Development and \textit{in vitro} evaluation of floating multiparticulate system of Repaglinide

Muddana Eswara Bhanoji RAO*, Suryakanta SWAIN**, Chinam Niranjan PATRA*, Jammula SRUTI*, Subhasmita PATRA*

\textbf{Summary}
Floating multi-particle system for Repaglinide was prepared by melt granulation method using Gelucire 43/01 as the binder. Inclusion in 1:1 complex ratio of $\beta$-CD and HP-$\beta$-CD enhanced the solubility of Repaglinide by 4 to 5 folds. In vitro release studies, using release modulators such as HPMC K4M, HPMC K15M, HPMC K100M and ethyl cellulose (20cPs) in different ratios with the drug showed extended release of up to 12h following zero order. Even though Gelucire 43/01 based drug-$\beta$-CD complexes showed good floating property but the release was associated with an initial burst release followed by a sustained release that is less than 50% within 12h. The hydrophobic to hydrophilic seems to be critical in the controlled release of the drug. FT-IR studies indicated that there was no interaction of drug and excipients. X-ray diffraction study of the optimized formulation showed hollow shaped spectrum with complete absence of peaks, proving that Repaglinide was in amorphous form in the inclusion complex. There was no significant change in the drug content and floating property from the granules stored at conditions as per ICH guidelines confirming that the Repaglinide multiparticulate system were stable.

\textbf{Key Words:} Melt granulation, Gelucire 43/01, Phase solubility study, Powder X-ray Diffraction, In vitro drug release profile

\textbf{Anahtar Kelimeler:} Eritme granülasyon, Gelucire 43/01, Faz çözünürlük çalışması, Toz X-ray Diffraksiyon, In vitro ilaç sahipsizlik profil

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INTRODUCTION

Repaglinide is a new class of oral hypoglycemic, designed to normalize the meal time glucose excursions and is indicated only in type II diabetes mellitus as an alternative to sulfonylurea, or to supplement metformin or long acting insulin. Though it is not a sulfonylurea, it acts in an analogous manner by binding to sulfonylurea as well as to other distinct receptors closer of ATP dependent K⁺ channel leading to depolarization and the release of the insulin. It induces rapid onset short lasting insulin release and is administered before each major meal to control postprandial hyperglycaemia (1). It is practically insoluble in water, but freely soluble in dichloride methane and methyl alcohol, (S)-(+-)-2-ethoxy-4-[2-(3-methyl-1-[2-(piperidin-1-yl) phenyl butylamino)-2-oxoethyl]-benzoic acid (2).

Granulation is an example of particle design intended to produce improved performance through the combination of formulation composition and manufacturing processes, and modified particle morphology is achieved through the use of a liquid acting on the powder blend to form inter-particle bonds which then result in formation of granules of varying sizes. Granulation is the process in which primary powder particles are made to adhere to form larger, multiparticle entities called the granules. The desired attributes of the granules are controlled by a combination of formulation and processes. There, an optimum range of granule size will exist, for a particular formulation. Pharmaceutical granules typically have size ranging between 0.2 and 4.0 mm, depending on their subsequent use. Granulation is used mainly to improve flow and compressibility of powders, and to prevent segregation of the blend components. Particle size of the granules is mainly affected by the quantity and feeding rate of the granulating liquid. Melt granulation is one of the most widely applied processing techniques in the array of pharmaceutical manufacturing operation of granules over other conventional techniques due to their certain advantages such as minimum processing steps needed, less time required for drying operation. Other advantages of this technique is that the active ingredients do not need to be compressed, yet the entire procedure is simple, continuous, and efficient and a uniform dispersion of the fine particle occurs. The major drawback about this method is that it requires high energy input, and should not to be applied to heat-sensitive materials due to the elevated temperatures involved. Lower-melting-point binder risks the situations where melting or softening of the binder occurs during the handling and storage of the agglomerates, higher-melting-point binders require high melting temperature and can contribute to instability problems especially for heat-labile material (3, 4). The basic elementary mechanism involved in melt granulation method are the distribution and immersion. In agglomeration by distribution mode, a distribution of the molten binding liquid occurs on the surfaces of primary particles, and agglomerates are formed via coalescence between the wetted nuclei. In agglomeration by immersion mode, nuclei are formed by immersion of the primary particles onto the surface of a droplet of molten binding liquid. Distribution of the molten binding liquid to surfaces of nuclei has to be effected by densification prior to coalescence between the nuclei. Depending on the relative size between the solid particles and the molten binding liquid droplets, the distribution will be a dominant mode when the molten binding liquid droplets are smaller than the solid particles or of a similar size. On the other hand, the immersion mode will dominate when the molten binding liquid droplets are larger than the solid particles. The distribution mode is promoted by a low molten binding liquid viscosity. In case of the immersion, it is favored for the molten binding liquid of high viscosity, as it could resist the breaking up by the dispersive forces (5). Different techniques for the melt granulation are: spray congealing, which involves spraying a hot melt of wax, fatty acid or glycercide into an air chamber below the melting point of the materials, or at a cryogenic temperature and the resulting spray-congealed particles (10–3000 µm in diameter) are obtained upon cooling, where as in case of the tumbling melt granulation method, spherical beads are prepared by considering the optimum value of viscosity and particle size of the melting materials. The particle size of a melting material should be 1/6 or lower than the diameter of the seeds. High-viscosity melting materials should not be employed to avoid agglomeration of seeds and producing beads of low sphericity (6).

Repaglinide belongs to BCS class II drug with low bioavailability (50%) and poor absorption in the upper intestinal tract. Poor solubility in gastrointestinal fluids gives rise to variations in its dissolution rate and incomplete bioavailability. Enhancement of the dissolution rate of water-insoluble drugs remains one of the most challenging tasks of drug development, as the enhanced dissolution rates can increase the drug’s oral bioavailability. Although Repaglinide is rapidly absorbed after oral administration, it
is critical to improve the dissolution rate of Repaglinide in order to enhance the bioavailability due to its low solubility (7). Hence, the major objectives of the study are to improve the solubility and to develop floating granules of Repaglinide in terms of increasing the gastric retention time. Apart from that, the formulations also modulate or control the drug release for a sustained action. Consequently, considering the above objectives, to prepare and to evaluate the floating multiparticulate drug delivery system of Repaglinide by melt granulation technique using β-CD and HP-β-CD.

MATERIALS AND METHODS

Materials
Repaglinide was obtained as a gift sample from Ranboxy Laboratories Ltd., Hyarayana, India. The excipients ethyl cellulose and Gelucire 43/01 were from M/s Gattefoss, Mumbai, India, whereas, β-CD and HP-β-CD were from M/s Evonik, Mumbai, India. All other chemicals were of analytical grade.

Methods

Pre-formulation studies for Repaglinide

Solubility data
A cleaned and dried graduated test tube (20 mL) was taken and 20 mL of freshly prepared simulated gastric fluid without enzyme (pH 1.2) was poured into it. Then a small, unknown quantity of Repaglinide was added to it and dissolved properly, shaking by hand. The above procedure of addition of the drug and then shaking was continued until the drug was dissolved completely, that means until a clear solution was obtained. When the drug remained undissolved even after shaking by hand thoroughly, which indicated a supersaturated solution, the test tube containing the drug with the solvent was shaken in a mechanical shaker at 25 ± 0.5°C for 24 h. The above solution was then filtered, diluted, and absorbance was noted in λ\text{max} 243 nm by using a UV spectrophotometer (Shimadzu, model no. 1800, Switzerland). Likewise, solubility was determined in at pH 6.8 and 7.4 as shown in Table 1 (8).

Melting point
The melting point of Repaglinide was determined by taking small amount of drug in a capillary tube closed at one end and the temperature at which the drug melted, was noted (9). The average of the triplet melting reading were determined as 128.63 ± 0.23°C.

Micromeritic properties of the pure drug
The flowability of Repaglinide was investigated by determining the angle of repose, bulk density,

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Repaglinide</th>
<th>β-CD</th>
<th>HP β-CD</th>
<th>Water : Methanol (50 mL : 50 mL)</th>
<th>Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>β-CD</td>
</tr>
<tr>
<td>B1</td>
<td>1</td>
<td>0</td>
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<td>-</td>
<td>4.251±0.22</td>
</tr>
<tr>
<td>B2</td>
<td>1</td>
<td>0.25</td>
<td>_</td>
<td>q.s</td>
<td>_</td>
</tr>
<tr>
<td>B3</td>
<td>1</td>
<td>0.5</td>
<td>_</td>
<td>q.s</td>
<td>_</td>
</tr>
<tr>
<td>B4</td>
<td>1</td>
<td>0.75</td>
<td>_</td>
<td>q.s</td>
<td>_</td>
</tr>
<tr>
<td>B5</td>
<td>1</td>
<td>1</td>
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<tr>
<td>B6</td>
<td>1</td>
<td>1.25</td>
<td>_</td>
<td>q.s</td>
<td>_</td>
</tr>
<tr>
<td>B7</td>
<td>1</td>
<td>1.5</td>
<td>_</td>
<td>q.s</td>
<td>_</td>
</tr>
<tr>
<td>B8</td>
<td>1</td>
<td>_</td>
<td>0.25</td>
<td>_</td>
<td>q.s</td>
</tr>
<tr>
<td>B9</td>
<td>1</td>
<td>_</td>
<td>0.5</td>
<td>_</td>
<td>q.s</td>
</tr>
<tr>
<td>B10</td>
<td>1</td>
<td>_</td>
<td>0.75</td>
<td>_</td>
<td>q.s</td>
</tr>
<tr>
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<td>1</td>
<td>_</td>
<td>q.s</td>
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<tr>
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<td>_</td>
<td>1.25</td>
<td>_</td>
<td>q.s</td>
</tr>
<tr>
<td>B13</td>
<td>1</td>
<td>_</td>
<td>1.5</td>
<td>_</td>
<td>q.s</td>
</tr>
</tbody>
</table>
Carr’s index and the Hausner’s ratio. The angle of repose was determined by the fixed height method \((\tan \theta = h/r)\). Repaglinide was tapped using USP tapped density tester (Electrolabs, Mumbai) for 1000 taps in a cylinder and the change in the volume was measured (10). Carr’s index and Hausner ratio were calculated using the equation.

\[
\text{Carr’s index} = \frac{\text{Tapped density} - \text{Bulk Density}}{\text{Tapped density} - \text{Bulk Density}} \times 100 \quad \text{(1)}
\]

\[
\text{Hausner’s ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}} \times 100 \quad \text{.............................(2)}
\]

**Preparation of the solid dispersions**

The solid dispersions were prepared by the kneading method separately, gradually increasing the molar concentration of \(\beta\)-CD and HP- \(\beta\)-CD with a fixed concentration of Repaglinide as depicted in Table 1. The required amounts of \(\beta\)-CD and HP \(\beta\)-CD were triturated in 50% of methanol and water solution in a mortar and pestle, and the drug was added and mixed for up to 1 h, to get slurry like consistency. The prepared solid dispersions were air dried, pulverized and sifted though a sieve number #100 and then stored in desiccators (11).

**Measurement of the solubility**

To evaluate the solubility of drug, in the presence and absence of \(\beta\)-CD and HP\(\beta\)-CD, saturation solubilities were measured (Table 1). An excess amount of pure drug and dispersion powder were added to 20 mL of freshly prepared simulated gastric fluid without enzyme in clean vials, continuously shaking at 25 ± 0.5°C for 24 h to achieve the equilibrium. The filtered solutions were suitably diluted and analyzed at \(\lambda_{\text{max}}\) 243 nm by using a UV spectrophotometer (Shimadzu, model no. 1800, Switzerland) (12).

**Phase-solubility study of Repaglinide using cyclodextrin**

Solubility determination was performed in triplicate according to the method of Higuchi’s and Connors. “An excess amount of drug was placed in a screw-cap glass vial to which 20 mL of SGF (pH1.2) solution containing various concentrations (0, 2, 4, 6, 8 and 10%w/v) of cyclodextrin was added”. The samples were shaken in a mechanical shaker at 37 ± 0.5 °C for 72 h. After 72 h, the samples were filtered through 0.45μm membrane filters (11). The filtrate was diluted 10 times and analyzed spectrophotometrically at \(\lambda_{\text{max}}\) 243 nm using a UV-visible spectrophotometer (ShimadzuUV-1800, Switzerland). The value of the apparent stability constant (Ks) for drug–carrier combinations was computed from the phase-solubility profiles, as show in Table 1 & Figure 1 (A-C):

\[
Ks = \frac{\text{Slope}}{\text{Intercept} (1 – \text{slope})} \times 100 \quad \text{(3)}
\]

The stability constant between 100 and 1000 M\(^{-1}\) is considered as ideal, whereas the smaller values indicate weak interaction between drug and cyclodextrin, while the larger values indicate incomplete drug release from the inclusion complex.

The values of Gibb’s free energy of transfer (\(\Delta G_{\text{tr}}\)) of drug from aqueous solution of the carriers were calculated according to the following relationship (Table 2):

<table>
<thead>
<tr>
<th>Concentration of cyclodextrin (% w/v)</th>
<th>Absorbance</th>
<th>Conc. of Repaglinide (mg/mL)</th>
<th>(\Delta G) (J/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.032</td>
<td>0.590909</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.038</td>
<td>0.863636</td>
<td>-940.383</td>
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<tr>
<td>4</td>
<td>0.046</td>
<td>1.227273</td>
<td>-1811.15</td>
</tr>
<tr>
<td>6</td>
<td>0.054</td>
<td>1.590909</td>
<td>-2454.23</td>
</tr>
<tr>
<td>8</td>
<td>0.062</td>
<td>1.954545</td>
<td>-2964.33</td>
</tr>
<tr>
<td>10</td>
<td>0.071</td>
<td>2.363636</td>
<td>-3435.26</td>
</tr>
</tbody>
</table>
ΔG_{tr} = -2.303 RT \left( \log \frac{S_0}{S_s} \right) \times 100 \quad \text{(4)}

Where, $S_0/S_s$ is the ratio of molar solubility of drug in aqueous solution of cyclodextrin to that in the same medium without cyclodextrin (13).

**Preparation of floating granules using Gelucire 43/01**

Floating granules of the drug were prepared using the melt granulation technique. The drug: lipid ratios used to prepare the different formulations were 1:1, 1:3 and 1:5 respectively as highlighted in Table 3. The lipids were melted at 50°C and solid dispersions equivalent to 100 mg were added, mixed well, and cooled to the room temperature. The masses were passed though a #16 sieve, in order to obtain uniform size granules.

**Table 3.** Composition of the floating granules using Gelucire 43/01 on Repaglinide.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid dispersion</td>
<td>403</td>
<td>403</td>
<td>403</td>
</tr>
<tr>
<td>Gelucire 43/01</td>
<td>100</td>
<td>300</td>
<td>500</td>
</tr>
</tbody>
</table>
Preparation of floating granules with different release modulators

Floating granules of drug were prepared by using the melt granulation technique. The drug: lipid ratio used to prepare the different formulations was 1:1. Different grades of HPMC and ethyl cellulose were added separately to the formulations as release rate modifier. The lipid was melted at 50°C temperature, and the drug or drug and additives mixture was added, mixed well, and cooled to the room temperature. The mass was passed through a sieve of size #16 to obtain uniform size granules as shown in Table 4.

Micromeretic properties of the prepared granules

Angle of repose

The angle of repose is defined as the maximum angle possible between the surface of the pile of the powders and the horizontal plane. Improper flow is due to the frictional forces between the particles. These forces are quantified by angle of repose (14).

\[ \tan \theta = \frac{h}{r} \] ........................(5)

Where, \( h \) = height of pile, \( r \) = radius of the base of the pile and \( \theta \) = angle of repose

The lower the angle of repose, better the flow property. Rough and irregular surface of particles gives higher angle of repose.

Bulk density and tapped density

An accurately weighed quantity of the powder (\( W \)) was carefully poured in to a graduated cylinder and the volume (\( V_0 \)) was measured. Then, the graduated cylinder’s lid was closed, set into a density determination apparatus (Bulk density apparatus, Electro lab, Mumbai). The density apparatus was set for 100 taps and after that the volume (\( V_f \)) was measured and continued operation till the two consecutive reading were equal (15). The bulk density and the tapped density were calculated using the following formulas:

Bulk density = \( \frac{W}{V_0} \) ........................(6)

Tapped density = \( \frac{W}{V_f} \) ........................(6)

Where, \( W \) = weight of the powder, \( V_0 \) = initial volume and \( V_f \) = final volume of the powder

Carr’s index

The Carr’s index is an important measure that can be obtained from the poured density of the material to the tapped density. It was calculated by using the following formula:

Carr’s index = \( \frac{Tapped
density – Bulk
density}{Tapped
density} \times 100 \) ........................(6)

Table 4. Composition of the floating granules with different release modulators.
Carr’s index = \( \frac{Tapped\ Density - Bulk\ Density}{Tapped\ Density} \times 100 \) ........(8)

Carr’s index values for pure drug and polymers were determined by measuring the initial volume (V0) and final volume (Vf) of the known weight (W) of the material after subjecting to 100 tapping in a graduated measuring cylinder. From these volumes, the poured density (W/V0) and the tapped density (W/Vf) values were calculated and substituted in the above equation to determine the Carr’s index (15).

Hausner’s ratio
It indicates the flow properties of the powder and is measured as the ratio of the tapped density to the bulk density.

\[ Hausner’s\ ratio = \frac{Tapped\ Density}{Tapped\ Density} \] ..............(9)

Determination of particle size of the prepared granules
The particle size of a pharmaceutical substance is strictly maintained in order to get optimal biological activity (14).

Sieving method
Standard sieves such as mesh number # 10, # 22, # 44, # 85 and # 120 are available commercially as per the specifications of IP and USP used in this method. Sieves are arranged in a nest, with the coarsest at the top and finest at the bottom. A sample (500mg) of the powder is placed in the top sieve. This sieve set is fixed to a mechanical shaker apparatus and shaken for a certain period of time (10 minutes) and the materials with diameters smaller than the mesh opening pass through the sieves. The powder retained in each sieve is weighed. The results are presented in a graph of percent passing verses the sieve size.

\[ Percentage\ retained = \frac{W_{sieve}}{W_{total}} \times 100 \] ............(10)

Where, \( W_{sieve} \) is the weight of the aggregate in the sieve and \( W_{total} \) is the total weight of the aggregate.

Then, the cumulative percent of aggregate retained in each sieve was calculated by adding the total amount of aggregate that is retained in each sieve and the amount in the previous sieves. The cumulative percent passing of the aggregate is found by subtracting the percent retained from 100%.

Percentage cumulative passing = 100% – % Cumulative retained ..(11)

He values are then plotted on a graph with cumulative percent passing on the y axis and logarithmic sieve size on the x-axis. The results of this test are provided in graphical form to identify the type of gradation of the aggregate (16).

Drug content
Granules equivalent to the dose of drug were added to 100 mL of simulated gastric fluid without enzyme, heated to 60°C, and allowed to cool to the room temperature. Upon cooling, the gelucire solidified, and the drug in SGF was filtered through a whatman membrane filter. Filtrate samples were suitably diluted, and the drug content was estimated by using UV spectrophotometer at \( \lambda_{max} \) 243 nm (17).

In vitro buoyancy study
The in vitro buoyancy was characterized by floating lag time and floating time. The test was performed using a USP-II paddle type dissolution rate test apparatus utilizing a 900 mL of SGF at paddle rotation of 100 rpm at 37 ± 0.5°C temperature. The time required for the granules to rise to the surface of the dissolution medium and the duration of time the granules constantly floated on the dissolution medium were noted as floating lag time and floating time respectively (18).

In vitro dissolution study
1 mg equivalent weight of Repaglinide granules was taken in 900 mL of dissolution medium, that is SGF without enzyme (pH 1.2) using USP-II paddle type apparatus with rotational speed of 100 rpm at 37 ± 0.5°C temperature up to 12 h. Then, 5 mL of sample was withdrawn at a pre-determined time interval and 5 mL of fresh medium was added for maintaining sink condition (drug concentration in solution maintained
constant at a low level). The samples withdrawn were filtered using whatman filter paper, diluted suitably and analyzed at $\lambda_{max}$ 243nm spectrophotometrically for the drug release (18).

**Kinetic modeling of the drug release**

In order to establish the mechanism of drug release from the matrix tablets, the experimental data were fitted to different kinetic models. The drug release data were subjected to various mathematical kinetic models like zero order and first order. The data were also subjected to Higuchi’s model and Korsmeyer’s model, when the release mechanism is not well known or when more than one type of release phenomena could be involved. The data were evaluated according to the following equations (19).

First order model:

$$\ln M_t = \ln M_0 + K_1 t$$ (12)

Zero order model:

$$M_t = M_0 + K_0 t$$ (13)

Higuchi’s model:

$$M_t = M_0 + K_o t^{0.5}$$ (14)

Korsmeyer – Peppa’s model:

$$M_t = M_0 + K_k n^{t}$$ (15)

Where, $M_t$ is the amount of drug released in time $t$, $M_0$ is the initial amount of drug, $K$ is respective release constant, $n$ is the release exponent, which characterizes the mechanism of drug release. The ‘$n$’ value could be used to characterize different release mechanisms. The best-fit model was determined statistically employing comparison of correlation coefficients. The preparation of graphs and statistical calculations were carried out with the help of Microsoft Excel® software.

**FT-IR spectral studies**

FT-IR spectra of pure drug, gelucire 43/01, solid dispersion and physical mixture were recorded on a Shimadzu FT-IR 8400 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Potassium bromide pellet method was employed and the background spectrum was collected under identical situation. Each spectrum was derived from single average scans collected in the region 400 to 4000 cm$^{-1}$ at spectral resolution of 2 cm$^{-2}$ and ratio against background interferogram (20).

**X-ray diffraction studies**

X-ray powder diffraction patterns were obtained at room temperature using an XPERT-PRO with a Cu anode and a graphite monochromator, operated at 35 kV and 20 mA. The samples were analyzed in the 20 angle range of 5–50°, and the process parameters were set at a scan-step size of 0.02° (20) and a scan-step time of 25 seconds (20).

**Short term stability study**

The stability study of the optimized formulations was carried out according to the ICH guideline. The stability studies of the optimized formulations were performed at 40°C/75% RH for 3 months in air tight sealed vials. At the end of the storage period, the formulation was observed for physical appearance, size, shape, and surface morphology, *in vitro* drug release to find out whether any significant difference was there or not. An ideal controlled release dosage form apart from other requirements should provide constancy of drug release throughout its shelf life. The storage condition for accelerated testing (as per ICH and WHO) are 40±5% RH and 75%±5% RH for solid dosage form for 6 months. If the product is unstable in the above conditions, then intermediate conditions (30±2°C, 60±5% RH) are recommended (21).

**RESULTS AND DISCUSSION**

**Measurement of solubility**

The solubility of Repaglinide in SGF (pH 1.2), pH 6.8 and pH 7.4 was found to be 4.251±0.22 mg/mL, 0.682 ±0.23 mg/mL and 0.334±0.29 mg/mL, respectively. The study shows that solubility of Repaglinide decreases as the pH increases. The maximum solubility was observed at the pH level of 1.2 (Table 1). In the present research work, an initial improvement in solubility was attempted by preparing solid dispersion with β-CD and HP-β-CD,
followed by developing granules for drug as shown in Figure 1(A-C). The rationale behind such an approach was to make drug release from granule as the rate limiting step but not both drug release and dissolution as rate limiting step. From the solubility measurement data given in Table 1, it was observed that 1:1 inclusion complex showed higher aqueous solubility in case of both β-CD and HP-βCD. The drug solubility in 1:1 complex with β-CD and HP-βCD was 1.954±0.33 mg/mL and 2.627±0.76 mg/mL, respectively. The data indicated that HP-βCD showed better solubility enhancement than β-CD by 5 folds increase in solubility of the drug at 1:1 ratio. The enhanced solubility of drug from solid dispersions prepared with HP-βCD using kneading method could be due to the formation of water soluble inclusion complex. The presence of solvent during the preparation of solid dispersions allows intimate contact between the drug and carrier, thus providing better molecular association (12).

**Phase solubility study**

The phase solubility graph of Repaglinide with β-CD and HP-β-CD is shown in Figure 1(A & B). The plot showed that aqueous solubility of the drug increased linearly as a function of cyclodextrin concentration. The phase-solubility diagram investigated in simulated gastric fluid was linear over a wide range of β-CD and HP β-CD concentrations and corresponded to A1-type profiles. As the slope of the line was less than unity, it was assumed that the increase in solubility observed was due to the formation of 1:1 complex. The stability constant was 0.401 mg/mL (11). The value of the stability constant depends on the slope. The greater the slope, the greater is the capacity of the polymer to solubilize the drug. Increases in the solubility of the drug may occur due to the formation of the water soluble inclusion complex with HP-βCD. The values of Gibb’s free energy deviation are an indication of the process of transfer of the drug from pure water to aqueous solution of HP-βCD (Figure 1C). Table 2 represents the values of the Gibb’s free energy associated with the aqueous solubility of drug in the presence of HP-βCD. The ΔG° values were all negative for HP-βCD at various concentrations, indicating the spontaneous nature of the drug solubilization (22).

**FT-IR spectral study**

The compatibility of the drug with selected polymers and their combinations are depicted in Figures 2&3, respectively. The characteristic absorption peaks of

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**Figure 2.** FT-IR spectra of Repaglinide and HP-β-CD solid dispersions.
the drug were obtained at 1687.36 cm⁻¹, 3472.92 cm⁻¹, 2918.30 cm⁻¹, and 1149.57 cm⁻¹. The IR spectra of the drug and polymer combinations were compared with the spectra of the pure drug and individual polymers. The principle peaks obtained for the combinations were almost similar to those of the pure drug. The FT-IR spectra of the drug with HP-β-CD, drug with gelucire 43/01 and drug with HP-β-CD + HPMC did not show any change. The possibility of interaction was ruled out as there was no major shift in the absorption bands of drug and the optimized formulations (23).

Micromeritic properties

**Angle of repose**
The pure drug had an angle of repose 35.12±0.98° and exhibited poor flow where as the angle of repose for all the formulations showed excellent flowability and ranged from 20±0.32° to 24.1±0.65° (Table 5).

**Carr’s index**
The Carr’s index for all the formulations varied from 10.5±0.23% to 14.6±0.57%, showing good flowability when compared to the pure drug as shown in Table 5. The Carr’s index of pure drug was 32.2±0.78%, indicating poor flow ability before formulating the granules.

**Hausner’s ratio**
Hausner’s ratio of pure drug was 1.42±0.01, which indicates poor flow ability but up on formulating granules good flow property was achieved as indicated in Table 5.

**Particle size distribution**
Particle size distribution of the granules was determined by the sieving method. The results are noted in Tables 6 and 7, respectively. Figures 4, 5, 6 and 7 showed that aggregate of all the formulations showed narrow gradation or uniform gradation as the curve on the gradation graph was very steep, and occupied a small range of the aggregate. It indicated that all the formulations had aggregate of approximately the same size. It was found that maximum aggregate retained in sieve no 20 (840µm). Therefore size of all the aggregate was uniform and found to be 840 µm (16).
Drug content
The content of the drug in granules was found to be in the range of 77 to 86%. The highest and lowest drug contents were found to be 86.25 ± 0.46% and 77.25 ± 0.14%, as shown in Table 5 (24).

In vitro buoyancy study
The results showed that all the formulations remained floating beyond 12h, reflecting excellent floating ability of the granules, as noted in Table 5. The surface hydrophobicity imparted to the drug particle by the hydrophobic lipid coat could be responsible for floating behavior and also the low density of gelucire 43/01 (True density 0.0856 g/cm³) might have played
a role in floating ability of granules. In contrast to most conventional floating systems (including gas-generating ones), these granules floated immediately upon contact with the release medium, showing no lag time in floating behavior because the low density prevailed from the beginning (t=0) (25).

**In vitro release study**

**Repaglinide: Gelucire granules**

From the *in vitro* release profile for the granules (G1, G2, and G3) it can be observed that there was a burst release of 32% in the initial stage and approximately 50% drug was released at the end of 12 h, which indicates that only 18% of the drug was released in the second 12h, as shown in Figure 8. The initial effect might be due to the fact that the drug that was not coated by the lipid and that found in channels formed by dissolution of particles percolating the granules. From the above reasons, only a small part of the drug was released from the lipid coated or covered drug particle. Jain et al., 2007, studied that the drug released from gelucire 43/01 showed

<table>
<thead>
<tr>
<th>Mean of size range (µm)</th>
<th>F1 (%)</th>
<th>F2 (%)</th>
<th>F3 (%)</th>
<th>F4 (%)</th>
<th>F5 (%)</th>
<th>F6 (%)</th>
<th>F7 (%)</th>
<th>F8 (%)</th>
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<td>79.4</td>
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<td>16.2</td>
<td>16.2</td>
<td>10.8</td>
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<td>1.2</td>
<td>0</td>
<td>2.8</td>
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<td>2.2</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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</tr>
</tbody>
</table>
biphasic and characterized by an initial burst release in 30 min followed by a period for constant release. The fast effect, namely the amount of encapsulated compound released at short times, is normally related to the drug embedded into or near the surface. Drug release was retarded significantly with the increased amount of gelucire (25). This could be attributed to the increase of lipid matrix density and in the length of the diffusion path, which the drug molecules had to transverse (26). Increases in lipid ratio above 1:1 caused significant retardation, making it unsuitable for gastro retentive release. Hence granules containing drug: lipid ratio 1:1 were used for further evaluation (17). However, there was no significant difference in the floating ability of granules containing different proportion of gelucire (25). This could be attributed to the increase of lipid matrix density and in the length of the diffusion path, which the drug molecules had to transverse (26). Increases in lipid ratio above 1:1 caused significant retardation, making it unsuitable for gastro retentive release. Hence granules containing drug: lipid ratio 1:1 were used for further evaluation (17). However, there was no significant difference in the floating ability of granules containing different proportion of gelucire (25). Granules of all the formulations were found to float beyond 12h with zero floating lag time. This may be due to the surface hydrophobicity imparted to the drug particle by the hydrophobic lipid coat and also due to low density of gelucire 43/01 (True density: 0.0856 g/cm³), which plays an important role in floating ability of granules. Gelucire can be used as extended delivery excipient; however, it is necessary to add a release modifying agent to improve the drug release kinetics. As an attempt to reduce initial drug release, different grades of HPMC, ethyl cellulose were evaluated as release-retardant additives (25).

**Repaglinide: Gelucire granules containing release modifiers**

Although gelucire is recommended as a sustained release agent, due to the above mentioned burst release and subsequent exceedingly slow drug release effect, it was necessary to add a release modifying agent to improve the drug release kinetics. The granules were prepared by the melt granulation technique employing gelucire 43/01 as a hydrophobic binder and various release modifying agents such as different grades of HPMC and ethyl cellulose. In formulation F1 to F4 HPMC K4M, in F5 to F8 HPMC K15M, in F9 to F12 HPMC K100M and in F13 to F16 ethyl cellulose were used as release modifying agents in four different ratios, i.e. 1:0.05, 1:0.1, 1:0.15, and 1:0.2 respectively. It was found that in case formulation F2(1:0.1) containing HPMC K4M showed 98.18 ± 1.78% of drug was released within 12h and 19.09 ± 1.75% was released within 1h. For formulation F1, F3, and F4 the cum. % drug release was found to be 84.54 ± 3.21%, 98.92 ± 1.87%, and 99.13 ± 3.45%, in 12h, 10h, and 8h respectively, as shown in Figure 9. In case of HPMC K15M formulation F6 (1:0.1) 100.23 ± 1.87%, the drug was observed to be released in 12h and 19.09 ± 1.75% was released within 1h. For formulation F1, F3, and F4 the cum. % drug release was found to be 84.54 ± 3.21%, 98.92 ± 1.87%, and 99.13 ± 3.45%, in 12h, 10h, and 8h respectively, as shown in Figure 9. In case of HPMC K15M formulation F6 (1:0.1) 100.23 ± 1.87%, the drug was observed to be released in 12h and 16.36 ± 1.89% was released in first 1h. For formulations, F5, F7, and F8 the cum. % drug release was found to be 81.85± 4.98%, 98.18±1.34%, 99.12±2.09% in 12h, 10h, and 8h respectively, as shown in Figure 10. Where as in case of HPMC K100M based formulation F9 (1:0.05), 98.18±1.32% of the drug release was obtained in 12h and 16.65±1.87% of the drug was released within 1h. For formulation F10, F11, and F12 the cum. % drug release was 100.09±4.76%, 98.82±1.89%, 98.18±1.69% in 10h, 8h and 6 h, respectively (Figure 11). In case of ethyl cellulose formulation F13 (1:0.05) showed a drug release of 98.82±3.56% in 12h and 16.36±4.23% in 1h. For formulation F14, F15, and F16 the cum % drug

**Table 7. Cumulative frequency plot data of formulations HPMC K100M and Ethyl cellulose.**

<table>
<thead>
<tr>
<th>Mean of size range (µm)</th>
<th>F9</th>
<th>F10</th>
<th>F11</th>
<th>F12</th>
<th>F13</th>
<th>F14</th>
<th>F15</th>
<th>F16</th>
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<tr>
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<td>77.2</td>
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<td>75.2</td>
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<td>14.8</td>
<td>13.6</td>
<td>17.0</td>
<td>26.6</td>
<td>8.2</td>
<td>12.6</td>
<td>2.4</td>
<td>8.2</td>
</tr>
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<td>2.62324929</td>
<td>9.0</td>
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<td>5.6</td>
<td>9.0</td>
<td>4.0</td>
<td>4.2</td>
<td>0.0</td>
<td>1.8</td>
</tr>
<tr>
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<td>0.0</td>
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</table>
release was 89.77±3.72%, 79.15±1.48%, 68.72±3.84%, in 12h, as shown in Figure 12. From the above results it may be seen that the low viscosity grades of HPMC such as K4M and K15M needed higher proportion, compared to high viscosity such as K100M and hydrophobic polymer ethyl cellulose to control the drug release of about 99% in 12h. The release of the drug from the granules seemed to depend on the relative proportion of the hydrophobic polymer (gelucire 43/01) and gel forming release modifier as well as the total proportion of the polymers in the formulation as reported by Juarez-soberanez et al., 2011. HPMC is a hydrophilic gel forming polymer commonly used as a controlled release carrier. There is a gradual and well controlled dissolution when HPMC is used as a release enhancing agent because of its dual function as a swelling and as a release retarding agent. The hydrophilic polymer, HPMC plays two different roles in a gelucire 43/01 sustained release matrix, that of a swelling polymer breaking off the domain of a gelucire matrix, and that of a release retarding polymer due to formation of a gelled matrix that can be obstructed by the presence of a hydrophobic material that is gelucire. The end effect of the hydrophilic polymer particles in a hydrophobic matrix seems to depend on the possibility of these particles percolating the matrix. At low HPMC proportions the particles are isolated or form small particle clusters and the observed effect is that of swelling. When the hydrophilic polymer can form a chain of particles percolating the waxy matrix the release retarding effect begins. Ethyl cellulose has been used as a release retardant polymer in controlled release dosage forms. EC reduces the drug release due to reduction in penetration of the SGF (pH 1.2) into the system because of the hydrophobic nature of ethyl cellulose present on the surface of the granules i.e. the rate of release is controlled by the permeability of the matrix structure (27).

**Drug release kinetics**

The mean correlation coefficients were determined for zero, first, Higuchi’s model which indicated that the drug release from all the formulations
followed zero order kinetics (Table 8). Formulations F1 to F12 showed a higher correlation for Higuchi’s equation indicating a good fit for Higuchi’s model which suggests that diffusion is the predominant mechanism of drug release where as formulations F13 to F16, showed a higher correlation to the zero order. The release exponent values obtained from Korsmeyer-Peppa’s equation for formulations F1 to F4 and F9 to F12 showed that drug release from SR granules followed non-Fickian anomalous diffusion as the value of n was 0.5 < n > 1. Where as in case of formulations F5 to F8 and F13 to F16, release followed super case-II transport mechanism as the value of n was > 1.

**Table 8. Correlation coefficients of the granules containing HPMC K4M, HPMC K15M, HPMC K100M and ethyl cellulose**

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Zero order plot</th>
<th>First order plot</th>
<th>Higuchi plot</th>
<th>Korsmeyer-Peppa’s Diffusion exponent (n) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.977</td>
<td>0.372</td>
<td>0.978</td>
<td>0.979</td>
</tr>
<tr>
<td>F2</td>
<td>0.981</td>
<td>0.366</td>
<td>0.988</td>
<td>0.992</td>
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<tr>
<td>F3</td>
<td>0.932</td>
<td>0.351</td>
<td>0.975</td>
<td>0.975</td>
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<tr>
<td>F4</td>
<td>0.896</td>
<td>0.391</td>
<td>0.981</td>
<td>0.981</td>
</tr>
<tr>
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<td>0.945</td>
<td>0.341</td>
<td>0.983</td>
<td>1.040</td>
</tr>
<tr>
<td>F6</td>
<td>0.974</td>
<td>0.379</td>
<td>0.993</td>
<td>1.065</td>
</tr>
<tr>
<td>F7</td>
<td>0.948</td>
<td>0.421</td>
<td>0.985</td>
<td>1.059</td>
</tr>
<tr>
<td>F8</td>
<td>0.897</td>
<td>0.394</td>
<td>0.968</td>
<td>1.016</td>
</tr>
<tr>
<td>F9</td>
<td>0.964</td>
<td>0.372</td>
<td>0.978</td>
<td>0.686</td>
</tr>
<tr>
<td>F10</td>
<td>0.922</td>
<td>0.339</td>
<td>0.971</td>
<td>0.640</td>
</tr>
<tr>
<td>F11</td>
<td>0.890</td>
<td>0.330</td>
<td>0.954</td>
<td>0.622</td>
</tr>
<tr>
<td>F12</td>
<td>0.956</td>
<td>0.372</td>
<td>0.962</td>
<td>0.684</td>
</tr>
<tr>
<td>F13</td>
<td>0.988</td>
<td>0.321</td>
<td>0.934</td>
<td>1.058</td>
</tr>
<tr>
<td>F14</td>
<td>0.978</td>
<td>0.437</td>
<td>0.897</td>
<td>1.037</td>
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<tr>
<td>F15</td>
<td>0.977</td>
<td>0.458</td>
<td>0.941</td>
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<tr>
<td>F16</td>
<td>0.993</td>
<td>0.409</td>
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</tr>
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</table>

**Powder X-ray diffraction study**

Powder X-RD study of the drug and solid dispersions of the drug with HP-β-CD (1:1) as shown in Figure 13, confirmed physical nature of the drug. The presence of numerous distinct peaks in the powder X-RD spectrum of the drug indicated that drug was present as crystalline form with major characteristic diffraction peaks appearing at a diffraction angle of 2θ at 7, 10, 11, 13, and 14. The diffraction patterns of optimized formulations showed hollow shape spectrum with complete absence of diffraction peaks which indicated that the drug was present in amorphous form in the inclusion complex of HP-βCD (11).

**Short term stability study**

The stability study of the formulations (F2, F6, F9 and F13) was performed at 45°C/75% RH for 3 months in the airtight sealed vials and the observed data are depicted in Table 9. Which indicated that there was no significant change in drug content, hence the formulations were stable.

**Figure 13. Powder X-RD study of Repaglinide and solid dispersion of drug with HP-β-CD (1:1).**
CONCLUSION

Multiparticulate systems as drug carriers have attracted much attention over the past few years compared to single unit systems. The floating multiparticulate systems have several advantages including increase in gastric retention time, better drug release and absorption which improve the control over, patient compliance and reduce toxicity. The present work was aimed to develop multiparticulate drug delivery system for Repaglinide by the melt granulation method for a controlled release. The drug has low bioavailability (56%), poor absorption in the upper gastrointestinal tract and short half life (1h). However, it is completely absorbed following oral administration, hence is a suitable candidate for formulation of a controlled drug delivery system. The solubility of drug was improved by inclusion complexes using HP-β-CD and β-CD. The inclusion complex with HP-β-CD, in 1:1 ratio gave the best result which was employed for the development of granules. Using gelucire 43/01 as the hydrophobic binder, granules were prepared for the drug complex, which exhibited excellent floating property with zero lag time and floating time more than 12h. As gelucire 43/01 could not control the drug release effectively, as burst effect was seen with 30-35% initial drug release followed by very slow drug release of less than 50% in 12h so, release modulators such as HPMC K4M, HPMC K15M, HPMC K100M and ethyl cellulose (20cPs) were incorporated in the granules in 0.05, 0.1, 0.15 and 0.2 ratio with respect to gelucire 43/01. Among these HPMC K4M and HPMC K15M in 0.1 and HPMC K100M and ethyl cellulose in 0.05 ratio with gelucire 43/01 effectively controlled the drug release for up to 12h, the hydrophobic to hydrophilic ratio seems to be critical in the controlled release of the drug, uniform particle size distribution was achieved by melt granulation with mean particle size of 840 µm. FT-IR spectral study revealed that there is no interaction between drug and polymers like different grades of HPMC and ethylcellulose. The powder X-RD study revealed that the drug was present in amorphous state in the inclusion complex and short term stability test showed no significant change in drug content. Hence it can be concluded that the floating multiparticulate system of drug were stable.

Acknowledgements

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Conflicts of interest

Authors have no conflicts(s) of interest.

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