DEVELOPMENT AND CHARACTERIZATION OF GROUND MIXTURES OF ARIPIPRAZOLE WITH HYDROPHILIC CARRIERS

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Abstract: The aim of the present study was to improve the solubility of poorly water soluble drug Aripiprazole (ARP) by grinding (ground mixture - GM) dispersion - SD technique using guar gum (GG) & modified guar gum (MGG) as a carriers. GM containing GG & MGG were further characterize by Scanning electron microscopy (SEM), differential scanning colorimetry (DSC), fourier transform infrared spectroscopy (FTIR) and X ray Diffraction study (XRD). Effect of polymer concentration and methods of preparation on solubility enhancement were studied using solubility and dissolution studies, respectively. In vivo study was performed by measuring 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG Co-A) reductase inhibition activity. The MGG was modified by heating and there observed irreversible decrease in viscosity, whereas swelling property remains unaffected. The result of solubility study showed increase in solubility of ARP with increase in concentration of MGG. Solubility of ARP was found to be more in MGG mixture than GG mixture. FTIR & DSC studies revealed that drug & carriers were in compatible state. Dissolution study revealed that the mixture prepared by grinding method with MGG was effective method for solubility enhancement of ARP, than GG. A significant reduction in 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG Co-A) reductase activity was observed in MGG than GG. In conclusion, grinding method with MGG could be used as a potential method & carrier in enhancing the dissolution rate and bioavailability of ARP.

Keywords: Co-ground mixtures, Dissolution rate enhancement, Hydrophilic carriers, Aripiprazole, Poorly water soluble drugs.

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INTRODUCTION

Solid dispersions, which are generally prepared by melting or solvent methods, possess several challenges\(^1\). Finely dispersed drug particles in solid dispersion systems are usually in the thermodynamically unstable amorphous form\(^2\). This could lead to crystallization upon storage, especially under humid conditions, resulting in changes in drug dissolution/bioavailability\(^3\). There is a possibility of thermal degradation of drug due to used melting technique. The solvent methods suffer from the undesirable environmental impact of many organic solvents and their potential toxic and carcinogenic effects\(^4\).

Poor biopharmaceutical properties are greatest challenges to the therapeutic efficacy of the potent drug. Micronization of drug powders results in a considerable decrease of particle size. However, the products tend to agglomerate, which leads to a considerable reduction of specific surface area and formation of a cohesive powder with poor flow properties\(^5,6\). Co-grinding of poorly soluble drugs with hydrophilic carriers is an interesting technique for the production of micronized and stable drug particles. Many authors have reported the use of this method for the enhancement of dissolution rates of various drugs such as Aceclofenac, Neusilin, Indomethacin, Furosemide and Naproxen\(^7-8\). Amorphous solid dispersion solution exhibits improvement in wettability of drug as well as decreases the particle size of the drug to the molecular level, if the carriers are easily wetted\(^9\). Availability of more surface area helps for easy mass transfer and showed increased dissolution rate according to modified Noyes Whitney equation\(^10\). Furthermore less enthalpy required to separate drug molecule from carrier molecules or each other, as compared to the energy required to separate drug molecules within a crystalline structure. Gibb’s free energy enhanced the solubility of amorphous drugs\(^11\). Some of the amorphous drugs mix with small quantities of plasticizer (water), they tend to revert to the crystalline state\(^12\).

Aripiprazole,7-[4-{4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy}-3,4-dihydro-2-(1H) quinolinone, is a second generation antipsychotic drug indicated for the treatment of schizophrenia and related psychoses\(^13\). It is a weakly basic drug (pKa= 3.55), poorly water soluble, supplied as crystals having a melting range 136-138°C, low and irregular bioavailability following oral administration\(^14\). Two metastable polymorphs as well as four different solvates crystallized forms were reported from more than 20 patent applications\(^15\). The objective of the present study was to improve the dissolution of poorly water soluble drug ARP using grinding technique with pharmaceutically acceptable hydrophilic carriers GG & MGG.
MATERIALS AND METHODS

Materials

Aripiprazole, was obtained as gift sample from Pfizer, India. # Guar gum I.P., and other chemicals supplied by S.D fine chemicals, Mumbai, India. All the reagents were of analytical and HPLC grade unless stated otherwise. Milli-Q-Water was used throughout the study. Potassium dihydrogen phosphate, Hydrochloric acid, Sodium acetate, Sodium Hydroxide, Ethanol absolute, Acetonitrile, Hydrogen Peroxide, Formic acid (Merck, Mumbai, India) were used. 0.05M Phosphate buffer was prepared by dissolving Potassium dihydrogen phosphate in water and pH of the resultant solution was adjusted to 3.2 with formic acid. Standard stock solution was prepared by dissolving 25 mg of Aripiprazole working standard in 25 ml of mobile phase. From the above stock solution, a series of solutions were prepared at concentration levels ranging from 80% to 120% of target concentration. Measured the peak area responses of solutions at all levels in duplicate. 100 mg of accurately weighed Aripiprazole powder and transferred into a 100 ml volumetric flask, few ml of mobile phase was added and sonicated to dissolve and made up the volume with mobile phase. The above solution was filtered and diluted to get a final concentration of 100 μg/ ml.

The chromatographic conditions used for the analysis were given as below.

Detection wavelength: 227 nm, Column: Phenomenex Luna C18, 150 x 4.6mm, 5μm, Flow rate 1.0 ml/ min and Injection volume 20μL. The HPLC system used was a Waters 2695 separation module with an auto injector and waters 2996 PDA Detector. The output signal was monitored and integrated using Empower software. Phenomenex Luna C18, 150 x 4.6mm, 5μm column was used.

Preparation of Modified Guar Gum

Preparation of MGG was done by heating method. Briefly, powdered gum was taken in a porcelain bowl and subjected to heating using sand bath for different time periods at different temperatures. The results of swelling capacity and viscosity studies revealed that the modified forms possessed swelling property similar to GG, but viscosity was decreased as a function of temperature and time period of heating. However, it was observed that GG samples were charred, when heated above 150 °C. In the preparation of modified form of GG, no further change in viscosity of GG was observed by heating at 125 °C for more than 2 h. Hence, the conditions of heating at 125 °C for 2 h were selected to prepare modified form of GG. The prepared modified form of GG was stored in airtight container at 25 °C.
Characterization of GG & MGG

Swelling Index

GG/MGG powder (1 gm) was accurately weighed and transferred to a 100-ml stoppered measuring cylinder. Initial volume of the powder in the measuring cylinder was noted. The volume was made up to 100-ml mark with distilled water. The volume occupied by the gum sediment was shaken gently and set aside for 24 h at room temperature and ambient humidity (1). The volume occupied by the gum sediment was noted after 24 h. Swelling capacity of GG/MGG was expressed in terms of swelling index. Swelling index was expressed as a percentage and calculated according to the following equation:

\[ SI = \left( \frac{X_t - X_o}{X_o} \right) \times 100 \]  

Where \( X_o \) is the initial height of the powder in graduated cylinder and \( X_t \) denotes the height occupied by swollen gum after 24 h.

Viscosity Measurement

The viscosity of 1% (w/v) GG/MGG solution was measured according to the US Pharmacopeia (USP) specification, using Brookfield DV-IIE Viscometer (Middleboro, MA, USA).

Hydration Capacity

Weighed quantity (1.0 g) of powdered (GG&MGG) was taken in the 15-ml tare centrifuge tube. Then, 10 ml of distilled water was added to it and allowed to centrifuge for 10 min at 1,000 rpm. After the centrifugation process, the tare centrifuge tube was taken out and inverted to remove the supernatant. The decanted tube then weighed on digital balance (AUX 120, Shimadzu, Japan) and the hydration capacity was calculated using the following equation:

\[ HC = \frac{\text{Weight of hydrated sample}}{\text{Weight of dry sample}} \] 

Moisture Sorption Capacity

Moisture sorption study was performed using programmable environmental test chamber (Remi Instruments, Mumbai, India). One gram of powdered GG/MGG was taken in a Petri dish of 9 cm in diameter and spread uniformly. Then, it was kept in programmable environmental test chamber at 37±1°C and 100% relative humidity for 2 days. The moisture sorption was calculated by recording weight difference of the sample before and after exposure to programmable environmental test chamber.
Preparation of ground mixtures

Ground mixtures (GM) were prepared by mixing ARP with the hydrophilic carriers gaur gum (GG) & modified gaur gum (MGG) at different compositions (Table 1) for 30 min, until a homogeneous mixture was obtained. GM was grounded using metal ball mill (MBM) consisted of a 40 ml jar with eight metal balls, a cooling attachment and samples were ground at 70 rpm for 30 min at ambient conditions. The resulting mixtures were stored in a screw cap vials at room temperature until use.

Solubility of ARP

1mg of pure ARP, ground mixtures containing GG & MGG, were transferred individually into test bottles contain 10ml of dissolution medium (pH 7.0 phosphate buffer without pancreatin - simulate intestinal fluid). The samples were sonicated (Sonica, ME5.5, USA) for 2 h at room temperature. Further the capped test tubes were shaken at 37 ± 0.1°C for 48 h in water bath (this duration was sufficient to reach equilibrium). Subsequently, the suspensions were filtered through a 0.22 µm membrane filter. The filtrate was suitably diluted and analyzed using a HPLC method. All solubility experiments were carried out in triplicate.

Determination of drug content

For determination of drug content, 100 mg of optimized formulations (individually) were dissolved in 100 ml of methanol. The resulted solution was analyzed HPLC.

Dissolution Studies

Dissolution rates from optimized formulations (individually) were determined in 900 ml of pH 7.0 phosphate buffer (without pancreatin ) at 37±0.5°C with a stirrer rotation speed of 100 rpm using the USP dissolution test apparatus (TDT 08L— ELECTROLAB, Mumbai, India) employing a basket stirrer (method I). A 10 ml aliquot of dissolution medium was withdrawn periodically using guarded sample collectors at regular intervals 30, 60, 90, 120, 180, 240, 300, 360, 420, 480 min with a pipette. The withdrawn sample was filtered through a 0.45µm membrane filter and after appropriate dilution estimated for drug concentration HPLC. Each dissolution rate test was repeated three times.

Drug release kinetics was fit into Peppas model:

\[
\frac{M_t}{M_\infty} = k t^n
\]

where, \( \frac{M_t}{M_\infty} \) is the fraction of drug released at time t, k is a constant incorporating the structural and geometric characteristics of the matrix tablets, n is the release exponent, indicative of the drug release mechanism. In case of Fickian release (diffusion controlled-
release), the n has the limiting values of 0.45 for release from cylinders. Case II transport or relaxation controlled delivery; the exponent n is 0.89 for release from cylinders.

A differential factor (f₁) and similarity factor (f₂) were calculated from dissolution data according to the following equations;

\[
 f_1 = \frac{\sum_{i=1}^{n} (R_t - T_t)^2}{\sum_{i=1}^{n} R_t} \times 100 
\]

\[
 f_2 = 50 \log \left\{ \left[ 1 + \left( \frac{1}{n} \right) \sum_{i=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}
\]

where, f₁ - differential factor, f₂ - similarity factor, n - number of time point, Rₜ - dissolution value of the reference at time, 't' and Tₜ - dissolution value of test formulation at time 't'. The acceptable range for differential factor, f₁ & similarity factor, f₂ were in the range of 0 to15& 50 – 100, respectively

Contact Angle Measurements

The ground mixtures were compressed to flat-faced pellets (diameter: 13 mm and weight: 200mg) with a hydraulic press (P/N 25.011, Specac, Orpington, England) at 10 KN for 5 sec. The compressed pellets were placed onto an adjustable platform of the contact angle goniometer (Kruss G1 goniometer, Hamberg, Germany). Using a micro- syringe, 2 µl distilled water was applied on the surface of the mixtures. The angles were measured after 30 sec wetting of the samples (n= 3).

Scanning Electron Microscopy (SEM) and Internal pore structure

The shape and surface characteristics of the optimized formulations were determined by scanning electron microscopy (model-LV 5600, Jeol, USA) and photomicrographs were recorded, by suitable magnification.

Differential Scanning Calorimetry (DSC)

DSC studies (DuPont thermal analyzer with 2010 DSC module) was carried out to study the thermal behaviors of pure drug & optimized formulations. Calorimetric measurements were made with the help of an empty cell (high purity alpha alumina disc) as the reference. The instrument was calibrated using high purity indium metal as standard. The DSC scans of the
samples were recorded in nitrogen atmosphere at a heating rate of 10 °C/min from room temperature to 200 °C.

**Fourier transform- infrared spectroscopic analysis (FT-IR)**

FTIR spectra of pure drug & optimized formulations were recorded using KBr pellet method (applying pressure of 6000 kg/cm²). Spectral measurements were obtained by powder diffuse reflectance on a FTIR spectrophotometer (Shimadzu, Model 8400S, Japan) in the wave number region of 400-4000 cm⁻¹ to study drug excipient interactions if any.

**Powder X-Ray Diffractometry**

X-ray diffraction patterns of pure drug & optimized formulations were recorded using (Phillips PW 1710, Tokyo, Japan) X-ray diffractometer with a copper target, voltage of 40 K, current 30 MA at a scanning speed of 0.30 °C/min.

**Stability of the optimized formulations**

Optimized formulations were subjected for stability studies at 25 °C/60% RH, 30 °C/65% RH and 40 °C/75% RH for 90 days and the above formulations were evaluated for drug content.

**In vivo study**

Male albino rats weighing (200 -250 g) were selected and rats were divided into three different groups (control, standard, and formulation) of five rats each. A written approval was obtained from the Institutional ethical committee of JSS College of Pharmacy, Mysore, India. Detailed verbal and written information on the study was provided to the Veterinary Surgeon, Central Animal Facility, JSS Medical College Hospital and permission was obtained. All the animals were kept on a standard diet. For 7 days, control group was given the standard diet only. The standard group was given 0.2 mg/kg dose of ARP with standard diet and the formulation group was given optimized formulation B6 (equivalent to 0.2 mg/kg of ARP). After 7 days, the liver tissue was removed as immediately and a 10% homogenate was prepared in saline arsenate solution. The homogenate was deproteinized using an equal volume of dilute perchloric acid and by centrifugation. This was then allowed to stand for 5 min and filtered. To 1 ml of this filtrate, 0.5 ml of freshly prepared hydroxylamine reagent (alkaline hydroxyl amine in case of HMG-Co A) was added and mixed, to this 1.5 ml of ferric chloride reagent was added after 5 min. The absorbance was read after 10 min at 540 nm versus a similarly treated saline arsenate blank. The ratio of HMG-Co A / mevalonate was calculated.
### Table 1: Formulations with polymeric carriers & drug solubility

| Code | formulations (Drug : Polymer) | Solubility μg/ml | | | | Gastric fluid | Intestinal Fluid |
|------|------------------------------|------------------|---|---|---|---|
| A    | Pure drug                    |                  |   |   | 0.67 | 1.92 |
| A1   | ARP:GG (1:1)                 | 1.01             |   |   | 1.27 |
| A2   | ARP:GG (1:3)                 | 1.12             |   |   | 2.11 |
| A3   | ARP:GG (1:6)                 | 1.33             |   |   | 2.32 |
| B4   | ARP:MGG (1:1)                | 1.60             |   |   | 3.53 |
| B5   | ARP:MGG (1:3)                | 1.82             |   |   | 3.74 |
| B6   | ARP:MGG (1:6)                | 1.98             |   |   | 4.64 |

*a mean ± standard deviation n= 3

### RESULTS AND DISCUSSION

Evidence have shown in the recent years that hydrophilic carriers have the physical properties and behavior suitable to prepare ground mixtures to release the mixed drug in the gastrointestinal tract. Grinding is a relatively simple and effective method to prepare drug formulation systems with an enhanced dissolution rate. Extensive literature review showed that, none of them succeeded to prepare ground mixtures of GG & MGG using ARP, poorly water soluble. In the present study, blend of GG & MGG were used to prepare ground mixtures by different ratio using non toxic solvent presented in Table 1.
Table 2. Characterization of LBG and MLBG

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GG</th>
<th>MGG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (cps)</td>
<td>4850</td>
<td>2526</td>
</tr>
<tr>
<td>Swelling index (%)</td>
<td>1754</td>
<td>1823</td>
</tr>
<tr>
<td>Water retention capacity (ml)</td>
<td>26.13</td>
<td>26.23</td>
</tr>
<tr>
<td>Hydration capacity</td>
<td>2.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Moisture sorption capacity (%)</td>
<td>6.0</td>
<td>6.1</td>
</tr>
</tbody>
</table>

*a mean ± standard deviation n= 3

The results of characterization of GG and MGG are given in Table 2. The results indicated that the viscosity of MGG was markedly lower. Water retention capacity of carrier is the amount of water retained in it which indicates the ability of carrier towards hydrophilic nature. Water retention capacity, hydration capacity & moisture sorption capacity of MGG was not reduced significantly comparing to that of GG. Due to the swelling nature of the carrier, the extensive surface of the carrier was increased during dissolution, and the dissolution rate of drug was markedly enhanced. The result of swelling capacity and viscosity studies revealed that the MGG possessed swelling properties similar to that of GG, but viscosity of MGG was decreased as a function of temperature and exposure time. However, it was observed that GG & MGG samples were charred when heated at 125°C & 150°C, respectively. In the preparation of MGG, no further change in viscosity was observed by heating it at 125°C for 2 h. Hence, these conditions of heating at 125°C for 2 h were selected to prepare MGG.

Solubility studies

The solubility data are given in Table 1. The optimization of drug/polymer ratio was done by solubility measurement of different drug/polymer ratios as shown in Table I. From solubility data show that the MGG enhance the solubility of ARP than GG. It was observed that, as the concentration of GG & MGG increases, the solubility of ARP increases. ARP can be considered as practically insoluble drugs at pH 1.2 and very slightly soluble at pH 7.2. Formulations B6 & A3 were exhibited better solubility of ARP and showed more contact angle than formulations A1, A2, B4 & B5 presented in Table 3. Because particles produced from grinding method showed less particle size, reduces the boundary layer thickness & covers the more surface area & improve the apparent solubility of the drug under physiologically relevant conditions. This indicates grinding method considered as efficient method. On the basis of solubility data,
formulations B6 & A3 (grinded particles with MGG & GG) were selected as optimized formulations for further studies.

Figure 1 shows in vitro dissolution profile of Pure ARP, (B6) & (A3) prepared by grinding using MGG & GG. Dissolution rate of ARP from formulations B6 showed faster dissolution rate than formulation B3 & pure ARP. The order of dissolution rate was found to be pure ARP < GG < MGG. From the above result grinded MGG exhibits higher dissolution rate due to decrease in particle size & higher contact angle. In addition, MGG could inhibit the recrystallization of amorphous drug in the dissolution fluid. SEM photographs showed decrease in crystallinity of ARP. These observations further confirmed by the results of DSC and XRD studies. The DSC thermograms of B6 & B3 showed identical peaks corresponding to pure drug ARP, indicated that drug and carriers were in intact form. Further, decrease in sharpnes of ARP endothermic peak in both the formulations B6 & B3 may be due to decrease in crystallinity of ARP. FTIR studies support the same hypothesis & confirmed by X-ray diffractometry. XRD spectra of ARP showed sharp peak at different diffraction angles. All major characteristic crystalline peaks appear in the diffractogram of ground mixture, but of low intensity. This proves decrease in crystallinity of ARP as some of the drug gets converted to amorphous form during grinding. From IR spectra of mixture, it was found that there was shift in the O-H and C=O group, because of weak hydrogen bonding leading to the increased rate of dissolution. Pure ARP is an organic compound having pKa 3.5, GG & MGG are natural gums and there is no dipole dipole interactions. It was proved that as the viscosity of the carrier increased, the dissolution rate was
decreased. During the process of dissolution, as soon as the drug carrier particle comes in contact with dissolution fluid, seeping in of dissolution medium in to the drug-carrier particle takes place, which initiated the formation of gel layer of carrier around the particle. The diffusion of dissolved drug through the gelatinous layer is determining factor in the enhancement of dissolution rate and diffusion coefficient is inversely proportional to viscosity. The viscosity of 1% w/v solution of MGG at 30°C is lower than that of GG. During the dissolution process, the drug-carrier particles are to be dispersed rapidly throughout the dissolution medium to promote the drug release. It was observed that the GG which is more viscous than MGG resulted in formation of lumps of drug-carrier particles during dissolution, whereas ARP-MGG particles dispersed rapidly. On the basis of the results obtained, the method of preparation also influences the rate of dissolution of ARP, because grinding method decreases crystallinity of ARP & improves dissolution rate. The trend of dissolution rate enhancement of the different hydrophilic carriers was in the order of MGG > GG. n value obtained for formulation B6 & A3 was 0.4234 & 0.4414, indicating that the release mechanism was non-Fickian or anomalous release. It can be inferred that the release was dependent on both drug release was dependent on both drug diffusion and polymer relaxation. Drug release was based on diffusion and diffusion helps to transport the drug into the in vitro fluid depending on the size and wetting of the particles. As gradient varies, the drug is released, and the distance for diffusion increases

The obtained correlation coefficient, $R^2$ for the ARP loaded MGG & GG was 0.981 & 0.997. However, effect of diffusion on the drug release was more than the effect of polymer relaxation as the values of $n$ were nearer to 0.5. Differential factor ($f_1$) and similarity ($f_2$) factor were calculated from dissolution profile and the results were compared to the formulations B6 and A3. The differential factor ($f_1$) for formulations B6 (9.12) and A3 (9.12) and similarity factor ($f_2$) for formulations B6 (69.132) and A3 (71.65) obtained from dissolution profile indicates that the formulations B6 & A3 were similar.

Generally multi particulate drug delivery systems are formulated as single dosage form (in the form of capsule or tablet), such systems posses better and adequate micromeritic properties. The relationship between angle of repose ($\theta$) and flow of powder should be < 25 is excellent, 25 - 30 is good, 30 – 40 is passable and > 40 is very poor flow. The values of angle of repose ($\theta$), tapped density, & % Carr's index (Table 3) for formulations B6 & A3 were, 25.65 & 30.67, 0.834 & 0.845 g/cm$^3$, 8.25 & 8.45%, respectively. The above micromeritic values were well within the limits and formulations B6 & A3 were exhibits good flow properties. Pure drug ARP exhibits poor flow & other formulations exhibits passable flow, due to inter particle friction (particles were dumb bell shaped with protruding surfaces confirmed from SEM Photo – Fig. 1a). From the above result it was observed that B6 & A3 were suitable for pharmaceutical purpose.
Table 3: Micromeritic properties, contact angle & drug content

<table>
<thead>
<tr>
<th>Code</th>
<th>Angle of repose(^a)</th>
<th>Tapped density(^a) (g/cm(^3))</th>
<th>%Carr's Index</th>
<th>Contact Angle(^a)</th>
<th>Drug content % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>42.35</td>
<td>1.12</td>
<td>10.76</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>A1</td>
<td>33.65</td>
<td>0.967</td>
<td>9.09</td>
<td>24</td>
<td>95.86</td>
</tr>
<tr>
<td>A2</td>
<td>34.78</td>
<td>0.932</td>
<td>9.99</td>
<td>28</td>
<td>96.12</td>
</tr>
<tr>
<td>A3</td>
<td>30.67</td>
<td>0.845</td>
<td>8.45</td>
<td>31</td>
<td>96.22</td>
</tr>
<tr>
<td>B4</td>
<td>28.65</td>
<td>0.921</td>
<td>8.76</td>
<td>37</td>
<td>97.56</td>
</tr>
<tr>
<td>B5</td>
<td>26.13</td>
<td>0.910</td>
<td>8.03</td>
<td>42</td>
<td>98.22</td>
</tr>
<tr>
<td>B6</td>
<td>25.65</td>
<td>0.834</td>
<td>8.25</td>
<td>47</td>
<td>98.65</td>
</tr>
</tbody>
</table>

\(^a\) mean ± standard deviation n= 3

Contact Angle Measurements

Measured contact angle found for formulations B6 & A3 as 47º & 31º respectively. Maximum contact angle was observed for B6 than A3 as compared to untreated pure drug particles (Table 3). Because less viscous MGG exhibits more contact angle \(^a\) than GG and improved wetting capacity was observed more for MGG

Determination of drug content

Drug content for the prepared formulations by using GG & MGG presented in the Table 3. Drug content for the formulations containing MGG & GG were in the range of 95.86 to 98.65 % w/w. Drug content was least in formulations containing GG and more for formulation containing MGG. Interestingly as the GG & MGG ratio increases drug content was found to be more. This might be due to increased relative surface area of the particles by grinding and wetting capacity of the carriers leads to more solubility of drug.

Scanning Electron Microscopy (SEM)

SEM photomicrographs of the pure drug (crystalline), B6 & A3 as shown in Fig. 2(a), 2(c), & 2(e). The crystalline drug (Fig. 2(b)) in the form of needles and has rough surface. Grinding resulted as development of cracks in the crystals & reduction in particle size. It was observed that some
of the size reduced particles have shown tube like structure pores with smooth surface [Fig. 2(d)]. The tubular structures formed due to applied shear during the grinding process. Along with tubes, some plates and fine particulate matter was also observed. The tubular structures when analyzed at higher magnification have shown bends at different places. The broken tube showed an opening, which revealed the formations of channels. The hallow tube opening may be clearly observed in the fractured particles. Applied energy to the drug crystal is responsible for occurrence of significantly different crystal structures such as hollow tubes and plates. SEM photographs showed decrease in crystallinity of ARP. These observations further confirmed by the results of DSC and XRD studies.

Figure 2. SEM photomicrographs of; (a) pure crystalline drug and (b) Fractured agglomerates (c) Crystalline drug in the form of needles and has rough surface, 
(d) Agglomerates has shown tube like structure and sintered crystals.
Figure 3. DSC thermograms of peak A = Pure ARP, peak B= B6 & peak C = A3

Pure ARP = Crystalline ARP, B6 = ARP:MGG (1:6) & A3= ARP:GG (1:6)

Differential Scanning Calorimetry

DSC thermograms of the pure drug, GM (B6) & (B3) were presented in Fig. 3. Samples B6 (142.36 °C) & A3 (142.26 °C) showed relatively broad melting endothermic peak and pure drug exhibits sharp endothermic peak at 140.43 °C. The DSC thermograms of GM B6 & B3 showed identical peaks corresponding to pure drug. Further, the peak intensity of ARP was found to be decreases. Because grinding method convert the crystalline form of drug into amorphous state.
These results indicate that only a small fraction of the drug substance existed in the crystalline state. Reduction in the melting point and enthalpy of the melting endotherm was observed. Small sized particles lead to high surface energy, which creates an energetically suboptimal state causing a decrease in the melting point. The changes in the melting point due to lattice defects created during grinding\textsuperscript{23}. The slow decrease in heat capacity may be due to the defects in crystalline structure. This wide variety of crystalline structure may require different energies for melting resulting in asymmetric endothermic peak.

**Fourier transform- infrared spectroscopic analysis**

From the FTIR studies (Fig. 4), the characteristic bands for important functional group of pure drug, B6 & B3 (GM) were identified. It was observed that the peaks at 3500 cm\(^{-1}\) due to N-H stretching, 3192 cm\(^{-1}\) due to Ar-H stretching, 2946 cm\(^{-1}\) due to C - H stretching, 1682 cm\(^{-1}\) due to C - O stretching, 1577 cm\(^{-1}\) due to C - O stretching, 1446 cm\(^{-1}\) due to C - H stretching, 1273 cm\(^{-1}\) due to C - O - C stretching and 863 cm\(^{-1}\) due to O - C - H stretching. FTIR spectra showed that the characteristics bands of ARP were not shifted after successful mixing of drug with polymers without any change in their position, indicating no chemical interactions between the drug and polymers used. A comparison and interpretation of this region in our spectra agrees with the results reported elsewhere\textsuperscript{24}.
Figure 4. X-ray powder diffraction patterns of pure ARP, B6 & A3

Pure ARP = Crystalline ARP, B6 = ARP:MGG (1:6) & A3 = ARP:GG (1:6)

Crystal morphology influences various pharmaceutical engineering and biopharmaceutical parameters such as flowability, packing, compaction, compressibility, solubility and dissolution characteristic of drug. XRD studies were undertaken to investigate the effect of grinding on the drug within the mixtures. Crystalline nature of ARP was indicated by the presence of multiple sharp peaks (Fig. 4). The powder diffractograms allow a clear and fast identification of the ARP, especially by the peaks at 2θ between 2° and 25°. X-ray diffraction patterns of ground mixtures of ARP with GG & MGG indicated the characteristic peaks of ARP were of significantly lower intensity. Garekani et al. have reported that decrease in the intensity to the changes in crystal habit of drug. Pure ARP existed in a less crystalline state in the ground mixtures with GG and MGG as compared to the corresponding physical mixtures. Small peaks were observed for fine ground drug particles, which could affect the drug release positively. X-ray diffractograms of pure ARP showed principle peak at 19.87° and intense peaks at 3.9°, 8.5°, 12.2°, 17.3°, 19.7°, 21.4°, 23.8° and ARP contain mixture (A6, B6 & C6) showed intense peaks at 3.9°, 8.6°, 12.9°, 17.7°, 19.9°, 21.5° and 23.9° as presented in Fig. 4. There was a significant decreases in the intensities of the peaks in the region of lower 2θ values (up to 18.2°), whereas peaks in the region above 2θ values (25°) have been broadened. There was no change observed in the d-spacing values of various samples. The broad and asymmetric endotherm peaks in DSC thermograms and significantly shifts in the XRD patterns may be attributed to this wide variety of crystalline structures. As observed in SEM these include plates and tubes along with fine drug crystallites and some sintered crystals, formed during grinding. The defects in the crystal structure produced due to ultrasonic energy may be responsible for these changes and also changes in the melting point due to lattice defects created during grinding and melt quenching. The slow decrease in heat capacity may be due to these defects in crystalline structure. Powder
Diffractograms revealed the crystalline nature of pure ARP. XRD of ARP showed number of sharp and intense peaks. This may be attributed to the incorporation of ARP between parts of the crystal lattice of the GG & MGG leading to a change in the degree of crystallinity of the ARP.

Figure 5. In vivo evaluation of ground mixture

In vivo study

From the above result formulation B6 was selected as optimized formulation and used for in vivo study. An indirect method was used for assessing variation in 3-hydroxy-3-methylglutaryl-coenzyme A reductase (NADPH) activity in liver tissue. 3-Hydroxy-3-methylglutaryl-Co A and mevalonate concentrations in the tissue homogenate were estimated in terms of absorbance and the ratio between the two was taken as an index of activity of the enzyme, which catalyzes the conversion of 3-hydroxy-3-methylglutaryl-Co A to mevalonate. The HMG Co-A/mevalonate ratio was measured in liver tissue of male albino rats. Result of in vivo study is given in Fig. 9. HMG Co A reductase inhibition activity was measured in terms of absorbance in all three groups. One-way analysis of variance was applied for comparison. All results are shown in mean ± standard error. HMG Co A to mevalonate ratio for formulation B6 (MGG) was found to be 3.2 ± 0.13 which was more than that of ARP 1.9 ± 0.89 & P value for was less than 0.05. HMG-CoA and mevalonate concentrations in the tissue homogenate were estimated in terms of absorbance and the ratio between the two is taken as an index of activity of the enzyme, which catalyzes the conversion of HMG Co A to mevalonate. HMG Co A/mevalonate ratio for formulation was higher than that of ARP. HMG Co A/mevalonate is inversely proportional to activity of HMG Co A reductase enzyme. Hence, the activity of the enzyme was significantly
decreased by using ground mixture (B6) in comparison with that of plain drug ARP which indicates better performance of B6 than pure ARP.

CONCLUSION

In conclusion, method employed was simple, rapid, and economical and does not imply the use of toxic organic solvents. Our studies showed that MGG could be used as a potential carrier in the dissolution rate enhancement of ARP. The dissolution rate of ARP from ground mixture of GG low when compared with ground mixture of MGG because of high viscosity of GG. Hence, various ground mixture were prepared using MGG than GG. Increase in apparent solubility of ARP from ground mixture increases the dissolution rate of ARP. Increased wettability, dispersibility, and solubilization effect of GG and MGG enhances the solubility of ARP. The results demonstrated that the optimum ARP/MGG ratio is 1:6. Result of in vivo study indicates better performance of MGG than GG as there observed significant reduction in activity of HMG Co A reductase enzyme.

References


