Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 1268–1273
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Voltammetric assay of the anthelmintic veterinary drug nitroxynil in bulk form and formulation at a mercury electrode

M.M. Ghoneim [a,*], M. El-Ries [b], A.M. Hassanein [a], A.M. Abd-Elaziz [a]

[a] *Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt*
[b] *National Organization for Drug Control and Research Corporation, Cairo, Egypt.*

Received 5 November 2005; received in revised form 18 March 2006; accepted 21 March 2006
Available online 9 May 2006

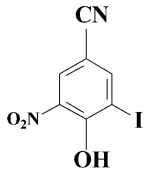
Abstract

The electrochemical behavior of the anthelmintic veterinary drug nitroxynil at the mercury electrode was studied in a series of Britton-Robinson universal buffer of pH 1.9–11 containing 20% (v/v) ethanol using dc-polarography cyclic voltammetry and controlled-potential coulometry. The voltammograms exhibited two irreversible cathodic steps over the pH range 1.9–10.2; the height of the first step is double that of the second one. Controlled-potential coulometry in the B–R universal buffer of pH 1.9–10 at a mercury pool working electrode revealed the consumption of four and two electrons via the first and second reduction steps, respectively, which attributed to reduction of the NO_2 group to the hydroxylamine stage (first step), and then to the amine stage (second step). Three voltammetric analytical procedures including dc-polarography, differential-pulse adsorptive stripping voltammetry and square-wave adsorptive stripping voltammetry were optimized for the direct determination of bulk nitroxynil. The three proposed procedures were applied for analysis of bulk nitroxynil with limits of detection of 3×10^{-5}, 1.31×10^{-8} and 8.4×10^{-10} M and limits of quantification of 1×10^{-5}, 4.36×10^{-8} and 2.80×10^{-9} M, respectively. The three procedures were successfully applied to the determination of nitroxynil in formulation (Dovenix, 25% nitroxynil injection solution) without the necessity for sample pretreatment and/or time-consuming extraction steps prior to the analysis.

Keywords: Nitroxynil; Dovenix; Electrochemical behavior; Dc-polarography; Cyclic voltammetry; Differential-pulse; Square-wave voltammetry

1. Introduction

Nitroxynil (4-hydroxy-3-iodo-5-nitrobenzenonitrile), 25% solution, is an anthelmintic veterinary drug widely used in prophylaxis and treatment of hepatic distomatosis, and fascioliasis (infestations of mature and immature fasciola hepatica) in cattle and sheep. It is also effective, at the recommended dose rate, against adult and larval infestations of haemonchus contortus in cattle and haemonchus placei, oesophagostomum radiatum and bunostomum phlebotomum in cattle [1,2].



(Structure of nitroxynil)

Studies of the therapeutic effects of nitroxynil drug required the use of sensitive methods for its determination at trace levels and also for testing content uniformity of nitroxynil dosage form. Several analytical methods have been described for the assay of nitroxynil in bulk, cattle muscle, edible tissues and milk. These methods include reversed-phase liquid chromatography-dual-electrode coulometric detection [3,4], high-performance liquid chromatography–UV spectroscopy [5], high-performance liquid chromatography–thermo-spray mass-spectrometry [6] and gas chromatography [7]. Nitroxynil has been also determined in pharmaceutical parenteral solutions using ac- and dc-polarography [8]. However, the electrochemical behavior of nitroxynil at the mercury electrodes still required more clarification. Moreover, the reported analytical methods did not offer a sufficient quantification limit of nitroxynil and neces-

* Corresponding author. Tel.: +2 106632694; fax: +2 403350804.
E-mail address: mmghoneim@usa.net (M.M. Ghoneim).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.03.022

sitate samples pretreatment and/ or time-consuming extraction or evaporation steps prior to the analysis. Besides expensive reagents and equipment are required, which are not economically feasible for routine analysis.

No stripping voltammetric method is reported in literature to date for assay of nitroxynil in formulation. Adsorptive cathodic stripping voltammetry has been shown to be an efficient analytical technique for determination of sub-nanomolar level of a wide range of drugs which have an interfacial adsorptive character onto surface of the working electrode [9]. This is due to its simplicity, sensitivity, precision and most of the excipients used do not interfere in the subsequent determination. In addition, the technique is easy to use and save of both time and costs.

The present work aimed to study the electrochemical behavior of nitroxynil at a mercury electrode using dc-polarography, cyclic voltammetry and controlled-potential electrolysis. It is also aimed to develop electroanalytical procedures for quantification of nitroxynil in bulk form and pharmaceutical formulation.

2. Experimental

2.1. Materials.

Nitroxynil, 4-hydroxy-3-iodo-5-nitrobenzenonitrile, was kindly supplied by the National organization for drug control and Research Corporation, Cairo, Egypt. The Pharmaceutical formulation: "Dovenix injection solution" is a 25% sterile solution of the *N*-ethylglucamine salt of nitroxynil in water (Merial Co., Lyon, France).

2.2. Reagent and solutions

A stock standard solution of bulk nitroxynil (1×10^{-3} M) was prepared in ethanol (Merck). Working solutions of 1×10^{-6} to 1×10^{-4} M bulk nitroxynil were prepared by dilution with ethanol.

Aliquots of "Dovenix" injection solution (a 25% solution of the *N*-ethylglucamine salt of nitroxynil in water) were diluted with a B–R universal buffer of pH 6 to obtain 1×10^{-6} to 1×10^{-4} M nitroxynil working solutions. The prepared solutions were kept at 4 °C. A digital micro-pipetter (Volac, John Poulten Ltd., Essex, UK) was used for transferring the solutions during the electrochemical measurements.

A series of Britton–Robinson (B–R) universal buffer of pH 1.9–11 (a mixture of 0.04 M solution of each of acetic, *ortho*-phosphoric and boric acids adjusted to the required pH with 0.2 M sodium hydroxide solution) was prepared [10] and used as supporting electrolyte. All the chemicals used were of analytical reagent grade.

A pH-meter incorporated with combined glass and saturated calomel electrodes (Crison, Barcelona, Spain) was used for the pH measurements. A Mettler balance (Toledo-AB104, Switzerland) was used for weighing the solid materials. The de-ionized water was supplied from a Purite-Still Plus de-ionizer connected to an Aqua-Matic double-distillation water system (Hamilton Laboratory Glass Ltd., Kent, UK).

2.3. Apparatus

A polarograph (Model 4001, Sargent-Welch, USA) was used for the polarographic measurements. A polarographic cell incorporated with a dropping mercury electrode as a working electrode ($m = 1.23$ mg s^{-1}, $t = 3.4$ s at mercury height = 60 cm) and a saturated calomel electrode (SCE) as a reference electrode was used.

Cyclic voltammetry, differential-pulse and square-wave stripping voltammetric measurements were carried out using a computer-controlled potentiostat (Model 273A-PAR, Princeton Applied Research, Oak Ridge, TN, USA) with the software 270/250-PAR together with the electrode assembly 303A-PAR incorporated with a three-electrode cell comprising of a hanging mercury drop electrode (HMDE) of area 0.026 cm^2, an Ag/AgCl/KCl$_s$ reference electrode and a platinum wire as a counter electrode.

A potentiostat/galvanostat (Model 173-PAR) incorporated with a digital coulometer (179-PAR) was used for controlled-potential electrolysis. A micro-cell incorporated with a platinum wire sealed through the cell bottom for contact with a mercury pool working electrode, a saturated calomel electrode as a reference electrode and a platinum gauze as a counter electrode was used. The potential selected was adjusted to be equal the $E_{1/2}$ of the reactant polarographic wave plus −0.1 V or at the beginning of the polarographic limiting current. The total charge (Q) passed during the exhaustive electrolysis of nitroxynil was obtained by integrating the current electronically. Using Faraday's equation: $N = Q/nF$ (where N is the number of moles of substance being electrolyzed) the number of electrons (n) transferred per reactant molecule was estimated.

2.4. Analytical procedure

Ten milliliters of the B–R universal buffer of pH 2 containing the appropriate concentration of nitroxynil were introduced into a micro-electrolysis cell, then deoxygenated with nitrogen for 5 min. An accumulation potential of −0.1 V (versus Ag/AgCl/KCl$_s$) was applied to the working electrode for 90 s, while the solution is stirred. After accumulation time elapsed, the stirrer was stopped and the solution allowed to be quiescent about 10 s, then the voltammogram was recorded by scanning the potential towards the negative direction using the differential-pulse or the square-wave mode. Calibration curves were obtained under the optimized conditions of the procedure. Quantification of nitroxynil in bulk form and formulation was performed by means of both calibration curve and standard addition methods.

3. Results and discussion

3.1. Dc-polarography

Polarograms of 2×10^{-4} M nitroxynil in Britton–Robinson universal buffer (pH 1.9–10.2) containing 20% (v/v) ethanol exhibited two irreversible cathodic waves; the height of the first wave was double that of the second ones (Fig. 1). Results of

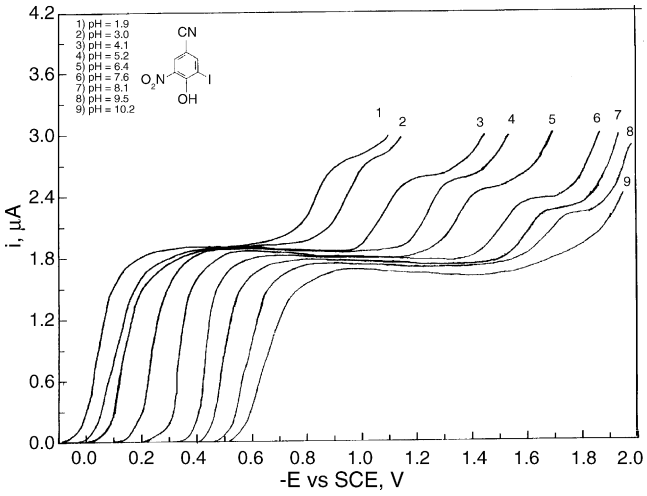


Fig. 1. Dc-polarograms of 2.5×10^{-4} M of nitroxynil in the B–R universal buffer of various pH values.

controlled-potential electrolysis experiments over the whole pH range revealed that four and two electrons were transferred via the first and second reduction waves of nitroxynil, respectively. Accordingly, this behavior may be attributed to the reduction of NO_2 group to the hydroxylamine stage via the consumption of four electrons (first wave), then to the amine stage via the consumption of two more electrons (second wave) over the pH range 1.9–10.2. This behavior is contradictory to that reported by Alvarezlueje et al. [8] for reduction of nitroxynil in buffered solutions containing 10% dimethylformamide, since they stated that the second wave disappeared at pH > 6. The shift of the half-wave potentials ($E_{1/2}$) of the cathodic waves of nitroxynil with the increase of pH indicated the involvement of protons (H^+ ions) in the electrode reaction and that the proton-transfer reaction precedes the electrode process proper [11]. The limiting current was pH-independent which disproved the concept of kinetic or catalytic control of the limiting current.

Logarithmic analysis of the two polarographic waves of 2×10^{-4} M nitroxynil was performed by plotting E_{de} versus log $(i/i_l - i)$ [12] at various pH values. Straight lines were obtained with slope values S_1 (S_1, mV = $59/\alpha n_a$) reported in Table 1; from which values of αn_a (0.8–1.14) and transfer coefficient α (0.40–0.57) were estimated at various pH values, for the number of electrons n_a transferred in the rate-determining step equals 2. The $E_{1/2}$–pH plots of the two polarographic waves were straight lines of slope values S_2 $\{\partial E_{1/2}/\partial pH = S_2$, mV = $59(Z_{H^+}/\alpha n_a)\}$ reported in Table 1. The number of protons (Z_{H^+}) participated in the rate-determining step was estimated as: $Z_{H^+} = \{(\partial E_{1/2}/\partial pH)/(59/\alpha n_a)\}$ [12]. Since S_1, mV = $(59/\alpha n_a)$ and $S_2 = (\partial E_{1/2}/\partial pH)$, the number of protons was estimated using the simplified relation: $Z_{H^+} = S_2/S_1$ [13] and found to equals one over the entire pH range (Table 1).

Polarograms for different concentrations of bulk nitroxynil were recorded in a B–R universal buffer of pH 2–6 and a linear variation of the total limiting current (i_l) with nitroxynil concentration (C) was obtained which following a straight-line equation: i_l (µA) = 0.065C (mM) + 0.33 (r = 0.991 and n = 12). Three replicate calibration curves were obtained over the concentration

Table 1
Dc-polarographic data for 2×10^{-4} M of nitroxynil in the B–R universal buffer of various pH values containing 20% (v/v) ethanol

pH	S_1 (mV)	αn_a	α (n_a = 2)	S_2 (mV)	$Z_{H^+} = (S_2/S_1)$
First wave					
1.9	58	1.02	0.51		1.36
4.1	77	0.77	0.39		1.03
5.2	80	0.74	0.37		0.99
6.4	62	0.95	0.48	79	1.27
7.6	60	0.98	0.49		1.32
8.5	62	0.95	0.48		1.27
9.5	67	0.88	0.44		1.18
Second wave					
1.9	91	0.65	0.32		1.28
4.1	88	0.67	0.43	116	1.32
5.2	88	0.67	0.34		1.32
6.4	86	0.69	0.34		1.35

range 3×10^{-5} to 2.5×10^{-4} M nitroxynil. For 6×10^{-5} M of nitroxynil a mean percentage recovery of 98.1 ± 1.95 (n = 3) was achieved by the proposed dc-polarographic procedure. Limits of detection (LOD) and quantification (LOQ) of 3×10^{-6} M and 1×10^{-5} M nitroxynil were estimated using the relations [14]:

$$\text{LOD} = 3\,\text{S.D.}/b \quad (1)$$

$$\text{LOQ} = 10\,\text{S.D.}/b \quad (2)$$

respectively, where S.D. is the standard deviation of the intercept of calibration curves (or the blank) and b is the slope of the calibration curve.

3.2. Cyclic voltammetry

Cyclic voltammograms of 1×10^{-4} M nitroxynil displayed two irreversible cathodic peaks over the pH range 1.9–10.2. As suggested from the dc-polarographic and controlled-potential electrolysis measurements, the two peaks were attributed to reduction of NO_2 group to the hydroxylamine stage via the consumption of four electrons (first peak) and then to the amine stage via the consumption of two more electrons (second peak). The sharpness of the peak suggests the adsorptive character of nitroxynil onto the surface of the mercury electrode.

The adsorptive character of nitroxynil onto the mercury surface was identified by recording the cyclic voltammograms of 2×10^{-6} M at 100 mV/s in a B–R universal buffer of pH 7 after its accumulation onto the HMDE at open circuit (Fig. 2, curve a) and at E_{acc} = 0.0 V (versus Ag/AgCl/KCl_s) for 30 s (curve b) and then its repetitive cycle at the same mercury drop (curve c). The peak current obtained in the repetitive cycle (curve c) decreased rapidly in comparison to that of the first cycle (curve b), which may be attributed to desorption of the nitroxynil from the mercury electrode surface. A plot of the logarithm of peak current (i_p) versus logarithm of scan rates (ν) of 25–500 mV s^{-1} gave a straight line following the equation: $\log i_p = 0.96 \log \nu + 0.81$ (r = 0.999 and n = 7), its slope value (0.96) is very close to the expected theoretical value 1.0 for an ideal reaction of surface species [15].

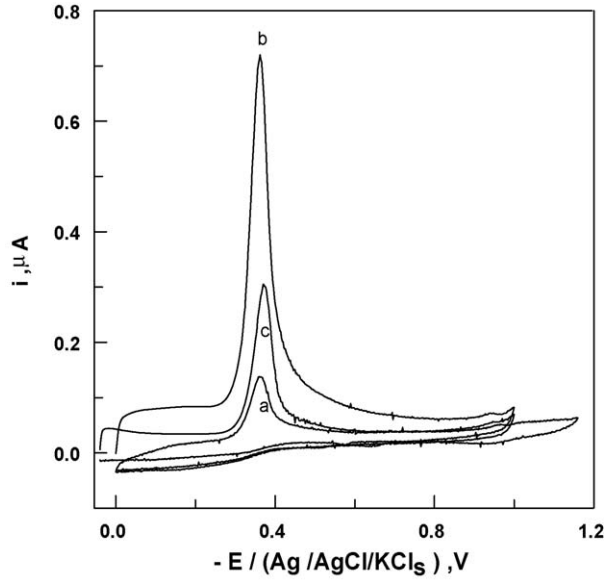


Fig. 2. Cyclic voltammograms for 2×10^{-6} M nitroxynil in a B–R universal buffer of pH 7: following accumulation onto the HMDE at open circuit (a) and at $E_{acc} = 0$ V. for 30 s (b) then its repetitive cycle at the same mercury drop (c); scan rate = 100 mV s^{-1}.

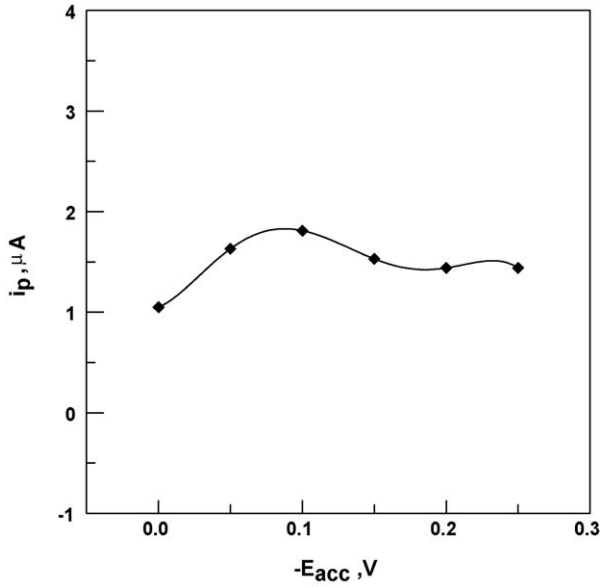


Fig. 3. Effect of accumulation potential E_{acc} on the SWAdCS voltammetric peak current i_p of 2×10^{-7} M nitroxynil in a B–R universal buffer of pH 6 following accumulation for 30 s, pulse-height $a = 50$ mV, frequency $f = 100$ Hz and scan increment $\Delta E = 8$ mV.

The electrode surface coverage of nitroxynil Γ_0 (mol cm^{-2}) in a B–R universal buffer of pH 7 was calculated using the equation: $\Gamma_0 = Q/nFA$, where Q is the charge consumed by the surface processes over the two peaks which estimated by the integration of the area under the two peaks corrected to the residual current [16], n the total number of electrons consumed in the reduction processes via the two peaks ($n = 6$), F the Faraday constant and A is the surface area of the working electrode (0.026 cm^2). On dividing the amount of charge (Q) consumed by the surface process, 1.22 μC, by the conversion factor (nFA) a monolayer surface coverage of 1.217×10^{-10} mol cm^{-2} was obtained. Each adsorbed nitroxynil molecule thus occupied an area of 1.318 nm^2.

3.3. Stripping voltammetry applying different waveforms

3.3.1. Differential-pulse

Differential-pulse adsorptive cathodic stripping voltammogram of 2×10^{-6} M bulk nitroxynil in the B-R universal buffer (pH 2–10), following accumulation onto the HMDE for 30 s, exhibited a main single peak over the entire pH range. A sharper peak and a much developed peak current were achieved at pH 6–7. The optimum experimental and instrumental conditions for analytical determination of nitroxynil using differential-pulse adsorptive cathodic stripping (DPAdCS) voltammetry were identified by studying the effect of each of accumulation potential E_{acc}, accumulation duration t_{acc}, scan rate ν and pulse-height a on the peak current intensity (i_p) at pH 6. The results indicated that the optimal procedural conditions which generated a well-shaped and much developed peak current were $E_{acc} = -0.1$ V, $t_{acc} = 30$ s, $\nu = 4$ mV s^{-1} and $a = 90$ mV. Variation of the peak current i_p (μA) with concentration (C) of nitroxynil was a linear relation: i_p (μA) = 0.057C (M) ± 0.08, $r = 0.995$. The achieved LOD and LOQ were 1.31×10^{-8} M and 4.36×10^{-8} M bulk nitroxynil, respectively.

3.3.2. Square-wave

The square-wave adsorptive cathodic stripping (SWAdCS) voltammogram of 2×10^{-7} M bulk nitroxynil in the B–R universal buffer of pH 2–10 following accumulation onto the HMDE for 30 s exhibited mainly a well-defined single peak over the entire pH range. A much developed peak current was achieved at pH values 6–7. The peak potential was shifted to more negative value on the increase of pH of the medium. The dependence of the stripping peak current on the accumulation potential was examined over the potential range 0 to −0.25 V (Fig. 3). A much developed peak current was obtained at −0.1 V (versus Ag/AgCl/KCl$_s$). In stripping analysis employing film formation, the peak current is almost proportional to the accumulation duration (Fig. 4). In the present study, an accumulation duration of 90 s was applied for assay of nitroxynil.

The peak current for 2×10^{-7} M nitroxynil in a B–R universal buffer of pH 6 following accumulation for 90 s at $E_{acc} = -0.1$ V was optimized by changing the pulse-amplitude (a), frequency (f), scan increment (ΔE_s) within the ranges 10–100 mV, 10–120 Hz and 2–10 mV, respectively. Although the SWAdCS voltammetric peak current of nitroxynil was almost directly proportional to each of a, f and ΔE_s, however a sharper peak was obtained under the following conditions $a = 70$ mV, $f = 120$ Hz and $\Delta E_s = 8$ mV. So the optimal square wave procedural conditions for the assay of nitroxynil were: $E_{acc} = -0.1$ V, $t_{acc} = 90$ s, $a = 70$ mV, $f = 120$ Hz, $\Delta E_s = 8$ mV and a B–R universal buffer of pH 6 as a supporting electrolyte.

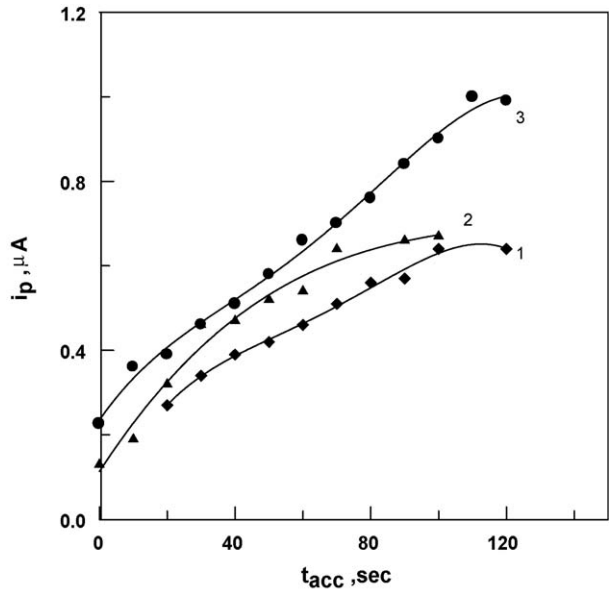


Fig. 4. Plots of i_p vs. t_{acc} for: 8×10^{-9} M (1), 3×10^{-8} M (2), and 1×10^{-7} M (3) nitroxynil in a B–R universal buffer of pH 6 following accumulation at $E_{acc} = -0.1$ V, $a = 50$ mV, $f = 100$ Hz and $\Delta E = 8$ mV.

3.4. Methods validation

Validation of the proposed stripping voltammetry procedures for trace assay of nitroxynil was examined via linearity, limit of detection (LOD), limit of quantitation (LOQ), repeatability, recovery, robustness and intermediate precision. The linear variation of SWAdCS voltammetric peak current (i_p) versus concentration (C) of bulk nitroxynil (1×10^{-9} to 3×10^{-7} M) was represented by a straight line equation: i_p (μA) = 0.039C (M) + 2.37 ± 0.1 ($r = 0.998$ and $n = 12$). Using Eqs. (1) and (2), the achieved LOD and LOQ were 8.4×10^{-10} and 2.8×10^{-9} M bulk nitroxynil, respectively. The obtained results confirmed the reliability of the proposed procedures for the trace quantitation of bulk nitroxynil. Repeatability of results of SW and DP-AdCS voltammetry procedures was examined by performing four replicate measurements for (2.7 and 5) $\times 10^{-8}$ M bulk nitroxynil and mean percentage recoveries of 98.25 ± 0.3 and 98.4 ± 0.05, respectively, were achieved.

The selectivity [17] of the described procedures was tested by analysis of solutions of a 5×10^{-8} M bulk nitroxynil and a standard formulation solution (Dovenix injection solution) containing 5×10^{-8} M nitroxynil, following accumulation of nitroxynil onto the HMDE at −0.1 V for 30 s in both cases. No significant differences in the recoveries or the relative standard deviations were obtained in the absence and presence of the excipient (*N*-ethyglucamine). Thus, the proposed procedure can be considered selective.

Table 2
Influence of small variation in some of the conditions of SWAdCS voltammetric procedure on recovery of 2×10^{-7} M nitroxynil

Variable parametres	Conditions	$R\%$ ± R.S.D. ($n = 3$)
pH		
5.5	$E_{acc} = -0.1$ V, $t_{acc} = 90$ s	95.01 ± 0.15
6.0		98.40 ± 0.05
6.5		95.23 ± 0.63
Accumulation potential (E_{acc})		
−0.05	pH 6, $t_{acc} = 90$ s	95.23 ± 0.12
−0.10		98.40 ± 0.05
−0.15		96.23 ± 0.24
Accumulation time (t_{acc})		
85	pH 6, $E_{acc} = -0.1$ V	94.00 ± 0.20
90		98.40 ± 0.05
95		94.23 ± 0.25

The robustness [17] of the proposed SWAdCS voltammetry procedure was examined by studying the effect of variation of some of the neck procedural conditions such as pH (5.5–6.5), accumulation potential (−0.05 to −0.15 V) and accumulation duration (85–95 s). As shown in Table 2, the mean percentage recoveries based on four replicate measurements were not significantly affected and consequently the optimized procedure was reliable for assay of nitroxynil and could be considered robust.

The intermediate precision [17] of measurements was examined by applying the proposed SWAdCS voltammetry procedure to assay of nitroxynil using two different potentiostats, Model 273A-PAR, Lab. 1 (98.40 ± 0.05) and Model 263A-PAR, Lab. 2 (97.95 ± 0.08) were found reproducible, since there was no significant difference in the mean percentage recoveries or the relative standard deviations.

3.5. Assay of nitroxynil in pharmaceutical formulation

The proposed dc-polarographic, differential-pulse and square-wave cathodic stripping voltammetric procedures were successfully applied to the determination of nitroxynil in its pharmaceutical formulation (Dovenix injection solution) without the necessity for samples pretreatment or time-consuming extraction steps prior to the analysis. Mean percentage recoveries of 99.8 ± 1.69 to 100.6 ± 1.66, based on four replicate measurements, were achieved (Table 3) using both the calibration curve and the standard addition methods.

Table 3
Recovery of nitroxynil in bulk form and formulation (Dovenix injection solution) applying the proposed SWAdCS voltammetric procedure using the calibration curve (A) and standard addition (B) methods

Sample	[Taken] (M)	R (%) (A)	$R\%$ (B)	R.S.D. (%) (A)	R.S.D. (%) (B)
Bulk nitroxynil	2.7×10	98.5	99.1	1.87	1.2
	5.0×10	100.1	99.6	1.45	1.6
Dovenix	2.7×10	99.8	98.4	1.69	1.3
	5.0×10	100.6	99.4	1.66	1.1

4. Conclusion

The electrochemical behavior of nitroxynil was studied in buffered solutions at the mercury electrode surface using dc-polarography, cyclic voltammetry and controlled-potential electrolysis. Dc-polarography, differential-pulse stripping voltammetry, and square-wave stripping voltammetry procedures were optimized and successfully applied for quantification of nitroxynil in bulk form and its pharmaceutical formulation.

Acknowledgements

The authors express their gratitude to the Alexander von Humboldt Foundation (Bonn, Germany) for donating the Electrochemical Analyzer (263A-PAR), and the personal computer used in the present study to M.M. Ghoneim.

References

[1] R.J. Martin, Vet. J. 154 (1997) 11–34.
[2] M Windholz, Merck Index, 10th ed., 1983, p. 954.
[3] K. Takeba, M. Matsumoto, H. Nakazawa, J. Chromatogr. 596 (1992) 67–71.
[4] K. Takeba, T. Itoh, M. Matsumoto, H. Nakazawa, S. Tanabe, J. AOAC Int. 79 (1996) 848–852.
[5] J.A. Tarbin, G. Shearer, J. Chromatogr. 613 (1993) 347–353.
[6] W.J. Blanchflower, D.G. Kennedy, Analyst 114 (1989) 1013–1015.
[7] M. Kazacos, V. Mok, Aust. J. Dairy Technol. 41 (1986) 82–84.
[8] A.F. Alvarezlueje, M. Bastias, S. Bollo, L.J. Nunezvergara, J.A. Squella, J. AOAC Int. 78 (1995) 637–641.
[9] J. Wang, Analytical Electrochemistry, 2nd ed., Wiley/VCH, New York, 2000.
[10] H.T.S. Britton, Hydrogen Ions, 4th ed., Chapman and Hall, London, 1952, pp. 113–114.
[11] P. Zuman, The Elucidation of Organic Electrode Processes, Academic Press, 1969.
[12] L. Meits, Polarographic Techniques, 2nd ed., Interscience Publishers, New York, 1965, pp. 232–248.
[13] M.M. Ghoneim, M.A. Ashy, Can. J. Chem. 57 (1979) 1249–1298.
[14] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 3rd ed., Ellis Harwood Series, Prentice Hall, New York, 1993, p. 119–120.
[15] E. Laviron, J. Electroanal. Chem. 112 (1980) 1–9.
[16] A. Webber, M. Shah, J. Osteryoung, Anal. Chim. Acta 157 (1984) 1–16.
[17] The USA Pharmacopoeia, The National Formulary, USP 26, Convention Inc., 2003, p. 2446.