, 15] using three different electrode materials – bare glassy carbon electrode, mercury thin film electrode and DNA-biosensor – that hydroxylamine formation involves four electrons and is pH dependent. Our results are in good agreement with previous work using the dropping mercury electrode [22, 23]. Moreover, in our work the DNA-biosensor showed the advantage of preconcentration of metronidazole on the electrode surface and a good linear working range of 1.0 to 54.3 $\mu$mol/L when using 2 min preconcentration at 0.0 V with stirring was obtained [15]. The preconcentration consists in accumulating the electroactive species onto the electrode surface at a predetermined applied potential during an optimized length of time, which will incorporate either metronidazole or its reduction products on the DNA-biosensor and will enable the detection of lower concentration levels of the electroactive analyte in solution when a potential scan is applied in the determination step.

The DNA-biosensor, developed by us [12, 14, 15] enables a new perspective in the research and study of the mechanism of action of nitroimidazoles with DNA, and which is explored in this article.

## 2. Experimental

Metronidazole (MTZ) and secnidazole (SCZ) were supplied by Rhodia Farma Lda, nimorazole (NMZ) by Abbott Laboratórios do Brasil Lda, and tinidazole (TNZ) by Laboratórios Pfizer Lda. Calf thymus DNA (sodium salt, type I), was obtained from Sigma Chemical Co. and was used without further purification. Single stranded DNA (ssDNA) was prepared by treating an accurately weighed sample of approximately 4 mg of DNA with 0.5 mL of

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Table 1. Comparison of the electrochemical data for the differential pulse reduction peak potentials of a group of nitroimidazoles at a bare glassy carbon electrode (GCE) and at the DNA-biosensor, pH 4.5 acetate buffer, and the calibration plot data obtained using the DNA-biosensor.

| *Compound* | *GCE* | *DNA-biosensor* | *Calibration plots (DNA-biosensor)* |
|---|---|---|---|
| Secnidazole | −0.634 V | −0.436 V | $I_p[\mu A] = 0.138\ C[\mu M] + 0.0655$ ($r = 0.999$) |
| Tinidazole | −0.642 V | −0.432 V | $I_p[\mu A] = 0.155\ C[\mu M] - 0.0185$ ($r = 0.999$) |
| Nimorazole | −0.571 V | −0.410 V | $I_p[\mu A] = 0.249\ C[\mu M] - 0.0193$ ($r = 0.999$) |
| Metronidazole | −0.692 V | −0.472 V | $I_p[\mu A] = 0.123\ C[\mu M] + 0.00399$ ($r = 0.999$) |

60 % pure perchloric acid; after dissolution, 0.5 mL of 9M NaOH was then added to neutralize the solution followed by 49 mL of pH 4.5 acetate buffer. Acetate buffer solutions of ionic strength 0.2 at pH 4.5 were used in all experiments, and were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system. All experiments were done in deoxygenated solutions and at room temperature.

The DNA-biosensor was prepared by covering a glassy carbon electrode (Tokai, GC20, area 0.07 $cm^2$) with 3 mg of DNA dissolved in 80 $\mu$L of pH 4.5 acetate buffer and leaving the electrode to dry. After drying, the electrode was immersed in acetate buffer solution and a constant potential of +1.4 V applied during 5 min. A differential pulse scan was then done from −0.9 V to +1.4 V (vs. SCE) to check that no electrochemical reaction occurs on the surface of the DNA-biosensor in supporting electrolyte. The electrode was then transferred to a solution containing single-stranded DNA and differential pulse voltammograms were recorded in the range 0 to +1.4 V until stabilisation of the peak currents that correspond to adenine and guanine electrooxidation occurred. The counter electrode was a Pt wire, and the reference electrode was a SCE, all contained in a one-compartment cell.

Voltammograms were recorded using a $\mu$Autolab potentiostat/galvanostat running with GPES version 3 software, from Eco-Chemie, Utrecht, Netherlands. Differential pulse voltammetry conditions were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV $s^{-1}$.

## 3. Results and Discussion

In the present work the DNA-biosensor was used to study the electrochemistry of metronidazole in more detail and also that of three other nitroimidazoles: secnidazole (SCZ), tinidazole (TNZ) and nimorazole (NMZ) which follow a similar reduction mechanism involving a four electron transfer to form the corresponding hydroxylamines [1, 3, 22, 24] as occurs with metronidazole.

The electrochemical reduction was studied at pH 4.5 using differential pulse voltammetry. These drugs were studied with a bare glassy carbon electrode and with the DNA-biosensor and, as occurred for metronidazole [14, 15], there was a difference of ca. ~190 mV between the potentials obtained using these two types of electrode for the four nitroimidazoles, Table 1. However, the values of the potentials of the reduction peaks are not very different, within 60 mV, which prevents simultaneous measurements of these nitroimidazoles. So they have always been studied independently and show similar behavior, leading to good calibration plots and linear working range as found for metronidazole [14, 15]. The equations of the calibration plots and corresponding errors are shown in Table 1, demonstrating high precision.

The DNA-biosensor presents the exceptional possibility of preconcentration of the nitroimidazole onto the electrode enabling in situ electrochemical evaluation of the interaction and damage to the DNA immobilized on the surface of the glassy carbon electrode. However, one has to bear in mind that the current peaks in the voltammetric profile probe electron transfer reactions; conformational damage will not be easily detected. On the other hand nonelectrochemical methods will not be able to explain the mechanism involving electron transfer reactions between damaging radicals and DNA.

Several mechanisms for the interaction have been proposed [1, 3, 4] and they all suggest that the reduction of the nitro group forming the radical $R-NO_2^{-\cdot}$ [1, 2, 4] or highly reactive hydroxylamine [16, 18, 25] is responsible for the damage to DNA, hydroxylamine being highly specific for exposed cytosine residues at neutral and acid pH [26].

The interaction of MTZ with DNA is shown in the cyclic voltammograms of Figure 1, in a 1.0 mM MTZ pH 4.5 acetate buffer solution, using three different electrodes: (a) bare glassy carbon electrode; (b) glassy carbon electrode with double-stranded DNA immobilized on the surface; and (c) DNA-biosensor. This figure shows very clearly the differences between the three electrodes. With a bare glassy carbon electrode or a glassy carbon electrode covered by double stranded-DNA the reduction of the nitro group to form the hydroxylamine occurs at, −0.809 V and

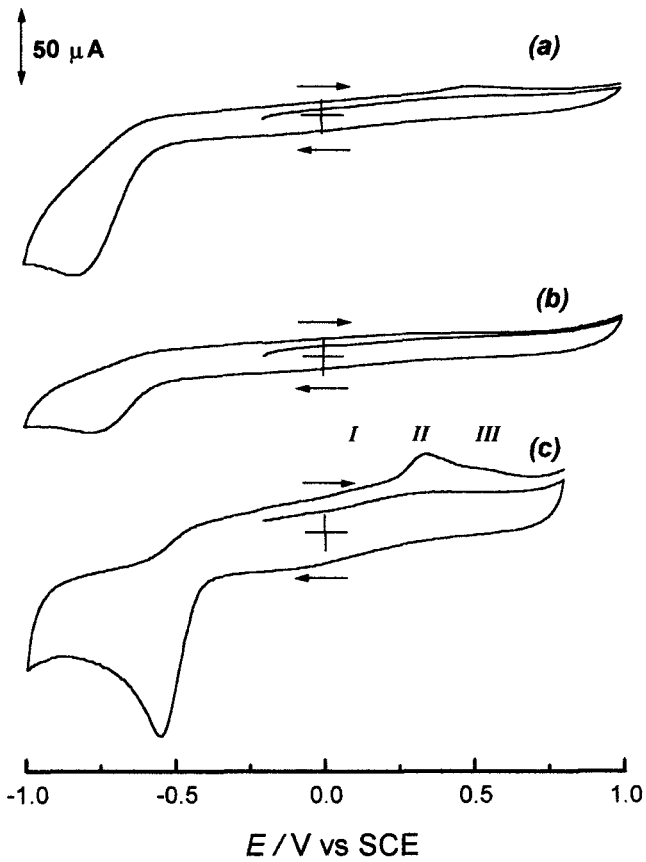



Fig. 1. Cyclic voltammograms of 1.0 mM MTZ in pH 4.5 acetate buffer at: a) GCE; b) GCE with double-stranded DNA immobilized on the surface; c) DNA-biosensor. $\nu = 100$ mV $s^{-1}$; $E_{dep} = 0.0$ V; $t_{dep} = 2$ min with stirring and $E_i = -0.2$ V (vs. SCE).

−0.762 V, respectively and nothing relevant occurs during positive sweep. No preconcentration is possible with either of these working electrodes.

When the DNA-biosensor is used the reduction potential is more positive, −0.556 V, the peak current higher and the peak shape much more well defined. Moreover, three oxidation peaks can be visualized, the first being reversible. These changes in the cyclic voltammogram can only be due to interaction with DNA. There is obviously a great advantage in using the DNA-biosensor as in its presence it is possible to study the mechanism of the irreversible damage. Unfortunately the damage to DNA is irreversible as shown by peak III of the cyclic voltammogram (Fig. 2); the reversible peak I corresponds to the hydroxylamine.

In order to investigate the oxidation of the reduced nitro compound, the preconcentration potential was changed from 0.0 V to −0.6 V (Fig. 2). Preconcentration at a potential lower than the reduction potential of MTZ leads to preconcentration of the reduction products rather than of the starting compound.

The identification of the peaks in Figure 2 is based on the electrochemical behavior of nitrobenzene [23, 27]. The reduction product of MTZ at −0.6 V is the corresponding hydroxylamine which was preconcentrated on the DNA-biosensor surface when a constant potential was applied for 2 min, consequently permitting interaction with the DNA. The cyclic voltammetric scan was started in the positive direction at −0.2 V so that the oxidation of the nitro compound reduction products could be detected. The first wave, *I*, corresponds to the oxidation of the hydroxylamine to a nitroso derivative ($E_p = 0.081$ V); which can be oxidized to an azoxy-compound [16, 28, 29] peak II ($E_p = 0.358$ V), or, in the cyclic voltammetry reverse scan, reversibly reduced to hydroxylamine [23, 27, 30] peak Ia ($E_p = -0.037$ V), and the reduction of the MTZ in solution corresponds to peak *IV* ($E_p = -0.556$ V).

The reduction mechanism of the nitroheterocyclic derivatives,

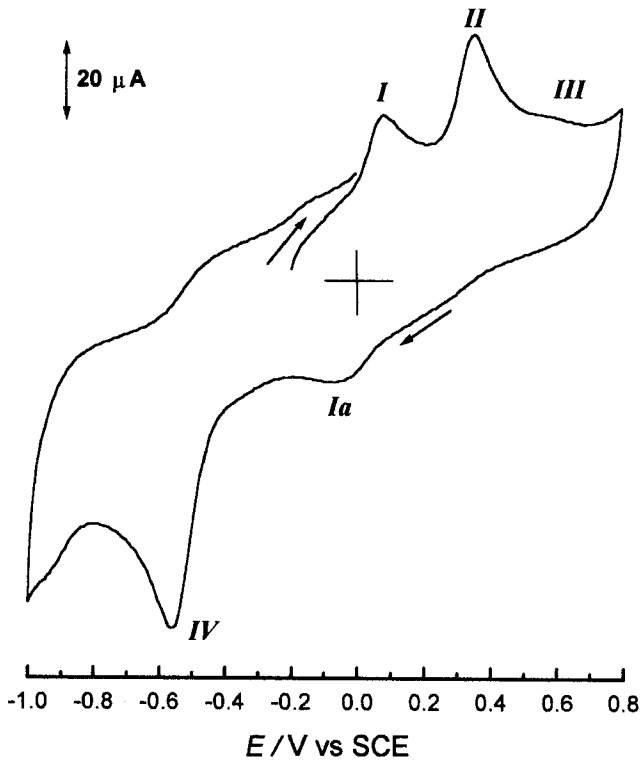
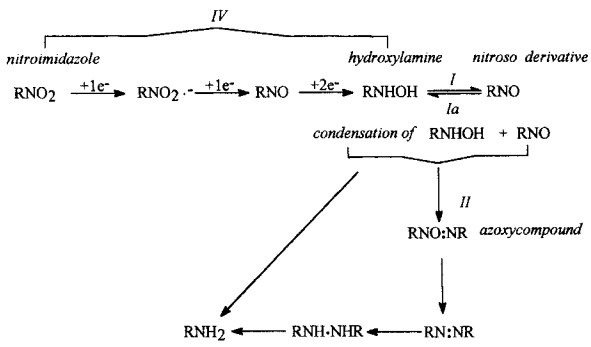



Fig. 2. Cyclic voltammograms of 1.0 mM MTZ in pH 4.5 acetate buffer at a DNA-biosensor. $\nu = 100$ mV s$^{-1}$; $E_{dep} = -0.6$ V; $t_{dep} = 2$ min with stirring and $E_i = -0.2$ V (vs. SCE).




Scheme 2.

responsible for biological activity, and the consequent formation of bimolecular azoxy, azo and hydrazo derivative compounds can schematically be described by Scheme 2.

This shows the reversible oxidation of the hydroxylamine derivative (RNHOH) to the corresponding nitroso derivative (RNO) and a condensation reaction between the hydroxylamine and nitroso derivatives to form the azoxycompound (RNO:NR). The amount of azoxycompound formed depends on the concentration of the nitroso derivative, which reduces rapidly to hydroxylamine [28–30]. Whereas in alkaline solution the hydroxylamine leads to the azoxyderivative and amine, in acid solution formation of the amine dominates.

Concerning peak III ($E_p = 0.595$ V), it is well known that

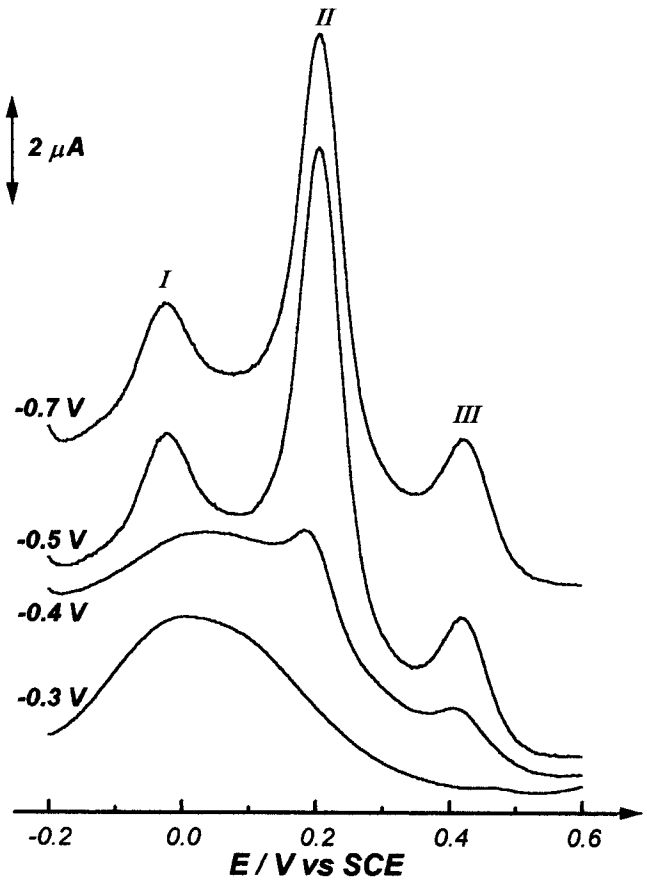



Fig. 3. Differential pulse voltammograms of 0.3 mM MTZ, in pH 4.5 acetate buffer at the DNA-biosensor and at four different preconcentration potentials, $E_{dep} = -0.3$, −0.4, −0.5 and −0.7 V (vs. SCE), $t_{dep} = 2$ min with stirring. $\nu = 5$ mV s$^{-1}$; $\Delta E = 50$ mV.

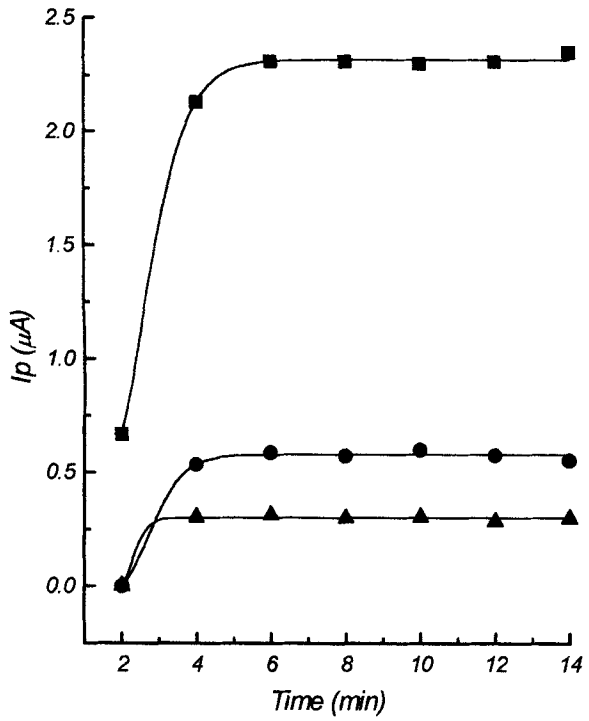



Fig. 4. Effect of preconcentration time on the currents of the anodic stripping peaks of 0.5 mM MTZ in pH 4.5 acetate buffer using the DNA-biosensor: ●) peak I; ■) peak II and ▲) peak III. $\nu = 5\,mV\,s^{-1}$; $\Delta E = 50\,mV$; $t_{dep} = 2\,min$ with stirring and $E_{dep} = -0.5\,V$ (vs. SCE).

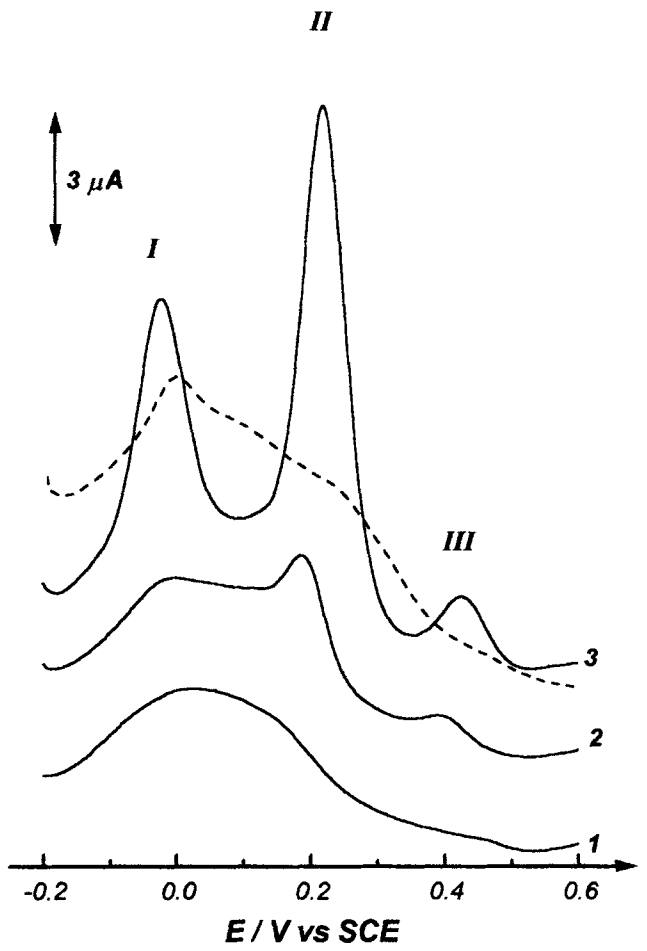



Fig. 5. Anodic stripping differential pulse voltammograms in pH 4.5 acetate buffer, $\nu = 5\,mV\,s^{-1}$; $\Delta E = 50\,mV$; $t_{dep} = 2\,min$ with stirring of MTZ: (——) 1) 0.0 mM; 2) 0.2 mM and 3) 0.5 mM and $E_{dep} = -0.5\,V$ (vs. SCE) using the DNA-biosensor; (– – –) 1.0 mM and $E_{dep} = -0.7\,V$ (vs. SCE) using a bare glassy carbon.

hydroxylamine displays a large difference in reactivity for single-stranded versus double-stranded DNA [26, 31, 32] which makes it a sensitive detector of H-DNA.

H-DNA is the recently discovered nucleic acid triplex structure [26]. The kinetics of formation of triple-stranded structures is slower than that of double-stranded structures and is dependent on the ionic strength of the medium. The recognition of the potential biological roles of H-DNA and the interest in the triple-helical nucleic acid research includes also genetic applications. This H-DNA conformation, formed by mirror-symmetric homopurine-homopyrimidine sequences, consists of an intramolecular PyPuPy triple-stranded region, i.e, the single-strand of an unwound region folds back on the double-strand forming Hoogsteen hydrogen bonds.

Since the DNA-biosensor was prepared by covering a glassy carbon electrode with double-stranded DNA and then transferring it to a solution containing single-stranded DNA for electrochemical conditioning, it is possible that the electrode contains a small amount of de H-DNA segments which could be detected by the hydroxylamine. Further evidence for this hypothesis was given by the same experiments using a glassy carbon modified electrode with either only ssDNA or only dsDNA: an irreproducible electrochemical response was obtained showing no peak III. So it can be suggested that peak III could correspond to the oxidation of a product of MTZ interaction with H-DNA caused probably by the highly reactive hydroxylamine which is at present under investigation. The differential pulse potential of peak III (Fig. 3), is very similar to that of uric acid $E_p = +0.424\,V$ (vs.SCE) in pH 4.5 acetate buffer measured at a newly prepared DNA-biosensor [12]. In fact, this comparison is not speculative and is in agreement with the literature that presents the purine bases as the principal target of the oxidative lesion of DNA [18, 25]. We can therefore assume that peak III corresponds to the formation of deoxypurinic acid derivative [33] formed by reaction of the MTZ reduction intermediates with the DNA multilayer immobilised onto the surface of the glassy carbon electrode. The immobilization of DNA on solid substrates by means of electrostatic interactions has been shown [34] to have clear advantages compared with chemical bonding.

The electrochemical reduction of nitroimidazoles using the DNA-biosensor enables a new perspective of the mechanism of action of these compounds because of the possibility of preconcentration of either the starting materials or the reduction products, thus permitting the probing of the presence of short lived intermediates and their damage to DNA. Four different preconcentration potentials: −0.3 V (before), −0.4 V (just at the beginning of), −0.5 V (just after), and −0.7 V (after) the reduction peak, were chosen to study the effect of applied potential on the formation of the reduction products of MTZ (Fig. 3). It is clear that a preconcentration potential, $E_{dep}$, just negative of the peak for the reduction of MTZ is the best and so −0.5 V was used during the rest of this work.

The values for the differential pulse peak potentials are: for the first oxidation wave, I, corresponding to the oxidation of the hydroxylamine to the nitroso derivative ($E_p = -0.021$ V), for the oxidation of the azoxycompound formed in a coupling reaction peak II ($E_p = 0.224$ V), and for the interaction with DNA peak III ($E_p = 0.429$ V).

An important parameter is the preconcentration time because of its relation with the formation of hydroxylamine that will interact with DNA. Figure 4 depicts the influence of the preconcentration

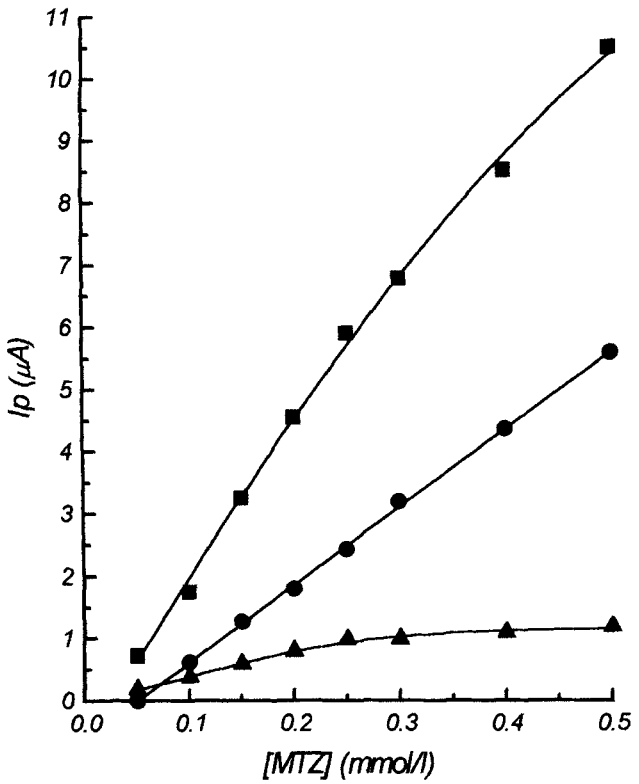



Fig. 6. Anodic stripping peak currents corresponding to ●) peak I, $E_p = -0.021$ V; ■) peak II, $E_p = +0.223$ V and ▲) peak III, $E_p = +0.429$ V using the DNA-biosensor during standard additions of MTZ, in pH 4.5 acetate buffer. $E_{dep} = -0.5$ V (vs. SCE), $t_{dep} = 2$ min and stirring, $E_i = -0.2$ V.

time on the peak currents for the three oxidation peaks; two minutes preconcentration with stirring, was found to be the best. Figure 5 shows the anodic stripping differential pulse voltammograms of MTZ for different concentrations. This figure also includes, for comparison, the results at a bare glassy carbon electrode; because it is not possible to preconcentrate when using this electrode a much higher concentration, 1 mM, of MTZ was used, nevertheless no peak III appeared. However, peak III can already be seen for a concentration of 0.2 mM when using the DNA-biosensor. This confirms the assumption that peak III is due to interactions of the reduction intermediates with the DNA. The effect of increasing the metronidazole concentration can be followed better in Figure 6 where the increase in current is proportional to concentration for peak I, oxidation of hydroxylamine, and peak II, azoxycompound formation, whereas peak III very quickly reaches saturation. In fact, the concentration of bases is limited by the amount of nucleic acid, in the double-stranded DNA or small segments of H-DNA form, immobilized on the electrode surface. This observation also confirms the link of peak III to the interaction of the metronidazole reduction intermediates with the DNA on the electrode.

In order to better understand the biological activity of this type of

Table 2. Electrochemical oxidation data at the DNA-biosensor of the reduction products of three nitroimidazoles: metronidazole, secnidazole, tinidazole.

| *Compound* | $E_p$ [V] | | | $I_p$ [μA] | | |
|---|---|---|---|---|---|---|
| | *I* | *II* | *III* | *I* | *II* | *III* |
| Metronidazole | −0.021 | 0.224 | 0.429 | 5.59 | 10.5 | 1.18 |
| Secnidazole | 0.0039 | 0.238 | 0.454 | 8.25 | 8.13 | 1.13 |
| Tinidazole | −0.0027 | 0.228 | 0.448 | 1.25 | 5.38 | 2.50 |

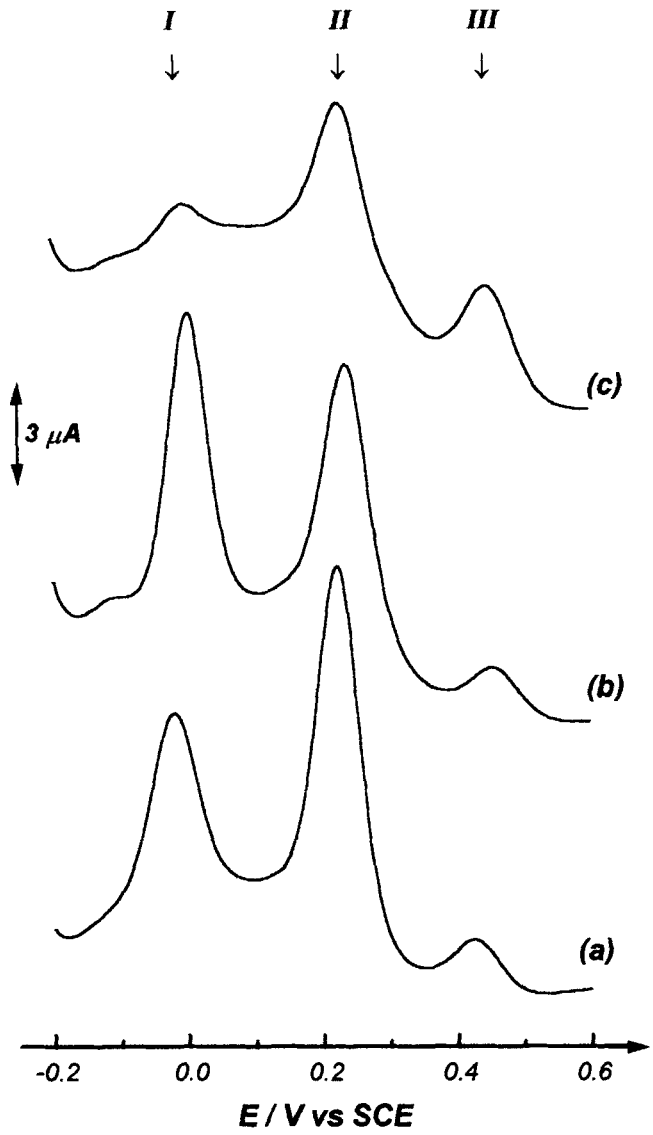



Fig. 7. Anodic stripping differential pulse voltammograms in pH 4.5 acetate buffer using a DNA-biosensor of three nitroimidaloles, 0.5 mM: a) metronidazole; b) secnidazole; c) tinidazole. $\nu = 5$ mV/s; $\Delta E = 50$ mV; $t_{dep} = 2$ min with stirring and $E_{dep} = -0.5$ V (vs. SCE), $E_i = -0.2$ V.

drugs similar voltammetric experiments using the DNA-biosensor were carried out for two other nitroimidazoles, secnidazole and tinidazole (Fig. 7 and Table 2). There is no appreciable difference in the peak potentials; the differences in the peak currents for peaks I and II can be explained by the difference in the substituent groups in the N1 position. The reduction intermediates' interaction with DNA can be assigned to peak III with the same explanation as for MTZ; the nitro radical is always that principally responsible for the biochemical lesion on DNA of anaerobic microorganisms.

Nevertheless, despite the importance of the nitro group for the biological activity of the nitroimidazoles, their liposolubility can vary with changes in the side chain [35] which alters the absorption rate of the drug, and which is therefore an additional factor to be taken into account.

## 4. Conclusions

The mechanism of reduction of metronidazole and other nitroimidazoles can be investigated using the DNA-biosensor in a

new way as it is possible to preconcentrate the analyte on the electrode surface and study either its reduction or the oxidation of the reduction products retained on the electrode surface.

In this sense the DNA-biosensor opens a wide perspective to the study of the mechanisms of interaction of drugs and the possibility of prescreening the damage they will cause to DNA integrity.

## 5. Acknowledgements

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## 6. References

[1] D.I. Edwards, in *DNA binding and nicking agents*, Comprehensive Medicinal Chemistry, Vol. 2, 5th ed. (Ed: C. Hansch), Pergamon Press, New York, **1990**.
[2] D.I. Edwards, *J. Antimicrob. Chemother.* **1993**, *31*, 9.
[3] P. Zuman, Z. Fijalek, *J. Electroanal. Chem.* **1990**, *296*, 538.
[4] J.H. Tocher, D.I. Edwards, *Biochem. Pharmac.* **1994**, *48*, 1089.
[5] Y.W. Chien, S.S. Mizuba, *J. Med. Chem.* **1978**, *21*, 374.
[6] A.Z.A Zuhri, S.I. Al-Khalil, M.S. Suleiman, *Anal. Lett.* **1986**, *19*, 453.
[7] Z. Wang, H. Zhou, S. Zhou, *Talanta* **1993**, *40*, 1073.
[8] E. Palecek, *Electroanalysis* **1996**, *8*, 7.
[9] S.R. Mikkelsen, *Electroanalysis* **1996**, *8*, 15.
[10] J. Wang, M. Chicharro, G. Rivas, X. Cai, N. Dontha, P.A.M. Farias, H. Shiraishi, *Anal. Chem.* **1996**, *68*, 2251.
[11] J. Wang, G. Rivas, D. Luo, X. Cai, F.S. Valera, N. Dontha, *Anal. Chem.* **1996**, *68*, 4365.
[12] A.M. Oliveira-Brett, S.H.P. Serrano, T.A. Macedo, D. Raimundo, M.H. Marques, M.A. La-Scalea, *Electroanalysis* **1996**, *8*, 992.
[13] C. Teijeiro, P. Perez, D. Marín, E. Palecek, *Bioelectrochem. Bioenerg.* **1995**, *38*, 77.
[14] A.M. Oliveira-Brett, S.H.P. Serrano, I. Gutz, M. A. La-Scalea, *Bioelectrochem Bioenerg* **1997**, *42*, 175.
[15] A.M.Oliveira-Brett, S.H.P. Serrano, I. Gutz, M.A. La-Scalea, *Electroanalysis* **1997**, *9*, 110.
[16] G.F. Whitmore, A. J. Varghese, *Biochem. Pharmac.* **1986**, *35*, 97.
[17] R.A. McClelland, R. Panicucci, A.M. Rauth, *J. Am. Chem. Soc.* **1987**, *109*, 4308.
[18] P.J. Declerck, C.J. De Ranter, *J. Chem. Soc., Faraday Trans 1* **1987**, **83**, 257.
[19] D. Dumanovic, J. Volke, V. Vajgand, *J. Pharma. Pharmac.* **1966**, *18*, 507.
[20] D. Dumanovic, J. Ciric, *Talanta* **1973**, *20*, 525.
[21] J.M.L. Fonseca, M.C.G. Rivera, J.C.G. Monteagudo, *Anal. Lett.* **1973**, *26*, 109.
[22] P. Zuman, Z. Fijalek, D. Dumanovic, D. Suznjevic, *Electroanalysis* **1992**, *4*, 783.
[23] C. Karakis, P. Zuman, *J. Electroanal. Chem.* **1995**, *396*, 499.
[24] P.J. Declerck, C.J. De Ranter, *Analusis* **1987**, *15*, 148.
[25] P.J. Declerck, C.J. De Ranter, *Biochem. Pharmac.* **1986**, *35*, 59.
[26] V. N. Soyfer, V.N. Potaman, *Triple-Helical Nucleic Acids*, Springer, Berlin **1995**.
[27] I. Rubinstein, *J. Electroanal. Chem.* **1985**, *183*, 379.
[28] N.V. Sidgwick, *Organic Chemistry of Nitrogen*, Clarendon Press, Oxford, **1937**.
[29] Z. Fijalek, M. Pugia, P. Zuman, *Electroanalysis* **1993**, *5*, 65.
[30] R. Carlier, E. Raoult, A. Tallec, V. Andre, P. Gauduchon, J.-C. Lancelot, *Electroanalysis* **1997**, *9*, 79.
[31] B. Johnston, *Meth. Pharmacol.* **1992**, *212*, 180.
[32] E. Freese, E.B. Freese, *Biochemistry* **1965**, *4*, 2419.
[33] G. Dryhurst, *J. Electrochem. Soc.* **1969**, *116*, 1411.
[34] G.B. Sukhorukov, M.M. Montrel, A.I. Petrov, L.I. Shabarchina, B.I. Sukhorukov, *Biosens. Bioelectron.* **1996**, *11*, 913.
[35] A. Korolkovas, *Essentials of Medicinal Chemistry*, 2nd. ed., Wiley, New York **1988**.