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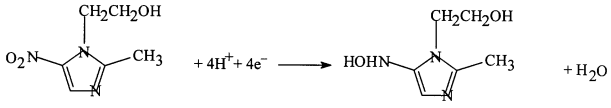
Quantitative assay of metronidazole by capillary zone electrophoresis with amperometric detection at a gold microelectrode

Capillary zone electrophoresis was employed for the determination of metronidazole using end-column amperometric detection with a gold microelectrode at a constant potential of –0.52V *vs.* saturated calomel electrode. To overcome interference of oxygen in the solution, a deaeration injector and a deaeration protector at the detection cell were used. The optimum conditions of separation and detection are 1.0×10^{-3} mol/L potassium dihydrogen citrate ($KH_2C_6H_5O_7$) for the buffer solution, 20 kV for the separation voltage, and 5 kV and 10 S for injection voltage and injection time, respectively. The limit of detection is 6.0×10^{7} mol/L or 0.78 fmole (S/N = 3). The relative standard deviation is 3.9% for the electrophoretic peak current. The method was applied to the determination of metronidazole in human urine.

Keywords: Capillary electrophoresis / Electrochemical detection / Metronidazole EL 3852

1 Introduction

Metronidazole, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole, is a drug widely used in the treatment of trichomonas, amoeboid, and anaerobic infections. Metronidazole has been measured by various assay methods involving the use of chromatographic techniques, such as gas chromatography [1, 2], thin-layer chromatography [3], and high performance liquid chromatography [4–8]. Recently, capillary zone electrophoresis (CZE) has rapidly become a powerful instrumental technique suitable for rapid separation and detection of a numer of complex mixtures [9–11]. The primary strength of CZE is its ability to provide extremely high separation efficiencies in short times and to do so with relatively small samples and simple instrumentation. McGrath *et al.* [12] investigated the capillary zone electrophoretic behavior of metronidazole using a UV-Vis diode fast-scanning detector. The detection limit obtained is 4.66×10^{6} mol/L. Amperometric detection provides excellent sensitivity for the small dimensions associated with CZE, with a degree of selectivity toward electroactive species as well as low cost [13]. In our laboratory this technique has been applied to cysteine [14], glutathione [15], and purine bases [16–18], as well as bovine serum albumin [19] and cytochrome *c* [20]. The theory concerning the current for the end-column amperometric detector in CZE has been investigated [21, 22]. Nevertheless, there are no reports on the determination of metronidazole by CZE with electrochemical detection. Metronidazole which is electrochemically active, can be reduced at the mercury electrode [23]. The reaction is



It can also be reduced at the gold electrode [24]. In principle, it can be determined by CZE with amperometric detection. However, in this case the interference of coexisting oxygen in the solution has to be considered, because it can also be reduced at the same working electrode. Therefore, the buffer has to be deaerated before and during each run for determination of metronidazole by CZE with amperometric detection.

In this article we developed a method to detect metronidazole with end-column amperometric detection at a gold microelectrode. A set of deaeration devices is used at both ends of the separation capillary. The separation was performed in a 20 μm ID fused-silica capillary. The detection was carried out using potentiostatic control of the electrode potential by means of a three-electrode system. The method has been used to determine metronidazole in human urine.

2 Materials and methods

2.1 Apparatus

2.1.1 Cyclic voltammetry

A voltammetric analyzer (Model 83–2.5; Ningde Analytical Instruments, Ningde, China) coupled with an *x-y* recorder

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Abbreviation: SCE, saturated calomel electrode

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(Model 3086–11; Yokogawa Hokuskin, Tokyo, Japan) was used. In connection with a cell, it used potentiostatic control of the electrode potential by means of a three-electrode system, which consisted of a gold microelectrode as the working electrode, a Pt wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. The reference electrode was connected to the analyte *via* a salt bridge filled with the same supporting electrolyte as in the cell.

2.1.2 CZE system

The CZE system without deaeration used here was similar to that described by Jin and Wang [15]. Briefly, the apparatus consisted of a fused-silica capillary of 20 μm ID, 330 μm OD (Yongnian Optical Conductive Fiber Plant, Yongnian, China), which was cut to a length of 30 cm and placed between two buffer reservoirs. A high voltage was applied at the injection end, while the reservoir containing the electrochemical detection cell was held at ground potential. Separations were carried out at an applied voltage of 20 kV with a high-voltage power supply (Model GDY; Shandong Institute of Chemical Engineering and School of Chemistry, Shandong University, China). The electrochemical detection at a constant potential was performed using the end-column amperometric approach with a microcurrent voltammeter (Model 901-pA; Ningde Analytical Instruments). The detection cell and detector were housed in a Faraday cage in order to minimize the interference from external sources of noise. Electrochemical detection was carried out with a three-electrode system, consisting of a gold microelectrode as the working electrode, a coiled Pt wire as the auxiliary electrode, which also served as the ground for the high potential drop (mentioned above) across the capillary, and an SCE as the reference electrode. For a detailed illustration of the electrochemical detection cell see [19]. The CZE system with deaeration is identical to the CZE system without deaeration. A deaeration injector (Fig. 1) and a deaeration protector (Fig. 2) of the detection cell are used. Both were made of plexiglass. For the deaeration injector there is a rubber stopper with two holes on the top of a cubic box, in which the anode and the separation capillary are inserted. In the cube at the center there are two cylindrical reservoirs for the buffer and the sample, respectively. Between the two reservoirs there is a duplicating slide, which is higher than the reservoirs, to prevent the buffer and the sample from spattering on each other while nitrogen is passed through the buffer or the sample. The nitrogen is added to the buffer or sample reservoir, or to the cubic box, at 16 mL/min from different ducts (Fig. 1). As shown in Fig. 2, the deaeration protector is placed on the detection cell. It consists of a ring-shaped bottom and a cup-shaped cover. The nitrogen is added to 84 mL/min from the duct at the bottom and out from a small hole at the top of the cover.

2.1.3 Gold microelectrode

The gold microelectrodes were constructed using a 100 μm diameter, *ca.* 7 cm gold wire, which was inserted carefully through an approximately 4 cm × 0.3 mm ID × 0.8 mm OD glass capillary until it protruded approximately 0.5 cm from the end. Then epoxy resin was applied to seal the fine gold wire to the junction of the glass capillary. The *ca.* 2.5 cm long gold wire at the other end was wound onto a copper lead. Then the gold wire and the copper lead were bound together with a copper wire of *ca.* 400 μm. Finally the copper lead and the glass capillary were bound together using a piece of rubberized fabric in order to protect the electrical junction. Before use, the gold wire was cut to *ca.* 1.5 mm for cyclic voltammetry or *ca.* 0.5 mm for CZE under a microscope. For cyclic voltammetric experiments, a glass tube (1 mm ID × 1 cm OD) was placed outside the glass capillary with the gold wire and the copper lead. The glass capillary was bonded to the glass tube at the two ends of the glass tube using epoxy resin, in order to protect the glass capillary.

2.2 Reagents and solutions

A 1.00×10^{-2} mol/L stock solution of metronidazole was prepared by dissolving an appropriate amount of metronidazole (Jinan Second Pharmaceutical Factory, Jinan, China) in water and then stored at 4°C in a refrigerator. Dilute solutions were obtained by serial dilution of the stock solution. All solutions were prepared with double distilled water. The running buffer consisted of 1.3×10^{-3} mol/L potassium dihydrogen citrate ($KH_2C_6H_5O_7$).

2.3 Procedure

For cyclic voltammetry, the supporting electrolyte was deaerated for 10 min with pure nitrogen in a cell of 1 mL. A certain amount of metronidazole was added to the cell. After passing pure nitrogen through the solution for 3 min, the cyclic voltammogram was recorded. In CZE, the gold microelectrode was cemented onto a microscope slide, which was placed over a homemade XYZ micormanipulator and glued in place such that the end containing the exposed gold protruded from the edge of the slide. The position of the gold microelectrode was adjusted (under a microscope) against the end of the capillary, so that the electrode and the capillary were in contact. This arrangement allowed one to easily remove and realign both the capillary and the electrode. The other end of the capillary

was inserted into a plastic syringe tip (the metal needle was previously removed) and glued in place with a small amount of epoxy glue. Before each run, the capillaries were flushed with double distilled water, 0.1 mol/L NaOH, double distilled water, and the corresponding separation electrolyte, by means of a syringe. In addition, the electrolyte solution in the electrochemical cell was also replaced before each run. If deoxygenation was required, prior to the run, the separation electrolyte (buffer or sample) was deaerated by bubbling pure nitrogen from the nitrogen ducts shown in Fig. 1, part 8 or 9, through the solution for 60 min, until the current was no longer reduced. Then pure nitrogen from the nitrogen duct shown in Fig. 1, part 10, was passed through the cubic box instead of the deaeration solution. At the same time, nitrogen was passed through the deaeration protector for the detection cell shown in Fig. 2. The arrangement of the detection cell was previously illustrated [19] in detail. During the experiments the separation voltage was applied across the capillary, the detection potential was applied at the working electrode, and the deaeration injector and the detection cell were under nitrogen atmosphere by means of passing nitrogen. After the electroosmotic current reached a constant value (after 20 min), the electromigration injection was carried out and the electropherogram was recorded. The separation electrolyte in the capillary was replaced after 5 or 6 runs. All potentials were measured *vs.* SCE.

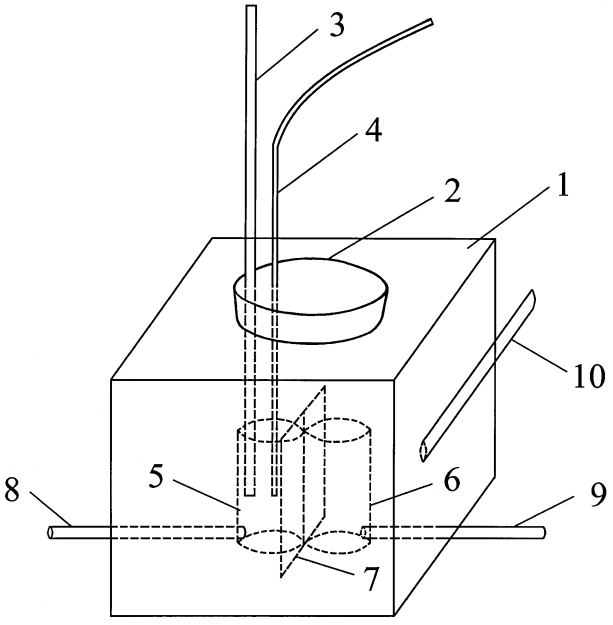

Figure 1. Schematic diagram of the deaeration injector. 1, cubic box; 2, rubber stopper; 3, anode; 4, separation capillary; 5, buffer reservoir; 6, sample reservoir; 7, duplicating slide; 8, nitrogen duct for buffer; 9, nitrogen duct for sample; 10, nitrogen duct for nitrogen atmosphere.

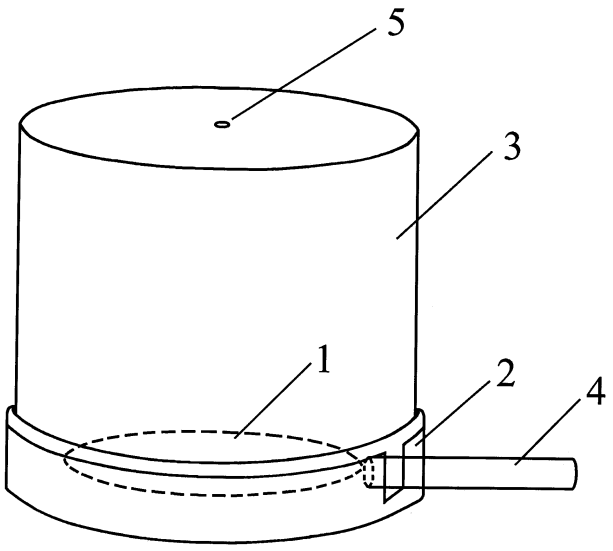

Figure 2. Schematic diagram of the deaeration protector for detection cell. 1, detection cell; 2, ring-shaped bottom; 3, cup-shaped cover; 4, nitrogen duct; 5, hole.

3 Results and discussion

3.1 Cyclic voltammogram of metronidazole

A typical cyclic voltammogram of metronidazole in 0.2 mol/L $KH_2C_6H_5O_7$ with a scan rate of 60 mV/s is shown in Fig. 3. It was found that metronidazole can also be reduced at the gold electrode in this buffer. A reduction peak of metronidazole at *ca.* –0.40 V is observed. However, there is no oxidation peak in the cyclic voltammogram. This means that the reaction of metronidazole at the gold electrode is irreversible.

3.2 Effect of oxygen

Table 1 lists the steady-state current of oxygen, i_s, at different detected potentials, E_d. The more negative E_d is, the higher i_s is. Table 2 lists i_s at different deaeration times, t_d; i_s decreases with increasing t_d. After deaeration for 60 min i_s drops to 1/12 of the original current (from 220 nA to 18 nA). These results indicate that the deaeration at both the injection end and the detection end is effective.

3.3 Optimum conditions of CZE with end-column amperometric detection

The effect of the buffer concentration, C_B, on the migration time, t_m, the peak current, i_p, the width at the half-peak, $W_{1/2}$, and the number of theoretical plates, N, in $KH_2C_6H_5O_7$ is listed in Table 3. N was calculated according to the following equation:

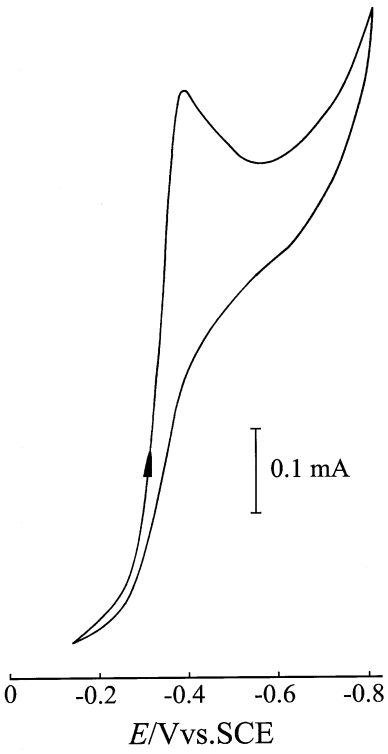

Figure 3. A typical cyclic voltammogram of 2.34×10^{-3} mol/L metronidazole at the gold electrode in 0.2 mol/L $KH_2C_6H_5O_7$. $v = 60$ mV/S.

Table 1. Steady-state current of oxygen at different detected potentials

E_d(V)	–0.35	–0.40	–0.45	–0.58	–0.52	–0.55	–058	–0.61	–0.65
i_s(nA)	165	175	185	190	220	240	260	270	285

1.0×10^{-3} mol/L $KH_2C_6H_5O_7$, capillary, 30 cm, 20 μm ID; separation voltage, 20 kV; detection potential, –0.52 V

Table 2. Steady-state current of oxygen at different deaeration times.

t_d (min)	0	10	20	30	40	50	60
i_s(nA)	220	119	71	47	29	20	18

Conditions as in Table 1

Table 3. t_m, i_p, $W_{1/2}$ and N at different C_B

10^3 C_B (mol/L)	t_m (s)	i_p (nA)	$W_{1/2}$ (s)	$\times 10^{-4}$ N
3.0	127	15.5	1.8	2.76
6.0	151	25.4	1.9	3.50
9.0	180	26.5	1.9	4.98
15.0	260	25.1	2.1	8.49
24.0	334	19.5	2.1	14.0
30.0	372	19.0	2.2	15.8

1.0×10^{-3} mol/L $KH_2C_6H_5O_7$; injection voltage 5 kV; injection time, 10 s; other conditions as in Table 1

$$N = 5.54\left(\frac{t_m}{W_{1/2}}\right)^2 \quad (1)$$

where t_m, $W_{1/2}$, and N increase with increasing C_B. The migration velocity of the substance depends mainly on the electroosmotic velocity, v_{eo}, of the buffer, which is proportional to the ζ potential [25]. With increasing buffer concentration, the thickness of the electric double layer becomes thinner and the ζ potential becomes smaller. Therefore, v_{eo} decreases and t_m increases. The N increases because t_m increases and $W_{1/2}$ changes very little with increasing buffer concentration (see Eq. 1). There is a highest value of i_p at around $C_B = 9.0 \times 10^{-3}$ mol/L. In our experiments 1.0×10^{-2} mol/L $KH_2C_6H_5O_7$ was used because of the higher i_p. Figure 4 shows the relationship between i_p and E_d. When E_d is between –0.35 and –0.52 V, i_p increases with increasing E_d. When E_d is more negative than – 0.52 V, i_p decreases rapidly. Therefore, E_d of –0.52 V is suitable for the detection because of the high peak current. Figure 5 shows the typical electropherograms of 1.00×10^{-5} mol/L and 1.00×10^{-6} md/L standard solutions of metronidazole at the optimum conditions. Small peak width and little tailing of the peak were obtained.

3.4 Linear range, limit of detection and reproducibility

The logarithmic relationship between the peak current and the concentration of metronidazole, with and without

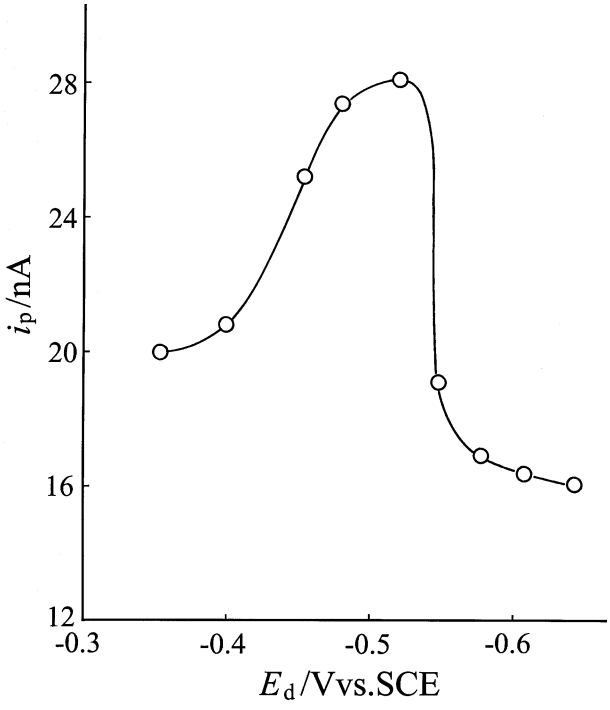

Figure 4. Relationship between detected peak current and detection potential. Other conditions as in Table 3.

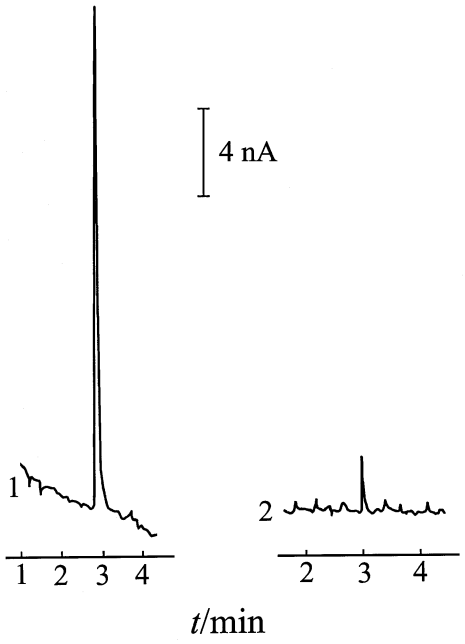

Figure 5. Typical electropherograms of metronidazole. Concentration of metronidazole: (1) 1.00×10^{-5} mol/L; (2), 1.00×10^{-6} mol/L. Conditions as in Table 3.

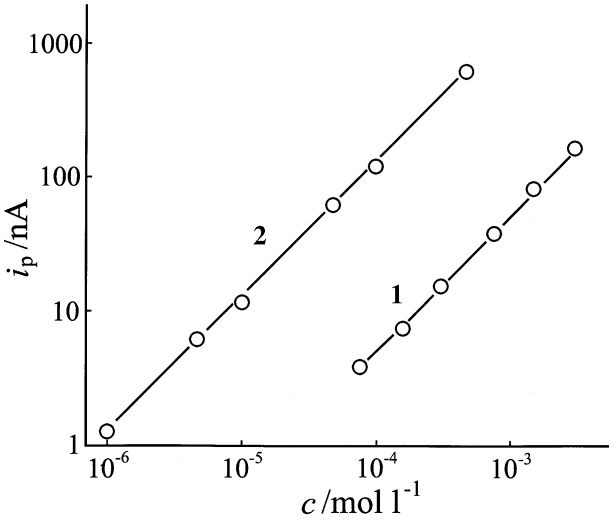

Figure 6. The relationship between the peak current and the concentration of metronidazole. (1) Without deaeration; (2) with deaeration. Conditions as in Table 3.

deaeration is shown in Fig. 6. A linear relationship holds between the peak current detected and concentration in the range of 7.50×10^{-5} to 3.00×10^{-3} mol/L for no deaeration and 1.00×10^{-6} to 5.00×10^{-4} mol/L for deaeration. Least-squares treatment of these data yields a slope of 52.6 pA $\mu mol^{-1}L$ and a correlation coefficient of 0.9998 for no deaeration, and a slope of 1.23 nA $\mu mol^{-1}L$ and a correlation coefficient of 1.000 for deaeration. Although there is a linear relationship between the peak current and the concentration for both situations, the concentration of metronidazole at the low end in the linear range for deaeration is much lower than for deaeration. The limit of detection is 3.8×10^{-5} mol/L or 49 fmole for no deaeration and 6.0×10^{-7} mol/L or 0.78 fmole for deaeration (according to S/N = 3). The detection limit under deaeration conditions is 1/100 of that under no deaeration. The response for a series of six injections of 5.00×10^{-4} mol/L metronidazole resulted in a relative standard deviation of 3.9% for i_p.

3.5 Determination of metronidazole in human urine

A synthetic human urine sample containing 6.00×10^{-5} mol/L metronidazole, which consisted of urine from an adult volunteer and the standard metronidazole, was used to verify the possibility of the standard addition method. After the sample solution of 1.0 mL was diluted to 1 mL, it was injected into the CZE-electrochemical system. The electropherograms of human urine sample with and without the standard solution of metronidazole are shown in Fig. 7. The concentration of metronidazole in the human urine sample obtained by the standard addition method is 6.05×10^{-5} mol/L, which agrees with the value in the human urine sample. The recovery is about 93%.

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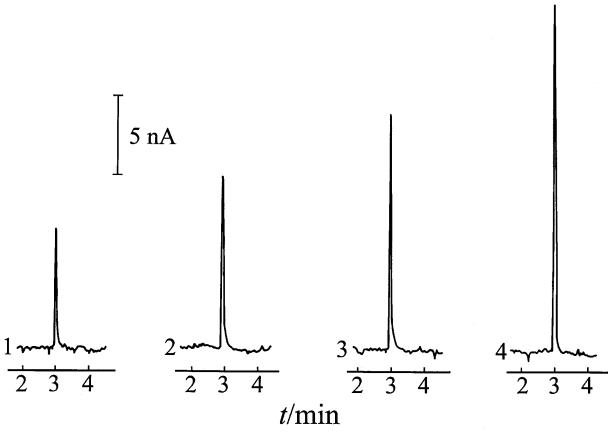

Figure 7. Electropherograms of metronidazole in the sample of human urine. Concentration of metronidazole (mol/L): (1) sample; (2) sample + 3.00×10^{-6}; (3) sample + 6.00×10^{-6}; (4) sample + 1.20×10^{-5}. Conditions as in Table 3.

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