Kinetics of Acid Degradation of Proton Pump Inhibitors in the Presence of a Thiol

ALI KH. AL-MATAR,[1] BASSAM EL-ESWED,[2] MAHA F. TUTUNJI[3]

[1]*Chemical Engineering Department, Faculty of Engineering and Technology, University of Jordan, Amman, Jordan*
[2]*Zarka University College, Al-Balqa Applied University, P. O. Box 313, Zarka, Jordan*
[3]*Chemistry Department, Faculty of Science, University of Jordan, Amman, Jordan*

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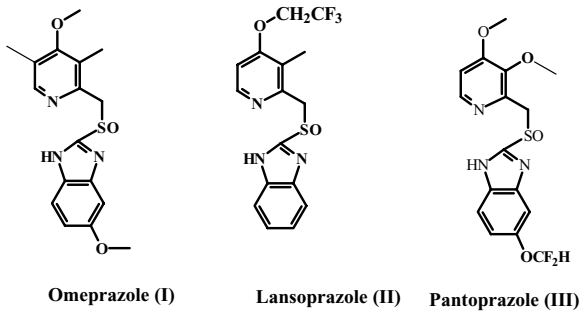
ABSTRACT: An in vitro investigation of the kinetics of the complex system of acid-catalyzed conversions and subsequent reactions of proton pump inhibitors (PPIs; omeprazole, lansoprazole and pantoprazole) was carried out using differential pulse polarography at the static mercury drop electrode. Reactions were investigated in the presence of 2-mercaptoethanol, in solutions buffered to pH values ranging from 2.0 to 5.0. The first-order reaction network was proposed for all conversions. The rate of degradation of PPIs and subsequent reactions with 2-mercaptoethanol were found to follow the following general order: lansoprazole > omeprazole > pantoprazole. The rate of conversion of PPIs into sulfenic acid was found to be directly dependent on the basicity of benzimidazole nitrogen of PPIs, which determines the electrophilic reactivity of the adjacent carbon (C2). The rate of conversion of the sulfenic acid of PPIs into the disulfide (the inhibition reaction) was found to be dependent on the electrophilicity of the sulfur atom of the sulfenic acid. © 2009 Wiley Periodicals, Inc. Int J Chem Kinet 41: 498–506, 2009

INTRODUCTION

The chemistry of proton pump inhibitors (PPIs; omeprazole (I), lansoprazole (II), and pantoprazole (III); see Scheme 1) led to a new era in the effective therapy of acid-peptic diseases. This chemistry is specifically responsible for (1) its accumulation in the most acidic space in the body, (2) the acid-catalyzed conversion into the active inhibitor, and (3) covalent inhibition of the acid pump as a result of the formation of the disulfide; by the reaction of the cyclic sulfenamide intermediate with the cystein of the gastric ATPase [1].

Among these PPIs, omeprazole was the most extensively studied [2–8]. Except for the work of Shin et al. [9], there is no in vitro comparison between the PPIs. Following the authors' work on the kinetics of acid degradation of omeprazole in the presence of 2-mercaptoethanol [10], the present work investigates the kinetics reactions of lansoprazole and pantoprazole under the same experimental conditions using differential pulse polarography (DPP) at the static mercury drop electrode (SMDE). Current (nA)–time (s) profiles for the three PPIs and their degradation products and subsequent reactions with thiol were compared.

Correspondence to: Ali Kh. Al-Matar; e-mail: aalmatar@ju.edu.jo



Scheme 1 Chemical structures of PPIs.

MATERIALS AND METHODS

Chemicals and Reagents

Omeprazole, lansoprazole, and pantoprazole were kindly donated by Dar Al Dawa Pharmaceuticals, Na'ur, Jordan. Methanol was HPLC-grade from Merck (Darmstadt, Germany). Phosphoric acid, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and trisodium phosphate were AR from Merck. 2-Mercaptoethanol was 99% GC from Across (Geel, Belgium). The cyclic sulfenamide (D^+) was prepared at the Pharmaceutical Research Unit, Royal Scientific Society (Amman, Jordan) using a reported method [2]. Authentication of D^+ was concluded using ^{1}H NMR, FTIR, mass, and UV-spectroscopy was reported previously [12].

Phosphate buffer was used after investigating the formal potentials of the analytes in several buffer solutions including Britton Robinson buffer and phosphate and acetate buffers. In addition, a solution of KCl in HCl and NaCl as well as sodium perchlorate solutions were also investigated. Phosphate buffer solutions were preferred because the polarograms demonstrated optimum peak resolution.

All other reagents were used without further purification. Ultra pure water was obtained by initially passing through two reverse osmosis cycles, before initiating a distillation followed by a deionization step. Oxygen-free nitrogen was used for dearation.

Instruments and Apparatus

A Metrohm 746 VA processor was used; it includes a potentiostat with a measuring amplifier, broad banded, and low noise with a piezoelectric keypad, in addition to a backlit LCD screen, which shows methods and routines. A Metrohm 747 VA stand with a multimode electrode, comprising a SMDE as a working electrode, an auxiliary platinum electrode, and a reference electrode (double junction type (Ag/AgCl saturated with a 3.0 M KCl solution)) completed the three-electrode cell.

Differential pulse polarograms at the SMDE were scanned using the following experimental conditions: a mercury drop area of 0.90 mm^2, t drop of 0.6 s, the voltage step was standardized at 13.33 mV/s sweep rate, and a pulse amplitude of 50 mV. A current potential range (ΔU) from 0.00 to −2.00 V versus Ag/AgCl was fixed for all measurement cycles. Unless otherwise specified, the temperature was adjusted using a thermostated polarographic vessel connected to a water bath equilibrated at 37°C.

Procedures

After adjusting the pH of each phosphate buffer solution (0.50 M) to the experimental value, a 10.0-mL aliquot was pipetted into the polarographic vessel. An aliquot of 2-mercaptoethanol was pipetted into the buffer solution to make up the specified final concentrations. Dearation was immediately started by passing an oxygen-free nitrogen gas for 8 min, prior to introducing PPI. This was immediately followed by initiating successive recordings of individual differential pulse polarograms. Recordings were sequentially repeated until the PPI signal was not detectable. DPP voltammograms were scanned between 0.00 and −2.00 V versus Ag/AgCl, 3.0 M KCl. Nine cathodic reduction cycles, each lasting 153 s, were recorded in each group of nine measurements. Peak currents (nA), peak potentials (volts versus Ag/AgCl, 3.0 M KCl), and time (minutes) were tabulated for all measurements in each group. Representative DPPs are illustrated in Fig. 1 for acid degradation of PPIs in the presence of thiol.

Reactions of each PPI were investigated in the presence of 2-mercaptoethanol in solutions buffered to different pH values including 2.0, 3.0, 4.0, and 5.0.

Chemical Reactions of PPI in the Presence of 2-Mercaptoethanol

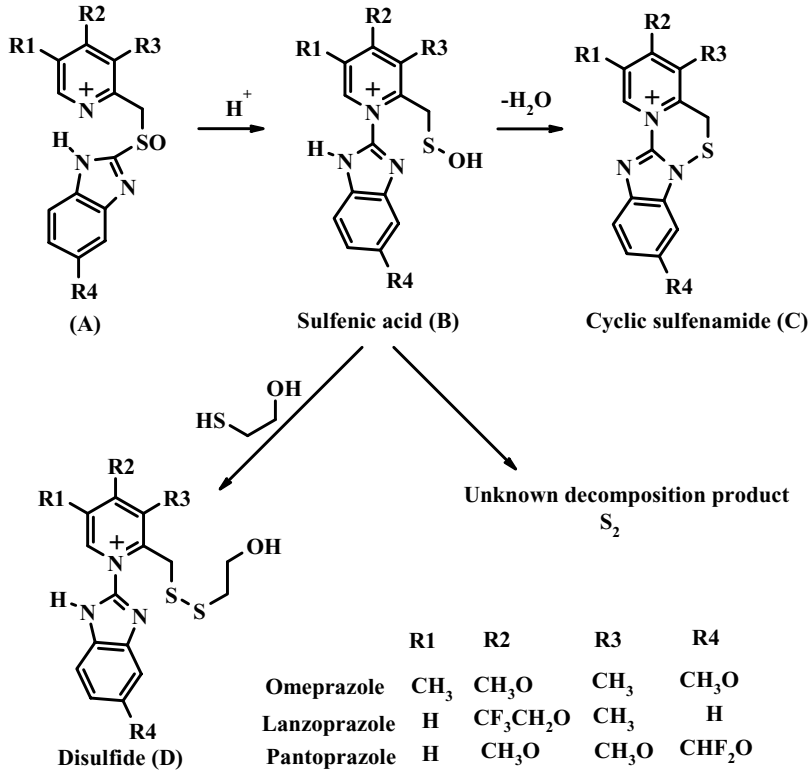
The proposed mechanism for the degradation of PPIs in the presence of 2-mercaptoethanol is illustrated in Scheme 2. The sulfenic acid was assumed to be the key intermediate for formation of the cyclic sulfenamide, disulfide, unknown degradation product, and the disulfide. This proposed scheme (Scheme 2) is suggested depending on previous work [10–13].

Derivation of Rate Equations

Based on the above reaction scheme, a first-order reaction network is proposed. The following rate expressions for the reaction network are, therefore, obtained:

$$r_A = -k_{AB}[A]$$

$$r_B = k_{AB}[A] - k_{BC}[B] - k_{BD}[B] - k_{BS_2}[B]$$



Scheme 2 General degradation reactions of PPIs.

$$r_C = k_{BC}[B]$$

$$r_D = k_{BD}[B]$$

$$r_{S_2} = k_{BS_2}[B]$$

The concentration of omeprazole [A] represents the total concentration of all protolytic species.

Solution Methodology

The above set of reaction rates represent a system of initial value ordinary differential equations; these can be solved to evaluate the concentrations of various species as a function of time. To obtain the rate constants from the experimental data, the authors implemented a stiff solver for the above set of equations. This was combined with Marquardt–Levenberg optimization algorithm to minimize the differences between the predicted and the experimental results. The objective function to be minimized was defined as

$$\text{Minimize } F = \sum_{j=1}^{N_{\text{Species}}} \sum_{i=1}^{N_j} \left(I_{\text{exp},ij} - I_{\text{model},ij}\right)^2$$

where I is the signal strength in nA, N_{Species} refers to the number of species that have experimental data points included in the optimization, N_j refers to the number of data points of a particular species, subscript i refers to the index of the data point, and subscript j refers to the component to which that data point belongs.

Identification of Polarographic Peaks

Representative voltammograms of omeprazole decay and appearance of degradation and reaction products with 2-mercaptoethanol are illustrated in Fig. 1. The assignment of peaks for omeprazole, lansoprazole, pantoprazole, cyclic sulfenamide, and 2-mercaptoethanol was done by recording their DPP voltammograms immediately after addition of each one individually to the buffer solution. The assignment of the disulfide peak was done depending on the fact that this peak was observed only after the addition of 2-mercaptoethanol to omeprazole. Therefore, it is rational to assume that this peak is due to their reaction product.

The peak at 0.65 V was assigned in our previous work [11–13] to omeprazole dimer because it is the major product expected to result from the acid decomposition of omeprazole. However, in our recent work [10] and in this work we are inclined to assign this peak to an unknown acid decomposition product, referred to as S_2, of omeprazole. It is worth mentioning that this peak was observed in the absence and presence of 2-mercaptoethanol.

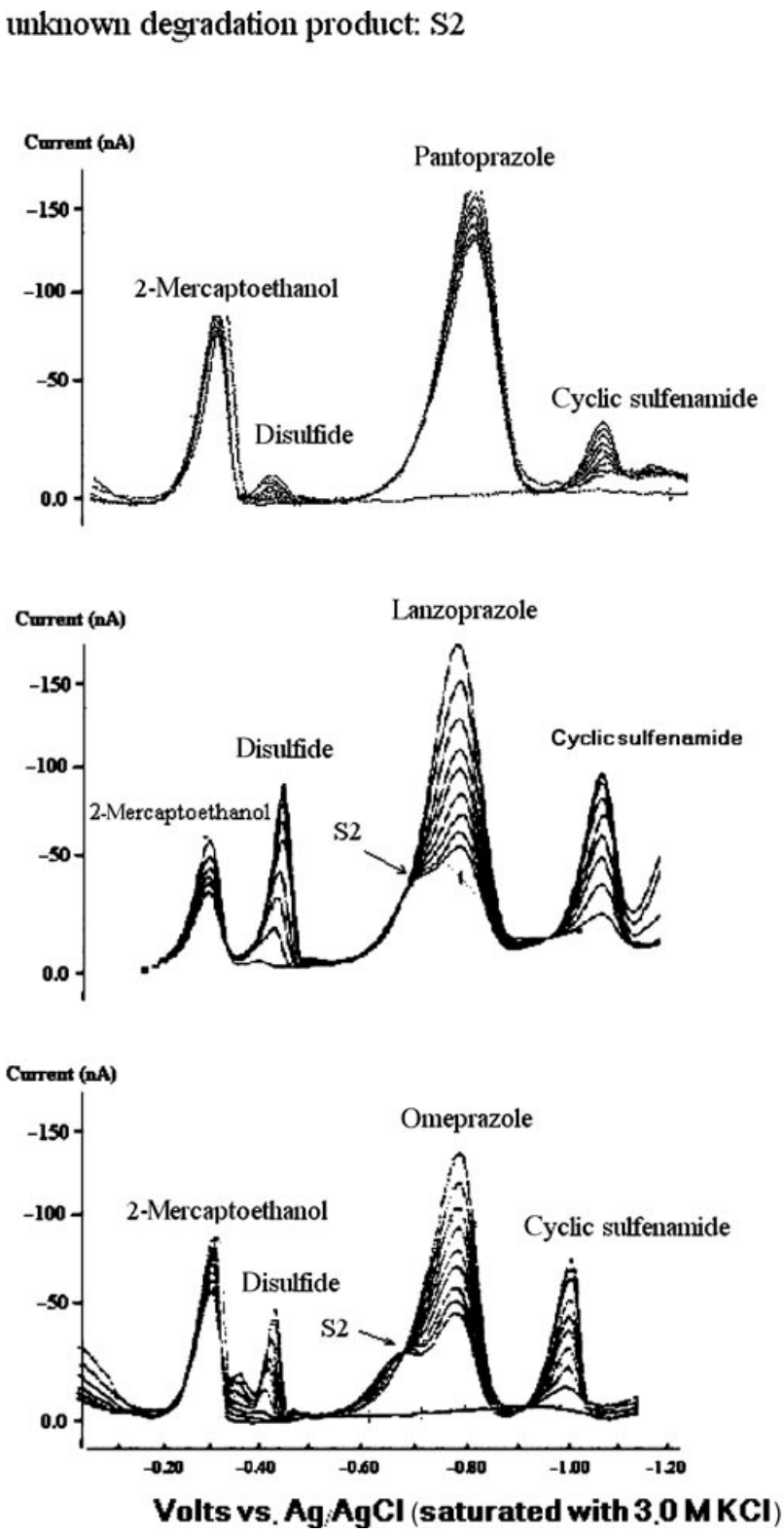


Figure 1 Representative differential pulse polarograms at the static mercury drop electrode for PPIs (3.00×10^{-5}) in a buffered solution (0.05 M, pH 4), illustrating the decay curves of: PPI and 2-mercaptoethanol and the appearance of the cyclic sulfenamide, unknown degradation product, and disulfide. Scanned between 0.00 and 19.0 (min).

RESULTS AND DISCUSSION

Effect of pH on the Rate Constants

Figures 2–7 illustrate signals (current: nA) versus time (s) profiles for the measured electroactive analytes monitored during the chemical reactions and conversions of PPIs (3.0×10^{-5} M). The continuous lines are those predicted by the kinetic model, whereas the scatter symbols are the experimental data points.

The rate constants (Tables I–III) were determined for the following reactions: (1) PPI conversion to sulfenic acid (the active inhibitor), (2) the conversion

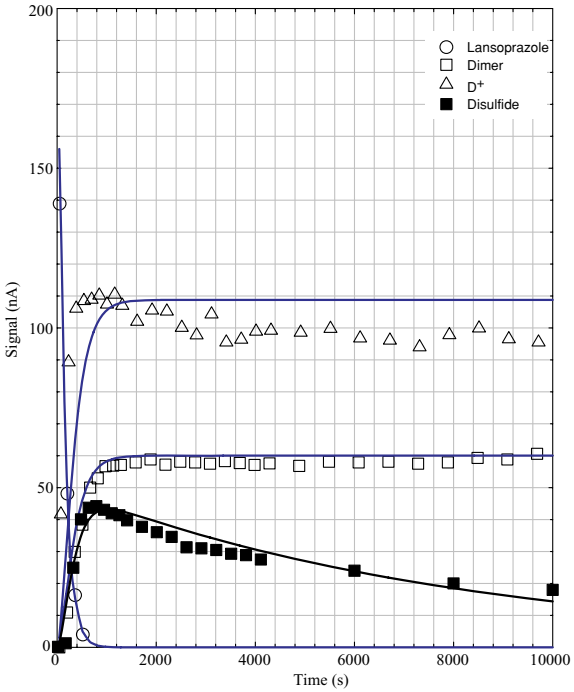


Figure 2 Current (nA)–time (s) profiles for (1) lansoprazole, (2) lansoprazole cyclic sulfenamide (D^+), (3) unknown degradation product, and (4) disulfide measured from a buffered solution (pH 4.0, 37°C) in the presence of 2-mercaptoethanol (1:1 mole ratio).

Table I Summary of Rate Constants Measured in Solutions Buffered to Different pH Values (37°C) for the Conversion of Omeprazole and Subsequent Reactions in the Presence of 2-Mercaptoethanol

pH	k_{AB} (s^{-1})	k_{BC} (s^{-1})	k_{BD} (s^{-1})	k_{BS2} (s^{-1})
2	0.01145	0.00539	[a]	0.00230
3	0.00803	0.00539	[a]	0.00198
4	0.00638	0.00223	0.000551	0.000812
5	0.00142	0.00138	0.000994	0.000293

[a] The signal of disulfide was not detected.

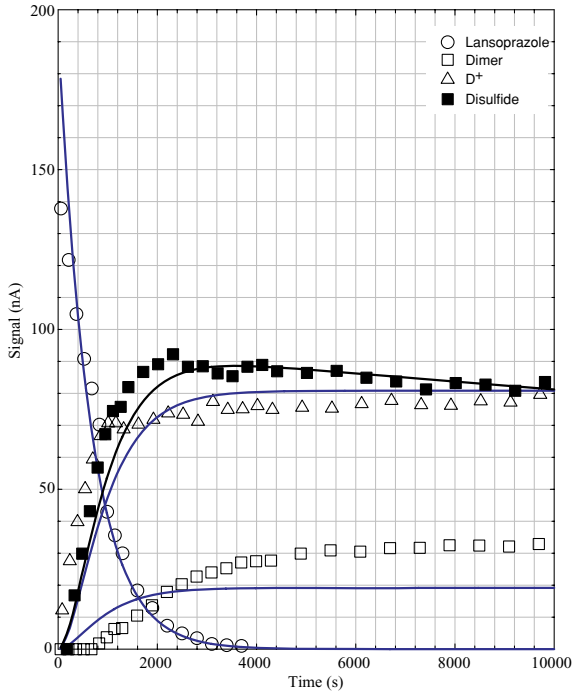


Figure 3 Current (nA)–time (s) profiles for (1) lansoprazole, (2) lansoprazole cyclic sulfenamide (D^+), (3) unknown degradation product, and (4) disulfide measured from a buffered solution (pH 5.0, 37°C) in the presence of 2-mercaptoethanol (1:1 mole ratio).

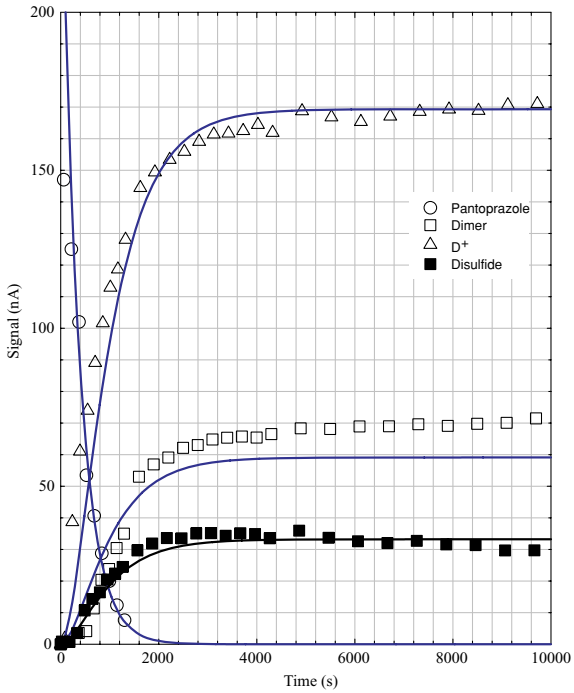


Figure 4 Current (nA)–time (s) profiles for (1) pantoprazole, (2) pantoprazole cyclic sulfenamide (D^+), (3) unknown degradation product, and (4) disulfide measured from a buffered solution (pH 4.0, 37°C) in the presence of 2-mercaptoethanol (1:1 mole ratio).

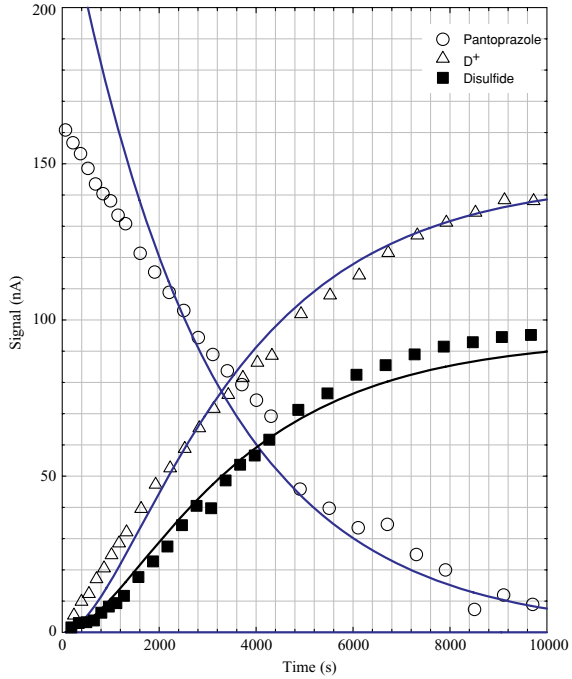


Figure 5 Current (nA)–time (s) profiles for (1) pantoprazole, (2) pantoprazole cyclic sulfenamide (D^+), (3) unknown degradation product, and (4) disulfide measured from a buffered solution (pH 5.0, 37°C) in the presence of 2-mercaptoethanol (1:1 mole ratio).

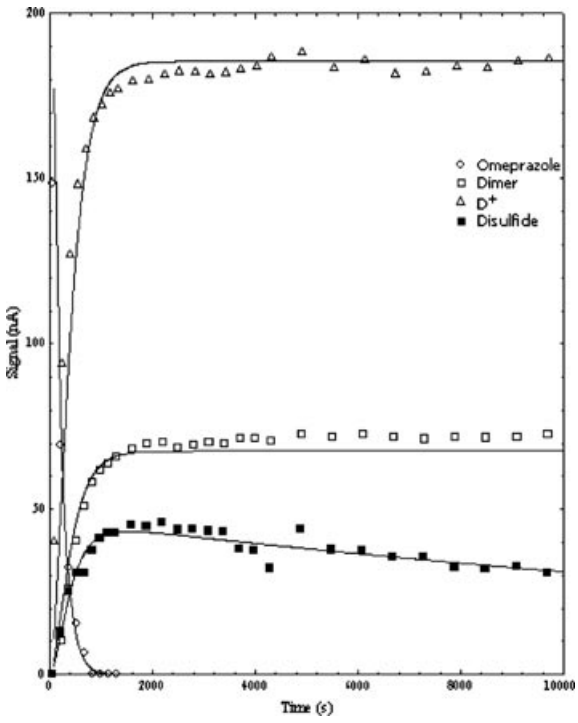


Figure 6 Current (nA)–time (s) profiles for (1) omeprazole, (2) omeprazole cyclic sulfenamide (D^+), (3) unknown degradation product, and (4) disulfide measured from a buffered solution (pH 4.0, 37°C) in the presence of 2-mercaptoethanol (1:1 mole ratio).

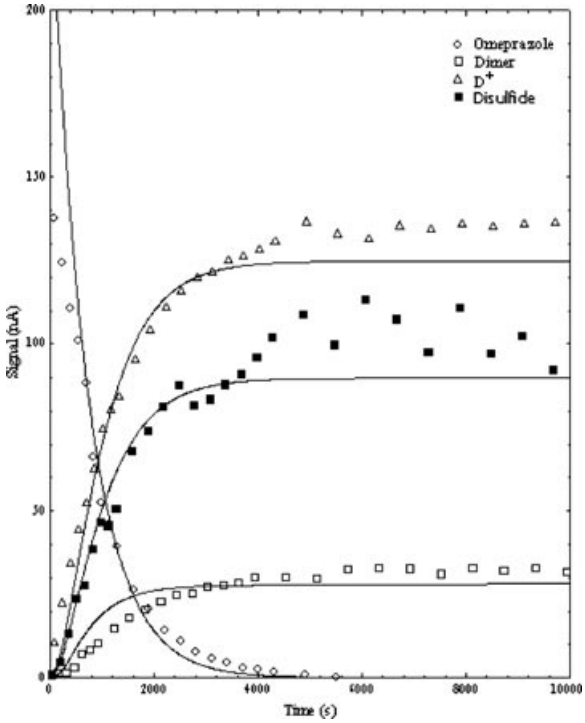


Figure 7 Current (nA)–time (s) profiles for (1) omeprazole, (2) omeprazole cyclic sulfenamide (D^+), (3) unknown degradation product, and (4) disulfide measured from a buffered solution (pH 5.0, 37°C) in the presence of 2-mercaptoethanol (1:1 mole ratio).

Table II Summary of Rate Constants Measured in Solutions Buffered to Different pH Values (37°C) for the Conversion of Pantoprazole and Subsequent Reactions in the Presence of 2-Mercaptoethanol

pH	k_{AB} (s^{-1})	k_{BC} (s^{-1})	k_{BD} (s^{-1})	k_{BS2} (s^{-1})
2	0.007797	0.004285	[a]	0.001754
3	0.004569	0.003618	[a]	0.00150
4	0.00277	0.000903	0.000177	0.000315
5	0.000346	0.000608	0.000394	3.75E-68

[a]The signal of disulfide was not detected.

Table III Summary of Rate Constants Measured in Solutions Buffered to Different pH Values (37°C) for the Conversion of Lansoprazole and Subsequent Reactions in the Presence of 2-Mercaptoethanol

pH	k_{AB} (s^{-1})	k_{BC} (s^{-1})	k_{BD} (s^{-1})	k_{BS2} (s^{-1})
2	0.013371	0.004179	0.001774	0.002114
3	0.013709	0.003762	0.001735	0.001659
4	0.007352	0.002202	0.00098	0.001215
5	0.001538	0.001122	0.001291	0.000266

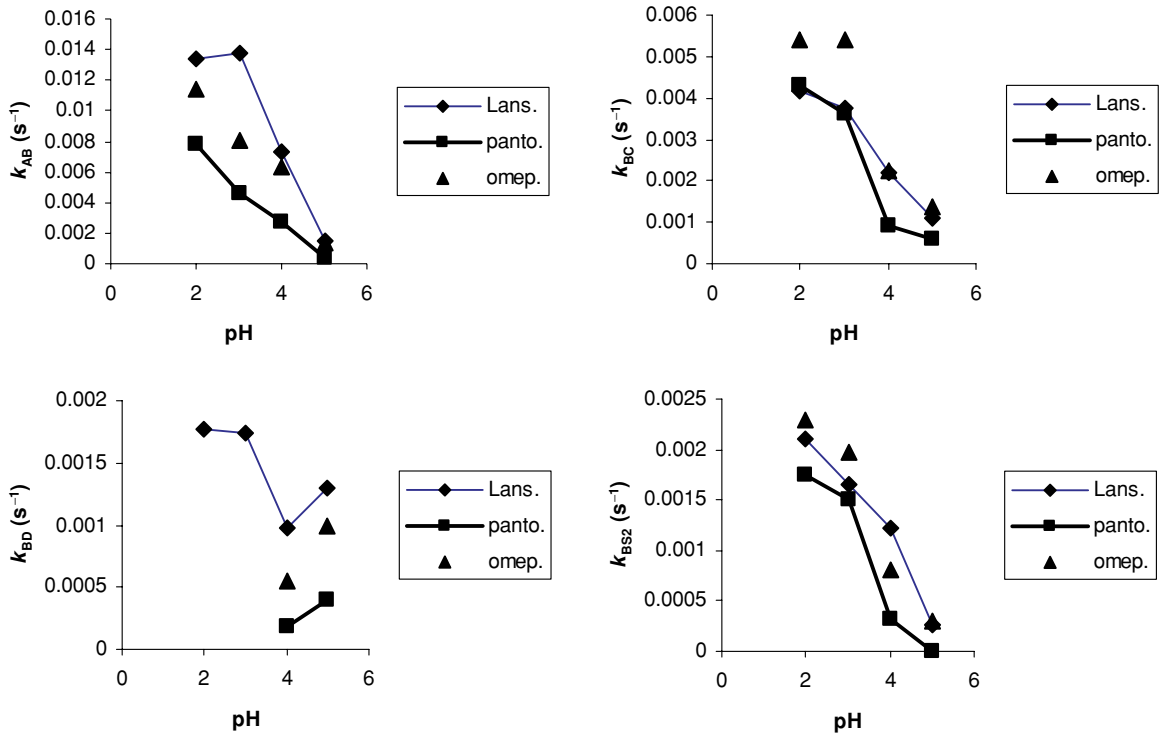


Figure 8 Comparison of the first-order rate constants for the conversion of PPIs.

of sulfenic acid to the cyclic sulfenamide, (3) degradation to an unknown product, and (5) the reaction of PPI sulfenic acid with 2-mercaptoethanol to form the disulfide. The rate constants were evaluated for solutions buffered to pH values of 2.0, 3.0, 4.0, and 5.0.

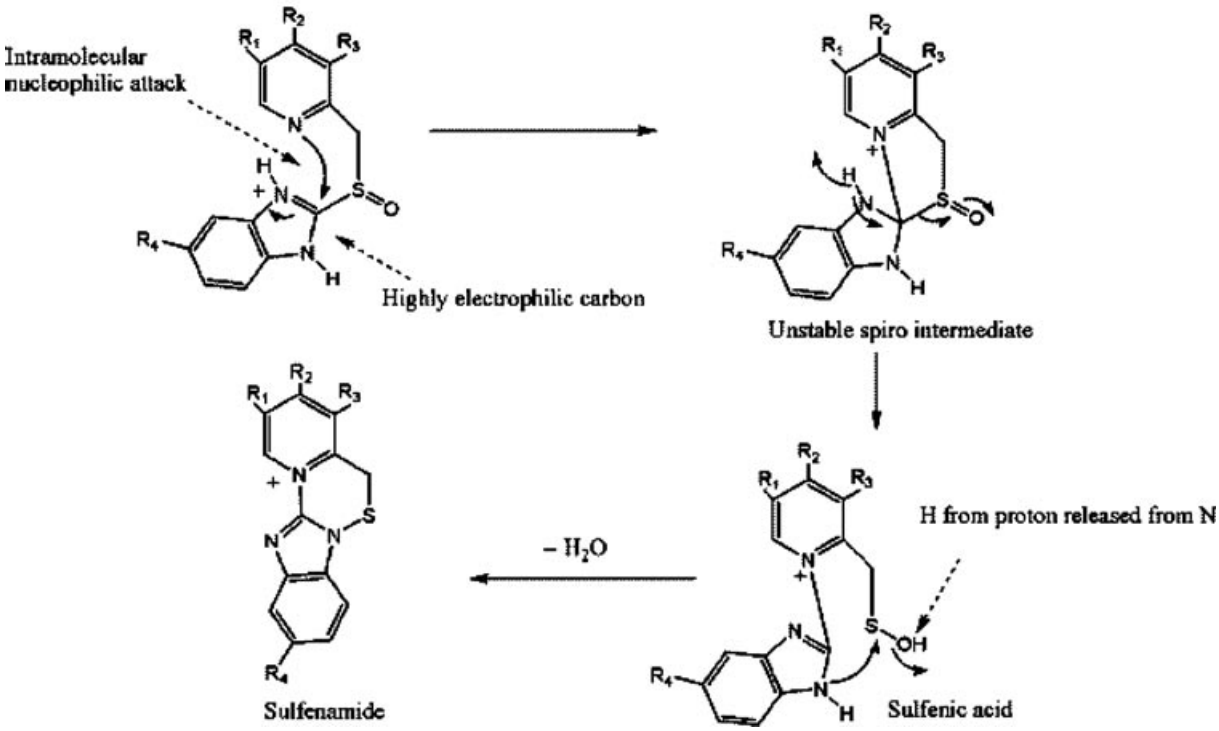
Figure 8 illustrates that the rate of degradation of PPI to sulfenic acid, the formation of the cyclic sulfenamide, and unknown degradation product, all decrease with the increase of pH from 2 to 5. The formation of sulfenic acid from PPI involves protonation of the benzimidazole nitrogen followed by the attack of pyridinic nitrogen on the C2 of benzimidazole ring-forming spiro compound [2] that restores aromaticity by forming sulfenic acid (Scheme 3). Furthermore, the formation of the cyclic sulfenamide from sulfenic acid involves elimination of water, which is facilitated by acid.

Although the acidic medium is necessary for the formation of sulfenic acid, the key intermediate believed to be the active inhibitor [12–14], the acid also converts this sulfenic acid into degradation products that are unreactive toward 2-mercaptoethanol. Thus, it is interesting to note that the rate of formation of the disulfide from the reaction of 2-mercaptoethanol with sulfenic acid decreases with the increase of pH from 4 to 5 (Fig. 8). No disulfide was observed at pH 2.0 and 3.0 in the case of omeprazole and pantoprazole.

Another interesting point is that the rate of transformation of sulfenic acid into degradation products is higher than the rate of reaction of sulfenic acid with thiol (Tables I–III). This gives an indication that most of the PPIs are consumed as degradation product and not as inhibitor of the sulfhydryl group of the enzyme.

Comparison of the Kinetics of PPIs

Conversion of PPIs into Sulfenic Acid. In the pH range 2.0–5.0, the rate of degradation of PPIs into sulfenic acid (k_{AB}) follows the following order: lansoprazole > omeprazole > pantoprazole (Fig. 8). The methoxy group of omeprazole is not in direct resonance with the nitrogens of benzimidazole. Consequently, due to the inductive effect (the electron-withdrawing effect), it is expected to decrease the basicity of the benzimidazole nitrogen compared with lansoprazole, which has no substituent on the benzimidazole ring. The benzimidazole ring of pantoprazole is expected to have the least basic nitrogen because of its more electron-withdrawing substituents on the benzimidazole ring (OCF_2H) than omeprazole. One may, therefore, arrange the theoretical basicity of benzimidazole nitrogen, with respect to the inductive effect of substituents, in the following order:

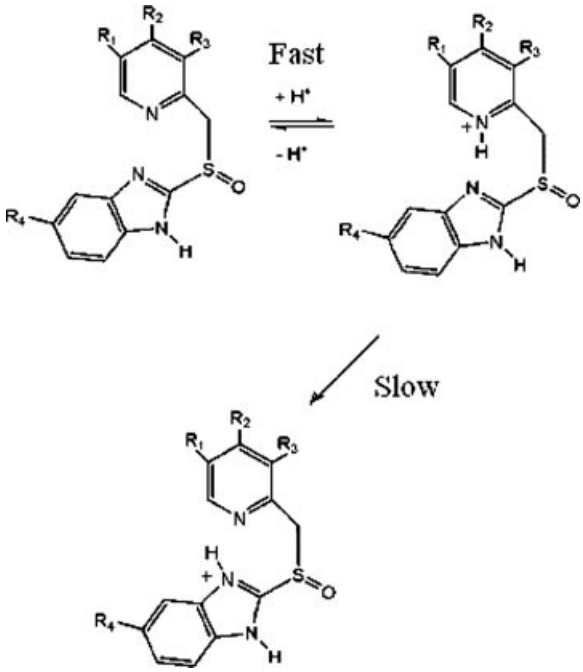


Scheme 3 Mechanism of formation of sulfenic acid/sulfenamide.

lansoprazole > omeprazole > pantoprazole. This order was consistent with the order of decreasing the observed rate of degradation of PPIs (k_{AB}).

Attempts to correlate the rate constants of degradation of PPIs into sulfenic with the basicity of pyridinic PPIs were without success because the order of basicity of pyridinic PPIs follows the order: omeprazole (4.06) > pantoprazole (3.83) = lansoprazole (3.83) as predicted from their pK_a values. The protonation of the pyridine nitrogen is rapid compared with benzimidazole because of the higher pK_a value of the pyridine nitrogen [14]. Consequently, we can, safely, assume that proton exchange with benzimidazole is the rate-limiting step, which determined the degradation reactions (Scheme 4).

The Formation of Disulfide. As shown in Fig. 8, the rate of reaction of sulfenic acid with 2-mercaptoethanol to form the disulfide follows the following order: lansoprazole > omeprazole > pantoprazole. At low pH values (2 and 3), and in contrast with omeprazole and pantoprazole, the reaction between lansoprazole and 2-mercaptoethanol was significant (Fig. 8). The electrophilicity of the sulfur atom of sulfenic acid is expected to be the limiting factor that determines the reaction sulfenic acid with nucleophilic thiol. The sulfur atom of lansoprazole sulfenic acid is the most electrophoric because of the presence of the strong electron-withdrawing group (OCH_2CF_3) on the pyridine group.



Scheme 4 Protonation reactions of PPIs.

Conversion of the Sulfenic Acid into the Cyclic Sulfenamide. In general, and as shown in Fig. 8,

pantoprazole always shows the lowest rates of conversion into cyclic sulfenamide (k_{BC}). However, the rates of omeprazole and lansoprazole are interchangeable. As in the case of formation of disulfide, it seems that the electrophilicity of the sulfur atom is important for the attack of pyridine nitrogen (Scheme 3).

CONCLUSION

An in vitro investigation of the kinetics of the acid-catalyzed conversions, and subsequent reactions of PPIs have been carried out. The kinetic model facilitated the estimation of the rate constants in the presence of 2-mercaptoethanol for (1) the acid-catalyzed degradation of PPI, (2) formation of sulfenic acid, (3) formation of the cyclic sulfenamide, (4) degradation to an unknown degradation product, and (5) reaction between sulfenic acid and 2-mercaptoethanol to form the disulfide.

Decreasing pH of solution facilitates the formation of sulfenic acid of PPIs and its conversion into degradation products that are unreactive toward 2-mercaptoethanol. On the other hand, decreasing pH decreases the rate of formation of the disulfide from the reaction of 2-mercaptoethanol with sulfenic acid.

The rate of degradation of PPIs and subsequent reactions with 2-mercaptoethanol were found to follow the following general order: lansoprazole > omeprazole > pantoprazole. The acid-catalyzed degradation of PPIs into sulfenic acid depends mainly not on pyridine nitrogen protonation but on protonation of the benzimidazole nitrogen. The basicity of the benzimidazole nitrogen seems to be the factor that plays a major role in determining the rate of degradation of the investigated PPIs. Protonation of this nitrogen is a prerequisite for the nucleophilic attack of pyridine nitrogen on the C-2 benzimidazole.

The rate of conversion of the sulfenic acid of PPIs into the disulfide (the inhibition reaction) was found to be dependent on the electrophilicity of the sulfur atom of the sulfenic acid, which is determined by the substituents on the pyridine ring. Thus in designing of effective PPI, substituents must be controlled so that maximum basicity of the benzimidazole ring and maximum electrophilicity of the sulfur is achieved.

The rate of transformation of sulfenic acid into degradation products is higher than the rate of reaction of sulfenic acid with thiol. This gives an indication that most of the PPIs are consumed as degradation product and not as inhibitor of the sulfhydryl group of the enzyme.

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