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International Journal of Pharmaceutics 323 (2006) 110–116
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

An in vitro investigation on acid catalyzed reactions of proton pump inhibitors in the absence of an electrophile

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Received 15 March 2006; received in revised form 6 May 2006; accepted 25 May 2006
Available online 2 June 2006

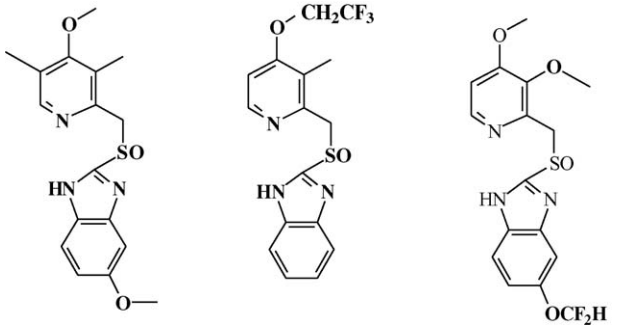
Abstract

An in vitro investigation of the complex system of acid catalyzed conversions of some proton pump inhibitors (PPI) have been carried out using differential pulse polarographhy (DPP) at the static mercury drop electrode (SMDE). Reactions were investigated in solutions buffered to pH values 2.0–8.0. The employed technique facilitated fast and timely monitoring of each PPI, in addition to its electroactive degradation products. Unlike previous techniques, which employed HPLC and UV spectroscopy, alone, or in combination, DPP facilitated fast and simultaneous recordings of all analytes in situ. This resulted in well-defined current–time profiles of individual electroactive degradation products, in addition to those of the starting materials. The results demonstrated that the rates of degradation of the investigated PPIs can be arranged in the following order: lansoprazole > omeprazole > pantoprazole. Furthermore, the rate of degradation of PPIs decreased with decreasing basicity of the corresponding benzimidazole nitrogen of PPIs, as predicted by the effect of individual substituents on each of the benzimidazole rings. The present study demonstrated that lansoprazole may have the fastest accumulation rate in the parietal cells, in addition to demonstrating the highest rate of conversion into the active inhibitor. At pH 6, however, pantoprazole was found to be the most stable, whereas lansoprazole and omeprazole underwent significant acid catalyzed decomposition. The cyclic sulfenamide of pantoprazole demonstrated highest stability among the three PPIs. Omeprazole, however, fell in the middle in terms of its stability and the stability of its corresponding cyclic sulfenamide. The present study may provide an insight for designing more potent new proton pump inhibitors.

Keywords: Proton pump inhibitor; Degradation; Polarography

1. Introduction

The chemistry of proton pump inhibitors (PPIs) including: omeprazole (I) lansoprazole (II) and pantoprazole (III) led to a new era in the effective therapy of acid-peptic diseases. Their accumulation in the most acidic space in the body, together with their acid catalyzed conversions into their corresponding active inhibitors (Lindberg et al., 1986; Brändström et al., 1989a,b,c,d,e,f; Shin et al., 2004) has been correlated with their intriguing chemistry.



Omeprazole (I) **Lansoprazole (II)** **Pantoprazole (III)**

PPIs have different pryridinic and benzimidazole substituents which directly impact their mechanism of action and directly affect their rates of reactions. The pK_a of these PPIs are 3.8 for pantoprazole, 3.9 for lansoprazole and 4 for omeprazole.

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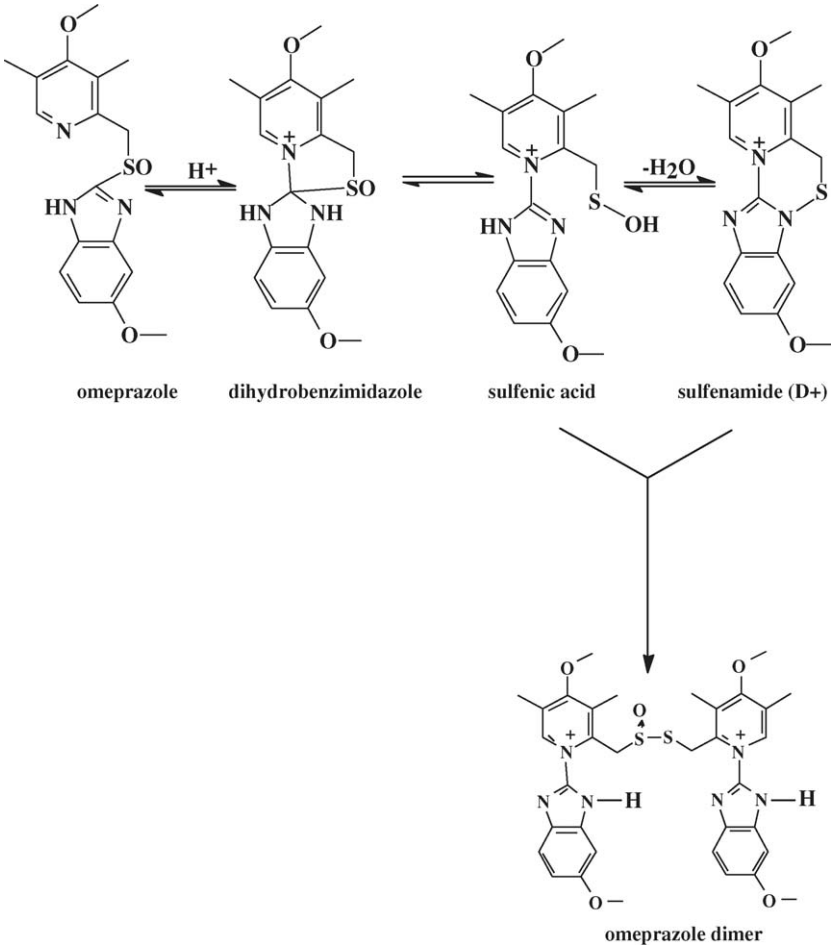
doi:10.1016/j.ijpharm.2006.05.057

Except for the work of Shin et al. (2004), no in vitro comparisons between the acid conversions of PPIs have been published so far. Shine et al investigated only the rates of degradation of PPIs using UV spectroscopy, no attempt was made to quantitatively evaluate or monitor the formation of degradation products. In particular, no emphasis was given to evaluate the kinetics of formation or the subsequent reactions of the resulting active inhibitors.

Following the author's work on the acid degradation of omeprazole (Qaisi et al., 2006), and on the reactions of sulfenic acid with 2-mercaptoethanol; which investigated the mechanism for the inhibition of gastric (H^+, K^+)-adenosine triphosphate by omeprazole (Tutunji et al., 2006), the authors investigated acid degradation 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 were then compared. Unlike other reports, the present work evaluated the rates of degradation of the three investigated PPIs in vitro, at different pH values, in addition to comparing current–time curves for all electroactive degradation and reaction products in situ.

The following scheme of acid catalyzed degradation of omeprazole, proposed by previous workers (Lindberg et al., 1986; Brändström et al., 1989a,f; Qaisi et al., 2006) was employed in the present study:



Experimental evidence demonstrated that the same scheme can also adopted for lansoprazole and pantoprazole.

2. Experimental

2.1. Chemicals and reagents

Omeprazole, pantoprazole and lansoprazole working standards 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 (Darmstadt, Germany).

All other reagents were used without further purification. Ultrapure water was obtained by initially passing through two reversed osmosis cycles before initiating a distillation followed by a deionization step. Oxygen free nitrogen was used for dearation, of each test solution, prior to initiating successive DPP cycles.

2.2. 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 multi-mode electrode (MME) comprising a static mercury drop electrode (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 (DPP) at the static mercury drop electrode (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.

2.3. Procedures

After adjusting the pH of individual phosphate buffer solutions (0.05 M) to the experimental value, a 10.0 mL aliquot was pipetted into the polarographic vessel. Dearation was started by passing nitrogen gas for 8 min, a background signal was measured prior to introducing PPI (100 μL, 3.0×10^{-3} M). This was immediately followed by initiating successive recordings of individual differential pulse polarograms. Recordings were repeated until the PPI degraded completely and no analytical signal (current, nA) was detected. DPP profiles were scanned between 0.00 and −2.00 V versus Ag/AgCl. Nine cathodic reduction cycles (each lasting for 153 s) were repeatedly recorded in each of the four measurement groups. Peak potential(s) (volts versus Ag/AgCl saturated with a 3.0 M KCl solution) and peak current(s) (nA) were recorded. The same procedures were followed using constant concentrations of individual PPI's (3.0×10^{-5} M). Each solution was buffered to a different pH value including: 2.0, 3.0, 4.0, 5.0, 6.0, 7.5 and 8.0. Peak currents (nA), peak potentials (volts versus Ag/AgCl), and time (minutes) were tabulated for all cycles in each group. Representative differential pulse polarogram for omeprazol, lansoprazole and pantoprazole are illustrated in Fig. 1 for four groups covering the time intervals between 0.00 and 19.00 min.

3. Results and discussion

3.1. Identification of polarographic peaks

The three PPIs and their degradation products demonstrated similar reduction peaks (Fig. 1A–C) identification of omeprazole, lansoprazole, pantoprazole and their corresponding cyclic sulfenamides were identified using the standard addition technique. Each cyclic sulfenamide (D^+) was prepared and isolated from a methanolic solution of individual PPI treated with perchloric acid. Authentication of D^+ was performed using Mass spectroscopy, ^1H NMR, FTIR, and UV-spectroscopy. The mass spectrum of the omeprazole cyclic sulfenamide gave an M^+ peak at 328. The ^1H NMR spectrum of the cyclic sulfenamide (two isomers) in deuterated methanol was characterized by the an extreme down field shift of the signal of proton of pyridino moiety ($\delta = 9.37$) compared to 8.1 ppm for omeprazole. The

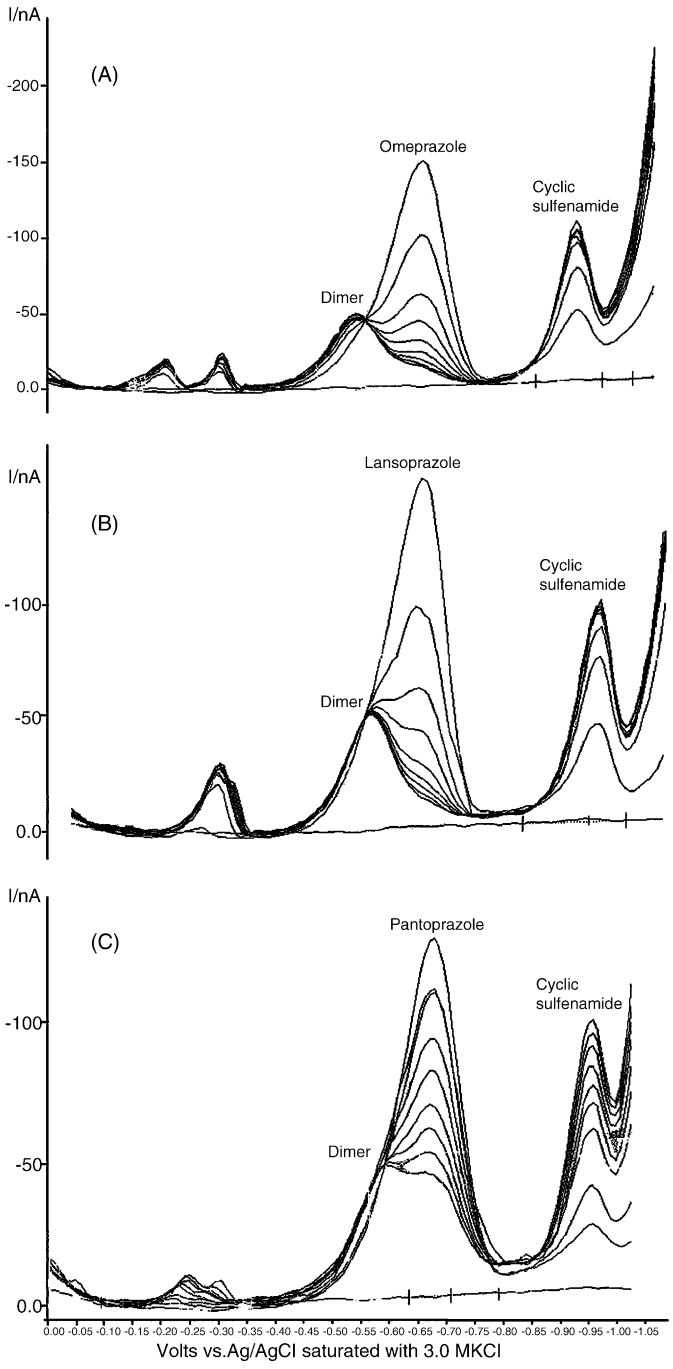


Fig. 1. Representative differential pulse polarograms (DPP) at the static mercury drop electrode (SMDE) for (A) omeprazole, (B) lansoprazole and (C) pantoprazole (3.00×10^{-5}) in a buffered solution (0.05 M, pH 4) illustrating the decay curves of: omeprazole and the appearance of the cyclic sulfenamide and dimer scanned between 0.00 and 19.0 min.

chemical shifts of the two-methyl groups of the pyridine group of D^+, were observed at 2.4 and 2.6 ppm, compared to 2.1 and 2.2 ppm for the original omeprazole. The observed downfield shift of substituents on the pyridine ring of D^+ confirmed that the pyridine ring was indeed positively charged. Further evidence to characterize D^+ was the observed upfield chemical shift of CH_2–S of D^+ at 4.3 ppm, compared with 4.7 ppm in the case of CH_2–S=O of the omeprazole. IR spectra demonstrated that

omeprazole and D^+ spectra were significantly different, the latter has a characteristic absorption at 1621 cm^{-1}. The UV spectrum of a methanolic solution of D^+ gave two peaks at 26 and 360 nm in methanol (Qaisi et al., 2006).

The yellow crystalline product was subsequently used to confirm that the assignments of the corresponding cyclic sulfenamide peak (D^+) for individual PPIs. Similarly the same procedures were done for lansoprazole and pantoprazole.

The dimer was assigned to the peak shown in Fig. 1 because it is the major product expected to result from the acid decomposition of each PPI as reported in the authors previous work (Brändström et al., 1989a; Qaisi et al., 2006).

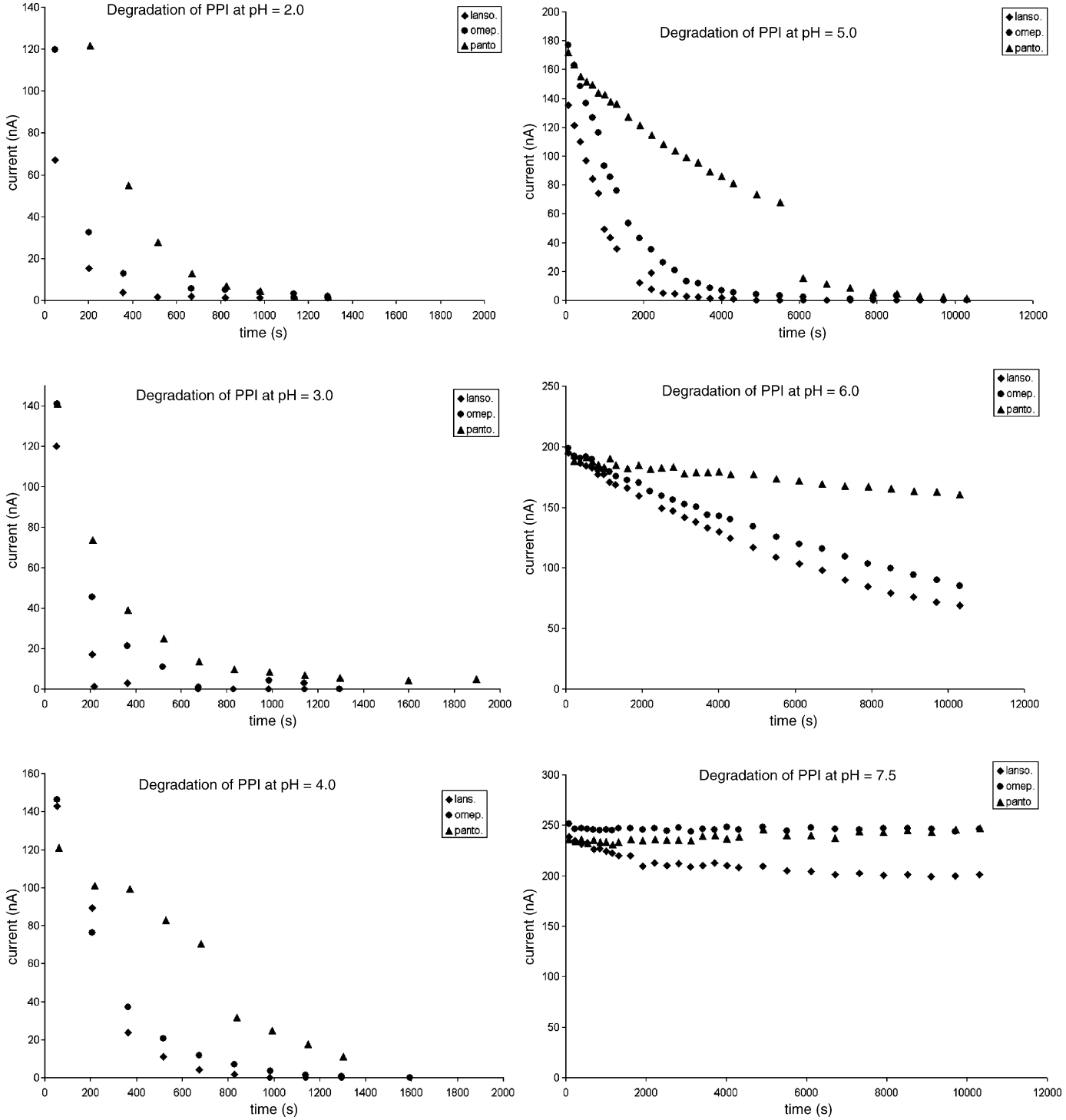


Fig. 2. Current (nA)–time (s) decay curves of omeprazole, lansoprazole and pantoprazole (3.00×10^{-5}) in solutions individually buffered to pH values of: 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0.

3.2. Electrochemical behavior of PPI and its related acid decomposition products

The electrochemical behavior of PPIs (3.00×10^{-5} M) in a 0.05 M phosphate buffer gave rise to well defined cathodic reduction peaks. As shown in Fig. 1A and B, the behavior of PPIs were comparable. Since the working electrode was a static mercury drop, the observed reduction peaks were believed to be due to sulfur adsorption prior to electrochemical reduction of each analyte. Adsorption is believed to be a prerequisite for observing the reduction peaks. Three other observations contributed to this conclusion, (1) the three PPIs, which differed only in substituents on the pyridine and on benzimidazole, demonstrated similar reduction peaks (2) the 2-mercaptoethanol, which has no pyridine or benzimidazole moieties gave a well defined reduction peak, using the same experimental conditions (3) the cyclic voltammograms of freshly prepared omeprazole, lansoprazole and pantoprazole solutions in a phosphate buffer solutions (1.23×10^{-3} M, pH 8) demonstrated irreversibility, when measured at different sweep rates: 24, 12, 6, 3 and 1.5 V/s (Qaisi et al., 2006). It is worth mentioning that even though the electrode reactions are irreversible, carry over effects were not anticipated, since the mercury drop electrode was automatically renewed prior to individual recordings of the successive voltammograms.

3.3. Effect of pH on peak potential(s) of each PPI and theirs related acid decomposition products

Similar to omeprazole (Qaisi et al., 2006; Tutunji et al., 2006), it was observed that the reduction potential(s) of lansoprazole and pantoprazole together with their degradation products shift cathodically, as solution pH increases from 2.0 to 8.0. This indicates that protonation of the electroactive site of the molecule affect the overall electrode reaction mechanism.

3.4. Acid degradation of PPIs

In the pH range 2.0–6.0, the rates of degradation of PPIs demonstrated the following order: lansoprazole > omeprazole > pantoprazole. A similar trend was observed by previous workers (Shin et al., 2004). At a pH value of 6.0, pantoprazole was stable whereas lansoprazole and omeprazole degraded significantly. At a pH value of 7.5, pantoprazole and omeprazole were stable while lansoprazole degraded slightly (Fig. 2).

The methoxy group of omeprazole is not in direct resonance with the nitrogens of benzimidazole. Consequently, due to inductive effect (electron withdrawing effect), it is expected to decrease the basicity of the benzimidazole nitrogen compared with lansoprazole, which has no substituent on the benzimida-

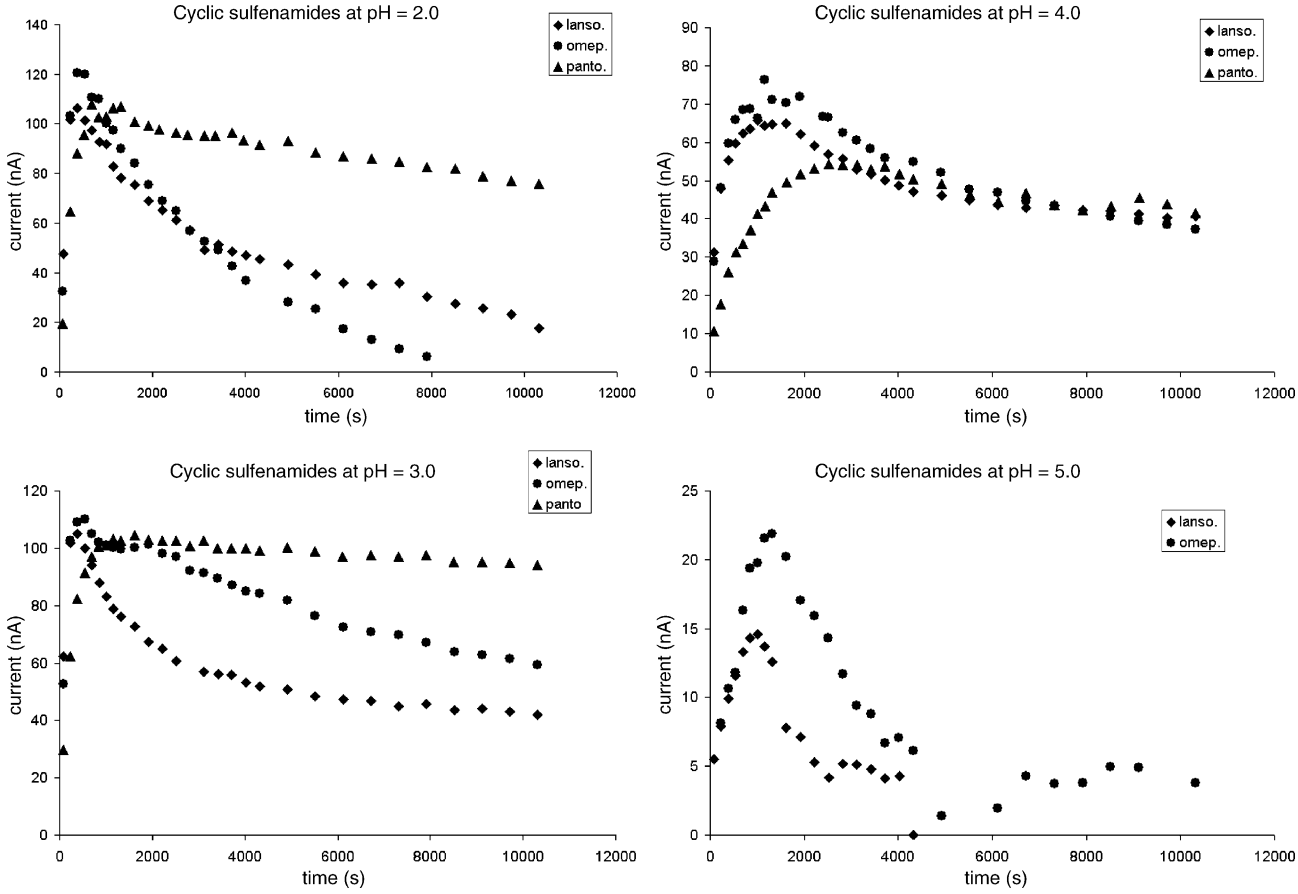


Fig. 3. Current (nA)–time (s) profiles for the corresponding cyclic sulfenamide of omeprazole, lansoprazole and pantoprazole (3.00×10^{-5} M) in solutions were individually buffered to pH values of 2.0, 3.0, 4.0 and 5.0.

zole 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) compared with omeprazole. One may, therefore, arrange the theoretical basicity of benzimidazole nitrogen, with respect to the inductive effect of substituents, in the following order: lansoprazole > omeprazole > pantoprazole. This was consistent with the observed rates of degradation of the investigated PPIs.

Acid-catalyzed degradation of PPIs depends mainly on protonation of the imidazole nitrogen. The basicity of the benzimidazole nitrogen seems to be the factor which plays a major role in determining the rate of degradation of each PPI. Protonation of this nitrogen is a pre-requisite for the nucleophilic attack of pyridinic nitrogen on the C-2 benzimidazole. In spite of the fact that protonation steps are usually fast in organic reactions, protonation of the benzimidazole nitrogen seems to be the rate-limiting step in the degradation of the investigated PPIs. This observation is justified if we note that (i) protonation of hydrophobic molecules such as PPIs is difficult (ii) there is a strong competition from the pyridinic basic nitrogen.

3.5. The cyclic sulfenamides of PPIs

As shown in Fig. 3, the stability of the cyclic sulfenamides resulted in the following order at pH values 2 and 3: pantoprazole > omeprazole > lansoprazole. This may be due to the fact that the cyclic sulfenamides of omeprazole has more electron-donating groups on the pyridinic moiety compared to that of pantoprazole. The latter has more electron donating groups compared to lansoprazole. The sum of Hammett σ values for the substituents on the pyridinic ring of PPIs follow the following order of decreasing electron donating power: omeprazole (-0.41) > pantoprazole (-0.15) > lansoprazole (0.09) (Cary and Sundberg, 1983). Since electron-donating groups are able to stabilize the positive charge on the sulfonam ring of the cyclic sulfonamide, the cyclic sulfonamide of omeprazole should demonstrate highest stability, whereas lansoprazole should demonstrate the lowest.

In solutions buffered to pH 4, the rates of formation of the cyclic sulfonamide of individual PPIs (Fig. 3) were consistent with the order of degradation of PPIs: lansoprazole > omeprazole > pantoprazole. PPIs, therefore, degraded to their corresponding cyclic sulfenamides. This trend was also observed in solutions buffered to pH values 2.0 and 3.0 since the fast formation of the cyclic sulfonamide from the investigated PPIs was observed (Fig. 3).

3.6. Appearance of the dimers

The rates of formation and stability of the dimers of the investigated PPIs (Fig. 4) follow the same trend as those of the cyclic sulfenamide, since the stability of both the dimer

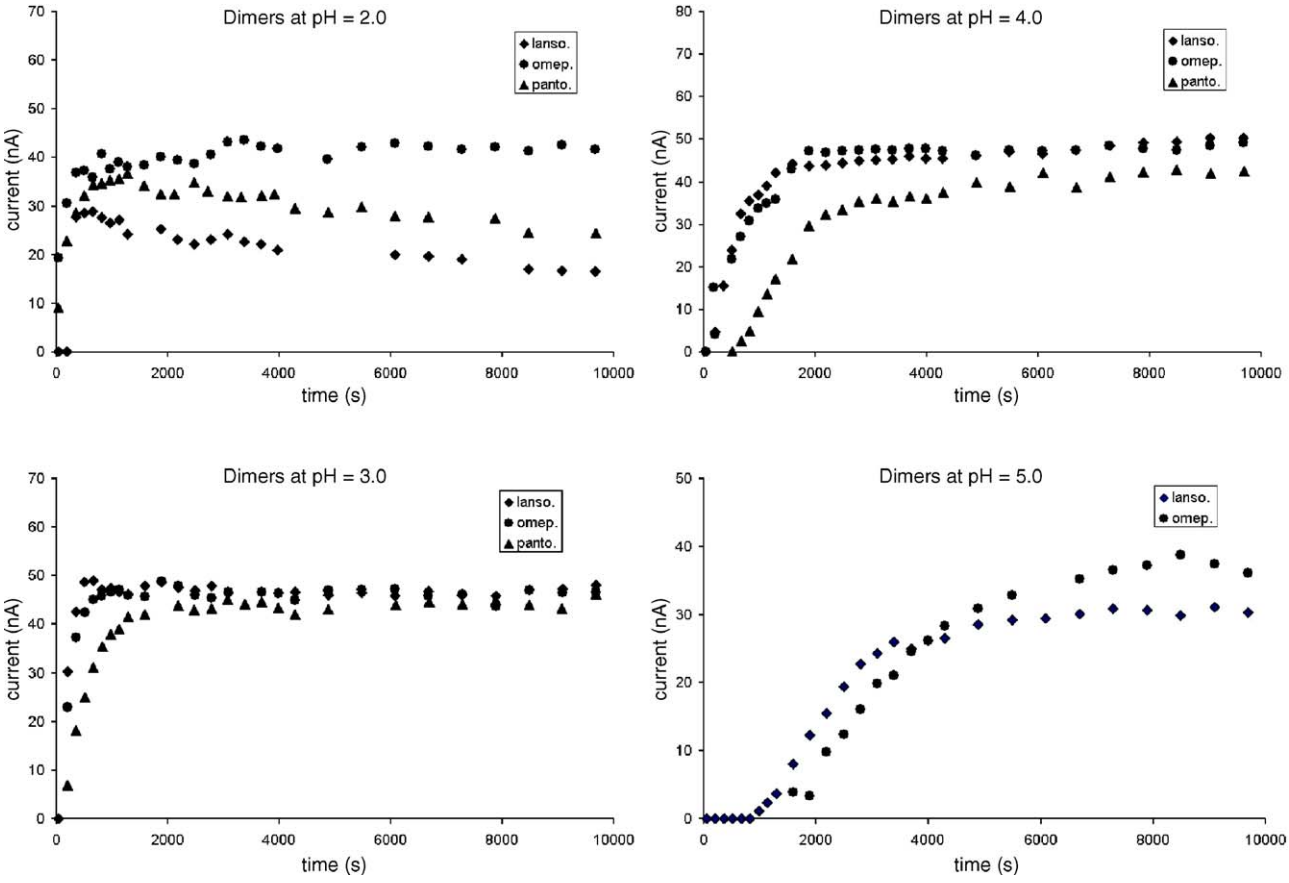


Fig. 4. Current (nA)–time (s) profiles for the dimers of omeprazole, lansoprazole and pantoprazole (3.00×10^{-5} M) in solutions were individually buffered to pH values of 2.0, 3.0, 4.0 and 5.0.

and cyclic sulfonamide are governed by the same electronic factors.

4. Conclusions

Contrary to previous studies which employed UV spectrophotometry, the present electroanalytical technique facilitated monitoring of the degradation of PPIs in addition to the appearance of all electroactive degradation products. The rates of degradation of the investigated PPIs were found to be directly dependant on the basicity of benzimidazole nitrogen of individual PPIs.

Lansoprazole and omeprazole undergo significant degradation even in solution having relatively high pH values (pH 6).

The present work clearly demonstrated that the addition of electron withdrawing groups to the benzimidazole ring increased the stability of proton pump inhibitors and therefore decreased the rates of the degradation.

As a consequence, decrease in the rate of transformation into the active inhibitor was observed. For the same reason, the basicity of the PPI decreased, which may cause a decrease in the rate of accumulation in the parietal cells.

The authors believe that the results of the present work provide further insight into the design of a more potent PPI.

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