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Short Communication

D.C. polarography for mebendazole analysis

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Introduction

Mebendazole, (5-benzoyl-1H-benzimidazol-2-yl)-carbamic acid methyl ester, is a highly effective agent against a wide range of human gastrointestinal helminth pathogens. Recent encouraging clinical observations on the oral chemotherapy of echinococcosis with chronic administration of mebendazole [1–5] have led to considerable interest in this drug. Relatively large amounts of mebendazole can be determined by non-aqueous titrimetry [6], while for smaller amounts UV spectroscopy [6] and HPLC [7–9] have been proposed. Since the polarographic behaviour of mebendazole has not been reported, we describe here the d.c. polarographic reduction of the drug at the dropping mercury electrode, and the optimum conditions for its quantitation in tablet dosage form.

Experimental

Reagents and chemicals

Mebendazole and two known human urinary metabolites [8, 10], 2-amino-5-benzoyl-1H-benzimidazole (I) and [5-(α-hydroxybenzyl)-1H-benzimidazol-2-yl]-carbamic acid methyl ester (II), were used as received from Janssen Pharmaceutica, Belgium. Mebendazole tablets were purchased in pharmacies. All other chemicals were analytical reagent grade. A pH 2.6 McIlvaine buffer was prepared by mixing 2.18 vol. 0.2 M sodium phosphate with 17.82 vol. 0.1 M citric acid. Freshly prepared stock solutions of mebendazole were prepared by dissolving 25 mg of the drug in 4.3 ml of 70% perchloric acid in a 50 ml volumetric flask and diluting to volume with doubly-distilled water. Formic acid, 10 ml, can be used instead of 70% perchloric acid.

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Apparatus

Measurements in the sampled d.c. mode were performed with a Metrohm E 506 recording polarograph equipped with a polarography stand (Metrohm E 505). Three-electrode operation was employed with a dropping mercury electrode, a platinum wire auxiliary electrode and a silver–silver chloride reference electrode (Metrohm EA 427). A forced drop time of 1 s and a scan-rate of 4 mV/s were used. Dissolved oxygen was removed by bubbling oxygen-free nitrogen through the cell for 15 min and passing it over the solution during the electrolysis. Cyclic voltammetry experiments were performed with a Tacussel modular polarographic apparatus and three-electrode operation. A Metrohm EA 290 hanging mercury drop system was used as working electrode. The number of electrons involved in the reduction was calculated at a mebendazole concentration of 1×10^{-4}M using a solid-state controlled-potential coulometric apparatus constructed in our laboratory. The experiments were carried out in the absence of air in a 5 ml cell (Metrohm EA 875-5) with a mercury pool as the working electrode and a fixed potential of −0.9 V vs Ag/AgCl.

Procedure

A calibration graph was prepared (average of four determinations) by pipetting, with a 5 ml piston microburette, appropriate aliquots of mebendazole stock solution into a series of 50 ml volumetric flasks to achieve final concentrations of 5–50 μg/ml, adding sufficient 1M perchloric acid to make the volume 5 ml, and diluting to volume with the McIlvaine buffer. The pH of these working standard solutions was 1.2. About 20 ml of solution was transferred into the polarographic cell and after deaeration it was polarographed at 25°C at a starting potential of −0.6 V with a sensitivity scale typically set at 2.5 nA/mm.

Tablet analysis

Six tablets were weighed (about 0.3 g) and finely ground. According to the weight per tablet and the labelled potency (100 mg mebendazole), a quantity of the powder equivalent to 50 mg of mebendazole was transferred into a 100 ml volumetric flask. Then 8.6 ml of 70% perchloric acid was added and the mixture stirred for 10 min and diluted to volume with water. The suspension was filtered under suction through a fine porosity glass crucible. A 2 ml portion of the filtrate was transferred to a 50 ml volumetric flask, 3 ml of 1 M perchloric acid was added and the solution diluted to volume with the specified buffer. A 20 ml aliquot of this solution (corresponding to 0.4 mg of mebendazole) was transferred into the polarographic cell. Deaeration and polarography were performed as previously described, using the standard addition technique (addition of 0.5 ml of a standard mebendazole solution prepared by dissolving 40 mg of drug in 4.3 ml of 70% perchloric acid and making up to volume with water in a 50 ml volumetric flask) [11].

Results

The d.c. polarographic assay provided precise and accurate mebendazole determinations, in pure solution or in tablet dosage form, in the concentration range 5–50 μg/ml. Analyses were performed within a few minutes and with low-cost apparatus. The calibration graph obtained for the above concentration range was rectilinear (slope = 10.06, intercept = 0.12, r = 0.9999) with an average recovery (8 determinations) at the 20 μg/ml level of 100.8 ± 1.6%.

Commercial tablets were assayed by the method of standard additions (Fig. 1b) without interference from labelled excipients (microcrystalline cellulose, talc, rice starch, magnesium stearate, hydrogenated vegetable oil, sodium saccharin, sodium lauryl sulphate, colloidal silicon dioxide and sunset yellow FCF). The temperature coefficient was less than 1.5%/degree, so no thermostatting of the cell was necessary, all solutions being analysed at room temperature. On six samples of the same lot the average recovery was 100.1 ± 1.0%. A comparative spectrophotometric determination [6] gave an average recovery of 101.0%.

Discussion

Mebendazole is very sparingly soluble in water and in common organic solvents. Stable solutions can be obtained in perchloric, formic or phosphoric acids at pH values lower than 2.3. To obtain controlled pH values in the presence of perchloric or formic acid, a McIlvaine buffer was selected as supporting electrolyte. Polarograms recorded in these conditions exhibited a single well defined d.c. reduction wave (Fig. 1a). Preliminary experiments showed that a catalytic wave was obtained with a supporting electrolyte containing nitrate. The limiting current was independent of pH in the range 0.9–2.3 and little influenced by changes of buffer concentration. These considerations and the constancy of the products $i_d\ C^{-1}$ (in the concentration range 1.7×10^{-5} to 1.7×10^{-4}M) and $i_d\ h^{-1/2}$, with a temperature coefficient of 1.36%/degree (over the range 18–42°C), indicated that the wave was diffusion controlled.

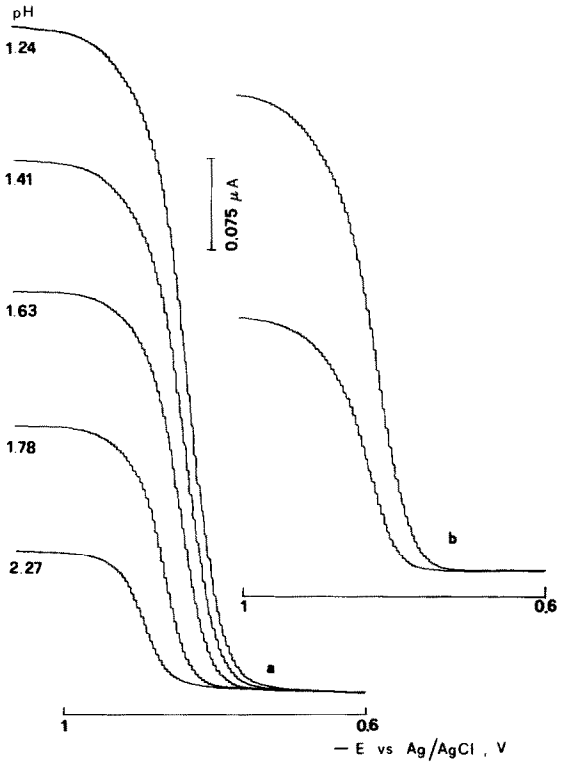


Figure 1
Polarographic analysis of mebendazole. (a) Polarograms of pure samples (1, 2, 3, 4 and 5 mg/100 ml) at various pH values in citric acid–sodium phosphate buffer containing different amounts of perchloric acid. (b) Polarogram of tablet assay before and after standard addition.

The number of electrons consumed can be estimated from data of wave height and the use of Ilkovic equation [12], $n = I/607\ D^{1/2}$; since the diffusion current constant I for the mebendazole wave was 2.95 μA $mmol^{-1}l\ mg^{-2/3}\ s^{1/2}$, and assuming a typical value of $6 \times 10^{-6} cm^2/s$ for D, the wave could represent a two-electron reduction. This was confirmed by coulometric reduction of mebendazole at controlled potential, which gave $n = 2.10$ at pH 1.2.

The cathodic wave was irreversible on the basis of several criteria: this was confirmed by cyclic voltammetry experiments (Fig. 2) in which no anodic peak was observed at any scan rate in the potential range studied.

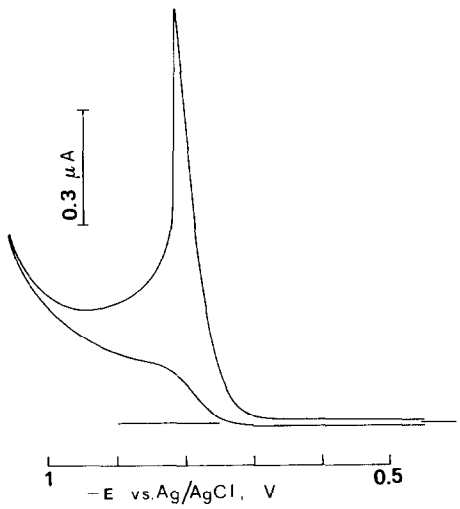


Figure 2
Cyclic voltammogram of 1×10^{-4}M mebendazole at pH 1.24 in citric acid–sodium phosphate buffer in the presence of perchloric acid. Scan-rate 15 mV/s.

A moderate positive shift of the half-wave potential with increasing mebendazole concentration (from −0.851 V at 0.169×10^{-4}M to −0.835 V at 1.69×10^{-4}M, pH 1.19) seemed to be related to an irreversible charge transfer with adsorption of reactant [13]. Similarly, the electrocapillary curve of the supporting electrolyte in the presence of mebendazole showed a large decrease in the drop-time over a considerable potential range and the characteristic shape of a major reactant adsorption with respect to the product (Fig. 3).

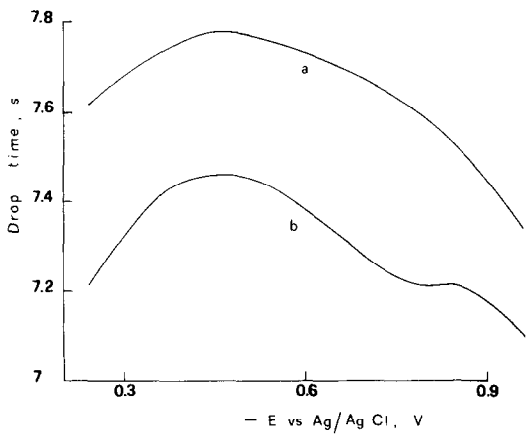
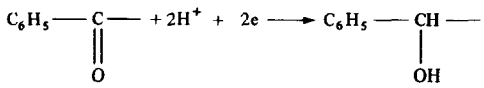


Figure 3
Electrocapillary curves at pH 1.24 of the supporting electrolyte (citric acid–sodium phosphate buffer and perchloric acid) in the absence (a) and in the presence (b) of 1.7×10^{-4}M mebendazole.

The shift of the half-wave potential to more negative values with increasing pH (−0.832 V at pH 0.94, −0.898 V at pH 2.19, see also Fig. 1a) suggested that hydrogen ions were consumed in the reduction. The polarographic wave is probably due to the reduction of the benzoyl group:



This interpretation agrees with the polarographic behaviour of two mebendazole metabolites (Fig. 4). Only mebendazole and its decarbamated metabolite (I) exhibited two-electron reduction waves, while the unconjugated metabolite (II) was not polarographically reducible.

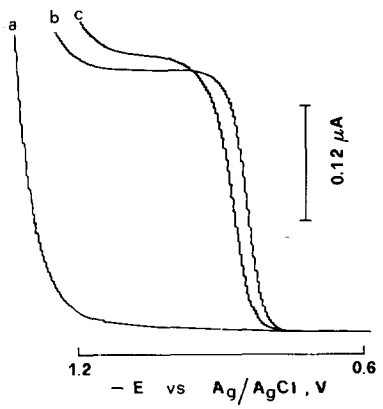


Figure 4
Polarograms of 7.3×10^{-5}M metabolite (II): (a) 1.0×10^{-4}M mebendazole: (b) and 1.0×10^{-4}M metabolite (I): (c) at pH 2.13 in citric acid–sodium phosphate buffer in the presence of formic acid.

Other preliminary experiments carried out in the differential-pulse mode indicated that mebendazole analysis could be performed with a limit of detection of 100 ng/ml; however, this limit compares unfavourably with that (10–20 ng/ml) described for HPLC determinations [8, 9]. On the other hand the HPLC method has recently been criticized [10] for its lack of sensitivity and the laborious extraction procedures necessary in the determination of the low plasma or tissue mebendazole levels after therapy. Only radioimmunoassay procedures [10, 14] seem to be capable of giving sufficient sensitivity for pharmacokinetic studies of mebendazole in animals and in man.

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