Polarographic Behaviour and Determination of Ranitidine in Pharmaceutical Formulations and Urine

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A polarographic method for the determination of ranitidine is described, based on the reduction of the nitro group at a dropping-mercury electrode. The proposed method permits the drug to be determined, without any prior separation or extraction, in pharmaceutical formulations and urine at levels at which the unchanged drug is excreted. The current is diffusion controlled and proportional to concentration from 3.58×10^{-3} to 1.50 mmol l^{-1}. The detection limit is 1.07×10^{-3} mmol l^{-1}. The reduction process was studied at different pH values and a reduction mechanism is proposed in which the importance of the homogeneous chemical reactions associated with the electron-transfer steps are indicated.

Keywords: Ranitidine determination; polarography; pharmaceutical formulation; urine

Introduction

Ranitidine, N-(2-{[(5-dimethylamino)methyl]-2-furanil}-methylthioethyl)-*N'*-methyl-2-nitro-1,1'-ethenediamine, is the active compound of the pharmaceutical formulation Zantac. It competitively inhibits the action of histamine on the H_2 receptors of parietal cells, reducing gastric acid secretion under daytime and nocturnal basal conditions and also when stimulated by food, insulin, histamine or pentaglandin. The drug is used for the short-term treatment of active duodenal ulcer, active and benign gastric ulcer, for the treatment of pathogenic gastrointestinal hypersecretory conditions and to provide short-term symptomatic relief of gastroesophageal reflux.

Ranitidine (Fig. 1) is metabolized in the liver to ranitidine *N*-oxide, desmethylranitidine and ranitidine *S*-oxide, and approximately 70% of a dose of the drug is excreted in urine as the unchanged drug.[1]

This nitroalkene has been determined by spectrophotometry, NMR spectrometry,[2] liquid chromatography[3] and differential-pulse adsorptive stripping voltammetry.[4] An a.c. polarographic method, based on the reduction of the aliphatic nitro group, has also been reported for its determination in pharmaceutical formulations.[5] Three a.c. peaks were observed and assigned to the reduction of the nitro group, and probably to the protonated hydroxylamine and other protonated structures.

The reduction of olefinic nitro compounds has been studied previously using polarographic methods.[6–14] β-Nitrostyrene, the most typical compound studied of this nature, is reduced in a four-electron polarographic wave between pH 1 and 11. A second two-electron process was observed over the pH range 1–3. Holleck and Jannakoudakis[9] stated that the nitro group of β-nitrostyrene shows some properties characteristic of both aliphatic and aromatic nitro compounds. They considered that the two polarographic waves led to β-hydroxylaminestyrene and β-aminostyrene, respectively. In contrast, Masui and co-workers[10,11] postulated that the two reduction products were the oxime of phenylacetaldehyde and β-hydroxylaminoethylbenzene, respectively. The effect of different substituents on the reduction of some olefinic nitro compounds has also been studied by polarography.[12,13]

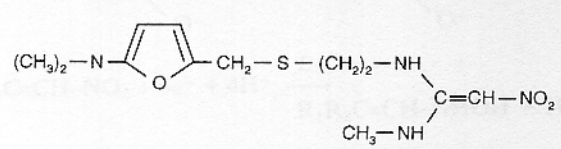

Fig. 1 Structure of ranitidine.

This paper reports a study on the electrode process of ranitidine and a direct polarographic method for its determination in pharmaceuticals and urine.

Experimental

Reagents

All chemicals were of analytical-reagent grade. Ranitidine was kindly provided by Laboratorio Chile, Santiago, Chile.

Stock standard solutions (0.01 mol l^{-1}) of ranitidine were prepared by dissolving the appropriate amount of the drug in distilled water. Tablets containing ranitidine were also dissolved in distilled water and assayed polarographically. Gelatin (0.05%) was used to eliminate the polarographic maxima. The supporting electrolyte used for the determinations was phosphate buffer (0.1 mol l^{-1} NaH_2PO_4–0.1 mol l^{-1} Na_2HPO_4) of pH 7.

Apparatus

Polarographic and cyclic voltammetric assays were performed using a CV-27 Voltammograph (Bioanalytical Systems, Lafayette, IN, USA). A laboratory-made damping assembly was constructed for d.c. polarographic measurements. A dropping-mercury electrode (DME) and a hanging mercury drop electrode (HMDE) were used as the working electrodes in polarography and cyclic voltammetry, respectively, together with a saturated calomel electrode (SCE) as the reference electrode and a platinum coil as the counter electrode. A mercury pool was employed for controlled-potential coulometric measurements.

Spectral measurements were made on Shimadzu UV-160 spectrophotometer.

An Orion Research Digital ion Analyser 701 with glass and SCE electrodes was used for pH determinations.

Techniques

Aliquots of the standard solutions were diluted with the supporting electrolyte, de-oxygenated with oxygen-free nitrogen for 5 min and analysed using the d.c. polarographic

mode. The mercury flow rate, m, and the drop time, t, were determined as different heights of the mercury column, h. The diffusion-controlled character of the current and the dependence of the diffusion-limited current on the depolarizer concentration were established.

Cyclic voltammetric experiments were carried out under identical experimental conditions. Voltammograms were recorded at scan rates between 0.02 and 0.4 V s^{-1}.

All measurements were performed at 25 ± 1 °C.

Determination in Pharmaceutical Formulations

Tablet formulations containing a nominal 300 mg of ranitidine in a total mass of approximately 660 mg were analysed. Twenty tablets were thoroughly ground and mixed. Samples of approximately 20 mg of ranitidine were accurately weighed, dissolved in water, transferred into separate 10 ml calibrated flasks and diluted to the mark with water. The contents of the flasks were shaken for 20 min and then allowed to settle. A 100 μl aliquot of the supernatant was diluted to 25 ml with the supporting electrolyte and subjected to polarography. The contents of the drug in the tablets were determined using the standard additions technique. In order to establish the reliability of the proposed method, ranitidine was determined in synthetic samples containing the drug in common tablet excipients (magnesium stearate, gelatin, lactose and starch).

Determination in Urine

Urine samples were obtained from patients at specific time intervals during single-dose administration. The ranitidine excreted as unchanged drug was determined using the standard additions technique.

Results and Discussion

The effect of pH on the d.c. polarograms was investigated by recording the current–voltage curves for aqueous solutions of ranitidine at a concentration of 0.278 mmol l^{-1} in different buffer systems (Britton–Robinson, McIlvaine and phosphate).

Irrespective of the buffer system used, ranitidine exhibits one d.c. polarographic wave at pH > 6.0, corresponding to the reduction of the nitro group to hydroxylamine. This wave is split into two waves at pH < 6.0 (Fig. 2). The ratio of the two wave height changes with pH [Fig. 2(*a*) and (*b*)] but the total height of these waves remains almost constant [Fig. 2(*c*)]. The decrease in wave A and increase in wave B in the shape of a dissociation curve with inflection point at about pH 2.6 is consistent with the following reactions:

$$R_1R_2C{=}CH{-}NO_2 \rightleftharpoons R_1R_2C{=}\bar{C}{-}NO_2 + H^+$$

$$R_1R_2C{=}C{=}\overset{+}{N}\begin{matrix}OH\\ O^-\end{matrix} \rightleftharpoons R_1R_2C{=}C{=}\overset{+}{N}\begin{matrix}O^-\\ O^-\end{matrix} + H^+$$

$$R_1R_2C{=}CH{-}NO_2 + 4e^- + 4H^+ \xrightarrow{E_1} R_1R_2C{=}CH{-}NHOH + H_2O$$

$$R_1R_2C{=}\bar{C}{-}NO_2 + 4e^- + 5H^+ \xrightarrow{E_2} R_1R_2C{=}CH{-}NHOH + H_2O$$

where E_1 (wave A) is more positive than E_2 (wave B) [Fig. 2(*a*) and (*b*)]. The pK_a value of ranitidine (2.7), where the acidity of the =CH– group is increased by the presence of the nitro group, and the $=C(NHR)_2$ group, resemble the pK_a of dinitromethane ($pK_a = 3.6$). In this last instance, at pH > pK_a a carbanion is also stabilized owing to the presence of two nitro groups.

In order to confirm the assignment of the polarographic waves for ranitidine, the change in the UV spectrum was followed in the pH range 1–4. As can be seen in Fig. 3, the appearance of an absorption band at 313 nm as the pH increases is in accord with the carbanion–nitronate formation, which confirms the above scheme. A similar spectroscopic behaviour was observed in both nitro- and dinitromethane at pH values close to their respectives pK_a values.

On the other hand, agreement of $pK_a' = 2.6$ from $i = f(pH)$ [Fig. 2(*c*)] with $pK_a = 2.7$ of ranitidine indicates that the acid–base equilibrium is established slowly, as expected for carbanion formation. This assumption was confirmed by studying the effect of the mercury pressure at pH 3.5. It was observed that even the small wave A was found to be diffusion controlled and proportional to $h^{\frac{1}{2}}$. Hence at pH > pK_a the carbanion nitronate predominates in the solution and protonation on the carbanion occurs slowly.

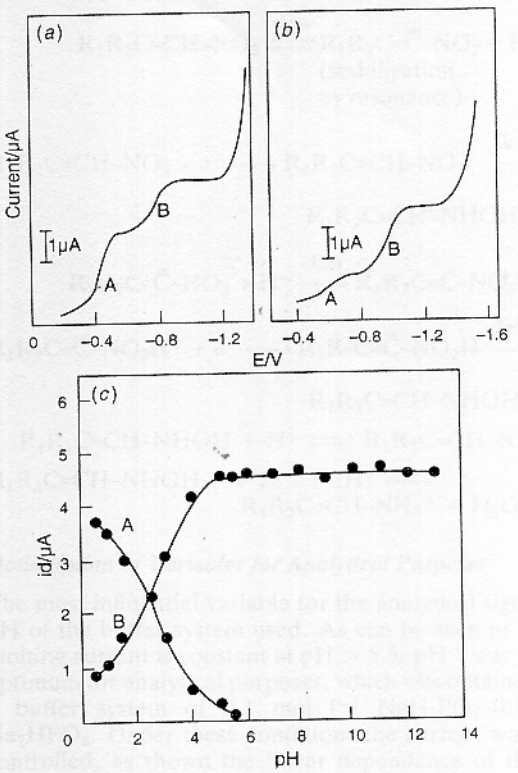

Fig. 2 pH Dependence of the reduction waves of 0.278 mmol l^{-1} ranitidine. (*a*) pH 1.5; (*b*) pH 3.5. (*c*) Diffusion-limited current *versus* pH graph. For details see text.

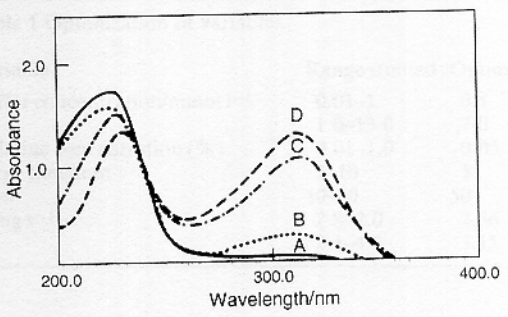

Fig. 3 Absorption spectra of ranitidine at 0.1 mmol l^{-1} measured against the blank. A, pH = 1.2; B, pH = 2.0; C, pH = 2.8; and D, pH = 4.

As the pH was gradually increased, the half-wave potential of the two waves shifted towards more negative values. The pH dependence of $E_{\frac{1}{2}}$ of wave B (Fig. 4) shows two linear segments intersecting at pH 3, confirming the presence of the above acid–base equilibrium. The pH-dependent segments at pH > 3 indicates protonation of the predominating species in solution (carbanion nitronate). As protonation of the carbanion is slow and proton transfer causing a dependence of $E_{\frac{1}{2}}$ on pH is fast,[15] the most probable process occurring at the electrode surface is

$$R_1R_2C=\bar{C}-NO_2 + H^+ \longrightarrow R_1R_2C=\bar{C}-NO_2H^+$$

and it is the protonated nitro group in the resulting zwitterion that is reduced at the mercury electrode. The slopes of the lines of the $E_{\frac{1}{2}}$ *versus* pH graph (Fig. 3) were found to be −21.67 mV at 0 < pH < 3, −67.00 mV at 3 < pH < 8 and −47.50 at pH > 8.

A third, more negative, polarographic wave ($E_{\frac{1}{2}}$ = −1.3 V, at pH 4.0) was observed below pH 5.5, but it was well defined only between pH 3.5 to 5.0. According to the wave height (i_{lim} = 2.15 μA at 0.278 mmol l^{-1}), wave shape and change of $E_{\frac{1}{2}}$ to more negative values with increase in pH, it corresponds to the reduction of the protonated hydroxylamine to the amine. Hence the hydroxylamine must be the product of the half-reaction responsible for the first two waves (A and B).

The coulometric *n* value was determined at pH 7 using a potential of −1.40 V, and the value obtained of 4.09 confirms reduction to hydroxylamine as the final product in waves A and B.

However, it is evident that the electrochemical behaviour of this compound differs considerably from that of nitroaromatic drugs because in this instance the stabilizing effect of the aromatic π-system on the initial (anion radical) and other intermediate reduction products is missing. As a result, reduction of ranitidine, as with other aliphatic nitro compounds, occurs at higher negative potentials than that of nitroaromatic compounds.

According to Seagers and Elving,[7] olefinic nitro compounds are reduced at more positive potentials than nitrobenzene. In the electrode process of ranitidine, the more negative reduction in wave B is due to the presence of the negative charge on the carbon adjacent to the nitro group and stabilization by resonance as indicated above. Reduction of the conjugate acid at pH < 3 in wave A at −0.4 V at pH 1.5 occurs in a similar range to nitrobenzene at the same pH. Likewise, conjugation with the π-electron of both nitrogens in the part of the structure resembling urea makes the nitro group more difficult to reduce by increasing the electon density on the nitro group.

The totally irreversible nature of the reduction process was confirmed by cyclic voltammetry. Cyclic voltammograms at all concentrations (1–0.1 mmol l^{-1}), scan rates (0.02–0.4 V s^{-1}) and pH values (1–10) studied showed only cathodic peaks for the reduction of the nitro group, even when wave clipping (reversal of the scan direction) was carried out after each peak. In addition, the current at the foot of the wave is independent of the scan rate, which, according to Reinmuth,[16] indicates that the electron transfer is electrochemically unidirectional. It was found, on the other hand, that the slope of the logarithmic graph of *E versus* log $[i/(i_d-i)]$ corresponding to the polarographic wave, appreciably exceeded 59.2/*n* mV, where *n* is the number of electrons involved in the over-all reduction process, when the electrode reaction is reversible.

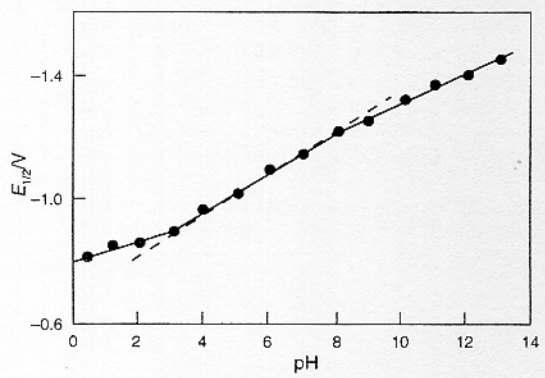

Fig. 4 Effect of pH on the half-wave potential of the ranitidine reduction wave (wave B) at 0.278 mmol l^{-1}.

The αn_a value and the number of protons (*P*) corresponding to the rate-determining step (RDS) were calculated at different pH values. In the pH range 1–11, αn_a was found to be 0.6 ± 0.1 for waves A and B, which indicates that one electron is involved in the rate determining step.

By using the expression

$$\Delta E_{\frac{1}{2}}/\Delta pH = 0.059\ P/\alpha n_a$$

P was found to be 0.83, showing that the rapid protonation of the nitro group occurs prior to the electron transfer at pH > 3.0.

The following mechanism can be assigned for the reduction of ranitidine:

$$R_1R_2C=CH-NO_2 \overset{\text{slow}}{\rightleftharpoons} R_1R_2C=\bar{C}-NO_2 + H^+$$
(stabilization by resonance)

$$R_1R_2C=CH-NO_2 + e^- \longrightarrow R_1R_2C=CH-NO_2^- \xrightarrow[-H_2O]{3e^-,\,4H^+} R_1R_2C=CH-NHOH \text{ (Wave A)}$$

$$R_1R_2C=\bar{C}-NO_2 + H^+ \overset{\text{fast}}{\longrightarrow} R_1R_2C=\bar{C}-NO_2H^+$$

$$R_1R_2C=\bar{C}-NO_2H^+ + e^- \longrightarrow R_1R_2C=\bar{C}-NO_2H \xrightarrow[-H_2O]{3e^-,\,4H^+} R_1R_2C=CH-NHOH \text{ (Wave B)}$$

$$R_1R_2C=CH-NHOH + H^+ \rightleftharpoons R_1R_2C=CH-NHOH_2^+$$

$$R_1R_2C=CH-NHOH_2^+ + 2e^- + 2H^+ \longrightarrow R_1R_2C=CH-NH_3^+ + H_2O \text{ (Wave C)}$$

Optimization of Variables for Analytical Purposes

The most influential variable for the analytical signal was the pH of the buffer system used. As can be seen in Fig. 2, the limiting current is constant at pH > 5.5. pH 7 was selected as optimum for analytical purposes, which was obtained by using a buffer system of 0.1 mol l^{-1} NaH_2PO_4–0.1 mol l^{-1} Na_2HPO_4. Under these conditions the current was diffusion controlled, as shown the linear dependence of the limiting current on $h^{1/2}$ and on the depolarizer concentration.

Under the working conditions given in Table 1, a series of standards with concentrations ranging from 5 × 10^{-4} to 2

Table 1 Optimization of variables

Variable	Range studied	Optimum value
Buffer concentration/mmol l^{-1}	0.01–1	0.1
pH	1.0–13.0	7.0
Gelatine concentration (%)	0.01–1.0	0.05
Purge time/min	1–10	5
h/cm	10–60	50
m/mg s^{-1}	2.8–2.0	2.36
t/s	2.1–4.5	3.15

mmol l^{-1} were polarographed. The linear relationship between diffusion-limited current and depolarizer concentration was expressed by the least-squares method as

$$i_d = 16.19\ [(\text{ranitidine (mmol } l^{-1})] - 0.075\ (r = 0.9998)$$

The determination limit was between 3.58×10^{-3} and 1.5 mmol l^{-1}. The detection limit, calculated using the 3σ criterion, was 1.07×10^{-3} mmol l^{-1}. The precision, expressed as the relative standard deviation, for 11 samples containing 0.028 and 0.708 mmol l^{-1} of ranitidine was 2.3 and 0.98%, respectively.

Applications

Ten determinations were carried out on a synthetic mixture containing ranitidine in common tablet excipients (magnesium stearate, gelatine, lactose and starch). The recovery was found to be 100.8 ± 0.8%, indicating that the method is free from these interferences.

On the basis of these results, ranitidine was determined in pharmaceutical formulations. Ten assays on tablets containing a nominal 300 mg of the drug per tablet gave a mean value of 298.5 mg per tablet with a relative standard deviation (s_r) of 1.3%.

The reliabilty of the method for the determination of ranitidine in urine was checked using different spiked urine samples in conjunction with the standard additions method. Five different samples were spiked with standards in the concentration range at which the unchanged drug is excreted.[1] The recovery was found to be 101% with $s_r = 2\%$ at the lowest concentration levels and 99.8% with $s_r = 0.8\%$ at concentrations of 10 μg ml^{-1} and higher.

Urine samples were obtained from patients at specific time intervals during single-dose administration. It is known that the portion of an orally administered dose that is excreted unchanged in urine is dose dependent. However, it was observed that after administration of a single oral dose of 300 mg, the drug concentration in urine increases until it reaches 15% of the initial dose at 3 h and then begins to decrease. On the other hand, approximately 30% of a dose is excreted within 7 h. After 10 h, the polarographic signal disappears and only unchanged drug is excreted below the limit of detection of the method together with polarographically inactive ranitidine metabolites.

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