The voltammetric study and determination of ramipril in dosage forms and biological fluids

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Abstract

The voltammetric behavior of ramipril was studied using cyclic voltammetry, direct current polarography (DCt), differential pulse polarography (DPP) and alternating current polarography (ACt). Ramipril developed well-defined cathodic waves in Britton–Robinson buffers over the pH range 6–12. The waves were characterized as being diffusion-controlled, irreversible and partially affected by adsorption phenomenon. The diffusion–current constant (Id) was 1.24 ± 0.02. The current–concentration plots were rectilinear over the range 10–50, 4–40 and 0.16–12 μg/ml in the DCt, DPP and ACt modes, respectively, with a minimum detectability (S/N = 2) of 0.02 μg/ml (4.8 × 10⁻⁸ M) using the latter mode. The proposed method was successfully applied to the determination of ramipril in commercial tablets. Hydrochlorothiazide, which is frequently co-formulated with ramipril, did not interfere with the assay. Furthermore, the proposed method was applied to the determination of ramipril in urine and plasma adopting the ACt technique. The percentage recoveries were 97.12 ± 0.56 and 94.97 ± 0.62%, respectively. A pathway for the electrode reaction was proposed. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Ramipril; Voltammetry; Polarography; Dosage forms; Biological fluids

1. Introduction

Ramipril, 2-[N-[(S)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl]-[(S,S,S)-2-azabicyclo[3.3.0]octane-3-carboxylic acid, is an orally active inhibitor of angiotensin converting enzyme (ACE) with antihypertensive activity [1]. It is used in the treatment of all forms of hypertension, heart failure and following myocardial infarction to improve survival in patients with clinical evidence of heart failure [2].

Despite the importance of ramipril, little has been published concerning its determination, viz: GC [3,4], HPLC [5,6], enzymatic assay with GC or HPLC [7], radioimmunoassay [8], derivative spectroscopy [9], ion-selective electrode potentiometry [10,11] and atomic absorption spectroscopy [12]. All the reported methods are laborious, time-consuming and require highly sophisticated instrumentation [3–8]. In addition, ramipril is characterized by its very low ability to absorb light in the UV region with barely discernible maxima at about 257 nm (molar absorptivity in methanol at 257 nm is about 290 l/mol·cm). Therefore, interest in electrochemical methods has increased. Reviewing the literature revealed that nothing has been reported either on its electrochemistry in general, or its voltammetry in particular. This led us to study its polarographic behavior in an attempt to develop a simple, sensitive and reliable method for its quantitative determination in dosage forms and biological fluids, and the results were promising. The high sensitivity attained adopting the ACt mode renders the method as an alternative substitute for HPLC.
2. Experimental

2.1. Materials and reagents

Ramipril (Batch no. HA-05326), Tritace 5 (Batch No. 40B 414) and Tritace 2.5 (Batch No. 204) were kindly provided by Hoechst, AG, Frankfurt/Main, Germany. Plasma was obtained from King Khalid University Hospital, Riyadh, Saudi Arabia. Urine was obtained from healthy volunteers.

- Britton Robinson buffers (BRb), 0.08 M, pH range 2.1–12.0 [13].
- Hydrochloric acid, 0.1 M and 1.0 M solutions.

A stock solution of ramipril (4 × 10⁻² M) was prepared in methanol and further diluted with the same solvent to give the appropriate concentrations for the working range. The solutions were purged with pure nitrogen gas before being polarographed at ambient temperature.

2.2. Apparatus

The polarographic study and the DPP measurements were carried out using the Polarecord E505 Metrohm (Herisau, Switzerland). The drop time of 1 s was electronically-controlled using a 506 Stand from the same company. The polarograms were recorded using a potential scan rate of 10 mV/s. A three-electrode system consisting of a Dropping Mercury Electrode (DME) as the working electrode, an Ag⁺/AgCl reference electrode and a platinum wire as the auxiliary electrode, was used. Phase-selective alternating current (ACₜ) polarograms of 1 × 10⁻⁴ M solutions of ramipril were recorded using the same instrument, the superimposed alternating voltage being 30 mV at a frequency of 75 Hz, a drop time of 1.4 s and a phase angle of 90°. The cyclic voltammograph consisted of an Oxford potentiostat equipped with a Phillips PM 8043 X-Y recorder. A three-electrode system consisting of a platinum wire as the working electrode, an Ag⁺/AgCl reference electrode and glassy carbon auxiliary electrode were used. Dimethylformamide (DMF) was the solvent, and tetrabutylammonium bromide (TBAB) was the supporting electrolyte.

2.3. Procedures

2.3.1. Procedure for the tablets

Weigh and pulverize 20 tablets, then mix well. Weigh out a quantity of the powder equivalent to 10 mg of ramipril. Extract with 3 × 7 ml of methanol and filter the extracts into a 25-ml standard flask. Wash the filter and transfer the washings into the same standard flask. Complete to the mark with the same solvent. Transfer 1.0 ml of the methanolic extract into a 25-ml standard flask, and complete to the mark with BRb of pH 9. Transfer the whole contents of the flask into the polarographic cell then bubble through nitrogen gas for 5 min. Record the DCₜ, DPP or ACₜ polarograms over the range −0.8 to −1.6 V. Calculate the nominal content of the drug from the corresponding regression equation.

2.3.2. Procedure for spiked urine

Transfer 1.0 ml of the spiked urine into a 25-ml standard flask. Complete to the mark with BRb of pH 9. Transfer the whole contents of the flask into the polarographic cell. Proceed as described above adopting the ACₜ mode. Determine the nominal content of ramipril from the corresponding regression equation.

2.3.3. Procedure for spiked plasma

To 1.0 ml of the spiked plasma, add 1 ml of methylethylketone then shake for 5 min. Centrifuge for 5 min, then transfer 0.5 ml of the supernatant into an evaporating dish. Evaporate under nitrogen. Dissolve the residue in 2 ml of methanol then transfer into 25-ml standard flask, and complete to the mark with BRb of pH 9. Transfer the whole contents of the flask into the polarographic cell then proceed as described above adopting the ACₜ mode. Calculate the ramipril content from the corresponding regression equation.

3. Results and discussion

3.1. Effect of pH on the development of the polarographic waves

Ramipril was found to be reduced at the DME producing well-defined cathodic waves. Fig. 1 shows a typical polarogram of 7.5 × 10⁻³ M solution of ramipril in BRb of pH 9. The waves started to develop in acid medium (pH 0 and 1.0) then completely disappeared up to pH 6 where an ill-defined wave began to appear. As the pH of the solution increased, the wave steepness increased up to pH 9, then its height began to decrease (Fig. 2). The waves exhibited positive shift upon increasing the pH up to pH 9, after which it remained almost constant. Logarithmic analysis of the waves obtained in BRb of different pH values resulted in straight lines. Assuming that the rate-determining step involves the transfer of two electrons (a free-radical, one-electron transfer is not likely to occur) the values of the slopes denote that, the reduction process is irreversible. The zn values were calculated according to the treatment of Meites and Israel [14] and are listed in Table 1. It is clear that, the degree of reversibility increases as the pH value increases up to pH 9, after which it decreases again. This observation was further confirmed by cyclic voltammetric measurements using different scan rates. Fig. 3 shows the cyclic voltam-
mograms of ramipril (1 × 10⁻³ M) in DMF in presence of TBAB as the supporting electrolyte. One cathodic peak was obtained using scan rates of 50–500 mV/s. The peak potentials (Eₚ) displayed cathodic shifts upon increasing the scan rate (Fig. 3), thus revealing the irreversible nature of the reduction process [15]. Upon plotting peak potentials, Eₚ versus ln V (scan rate), a linear relationship was obtained. The number of pro-

tons, Z, consumed in the electrode reaction is given by the following formula [16].

\[ \Delta E_{1/2}/\Delta pH = -0.059Z/zn_a \]

where \( z \) is the transfer coefficient. The value of \( zn_a \) is calculated from the following equation:

\[ E = E_{1/2} - (0.059/zn_a) \log[iid - i] \]

where \( iid \) is the diffusion current. At pH 9, Z was found to be 0.31, i.e. one proton is probably consumed in the rate-determining step of the electrode reaction.

### 3.2. Study of the wave characteristics

Increasing the mercury height (\( h \)) resulted in a corresponding increase in the waveheight (\( w \)), a plot of \( \sqrt{h} \) versus \( w \) gave a straight line. Also, a plot of \( \log h \) versus \( \log w \) gave a straight line with a slope of about 0.5. Changing the buffer concentration over the range 0.01–0.08 M resulted in a negligible increase in wave height. These two characteristics point to a diffusion-controlled process partially affected by adsorption phenomenon.

#### Table 1
Effect of pH on the polarographic behavior of ramipril

<table>
<thead>
<tr>
<th>pH</th>
<th>( E_{1/2} ) (mV)</th>
<th>( \Delta pH )</th>
<th>( \Delta E_{1/2}/\Delta pH )</th>
<th>( zn_a )</th>
<th>( W_2 ) (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>-1370</td>
<td>1.0</td>
<td>140</td>
<td>0.34</td>
<td>1.70</td>
</tr>
<tr>
<td>7.0</td>
<td>-1230</td>
<td>1.0</td>
<td>35</td>
<td>0.36</td>
<td>1.65</td>
</tr>
<tr>
<td>8.0</td>
<td>-1195</td>
<td>1.0</td>
<td>25</td>
<td>0.68</td>
<td>1.50</td>
</tr>
<tr>
<td>9.0</td>
<td>-1170</td>
<td>1.0</td>
<td>0.0</td>
<td>0.73</td>
<td>1.40</td>
</tr>
<tr>
<td>10.0</td>
<td>-1170</td>
<td>1.0</td>
<td>0.0</td>
<td>0.71</td>
<td>1.70</td>
</tr>
<tr>
<td>11.0</td>
<td>-1170</td>
<td>1.0</td>
<td>0.0</td>
<td>0.55</td>
<td>1.80</td>
</tr>
<tr>
<td>12.0</td>
<td>-1170</td>
<td>1.0</td>
<td>0.0</td>
<td>0.54</td>
<td>1.80</td>
</tr>
</tbody>
</table>

\( * \) \( W_2 \), half-peak width in DPP mode; \( z \), the transfer coefficient; \( zn_a \), the number of electrons transferred in the rate-determining step.

Fig. 1. Typical polarogram of ramipril (7.5 × 10⁻³ M) in BRb of pH 9.

Fig. 2. Effect of pH on the development of the polarographic waves of ramipril (1 × 10⁻⁴ M).
This observation was further confirmed by an AC study.

The alternating current behavior of a $1 \times 10^{-4}$ M solution of ramipril was studied using a phase-selective angle of 90°. In BRb of pH 7 and 9, the summit potentials were 70 and 90 mV, respectively, more negative than the corresponding $E_{1/2}$ values. Fig. 4 demonstrates that, at pH 5, 7 and 9, the depolarizer is strongly adsorbed to the surface of a mercury drop.

3.3. Analytical applications

The relationship between the diffusion-current, $i_d$ (µA) and the concentration, $c$ (µg/ml) was found to be rectilinear over the range 10–50, 4–40 and 0.16–12 µg/ml in the DC, DPP and AC modes, respectively. The corresponding regression equations are listed in Table 2. The minimum detectability using the AC mode, taking $S/N = 2$, was found to be 0.02 µg/ml (4.8 $\times$ 10$^{-8}$ M). The diffusion-current constant ($I_d$) was calculated at 25°C and was found to be 1.24 ($\pm$ 0.02) based on eight different measurements. No maxima was developed, therefore, no maximum suppressor was needed.

Polarograms of ramipril in BRb of pH 9 exhibit well-defined cathodic waves. The current is mainly diffusion-controlled and is proportional to the concentration over a convenient range. Both DC and DPP were successfully applied to the determination of ramipril in commercial tablets. Hydrochlorothiazide, which is frequently co-formulated with ramipril, did not interfere with the assay in the DC and DPP modes only, while in AC it gave higher results. The results obtained (Table 3) were compared to those given by a reference HPLC method (5). Common tablet excipients such as talc, starch, lactose avisil and magnesium stearate did not interfere with the assay. Statistical analysis of the results obtained by both methods showed no significant

Fig. 3. Cyclic voltammograms of ramipril ($1 \times 10^{-3}$ M) in DMF containing tetrabutylammonium bromide. Scan rate: (A) 50 mV/s; (B) 100 mV/s; (C) 200 mV/s; (D), 500 mV/s.
difference in the performance of the two methods with regards to accuracy and precision [17]. The high sensitivity of the proposed method allowed it to be successfully applied to the determination of ramipril in biological fluids adopting the ACt mode; lower concentration range could be achieved (0.16–12 µg/ml) using this technique. The blood level concentration of ramipril following an oral dose of 5 mg is typically 0.15 µg/ml. This value lies within the working range (0.16–12 µg/ml), thus it could be determined adopting the ACt technique. The urine content did not interfere with the determination, therefore, no prior extraction step was needed. As for the plasma, extraction with methylethylketone was necessary to eliminate the interference produced by plasma proteins. The results in Table 4, show that the method is satisfactorily accurate and precise.

3.4. The number of transferred electrons

The number of electrons transferred during the electrode process could be accomplished through comparing the waveheight of ramipril in BRb of pH 9 with that produced from an equimolar solution of a compound having the same reducible functional group, the carbonyl group namely, flumequime [18]. From the comparison it is concluded that two electrons are involved in the electrode process.

3.5. Mechanism of the electrode reaction

Based on the observed transfer of two electrons, and depending on the presence of carbonyl group, activated by the neighboring nitrogen, it is postulated that the electrode reaction is as follows:

\[ \text{O} \]
\[ \text{R}^-\text{C}^-\text{R}^- + 2e + 2\text{H}^+ \rightarrow \text{H} \]
\[ \text{R}^-\text{C}^-\text{R}^- \]
\[ \text{OH} \]

However, the exact electrode reaction, and the isolation and identification of the reduction product is the subject of a next communication.

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Performance data for the proposed method</strong> *</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mode</th>
<th>Working range (µg/ml)</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCt</td>
<td>10–50</td>
<td>( C = -0.65 + 290 \times i_d )</td>
<td>0.9998</td>
</tr>
<tr>
<td>DPP</td>
<td>04–54</td>
<td>( C = 0.11 + 546 \times i_d )</td>
<td>0.9985</td>
</tr>
<tr>
<td>ACt</td>
<td>0.16–12</td>
<td>( C = 0.037 + 8 \times i_d )</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

* Where \( C = \text{concentration in µg/ml and } i_d = \text{the diffusion current in µA.} \)
Table 3
Application of the proposed method to the determination of ramipril in tablets.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC&lt;sub&gt;c&lt;/sub&gt;</td>
<td>DPP</td>
</tr>
<tr>
<td>Tritace (ramipril, 5 mg/tablet) *</td>
<td>94.41 ± 0.93</td>
</tr>
<tr>
<td>Tritace 2.5 mg (ramipril 2.5 mg/tablet)*</td>
<td>99.05 ± 0.66</td>
</tr>
<tr>
<td>Tritace comp. (ramipril, 5 mg + hydrochlorothiazide, 25 mg/tablet)*</td>
<td>98.33 ± 0.83</td>
</tr>
</tbody>
</table>

<sup>*</sup> Product of Hoechst, AG. (Frankfurt, Germany).
<sup>b</sup> The results are the average of six separate determinations.

Table 4
Application of the proposed method to the determination of ramipril in biological fluids adopting the AC<sub>c</sub> mode.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Recovery&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Urine</td>
<td>94.12 ± 0.56</td>
</tr>
<tr>
<td>2 Plasma</td>
<td>94.97 ± 0.62</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each result is the average of six separate determinations.

4. Conclusion

A simple and sensitive method was developed for the determination of ramipril in dosage forms and biological fluids. It has some distinct advantages over existing methods regarding sensitivity, time saving and minimum detectability, moreover, it can be directly applied to the determination of ramipril in urine without prior extraction, and this is an advantage over HPLC which necessitates a clean-up procedure before application. The method is sensitive enough to monitor the drug level after therapeutic doses.

Acknowledgements

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References