Development and in vivo evaluation of mucoadhesive tablets of Rebapimide

Ganesh Kumar Gudas *, A. Jaswanth1 and D.V.R.N. Bhikshapathi*2

*Srikrupa Institute of Pharmaceutical Sciences, Siddipet, India
1Procadance Institute of Pharmaceutical Sciences, Gajwel, India
2CMR College of Pharmacy, Kandlakoya, Hyderabad-501401, Telangana, India

*Correspondence Info:
Mr. Ganesh Kumar Gudas
Srikrupa Institute of Pharmaceutical Science,
Siddipet, Telangana, 502277.
E-mail: gkganeshpharmaco@gmail.com

Abstract

The aim of the present work was in vitro and in vivo evaluation of mucoadhesive tablets of rebapimide to prolong the gastric residence time after oral administration. The solubility of rebapimide was enhanced by kneading technique with that mixture formulations were prepared by using 3 factorial designs to explore the effects of gum Kondagogu, gum Olibanum and Guar gum (as independent variables) on mucoadhesive strength, drug release and Ex vivo residence time (as dependent variables) was studied and published in the earlier research paper.

In this investigation the formulated mucoadhesive tablets which was optimized through in vitro studies was selected and performed the in vivo studies on human volunteers. The drug-polymer interaction was also studied by conducting FTIR and DSC tests. The in vitro release kinetics studies reveal that all formulations fits well with zero order, followed by Korsmeyer-Peppas, Higuchi and the mechanism of drug release is erosion. After analysis of different evaluation parameters and drug release kinetics, formulation code F13 was selected as a promising formulation for delivery of rebapimide as a mucoadhesive gastroretentive tablet with best mucoadhesive strength and 99.34% drug release at 12th hour. Radiological evidences suggest that, a formulated tablet was well adhered for >10 h in human stomach. The bioavailability studies of F13 containing rebapimide was carried out which exhibited increased pharmacokinetic parameters of Cmax (427.01±73), Tmax (4.00±1.23 h) and AUC0-t (2242±18.24) as compared to marketed formulations which indicates improved bioavailability of formulations.

Keywords: Rebapimide, Mucoadhesive, Radiographic studies, In vivo bioavailability studies.

1. Introduction

Oral administration is the most convenient, widely utilized, and preferred route of drug delivery for systemic action. However, when administered orally, many therapeutic agents are subjected to extensive presystemic elimination by gastrointestinal degradation and or first pass hepatic metabolism, as a result of which low systemic bioavailability and shorter duration of therapeutic activity. Much attention has been focused, recently on targeting a drug delivery system to a particular region of the body for extended period of drug release, not only for local targeting of drugs but also for the better control of systemic delivery [1].

Naturally occurring polymers, being biocompatible and biodegradable, are currently extensively researched for the development of novel drug delivery systems. There are number of drugs like domperidone, ranitidine, theophylline those have narrow absorption window from upper GIT i.e. stomach. Due to short gastric resident time less than 3 hr these drug reaches the non absorbing distal parts of intestine. Therefore main challenge is to prolong the resident time of drug in stomach. Gastro retentive drug delivery techniques are primarily controlled release drug delivery systems, which gets retained in the stomach for longer period of time, thus helping in absorption of drug for the intended duration of time. It helps to improves bioavailability, reduces drug wastage, improve solubility of drugs that are less soluble at high pH environment (e.g. weakly basic drugs like domperidone, papaverine) [2].

Rebapimide, (+)-2-(furfurylsulfinyl)-N-(4-[4-(piperidinomethyl)-2-pyridyl]oxy-(Z)-2-butenyl) acetamide is a newly developed 2nd generation histamine H2-receptor antagonist. It is used in the treatment of gastric ulcers, duodenal ulcers, and gastric mucosal lesions associated with acute gastritis and acute exacerbation of chronic gastritis. It is absorbed in the small intestine, reaches gastric cells via the systemic circulation, and rapidly binds to gastric cell histamine H2 receptors, resulting in immediate inhibition of gastric acid secretion [12].
2. Materials and Methods

2.1 Materials

The Rebapimide was obtained as a gift sample from splendid laboratories, Pune. Gum Kondagugu, gum Olibanum and Guar gum were obtained from Girijan Co-operative corp. Ltd, Hyderabad. PVP-K30 was gifted from MSN Labs Ltd, Hyderabad. All other chemicals used were of analytical grade.

2.2 Preparation of mucoadhesive tablets

2.2.1 Wet granulation method

Mucoadhesive tablets of rebapimide solid dispersion were prepared by wet granulation technique using different concentrations of gum Kondagugu, gum olibanum and Guar gum. All the ingredients were passed through sieve no 85#. and were mixed uniformly. Granulation was carried out with sufficient quantity of binder solution (PVP K 30 - 5% in isopropyl alcohol). Wet mass was passed through sieve no 12# and dried at 45-55 °C for 1 hr. Dried granules were sized by sieve no.18#. Add magnesium stearate and talc. Granules obtained were compressed with 9 mm flat punch (Cadmach, Ahmedabad, India) [3].

The formulations are made by using design of experiment method (factorial designs)

Study type: Response surface
Design type: Central Composite
Design mode: Quadratic

<table>
<thead>
<tr>
<th>F.NO</th>
<th>Rebapimide Solid Dispersion (mg)</th>
<th>GK (mg)</th>
<th>GO (mg)</th>
<th>GG (mg)</th>
<th>MCC (mg)</th>
<th>PVP K-30 (mg)</th>
<th>Talc (mg)</th>
<th>Magnesium Stearate (mg)</th>
<th>Total Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>200</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>122</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F2</td>
<td>200</td>
<td>45</td>
<td>15</td>
<td>30</td>
<td>117</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F3</td>
<td>200</td>
<td>15</td>
<td>45</td>
<td>30</td>
<td>117</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F4</td>
<td>200</td>
<td>45</td>
<td>45</td>
<td>30</td>
<td>72</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F5</td>
<td>200</td>
<td>15</td>
<td>30</td>
<td>30</td>
<td>139</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F6</td>
<td>200</td>
<td>45</td>
<td>30</td>
<td>30</td>
<td>94</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F7</td>
<td>200</td>
<td>30</td>
<td>15</td>
<td>30</td>
<td>139</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F8</td>
<td>200</td>
<td>30</td>
<td>45</td>
<td>30</td>
<td>94</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F9</td>
<td>200</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>116</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F10</td>
<td>200</td>
<td>15</td>
<td>15</td>
<td>60</td>
<td>132</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F11</td>
<td>200</td>
<td>45</td>
<td>15</td>
<td>60</td>
<td>87</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F12</td>
<td>200</td>
<td>15</td>
<td>45</td>
<td>60</td>
<td>87</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F13</td>
<td>200</td>
<td>45</td>
<td>45</td>
<td>60</td>
<td>42</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F14</td>
<td>200</td>
<td>15</td>
<td>30</td>
<td>60</td>
<td>109</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F15</td>
<td>200</td>
<td>45</td>
<td>30</td>
<td>60</td>
<td>64</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F16</td>
<td>200</td>
<td>30</td>
<td>15</td>
<td>60</td>
<td>109</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F17</td>
<td>200</td>
<td>30</td>
<td>45</td>
<td>60</td>
<td>64</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F18</td>
<td>200</td>
<td>30</td>
<td>30</td>
<td>60</td>
<td>86</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F19</td>
<td>200</td>
<td>15</td>
<td>15</td>
<td>90</td>
<td>87</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F20</td>
<td>200</td>
<td>45</td>
<td>15</td>
<td>90</td>
<td>42</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F21</td>
<td>200</td>
<td>15</td>
<td>45</td>
<td>90</td>
<td>42</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F22</td>
<td>200</td>
<td>45</td>
<td>45</td>
<td>90</td>
<td>03</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F23</td>
<td>200</td>
<td>15</td>
<td>30</td>
<td>90</td>
<td>64</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F24</td>
<td>200</td>
<td>45</td>
<td>30</td>
<td>90</td>
<td>19</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F25</td>
<td>200</td>
<td>30</td>
<td>15</td>
<td>90</td>
<td>64</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F26</td>
<td>200</td>
<td>30</td>
<td>45</td>
<td>90</td>
<td>19</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>F27</td>
<td>200</td>
<td>30</td>
<td>30</td>
<td>90</td>
<td>41</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
</tbody>
</table>


2.2 In-vitro dissolution studies:

The USP dissolution test apparatus (apparatus II paddle type) was used to study the drug release from the tablets. The dissolution medium was 900 ml of 0.1N HCl buffer pH 1.2. The release was performed at 37 ± 0.5°C, with a rotation speed of 100 rpm. 5 ml samples were withdrawn at predetermined time intervals and replaced with fresh medium. The samples were filtered through whatmann filter paper and analyzed after appropriate dilution by UV spectrophotometer at 220 nm and drug release was determined from standard curve. [4]

Dissolution Parameters:
Dissolution medium: 900 ml of 0.1 N HCl buffer with pH 1.2
RPM: 100
Temp: 37 ± 0.5°C
Sample volume withdrawn: 5ml sample
λ max : 227 nm
Time interval: 0, 1, 2, 3, 4, 6, 8, 10 & 12 h.

www.ssjournals.com
2.3 Drug Excipient Compatibility Studies

The drug excipient compatibility studies were carried out by Fourier transform infrared spectroscopy (FTIR), DSC and SEM.

2.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer. The samples were dispersed in KBr and compressed into disc/pellet by application of pressure. The pellets were placed in the light path for recording the IR spectra. The scanning range was 400-4000 cm⁻¹ and the resolution was 1 cm⁻¹.

2.3.2 Differential Scanning Calorimetry (DSC)

DSC studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Samples were accurately weighed and heated in sealed aluminium pans at a rate of 10°C/min between 25 and 350°C temperature range under nitrogen atmosphere. Empty aluminium pan was used as a reference.

2.3.3 SEM studies

The surface and shape characteristics of tablets were determined by scanning electron microscopy (SEM) (HITACHI, S-3700N). Photographs were taken and recorded at suitable magnification.

2.4 Stability studies

The stability study of the optimized formulation was carried out under different conditions according to ICH guidelines. The optimized tablets were stored in a stability chamber for stability studies (REMI make). Accelerated Stability studies were carried out at 40 °C / 75 % RH for the best formulations for 6 months. The tablets were characterized for hardness, mucoadhesive strength and cumulative % drug released during the stability study period[6].

2.5 In-vivo bioavailability studies:

In vivo study protocol:

Twelve healthy male subjects with a mean age of 27.13±3.60 years (ranging from 24 to 34 years), mean weight 66.33±7.61Kg (ranging from 61 to 74 Kg) and a mean height 168.17 ± 10.46 cm (ranging from 157 to 179 cm) were directly obtained from concentration time data. In the present study, AUC₀ₐ refers to the AUC from 0 to 24h, which was determined by linear trapezoidal rule and AUC₀→ₐ refers to the AUC from time at zero hours to infinity. The AUC₀→ₐ was calculated using the formula AUC₀→ₐ = [Cₐ开发利用] / K where Cₐ开发利用 is the concentration in ng/ml at the last time point and K is the elimination rate constant. Various pharmacokinetic parameters like area under the curve [AUC], elimination half life (t½), Volume of distribution (Vₐ开发利用), total clearance (Clₐ开发利用) and mean residence time for each subject using a non compartmental pharmacokinetic program. The pharmacokinetic parameters were performed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean ±SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA)
using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant. [17].

2.8 *In-Vivo* radiographic studies

The bio-study protocol for radiographic studies was approved by Institutional Human Ethics Committee, No: IHEC/VGOPC/053/2015. From the formulations 100 mg drug was changed with barium sulfate to make them x-ray opaque. The subjects were given these tablets with breakfast. The volunteers were given 200 mL of water at zero time, to ensure the absence of radio-opaque material in the stomach. X-ray images were taken using (Genesis 50, Josef Bets chart AG, Brunnen, Switzerland) in standing position after 0.5, 2, 4 and 10 hrs post-administration of tablets. From the X-ray films gastric residence and position was interpreted.

3. Results & Discussion

3.1 Physico-chemical parameters of Rebapimide mucoadhesive tablets

The prepared tablets were evaluated for different physico-chemical properties and the results are found to be within the pharmacopoeial limits.

3.2 Kinetic modeling of drug release:

To explore the mechanism of drug release from mucoadhesive tablets, various kinetic models like zero order, first order, Higuchi and Korsmeyer-Peppas equations were applied to the different formulations. The release kinetics of best formulation (F13) was shown in Table 2.

| Table: 2 Release kinetics of optimized formulation of rebapimide mucoadhesive tablets |
|-----------------------------------------------|---------------|---------------|---------------|---------------|
| Formulation Code | Zero Order | First Order | Higuchi | Korsmeyer-Peppas |
| | $R^2$ | $K$ | $R^2$ | $K$ | $R^2$ | $K$ | $R^2$ | $N$ |
| F13 | 0.993 | 7.873 | 0.766 | 0.131 | 0.953 | 29.08 | 0.554 | 2.175 |

From the above results it is apparent that the regression coefficient value closer to unity in case of zero order plot i.e.0.993 indicates that the drug release follows a zero order mechanism Table No:2. This data indicates a lesser amount of linearity when plotted by the first order equation. Hence it can be concluded that the major mechanism of drug release follows zero order kinetics.

Further, the translation of the data from the dissolution studies suggested possibility of understanding the mechanism of drug release by configuring the data in to various mathematical modeling such as Higuchi and Korsmeyer-Peppas plots. The mass transfer with respect to square root of the time has been plotted, revealed a linear graph with regression value close to one i.e. 0.946 starting that the release from the matrix was through diffusion. Further the $n$ value obtained from the Korsmeyer-Peppas plots i.e.0.554 suggests that the drug release from tablets was anomalous Non fickian diffusion.

3.3 Drug excipient compatibility studies

3.3.1 FTIR Studies

![Figure: 2 FT-IR spectrum of pure drug rebapimide](image1.png)

![Figure: 3 FT-IR spectrum of optimized formulation F13](image2.png)
Possible interactions between drug and polymer in formulations were investigated by FTIR. FTIR spectra of rebamipide and optimized formulation F13 were examined. FTIR spectrums are properly labelled and shown in (Fig.2 ). FTIR of pure rebamipide characteristic sharp peaks of amine stretching (=N–H and CH₂) vibration at 3420.32–3379.48 cm⁻¹ and alkane stretching (–CH₃, –CH₂ and –CH) vibration at 2938.73 cm⁻¹. Also exhibited C=O stretch at 1740.2 cm⁻¹ due to aldehydes and C=O–NH stretching at 1650.90 cm⁻¹. A selective stretching vibration at 1580.57 cm⁻¹ and 1525.80 cm⁻¹ for primary and secondary amine was also observed. For functional groups like –C–H bend alkanes and –C–H rock alkanes stretch showed vibrations at 1450.78 cm⁻¹ and 749.57 cm⁻¹ respectively.

Overall there was no alteration in peaks of rebamipide pure drug and optimized formulation, suggesting that there was no interaction between drug & excipients. There is additional peaks appeared or disappeared hence no significant changes in peaks of optimized formulation was observed when compared to pure drug, indicating absence of any interaction.

3.3.2 DSC studies

![DSC thermogram of Rebapimide](image1)

![DSC Thermogram of optimized tablets F13](image2)

DSC was used to detect interaction between rebamipide and excipients. The thermogram of rebamipide pure drug exhibited a sharp endotherm melting point at 305°C. The thermogram of optimized formulation F13 exhibited a sharp endotherm melting point at 300.5°C. The thermograms of gum Kondagogu, Guar gum and gum olibanum were shown in Fig: 5 & 6 respectively. There is no considerable change observed in melting endotherm of drug in optimized formulation. It indicates that there is no interaction between drug & excipients used in the formulation.

There is no considerable change observed in melting endotherm of drug in optimized formulation. It indicates that there is no interaction between drug & excipients used in the formulation.
3.3.3 SEM:

Fig: 6 Scanning Electron Microscopy of rebapimide mucoadhesive

Fig: 7 Scanning Electron Microscopy of rebapimide mucoadhesive

3.4 Radiographic studies:

3.4.1 Intragastric behavior of rebapimide mucoadhesive tablets

The radiographic images were taken at different periods post administration of the barium sulfate-loaded tablet in three human volunteers. It is clear that the tablet appears more or less at the same position for the initial 4 h. This could be related to its floating ability. Later on, the tablet was slightly moved downwards, yet, remained within the stomach till the end of 10 h. The increased gastric residence time favors increase in the bioavailability of drugs.

Fig: 8 Scanning Electron Microscopy of rebapimide mucoadhesive tablets

Fig: 9 Radiographic Images of a BaSO₄ loaded rebapimide mucoadhesive tablet (F 13) in the stomach
Fig: 10 Percentage drug release of rebapimide formulations F13 & Innovator

Fig: 11 Plasma concentrations at different time intervals for rebapimide optimized formulation and Marketed Product

Table: 3 Comparison of rebapimide optimized formulation and Marketed Product

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Rebamipide Optimized formulation</th>
<th>Marketed Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>427.01±73</td>
<td>315±1.41</td>
</tr>
<tr>
<td>$\text{AUC}_{0\rightarrow t}$ (ng. h/ml)</td>
<td>2015±23.14</td>
<td>1612±14.26</td>
</tr>
<tr>
<td>$\text{AUC}_{0\rightarrow \infty}$ (ng. h/ml)</td>
<td>2242±18.24</td>
<td>1815±18.12</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>4.00±1.23</td>
<td>1.50±0.24</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>3.153 ± 0.41</td>
<td>3.574 ± 0.01</td>
</tr>
<tr>
<td>Kel (h$^{-1}$)</td>
<td>1.033 ± 0.11</td>
<td>1.142 ± 0.33</td>
</tr>
</tbody>
</table>
3.5 Bioavailability parameters

Mean plasma concentration profiles of prepared rebapimide optimized formulation and marketed product are presented in Figure 10 rebapimide optimized formulation exhibited as sustained release in vivo when compared with marketed tablet. All the pharmacokinetics parameters displayed in Table 3 in this study in human subjects, prolonged drug absorption was achieved with the test formulation. The average peak concentration of the test formulation was significantly higher than that of the reference (427.01±73 ng/ml for the test formulation versus 315±1.41ng/ml for the reference).

In order to estimate the amount of drug absorbed from the test formulation, the relative bioavailability was calculated from the AUC of the reference and test formulations (1612±14.26ng.h/ml for the reference product versus 2242±18.24ng.h/ml for the test formulation). The results indicated that the test formulation could increase the bioavailability of rebapimide in humans effectively. In this study, the rebapimide mucoadhesive tablet produce higher bioavailability than that of a marketed product, this overall increase in bioavailability and increased gastric residence time due to mucoadhesion of tablet in the stomach region for 10 h.[17]

4. Conclusion

Rebapimide mucoadhesive oral tablets could be formulated using the drug, gum kondagogu, gum obilalanum and Guar gum with different proportions using 3³ full factorial designs. It can be seen that there is a synergistic effect when polymers are used in combinations. The in vitro release kinetics studies reveal that all formulations fits well with zero order, followed by Korsmeyer-Peppas, Higuchi and the mechanism of drug release is erosion.

From the formulations F1-F27 the formulation F 13 was selected as optimized formulation because it showed maximum release and the other properties such as swelling index was also low, mucoadhesion force shown good and the Post and pre compression parameters were found to be within the Pharmacopeial limits.

Radiological evidences suggest that, a formulated tablet was well adhered for >10 h in human stomach. The bioavailability studies of F 13 containing Rebapimide was carried out which exhibited increased pharmacokinetic parameters of Cmax, Tmax and AUC as compared to marketed formulations which indicates improved bioavailability of formulations.

Reference