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Voltammetry and determination of metronidazole at a carbon fiber microdisk electrode

P.N. Bartlett[a,*], E. Ghoneim[a,b], G. El-Hefnawy[b], I. El-Hallag[b]

[a] *School of Chemistry, University of Southampton, Southampton SO17 1BJ, UK*
[b] *Department of Chemistry, Tanta University, Tanta, Egypt*

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Abstract

The electrochemistry of metronidazole, 1-(hydroxyethyl)-2-methyl-5-nitroimidazole, was investigated at a carbon fiber microdisk electrode in pH 9 Britton Robinson buffer. Under these conditions, the reduction of metronidazole is controlled by both mass transport to the microdisk and adsorption with an equilibrium constant of 4×10^3 mol^{-1} dm^3 and a saturation coverage of 0.88×10^{-8} mol cm^{-2}. The adsorption and accumulation of metronidazole on the surface of the carbon fiber allows its determination at low concentrations by square wave adsorptive stripping voltammetry. A detection limit for metronidazole of 5×10^{-7} mol dm^{-3} and a R.S.D. of 3.7% at 1×10^{-6} mol dm^{-3} ($n = 4$) were obtained with a two electrode system with no stirring during the accumulation step. Based on this method, a simple procedure for the determination of metronidazole in urine is described which requires no pre-treatment of the sample before analysis.

Keywords: Metronidazole; Carbon fiber microdisk; Stripping voltammetry

1. Introduction

The unique properties of microelectrodes offer a number of particular advantages for their use in analytical applications. In the steady-state mass transport to the microdisk is dominated by quasi-hemispherical diffusion. As a consequence mass transport to and from the microdisk electrode surface is efficient, reproducible and insensitive to random convection. In analytical applications based on accumulation and stripping techniques this obviates the need for stirring during the preconcentration step, improves the precision of the measurement, and reduces analysis times. In addition, the small electrode surface area of the microdisk means that the double layer capacitance of the electrode is small, thus significantly reducing the charging current. As a consequence, the ratio of the steady-state Faradaic current to the non-Faradaic current is increased and the detection limit is enhanced [1]. The small size of the electrodes also means that the currents passed at the microelectrode are small resulting in negligible ohmic drop (*iR* drop) even in the absence of a supporting electrolyte [2,3]. This has dual advantages in analytical applications that sample preparation can be simplified and that the possibility of impurities from the added electrolyte is eliminated. Microelectrodes are thus particularly well-suited for trace analysis. Pt, Au, Hg and carbon microelectrodes have been used in stripping analyses for the determination of trace metals by a number of authors [4–8]. In contrast, studies using microelectrodes for the determination of organic species are far less numerous and are restricted to the use of carbon fiber microelectrodes [9–19]. We are not aware of any publications describing the use of carbon fiber microdisk electrodes for the determination of pharmaceutical drugs.

In this paper, we report the investigation of the electrochemistry of the drug metronidazole, 1-(hydroxyethyl)-2-methyl-5-nitroimidazole, at carbon fiber microdisk electrodes in buffered aqueous solution at pH 9. The adsorption of metronidazole at the carbon surface was studied together

* Corresponding author. Tel.: +44 2380 592373; fax: +44 2380 593781.
E-mail address: p.n.bartlett@soton.ac.uk (P.N. Bartlett).

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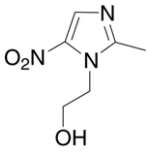

Fig. 1. The structure of metronidazole.

with the effects of concentration, accumulation time and scan rate on the reduction. Finally, low concentrations of metronidazole were determined by square wave adsorptive stripping voltammetry and the method was applied to the determination of metronidazole in urine.

Metronidazole is a nitroimidazole derivative and has been widely used for the treatment of protozoal diseases, including trichomoniasis and giardiasis [20]. The structure of metronidazole is shown in Fig. 1, it contains a nitro group, which is the electrochemically active reducible center. Generally, the reduction of the nitroimidazoles in alcoholic solution is a complex process, involving six electrons for complete reduction of the nitro group to the amine [21]. In the absence of oxygen, the reduction process for nitroimidazoles is similar to that for nitrobenzene [22]. The cytotoxicity of metronidazole is due to intermediates formed during the reduction [23–25] and voltammetric techniques have been applied to investigate the mechanism of action of nitroimidazoles as antimicrobial agents [26], for their determination in pharmaceutical [27–32] and clinical [33,34] matrices, and at DNA modified electrode surfaces [35–38]. The mercury electrode is the most widely used, but good results were obtained using solid electrodes when studying nitrobenzene [39,40] and metronidazole [37,38]. Recently, an amperometric sensor for the determination of metronidazole at metalloporphyrin modified carbon paste electrode was reported [41].

2. Experimental

2.1. Apparatus

Voltammetric experiments were controlled by an Autolab PGSTAT30. A Corning, model 145, pH-meter was employed for pH measurements.

2.2. Electrodes and electrochemical cells

Voltammetric experiments were carried out with a two electrode cell inside a Faraday cage. The working electrode was a 7.5 μm diameter carbon fiber microdisk and the reference electrode was a saturated calomel electrode, SCE, all potentials are referenced to this electrode.

2.3. Reagents and solutions

Metronidazole was kindly supplied by Squibb Company, Cairo, Egypt. A stock solution of 1×10^{-3} mol dm^{-3} of metronidazole in ethanol was prepared and used to prepare the other concentrations by dilution with Britton Robinson (BR) buffer (pH 9) as the supporting electrolyte. All solutions were freshly prepared using reagent-grade water (18 MΩ cm) from a Whatman RO80 system coupled to a Whatman "Still Plus" system. The buffer is composed of mixtures of 0.04 mol dm^{-3} acetic, orthophosphoric, and boric acids; adjusted to the required pH with 0.2 mol dm^{-3} sodium hydroxide solution [42], all reagents were of analytical grade. Ruthenium(III) hexamine trichloride (used for the characterization of the microelectrode) was obtained from Aldrich. All samples were purged with oxygen free argon (BOC Ltd.) to get rid of dissolved oxygen. The argon gas was presaturated with deionized water prior to purging to prevent evaporative losses from the sample solution. Prior to the first run, a purge time of 12–15 min was generally used to deoxygenate the sample solution.

2.4. Procedures

2.4.1. Carbon fiber microdisk fabrication and treatment

The carbon fiber microdisk electrodes were prepared by sealing short lengths of 7.5 μm diameter carbon fiber directly into glass. The microelectrode surface was polished mechanically with graded alumina powder of different sizes (1, 0.3–0.05 μm, Buehler, Lake Bluff, IL, USA) on a polishing cloth. In order to improve the repeatability of the measurements, an electrochemical cleaning of the electrode surface was also made by cycling the potential several times in buffer at a scan rate of 50 mV s^{-1} between −0.3 and −1.2 V, until the blank cyclic voltammograms were reproducible, finally the electrode was kept at −1.5 V for 60 s. The effective electrode radius was determined by recording the steady-state diffusion limiting current, I_L, from a 10 mmol dm^{-3} solution of ruthenium(III) hexamine trichloride in 0.2 mol dm^{-3} KCl. The radius of the electrode was estimated using the equation for the current at a microdisk [43,44]

$$I_L = 4nFDC^b r \quad (1)$$

where r is the radius of the microdisk, C^b the bulk concentration, D the diffusion coefficient of the $[Ru(NH_3)_6]^{3+}$ ($D = 0.9 \times 10^{-5}$ cm^2 s^{-1} [43]) and the other symbols have their usual meanings.

2.4.2. Adsorptive stripping measurements

All measurements were carried out in aqueous solutions de-oxygenated with argon. A two-electrode configuration, in which the reference and counter electrode connections of the potentiostat were both attached to the SCE reference electrode, was employed. The appropriate solutions were transferred into the electrochemical cell, and an accumulation potential of −0.4 V was applied to the pretreated carbon fiber electrode without stirring. When the accumulation time was completed, a square wave scan (50 Hz frequency, 10 mV scan increment, 25 mV pulse am-

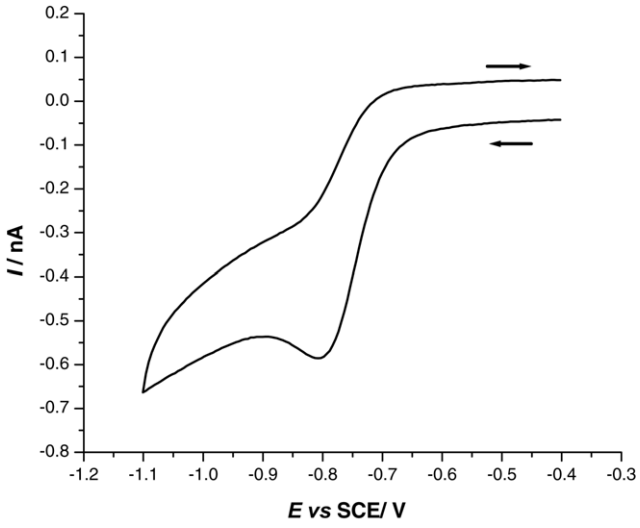

Fig. 2. Cyclic voltammogram recorded at a carbon fiber microdisk electrode, 7.5 μm diameter, in a solution of 1×10^{-4} mol dm^{-3} metronidazole in pH 9 Britton Robinson buffer. Scan rate, 50 mV s^{-1}; t_{acc}, 60 s; E_{acc}, −0.4 V.

plitude) was initiated towards more negative potential values.

3. Results and discussion

3.1. Electrochemical behaviour of metronidazole at the carbon fiber microelectrode

Fig. 2 shows a cyclic voltammogram of metronidazole in pH 9 buffer at a pre-treated carbon fiber electrode. For metronidazole, it was found that the pre-treatment of the electrode led to a slight improvement in the repeatability of the measurements. pH 9 buffer was chosen because preliminary experiments showed this to give the best resolved voltammetry. For this experiment, the electrode was held at −0.4 V for 60 s before initiating the scan to −1.1 V and back at 50 mV s^{-1}. On the forward scan the voltammogram shows a well defined peak at −0.8 V on top of the sigmoidal wave expected for a simple diffusion controlled process at a microdisk electrode. In contrast, on the return scan the wave has the expected sigmoidal shape with no evidence of a corresponding oxidation peak. The voltammetry in Fig. 2 indicates that during the period before the start of the voltammetric scan at −0.4 V, metronidazole adsorbs at the carbon electrode surface. Then during the cathodic scan, the adsorbed metronidazole is reduced, giving the peak, along with the mass transport limited reduction of metronidazole from the solution (giving the sigmoidal wave). To confirm this interpretation the effects of sweep rate, metronidazole concentration and accumulation time were studied.

Keeping the metronidazole concentration and accumulation time constant, the effect of the scan rate on the peak current, I_p, at −0.8 V was investigated over the range of 20–500 mV s^{-1}. I_p increases as the sweep rate increases but it does not follow a simple linear relationship or square root relationship indicating that the peak current is determined by a mixture of adsorption and diffusion. On the return scan from −1.1 to −0.3 V, adsorption does not play a significant role and the voltammetry gives a well defined sigmoidal wave of the type expected for a diffusion controlled processes at a microdisk electrode. Under these circumstances, the mass transport limiting current, I_L, is given by Eq. (1), where for metronidazole in pH 9 buffer, $n = 4$ for the four-electron reduction of the nitro group to the corresponding hydroxylamine [45] and D is the diffusion coefficient of metronidazole. Fig. 3A shows a set of voltammograms recorded for different concentrations of metronidazole when scanning the potential from −1.1 to −0.3 V at 50 mV s^{-1}. The corresponding limiting currents, I_L, increase linearly with the metronidazole concentration as expected from Eq. (1) and shown in Fig. 3C (curve a). From the slope of the plot we calculate the diffusion coefficient of the metronidazole to be 5.53×10^{-6} cm^2 s^{-1}. The theoretical limiting currents calculated from Eq. (1) using this value of the D value agreed to within 3.3% with the experimental limiting currents recorded at the carbon fiber microelectrode for concentrations reported in Fig. 3A and also for other concentrations. Fig. 3B shows the effect of the metronidazole concentration on the peak current, I_p, on the forward scan. The voltammograms were obtained by holding the potential of the working electrode at −0.4 V for 60 s then scanning the potential from −0.4 to −1.05 V at 50 mV s^{-1}. The peak currents are plotted against the metronidazole concentration in Fig. 3C (curve b). In this case, the plot is curved with the peak current increasing linearly with metronidazole concentration up to about 1.7×10^{-4} mol dm^{-3}, after which it levels off at higher concentrations.

To separate the contributions from adsorption and mass transport to the peak current we subtract the diffusion controlled current (curve a) from the peak current (curve b). In doing this we assume that the contributions to the current from the reduction of adsorbed metronidazole and from the metronidazole diffusing to the electrode surface are additive and that the adsorbed metronidazole does not block reduction of metronidazole in solution. Support for the latter assumption is provided by comparison of the shapes of the forward and reverse scans in the voltammograms in Figs. 2 and 3A and B. The result is plotted as curve c in Fig. 3C. It can be seen that the contribution from adsorption increases with concentration until full coverage of the electrode surface is attained at the higher concentrations. Analysis of the data using a simple Langmuir isotherm gives an equilibrium constant for the adsorption of metronidazole at the electrode surface of 4×10^3 mol^{-1} dm^3. The maximum surface coverage of metronidazole was calculated by integration of the area under the peak under saturating conditions assuming a four-electron reduction process for the adsorbed species. This gave a saturation coverage of 0.88×10^{-8} mol cm^{-2}; corresponding to an area per molecule of 0.02 nm^2 consistent with the formation of an adsorbed monolayer of metronidazole at the electrode surface.

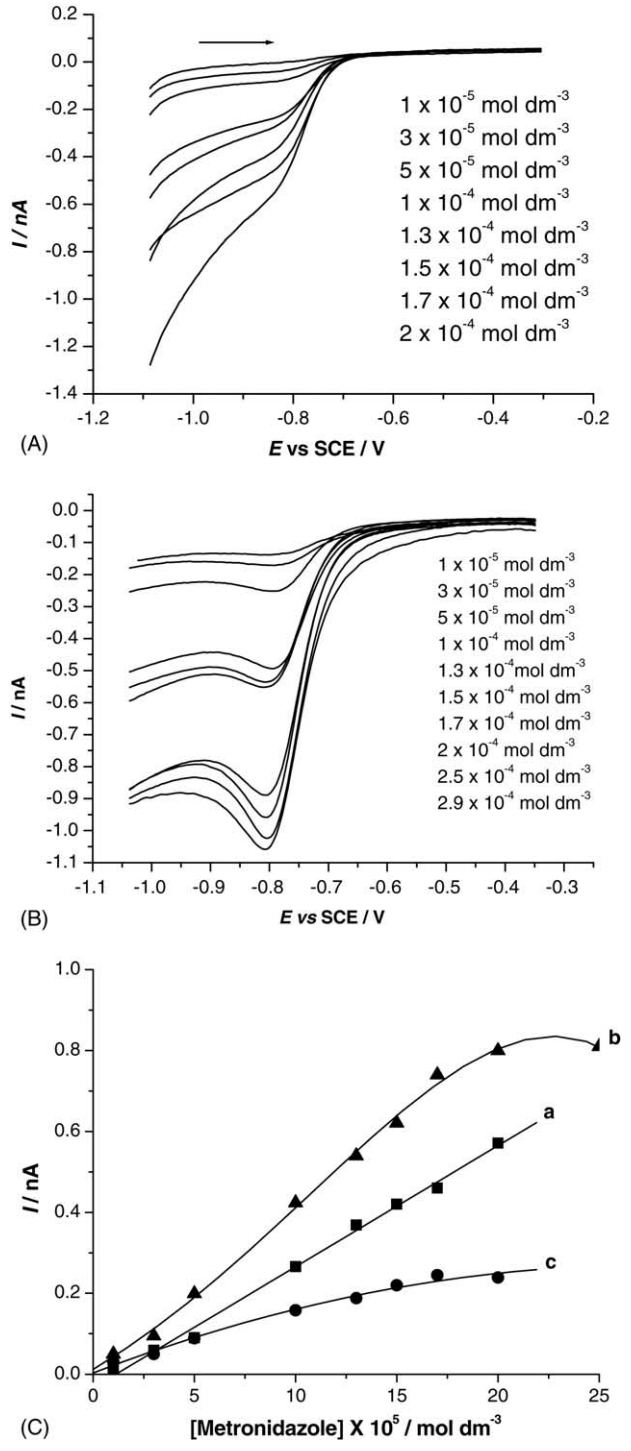

Fig. 3. Linear sweep voltammograms recorded at a carbon fiber microdisk, 7.5 μm diameter, in solutions containing various concentrations of metronidazole in pH 9 Britton Robinson buffer: scan rate, 50 mV s^{-1}. (A) Potential scanned from −1.2 to −0.4, with no accumulation time; (B) voltammograms obtained with t_{acc}, 60 s and E_{acc}, −0.4 V; (C) plots of current against concentration, curve a for I_L, curve b for I_p and curve c for $(I_p - I_L)$.

3.2. Effect of accumulation time

The effect of the accumulation time at −0.4 V on the peak current was studied by recording linear sweep voltammograms at various accumulation times in a solution of 1×10^{-4} mol dm^{-3} of metronidazole using a scan rate of 50 mV s^{-1}. Fig. 4 shows the plot of the peak current against the accumulation time. At short times, less than 60 s, the current linearly increases with the accumulation time; at longer accumulation times the surface of the electrode becomes saturated with adsorbed metronidazole and the current levels off and becomes constant. Note that the intercept at zero accumulation time corresponds to the mass transport limited current for metronidazole reduction under these conditions of 0.24 nA. The increase of the peak current with increasing accumulation time is consistent with our earlier data for the adsorption of the metronidazole at the carbon fiber electrode surface. Comparing the mass transport limited current for 10^{-4} mol dm^{-3} metronidazole at the microelectrode (0.24 nA) with the charge for saturation coverage of the electrode (1.5 nC) it is clear from the accumulation times in Fig. 4 that adsorption of metronidazole at the carbon surface occurs at less than the mass transport limited rate.

The surface coverage was calculated from the charge for saturation coverage at long times and was found to be 0.87×10^{-8} mol cm^{-2}. This value is in agreement with that calculated from the I_p concentration data.

3.3. Square wave adsorptive stripping analysis

The adsorption of metronidazole on the carbon fiber surface can be exploited to develop an analytical procedure for determination of the drug. Square wave adsorptive stripping was investigated for rapid low concentration determination of the metronidazole. The effect of accumulation time on the square wave peak was studied for a solution of

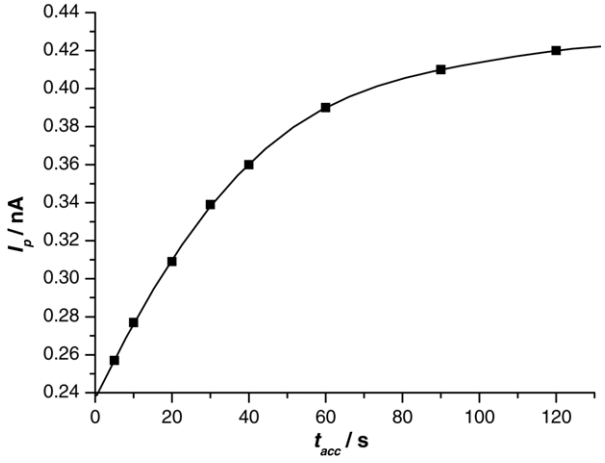

Fig. 4. Plot of the peak current, I_p, against the accumulation times plot for linear sweep voltammograms recorded at a carbon fiber microdisk, 7.5 μm diameter, in a solution of 1×10^{-4} mol dm^{-3} metronidazole in pH 9 Britton Robinson buffer; scan rate, 50 mV s^{-1}; E_{acc}, −0.4 V.

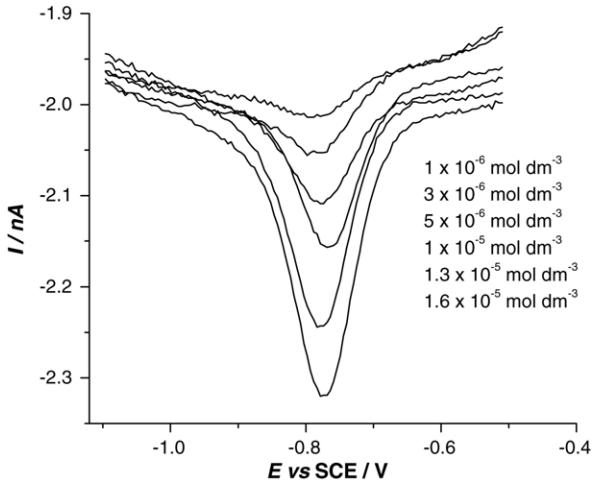

Fig. 5. Square wave adsorptive stripping voltammograms recorded at a carbon fiber microdisk, 7.5 μm diameter, in solutions containing various concentrations of metronidazole in pH 9 BR buffer; t_{acc}, 60 s; E_{acc}, −0.5 V; pulse height, 0.025 V; scan rate, 20 mV s^{-1}; frequency, 50 Hz.

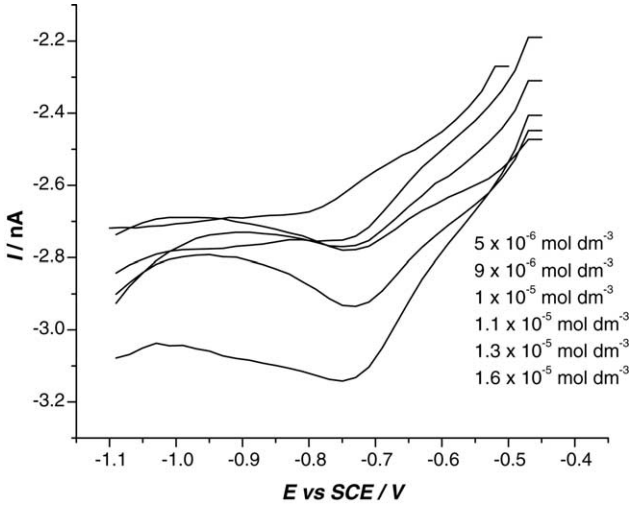

Fig. 6. Square wave adsorptive stripping voltammograms recorded at a carbon fiber microdisk, 7.5 μm diameter, in solutions containing various concentrations of metronidazole in a 10 cm^3 urine sample; t_{acc}, 60 s; E_{acc}, −0.45 V; pulse height, 0.025 V; scan rate, 20 mV s^{-1}; frequency, 50 Hz.

1×10^{-5} mol dm^{-3} metronidazole in pH 9 buffer. The plot of I_p versus t_{acc} produced a straight line over the range from 5 to 120 s. In addition, the optimum instrumental conditions were then chosen from a study of the variation of the peak current of the square wave voltammogram with frequency, scan increment and pulse amplitude. On increasing the frequency from 20 to 120 Hz, the peak current increases but the peak becomes less well-defined. Higher peak currents were observed on increasing the pulse amplitude from 25 to 100 mV but the background current also increased. The peak current increases linearly with the scan increment up to 10 mV. Thus, the best peak definition was obtained using 50 Hz frequency, 10 mV scan increment and 25 mV pulse amplitude.

Using these optimised instrumental conditions, square wave voltammograms for solutions with various concentrations of the metronidazole were recorded, Fig. 5. A plot of concentration versus the peak current gives a straight line over the concentration range of 1×10^{-6} to 2.2×10^{-5} mol dm^{-3} with a correlation coefficient, $r = 0.998$, a slope of 0.015 nA mol^{-1} dm^{-3} and an intercept of 0.0080 nA. The detection and the determination limits were calculated from the calibration curve using the $3s_b/m$ and $10s_b/m$ [46] criteria respectively, where m is the slope of the calibration curve and s_b the standard deviation of the intercept. In this way, a detection limit of 5×10^{-7} mol dm^{-3} and a limit of determination of 1×10^{-6} mol dm^{-3} were obtained. Furthermore, a relative standard deviation of 3.7% ($n = 4$) was obtained at the 1×10^{-6} mol dm^{-3} metronidazole concentration level.

3.4. Application to measurement in urine

Metronidazole is mainly excreted in the urine with about 40% excreted unchanged [47]. We therefore carried out preliminary studies to see if the method could be used for the determination of metronidazole in urine. The samples were prepared by adding known concentrations of metronidazole in ethanol to the untreated urine. No additional electrolyte or buffer was added. The pH of the urine was 8.3.

Using the optimised instrumental conditions described above square wave voltammograms for the untreated urine samples containing various concentrations of the metronidazole were recorded (Fig. 6). In comparison to the data shown in Fig. 5 above the square wave peaks for the urine samples are less well resolved with a larger background current presumably due to the absorption of other species from the urine at the electrode surface. Nevertheless, a plot of the metronidazole concentration against the peak current, measured after background correction, is linear over the range of 5×10^{-6} to 1.6×10^{-5} mol dm^{-3} with a correlation coefficient, $r = 0.995$, a slope of 0.015 nA mol^{-1} dm^{-3} and an intercept of 0.011 nA. Thus, a detection limit of 1×10^{-6} mol dm^{-3} and a limit of determination of 5×10^{-6} mol dm^{-3} were obtained.

A number of methods for the determination of metronidazole have been described in the literature including the use of HPLC [48,49], capillary zone electrophoresis with amperometric detection [50], flow injection biamperometric detection with on-line photodegredation [51], electrochemical detection at glassy carbon [27] or mercury macroelectrodes [34], and in vivo measurement using a fibre optic sensor [52]. These different methods have been applied to the measurement of metronidazole in pharmaceutical formulations [27,34,51], urine [34,50], plasma [48,49], gastric fluid [49] and cerebrospinal fluid [52]. The reported limits of detection for these methods range from 6×10^{-8} mol dm^{-3} for the HPLC measurement using 1 cm^3 of plasma [48] to around 2×10^{-6} mol dm^{-3} for electrochemical measurement [27]. All of these methods require some degree of sample pre-

treatment. In comparision, the method reported here gives comparable limits of detection but has some potential advantages. First, the measurements using the microelectrode can be carried out in small sample volumes ($\ll 1\,cm^3$) if required. Second, only simple equipment is required and the measurements are rapid. Third, as the preliminary measurements on the urine sample illustrate, the method can be carried out with no, or minimal, prior sample pre-treatment.

4. Conclusion

Our results show that in pH 9 buffer metronidazole adsorbs at the carbon fiber microdisk with an equilibrium constant of $4 \times 10^3\,mol^{-1}\,dm^3$ and a saturation coverage of $0.88 \times 10^{-8}\,mol\,cm^{-2}$. The adsorbed metronidazole is reduced on a cathodic scan at $-0.8\,V$ along with the mass transport limited reduction of metronidazole from solution. From the mass transport limited current at the microdisk we obtain a diffusion coefficient for metronidazole of $5.53 \times 10^{-6}\,cm^2\,s^{-1}$.

Square wave adsorptive stripping voltammetry can be used for low concentration determination of the metronidazole in buffer or untreated urine at the carbon fiber microelectrode with detection limits of 5×10^{-7} and $1 \times 10^{-6}\,mol\,dm^{-3}$, respectively.

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