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Electrochemical reduction and voltammetric determination of Metronidazole Benzoate on carbon paste electrode.

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ABSTRACT

Simple and sensitive electrochemical method for the determination of metronidazole benzoate, based on a carbon paste electrode (CPE), is described. Metronidazole benzoate yields a well defined reduction peak whose potential is −0.81V at the CPE in pH 3.0 BrittonRobinson buffer. Compared with bare and modified glassy carbon electrodes (GCE), the Carbon paste electrode (CPE) significantly enhances the reduction peak current of metronidazole benzoate. All the experimental parameters were optimized for the determination of metronidazole benzoate. The detection limit is 6×10−9 mol/l at 2 min accumulation. This method has been successfully used to determine metronidazole benzoate in the drugs. The recovery was found in the range from 99.56% to 100.26%. The relative standard deviation was found in the range from 0.429% to 0.845%. The proposed method possesses high sensitivity, accuracy and rapid response. Finally, this method was successfully used to determine metronidazole benzoate in tablets was described. Furthermore, results obtained by the proposed method have been compared with high performance liquid chromatographic method.

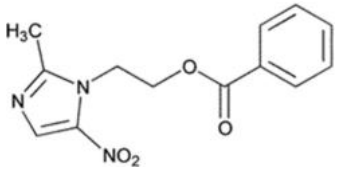
Keywords : Carbon paste electrode, Metronidazole benzoate, Differential pulse voltammetry.

Introduction

Metronidazole benzoate (MDB), which is a nitroimdazole derivative is selectively toxic to anaerobic bacterial and protozoarium. Metronidazole benzoate, 1-(2-benzyloxy ethyl) -5-nitro methyl imidazole, is one of the cytostatic drugs well known for its antimicrobial properties. It has been widely used for the treatment of protozoal diseases, including trichomoniasis, giardiasis and amoebiasis [1,2]. The chemical structure of metronidazole benzoate 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 [3]. In the absence of oxygen, the reduction process for nitroimidazoles is similar to that for nitrobenzene [4]. The cytotoxicity of metronidazole benzoate is due to intermediates formed during the reduction [5 -7] and voltammetric techniques have been applied to investigate the mechanism of action of nitroimidazoles as antimicrobial agents [8], for their determination in pharmaceutical [9-14] and clinical [15,16] matrices, and at DNA modified electrode surfaces [17-20]. The mercury electrode is the most widely used, but good results were obtained using solid electrodes when studying nitrobenzene [21,22] and metronidazole benzoate [19,20]. Metronidazole benzoate has been determined by spectrophotometry [23,24], gas chromatography [25] and high performance liquid chromatography [26].

From the electrochemical point of view, several works on the electro reduction of metronidazole derivative have been published. For example, polarographic determination of metronidazole benzoate with a detection limit of 1×10^{-6} mol/l has been proposed [27]. Otherwise, electrochemical reduction at mercury-free electrode such as DNA-modified glassy carbon electrode (GCE) [28], activated glassy carbon electrode[29], nanomaterial thin film coated glassy carbon electrode [30], carbon fiber microdisk electrode [31] has also been reported. However, the sensitivity of these developed methods is poor since the detection limit is about 10^{-7} mol/l.The aim of this work was to study the electro chemical behavior of Metronidazole benzoate at carbon paste electrode (CPE). It is found that metronidazole benzoate yields a very sensitive and well defined reduction peak at the CPE. Compared with the bare GCE, CPE shows significant enhancement effect on the reduction peak of metronidazole benzoate. A new procedure has been proposed for determining metronidazole benzoate and it possesses following advantages such as low detection limit, rapid response, excellent reproducibility and low cost. The development of this method is used for the direct determination of metronidazole benzoate in pharmaceutical tablets.

Figure 1 Structure of Metronidazole benzoate



Experimental

Apparatus

Voltammetric measurements were performed using CH instrument, USA (model 1100A series and 760C) electrochemical analyzer interfaced to a PC. The three-electrode system consisted of a carbon paste working electrode, Ag/AgCl(3M KCl) reference electrode and a Pt auxiliary electrode. Before each measurement, the carbon paste electrode was polished with the tissue paper and refilled the exposed portion with fresh paste of carbon . All the solutions examined were carried out at room temperature 25 ± 2° C. Operating conditions for the DPV were: pulse amplitude, 125mV; pulse width, 50 ms; scan rate, 20mV$s^{-1.}$ The pH was measured using a Schott Gerate pH meter (CG804 model).

Reagents

Pure Metronidazole benzoate (MDB) was obtained as a gift sample and the Metronidazole benzoate in tablet form were purchased from a local pharmacy. Other chemicals, all of analytical-reagent grade (Merck) were used. The stock solution of MDB (1×10^{-3} M) was prepared in DMF. The Britton Robinson buffer were prepared by using 0.04 mol L^{-1} of Boric acid, Ortho phosphoric acid and acetic acid and the pH were adjusted by 0.2 mol L^{-1} sodium hydroxide and used as supporting electrolyte [32]. The diluted solutions were prepared daily by accurate dilution with pH 4 BR- buffer solution before use and should be protected from light. All solutions were kept in the dark in a refrigerator and used within several hours to avoid hydrolysis. MDB solutions were stable and their concentration did not change with time. All measurements were carried out at ambient temperature of the laboratory (2327 °C).

Tablet assay procedure

Ten tablets were weighed accurately and finely powdered. A portion of the powder, equivalent to 1×10^{-3}M MDB, was transferred to a 100 ml volumetric flask and completed to volume with methanol and sonicated for15 min.

Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with selected supporting electrolyte. The nominal content of the tablet amounts were calculated from the corresponding regression equations of previously plotted calibration plots.

To study the accuracy of the proposed method and check the possible interferences from common excipients, recovery studies were carried out. For these experiments, known amount of the pure drug were added to the earlier analyzed tablet formulation of MDB. The recovery of the drug was calculated using the corresponding regression equations of previously plotted calibration plots.

Results and discussion

Electrochemical reduction behavior of MDB at carbon paste electrode.

The reduction of nitroimidazole is a complex process in which the nitro group can receive up to six electrons until complete reduction to corresponding amine [3]. Under anaerobic conditions or low oxygen pressure, the reduction of nitroimidazole occurs in a similar way to the reduction of nitrobenzene. The formation of the nitro derivatives (R-NO) and hydroxylamine (R-NHOH) requires a total of four electrons and four protons [33]. In the protonated form, reduction of the hydroxylamine to the corresponding amine requires two electrons. The electrochemical behavior of metronidazole benzoate at CPE have been examined using cyclic voltammetry at scan rate of 100 mV/s in 0.04mol L^{-1} Britton-Robinson buffer. Fig. 2 shows the cyclic voltammogram of 1 $x10^{-3}$mol/l metronidazole benzoate in BR buffer at a pre-treated carbon paste electrode. For the metronidazole benzoate, it is found that the pre-treatment of the electrode surface led to a sensitive and well defined reduction peak appears at -0.9V during the first cathodic sweep from 0 to -1.8V. At acidic condition, peak current and peak shape were evident but at neutral medium peak response was low. In basic medium, the peak was broadened. The background current was recorded for all pH range and subtracted properly in calculating the peak currents and the peak potential. Current of well defined cathodic peaks noticed in the cyclic voltammogram were considered for the study of effect of pH. Fig 3 shows the variation of peak potential with variation of pH from 2 to 9. Metronidazole benzoate exhibited maximum peak current response at pH 3.0

Fig. 2 Cyclic voltammogram of $1x10^{-3}$ mol L^{-1} metronidazole benzoate in 0.04 mol L^{-1} Britton-Robinson buffer

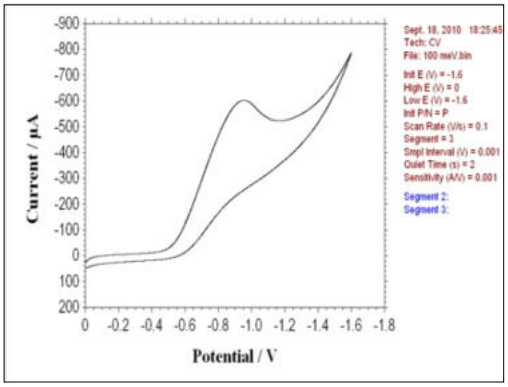
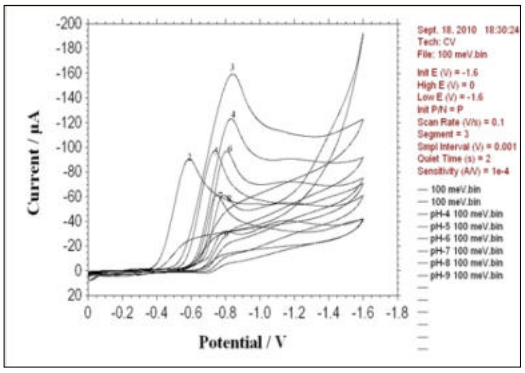


Fig -3: Cyclic voltammograms of 1 x 10^{-3} molL^{-1} metronidazole benzoate in BR buffer at various pH ranges from 2 9 at the CPE surface.

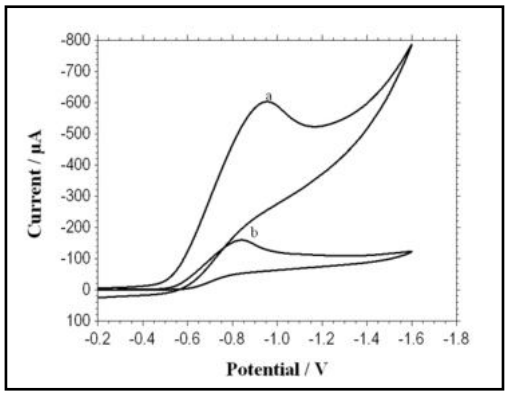


Moreover, the electrochemical behavior of metronidazole benzoate at different scan rates from 10 to 300mV/s were investigated by using CV. Only a reduction peak appears even at 10mV/s, and no corresponding oxidation peak is observed on the reverse scan. This suggest that the electrode reaction of metronidazole benzoate is totally irreversible. The reduction peak current is proportional to the scan rate, indicating that the reduction of metronidazole benzoate at the carbon paste electrode is adsorption control.

The reduction peak current in the second cyclic sweep decreases remarkably compared with that of the first cyclic sweep. After second cyclic sweep, the peak current decreases slightly and finally almost maintains unchangeable. This phenomenon is may be caused by the fact that the adsorption of metronidazole benzoate or its reductive product occurs at the electrode surface and therefore, inactivate the electrode surface.

The reduction of metronidazole benzoate at two different electrodes, i.e., bare GCE, and CPE, were compared by CV. It can be found that 5×10^{-7} mol/l metronidazole benzoate has less sensitive reduction signal at bare GCE . However, under the identical conditions, metronidazole benzoate yields a very sensitive reduction peak at CPE. The reduction peak potential is at -0.81V. Fig. 4 shows the remarkable enhancement in the reduction peak current is undoubtedly attributed to excellent adsorptive ability of carbon paste electrode.

Figure 4 : Cyclic voltammograms of 1 x 10^{-3} molL^{-1} metronidazole benzoate in pH-3- BR buffer at a) Bare CPE surface b) Bare GCE surface.



Effect of supporting electrolytes.

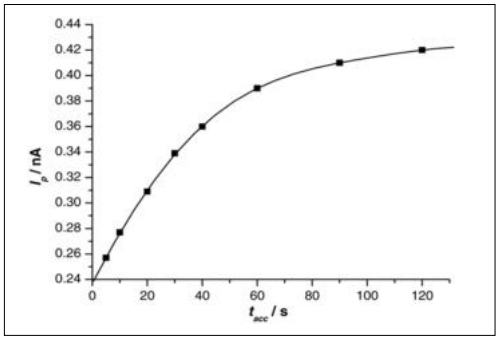
The electrochemical reduction of 5×10^{-7} mol/l metronidazole in various medium such as pH 5.08.0 phosphate buffer, pH 2.010.0 BrittonRobinson buffer (each 0.1 mol/l), were studied by CV. The excellent reduction response was obtained in pH 3.0 BrittonRobinson buffer for metronidazole benzoate since the peak current is highest and the peak shape is well-defined.In 0.1 mol/l BrittonRobinson buffer, the influences of pH on the reduction peak potential were examined by Differential pulse voltammetry (DPV). The reduction peak potential (*E*pc) shifts positively as pH from 10.0 down to 2.0. It is found that *E*pc obeys the following equations, *E*pc =−0.197 − 0.057pH (*r* = 0.998). The slopes of 57 mV/pH mean that equal electrons and protons involves in the electro reduction of metronidazole benzoate.

Accumulation behavior of metronidazole benzoate.

The reduction peak current of 1 × 10^{-7} mol/l metronidazole benzoate was compared after 2 min accumulation under different potential by DPV. The peak current almost kept unchangeable as accumulation potential shifting from 0.20 to −0.50V, revealing that the accumulation potential has no influence on the reduction peak current of metronidazole benzoate at the CPE.

Fig. 5 shows the effects of accumulation time on the reduction peak current of 1×10^{-7} mol/l metronidazole benzoate. The stripping peak current increases greatly within the first 2 min and then levels off, suggesting that the accumulation of metronidazole benzoate is very rapid to reach saturation at the CPE.

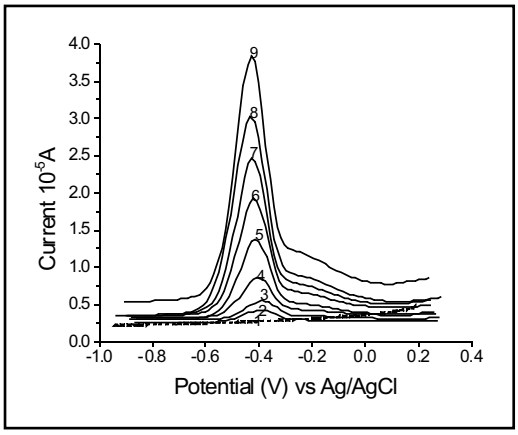
Fig. 5. Influences of accumulation time on the reduction peak current Of 1 × 10^{-7} mol/l metronidazole at CPE.



Calibration graph

The calibration curve for metronidazole benzoate in pH 3.0 BrittonRobinson buffer was measured by differential pulse voltammetry (DPV). The best parameters on the CPE are accumulation time = 2 min; pulse amplitude = 50mV, scan rate = 20 mV/s, pulse width = 50 ms. The DP voltammograms of various concentrations of metronidazole are illustrated in Fig. 6, and show good linearity. The linear segment increases from 6 × 10^{-9} to 1 × 10^{-5} mol/l (*r* = 0.997) with a regression equation of *i*p = 0.04 + 6.56 × 10^{6}C (*r* = 0.998, *C* in mol/l, *i*p in A). It is found that this method can detect 6×10^{-9} mol/l metronidazole benzoate after 2 min accumulation via experiment. The relative standard deviation (R.S.D.) of 4.8% for 1 × 10^{-7} mol/l metronidazole benzoate (*n* = 10) showed good reproducibility.

Fig. 6. DP voltammograms of different concentrations of metronidazol Benzoate in pH 3.0 BrittonRobinson buffer: (1) Blank ; (2) 6 × 10^{-9} ; (3) 2.5× 10^{-8}; (4) 7.5 x 10^{-8}; (5) 1.5×10^{-7}; (6) 3×10^{-7}; (7) 6×10^{-7}; (8) 1.2×10^{-6}; and (9) 1×10^{-5}



The long-term stability of the CPE was evaluated by measuring the current responses at a fixed metronidazole benzoate concentration of 1×10^{-7} mol/l over a period of 4 weeks. The CPE was used daily and stored in the air. The experimental results indicated that the current responses deviated only 5.3%, revealing that the CPE possesses long-term stability.

Analysis of metronidazole benzoate in drugs.

On the basis of above results, the DPV technique is applied to the direct determination of metronidazole benzoate in pharmaceutical dosage forms, using the related calibration straight lines. The proposed procedure was successfully applied to the analysis of three pharmaceutical products without the need for any pretreatment or extraction steps prior to the analysis.

The average mass of five tablets was determined and finely powdered, then the required amount of sample to prepare a solution of 10^{-3} mol/l was transferred into a 100 ml standard flask containing 80 ml of BrittonRobinson buffer (pH 3.0). The contents of flask were stirred magnetically for 15 min and then diluted to volume with the same supporting electrolyte. The solution was filtered and the first 20 ml of the filtrate was removed. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrate and diluting them with supporting electrolyte mentioned above. The accuracy of the method was determined by its recovery during spiked experiments. Recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulation of metronidazole benzoate. There is no any pharmacopoeia method related to tablet dosage form or pure drugs of metronidazole benzoate . Measurements of peak currents under above mentioned conditions were successfully applied, the determination of metronidazole benzoate in spiked tablets and no interference by excipients were observed. However, the accuracy of the proposed methods was determined by its recovery during spiked experiments. In order to detect interactions of excipients in this method, the standard addition technique was applied to the same preparations, which were

analyzed by calibration curve. The mean percentage recovery of metronidazole benzoate , based on the average of five replicate measurements, was favorably compared (Table 1) with that obtained by assay of the same tablets solution by means of a reported HPLC method [26]. The results reveal that the method had adequate precision and accuracy and consequently can be applied to the determination of metronidazole benzoate in pharmaceutical formulations without any interference from the excipients. There was no need for any precipitation, evaporation or extraction step priors to the drug assay.

Conclusion

Our results show that by utilizing the unique property of carbon paste electrode, such as high specific surface area, strong adsorptive ability, a CPE was described for the determination of metronidazole benzoate. The principal advantage of DPV techniques over the other techniques are that they may be applied directly to the analysis of pharmaceutical dosage forms and biological samples without the need for extensive sample preparation, since there was no interference from the excipients and endogenous substances. The proposed methods are rapid, requiring less than 5 min to run a sample and do not include time consuming extraction steps. The CPE shows highly effective accumulation to metronidazole benzoate. As a conclusion, results obtain in the determination of metronidazole benzoate using CPE showed a significant improvement in sensitivity and its possibilities of application to pharmaceuticals.

Table 1 : Determination of metronidazole benzoate in tablet samples by the proposed electrochemical method

Pharmaceutical product	Labeled amount mg / Tablet	DPV			Reference method HPLC		
		Found (mg / tablet)	Recovery (%)	RSD (%)	Found (mg / tablet)	Recovery (%)	RSD (%)
Brand – A	400	414.51 ± 2.788	103.63	0.673	404.14 ± 1.708	101.03	0.423
Brand – B	400	413.78 ± 1.666	103.45	0.403	404.97 ± 2.654	101.24	0.655
Brand – C	400	394.65 ± 1.550	98.66	0.393	392.39 ± 2.331	98.29	0.594
Mean = Standard deviation (n=6) RSD – relative standard deviation.							

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