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Differential pulse polarographic investigation of lansoprazole and rabeprazole using dropping mercury electrode

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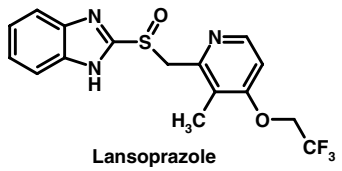
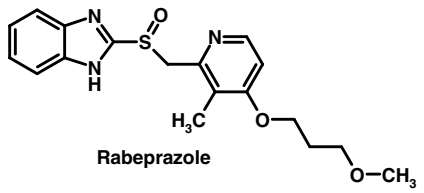
The electrochemical reduction of the proton pump inhibitors (PPI) lansoprazole and rabeprazole has been investigated by differential pulse polarography (DPP) using a dropping mercury electrode (DME). The results were compared not only among both substances but also among other proton pump inhibitors depending on the varying chemical structures of the agents. All investigations were carried out in Britton-Robinson buffer solutions with pH values from 3.0 to 11.0. It was shown that both PPI undergo an extensive decomposition decreasing with increasing pH values forming two main compounds, a cyclic sulfenamide and a dimer. In this case lansoprazole was found to be stable at pH 8.0 and rabeprazole at pH 9.0. The decomposition of rabeprazole ran considerably quicker and also up to higher pH values than those of lansoprazole. The peak currents varied linearly with the concentration of both PPI in the range from 1×10^{-6} M to 7×10^{-5} M at pH 9.0. Both substances showed similarities in reaction as well as individual differences based on their varying chemical structures and characteristics.

1. Introduction

The proton pump inhibitors (PPI) lansoprazole, 2-[(3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methylsulfinyl]-1 H-benzimidazole, and rabeprazole, 2-[(4-(3-methoxypropoxy)-3-methyl-pyridin-2-yl)methylsulfinyl]-1H-benzimidazole, are potent agents in the treatment of acid dependent gastro-intestinal diseases, such as peptic ulcer, reflux oesophagitis and Zollinger-Ellison syndrome. Their chemical structures consist of a benzimidazole linked by a methylsulfinyl chain with an individually substituted pyridine.



Lansoprazole



Rabeprazole

There are only a few reports describing the electrochemical reduction of lansoprazole in pharmaceutical dosage forms. A study using cyclic voltammetry and direct current as well as differential pulse polarography (Yardimci and Özaltin 2001) and an adsorptive stripping square-wave voltammetric study (Radi 2002) is described. Another investigation on acid catalyzed reactions is also reported using differential pulse polarography (Tutunji et al. 2006). There is a research using alternating current polarography (El-Enany et al. 2008). In addition, the electrochemical oxidation of lansoprazole is described at a carbon paste electrode by cyclic and differential pulse voltammetry (Radi 2003a). However, hardly any electrochemical determination of rabeprazole is reported. There is only one investigation about its oxidative reaction at a glassy carbon electrode using cyclic, linear sweep and differential pulse polarography (Radi et al. 2004).

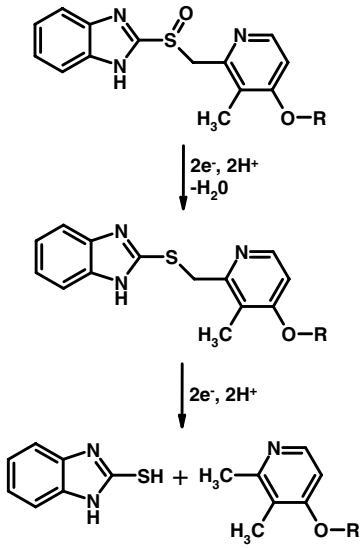
Therefore, the aim of the present study was to investigate the electrochemical reduction of lansoprazole and rabeprazole under the influence of several parameters, e.g. different pH values or concentrations of the analytes, by differential pulse polarography using the dropping mercury electrode. The results are going to be discussed not only among both substances but also among other PPI depending on the varying chemical structures of these agents.

2. Investigations and results

2.1. Proposed mechanism

The mechanism of the reduction of omeprazole has already been investigated (Oelschläger and Knoth 1998). Because of the same characteristic chemical structures of the agents it is also supposed to be valid for pantoprazole (Radi 2003b) and lansoprazole (Yardimci and Özaltin 2001, Radi 2002) as well as for rabeprazole (Scheme 1).

Scheme 1 Electrochemical reduction mechanism of lansoprazole and rabeprazole



The electrochemical active site of the PPI is located in the sulfoxide group. It is assumed that the four electron-four proton reduction process consists of two steps. First the sulfoxide group is being reduced under an uptake of two electrons and two protons forming a thioether derivative. This is followed by a reductive cleavage of the thioether bond using another two electrons and two protons to give rise to 2-mercaptobenzimidazole and an individually substituted 2-methylpyridine.

2.2. Influence of pH value

The influence of the pH value on the reduction of lansoprazole and rabeprazole at the DME has been studied in the pH range from 3.0 to 11.0. A 1×10^{-3} M standard solution was used for the measurements. Using the standard addition technique, 50 µl of each standard solution was added to the polarographic vessel five times, taking three measurements after every addition. Both lansoprazole and rabeprazole had well defined cathodic reduction peaks having characteristic peak potentials and peak currents for each pH value (Fig. 1).

The peak potentials shifted linearly to more negative values with increasing pH in the range from 3.0 to 10.0 by -78.6 mV/pH for lansoprazole ($R^2 = 0.9985$) and -71.2 mV/pH for rabeprazole ($R^2 = 0.9977$) as shown in Fig. 2. The same characteristic was already shown for omeprazole (Oelschläger and Knoth 1998; Qaisi et al. 2006; Tutunji et al. 2007) and pantoprazole (Knoth 2004). In the pH range from 10.0 to 11.0 the lansoprazole peak shifted contrary to more positive potentials while the rabeprazole peak moved on in the negative way. This could be due to the different symmetry of the peaks recorded in that pH range. It can also be assumed that the peak potentials become pH-independent from that point.

Analysing the peak current, it was found that this parameter is also affected by pH value. The peak currents increase with increasing pH values in the range from 3.0 to 9.0 and decrease afterwards (Fig. 3). The same characteristic was already shown for lansoprazole (Radi 2002), whereas the peak current of pantoprazole increases up to pH 7.0 and then decreases again (Radi 2003b). Both lansoprazole and rabeprazole reach its maximum peak current at pH 9.0 with -89.5 nA for lansoprazole and -91.4 nA for rabeprazole and a concentration of 5×10^{-6} M. Particularly in the acidic medium the reduction of rabeprazole led to significant lower peak currents than those of lansoprazole but adapted with rising pH values. Furthermore the measured peak currents decreased with any replication of each addition up to pH 5.0 for lansoprazole and pH 6.0 for rabeprazole. These facts show that there is an extensive degradation of the PPI in the acidic medium and rabeprazole is more sensitive than lansoprazole.

2.3. Acid degradation

It is well known that PPI are acid labile depending on their chemical structure, the prerequisite for their specifically effect on the H^+/K^+-ATPase in the parietal cells of the gastric mucosa. The scheme of acid catalyzed degradation, first proposed for omeprazole in previous studies (Lindberg et al. 1986; Brändström et al. 1989; Quaisi et al. 2006), is supposed to be valid for lansoprazole (Tutunji et al. 2006) and rabeprazole, too (Scheme 2).

In accordance with activating PPI in the acidic medium of the parietal cells of the gastric mucosa, the first step is the protonation of the nitrogen in the benzimidazole of the weak bases. This process seems to be rate-limiting and is the prerequisite for the nucleophilic attack of the pyridinic nitrogen on the carbon two of the benzimidazole forming a spiro derivative. After rearranging to a sulfenic acid and elimination of water, the characteristic cyclic sulfenamide results, which is due to be the active inhibitor of the H^+/

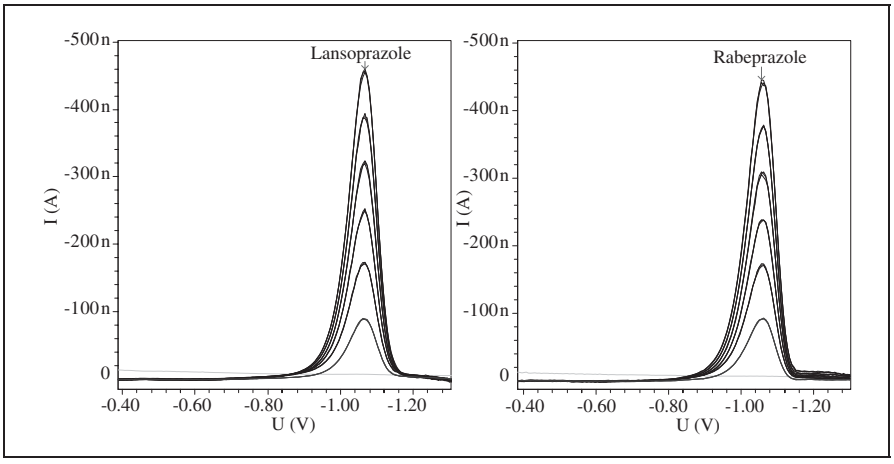


Fig. 1:
Differential pulse polarograms of lansoprazole and rabeprazole (5×10^{-6} M–3×10^{-5} M) at pH 9.0 in Britton-Robinson buffer solution

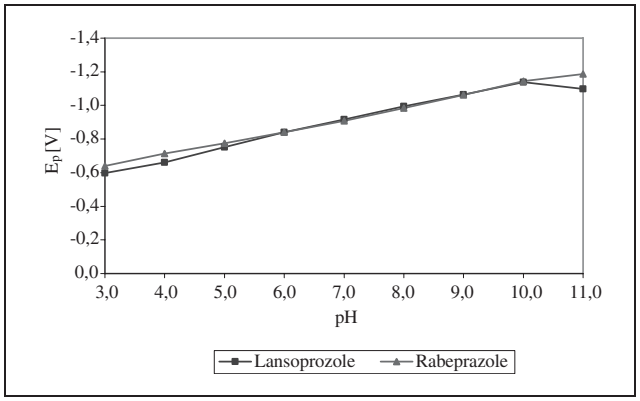


Fig. 2: Effect of pH on the peak potentials of lansoprazole and rabeprazole (5×10^{-6} M) in Britton-Robinson buffer solutions

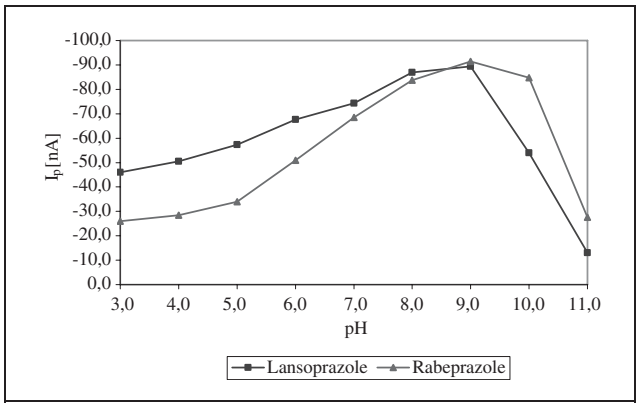
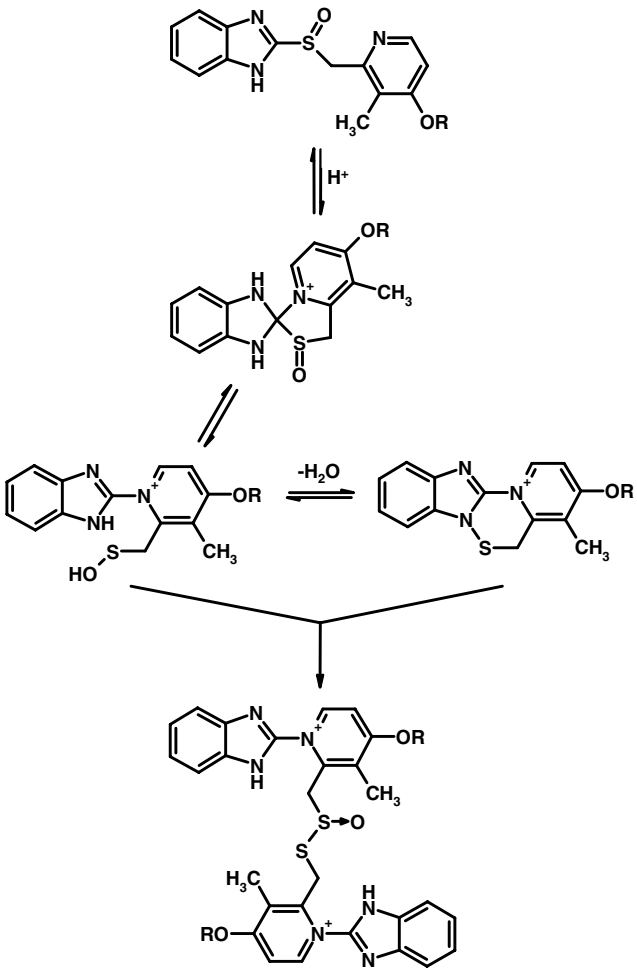


Fig. 3: Effect of pH on the peak currents of lansoprazole and rabeprazole (5×10^{-6} M) in Britton-Robinson buffer solutions

K^+-ATPase (Besancon et al. 1997). At last the sulfenamide and the sulfenic acid accumulate to a dimer connected by a disulfide bond. It is assumed that all these reactions are reversible steps with the exception of forming the final dimer.

In order to investigate the dimension of the acid catalyzed degradation of lansoprazole and rabeprazole, 1×10^{-3} M standard solutions were determined in the pH range from 4.0 to 9.0. The polarogram was recorded once after adding an adequate volume of the standard solution to the Britton-Robinson buffer solution and after every five minutes, respectively. At a pH value of 4.0 both PPI had a peak at -0.661 V for lansoprazole and -0.714 V for rabeprazole, which was quickly divided into two peaks with time, one increasing while the other was decreasing (Fig. 4). The decreasing peak shows the degraded PPI and

Scheme 2 Acid catalyzed degradation of lansoprazole and rabeprazole



the increasing one shows the developed dimer. In addition, a single peak rose over time in more negative potentials, which characterizes the developed cyclic sulfenamide.

Increased pH values led to lower degradation rates of the PPI. But the degradation of rabeprazole ran considerably quicker and also up to higher pH values than those of lansoprazole. In this case the dimer was recorded up to pH 5.0 for lansoprazole and up to pH 7.0 for rabeprazole. The cyclic sulfenamide was found up to pH 7.0 for lansoprazole and up to pH 8.0 for rabeprazole. After measurement duration of 5 min at pH 4.0 the concentration of rabeprazole was only 28% from that of the beginning whereas that of lanso-

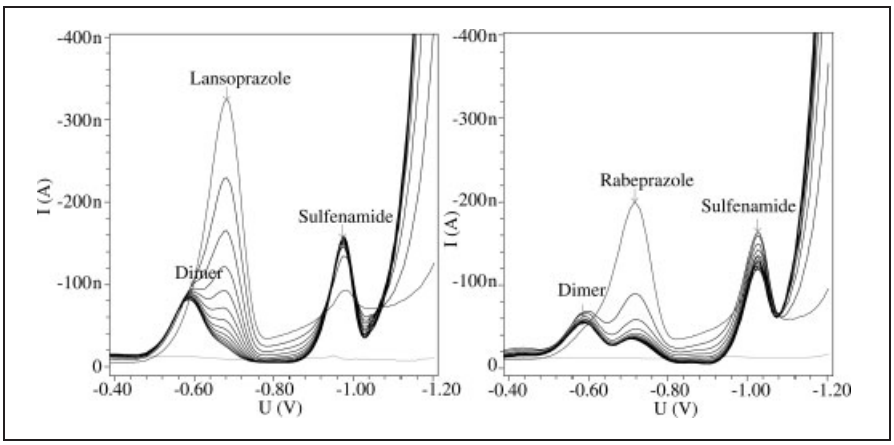


Fig. 4:
Differential pulse polarograms of lansoprazole and rabeprazole (2.5×10^{-5} M) at pH 4.0 scanned every 5 min between 0 and 60 min in Britton-Robinson buffer solution

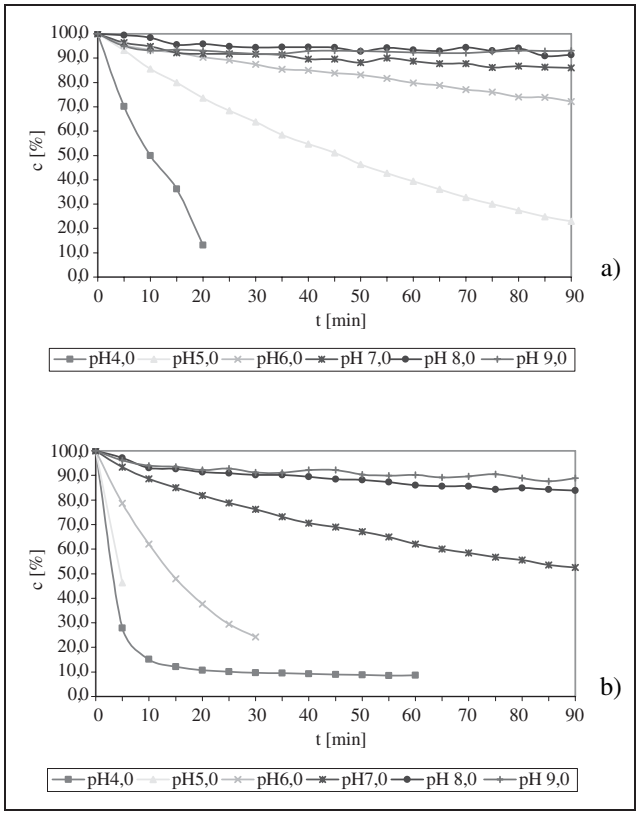


Fig. 5: Concentration-time decay curves of lansoprazole (a) and rabeprazole (b) in Britton-Robinson buffer solutions (the peak current of the t_0-scan was set to 100 %)

prazole was 70%. But after 20 min only 11% of rabeprazole and 13% of lansoprazole were left (Fig. 5). It is characteristic that the working solution in the polarographic vessel turned yellow over time in the pH range from 4.0 to 5.0, which is also a sign of the degradation. At pH 6.0 a concentration of only 24% was calculated for rabeprazole after 30 min of measuring whereas lansoprazole counted 72% even after 90 min. Lansoprazole showed stability from pH 8.0 onwards and rabeprazole from pH 9.0. For this reason a pH value of 9.0 should be chosen for further quantitative measurements of both agents.

These findings confirm previous studies, which reported that lansoprazole is slightly degraded at a pH value of 7.5. Furthermore it is described that pantoprazole shows stability at pH 6.0 and omeprazole at pH 7.5 (Shin et al. 2004; Tutunji et al. 2006).

2.4. Linear concentration range

In order to find out the influence of the concentration of the agents on the peak current, measurements were carried out in the pH range from 8.0 to 9.0. These conditions were chosen because of the described degradation at lower pH values and the highest determined signals. The studied concentrations ranged from 1×10^{-7} M to 7×10^{-4} M. Concentration levels lower than 1×10^{-6} M led to non-evaluable polarograms as the recorded peak currents were to small in relation to the background signal. In the concentration range from 1×10^{-6} M to 7×10^{-4} M a growing saturation of the electrode was observed since the peak currents did not rise with increasing concentrations of the agents. A linear range was only observed between 1×10^{-6} M and 7×10^{-5} M both for lansoprazole (pH 8.0 and pH 9.0 $R^2 = 0.9975$) and rabeprazole (pH 8.0 $R^2 = 0.9978$, pH 9.0 $R^2 = 0.9981$). Further quantitative measurements of the agents should be carried out in this concentration range using the described conditions.

A previous study of rabeprazole at a glassy carbon electrode showed linearity in peak current and concentration between 1×10^{-6} M and 2×10^{-5} M at pH 8.0 (Radi et al. 2004). For lansoprazole a linear concentration range between 1×10^{-7} M and 3×10^{-5} M was described using a static mercury drop electrode (Yardimci and Özaltin 2001), another investigation reported a range between 2×10^{-7} M and 5×10^{-5} M at a carbon paste electrode for lansoprazole and omeprazole (Radi 2003a). In addition, for pantoprazole a linear range was investigated at a carbon paste electrode between 1×10^{-7} M and 1×10^{-5} M (Radi 2003c) and at a glassy carbon electrode between 6×10^{-6} M and 8×10^{-4} M (Erk 2003).

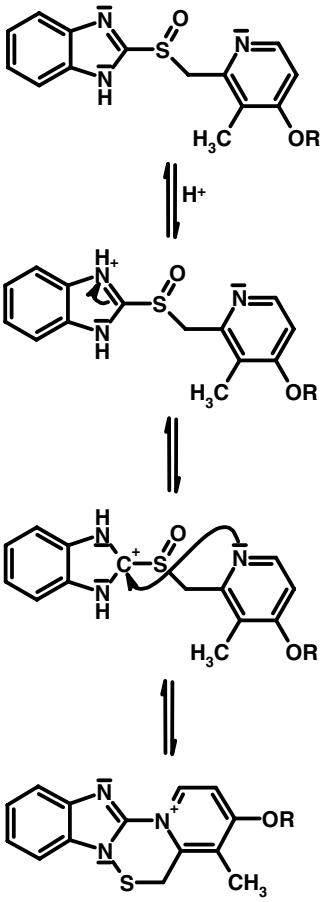
3. Discussion

The present study shows that there are a lot of similarities in the reduction of lansoprazole and rabeprazole at a dropping mercury electrode using differential pulse polarography. The further described investigations with omeprazole and pantoprazole also reported analogue characteristics in the polarographic and voltammetric determination of the agents. Not only the peak potentials shifted linearly to more negative values with increasing pH for all the studied PPI, but also the fact that the peak currents increase with increasing pH values and decrease afterwards was approved. The same linear concentration range was detected for lansoprazole and rabeprazole as described above. All PPI showed an acid catalyzed decomposition during their investigations. These characteristics are based on the same basic chemical structure of the agents, which is responsible for the similar reactions at the electrode.

But there are also differences between several features of the studied PPI, which are due to the individual substitutions of the heteroaromatic compounds of each agent, e.g. different side chains. First and foremost, there is a varying sensitivity on acid catalyzed degradation between the agents. The decomposition of rabeprazole ran considerably quicker and also up to higher pH values than those of lansoprazole. Therefore, less substance is to be reduced and lower peak currents are reached. In this case lansoprazole showed stability above pH 8.0 values and rabeprazole above pH 9.0. This is due to the greater basicity of the rabeprazole molecule ($pK_a = 4.90$) than that of lansoprazole ($pK_a = 4.01$). Furthermore the nitrogen of the pyridinyl component of the rabeprazole structure is more nucleophilic because of the differently configured side chains in both molecules (Scheme 3). The fluorine atoms in the trifluoroethoxy group of the lansoprazole structure exert an electron pulling effect on that nitrogen in contrast to the long aliphatic chain in the rabeprazole structure. This results in a greater electron density and nucleophilicity of the pyridinic nitrogen in the rabeprazole molecule. After the initial protonation of the nitrogen in the benzimidazole component, the nucleophilic attack of the pyridinic nitrogen on the carbon two of the benzimidazole is easier for rabeprazole than for lansoprazole, therefore. The following steps yield to the final cyclic sulfenamide.

In previous studies pantoprazole and omeprazole were reported to be less sensitive against acid catalyzed degradation than lansoprazole. Pantoprazole shows stability at pH 6.0 and omeprazole at pH 7.5, which is due to the substitution of the benzimidazole component and the asso-

Scheme 3 Reaction process of the acid catalyzed degradation of lansoprazole and rabeprazole



ciated basicity of its nitrogen. The more basic the nitrogen is, the faster it can be protonated. This is the rate-determining step of the acidic decomposition. The negative inductive effect of the difluoromethoxy group, which is connected with the benzimidazole of the pantoprazole structure, reduces the electron density of that nitrogen. This is why pantoprazole gets protonated worst and shows the greatest stability of all PPI. Omeprazole has a methoxy group instead, whose electron pulling effect is much weaker. In comparison with pantoprazole the nitrogen of the benzimidazole is more basic and protonation is easier. Lansoprazole and rabeprazole do not have any substituents at the benzimidazole component, so they have the most basic nitrogens and the acidic degradation runs best. Finally it is to point out that the acid catalyzed degradation sensitivity of the determined PPI is as follows: rabeprazole > lansoprazole > omeprazole > pantoprazole.

4. Experimental

4.1. Chemicals and reagents

Lansoprazole was obtained from Takeda (Aachen/Germany) and rabeprazole from Eisai (Frankfurt am Main/Germany). All other chemicals were of analytical reagent grade. Stock solutions of the analytes were prepared using methanol (Merck, Darmstadt/Germany). Britton-Robinson buffer solutions were used as supporting electrolyte consisting 0.04 M each of phosphoric acid, acetic acid and boric acid (Merck, Darmstadt/Germany). Different pH values were adjusted with appropriate volumes of 1.0 M sodium hydroxide solution. Distilled water was used for preparing the solutions. To run the electrode and deaerate the sample solutions, nitrogen gas with a reagent grade of 5.0 was obtained from Messer (Sulzbach/Germany). Mercury 99.9999% (VWR International, Darmstadt/Germany) was used to fill the electrode.

4.2. Instruments and apparatus

Polarographic determinations were made using a computer controlled 797 VA Computrace analyser (Metrohm, Herisau/Switzerland) with a multimode electrode (MME). A dropping mercury electrode (DME) as working electrode, an auxiliary platinum electrode and an Ag/AgCl reference electrode (saturated with a 3.0 M KCl solution) completed the three electrode cells. The pH measurements were carried out with a digital HI 9321 pH meter (Hanna Instruments, Kehl am Rhein/Germany).

4.3. Procedure

A 1×10^{-3} M stock solution of lansoprazole or rabeprazole was prepared daily by dissolving an appropriate amount of each analyte in methanol. Working standard solutions were obtained by diluting the stock solutions to the aspired concentrations also with methanol. Solutions of PPI are sensitive to light (DellaGreca et al. 2006). They were therefore kept in the dark. A 10.0 ml volume of the Britton-Robinson buffer solution with adjusted pH value was placed into the polarographic vessel and then deaerated by nitrogen gas for 5 min. The background signal was recorded before adding an adequate volume of the standard PPI solution. After a second deaeration by nitrogen gas for 15 s, an individual differential pulse polarogram of the analyte was obtained. The peak current was evaluated as the difference between each polarogram and the background signal. A scan rate of 14.9 mV/s, a pulse time of 0.04 s, a pulse amplitude of 50 mV, and a drop size of 0.29 mm^2 were chosen as operating parameters. All data were obtained at room temperature.

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