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ORIGINAL ARTICLE

Validated voltammetric method for the determination of some antiprotozoa drugs based on the reduction at an activated glassy carbon electrode

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Abstract A sensitive electrochemical procedure based on reduction of secnidazole (I), tinidazole (II) and ornidazole (III) at a glassy carbon electrode (GCE) was introduced. A study of the variation of the peak current with solution variables such as pH, ionic strength, concentration of drugs, possible interference, and instrumental variables such as scan rate, pulse amplitude, preconcentration time, accumulation potential, has resulted in the optimization of the reduction signal for analytical purposes. Linear calibration plots were obtained over the concentration ranges of 50–800, 50–750 μg mL^{-1} for I, and both (II, III) respectively, in Britton–Robinson buffer of pH 7. The relative standard deviations of five replicate measurements of 1.0 and 10.0 μg mL^{-1} of I, II and III concentrations were 4.7%, 4.9% and 5.3%, and 2.2%, 2.6% and 2.8%, respectively. The limits of detection (LOD) for I, II and III were found to be 2×10^{-10}, 3×10^{-10} and 2.5×10^{-10} mol L^{-1} and limits of quantification (LOQ) for I, II and III were found to be 4.0×10^{-8}, 1.2×10^{-8} and 4.4×10^{-8} mol L^{-1}, respectively. The optimal conditions were: $E_{acc} = -0.9$ V, $t_{acc} = 30$ s, scan rate = 20 mV s^{-1}, pulse-height = 90 mV and Britton–Robinson buffer of pH 7. The method was

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applied for the determination of the cited drugs both in raw materials and in pharmaceutical preparations with satisfactory results and compared with the official reference method. Complete validation of the proposed method was also done.

1. Introduction

Secnidazole (I), tinidazole (II) and ornidazole (III) (Fig. 1) are 5-nitroimidazole derivatives which have the effect of antibacterial, antiprotozoa and anticancer. They act selectively against anaerobic and microaeriophilic bacteria and protozoa, with a half life longer than that of metronidazole. They were used in the treatment of triochomoniasis of the genito-urinary tract in males and females without affecting the normal acidophilic flora of the vagina and they had no effect on candida species (Martendel, 2007). The mechanism of action of nitroimidazoles, as the cited drugs, involved interference with DNA by a metabolite in which the nitro group had been reduced. It seems that their cytotoxicity is not due to the final reduction products, but to an unstable anion radical intermediate formed at a lower reduction level (Edwards, 1986).

The studied mechanism of action of nitroimidazoles showed that the reduction pathway is more complex than is usually postulated for the electroreduction of aromatic nitro compounds (Declerck and de Ranter, 1987). For this reason, elucidation of the mechanism of the electroreduction of the drugs under investigation and related compound is of evident biological interest. Very few studies had been done on these compounds. Secnidazole (I) was determined using high performance liquid chromatography (Bakshi and Singh, 2004; Qi and Shan, 2002), electrochemical (El-Sayed et al., 2010) and spectrophotometric methods (Li et al., 2007). Methods for determination of tinidazole (II) included high performance liquid chromatography (Ouyang et al., 2010), electrochemical method (Wang et al., 2006), spectrophotometery (López-Martínez et al., 1997) and electrophoresis (Zhang et al., 2006). Studies on ornidazole (III) included high pressure liquid chromatography (Nranjane et al., 2008; Sun et al., 2007), high performance thin layer chromatography (Gandhimathi et al., 2006; Ranjane et al., 2010), electrophoresis (Zhang et al., 2006; Al Azzam et al., 2010) and spectrophotometeric methods (Maheshwari et al., 2009). Electrochemical methods are preferred over others because of its low detection limits, fast response time, low cost and simplicity.

This paper describes simple polarographic method for determination of some antiprotozoa drugs via their reduction at an activated glassy carbon electrode. The electrode process was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The influence of different experimental parameters such as pH, supporting electrolyte, accumulation time and scan rate was investigated to optimize the proposed method. The method is successfully applied for the determination of the cited drugs in tablets and the results obtained are compared with the official method.

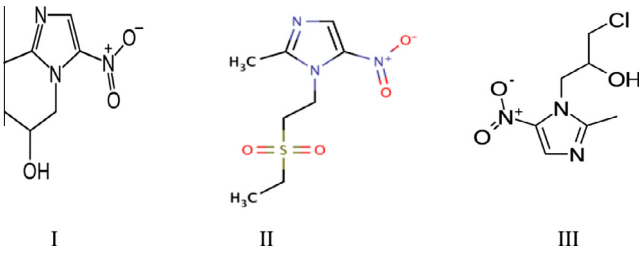

Figure 1 Structure of secnidazole (I), tinidazole (II) and ornidazole (III).

2. Experimental

2.1. Instruments, electrodes and chemicals

The electrochemical analyzer computerized system with 797VA computerized software (1.0) from Metrohm, Swizerland was used. A three-electrode cell was employed incorporating a glassy carbon stationary electrode as a working electrode (Mini glassy carbon disk electrode of the active zone: 2.8 mm, for ELCD 641/656), Ag/AgCl (3 mol L^{-1} KCl) reference electrode and a platinum wire counter electrode. The data were treated with micro origin (ver.5) software to transform the initial signal. A Mettler balance (Toledo-AB104) was used for weighing the solid materials. A cyberscane 500 digital (EUTECH instruments, USA) pH meter with a glass combination electrode was used to carry out pH measurements. A micropipette (Eppendorf-multipette plus) was used throughout the present experimental work.

Deionized water used throughout the present study was supplied from a purite still plus deionized connected to a Hamilton-Aqua-Metric deionized water system. For the application of the pretreatment to the glassy carbon electrode, a Wenking Model HP 70 potentiostat and exact-type 250 function generator were used.

The active ingredient pharmaceutical drugs, secnidazole, tinidazole and ornidazole and their pharmaceutical preparations; secnidazole were supplied from Egypt pharmaco co. (500 mg/tablet), protozol from El mehan eltebea co. and tibazole (500 mg/tablet) were provided from sigma co. Stock solutions of 1×10^{-3} mol L^{-1} were prepared by dissolving a calculated weight of the active ingredient drugs in deionized water and stored at 4 °C in PVC containers. More diluted solutions were freshly prepared daily by accurate dilution. Britton–Robinson (B–R) buffer solutions (pH 2–11) were used as supporting electrolytes. All solutions were prepared from analargrade reagents (Merck and Sigma) in doubly distilled water.

2.2. Preparations of pharmaceutical samples

For tablets solution, ten tablets of secnidazol, tibezole and protozol were weighed and the average mass per tablet was determined. A weighed portion of a finely grounded powder equivalent to the calculated weight of pharmaceutical preparations was dissolved to produce a 1×10^{-3} mol L^{-1} solution. The solution was filtered through a 0.45 μm millipore filter, in order to separate out the insoluble excipients and reject the first portion of the filtrate. The solution was directly analyzed,

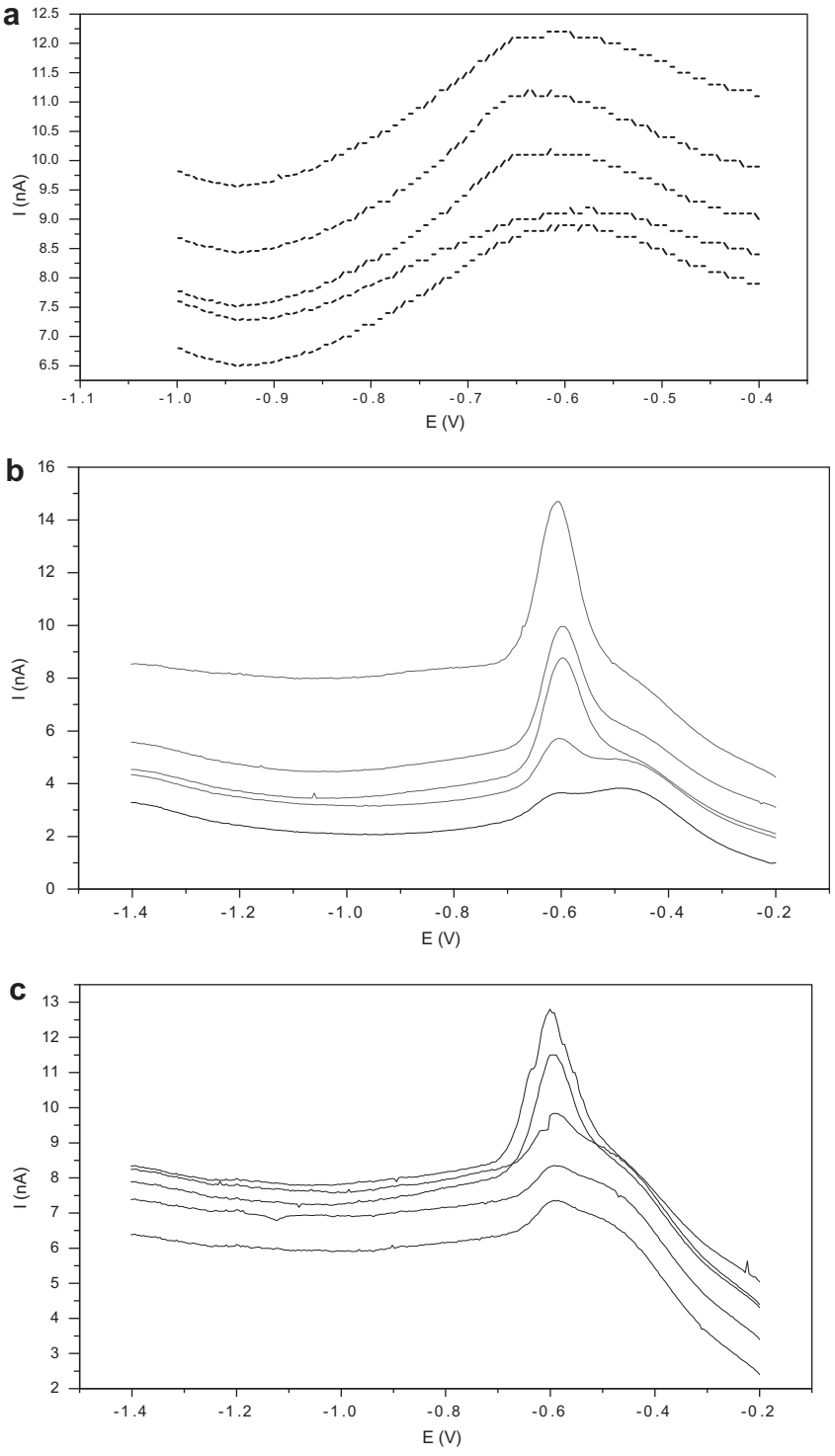

Figure 2 Cyclic voltamogram of 1×10^{-9} mol L^{-1} (a) secnidazole, (b) tinidazole and (c) ornidazole, scan rate 20 mV/s in Britton–Robinson buffer pH 7.

according to the general analytical procedures without the necessity for sample pretreatment or any extraction steps.

2.3. Pre-treatment of the glassy carbon electrode

The electrode was pretreated by cycling a square-wave potential with a frequency of 350 Hz between the potential limits of ± 6 V followed by the application of a triangular potential sweep between ± 6 V (frequency 3500 Hz) in 0.1 mol L^{-1} potassium nitrate solution. Finally, the electrode was subjected to an electrochemical pretreatment by applying a potential of $+1.5$ V for 5 min and then -1.0 V for 2 s in 0.1 mol L^{-1} potassium nitrate solution. These steps were repeated until the voltammetric response of the electrode became reproducible. At

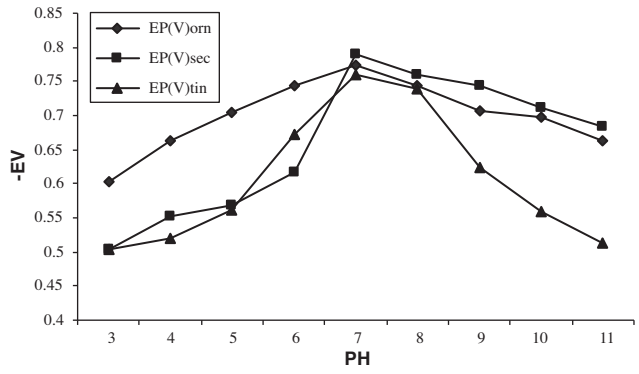

Figure 3 Effect of pH on the peak potential in B–R buffer of PH (2–11) for 1×10^{-3} mol L^{-1} solution of scindazole (I), tinidazole (II) and ornidazole (III) using CV mode.

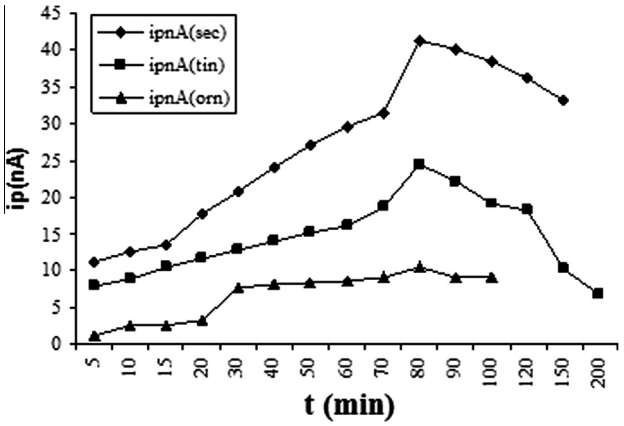

Figure 4 Effect of accumulation time on the peak current for 1×10^{-3} mol L^{-1} of scindazole (I), tinidazole (II) and ornidazole (III), in B–R buffer using CV mode.

the end of this procedure, the electrode surface was so stable that for 40 measurements, the electrochemical pretreatment alone was sufficient before each scan (Declerck and de Ranter, 1987).

3. Results and discussion

The cathodic cyclic voltammogram for the reduction of I, II and III drugs in Britton–Robinson buffer (pH 7.0) at GCE are shown in Fig. 2. In the forward scan, one cathodic peak owing to the reduction of nitro group is observed. In the reverse sweep no cathodic peak is observed which indicates that the reduction of the drugs under investigation (I, II and II) is irreversible. The reduction peaks observed were broad in nature with peak potentials −0.652, −0.624 and −0.664 mV for I, II and III, respectively.

Differential pulse voltammogram of I, II and III drugs exhibit one distinguishable cathodic peak at pH 7.0 corresponding to the cyclic voltammetric peaks. Differential pulse voltammograms of the GCE recorded at different pulse amplitudes (10–75 mV) and potential scan rates (2–20 mV s^{-1}) show that the peak increased, broaden and shifted to lower potential values on increasing the amplitude or the potential scan rate. The waveform employing amplitude of 25 and 10 mV s^{-1} was used in all subsequent work as a compromise between sensitivity, speed and resolution.

3.1. Influence of the pH of supporting electrolyte

The pH of the electrolyte medium is one of the variables that commonly and strongly influenced the shape of the voltammogram, and therefore it was important to investigate the effect of the pH on the electrochemical behavior of the drug. The effect of pH on the reduction of the drugs under investigation at activated glassy carbon electrode was studied over the pH range 2.0–12.0 at the same concentration (1×10^{-3} mol L^{-1}) by means of cyclic voltammetry. The three drugs yield one main irreversible reduction processes at −0.616, −0.624 and −0.609 V for I, II and III, respectively, which shifts towards more negative potentials as the pH increased. The E_{p1} versus pH plot (Fig. 3) exhibits two linear intervals with break approximately at pH 7.0. The peak current is also dependent on the pH, implying the involvement of protons in the current-limiting electrode process. This denotes that the proton – transfer reaction precedes the electrode process proper (Martendel, 2007). The peak current (i_{p1}) has its maximum value at pH 7.0, which was used in the subsequent examination of other dependencies.

The influence of ionic strength on the efficiency of the accumulation of 1×10^{-6} mol L^{-1} I, II and III solution was studied at GCE using CV mode. The ionic strength was varied by changing the NaCl from 0.001 to 0.05 mol L^{-1} in Britton–Robinson buffer of pH 7.0. The results showed that increasing ionic strengths were found to be of a less significance on the degree of accumulation. This indicates that the process responsible for accumulation of the drug at the electrode surface is not electrostatic in nature. The predominance of hydrophobic:hydrophilic interactions between the electrode surface and the drug may, therefore, be considered. The pretreatment procedure which was described for treating glassy carbon electrode offered improved responses in drug analysis (Biryol et al., 1995; Radi, 2001). It was seen by scanning electron microscopy that this activation created scratches and holes in the glassy carbon electrode which gave a higher active area comparing to the non activated glassy carbon electrode (Biryol and Ozkan, 1997).

In our present work we used the activated glassy carbon electrode to estimate the electroreduction of the cited drugs in the prepared buffer.

3.2. The effect of adsorption character

3.2.1. Effect of accumulation time and potential

The interfacial adsorptive character of drugs onto the activated glassy carbon were studied by both DPV and CV modes, and identified from the peak current, ip (nA). The dependence of ip developed in B–R buffer (pH 7) on the accumulation time was investigated as shown in Fig. 4 at a concentration level 1×10^{-3} mol L^{-1} for drugs investigated by DPV. From the plot of ip vs t_{acc} (accumulation time), the short preconcentration time of the drug in B–R buffer (pH 7) resulted in large cathodic peak. A full surface coverage was established after a certain accumulation time for each drug thus, the accumulation time of choice is dictated by the sensitivity.

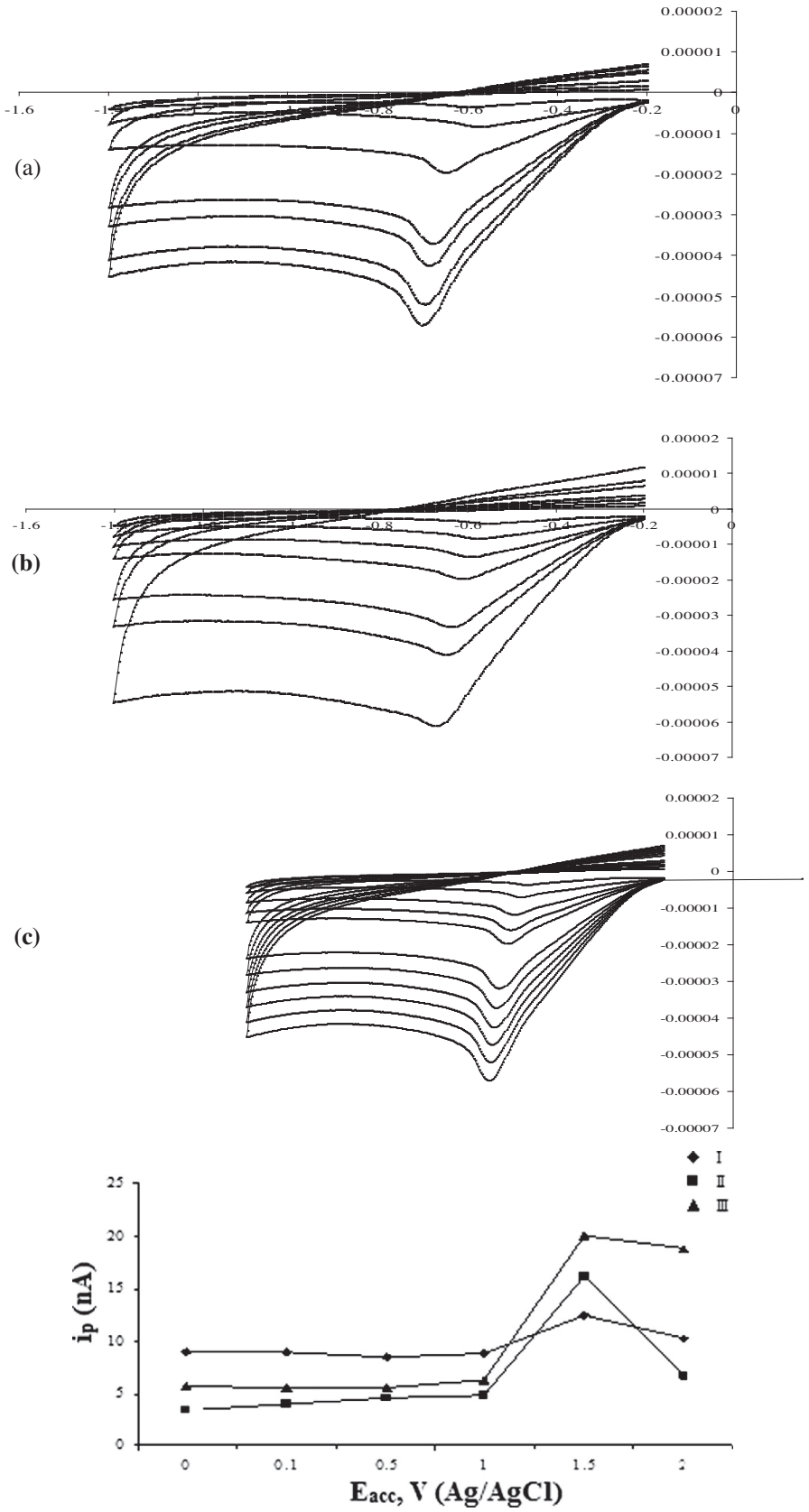

Figure 5 Effect of accumulation potential on the peak current for 1×10^{-3} mol L^{-1} of scindazole (I), tinidazole (II) and ornidazole (III), in B–R buffer pH 7 using CV mode.

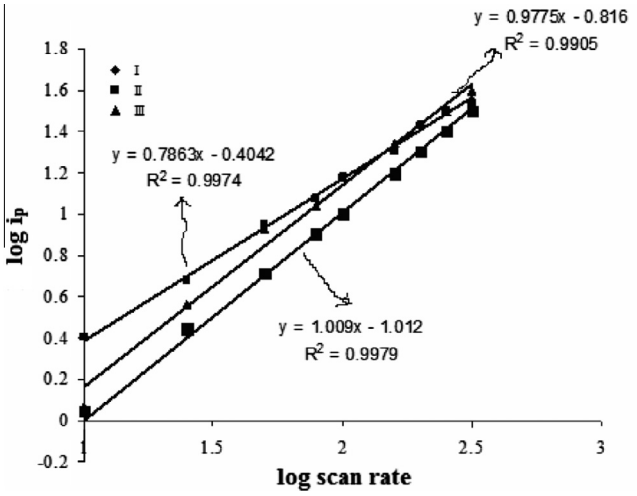

Figure 6 Relation between log i_p and log scan rate (V) for the reduction of investigated drugs at activated glassy carbon electrode.

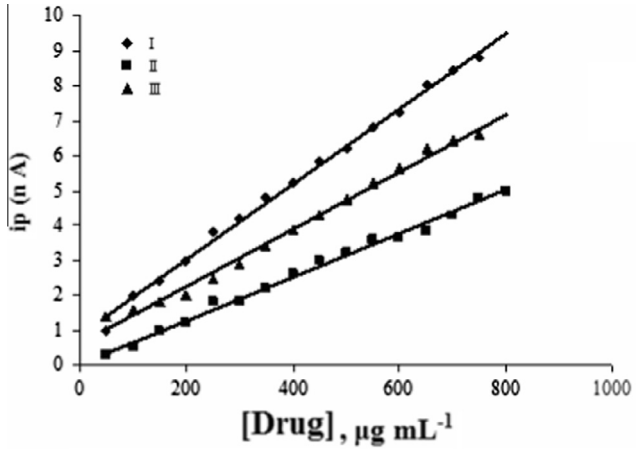

Figure 7 Calibration curves for I, II and III.

The effect of accumulation potential on the extraction efficiency was also investigated at a potential range from 0.0 to +0.2 V following 3 min preconcentration from 1×10^{-3} mol L^{-1} I, II and III solution at pH 7.0 (Fig. 5). The stripping peak currents at the GCE surface appear to be dependent on the accumulation potential. The effect of the accumulation potential as a function of peak current, ip, was studied over the range of 0–0.2 V under selected conditions suitable for each drug.

3.2.2. Effect of scan rate

The effects of the potential scan rate (v) on the peak current i_{p1} and peak potential E_{p1} was studied using 1×10^{-3} mol L^{-1} I, II and III in buffer of pH 7 within the range of 10–300 mV s^{-1}. The peaks potential (E_p) moved to a more negative potential with increasing the scan rate, which confirms the irreversibility of the process. A linear increase in the reduction peak current with the scan rate shows the adsorption control process. A plot of peak current versus the scan rate for the investigated drugs gave straight lines, with a simultaneous increase in the reduction peak current (ip) at a high scan rate. The plot of log i_p against log scan rate displayed a linear correlation (Fig. 6). The slop values are 0.7863, 0.9775 and 1.009 for I, III and II, respectively. These slops are expected for ideal reaction of the solution and the surface species (Martendel, 2007). In these study 100 mV s^{-1} was chosen as the scan rate because at this value the sensitivity was relatively high and the voltammetric curves were well-shaped with relatively narrow peak width (Abdel Ghani et al., 2007).

3.3. Analytical application

While the sensitivity enhancement associated with the interfacial accumulation is significant, the main advantage of the method is its inherent selectivity towards the surface-bound analyte. For this purpose the working electrode with the extracted drug was transferred from the complex sample to an electrolytic blank solution between the preconcentration and measurement steps.

3.3.1. Assay for determination of active ingredient materials

Calibration curves within the concentration levels of the cited drugs for the proposed method were attempted following the selected optimal conditions (Fig. 7). In order to verify the uniformity content of the drug in tablets, the proposed DPV method was applied for a tablets assay of the named commercial properties. The results obtained by developed DPV method was compared with that obtained by the official method (British pharmacopeia, 2009). The results of the statistical evaluation are given in Table 1. The results of F-test and t-test under a confidence limit of 95% showed that there are insignificant differences between the methods. Moreover, the content of all assayed tablets were found to be within the claimed amount.

3.3.2. Validation of the analytical procedure

The validation of the procedure for the quantitative assay of the cited drugs was examined by the evaluation of the LOD, LOQ selectivity, repeatability, recovery and robustness and ruggedness. Calibration curves within the concentration levels of 10^{-7}–10^{-9} mol L^{-1} were attempted. Both LOD (LOD = 2×10^{-10}, 3×10^{-10} and 2.5×10^{-10} mol L^{-1} for I, II and III, respectively) and LOQ (LOQ = 4.0×10^{-8}, 1.2×10^{-8} and 4.4×10^{-8} mol L^{-1} for I, II and III, respectively) values confirmed the high sensitivity of the proposed procedure compared with the official methods. The repeatability of the method was determined from multiple measurements at each of the studied samples by performing five replicates measurements (n = 5). A mean recovery of 99.6 ± 0.6 was achieved indicated a high precision of the proposed procedure for assay of the drugs.

The selectivity of the optimized procedure was examined in the presence of some common excipients to monitor the interference effect. A mean recovery of 2.0×10^{-9} mol L^{-1} of the cited drugs ranging from 98.5% to 101% was obtained. The proposed method can be considered to be selective. The robustness (The United State Pharmacopoeia, 2003) of the results of the procedure is the ability to remain unaffected by small change in its operational parameters such as pH (Table 2). In the present work this was examined by studying the effect of a variation of pH. The recovery values were not significantly affected by this variation and consequently the optimized procedure was reliable for the assay of drugs. It could be

Table 1 Statistical parameters of the pharmaceutical dosage forms assay of the investigated drugs by the proposed DPV method and official method.

Sample	Drug	[Drug] taken $\mu g\ mL^{-1}$	Proposed method ± %RSD, $n = 5$	Official method ± %RSD, $n = 5$	*F*-test	*t*-test
Ornidazole	Tibezole tablets (500 mg/tablet)	200	100.25 ± 2.13	99.41 ± 2.00	1.57	2.25
		400	99.55 ± 1.21	99.58 ± 1.42	1.31	2.14
Secnidazole	Secnidazole tablets (500 mg/tablet)	200	99.98 ± 0.95	99.75 ± 1.00	1.85	2.23
		400	100.16 ± 1.32	99.99 ± 1.42	1.35	2.10
Tinidazole	Protozol tablets (500 mg/tablet)	200	100.10 ± 1.16	99.99 ± 1.42	2.9	2.09
		400	99.98 ± 1.11	100.20 ± 1.32	1.78	2.45

Tabulated *t*-value at 95% confidence limit = 2.77, $n = 5$, degree of freedom = 4. Tabulated *F*-value at 95% confidence limit = 6.39, $n = 5$.

Table 2 The robustness and the ruggedness of the condition of the proposed procedure for the determination of investigated drugs at glassy carbon electrode.

Variables	Drug	Recovery % ± RSD
Robustness	Secnidazole	99.0 ± 0.40
Results	Tinidazole	98.5 ± 0.69
At pH = 7	Ornidazole	99.1 ± 0.59
Ruggedness	Secnidazole	98.3 ± 0.31
Analyst-1	Tinidazole	99.0 ± 0.70
	Ornidazole	98.3 ± 0.52
Ruggedness	Secnidazole	99.0 ± 0.52
Analyst-2	Tinidazole	98.8 ± 0.37
	Ornidazole	99.0 ± 0.63

robust. The ruggedness is the degree of reproducibility of the results obtained by analysis of the same sample under a variety of normal test conditions, such as different laboratories, analysts and lots of reagents. This was examined by applying the proposed procedures to an assay under experimental conditions using different analysis. The results obtained due to lab. (1) to lab. (2) and even day to day were found to be reproducible, since there is no significant difference between the recovery and SD values.

4. Conclusion

In conclusion, the present work demonstrates that highly sensitive electrochemical measurement of the drugs under investigation (secnidazole, tinidazole and ornidazole) is feasible utilizing its extraction on to GCE. The proposed method showed clear advantages, such as a short period of the real time of drug analysis and no pretreatment or time consuming in extraction steps were required prior to analysis. Moreover, because of its very limits of detection and quantification, the proposed method could be applied in clinical laboratories and pharmacokinetic studies.

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