Dissolution enhancement of ramipril using water soluble carrier by solid dispersion technology

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ABSTRACT

Ramipril and β-cyclodextrin (β-CD) solid dispersions were prepared with an intention to study the influence of β-CD on the solubility and dissolution rate of this poorly soluble drug. Phase-solubility profile indicated that the solubility of ramipril was significantly increased in the presence of β-CD and was classified as A1-type, indicating the possible 1:1 stoichiometric inclusion complex with a stability constant of 328.65 M⁻¹. Physical characterization of the solid dispersion were characterized by Fourier transform infrared spectroscopy (FT-IR), Differential scanning calorimetry (DSC) and X-ray diffraction studies (XRD). Effect of variable such as drug:carrier ratio were studied. These studies revealed that a distinct loss of drug crystallinity in the solid dispersion is ostensibly accounting for enhancement of dissolution rate in distilled water. The drug release from the prepared solid dispersion exhibited a first order kinetics. Solid dispersion of ramipril showed 4.83 times fold increase in dissolution rate over the pure drug.

Key words: Ramipril, β-cyclodextrin, solid dispersion, kneading method, dissolution, release kinetics.

INTRODUCTION

Poorly water soluble drugs are generally associated with slow drug absorption leading eventually to inadequate and variable bioavailability [1-3]. Approximately 40% of the new chemical entities currently being discovered are poorly water soluble drugs [4-6]. From an economic point of view, low oral bioavailability results in wastage of large portion of drug and adds to cost of drug therapy, especially when the drug is expensive one [7]. Attempts to enhance drug solubility of therapeutics agents correlate well with enhancement of their bioavailability [8-10]. However, solid dispersion technology was most widely used [11-12]. A number of insoluble drugs have shown to improve their dissolution character when converted to solid dispersion [12-13]. Solid dispersion technology is a well known process used to increase the dissolution kinetics and oral absorption of poorly water soluble drugs using water soluble inert carriers [10]. The use of hydrophilic polymers as carriers for the dissolution enhancement of poorly water-soluble drugs is of increasing interest [14-15]. Various hydrophilic carriers such as polyethylene glycol [16], polyvinylpyrrolidone [17] and sugars [18] have been extensively investigated for their improvement of dissolution characteristics and bioavailability of poorly aqueous soluble drugs.

Cyclodextrins are cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose, containing a relatively hydrophobic central and hydrophilic outer surface. During the past two decades, cyclodextrins and their derivatives have attracted considerable attention in the pharmaceutical field due to their potential in forming complexes with a variety of drug molecules. Cyclodextrins are used to increase the solubility of water insoluble drugs, through inclusion complexation [19-22]. Generally, the small drug molecules, and those compounds with the lowest water solubility showed a percent increase in solubility as a function of cyclodextrin concentration. Therefore, cyclodextrins have been used in pharmaceutical preparations in order to increase the stability and bioavailability of poorly water soluble drugs [23]. Natural cyclodextrins have been used extensively for this purpose. However, they are characterized by a relatively low solubility in water, which limits their application. Hence, a chemically modified cyclodextrins are gaining considerable attention to improve the physicochemical properties of cyclodextrin. Cyclodextrins are known to form an inclusion complex with many drugs of appropriate molecular size and polarity in hydrophobic drug molecules. The resulting complex generally leads to an improvement in some of the properties of drugs in terms of solubility, bioavailability and tolerability. Ramipril is {[(2S, 3aS, 6aS)-1-[(2S)-2-[(1S)-1-(ethoxy carbonyl)-3-phenylpropyl] amino]-1- oxopropyl] octahydrocyclo penta [b] pyrrole-2-carboxylic acid}, a potent antihypertensive agent with higher lipophilic nature (log P 3.32). The major drawback of this drug is its poor aqueous solubility (BCS-II Classification) [3.5 mg/L] and its oral bioavailability is 28-35% [24-25]. To overcome these difficulties, increase in the aqueous solubility of ramipril is an important goal. Hence, in this present investigation, inclusion complexation of ramipril was tried with an aim to improve its pharmaceutical properties such as aqueous solubility and dissolution properties.

In this study, an attempt was made to improve the solubility and dissolution rate of ramipril by complexing with β-CD thereby with a view of increasing its bioavailability and therapeutic efficacy. The characterization of the drug, β-CD and complex was done by using Differential scanning calorimetry (DSC), FTIR and Powder X-ray diffractometry (PX-RD). In vitro aqueous solubility and dissolution rate profiles of the complex were performed.

MATERIALS AND METHODS

Ramipril was obtained as a gift sample from Amanath Pharma, Puducherry, India. β-cyclodextrin (β-CD) was obtained from Sigma, USA. All other materials used in the study were of analytical grade.

Preparation of ramipril-β-cyclodextrin solid dispersion

A mixture of ramipril and β-cyclodextrin (1:1, 1:2, 1:3, 1:4, and 1:5 mol/mol) was wetted with a mixture of methanol and water (1:1) thoroughly for 30 min in a glass mortar by kneading method [26]. The paste formed was dried under vaccum for 24 h, dried powder was scrapped, crushed, pulverized, passed through sieve no 100 (ASTM-100, 150 µm) and stored in dessicator for further studies. The prepared solid dispersions were evaluated for their physicochemical parameters such as yield, angle of repose, bulk density,
compressibility, moisture uptake, drug content and in vitro dissolution studies.

SOLID STATE STUDIES

Fourier transform infrared (FTIR) spectroscopy
FTIR spectra were recorded for pure drug, drug with carrier samples prepared by kneading method in different ratio’s of carrier (w/w) in a KBr pellets using Shinadzu FTIR – 5300 (Tokyo, Japan). The scanning range was 450 to 4000 cm⁻¹ and the resolution was 4 cm⁻¹.

Differential scanning calorimetry (DSC)
DSC analysis was performed using Netzsch DSC 204, Tokyo, Japan. The samples were heated in a sealed aluminium pans at a rate of 10° C per/min in a 30 to 300° C temperature under nitrogen flow of 40 mL/min.

X-ray powder diffractometry (XRD)
X-ray powder diffraction patterns were recorded on a Jeol JDX 8030 X-ray diffractometer (Tokyo, Japan) using Ni-filtered, CuKa radiation, a voltage of 40 kV and a 25-mA current. The scanning rate employed was 1°/min over the 10 to 30° diffraction angle (2θ) range.

LIQUID STATE STUDIES

Phase solubility study
An excess of ramipril (50 mg) was added to screw capped bottles containing various concentrations of β-cyclodextrin solution (0.2, 0.4, 0.6, 0.8 and 1 mM×10⁻¹). Vials were shaken mechanically at 25±0.5° C for 24 h using rotary flask shaker[27]. After 24 h of shaking to achieve equilibrium, 5 mL of aliquots were withdrawn, filtered (0.45 µm pore size) and analyzed spectrophotometrically for drug content at 210 nm using UV 1700 spectrophotometer (Shimadzu, Japan).

Estimation of drug content
The content of ramipril in formulated solid dispersion was determined by UV spectrophotometry. An accurately weighed quantity of solid dispersion (100 mg) was transferred into a beaker containing known volume of mixture of methanol and phosphate buffer (pH 6.8) (1:10). The solution was stirred for 1 h using magnetic stirrer. The dispersion was filtered through Whatman filter paper (0.45 µm pore size) and assayed for drug content at 210 nm spectrophotometrically.

Dissolution rate studies
Dissolution rate studies were performed in double distilled water at 37±0.5°C, using USP XXII apparatus (Electrolab, Mumbai, India) with paddle rotating at 50 rpm. Solid products each containing 100 mg of drug was subjected to dissolution. At fixed time intervals, 5 mL samples were withdrawn, filtered through Whatman filter paper (0.45 µm pore size) and spectrophotometrically assayed for drug content at 210 nm. Dissolution efficiency (DE) was calculated from the area under the dissolution curve at time ‘t’ (measured using trapezoidal rule) and expressed as percentage of the area of the rectangle described by 100% dissolution in the same time [28].

RESULTS AND DISCUSSION

Physicochemical evaluation of ramipril solid dispersions

The physicochemical evaluations of the solid dispersions were shown in Table 1. All the pharmacotechnical parameters with respect to solid dispersions were within the official limits.

Table 1. Physicochemical Evaluation of Ramipril Solid Dispensions

<table>
<thead>
<tr>
<th>S. No</th>
<th>Batch Code</th>
<th>Drug:carrier Ratio</th>
<th>Yield (%)</th>
<th>Angle of repose (°)</th>
<th>Bulk density (g/cc)</th>
<th>Compressibility (%)</th>
<th>Moisture uptake (%)</th>
<th>Drug content (%)</th>
<th>DE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pure drug</td>
<td>—</td>
<td>—</td>
<td>21±1.3</td>
<td>0.79±0.03</td>
<td>15±1.0</td>
<td>5±0.9</td>
<td>99±0.9</td>
<td>19.88</td>
</tr>
<tr>
<td>2</td>
<td>RSD-I</td>
<td>1:1</td>
<td>93.34±1.1</td>
<td>22±0.8</td>
<td>0.78±0.02</td>
<td>15±1.2</td>
<td>6.2±0.8</td>
<td>96±1.4</td>
<td>31.01</td>
</tr>
<tr>
<td>3</td>
<td>RSD-II</td>
<td>1:2</td>
<td>94.47±1.3</td>
<td>22±0.7</td>
<td>0.82±0.04</td>
<td>16±1.1</td>
<td>6.2±0.9</td>
<td>97±1.3</td>
<td>43.27</td>
</tr>
<tr>
<td>4</td>
<td>RSD-III</td>
<td>1:3</td>
<td>96.87±0.9</td>
<td>22±1.3</td>
<td>0.81±0.05</td>
<td>16±1.5</td>
<td>6.3±1.1</td>
<td>96±1.3</td>
<td>60.41</td>
</tr>
<tr>
<td>5</td>
<td>RSD-IV</td>
<td>1:4</td>
<td>97.22±1.5</td>
<td>22±1.5</td>
<td>0.85±0.02</td>
<td>16±1.3</td>
<td>6.4±0.8</td>
<td>98±1.1</td>
<td>85.09</td>
</tr>
<tr>
<td>6</td>
<td>RSD-V</td>
<td>1:5</td>
<td>96.11±1.1</td>
<td>21±1.3</td>
<td>0.83±0.03</td>
<td>16±1.4</td>
<td>6.4±1.2</td>
<td>97±1.4</td>
<td>96.89</td>
</tr>
</tbody>
</table>

* Code of formulations
* Mean±SD (n=3)

Phase solubility study
Cyclodextrins have been explored to be a powerful solubilizing agent for many poorly soluble drugs. To enhance the solubility of ramipril, the phase solubility diagram of ramipril with β-CD in distilled water was examined. The results revealed that β-CD was found to be more effective as a solubilizing complex with ramipril. The aqueous solubility of ramipril is increased linearly (r²=0.9917) as a function of carrier concentration and corresponded to A₁ type. Since, the slope of the diagram is less than 1, the complex stoichiometry was assumed to be 1:1. As the purpose of the study was not to prove the stoichiometry of the complex, based on this assumption binary system of ramipril and β-CD were prepared using 1:1 molar proportion. The extent of complexation is characterized by the apparent 1:1 stability constant Kₛ, which was calculated based on the solubility diagram according to the equation

Kₛ = Slope/S 0 (1-Slope)

Where, 'S₀', is the solubility of ramipril in the absence of β-cyclodextrin...

The value of the stability constant Kₛ was 328.65 M⁻¹, which was adequately stable and well within the range of 100 to 1000 M⁻¹ considered to
be ideal \[29\]. A smaller the $K_s$ indicates too weak interaction, while a larger value indicates the possibility of limited release of drug from the complex by interfering with drug absorption.

**Fourier Transform Infrared Spectroscopy (FTIR)**

Figure 2a, 2b shows the FTIR spectrum of drug and its binary system with β-CD. The results depicted that there was no significant change in the spectrum of solid dispersion, as incorporation of ramipril into the β-CD did not modify the position of its functional groups. All the major characteristic peaks of ramipril observed at wave numbers 3367 cm$^{-1}$ (COOH-stretching), 3348 cm$^{-1}$ (NH-stretching) and 1743 cm$^{-1}$ (C=O-stretching) were retained in the binary systems at the same wave numbers indicating the lack of significant interaction between the drug and carrier in the solid dispersion. This may be an indicative of the drug monomeric dispersion, as a consequence of the interaction with β-CD through hydrogen bonding, which could result in its inclusion into the hydrophobic cavity of the cyclodextrin.

Shift of peaks from 1498.74 cm$^{-1}$ to 1464.02 cm$^{-1}$ (C-H aromatic bending) and 1375.29 cm$^{-1}$ to 1348.29 cm$^{-1}$ (C-H aliphatic bending) indicates weak interaction between drug and β-CD.

**Differential Scanning Calorimetry**

Thermogram of ramipril and corresponding drug carrier systems were illustrated in Fig 3a and 3b. The DSC curve of ramipril exhibit corresponding
endothermic peak (T peak = 117°C) corresponding to its melting point. However, the characteristic endothermic peak, corresponding to drug melting was broadened and shifted towards lower temperature, with reduced intensity in solid dispersions. This could be due to higher concentration and uniform distribution of drug in the crust of carrier resulting in its complete miscibility. Moreover, the data also indicates there seems to be no interaction between the components of binary systems. No significant difference in the DSC pattern of dispersions suggesting that the kneading process could not induce the interaction at molecular level and the solid dispersion formed as highly dispersed drug crystals in carrier.

**X-ray Diffractometry (XRD)**

X-ray diffractometry spectra of pure drug and binary systems with carriers are represented in Figure 4a & 4b. The X-ray diffractogram of ramipril has sharp peaks at diffraction angles (2θ) at 16.23, 17.98, 20.86 and 21.34 with intensities of 100, 77.8, 40.5 and 71 respectively showing a typical crystalline pattern. On the other hand, XRD of solid dispersion exhibited a significant decrease of crystallinity, as evident by the disappearance of sharp distinctive peaks. In addition, the intensity of ramipril peaks at same diffraction angles were 46.8, 25.6, 32.3 and 57.3, which reveals that the intensity of the peaks were remarkably reduced in solid dispersions indicating the amorphous state of the drug. The broadening diffraction peaks reveals that the inhibition of crystallization of ramipril and converting it into amorphous form. However, the intensity of crystalline peaks of ramipril in the solid dispersions was less than that of intact ramipril, indicating lower crystallinity of ramipril in the solid dispersions. Based on these data, we
Fig 4 (b). XRD of ramipril and β-cyclodextrin binary system.

Fig 5. In vitro dissolution profiles of solid dispersion of ramipril. Pure drug - (•), RSD-I (■), RSD-II(Δ), RSD-III (+), RSD-IV (×) and RSD -V (○). Samples were withdrawn at different time intervals and ramipril was determined by UV spectrophotometer.

can confirm that a structural modification occurred in molecular state of ramipril, the physical state of ramipril is crystalline, but that of carrier is amorphous.

Dissolution rate studies
Figure 5 illustrates the dissolution profiles plotted from the experimental values of pure ramipril and its binary systems. These binary systems exhibit faster dissolution rates over pure drug. A complete dissolution of drug from sample from RSD-V (97.12 ±1.1%) was achieved within 60 minutes from binary system, where as pure drug showed dissolution of 20.12±0.8%. Solid dispersions showed a 4.83 times increase in the dissolution rate over pure drug. The improvement in dissolution rate of ramipril from binary system is in accordance with the results of the solubility study (Figure 1). The T values of the batches prepared with 1:4 and 1:5 drug: carrier ratio were 26 and 19 min respectively. The batch prepared in the ratio of 1:5 (drug: carrier) showed better in vitro release and better T values, when compared with the in vitro release of pure drug. The enhancement of dissolution of ramipril from the carrier may be attributed to several factors such as lack of crystallinity, increased wettablility and dispersibility. Incorporation of a drug with hydrophilic carrier system offered an increased wetting and reduction in interfacial tension between hydrophobic drug and dissolution medium. The solid dispersion prepared using the molar ratio of 1:5(RSD-V) exhibited maximum dissolution rate of the drug. The “K” values of solid dispersion were found to be more than pure drug and followed first order kinetics.

CONCLUSION
Phase solubility profile revealed that the solubility of ramipril and its apparent stability constant was significantly increased in presence of β-Cyclodextrin. FT-IR and DSC studies showed no evidence of interaction between the drug and carrier. The XRD studies confirmed the amorphization of drug which offered an explanation for better dissolution rate of ramipril from solid dispersion. Solid dispersions showed a preferential increase in dissolution of ramipril over pure drug. The study shows that, the dissolution rate of ramipril can be improved to a greater extent by solid dispersions technique employing an industrially feasible kneading method. It is concluded that the solid dispersion of ramipril increased the solubility and dissolution rate of drug, suggesting a possible enhancement of its oral bioavailability.

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