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KINETIC STUDY ON OMEPRAZOLE ACID REACTION AT DIFFERENT PH VALUES BY USING HPLC

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ABSTRACT. High performance liquid chromatography (HPLC) has been used to monitor the time evolution of omeprazole under various pH conditions. First-order rate constants for the transformation into sulfenamide, the active inhibitor of the gastric secretion, were determined at 25^{0}C. They depend strongly on the acidity of the medium. From this dependence, the first pKa value of omeprazole was calculated, based on kinetic measurements. The acid-catalysed rate constant has been deduced.

Keywords*:* omeprazole, kinetics, pKa, acid catalysis.

INTRODUCTION

Omeprazole, 5-Methoxy-2-(4-methoxy-3,5-dimethyl-2-pyridimyl-methyl-sulfinyl)-1,4-benzimidazole (abbreviated as OPZ), with the chemical structure shown in figure 1 is a substituted benzimidazole that inhibits gastric secretion by altering the activity of H^-/K^+ATPase, which is the final common step of acid secretion in parietal cells [1-3]. OPZ itself is not an active inhibitor of this enzyme, but is transformed within the acid compartments of the parietal cells into the active inhibitor [4]. It has been used for more than two decades in the treatment of peptic ulcers [5], reflux esophagitis [6] and the Zollijger-Ellison syndrome [7]. The active inhibitor has been shown to be a cyclic sulphenamide (two isomers), which reacts with mercapto groups in the enzyme with the formation of a disulfide complex thus inactivating the H^+ and K^+-ATPase. These reactions lead to the blockade and have been studied both *in vivo* and *in vitro* [8]. Based on more than 200 million patient treatments it has been demonstrated that OPZ is a safe drug with no reported dose related side effects. OPZ is commercially available as enteric-coated granules encased in gelatine capsules, with a delayed-release of the medicine [9].

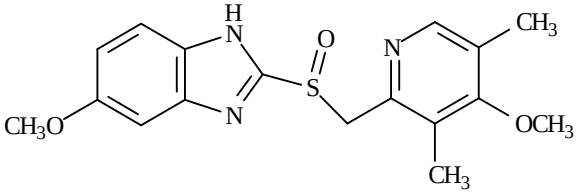


Fig. 1. Chemical structure of Omeprazole (OPZ)

The kinetics and the mechanism of the OPZ transformation have been studied by Arne Brändström and co-workers [10, 11] in the presence of 2-mercaptoethanol to simulate the formation of a disulfide bond with the enzyme.

In this study, we have followed the degradation of OPZ in buffered solutions of various pH and obtained the dependence of observed rate constant *vs.* hydrogen concentration. First acid dissociation constant has been determined on a kinetic base.

EXPERIMENTAL

Materials: Chemicals of analytical grade and ultra-pure water (de-ionised and tetra-distilled) were used throughout this study. A stock solution of 10 μg/mL of OPZ was prepared in methanol. The pH-buffer was prepared from K_2HPO_4 and citric acid. Working solution of OPZ (1 μg/mL) was prepared before each runs from 0.1 mL stock solution and 0.9 mL of previously prepared pH-buffer.

Apparatus: The HPLC system was an 1100 series model (Agilent Technologies, USA) consisted of a binary pump, an in line degasser, an auto sampler, a column thermostat and an UV detector. Data were achieved and computed by ChemStation software (ver. A.09.03). The detector wavelength was set as 303 nm. Chromatographic separation was performed in 3.5 minutes at 35 ^oC with a Zorbax SB-C18 100 x 3.0 mm I.D., 3.5 μm (Agilent Technologies, USA), protected by an on-line filter.

A Mettler-Toledo (Greifenseee, Switzerland) model MP225was used to adjust pH of sample solutions. The mobile phase consisted in an acetonitrile: sol K_2HPO_4 20 mmol/L (27:73 v/v). Kinetic fitting was performed with WinNonlin Professional (Pharsight, USA).

Procedures: A series of pH-buffer solutions were prepared. The values of the pH for these solutions were 2.3, 3, 3.3, 4, 4.3, 5, 5.3, 6, 6.3 and 7. Each component solution was degassed for 10 minutes in an Elma Transsonic 700/H (Singen, Germany) ultrasonic bath. Using these solutions, every sample mixture was prepared from 0.1 mL stock solution of OPZ and 0.9 mL of pH buffer. Temperature has been maintained constant at 25.0±0.1 C^0 by means of a Lauda M-20 thermostat. Aliquots of the mixture were taken at different time periods and analysed by HPLC. The injection volume was 10 μL. The pump delivered the mobile phase at a flow of 1 mL/min.

RESULTS AND DISCUSSIONS

It is know that the OPZ concentration decreases with time in acidic solution. The samples were performed at pH values between 2.2 and 7. OPZ concentrations were determined by using high-performance liquid chromatographic (HPLC) methods used by Kobayashi *et. all*. [12] and Amantea and Narang [13]. The HPLC method can be used in stability studies because there is no interference between the drug and its decomposition products [14, 15].

Figure 2 shows chromatograms for OPZ at time 0 and after 4 minutes

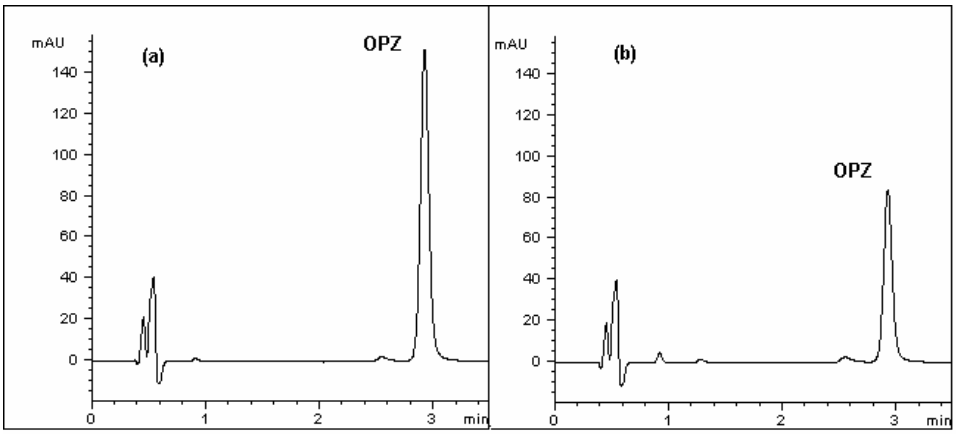


Fig. 2. Chromatograms of OPZ at t = 0 (a) and after 4 minutes (b)

Using chromatographic data it is possible to calculate the omeprazole concentrations at different time intervals elapsed after mixing. Figure 3 presents concentration dependence as a function of time for five pH values

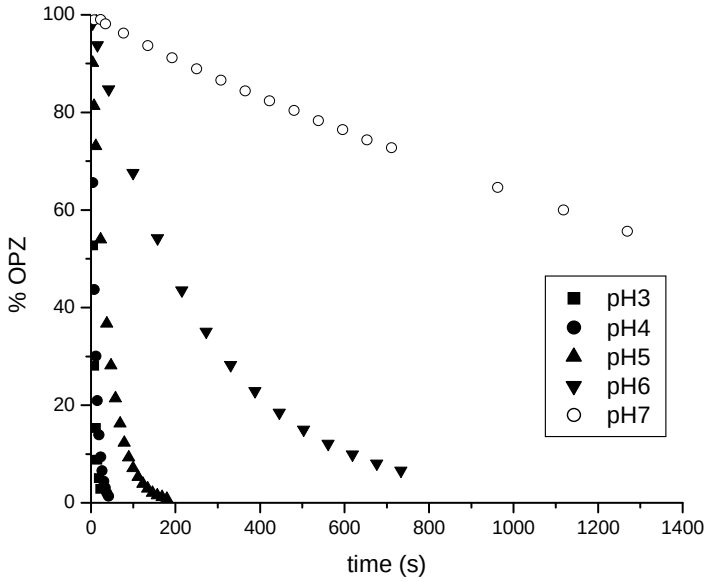


Fig. 3. The omeprazole concentration as a function of time

The concentration–time curves were well described by an exponential dependence:

$$[\mathrm{OPZ}] = [\mathrm{OPZ}]_0 \cdot \mathrm{e}^{-k_{obs} \cdot t} \quad (1)$$

where k_{obs} stands for the apparent first-order rate constant, involving decomposition of OPZ. Indeed, good correlation coefficients were obtained (0.990 - 0.9990) by using a least-square method for linear first-order dependence. However, we took advantage of a non-linear WinNonlin Professional (Pharsight, USA) method to compute first-order rate coefficients k_{obs} at different pH values. Data are presented in Table 1 as means of two or three replicate runs.

The omeprazole degradation exhibits a complex dependence on the acidity. A plot of k_{obs} *vs.* pH is a curve with an inflexion point at pH=4.32. Observed first-order rate constant dependence on hydrogen ion concentration is well described by the following equation:

$$k_{obs} = \frac{a[H^+]}{b + [H^+]} = \frac{(0.189 \pm 0.007)[H^+]}{[H^+] + (8.1 \pm 1.2) \cdot 10^{-5}} \quad (2)$$

with $\chi^2 = 9.1.10^{-5}$ and $r^2 = 0.9911$.

Table 1

Apparent first-order rate constant at various pH values.

pH	$[H^+]$ (mmol/L)	k_{obs} (min^{-1})
2.3	5.0119	0.2050
3	1.0000	0.1650
3.3	0.5012	0.1490
4	0.1000	0.1050
4.3	0.0501	0.0770
5	0.0100	0.0260
5.3	0.0050	0.0150
6	0.0010	0.0030
6.3	0.0005	0.0020
7	0.0001	0.0004

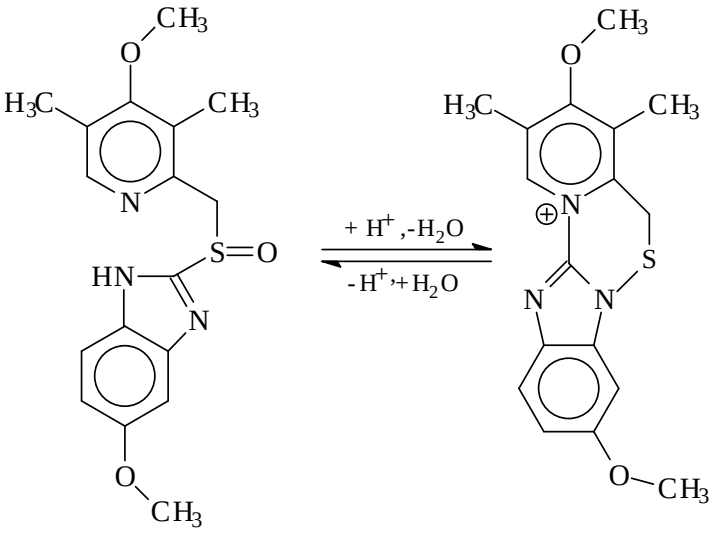
The form of experimental first-order rate constant in equation (2) also suggests the involvement of at least two consecutive reaction steps (two terms in the denominator). Kinetic data fit well with the following reaction scheme; the active species seems to be the protonated omeprazole:

$$OPZ + H^+ \Leftrightarrows OPZH^+ \longrightarrow Prod \qquad k_1, k_{-1}, k_2 \quad (3)$$

The first stable product is sulfenamide, with the equilibrium shifted to the right.

OPZ can accept a proton on either the pyridine ring or the benzinidazole ring. The cationic species suffers a ring closure with the elimination of a water molecule to form cyclic sulfonamide, also as a cation. The first step in (3) is a rapid one. A plot of $1/k_{obs}$ as a function of $1/[H^+]$ should be linear as in equation (4):

$$\frac{1}{k_{obs}} = \frac{b}{a[H^+]} + \frac{1}{a} = \frac{2.18 \cdot 10^{-4}}{[H^+]} + 20.05 \quad (4)$$



If the only reactive species is $OPZH^+$, than k_{obs} expressed by eq.2 has the form:

$$k_{obs} = \frac{k_2[H^+]}{[H^+] + k_{-1}/k_1} = \frac{k_2[H^+]}{[H^+] + Ka} \tag{5}$$

and the constants Ka and k_2 can be obtained using the above given equations or the Weighted Sum of Squares of Residuals program (WSSR). The calculated values are Ka= $8.1 \cdot 10^{-5}$ mole.L^{-1} and k_1= 0.19 L.$mol^{-1}min^{-1}$. Therefore, pKa = 4.1 for $OPZH^+$ is deduced from eqs (2) and (5). With the WSSR (WNL program) the best fit corresponds to pKa =4.38 and k_2= 0.16 L.$mol^{-1}min^{-1}$ respectively. The acidity constant is in agreement with the literature data [2,10, 11]. Maton, for example, has reported a value of pKa = 4.2.

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