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Detection of the damage caused to DNA by niclosamide using an electrochemical DNA-biosensor

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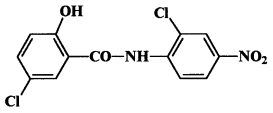
Abstract

Niclosamide is the only commercially available molluscicide recommended by the WHO for large-scale use in schistosomiasis control programs. The electrochemical reduction and oxidation mechanism of niclosamide was studied using cyclic, differential and square wave voltammetry, at a glassy carbon electrode. An indirect procedure for in situ quantification of niclosamide using batch injection analysis with electrochemical detection, possible to be used for in situ determinations in river streams and effluents, was developed. It enabled a detection limit of 8×10^{-7} M. The investigation of the niclosamide–DNA interaction using an electrochemical DNA-biosensor showed for the first time clear evidence of interaction with DNA and suggested that niclosamide toxicity can be caused by this interaction, after reductive activation.

Keywords: DNA biosensor; Niclosamide; Quantification; Reductive activation; Electrochemical methods; DNA damage

1. Introduction

The halogenated salicylanilides are a large group of compounds which have been developed mainly due to their antiparasitic activity in animals. Among them, the commercially available ethanolamine salt of Niclosamide (Bayluscide®) has several uses in agriculture, veterinary (Swan, 1999) and public health (Andrews et al., 1982; Datta and Bera, 2000).



Niclosamide

Niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) is the only commercially available molluscicide recommended by the WHO for large scale use in schistosomiasis control programs (WHO, 1993). Schistosomiasis is an endemic parasitic disease, affecting the tropical and subtropical regions of the world, and is second only to malaria in the havoc it causes to the social and economic development of countries located in these areas (Lardans and Dissous, 1998).

The use of molluscicides in prophylactic treatment promotes the rupture of the evolutionary cycle of the worm with the destruction of its intermediate host, the snail *B. glabrata* (Lardans and Dissous, 1998; Perrett and Whitfield, 1996). Niclosamide can kill *B. glabrata* adults at concentrations as low as 1.5 ppm after 2 h exposure, as well as the miracidia and cercariae, other larval stages of trematodes (Andrews et al., 1982).

Despite its general use, niclosamide is severely toxic to some aquatic organisms (Andrews et al., 1982; Lardans and Dissous, 1998; Oliveira and Paumgartten, 2000). Long-time administration also caused problems to terrestrial and aquatic plants (Andrews et al., 1982). The pharmacokinetic behaviour appears to play an important role in the efficacy and safety of these compounds (Swan, 1999).

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Many efforts have been made to elucidate the mode of action of niclosamide. However, the mechanisms of action of niclosamide are not completely elucidated (Andrews et al., 1982). The available data suggested that it can act mostly on respiration and carbohydrate metabolism and that the nitro group is fundamental for the activity. The reduced niclosamide, 2,5′-dichloro-4′-aminosalicylanilide, has been shown to lose all molluscicidal and cestocidal properties, being also ineffective in decoupling respiration and electron transport linked phosphorylation (Strufe, 1964).

Analytical methods used for its quantification in natural water and in fish tissues employed HPLC (Schreier et al., 2000; Van Tonder et al., 1996) and spectrophotometric methods (Daabees, 2000; Abdel-Fattah, 1997; Onur and Tekin, 1994; Emara, 1993).

The electrochemical reduction of niclosamide at a mercury electrode and for a wide pH range showed a reduction mechanism involving the transfer of four electrons (Sridevi and Reddy, 1991). The role of nitroreduction in the activation of niclosamide and 2-chloro-4-nitroaniline was investigated and the studies indicated that mutagenicity depends on both nitroreduction and transacetylation (Espinosa Aguirre et al., 1991).

The importance of niclosamide justifies the research of its still uncertain mechanism of action, in order to clarify the possible involvement of the reductive metabolism into the pharmacokinetics of this family of compounds, and also to verify the long time administration cytotoxicity on endobiotics.

With this in mind an electrochemical DNA-biosensor was used to investigate possible DNA damage caused by niclosamide. The electrochemical DNA-biosensor enables us to evaluate and predict DNA interactions and damage by health hazardous compounds, based on their binding to nucleic acids, exploring the use of voltammetric techniques for in situ generation of reactive intermediates, which react with DNA. It is also a complementary tool for the study of biomolecular interaction mechanisms. The electrochemical characteristics of these DNA-biosensors have been evaluated (Oliveira-Brett et al., 1999).

The mechanism of interaction of DNA–niclosamide at charged interfaces mimics better the in vivo DNA–niclosamide situation, where it is expected that DNA is in close contact with charged phospholipid membranes and proteins, rather than when the interaction is in solution.

This paper presents electrochemical studies of reduction and oxidation of niclosamide using a glassy carbon electrode, including an indirect procedure for its quantification and an investigation of the niclosamide–DNA interaction using an electrochemical DNA-biosensor. The results presented lead to the proposal of a possible mechanism through which niclosamide can cause direct in vivo damage to DNA.

2. Experimental

Niclosamide and Calf Thymus DNA (Sodium salt, Type I) were obtained from Sigma Chemical Co. 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 (conductivity < 0.1 μS cm^{-1}). All experiments were performed at room temperature.

Niclosamide was dissolved in a 1:1 mixture of pH 4.5 0.2 M aqueous acetate buffer and ethanol. Except where mentioned, no deoxygenation by bubbling an inert gas through the solution was necessary.

The DNA-modified electrode was prepared by covering a glassy carbon electrode with 80 μl of DNA solution (35 mg of DNA dissolved in 1 ml of pH 4.5 0.2 M acetate buffer) and leaving the electrode to dry for 24 h (Oliveira-Brett et al., 1997).

The pH measurements were carried out with a Crison Model micropH2001 pH-meter with a combined glass electrode.

Differential pulse voltammograms were recorded using an AUTOLAB PGSTAT 10 running with GPES version 4.3, software PG (Eco-Chemie, Utrecht, The Netherlands). The working electrode was glassy carbon ($d = 6$ mm), the counter electrode was a Pt coil, and the reference was a saturated calomel electrode (SCE), all contained in a one-compartment electrochemical cell, with a volumetric capacity of 10 ml. The glassy carbon electrode was polished with diamond spray (6 and 3 μm) on a polishing felt, resulting in a surface with a mirror-like appearance. After mechanical cleaning, electrochemical pretreatment involved a sequence of ten cyclic potential scans from 0 to $+1.5$ V in the acetate buffer solution. The differential pulse voltammetry conditions used were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s^{-1}. The square wave voltammetry conditions used were: frequency 50 Hz, potential increment 2 mV, and effective scan rate 100 mV s^{-1}. Some experiments were carried out under N_2.

Batch injection analysis (BIA) experiments (Brett et al., 1994) were carried out in an appropriate cell, using a glassy carbon electrode, deposition time, $t_d = 30$ s, and deposition potential, $E_d = -0.6$ V. Injections were performed using a programmable motorised electronic micropipette (EDP Plus 100, EP-1000, Rainin). This micropipette, with a maximum dispension volume of 100 μl, was used with the dispension speed of 24.5 μl s^{-1}, and injection of 50 μl of niclosamide.

3. Results and discussion

3.1. Electrochemistry of niclosamide

Cyclic voltammetry of niclosamide at a glassy carbon electrode (Fig. 1) presents the usual oxidation and reduction electron transfer reactions characteristic of this class of compounds. The irreversible reduction wave, Ic, at $E_{pIc} = -0.625$ V, corresponds to a four electron/four proton reaction, forming hydroxylamine, typical of a nitroreduction in aqueous acid solution (Tocher, 1997) (Scheme 1) and is similar to the behaviour of this compound observed at mercury, at the same pH (Sridevi and Reddy, 1991). Scheme 1 represents the usual pathway for the reduction of the aromatic nitro group. At neutral and less acid pH, the reduction product is mainly the hydroxylamine derivative (Tocher, 1997).

Three anodic peaks are observed. Two are easily identified, IIa, at $E_{pIIa} = -0.006$ V, and IVa, $E_{pIVa} = +0.783$ V, but the third, IIIa, $E_{pIIIa} = +0.420$ V, can only be distinguished with great difficulty. The first anodic wave, IIa, corresponds to the reversible oxidation of hydroxylamine to form the nitroso derivative which is itself reduced in IIc, at $E_{pIIc} = -0.060$ V, to form again the hydroxylamine. Wave IIIa, $E_{pIIIa} = +0.420$ V, probably corresponding to condensation reactions expected concerning the oxidation of the reduced intermediates formed, generating electroactive azoxy derivatives (Oliveira-Brett et al., 1997; Fijalek et al., 1993; Lund, 2001) and will be discussed later (Scheme 1). Wave IVa, $E_{pIVa} = +0.783$ V, is related to the irreversible oxidation of the phenolic group.

The reduction of niclosamide was also studied by square wave voltammetry (Fig. 2) and the reduction mechanism described earlier was confirmed. The irreversible reduction wave Ic, corresponding to a four

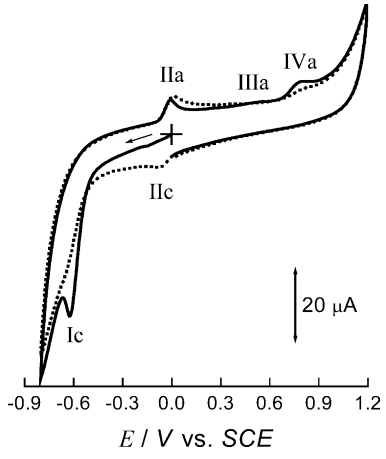
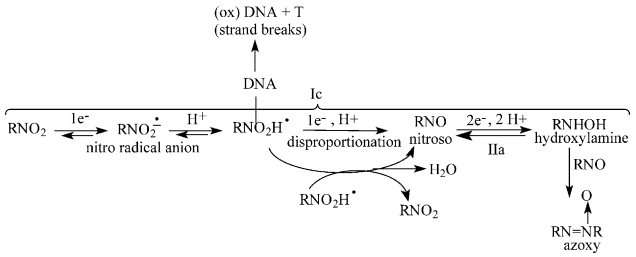


Fig. 1. Cyclic voltammogram of 5.0×10^{-5} M niclosamide in 1:1 mixture of pH 4.5 0.2 M aqueous acetate buffer and ethanol at a GCE: (—) first scan and (· · · · ·) second scan. $E_i = 0$ V, $v = 100$ mV s^{-1}.



Scheme 1. Reduction pathway of nitroaromatics (T is thymine). Adapted from Tocher (1997).

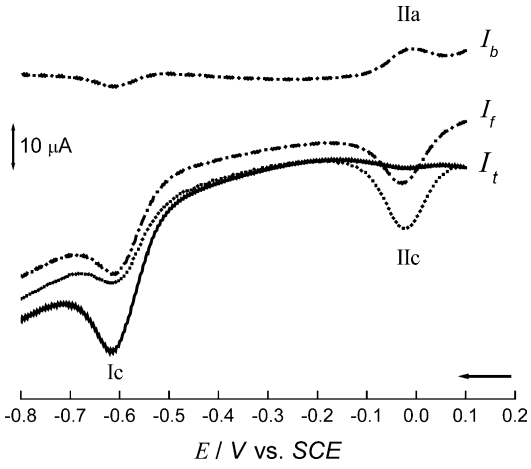


Fig. 2. Square wave voltammograms of 5.0×10^{-5} M niclosamide in 1:1 mixture of pH 4.5 0.2 M aqueous acetate buffer and ethanol at a GCE, under N_2: first scan, (—) total current, I_t; and second scan, (· · · ·) total, I_t, (-·-·-) forward, I_f, and backward, I_b, currents. $f = 50$ Hz.

electrons/four protons nitroreduction in aqueous acid solution, was present in the first scan but the reversible wave IIa/IIc corresponding to the pair hydroxylamine/nitroso derivative, only occurred in the second scan, as predicted. Square wave voltammetry forward and backward currents for the second scan clearly show the reversibility of the electrogenerated hydroxylamine/nitroso (IIa/IIc) system.

Successive differential pulse voltammograms for reduction of niclosamide showed the decrease of the reduction peak corresponding to the nitro group, Ic, and an increase of the peak for reduction of the nitroso derivative, IIc (Fig. 3). This behaviour is explained by reduction of niclosamide leading to its consumption on the electrode surface and electrogenerating hydroxylamine that accumulates onto the electrode surface and is reversibly oxidised to nitroso.

Successive differential pulse voltammograms for oxidation of niclosamide showed the decrease of all three oxidation peaks (Fig. 4). However, it is interesting to notice that peak IIIa ($E_{pIIIa} = +0.420$ V) only appears in the first scan. Condensation reactions are expected concerning the reduced intermediates formed, generating electroactive azoxy derivatives, responsible for peak

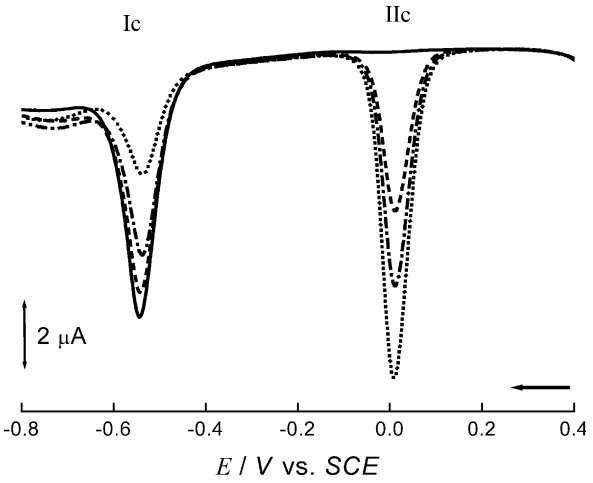


Fig. 3. Successive differential pulse voltammograms of reduction of 5.0×10^{-5} M niclosamide in 1:1 mixture of pH 4.5 0.2 M aqueous acetate buffer and ethanol at a GCE, under N_2: (—) first scan, (– – –) second scan, (–·–·–) sixth scan, and (·····) 15th scan. $v = 5$ mV s^{-1}.

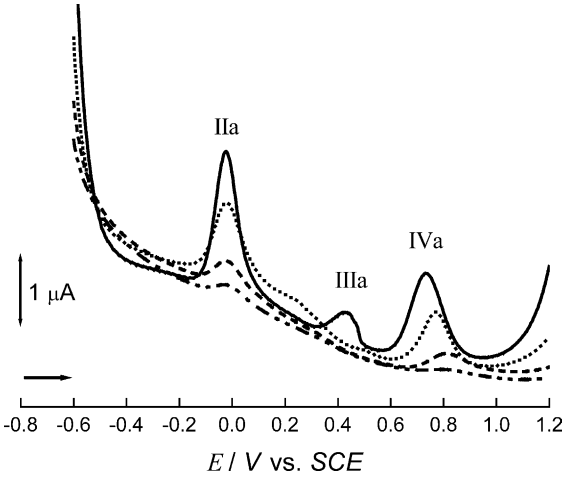


Fig. 4. Successive differential pulse voltammograms for oxidation of 5.0×10^{-5} M niclosamide in 1:1 mixture of pH 4.5 0.2 M aqueous acetate buffer and ethanol at a GCE, under N_2: (—) first scan; (·····) fifth scan; (– – –) tenth scan and (–·–·–) 16th scan. $v = 5$ mV s^{-1}.

IIIa (Scheme 1, Tocher, 1997) (Oliveira-Brett et al., 1997; Fijalek et al., 1993; Lund, 2001). The decrease in height of the peaks can be explained by the formation of non-electroactive oxidation products during the irreversible oxidation of the phenolic group, peak IVa, that causes phenol polymerisation which blocks the electrode surface. This is confirmed, because the first scan can be reproduced after cleaning the electrode surface.

Differential pulse voltammograms for oxidation of niclosamide starting at different potentials confirmed that peak IVa is due to the irreversible oxidation of the phenolic group, the only group able to undergo oxidation in niclosamide (Fig. 5 first scan), and that IIIa is generated only after niclosamide reduction at −0.6 V and is not stable, occurring only in the first scan, Fig. 5. Applying a potential of −0.6 V for 120 s as expected

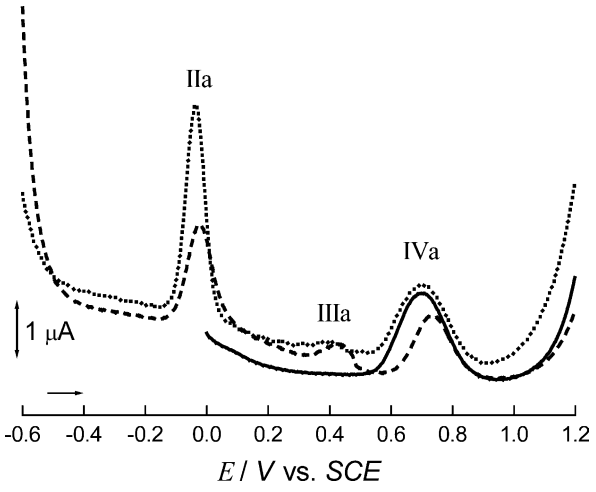


Fig. 5. Differential pulse voltammograms of 5.0×10^{-5} M niclosamide in 1:1 mixture of pH 4.5 0.2 M aqueous acetate buffer and ethanol at a GCE, under N_2: (—) $E_i = 0$ V; (– – –) $E_i = -0.6$ V; (·····) $E_i = -0.6$ V after $E_d = -0.6$ V applied for $t_d = 120$ s. $v = 5$ mV s^{-1}.

causes always an increase in the hydroxylamine peak, IIa.

The possibility of accumulating hydroxylamine or nitroso derivatives at the glassy carbon surface, as shown in Fig. 5, was used for the development of an indirect quantification procedure for niclosamide. Good linearity was found between peak current and concentration described by the equation $I_{p,c}$ (A) = 6.45432 + 1.7889 ([C] M^{-1}) ($R = 0.99351$, $n = 5$, S.D. = 2.68356), in a stationary cell, for niclosamide in the range $2–30 \times 10^{-6}$ M, applying a deposition time, $t_d = 30$ s, and a deposition potential, $E_p = -0.6$ V. The detection limit was 4.5×10^{-6} M.

BIA (Wang and Taha, 1991) enables the investigation of electrode reactions using extremely small volumes of analyte solution of 100 μl. In electrochemical amperometric detection, a sample is injected from a micropipette tip directly over the centre of a disk electrode. The electrode immersed in inert electrolyte is held at a constant applied potential corresponding to the electrode reaction and the injection of sample causes a peak. The determination of niclosamide using BIA (Brett et al., 1994) (Fig. 6) showed a much better correlation, $I_{p,c}$ (A) = 0.19594 + 0.28586 ([C] M^{-1}) ($R = 0.99863$, $n = 12$, S.D. = 0.07685) and enabled to attain a lower detection limit of 8×10^{-7} M. The reason for this can be attributed the hydrodynamic conditions during injection and to the lower contact time between sample and electrode.

This methodology is very simple and has great advantages, because it can be used for in situ analysis in a real situation for determination of niclosamide in water streams in the environment when this drug is applied. The detection limit is comparable to that currently obtained by chromatographic and spectro-

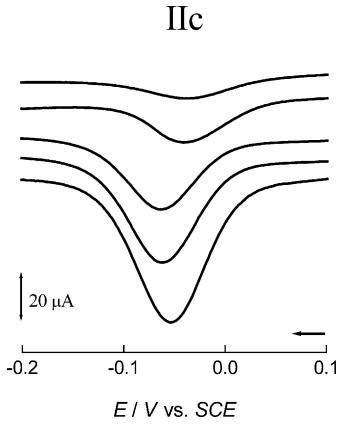


Fig. 6. Square wave voltammograms using BIA of 2, 6, 10, 20 e 30 μM niclosamide in 1:1 mixture of pH 4.5 0.2 M aqueous acetate buffer and ethanol at a GCE, under N_2. $f = 50$ Hz.

scopic methods (Schreier et al., 2000; Van Tonder et al., 1996; Daabees, 2000; Abdel-Fattah, 1997; Onur and Tekin, 1994; Emara, 1993; Sridevi and Reddy, 1991).

3.2. Niclosamide–DNA interaction

The niclosamide–DNA interaction was investigated using the DNA-biosensor prepared by immobilising DNA onto the glassy carbon surface as described in the Section 2.

The DNA gel on the electrode surface enables the accumulation of the analyte on the biopolymer matrix. The electrochemical reduction of niclosamide generates short-lived radicals that interact with DNA causing damage (Scheme 1).

The differential pulse voltammetry of niclosamide reduction using a DNA-biosensor (Fig. 7) shows that pre-concentration of hydroxylamine into the DNA matrix occurs, denoted by the increase, with successive scans, of the reduction peak for the nitroso derivative, IIc at $E_{pIIc} = +0.060$ V.

Differential pulse voltammetry of niclosamide oxidation using a DNA-biosensor (Fig. 8) shows no interaction with DNA whatsoever. After 15 scans from 0 to +1.4 V, no peak for the oxidation of the phenol group or of a purine base was observed. This clearly indicates that niclosamide which is not reduced does not interact and damage DNA. In fact, only after niclosamide is reduced, at $E_d = -0.6$ V for $t_d = 120$ s, does it generate radicals that interact with DNA (Fig. 8). This interaction was detected by electrochemical sensing of the oxidation of the DNA purine bases (Oliveira-Brett et al., 1999). The appearance of guanosine, $E_p = +0.97$ V, and adenosine, $E_p = +1.33$ V, peaks, demonstrates clearly that the damage by the radicals caused distortion of the double helix and exposure of the bases that can be oxidised.

It is interesting to observe that if the reduction of niclosamide was accomplished in successive cathodic cycling, from 0 to −0.8 V (15 scans) (Fig. 9) the damage caused was greater than when a fixed potential of −0.6 V was applied. The oxidation peak of 8-oxoguanine, a product of guanine oxidation, $E_p = +0.46$ V (Oliveira-Brett et al., 2000) could also be identified.

Comparison between the results obtained, after cycling 15 scans, from 0 to −0.8 V, and pre-concentrating reduced niclosamide radicals, using the glassy carbon bare electrode and modified with DNA, DNA-biosensor (Fig. 10) clearly shows the damage caused by the radicals to the DNA, in agreement with Scheme 1 (Tocher, 1997).

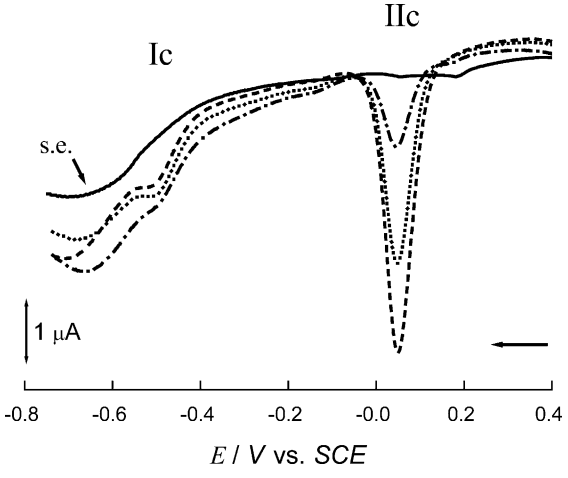


Fig. 7. Differential pulse voltammograms using the DNA-biosensor, under N_2: (—) in 1:1 mixture of pH 4.5 0.2 M aqueous acetate buffer and ethanol; and 5.0×10^{-6} M niclosamide in 1:1 mixture of pH 4.5 0.2 M aqueous acetate buffer and ethanol: after (–·–·–·–·–) ten scans, (·····) 20 scans and (– – – –) 55 scans. $v = 5$ mV s^{-1}.

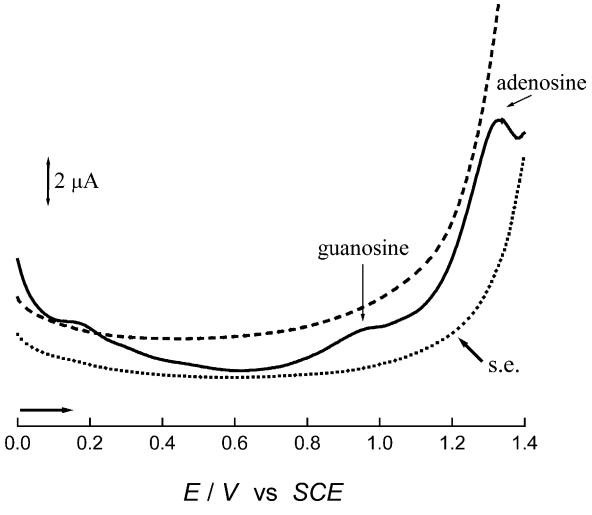


Fig. 8. Differential pulse voltammograms using the DNA-biosensor in 1:1 mixture of pH 4.5 0.2 M aqueous acetate buffer and ethanol: without (·····) and with 5.0×10^{-5} M niclosamide after: (– – – – –) 15 scans from 0 to +1.4 V; and (—) $t_d = 120$ s at $E_d = -0.6$ V. $v = 5$ mV s^{-1}.

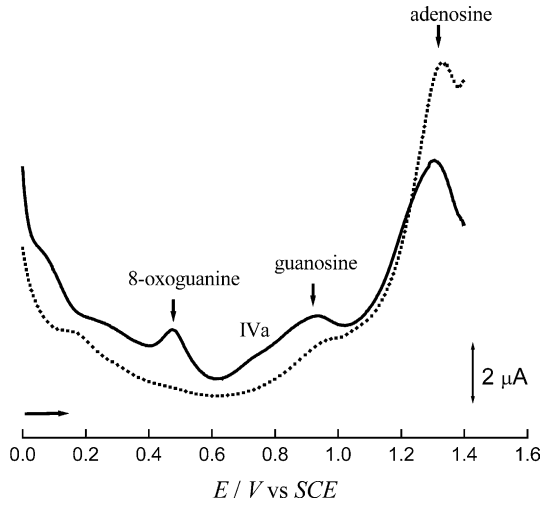


Fig. 9. Differential pulse voltammograms of 5.0×10^{-5} M niclosamide in 1:1 mixture of pH 4.5 0.2 M aqueous acetate buffer and ethanol using the DNA-biosensor after (—) 15 scans from 0 to −0.8 V; and after (·····) $t_d = 120$ s at $E_d = -0.6$ V. $v = 5$ mV s^{-1}.

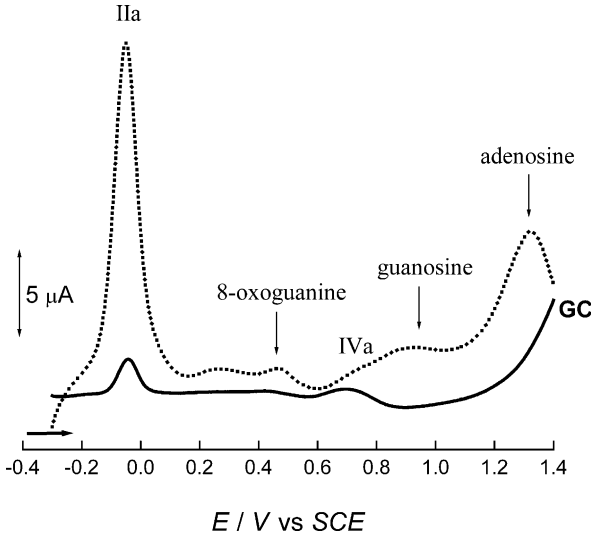


Fig. 10. Differential pulse voltammograms of 5.0×10^{-5} M niclosamide in 1:1 mixture of pH 4.5, 0.2 M aqueous acetate buffer and ethanol after 15 scans from 0 to +1.4 V using a: (—) glassy carbon (GC) bare electrode and (·····) DNA-biosensor. $v = 5$ mV s^{-1}.

As shown, the electrochemical DNA-biosensor enabled the investigation of DNA damage caused by niclosamide, by monitoring the occurrence of the oxidation peaks of guanosine, adenosine and 8-oxoguanine. Using the electrochemical DNA-biosensor it was possible to select the potential for the in situ electrogeneration of reactive intermediates during niclosamide reduction that can cause damage to the DNA, and to detect in situ the damage caused by those species on the DNA immobilised on the electrode surface.

4. Conclusions

This detailed study of the electron transfer reaction of niclosamide showed that it is possible to indirectly quantify this compound by quantifying the nitroso formed after oxidation of the hydroxylamine. This procedure can easily be used to monitor niclosamide in situ in the rivers where it is necessary to be controlled.

For the first time there is evidence of interaction of niclosamide and DNA, which suggests that niclosamide toxicity can be caused by this interaction, after reductive activation. The DNA-biosensor enabled a conclusive detection of niclosamide damage to DNA.

Acknowledgements

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References

Abdel-Fattah, S.A., 1997. Spectrophotometric determination of niclosamide using *p*-benzoquinone. Spectrosc. Lett. 30, 795–804.

Andrews, P., Thyssen, J., Lorke, D., 1982. The biology and toxicology of molluscicides, Bayluscide®. Pharmacol. Ther. 19, 245–295.

Brett, C.M.A., Oliveira-Brett, A.M., Mitoserin, L.C., 1994. Amperometric and voltammetric detection in batch injection analysis. Anal. Chem. 66, 3145–3150.

Datta, G., Bera, T., 2000. The effects of clofazimine, niclosamide and amphotericin B, on electron transport of *Leishmania donovani* promastigotes. Indian J. Med. Res. 112, 15–20.

Daabees, H.G., 2000. Selective differential spectrophotometric methods for determination of niclosamide and drotaverine hydrochloride. Anal. Lett. 33, 639–656.

Emara, L.H., 1993. Rapid and accurate method for determination of niclosamide released from molluscicidal formulations. JAOAC Int. 76, 847–850.

Espinosa Aguirre, J.J., Reyes, R.E., Denava, C.C., 1991. Mutagenic activity of 2-chloro-4-nitroaniline and 5-chlorosalicylic acid in *Salmonella-typhimurium* — two possible metabolites of niclosamidem. Mutat. Res. 264, 139–145.

Fijalek, Z., Pugia, M., Zuman, P., 1993. Polarographic and electrochemical studies of some aromatic nitro compounds: part VII. Polarographic, voltammetric, and controlled potential electrolytic reduction of meta- and para-nitrobenzaldehyde. Electroanalysis 5, 65–78.

Lardans, V., Dissous, C., 1998. Snail control strategies for reduction of schistosomiasis transmission. Parasitol. Today 14, 413–417.

Lund, H., 2001. Cathodic reduction of nitro and related compounds. In: Lund, H., Hammerich, O. (Eds.), Organic Electrochemistry, fourth ed. (Chapter 9). Marcel Dekker, New York, pp. 389–390.

Oliveira, E.C., Paumgartten, F.J.R., 2000. Toxicity of *Euphorbia milli* latex and niclosamide to snails and nontarget aquatic species. Ecotoxicol. Environ. Safety 46, 342–350.

Oliveira-Brett, A.M., Serrano, S.H.P., Gutz, I., La-Scalea, M.A., 1997. Voltammetric behaviour of nitroimidazoles at a DNA-biosensor. Electroanalysis 9, 1132–1137.

Oliveira-Brett, A.M., Serrano, S.H.P., Piedade, J.A.P., 1999. Electrochemistry of DNA. In: Compton, R.G. (Ed.), Comprehensive Chemical Kinetics, Applications of Kinetic Modelling, vol. 37 (Capítulo 3). Elsevier, Amsterdam, pp. 91–119.

Oliveira-Brett, A.M., Piedade, J.A.P., Serrano, S.H.P., 2000. Electrochemical oxidation of 8-oxoguanine. Electroanalysis 12, 969–973.

Onur, F., Tekin, N., 1994. Spectrophotometric determination of niclosamide and thiabendazole in tablets. Anal. Lett. 27, 2291–2301.

Perrett, S., Whitfield, P.J., 1996. Currently available molluscicides. Parasitol. Today 12, 156–159.

Schreier, T.M., Dawson, V.K., Choi, Y., Spanjers, N.J., Boogaard, M.A., 2000. Determination of niclosamide residues in rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*) fillet tissue by high-performance liquid chromatography. J. Agric. Food Chem. 48, 2212–2215.

Sridevi, C., Reddy, S.J.J., 1991. Voltammetric determination of niclosamide. J. Ind. Chem. Soc. 68, 263–266.

Strufe, R., 1964. Zum Wirkungsmechanismus des Bandwurmmittels Yomesan. Third International Congress of Chemotherapy, July 1963, Thieme Verlag, Stuttgart, pp. 1544–1547.

Swan, G.E., 1999. The pharmacology of halogenated salicylanilides and their anthelmintic use in animals. J. South Afr. Vet. Assoc.-Tydskrif van die Suid-afrikaanse Veterinere Vereniging 70, 61–70.

Tocher, J.H., 1997. Reductive activation of nitroheterocyclic compounds. Gen. Pharmacol. 28, 485–487.

Van Tonder, E.C., De Villiers, M.M., Handford, J.S., Malan, C.E.P., Du Preez, J.L., 1996. Simple, robust and accurate high-performance liquid chromatography method for the analysis of several anthelmintics in veterinary formulations. J. Chromatogr. A 729, 267–272.

Wang, J., Taha, Z., 1991. Batch injection-analysis. Anal. Chem. 63, 1053–1056.

WHO (World Health Organisation), 1993. The control of schistosomiasis: second report of the WHO Expert Committee, WHO Technical Report Series, WHO, Geneva, No. 830.