Electrochemical Redox Behavior of Omeprazole Using a Glassy Carbon Electrode

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Abstract
The electrochemical redox behavior of omeprazole (OMZ), a gastric acid pump inhibitor, was investigated at a glassy carbon electrode using cyclic, differential pulse and square-wave voltammetry over a wide pH range. The pH-dependent oxidation occurs in two irreversible consecutive charge transfer reactions. Adsorption of the non-electroactive product was also observed. The first oxidation involves removal of one electron, followed by deprotonation and leads to the formation of a hydroxylated species. The second oxidation process is related to the hydroxyl and amino groups in the benzimidazole moiety. The reduction is irreversible, also pH-dependent, and occurs in a single step at the sulfoxide group in a diffusion-controlled mechanism. The diffusion coefficient of omeprazole was calculated to be \( D_{\text{OMZ}} = 2.31 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1} \).

Keywords: Omeprazole, Electrochemistry, Oxidation, Reduction

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1. Introduction

Omeprazole (OMZ), 5-methoxy-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl]methyl] sulfanyl]-1H-benzimidazole, structural formula shown in Scheme 1, is a benzimidazole derivative which acts as proton pump inhibitor (PPI). It is a drug used for the effective treatment of gastric and duodenal (peptic) ulcers, reflux esophagitis, Zollinger-Allison syndrome, and other gastrointestinal conditions where gastric acid reduction is beneficial, and has found worldwide popularity over the past decade [1 – 4]. Administered as enteric-coated granules to prevent degradation within the oesophagus and stomach, OMZ enters the gastric parietal cell (via the blood system) and accumulates within the acidic secretory canaliculi where it is activated to sulfenamide. This activated form irreversibly binds to the \( \text{H}^+ \), \( \text{K}^+ \)-ATPase enzyme system to inhibit gastric acid production. OMZ contains a tricoordinated sulfur atom in a pyramidal structure and therefore can exist in two different optically active forms, \((S)\)- and \((R)\)-omeprazole. The \((S)\) isomer has a higher bioavailability resulting in higher plasma concentrations than those achievable with the \((R)\) isomer. Nevertheless, these two enantiomers have a similar inhibitory effect on acid formation in isolated gastric glands from rabbits.

Several methods have been used for the quantitative determination of OMZ in formulations and biological samples, including: spectrophotometry [4 – 6], high-performance liquid chromatography (HPLC) [7 – 9], thin layer chromatography (TLC) [10], capillary electrophoresis (CE) [11, 12], etc. The development of new techniques capable of determining drugs in pharmaceutical formulations is important. Electroanalytical methods are fast, highly sensitive and can allow direct measurement in biological samples with very little or no sample pretreatment. They have been successfully used for the detection and determination of several drugs [13 – 16].

The electrochemical reduction of OMZ using by polarographic techniques [2, 17 – 21], and alternating current (AC) polarography was studied [2]. The pathway for the electrode reaction involved reduction of the sulfonyl group to the corresponding thiol group at the dropping mercury electrode (DME). Several polarographic techniques and supporting electrolytes for determining OMZ were reported and the best results were obtained with differential pulse polarography (DPP) in borate buffer solution (pH9.0) [17]. The reactions of OMZ, in the absence of a nucleophile, were monitored using DPP at the static mercury drop electrode (SMDE) [18]. Experiments carried out in solutions buffered to pH values ranging from 2.0 to 8.0 showed that the DPP...
signals of OMZ and its degradation products are highly
dependent on pH. The adsorptive stripping voltammetric
(ASV) method was set-up using a multivariate strategy by
means of experimental design tools [19]. The optimized
method led to the determination of OMZ at low concen-
tration levels in biological fluids. DPP was used to monitor
the degradation of OMZ in acidic media to two main
products [20], and to follow the reactions of 2-mercapto-
tanol with the respective products of these degradations, as
simulations of their possible reactions in vivo. The polaro-
graphic behavior of OMZ was investigated and a pathway
for the reduction reaction was postulated [21].

The oxidation process and voltammetric method for
quantitative determination of OMZ has also been described
[3, 22]. The electrochemical oxidation of OMZ at a carbon
paste electrode was studied by cyclic and differential pulse
voltammetry [3]. A determination method was developed and
successfully applied to the analysis of OMZ in capsules.

The oxidation process of OMZ at a glassy carbon electrode
(GCE) in different supporting electrolytes was investigated
using cyclic voltammetry and DPV techniques [22]. A
proposed method in acetate buffer solution (pH 5.1) was
utilized for the determination of OMZ in drug enteric-
coated tablets.

Omeprazole has been oxidized by several forms of P450
in human liver microsomes in vitro and in vivo [25–31].
This drug is oxidatively metabolized prior to excretion and
the major metabolites in blood are omeprazole sulfone and
5-hydroxyomeprazole [32]. The major urinary metabolites
identified are 5-hydroxyomeprazole and its corresponding
carboxylic acid. Neither omeprazole nor omeprazole
sulfone are detected in urine. The metabolism of omepr-
zole to 5-hydroxyomeprazole co-segregates with 4'-hydro-
xylation of (S)-mephentoin [33], indicating a role for
the polymorphic CYP2C19 in this metabolic pathway. The
rate of omeprazole hydroxylation has been used as an
index for hepatic CYP2C19 activity in vivo [34, 35]. In vivo
studies suggest that CYP3A4 is mainly responsible for
omeprazole sulfoxidation [36]. Consistent with this, in vivo
studies have shown that the formation of omeprazole
sulfone is strongly inhibited by a CYP3A4 inhibitor,
ketoconazole [37] and increased after prolonged treatment
with a CYP3A4 inducer, carbamazepine [38]. Approx-
imately 3% of Caucasian and 15–20% of Oriental pop-
ulations are poor metabolizers of (S)-mephentoin. Ome-
prazole S-oxidation is thus the predominant metabolic
pathway in the poor metabolizer phenotype. Moreover, 5-
hydroxyomeprazole and omeprazole sulfone undergo
further oxidation to a secondary metabolite, 5-hydroxy-
imeprazole sulfone, mediated by CYP3A4 and CYP2C19,
respectively [39, 40, 29].

Although voltammetry has been exhaustively used for the
determination of OMZ, a systematic investigation of the
redox mechanism of the drug has not been undertaken.
Therefore, in this work the electrochemical redox behavior
of OMZ was studied on a GCE over a wide pH interval,
using cyclic, square-wave and differential pulse voltamme-
try.

2. Experimental

2.1. Chemicals

OMZ was obtained from Sigma and used without further
purification. All supporting electrolyte solutions (Table 1)
were prepared using analytical grade reagents and purified
water from a Millipore Milli-Q system (conductivity ≤
0.1 μS cm⁻¹). A stock solution of 60 μM OMZ was prepared
daily in different pH buffers. Solutions of different concen-
trations of OMZ were obtained by dilution of the appro-
priate volume in supporting electrolyte.

2.2. Apparatus

Voltammetric measurements were carried out using a
μAutolab Type III running with GPES 4.9 software, (Eco-
Chemie, Utrecht, The Netherlands) and a three-electrode
system in a 0.5 mL one-compartment electrochemical cell of
capacity 2 mL (Cypress System Inc., USA). GCE (d =
1.5 mm) was the working electrode, Pt wire the count-
er electrode and the Ag/AgCl (3 M KCl) reference electrode.
Microvolumes were measured using EP-10 and EP-100
Plus Motorized Microliter Pipettes (Rainin Instrument Co.
Inc., Woburn, USA). The pH measurements were carried
out with a Crison microPH 2001 pH-meter with an Ingold
combined glass electrode. A spectrophotometer SPE-
CORD S100 running with Aspect Plus Version 1.5 (Analytik
Jena GmbH, Jena, Germany) was used for UV-VIS
measurements.

2.3. General Procedures

The GCE was polished using diamond spray (particle size
3 μm) (Kemet, UK) before each electrochemical experi-
ment. After polishing, it was rinsed thoroughly with Milli-Q
water. Following this mechanical treatment, the GCE was
placed in buffer supporting electrolyte and differential pulse
voltammograms were recorded until a steady state baseline
voltammogram was obtained.

<table>
<thead>
<tr>
<th>pH</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>0.2 M HCl + 0.2 M KCl</td>
</tr>
<tr>
<td>3.5</td>
<td>0.2 M HAcO + 0.2 M NaAcO</td>
</tr>
<tr>
<td>4.0</td>
<td>0.2 M HAcO + 0.2 M NaAcO</td>
</tr>
<tr>
<td>5.3</td>
<td>0.2 M HAcO + 0.2 M NaAcO</td>
</tr>
<tr>
<td>5.9</td>
<td>0.2 M NaHPO₄ + 0.2 M NaHPO₄</td>
</tr>
<tr>
<td>6.9</td>
<td>0.2 M NaHPO₄ + 0.2 M NaHPO₄</td>
</tr>
<tr>
<td>8.0</td>
<td>0.2 M NaHPO₄ + 0.2 M NaHPO₄</td>
</tr>
<tr>
<td>8.9</td>
<td>2 M NH₃ + 2 M NH₄Cl</td>
</tr>
<tr>
<td>9.9</td>
<td>2 M NH₃ + 2 M NH₄Cl</td>
</tr>
<tr>
<td>10.5</td>
<td>2 M NH₃ + 2 M NH₄Cl</td>
</tr>
<tr>
<td>11.9</td>
<td>0.2 M NaOH + 0.2 M KCl</td>
</tr>
</tbody>
</table>
The experimental conditions for differential pulse voltammetry were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s\(^{-1}\). For square-wave voltammetry a frequency of 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate of 100 mV s\(^{-1}\) were used. This procedure ensured very reproducible experimental results.

UV-vis measurements were done every 10 min for the first 30 min, then at 1 h and 2 h. The last measurements were carried out after 4 days. All experiments were performed at room temperature (25 ± 1 °C).

2.4. Acquisition and Presentation of Voltammetric Data

The differential pulse voltammograms presented were background-subtracted and baseline-corrected using the moving average application with a step window of 5 mV included in GPES version 4.9 software. This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing any artifact, although the peak intensity is, in some cases, reduced (<10%) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks. The values for peak current presented in all plots were determined from the original untreated voltammograms after subtraction of the baseline.

3. Results and Discussion

The electrochemical behavior of OMZ was first studied by CV at 100 mV s\(^{-1}\) in electrolytes with different pH, N\(_2\) saturated, using a GCE. Cyclic voltammograms showed that OMZ undergoes oxidation and reduction (Fig. 1). However, these processes occur independently of each other and were investigated separately.

3.1. Oxidation

3.1.1. Cyclic Voltammetry

The oxidation process using 60 µM OMZ solution at a GCE, studied by CV in pH 7.0 0.2 M phosphate buffer at a scan rate \(v = 100 \text{ mV s}^{-1}\), showed two anodic peaks P1, at \(E_{\text{P1}}^p = +0.88 \text{ V}\) and P2, at \(E_{\text{P2}}^p = +1.13 \text{ V}\) (Fig. 2). On scanning in the negative direction, no reduction peak is observed, indicating that the oxidation processes are irreversible. Successive scans result in a large decrease of the oxidation currents for these peaks due to the adsorption of OMZ and/or its nonelectroactive oxidation products on the GCE surface.

By increasing the scan rate, the main peak P1 current increases (Fig. 2), following a linear relationship with scan rate as expected for an adsorbed species reaction. Such a kind of behavior was already described for OMZ oxidation on a carbon paste electrode [3].

3.1.2. Differential Pulse Voltammetry

The electrochemical oxidation of OMZ was studied using differential pulse voltammetry (DPV) in aqueous buffer supporting electrolytes over a pH range from 2 to 12. The differential pulse voltammograms, recorded in solutions of 3.0 µM OMZ, presented significant changes on altering the pH.

The main oxidation peak P1 appears at all studied pH values and its potential is shifted to more negative values with increasing pH (Fig. 3A). The slope of the \(E_{\text{P1}}^p\) vs. pH curve (Fig. 3B), \(-59 \text{ mV per pH unit}\), shows that the mechanism of this oxidation process involves the same number of electrons and protons. The number of electrons transferred, \(n\), was determined by the peak width at half height, \(W_{1/2} \approx 83 \text{ mV}\), and is close to the theoretical value of 90 mV, corresponding to an electrochemical reaction involving the transfer of one electron [23]. Consequently, it can be concluded that an oxidation process below pH 9 occurs with the transfer of one electron and one proton. The value found for \(pK_a \approx 9\) is in agreement with the literature.
For pH > 9, the oxidation of peak P1 does not depend on pH.

The oxidation peak P2 that only appears for 3 < pH < 7, also decreased with increasing pH. The plot of $E_p$ vs. pH showed a linear pH dependence with slope of $-59 \text{ mV per pH unit}$ (Fig. 3B). Therefore, it can also be concluded that the same number of protons and electrons was involved in this electrode process. Taking into consideration that the value found for $W_{1/2}$ of the OMZ peak P2 was 45 mV, this oxidation step involves the transfer of two electrons and two protons.

3.1.3. Square-Wave Voltammetry

Square-wave voltammetry (SWV) experiments were carried out over the same pH range. The advantages of SWV are greater speed of analysis, lower consumption of the electroactive species in relation to differential pulse voltammetry, and reduced problems regarding the blocking of the electrode surface [23]. Furthermore, it permits to see during only one scan if the electron transfer reaction is reversible or not. Since the current is sampled in both positive and negative-going pulses, peaks corresponding to the oxidation and reduction of the electroactive species at the electrode surface can be obtained in the same experiment.

This method showed similar results to DPV, i.e. the presence of the oxidation peak P1 in all buffer supporting electrolytes and of the oxidation peak P2 in a smaller pH interval. The irreversibility of both peaks was confirmed by plotting the forward and backward components of the total current obtained in solutions of 3.0 $\mu$M OMZ in pH 7.0 0.2 M phosphate buffer (Fig. 4).

The oxidation mechanism proposed, Scheme 2, is based on the electrochemical data described, and supported by the omeprazole structure, with two systems that are in resonance, separated by a methylene group.

Because the benzimidazole moiety is planar, the first oxidation step in the ortho position will be favored, via resonance, by the stabilization of the radical formed. Preferential attack is in the aromatic ring, where the extent of conjugation is greater, making removal of the first electron easier. In the first step, peak P1, one electron is removed, followed by deprotonation to produce a cation radical, which reacts with water and leads to the formation of hydroxylated species.

The second oxidation step, peak P2, with a two-electron and two-proton transfer, involves the hydroxylated and amino groups in the benzimidazole moiety leading to the formation of species similar to quinones.

![Scheme 2. Proposed oxidation mechanism for OMZ.](image)
3.2. Reduction

3.2.1. Cyclic Voltammetry

The reduction of OMZ at a GCE was first investigated in pH 7.0 0.2 M phosphate buffer, N₂ saturated solutions. CV recorded in 60 μM OMZ depicted only one cathodic peak P3, at \( E^0_{P3} = -1.04 \) V. No oxidation peak was observed on scanning in the positive direction, showing that the reduction of OMZ is an irreversible process (Fig. 5). This result was confirmed by SWV studies (not shown). The decrease of the cathodic current was observed with successive scans leading to the conclusion that adsorption of OMZ reduction products occurs at the GCE surface.

The effect of scan rate on peak P3 potential and current was investigated. The results showed that the peak potential was shifted to more negative values by increasing the scan rate. Also, the cathodic current of peak P3 increases by increasing the scan rate following a linear relationship with the square root of the scan rate. Therefore, it was concluded that the reduction of OMZ involves the sulfoxide group [2], and occurs with one-electron and one-proton transfer.

The electrochemical reduction of OMZ was studied by varying the pH of the supporting electrolyte from 2 up to 12 using DPV. The differential pulse voltammograms were all recorded in 6 μM OMZ saturated with N₂, Fig. 6A. The potential of peak P3, that appears in all pH values, is shifted to more negative values with increasing pH. This indicates that protonation of the electroactive site of the molecule affects the overall electrode reaction mechanism. The dependence of \( E^0_{P3} \) with pH is linear over the pH range between 2 and 9 (Fig. 6B). The slope of the dotted line shows that the same number of electrons and protons is involved in the reduction mechanism of OMZ. The width at half-height of P3 was \( W_{1/2} \approx 72 \) mV, which confirms that the reduction of OMZ involves the sulfoxide group [2], and occurs with one-electron and one-proton transfer.

3.2.2. Differential Pulse Voltammetry

Spectrophotometric measurements were carried out in order to complement the voltammetric studies. Thus, the UV-vis absorption spectra were recorded for 30 μM OMZ in aqueous buffer supporting electrolytes over a pH range from 2 to 12. Figure 7 shows absorption spectra for OMZ solutions at initial time, after 30 min and at the end of the 4-day study in three pH values. The results of the experi-
ments revealed that large changes occur in strong acid media. At pH values between 2 and 4, the degradation of OMZ was fast. Thus, the principal absorption bands of the parent molecule at 275 and 301 nm decreased and a broad absorption band at 370 nm arose in pH 2 solution, as shown in Figure 7A (curve 1). After 30 min, the band at 370 nm disappeared and a peak at 293 nm was formed (curve 2) and remained so for the next 4 days (curve 3).

It is well established that OMZ is stable at high pH but degrades under acidic conditions to produce the corresponding sulfenamide, sulfide, dimer and other products, which reduce at DME [18, 20]. Therefore, the degradation products may be due to omeprazole sulfenamide, which can suffer further degradation, and omeprazole sulfide [20]. On the other hand, omeprazole sulfenamide can react with sulfenic acid, another possible degradation product of OMZ, to yield an omeprazole dimer [18]. Degradation became slower as solution pH was increased, and the absorption spectrum of OMZ at pH 5 presented few changes after 30 min (Fig. 7B, curve 2). However, at the same time, the absorption bands of the parent molecule remain unchanged in a solution of pH 7 (Fig. 7C, curve 2), with some degradation only occurring after 3 days. At pH > 7, the absorption spectra of OMZ did not present changes within a 4-day interval. These results are in agreement with the literature [5, 18].

4. Conclusions

This electrochemical investigation carried out a wide pH range showed that OMZ undergoes oxidation and reduction at a GCE. The oxidation is pH-dependent and occurs in two irreversible steps leading to the formation of nonelectroactive products that adsorb on the electrode surface. The first oxidation involves a one-electron and one-proton transfer to yield a hydroxylated product. The second oxidation process corresponds to oxidation of the hydroxyl and amino groups in the benzimidazole moiety and involves the transfer of two electrons and two protons. The reduction is
also a pH-dependent irreversible process, occurs in a single step, with the same number of electrons and protons transferred and corresponds to reduction of the sulfoxide group.

The electroanalytical determination of OMZ in biological fluids is foreseen and would provide very important and useful data for clinicians.

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6. References
