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Square-wave adsorptive cathodic stripping voltammetry of pantoprazole

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Abstract

Adsorption and reduction of pantoprazole were investigated by cyclic and square-wave voltammetry on a hanging mercury drop electrode in Britton–Robinson buffers at pH 2.0–11.0. The reduction process gave rise to a single peak within the entire pH range. Study of the variation of the reduction signal with solution variables such as pH and concentration of pantoprazole and instrumental variables such as accumulation time and potential, frequency, pulse height and pulse amplitude, has resulted in optimization of the reduction signal for analytical purposes. The voltammetric procedure was applied successfully to give a rapid and precise assay of pantoprazole in a tablet dosage form.

Keywords: Stripping voltammetry; Square-wave; Pantoprazole; Dosage form

1. Introduction

Pantoprazole, 5-(difluoromethoxy)-2-{[(3,4-dimethoxy-2-pyridyl)methyl]sulfinyl}-1*H*-benzimidazole (Scheme 1), is a new proton pump inhibitor (PPI). Like other PPIs, pantoprazole acts by inhibiting H^+/K^+-adenosine triphosphatase, the proton pump, which is the terminal step in acid secretion by parietal cells of the gastric mucosa [1–4]. PPIs are substituted benzimidazoles, which accumulate in the highly acidic environment of the parietal cell and are activated by conversion to cyclic sulfenamides [3,4]. The activated sulfenamides subsequently inactivate proton pumps by covalent binding to cysteine residues. Pantoprazole is used for the treatment of variety of acid-related disorders of the upper gastrointestinal tract, including gastric and duodenal ulcers, and gastroesophageal reflux disease accompanied by oesophagitis [5,6].

A number of studies on the determination of pantoprazole in formulations or biological fluids have been described. Methods using spectrophotometry [7,8], high performance liquid chromatography [9–12] and capillary electrophoresis [13–15] have been published. Polarographic methods of determining benzimidazole PPIs other than pantoprazole are found in the literature [16–21].

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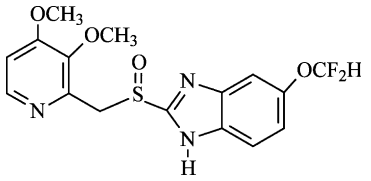

Scheme 1.

Pantoprazole contains a sulfoxide group, which could be reduced at mercury electrode.

This paper is concerned with the study of the voltammetric behavior of pantoprazole using the particularly rapid and sensitive technique of square-wave (SW) voltammetry at a hanging mercury-dropping electrode (HMDE). Pantoprazole is adsorbed on HMDE and this phenomenon was utilized to analytical advantage in the design of an adsorptive stripping method for its determination. The possibility of determining pantoprazole at ultra trace levels and the applicability of the method in determining pantoprazole in tablet dosage form (Controloc, 40 mg) have been demonstrated.

2. Experimental

2.1. Reagents

Pantoprazole sodium sesquihydrate and Controloc tablets were obtained from Byk Gulden (Konstanz, Germany). Stock solutions (1.0×10^{-3} M) of pantoprazole were prepared daily in methanol and stored under refrigeration. Britton–Robinson buffers (0.04 M in each of acetic, orthophosphoric, and boric acids, adjusted to the required pH with 0.2 M sodium hydroxide) were used as supporting electrolytes. All other reagents were of analytical-reagent grade; purified water obtained from an AquaMatic bi-distillation system attached to a Pur1-TE Still Plus deionizer system (Hamilton, England) was used throughout the work.

2.2. Apparatus

Voltammetric measurements were carried out using an EG&G PARC 394 Electrochemical Trace Analyzer. An EG&G PARC 303A stand was used in the hanging mercury drop (HMDE) mode. The three-electrode system was completed by means of a platinum wire as a counter electrode and an Ag/AgCl/saturated KCl reference electrode; all potentials are quoted relative to this electrode. A magnetic stirrer (par 305) and stirring bar provided the convective transport during accumulation. The whole procedure was automated and controlled through the programming capacity of the apparatus. All pH measurements were made with a CG 808 digital pH-meter with glass combination electrode (Schott Geräte, Germany).

2.3. Procedure

A 10 ml aliquot of buffer solution was placed in the voltammetric cell and the solution was purged with nitrogen for 10 min (30 s for each successive cycle). Then, the accumulation potential (usually -0.6 V) was applied to a working electrode while the solution was stirred continuously. Stirring was discontinued and, after 5 s, a negative potential scan was initiated and the resulting voltammogram was recorded. An aliquot of the standard pantoprazole solution was introduced with a micropipette and the adsorptive stripping cycle was repeated using a new mercury drop. Peak heights were evaluated as the differences between each voltammogram and the background electrolyte voltammogram.

2.3.1. Procedure for tablets

Ten Controloc tablets were powdered and thoroughly mixed. An accurately weighed amount of the tablet powder equivalent to approximately 40 mg of pantoprazole was transferred to a 5-ml calibrated flask and dissolved in methanol by sonication for 5 min. After the non-dissolved excipients settled at the bottom of the flask 100 μl of the clear supernatant were transferred to a voltammetric cell containing 10 ml of a supporting electrolyte solution (Britton–Robinson buffer pH 7.0) and the stripping procedure described above was applied. The amounts of pantoprazole in tablets were calculated by reference to the regression equation of the peak current vs. the pantoprazole concentration.

3. Results and discussion

3.1. Cyclic voltammetry

Pantoprazole species were readily adsorbed onto the mercury electrode. Fig. 1 (solid line) displays a cyclic voltammogram of 5.0×10^{-7} M pantoprazole in Britton–Robinson buffer at pH 7.0 on a HMDE following 60 s stirring at -0.6 V. A large definite cathodic peak, corresponding the oxidation of the adsorbed drug is observed at -1.178 V. Also shown (dotted line) is the analogous response without prior accumulation. The adsorption effect was also identified by a plot of log i_p vs. log v, giving a straight line which can be expressed by the equation: log i_p (μA) $= -2.09 + 0.91 \log v$ (mV s^{-1}). A slope close to 1.0 shows that the compound was adsorbed on the electrode surface [22]. On the reverse scan, no anodic peak was observed at scan rate of 25–300 mV s^{-1}, which is characteristic behavior for an irreversible process. The reduction peak was displaced to more negative potentials when the scan rate increased, confirming the irreversible nature of the reduction reaction [23].

The dependence of the peak potential on the logarithm of the scan rate was a straight line following the equation: E_p (mV) $= -1083.6 - 47.2 \log v$ (mV s^{-1}). Using the value of the slope [24], a value of $\alpha n = 0.80$ was obtained. Taking into account that the molecular structures of three benzimidazole sulfoxides, omeprazole, lansoprazole and pantoprazole, are similar, it is assumed that the reduction mechanism of the three benzimidazoles should be similar. The different substituents do not modify the electrochemistry of the three molecules, which is generally that of the sulfoxide electroactive site. The reduction signal can be assigned to an overall of 4e–4H$^+$ reduction process of pantoprazole, involving a reduction of the sulfoxide group in the 2e–2H$^+$ step to thioether function, followed by reductive cleavage of the thioether linkage in the 2e–2H$^+$ step giving rise to 3,4-dimethoxy-2-methylpyridine and 6-methoxybenimidazole-2-thiol [19,20]. The number of electrons n exchanged by the molecule was assumed to be 4. Hence, the value of the charge transfer coefficient is $\alpha = 0.20$.

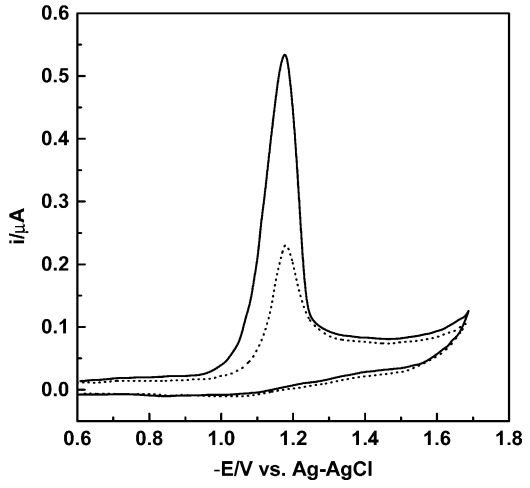

Fig. 1. Cyclic voltammograms for 5.0×10^{-7} M pantoprazole in Britton–Robinson buffer (pH 7.0) and scan rate $v = 100$ mV s^{-1} following 60 s accumulation at -0.6 V (solid line). An analogous voltammogram without accumulation (dotted line).

3.2. Effect of pH

The influence of pH on the SW voltammetric response of 1.0×10^{-7} M pantoprazole was examined between pH 2.0 and 11.0 (Fig. 2) without accumulation and after a 60-s accumulation time. Clearly, one can see that the response preceded by accumulation increased extensively at pH 7.0.

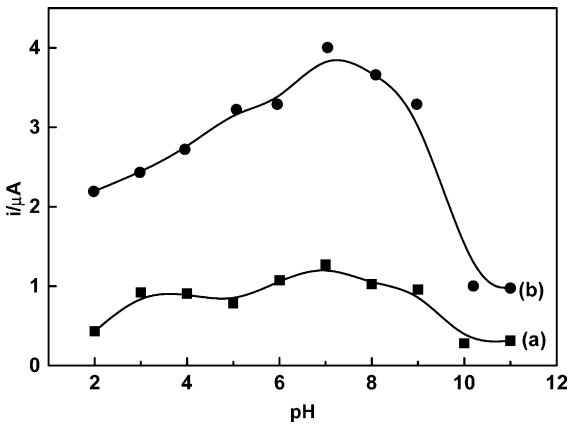

Fig. 2. Effect of pH on the accumulation of 1.0×10^{-7} M pantoprazole using SW voltammetry (frequency $f = 120$ Hz, scan increment $\Delta E = 10$ mV and pulse amplitude $\Delta U = 50$ mV) following the accumulation time, t_{acc}: (a) 0 and (b) 60 s at $E_{acc} = -0.6$ V and equilibration time 5 s.

Pantoprazole is an ampholyte with pK_a values of ≈ 4.0 (pyridine) and ≈ 8.5 (benzimidazole), the reactant being cation, anion or neutral molecule depending on pH of the solution. It follows that in the pH region where pantoprazole showed a major adsorptivity, the distribution of protolytic forms is shifted to the neutral one. The largest adsorptive peak current is attained at pH 7.0, which was chosen for the subsequent analysis.

The peak potential of the reduction process was shifted to more negative values with pH increasing up to 9.0 by 72 mV per pH unit. The peak potential was almost constant at pH > 9.0. This behavior clearly indicates that protons are involved in the electrode reaction [25].

3.3. Effect of accumulation potential

Fig. 3 shows the dependence of the SW stripping peak current of 5.0×10^{-8} M pantoprazole solution following t_{acc} of 60 s on the accumulation potential in the range from -0.1 to -1.0 V. The i_p reached the maximum values over the E_{acc} range from -0.4 to -0.7 V. The adsorbed species are most probably neutral molecules of the drug and the maximum of the peak current is reached in the potential range of zero charge of mercury electrode. At more cathodic values, a decrease in peak current was observed, indicating that the drug is no longer strongly adsorbed at potentials where the mercury is negatively charged with respect to the point of zero charge potential. The other dependences were therefore measured at a potential of accumulation of -0.6 V.

3.4. Effect of accumulation time

Different SW voltammograms with increasing accumulation times were recorded for solutions containing pantoprazole at two concentrations (1.0×10^{-8} and 5.0×10^{-8} M) using the selected conditions (Fig. 4). For 1.0×10^{-8} M pantoprazole solution, the peak current increased linearly with the accumulation time in the whole range of the accumulation times tested, while for 5.0×10^{-8} M solution, a rectilinear relation up to accumulation time of 300 s was obtained. Above this time, saturation of the mercury drop was observed. Hence, the choice of the accumulation time depends on the range of the analyte concentration being determined.

3.5. Square-wave parameters

The analysis of the effect of SW parameters on the analytical signal allows some kinetic aspects, as well as the optimization of these parameters for analytical application to be determined. The

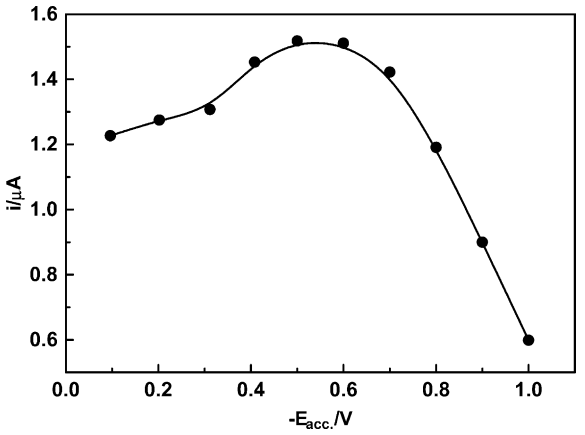

Fig. 3. Effect of accumulation potential (E_{acc}) on the SW stripping signal (i_p) for 5.0×10^{-8} M pantoprazole in Britton–Robinson buffer (pH 7.0) for $t_{acc} = 60$ s. For other conditions see Fig. 2.

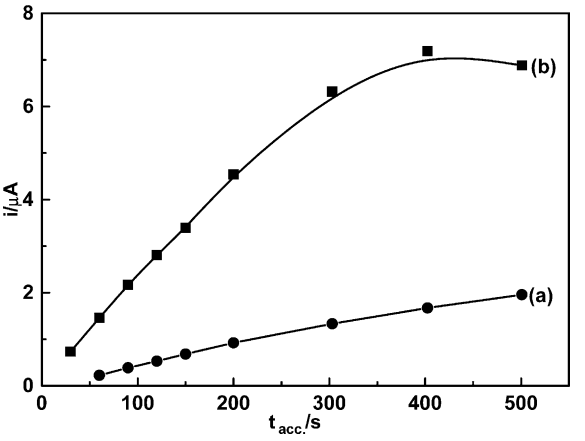

Fig. 4. Stripping peak current vs. accumulation time plots for (a) 1.0×10^{-8} M and (b) 5.0×10^{-8} M pantoprazole in Britton–Robinson buffer pH 7.0 following accumulation at $E_{acc} = -0.6$ V. For other conditions see Fig. 2.

analytical signal for pantoprazole (1.0×10^{-7} M, pH 7.0, $t_{acc} = 30$ s, $E_{acc} = -0.6$ V) was optimized by changing the pulse amplitude ΔU, scan increment ΔE and frequency f in the range 10–100 mV, 2–10 mV and 10–120 Hz, respectively. For a constant scan increment of 2 mV and pulse amplitude of 100 mV, the peak potential (E_p) shifted to more negative values with frequency increasing according to the equation: E_p (mV) = $-1122 - 71 \log f$ (Hz). The slope of this straight line was used to calculate the charge transfer coefficient for the electrochemical reaction. The variation of the peak potential with that of SW frequency conformed to the equation $\Delta E_p / \Delta \log f = -RT/Fn\alpha$ [26]. Assuming $n = 4$, a resulting charge transfer coefficient $\alpha = 0.21$ can be evaluated, which is very close to the value obtained by cyclic voltammetry.

The current intensity was a linear function of the logarithm of SW frequency in the whole range studied (10–120 Hz), conforming to the following equation: i_p (μA) = $0.1214 + 0.0167f$ (Hz). According to the equation $i_p = (5 \pm 1) \times 10^2 A\alpha n^2 F \Delta U f \Delta E \Gamma_{max}$, a coating index for the electrode can be calculated from the slope [26]. Using the values $\alpha n = 0.84$, $n = 4$ and $A = 0.026$ cm^2, one obtains $\Gamma_{max} = (1.98 \pm 0.40) \times 10^{-11}$. The relationship between i_p and f, on one hand, and between E_p and $\log f$ on the other is consistent with a diffusion-controlled adsorption process [27].

The influence of the pulse amplitude on the analytical signal was studied varying ΔU from 10 to 100 mV for a frequency of 120 Hz at scan increment of 2 mV. The peak current increased linearly with the SW amplitude in the range 10–50 mV, according to the equation i_p (μA) = $0.1041 + 0.0174\Delta U$. From the slope of this straight line, one obtains $\Gamma_{max} = (1.65 \pm 0.33) \times 10^{-11}$. The two independently calculated values of Γ_{max} were in fair agreement.

The influence of the scan increment on the analytical signal was studied varying ΔE from 2 to 10 mV for a frequency of 120 Hz at an amplitude of 100 mV. A linear relationship between peak intensity, i_p, and ΔE was found. A scan increment at 10 mV was adopted in the rest of the study.

3.6. Analytical application

Fig. 5 shows SW stripping voltammograms recorded using optimum conditions ($t_{acc} = 60$ s, $E_{acc} = -0.6$ V, $f = 120$ Hz, $\Delta E = 10$ mV and $\Delta U = 50$ mV) at different concentrations of pantoprazole. A linear calibration from 1.0×10^{-9} to 5.0×10^{-8} M was obtained. Least-squares analysis of the calibration graph yielded the equation i_p (nA) = $17.34 + 28.33C$ (nM) with a correlation coefficient of 0.996. The detection limit, 5.0×10^{-10} M, was calculated as the analyte concentration giving a signal equal to the blank signal y_B (intercept) plus three standard deviations of y-residuals $s_{y/x}$ [28]. The precision of the method, expressed as the value of the relative standard deviation calculated from ten independent assays on samples containing 1×10^{-8} M pantoprazole each, was 1.6%.

3.6.1. Pantoprazole assay in tablets

The validity of the proposed Ad-SWV was tested by determining pantoprazole in Controloc tablets. Each tablet was labeled to contain 45.1 mg pantoprazole sesquihydrate (equivalent to 40 mg pantoprazole) and inactive ingredients of magnesium carbonate, mannitol, crospovidone, polyvidone K90, calcium stearate, (hydroxypropyl)

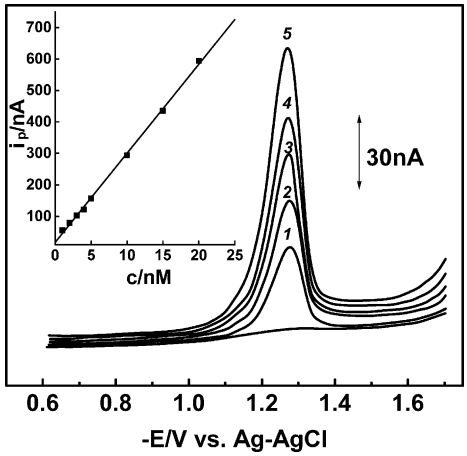

Fig. 5. Stripping SW voltammograms obtained for solutions of increasing pantoprazole concentration over the range (1.0–5.0) $\times 10^{-9}$ M (1)–(5); $t_{acc} = 60$ s; $E_{acc} = -0.6$ V. Dotted lines represent the blank; the inset is calibration plot.

Table 1
Statistical analysis of the results obtained by the Ad-SWV and spectrophotometric methods for Controloc tablets

Technique parameters	Ad-SWV	UV-spectrophotometry[7]
$\bar{x}$	39.91	39.79
S	0.91	0.77
s_r	2.28	1.94
CL	1.13	0.96
t-Test significance	0.23	t (tabulated) = 2.31
F-Test significance	0.72	F (tabulated) = 6.388

methylcellulose, 2910, polyvidone K25, titanium dioxide E171/C1 77891, yellow ferric oxide E 172/C1 77492, propylene glycol, eudragit L 30 D-55, sodium dodecyl sulfate, polysorbate 80, triethyl citrate and printing ink (opcode S-1-9210).

The results of the proposed Ads-SWV method were evaluated statistically as compared with a spectrophotometric method [7] (Table 1). According to the results of t- and F-tests, the variances between the two methods were found to be insignificant at 95% probability level, indicating that no significant differences exist between the performances of the two methods regarding their accuracy and precision.

As a conclusion, the proposed method is sensitive, precise, accurate and rapid enough to be used in the routine analysis of pantoprazole in pharmaceutical tests. Moreover, it can be used to selectively determine pantoprazole in pharmaceuticals after suitable dilution of the sample without interference from the ingredients of tablet matrix.

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