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Short communication

Cathodic stripping voltammetric behaviour of nitrofurazone and its determination in pharmaceutical dosage form, urine and serum by linear sweep voltammetry

Mahmoud Khodari *, Hesham Mansour, Gaber A.M. Mersal

Chemistry Department, Faculty of Science, South Valley University, Qena, Egypt

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1. Introduction

The antibacterial drug, nitrofurazone [5-nitro-2-furaldehyde semicarbazone] a synthetic nitrofuran derivative, was reported to possess good bacteriostatic and bactericidal properties, has an antibacterial action against a number of Gram-negative and Gram-positive micro-organisms [1,2] and is used for the treatment of burns [3] and trypanosomiasis [4]. In veterinary medicine nitrofurazone is used for the treatment and prophylaxis of coccidiosis in poultry and farm animals, and necrotic enteritis in pigs [1].

The determination of some nitrated heterocyclic compounds containing reduction sites similar to that of nitrofurazone was studied by Vignoli et al. [5] using Brittion–Robinson (BR) buffer pH of between 1.81 and 11.98.

Various methods including spectrophotometry [6,7], turbidity [8], colorimetry [9,10], conductimetry [11], paper chromatography [12] and high performance liquid chromatography [13,14] have determined nitrofurazone in its pharmaceutical preparations.

A little attention has been paid to the polarographic determination of nitrofurazone [15–18] or to the electrochemical behaviour of this compound [19,20]. A rotating platinum electrode was used to study nitrofurazone by a simple direct current procedure [21–24].

The present work is a continuation of our studies in the field of the determination of the antibacterial drugs using cathodic stripping voltammetry (CSV) at the hanging mercury drop electrode (HMDE) [25]. This technique offers a high senstivity with respect to the other analytical techniques [26]. The aims of this study are to establish the experimental conditions, to investigate the adsorptive voltammetric behaviour of nitrofurazone and to optimize the conditions for determination of the titled compound in pharmaceutical formulations present in urine and serum.

* Corresponding author.

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2. Experimental

2.1. Apparatus

Cathodic stripping and cyclic voltammetric measurements were performed using EG&G PAR (model 263 polarographic analyzer) with 250/270 research electrochemistry software version 4.0. A cell model 303A static mercury drop electrode (SMDE) was connected to the potentiostat, a HMDE was used as the working electrode, silver/silver chloride (saturated KCl) as a reference electrode, and a platinum wire as an auxiliary electrode.

2.2. Chemicals and preparations

Pure nitrofurazone was obtained from Sigma, MO and used without further purification. A stock solution of 1×10^{-3} mol dm^{-3} nitrofurazone was prepared daily by dissolving the required amount of this drug in DMF and stored in the dark; the test solutions containing various concentrations of nitrofurazone were obtained by dilution of the stock with twice distilled water. Three different buffer solutions, namely borax (pH 8–12), citrate (pH 8–12) and BR buffer (2–13) were prepared in twice distilled water. All the other chemicals used were either Analar grade from BDH or general-reagent grade from Merck.

2.3. Procedure for voltammetric behaviour

2.3.1. Stripping voltammetric procedure

A total volume of 10 ml supporting electrolyte-containing nitrofurazone was placed into the stripping cell for analysis. The solution was deoxygenated for 8 min with a stream of pure nitrogen before taking the voltammograms. After deareation, a hanging mercury drop was formed, the selected deposition potential was applied with stirring for a given time interval, while the accumulation of the analyse at the electrode proceeded. After a selected deposition time and a rest period of 15 s, the potential was scanned from positive to negative.

2.3.2. Assay procedure for applications

2.3.2.1. Interference studies. The voltammograms of nitrofurazone and a suitable amount of metal ions or amino acids were recorded by the method described above in order to study possible interference of metal ions and amino acids on nitrofurazone and their influence on the peak current response of the drug.

2.3.2.2. Analysis of pharmaceutical dosage form. A total of 1 g of the ointment was weighed and dissolved in 100 ml DMF with stirring for 20 min. Then the solution was filtered and 1 ml was transferred to the voltammetric cell containing BR buffer (pH 12). After degassed by nitrogen, and the voltammogram was recorded.

2.3.2.3. Urine treatment. A urine sample was filtered, and 1 ml was diluted with pH 12 buffer solution to 10 ml. Then the voltammograms were recorded using a scan rate of 100 mV s^{-1}, accumulation potential (E_{acc}) of -0.35 V and accumulation time (t_{acc}) of 15 s.

2.3.2.4. Serum treatment. A total of 1 ml of human serum was diluted to 10 ml with the selected buffer and the pH was adjusted to 12 with sodium hydroxide.

3. Results and discussion

In cyclic voltammetry using BR buffer pH 12, nitrofurazone showed a well-defined cathodic reduction peak due to the reduction of the nitro group to hydroxylamine, and a small anodic peak appears in a reverse scan due to the formation of the nitroso compound [20]. Nitrofurazone showed a rapid desorption of the adsorbed form in repetitive cyclic voltammograms; the reduction peak current decreased sharply in the second and third cycles. A linear sweep voltammetric technique with high scan rate is preferable in such cases.

3.1. Influence of supporting electrolyte and pH

The effect of supporting electrolyte was examined using different supporting electrolytes including: sodium acetate, sodium nitrate, sodium borate, potassium chloride, sodium phosphate, sodium citrate, borax and BR buffers. Only three buffers; borax, sodium citrate and BR showed adsorption of nitrofurazone onto the HMDE, while the other systems did not show this behaviour. BR buffer was selected for further work because it not only gave the highest peak current but also gave the best peak shape. The effect of pH on the peak current of nitrofurazone in BR (pH 2–13) was examined; the results showed that the peak current increased with increasing pH up to a pH of 12 (Fig. 1a). Beyond this value the peak current decreased. Nitrofurazone has its maximum stability at pH 12.

On plotting the relation between the peak potential against the pH value, the peak potential (E_p) of nitrofurazone was shifted to more negative values with increasing pH (Fig. 1b). A linear region was observed in the pH range from 4 to 12, with correlation coefficient of 0.996.

3.2. Influence of accumulation potential and scan rate

The influence of accumulation potential (E_{acc}) on the stripping peak current was examined over the range -0.1 to -0.45 V, by increasing the accumulation potential; the peak current increased to a maximum value at an accumulation potential of -0.35 V; thereafter the peak current began to decrease. An accumulation potential of -0.35 V, was used for further work.

The peak current of 1×10^{-8} mol dm^{-3} nitrofurazone in BR buffer pH 12, after a 15 s preconcentration time and -0.35 V accumulation potential, was enhanced by increasing the scan rate from 20 to 500 mV s^{-1}. But at higher values >400 mV s^{-1}, undefined peaks were observed. At preconcentration times >15 s, the peek shape was rapidly distorted on using a scan rate of more than 100 mV s^{-1}, so a scan rate of 100 mV s^{-1} was preferable for the study of this drug. By plotting $\log i_p$ against the log scan rate ($\log v$) a linear relation was observed ($r = 0.997$) and a slope of 0.923 was obtained. A slope of 1.00 is expected for an ideal reaction surface [27].

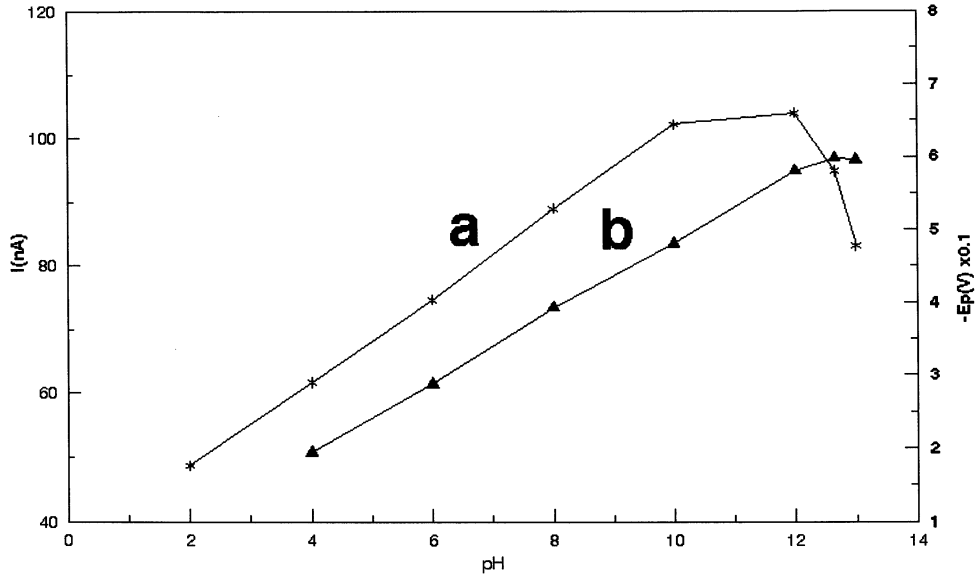

Fig. 1. Dependence of the peak current (a) and peak potential (b) on pH for 1×10^{-7} mol dm^{-3} nitrofurazone in BR buffer, $t_{acc} = 15$ s and scan rate $= 100$ mV s^{-1}.

Table 1
Characteristics of current-time curves established using different nitrofurazone concentrations in BR buffer (pH 12), scan rate of 100 mV s^{-1} and −0.35 V accumulation potential

Nitrofurazone (mol dm^{-3})	Linearity range (s)	Correlation coefficient	Slope (nA s^{-1})	Intercept (nA)
1×10^{-8}	0–23	0.997	5.690	34.583
5×10^{-8}	0–17	0.983	13.734	34.707
1×10^{-7}	0–10	0.999	20.886	46.456

3.3. Influence of accumulation time and calibration curves

The influence of accumulation time t_{acc} on the peek height was investigated at different times (0–60 s) for different concentrations of nitrofurazone ranging from 1×10^{-9} to 2×10^{-7} mol dm^{-3}. The obtained results showed that i_p (the peak current) increased with increasing accumulation time. On plotting the peak current (i_p) versus the accumulation time (t_{acc}) for different concentrations of nitrofurazone; the peak current increased linearly with accumulation times up to 23, 17 and 10 s for 1×10^{-8}, 5×10^{-8} and 1×10^{-7} mol dm^{-3}, respectively. A deviation from linearity was observed at acccumulation times longer than the mentioned ones. The collected data are illustrated in Table 1. On plotting the peak current i_p against the square root of time ($\sqrt{t}$), a straight line was observed with correlation coefficient of 0.971 and slope of 16.60; this behaviour is expected for mass transport controlled by adsorption [28].

The influence of different concentrations of nitrofurazone on the peak current has been examined at different preconcentration times ranged form 0 to 60s at accumulation potential −0.35 V. Nitrofurazone showed an increase in peak current by increasing its concentration and also by raising the accumulation time (t_{acc}). Fig. 2 represents the voltammograms for different concentrations of nitrofurazone (1×10^{-8}–2×10^{-7}) at 15 s accumulation time and a scan rate of 100 mV s^{-1} in BR buffer (pH 12). The results were treated statistically using different regression modes (linear, power, logarithmic and exponential) to select the most suitable one for the collected results (Table 2). From the table one can observe that the linear regression mode is the best to fit the collected results for the concentrations of 1×10^{-8} up to 2×10^{-7} mol dm^{-3} with 10 and 15 s accumulation times. Where the power regression mode is the most suitable one for the results of 1×10^{-8} up to 5×10^{-8} mol dm^{-3} with 20 s accumulation time.

3.4. Detection limit and reproducibility

A detection limit of 1×10^{-9} mol dm^{-3} nitrofurazone was detected in BR buffer pH 12 after 15 s accumulation time, −0.35 accumulation potential, and scan rate 100 mV s^{-1}. This value along with the reported ones are shown in Table 3.

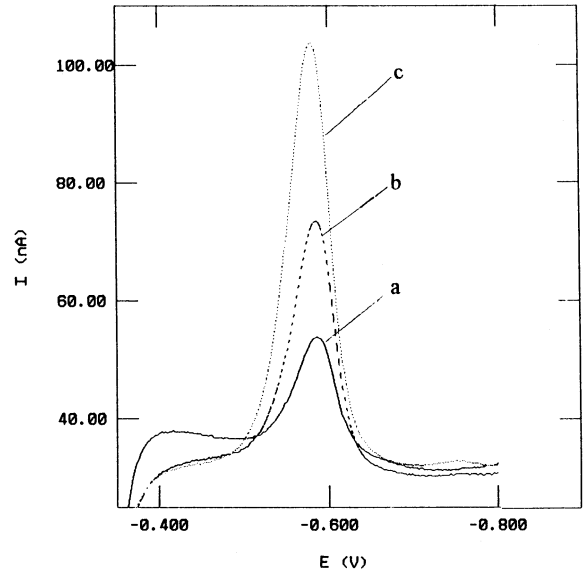

Fig. 2. Typical voltammograms of different nitrofurazone concentrations; scan rate = 100 mV s^{-1}, E_{acc} = −0.35 V, scan rate 100 mV s^{-1} and t_{acc} = 15 s in BR buffer (pH 12). (a) 5×10^{-8} (b) 1×10^{-7} (c) 2×10^{-7} mol dm^{-3}.

Table 2
Characteristics of the calibration curves established using different accumulation times, in BR buffer pH 12, scan rate of 100 mV s^{-1} and −0.35 V accumulation potential[a]

Regression modes	Accumulation time (s)	Nitrofurazone (mol dm^{-3})	Correlation coefficient	Slope (nA/s)	Intercept (nA)
Linear	10	$1\times10^{-8}-2\times10^{-7}$	0.9950	5.0600	42.896
$y=A+Bx$	15	$1\times10^{-8}-1\times10^{-7}$	0.9954	5.9460	44.828
$i=A+Bt$	20	$1\times10^{-8}-5\times10^{-8}$	0.9911	4.4450	47.588
Power	10	$1\times10^{-8}-2\times10^{-7}$	0.9518	0.0568	49.379
$y=Ax^{B}$	15	$1\times10^{-8}-1\times10^{-7}$	0.9867	0.0792	48.667
$i=At^{B}$ or	20	$1\times10^{-8}-5\times10^{-8}$	0.9972	0.0729	48.609
$\ln i=\ln A + B\ln t$					
Exponential	10	$1\times10^{-8}-2\times10^{-7}$	0.8263	30.312	31.407
$y=A\ e^{BX}$	15	$1\times10^{-8}-1\times10^{-7}$	0.8808	21.817	45.392
$i=A\ e^{Bt}$	20	$1\times10^{-8}-5\times10^{-8}$	0.9113	10.366	51.566
$\ln i=\ln A + \ln Bt$					
Logarithmic	10	$1\times10^{-8}-2\times10^{-7}$	0.9241	0.3680	41.457
$y=A+B\ln x$	15	$1\times10^{-8}-1\times10^{-7}$	0.9379	0.3011	48.317
$i=A+B\ln t$	20	$1\times10^{-8}-5\times10^{-8}$	0.9334	0.2171	51.815

[a] Peak current, i (nA); time (t) in second.

The reproducibility of the results was examined by eight successive measurements of 5×10^{-8} mol dm^{-3} nitrofurazoneat accumulation times 15 s, −0.35 V accumulation potential and 100 mV s^{-1} scan rate in BR buffer (pH 12). The relative standard deviation was calculated and was found to be 2.3%.

3.5. Interferences with some metal ions, amino acids and urea

The influence of divalent and trivalent metal ions on the stripping peak current of 5×10^{-8} mol dm^{-3} nitrofurazone was examined. Concentrations ranged from 1×10^{-9} to 5×10^{-8} mol dm^{-3} of Sn (II), Mg (II), Sr (II) and Mn (II) having no effect on the peak current of nitrofurazone, while a concentration of 1×10^{-7} mol dm^{-3} of these metal ions reduced the peak current by about 6.9, 7.0, 5.3 and 4.1%, respectively. Also 5×10^{-7} mol dm^{-3} of both Mg (II) and Mn (II) reduced the peek response of nitrofurazone by about 11.8 and 7.01%, respectively. Concentrations from 1×10^{-9} up to 1×10^{-7} mol dm^{-3} of Co (II), Ca (II), UO_2(II) and Cr (III) had no effect on the peak current of nitrofurazone but a concentration of 5×10^{-7} mol dm^{-3} reduced the peak current by about 3.9, 4.1, 5.8 and 9.4%, respectively. A 5×10^{-8} mol dm^{-3} concentration of Al (III) reduced the peak current of nitrofurazone by about 3.4%, while concentrations ranging from 1×10^{-9} to 5×10^{-7} mol dm^{-3} for Zn (II), Pb (II), Ni (II) and Ba (II) showed no influence on the peak response of nitrofurazone.

The addition of 1×10^{-8} mol dm^{-3} glycine and aspartic acid depressed the peak current of nitrofurazone by about 8.8 and 6.5%, respectively. The addition of 5×10^{-8} mol dm^{-3} of glycine or L-lucine also depressed the peak response by about 11.3 and 5.7%, respectively. The effect of L-valine on the peak response was studied over the range of 5×10^{-9}–5×10^{-7} mol dm^{-3} and no effect was observed. Urea with a concentration range from 1×10^{-9} to 5×10^{-7} mol dm^{-3} showed no effect on the peak current of 5×10^{-8} mol dm^{-3} nitrofurazone.

Table 3
Comparison between the detection limits nitrofurazone the present and other previous works

Technique	Supporting electrolyte	Working electrode	Reference electrode	E_p or $E_{1/2}$ (V)	Detection limit (M)	Reference
Cathodic stripping voltammetry	BR buffer (pH 12)	HMDE	Ag/AgCl	−0.404	1×10^{-9}	Present work
D.C. polarography	Pyridine-formic acid (pH 4.5)	DME	SCE	−0.18	1.24×10^{-6}	[18]
D.P. polarography	Aq. DMF	–	–	–	2×10^{-8}	[28]
D.P. Polarography	Universal buffer	DME	–	–	0.21×10^{-6}	[20]

4. Applications

From the results obtained using cathodic stripping voltammetry, nitroffirazone can be determined successfully in pharmaceutical formulations (ointment) and biological samples (urine and serum) using the following conditions; a scan rate of 100 mV s^{-1} and -0.35 V accumulation potential in BR buffer pH 12.

4.1. Application to pharmaceutical formulation

4.1.1. Determination of nitrofurazone in ointment sample

Using the method mentioned previously, the voltammograms of nitrofurazone were recorded after 15 s preconcentratrion time. The standard addition method was used to calculate the concentration of the drug. The measurements were repeated seven times. The corresponding concentration of nitrofurazone was calculated and a recovery of 95.14% was obtained.

4.2. Application to biological samples (urine and serum)

4.2.1. Determination of nitrofurazone in urine sample

To determine nitrofurazone in urine, different voltammograms were recorded after adding different concentrations of this drug to a voltammetric cell containing the urine sample and the buffer (1 ml urine and 9 ml BR buffer). The conditions used are: BR buffer pH 12; accumulation time = 15 s; accumulation potential = -0.35 V using a scan rate of 100 mV s^{-1}. Fig. 3 shows typical voltammograms recorded for the urine sample in the absence and presence of different concentration of the drug under investigation. The resulting peak currents showed a linear behaviour with concentrations over the range 2×10^{-6}–1×10^{-5} mol dm^{-3} with a correlation coefficient of 0.942.

4.2.2. Determination of nitrofurazone in serum sample

The method described above was used for the determination of nitrofurazone in serum samples at an accumulation time of 5 s and a deposition potential of -0.35 V. A linear behaviour was observed in concentrations ranged from 7×10^{-8} to 1×10^{-6} mol dm^{-3} with a correlation coefficient of 0.996.

5. Conclusion

Cathodic stripping voltammetric method improved the sensitivity of nitrofurazone determination. This method gave a selectivity and a lower detection limit of 1×10^{-9} mol dm^{-3} the other reported values are 1.24×10^{-6} [18], 2×10^{-8} [29] and 0.21×10^{-6} M [20]. This method was also successfully applied for the determination of this drug in pharmaceutical formulations (oint-

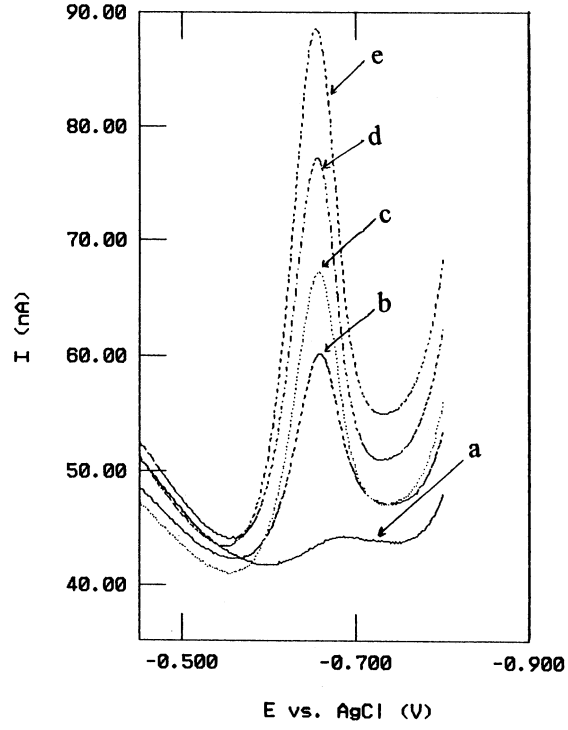

Fig. 3. Typical voltammograms for different nitrofurazone concentrations in urine samples, 15 s preconcentration time, 100 mV s^{-1} scan rate and -0.35 V accumulation potential. (a) Urine sample + BR buffer (pH 12); (b) urine sample + BR buffer (pH 12) + 2×10^{-6} mol dm^{-3} nitrofurazone; (c) urine sample + BR buffer (pH 12) + 4×10^{-6} mol dm^{-3} nitrofurazone; (d) urine sample + BR buffer (pH 12) + 6×10^{-6} mol dm^{-3} nitrofurazone; (e) urine sample + BR buffer (pH 12) + 8×10^{-6} mol dm^{-3} nitrofurazone.

ment) and biological samples (urine and serum), the optimum conditions were found to be: scan rate = 100 mV s^{-1}, accumulation potential = −0.35 V (vs. Ag/AgCl) and BR buffer pH 12.

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