Dissolution enhancement of glibenclamide by preparing drug nanoparticles
Part B by nanoprecipitation
Abstract

This study is to improve the dissolution characteristics of a poorly water-soluble drug glibenclamide (GLB), by preparing nanoparticles through liquid anti solvent precipitation. A Placket Burman screening design was employed to screen the significant formulation and process variables. Total 12 experiments were generated by Minitab 16 for screening 5 independent variables namely amount of poloxamer 188 (PX) (X1), amount of PVP S 630 D (PD) (X2), solvent to antisolvent volume ratio (S/AS) (X3), amount of GLB (X4) and speed of mixing (X5). Particle size (Y1), saturation solubility (Y2) and % DE$_{5\text{min}}$ (Y3) were selected as response variables. all of the regression models yielded a good fit with high determination coefficient and F value. The pareto chart depicted that all the independent variables except amount of drug had a significant effect on the response variables. The mathematical model for particle size generated from the regression analysis was given by $PS = 830 - 8.14 PX + 12.8 PD - 11.1 S/AS + 1.42 \text{Drug Conc.} - 0.676 \text{Speed}$ ($R^2$=93.5, $F_{\text{ratio}}$ = 17.28, $p<0.001$). Prepared GLB nanoparticles showed a significant improvement in the release as compared to pure GLB with the optimum formulation releasing almost 80 % drug within first five minutes. X ray diffraction studies concluded that the crystallinity of optimum batch nanoparticles was intact and the increased dissolution could be ascribed to conversion of un milled drug to nanosized drug.
7.1 Introduction

According to the food and drug administration nanoparticulate drug is not a generic drug rather is considered as “newdrug”. It is not bioequivalent to its microcrystalline or solubilized form, administered at the same dosage and therefore a nanoparticulate drug can be patented (Singare et al., 2010). It also offers fewer side effects, lower doses and faster onset of action. Nanosuspensions are liquid dispersion consisting of solid drug nanoparticles which are stabilized by polymer and or surfactant (Liversidge & Liversidge, 2011). Nanosizing has been proven to be an effective tool for an active moiety considered as “brick dust candidate”. Extensive review has been done on the various approaches for development and characterization of nanosuspensions, nevertheless briefly it has been classified in to two basic approaches i.e. top down technology and bottom up technology. The top down approach relies on mechanical attrition to render large crystalline particles into nanocrystals and their small size and increased surface area leads to an increased dissolution rate and increased bioavailability (Eerdenbrugh, Van den Mooter, & Augustijns, 2008). However, breaking drug particles to nanoparticles with size below 100 nm is extremely difficult with these methods since these methods are very time consuming and require significant energy, which might generate a large amount of amorphous particles, and contamination from milling media or homogenization chamber (Krause, Kayser, Ma’der, Gust, & Müller, 2000). The bottom up technology involving solvent antisolvent precipitation involves dissolving drug in a solvent which is then added to non-solvent to precipitate and control the growth of crystals which produces fine particles by starting at the atomic level (Bilati, Allemann, & Doelker, 2005). These methods give better control over particle properties such as, size, morphology and crystallinity as compared to top-down methods (Thorat & Dalvi, 2012). Other solvent removal methods such as, evaporative precipitation into aqueous solution (EPAS) (Kakran et al., 2010) and microemulsions have also been reported, though this are one-step processes they have certain but have disadvantages such as, low yield and degradation of heat sensitive (Zeng, Li, Zhang, & Dong, 2008). The supercritical fluid technology is another extensively researched bottom-up technology for precipitation of nanoparticles (Kim et al., 2008), however, these processes have inherent disadvantages of using extremely high pressures which require high pressure pumps, temperatures, and specially designed fine nozzles. High pressure pumps and specially
designed nozzles increase the cost of technology. Moreover, fine nozzles may get clogged anytime and pose operational problems.

![Figure 7.1 Glibenclamide (5-chloro-N-(2-(4-(cyclohexylcarbamoyl)amino)sulfonyl)phenyl)ethyl)-2-methoxybenzamide)](image)

Glibenclamide [GLB (5-chloro-N-(2-(4-(cyclohexylcarbamoyl)amino)sulfonyl)phenyl)ethyl)-2-methoxybenzamide] figure 7.1, is a potent sulfonylurea and has established potential benefits such as lower dose, rapid onset, lower insulin levels and less-pronounced glucagonotropic effects, insulin-sensitizing and insulin-mimetic affects. GLB lowers blood glucose level in patients with Type 2 diabetes by directly stimulating the acute release of insulin from functioning beta cells of pancreatic islet tissue by binding to the SUR1 subunits and block the ATP-sensitive K+ channel, however GLB is a poorly soluble drug (<8ug/ml in pH 7.4 phosphate buffer) (Seedher & Kanojia, 2009) with relatively high permeability through CaCo-2 cell monolayer's which warrants it to be classified under BCS Class II classification (Lindenberg, Kopp, & Dressman, 2004), Otoom et. al. concluded that glyburide administration under fasting condition significantly increases area under curve for 24 hours and increases maximum concentration of GLB in blood compared to its administration under feeding condition and the lag time was significantly reduced in fasting condition compared with feeding situation suggesting that it is effectively absorbed from the gastrointestinal tract, however, presence of food, certain dietary supplements interfere with its dissolution and in turn its absorption (Otoom, Hasan, & Najib, 2001). In view of the time required to reach an optimal concentration in plasma, GLB may be more effective if given 30 minutes before meal (Hardman & Limbird, 2001). Conversely, this might reduce patient
compliance since if after taking the drug the patient is not able to have the meal it would result in severe hypoglycemia and if taken with meal, food sequentially would interfere with its absorption. Hence, improving the dissolution characteristics of GLB might allow concomitant dosing of the drug with food. Various researchers have tried to improve the dissolution characteristics of GLB, but the application of nanotechnology drug delivery for improving the dissolution characteristics of GLB is still in the early hours. Recently Patravale and Bachhav prepared self microemulsifying drug delivery system (SMEDDS) and improved GLB dissolution characteristics but concluded that it gets degraded in the prepared SMEDDS (Bachhav & Patravale, 2009), more recently Singh and coworkers prepared a self nanoemulsifying drug delivery system (SNEDDS) of GLB and improved the dissolution characteristics of GLB (Singh, Verma, & Razdan, 2010). However the excessive use of surfactants to dissolve the drug may limit the application of the SNEDDS. The same groups of coworker also prepared and optimized a nanosuspension formulation using GLB as a model drug (Singh et al., 2011). However, a detailed investigation into the preparation of GLB nanoparticles for dissolution enhancement was lacking and in view of all this the present investigation was carried to develop, characterize and optimize GLB nanoparticles, to improve its dissolution characteristics. A Placket burman screening design was employed to screen various factors such as stabilizer type, stabilizer concentration, drug concentration, solvent and antisolvent volume ratio, milling speed for their effect on particle size, saturation solubility and % drug released.

7.2 Materials and methods

7.2.1 Materials

Glibenclamide (GLB) was obtained as gift sample from Cadila Pharmaceuticals Limited (Ahmedabad, India). PVP S 630 D (PD) was obtained as gift sample from International Specialty Products, Singapore. Poloxamer 188 (PX) was obtained as gift sample from Cadila Pharmaceuticals limited (Ahmedabad, India). Tween 80, Hydroxypropylmethylcellulose (HPMC), Hydroxypropylcellulose (HPC), Hydroxyethylcellulose (HEC) were purchased from S.D.Fine chemicals limited (Mumbai, India). Analytical grades of acetone, dichloromethane, ethyl acetate, isopropyl alcohol were purchased from S. D. Fine chemicals limited (Mumbai, India).
7.2.2 Preparation of GLB nanocrystals

GLB nanocrystals were prepared using an liquid antisolvent precipitation technique. GLB was dissolved in a solvent at definite concentration and sonicated for 20 seconds. The solution was filtrated through 0.22µm whatman filter paper to remove possible particulate impurities. The prepared GLB solution was injected by syringe onto tip of the antisolvent water containing each specific concentration of polymer and/or surfactant with stirring. Precipitation took place immediately upon mixing and formed a suspension with bluish appearance. The consequences of the formulation and process parameters, such as the types of type of solvent, the solvent / antisolvent ratio, the stirring rate, and the concentration of GLB on the properties of the formed nanoparticles were investigated. The freshly formed suspension was centrifuged at 5000 rpm (Remi Centrifuge, Remi Instruments Pvt. Ltd) for 10 minutes and washed twice with 5 ml of deionized water, the obtained nanoparticles were then dried at 50°C for 8h and stored in desiccators till further use.

7.2.3 Experimental design

A set of experiments with Plackett–Burman (PB) screening design was adopted to develop nanoparticles of GLB by nanoprecipitation method. PB designs are screening designs that involve a large number of factors and relatively few runs. They are resolution 3 designs, so they can estimate only main effects. They are typically used to identify a few significant factors out of a large set. A total of 12 experimental trials involving 5 independent variables were generated by Minitab 16 (USA). The independent variables screened were concentration of PX (X1), concentration of PD (X2), solvent to anti solvent volume ratio (S/AS) (X3), concentration of GLB (X4) and stirring speed (X5). Mean particle size (Y1), Saturation solubility (Y2) and DP 5min percentage of drug dissolved after 5 minutes (Y3), were selected as the response variables on the basis of trials taken during preliminary batches.

7.3 Characterization of GLB nanocrystals

7.3.1 Particle Size and Zeta Potential analysis.

Particle size, size distribution and zeta potential of GLB nanocrystals were determined using Zetatrac (Microtrac Inc., USA). Zetatrac utilizes a high frequency AC electric
field to oscillate the charged particles. The Brownian motion power spectrum is analyzed with modulated power spectrum (MPS) technique, a component of power spectrum resulting from oscillating particles. 100 mg of sample was suspended with sufficient water and suspension samples were directly placed into cuvette and measure particle size and zeta potential (Dabhi, Limbani, & Sheth, 2011)

7.3.3.2 Percentage yield and drug content

GLB content was determined by dissolving accurately weighed quantity of GLB nanocrystals in methanol. The solutions were filtered, diluted appropriately and samples were measured spectrophotometrically at 229.8 nm for the drug content.

7.3.3 Determination of saturation solubility

Saturation solubility measurements of GLB nanocrystals was carried out as follows: known excess amount of different formulations of GLB was added to 10 ml of 0.05 M phosphate buffer (pH 7.5). Samples were sonicated briefly (bath sonicator, Trans o Sonic,) for 5 second and stirred in a water bath (37 ± 0.3 °C) for 48 h. Samples were then centrifuged, filtered, diluted suitably and analyzed spectrophotometrically as described earlier.

7.3.4 In vitro dissolution studies

Dissolution studies were carried out in 500 ml pH 7.5 phosphate buffer at 37°C at 50 rpm (Paddle method, Electrolab Dissolution Tester TDT-06P, USP). Ten milligrams of GLB or its equivalent formulations were added to dissolution medium and five ml of sample were withdrawn at 5, 10, 20, 30, 45 and 60 minutes and replaced with fresh media. The solutions were filtered with whatman filter paper (0.22 µm) and assayed spectrophotometrically for the dissolved drug at 229.8 nm by the regression equation of standard curve developed in the same range in the linearity range of 2-18 µg/ml.

7.3.5 Powder X-Ray Diffraction Analysis

Powder X-Ray Diffraction (PXRD) patterns of pure GLB, GLB nanocrystal particles were obtained using Powder X-ray diffractometer (MiniFlex, Rikagu Inc., USA) with a copper tube anode over 1–40° 2θ range. The operation data were as follows:
generator tension (voltage) 45 kV; generator current 40 mA; scan step time 9 s\(^{-1}\) and scan step size of 0.008° (2\(θ\)).

7.3.6 Statistical evaluation

Dissolution profiles were compared using % DE\(_{5\text{min}}\) (Area under the dissolution curve within 0 and 5 min time interval) which was computed as follows

\[
\% DE = \left( \frac{\int_0^T t dt}{y_{100\%}} \right) 100
\]

... ... ... (1)

The experimental data obtained were validated by ANOVA combined with the F-test. The determination coefficient (R\(^2\), agreement between the experimental results and predicted values obtained from the model) and the model F-value (Fisher variation ratio, the ratio of mean square for regression to mean square for residual) were applied for statistical evaluation.

7.4 In vivo studies of optimized GLB nanocrystals

Approval to carry out in vivo study was obtained from Department of Pharmaceutical Sciences, Saurashtra University, Institutional Animal Ethics Committee, CPCSEA Reg. No. 1155/ac/07/CPCSEA and their guidelines were followed for the studies. Male wistar rats weighing (~250 gm) were kept on standard diet and fasted over night. Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Non-insulin-dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of STZ (45 mg/kg b.w.). 15 min later, the rats were given the intraperitoneal administration of nicotinamide (110 mg/kg b.w.). Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h. The animals with blood glucose concentration more than 250 mg/dL were considered to be diabetes and used for the experiment (Prabu, Kumarappan, Christudas, & Kalaichelvan, 2012)

The rats were divided into three groups after the induction of STZ-nicotinamide diabetes. Animals in the control group received 0.5% carboxy-methyl cellulose
GLB nanoparticles

(CMC) only. The test groups of animals were treated with the test samples suspended in the same vehicle in a parallel group design. Intragastric tubing was used to administer a single dose of 600µg/kg of the free drug or the equivalent amount of optimized GLB nanoparticles. Blood samples were collected at 1, 2, 3...8 and 24 h after the oral administration of test samples. Blood glucose level (BGL) was measured at different time intervals, up to 24 h the hypoglycemic response was evaluated as percentage decrease in blood glucose level:

\[
\text{Decrease in } \% \text{BGL} = \frac{BGL \text{ at } t = 0 - BGL \text{ at } t}{BGL \text{ at } t = 0} \times 100
\]

The pharmacodynamic parameter of the area under percentage decrease in BGL versus time curve (AUC0–8h) was calculated adopting the trapezoidal rule. Statistical analysis of the results was performed using one-way analysis of variance (ANOVA).
7.5 Results and Discussion

7.5.1. Preliminary studies

7.5.1.1. Effect of the types of solvent and anti-solvent

The preliminary study was aimed to screen the optimal solvent and antisolvent system that together could aid the formation of nanoparticles. The solubility of drug was found out in various organic solvents to find out the most suitable solvent which had maximum drug loading and provided high supersaturation resulting in rapid nucleation and precipitation. It was seen that amongst the various organic solvent assorted, acetone showed highest solubility for GLB (drug loading up to ~ 80 mg/ml) and was selected as the solvent phase. Water was then selected as the antisolvent phase, since acetone is highly miscible with water to go along with by GLB’s poor aqueous solubility assisting the precipitation process.

7.5.1.2. Effect of stabilizer and concentration of stabilizer

A suitable stabilizer has to be added to stabilize the nanosuspension system and prevent aggregation and Ostwald ripening (Sudhir Verma, Kumara, Gokhale, & Burgess, 2010). Screening for an optimal surfactant(s) and its amount is very important for the product quality. In view of this a series of stabilizers were assorted for preparing nanoparticles. Different formulations were prepared varying the type of stabilizer at a constant concentration of stabilizer. The prepared nanosuspensions were evaluated for particle size. As shown in figure 7.2, amongst the polymeric group PD showed highest particle size reduction while in the surfactant group PX showed highest size reduction. However as depicted from figure 7.3 no individual stabilizer showed sufficient zeta potential for developing nanoparticles and hence it was decided to employ both PD as well as PX to prepared GLB nanoparticles.
Figure 7.2 Effect of stabilizer on particle size reduction

Figure 7.3 Effect of stabilizer on zeta potential
7.5.2 Experimental Design:

Plackett Burman (PB) designs are screening designs that involve a large number of factors and relatively few runs. They are resolution 3 designs, so they can estimate only main effects. They are typically used to identify a few significant factors out of a large set. As shown in table 7.1 the selected response parameters showed a wide variation suggesting that the independent variables had a significant effect on the response parameters chosen. Figure 7.4 reveals speed had maximum standardized effect at 95% confidence interval, while the concentration of drug did not had a significant effect on particle size. Equation 2-4 suggests that all of the regression models yielded a good fit with high determination coefficient and F value. The determination coefficients (R2) are larger than 0.9, indicating that over 90% of the variation in the response could be explained by the model and the goodness of fit of the model was confirmed. The obtained F value is compared with the theoretical value (Fisher test critical value) \( F_{\alpha} \) \((p-1,N-p)\) (\(\alpha\), chosen risk, \(p\) the number of terms of the model, \(N\) the number of the experiments) to test the significance of the regression model. The theoretical value \(F_{0.05}(5,4)\) is 6.25. As shown in table 7.2, the F-ratio was found to be far greater than the theoretical value with very low probability of less than 0.001 for each regression model, indicating that the regression model is significant with a confidence level of 95%. The significance F and \(R^2\) suggested that there was a good linearity between the predicted and the observed values. All the
independent parameters significantly affected the response parameters except drug concentration.
<table>
<thead>
<tr>
<th>Batch Code</th>
<th>PX (mg) (X1)</th>
<th>PD (mg) (X2)</th>
<th>S/AS (X3)</th>
<th>Drug (mg) (X4)</th>
<th>Speed (rpm) (X5)</th>
<th>Particle Size (nm) (Y1)</th>
<th>Saturation Solubility (µg/ml) (Y2)</th>
<th>% DE&lt;sub&gt;5min.&lt;/sub&gt; (Y3)</th>
</tr>
</thead>
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<tr>
<td>PB1</td>
<td>30</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>300</td>
<td>316.2 ± 6.1</td>
<td>18.54 ± 1.2</td>
<td>31.52 ± 0.45</td>
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<tr>
<td>PB2</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>80</td>
<td>300</td>
<td>678.6 ± 12.6</td>
<td>13.99 ± 0.6</td>
<td>19.43 ± 0.14</td>
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<td>PB3</td>
<td>15</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>800</td>
<td>208.4 ± 4.6</td>
<td>23.98 ± 2.4</td>
<td>45.32 ± 0.68</td>
</tr>
<tr>
<td>PB4</td>
<td>30</td>
<td>10</td>
<td>20</td>
<td>80</td>
<td>300</td>
<td>345.6 ± 12.1</td>
<td>17.56 ± 1.2</td>
<td>29.02 ± 1.24</td>
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<td>30</td>
<td>20</td>
<td>10</td>
<td>80</td>
<td>800</td>
<td>286.1 ± 5.3</td>
<td>19.56 ± 0.8</td>
<td>35.17 ± 0.98</td>
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<td>PB6</td>
<td>30</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>800</td>
<td>214.3 ± 4.5</td>
<td>23.25 ± 0.7</td>
<td>41.26 ± 0.55</td>
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<tr>
<td>PB7</td>
<td>15</td>
<td>20</td>
<td>20</td>
<td>80</td>
<td>300</td>
<td>692.2 ± 6.6</td>
<td>13.78 ± 2.9</td>
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<td>PB8</td>
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<td>80</td>
<td>800</td>
<td>205.4 ± 3.1</td>
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<td>PB9</td>
<td>15</td>
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<td>10</td>
<td>80</td>
<td>800</td>
<td>279.9 ± 5.9</td>
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<td>PB10</td>
<td>30</td>
<td>10</td>
<td>10</td>
<td>40</td>
<td>800</td>
<td>109.2 ± 4.3</td>
<td>24.94 ± 0.7</td>
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<td>15</td>
<td>20</td>
<td>10</td>
<td>40</td>
<td>300</td>
<td>621.5 ± 10.2</td>
<td>13.48 ± 2.6</td>
<td>19.39 ± 0.21</td>
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<td>PB12</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>40</td>
<td>300</td>
<td>676.1 ± 14.5</td>
<td>13.86 ± 0.7</td>
<td>20.53 ± 2.55</td>
</tr>
</tbody>
</table>
Mathematical equation generated from the regression analysis

\[
% \, DE_{5\text{min}} = 13.4 + 0.156 \, PX - 0.578 \, PD + 0.513 \frac{S}{AS} - 0.0721 \, Drug \, concentration + 0.0387 \, Speed
\]

... ... ... ...

(2)

\[
PS = 830 - 8.14 \, PX + 12.8 \, PD - 11.1 \frac{S}{AS} + 1.42 \, Drug \, concentration - 0.676 \, Speed
\]

... ... ... ...

(3)

\[
SS = 9.87 + 0.0893 \, PX - 0.193 \, PD + 0.253 \frac{S}{AS} - 0.0352 \, Drug \, concentration + 0.0151 \, Speed
\]

... ... ... ...

(4)

<table>
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<th>Response variables</th>
<th>Regression analysis</th>
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<tr>
<td></td>
<td>$R^2$</td>
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<tr>
<td>PS</td>
<td>93.5</td>
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<tr>
<td>SS</td>
<td>96.4</td>
</tr>
<tr>
<td>% DE$_{5\text{min}}$</td>
<td>96.6</td>
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</tbody>
</table>
7.5.2.1 Effect of PX

The concentration of PX had a significant effect on the particle size. The negative value of the coefficient in equation 2 suggested that increase in the concentration of PX decreased the particle size. PX is responsible for the hydrophobic interaction with the drug molecule and the hydrophobic polypropylene oxide group (PPO) in the polymer serves to anchor the copolymer to the particle surface while the polyethylene oxide group has little affinity for the surface and is extended into the dispersion medium and thus improves the wettability of drug powder in the dispersion medium (Kumar, Rao, & Apte, 2008) which may contribute to a reduction in mean particle size due to the surface active properties of the PX. Visually also it was seen that when only PD was employed the GLB particles did not wetted adequately and particles floated on the aqueous vehicle while when PX was employed it increased the wettability of GLB particle and coated the newly formed surfaces more effectively and hence showed better particle size reduction.

7.5.2.2 Effect of PD

As shown in equation 2 the concentration of PD had a significant effect on the particle size. The positive value of the coefficient suggested that increase in the concentration of PD resulted in increased particle size. PD chemically is vinyl pyrrolidone/vinyl acetate copolymer, and is acknowledged to get adsorb on the surface of the drug particle and resist crystal growth, as when two particles surrounded by an adsorbed polymer layer approach each other, there will be a local increase in polymer concentration in that region which result in a increase in osmotic pressure locally and leads to an increase in total potential energy which might be responsible for preventing of agglomeration of the particles formed (Sudhir Verma, Huey, & Burgess, 2009). However, a further increase in the concentration of PD could result in a decrease in an appropriate diffusion of the solvent toward the antisolvent caused by the high viscosity of the solution, which in turn increases the particle size.

7.5.2.3 Effect of solvent to antisolvent volume ratio:

The effect of the S/AS volume ratio was investigated and from the ANOVA results as shown in equation 2 the coefficient for S/AS volume ratio has negative sign indicating that as S/AS as volume ratio was increased from 1:10 to 1:20, the decrease in particle
size was observed. As soon as the solvent is added to anti solvent nuclei are formed and growth of nuclei occurs simultaneously. For the consequent growth a high solvent to anti-solvent ratio would increase the diffusion distance for growth species and thus diffusion becomes the limiting step for nuclei growth. Hence increasing the S/AS volume ratio would decrease the particle size formed.

7.5.2.4 Effect of drug concentration.

The drug concentration was varied at two levels viz., 40 mg and 80 mg. As shown in figure 7.4 the p value for drug concentration was found to be less than 0.05 indicating that drug concentration did not affected the particle size significantly. This could be due to the high solubility of GLB in the solvent acetone which subsequently shows very less variation in the viscosity of the final drug solution.

7.5.2.5 Effect of speed

As shown in equation 2 the p value for speed was found to be > 0.001 which suggested that speed of mixing had a highly significant effect on particle size. The coefficient for effect of speed was negative indicating that increasing the speed of mixing decreased the particle size. The decreasing of the particle size can be explained by the intensification of the micromixing (i.e. mixing on the molecular level) between the multi-phases with the increasing of stirring speed. High micromixing efficiency enhanced the mass transfer and the rate of diffusion between the multiphase, which induced high homogenous supersaturation in short time and thus rapid nucleation to produce smaller drug particles.

7.5.3 Effect of independent variables on saturation solubility and DE$_{5\text{min}}$.

As shown in figure 7.5 and 7.6 a high correlation ($r^2 = 0.9587$) was obtained between size of nanoparticles and % drug released, it was seen that batch PB 10 with droplet diameter 109 nm released almost all the drug within five minutes with DE$_{5\text{min}}$ 47.74, while the formulation PB 7 having droplet diameter 692 nm showed 42.68 % drug release within 5 minutes, with DE$_{5\text{min}}$ 19.18. Nanoparticle size, which generally depends upon the formulation and process variables, is a significant part for improving drug release and the increased drug released as seen from dissolution studies with decreased nanoparticle size could be attributed to the reason that
according to Noyes Whitney equation increases the saturation solubility and hence improves dissolution characteristics.

However batch PB 9 could be considered as optimum batch, since it allows higher drug concentration at lower excipient level and as shown in figure 7 has particle size of 209 nm and as depicted in figure 8 releases almost 80 % drug within first 5 minutes which is significantly ($p<0.001$) higher as compared to pure GLB.
Moreover, comparing the dissolution profile of pure drug and optimized formulation in pH 7.5 phosphate buffer and pH 1.2 0.1 N HCl it was seen that there was no significant difference between the dissolution profile of optimized formulation while the dissolution profile for pure drug was significantly different (p < 0.05) in pH 7.5 phosphate buffer and 0.1 N HCl pH 1.2. Seedher and Kanojia concluded that GMP shows pH dependent solubility (Seedher and Kanojia, 2008; Seedher and Kanojia, 2009) and hence there was significant difference between the dissolution of pure drug.
in two different buffers, while the optimized formulation was able to release the drug independent of the pH of the dissolution medium.

As shown in figure 7.9 the PXRD pattern of pure GLB showed sharp and intense peaks at 2θ values of 11.68, 18.91, 20.96, 23.03 indicating its crystalline nature. While in GLB nanoprecipitates of batch PB 9 (bottom) the peaks are almost similar in intensity and position, indicating the crystallinity of the drug is intact and the increased dissolution as seen from dissolution studies could be attributed to a decrease in particle size from micrometer size of pure drug to nanometer size of nanoparticles. The zeta potential of the optimum batch was found to be -35.09 mV, which suggests that no aggregation of nanoparticles took place.

Figure 7.9 PXRD spectra of pure GLB b.) PXRD spectra of GLB nanocrystals.
7.5.4 In vivo studies of GLB nanoparticles

Figure 7.10 shows the mean percentage decrease in blood glucose level (BGL) in diabetic rats after administration of optimized GLB nanoparticles and pure GLB. It is evident that the area under effect curve (AUEC) -values for optimized formulation containing GLB were higher than the corresponding values of pure GLB (table 7.3). These differences were found to be significant (P < 0.05). The increase AUEC values could be attributed to the improved dissolution of GLB from optimum GLB nanoparticles formulation as compared to pure GLB.

Table 7.3 Area under effect curve value for different groups

<table>
<thead>
<tr>
<th>Group of Animal</th>
<th>AUEC (0-8h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.4</td>
</tr>
<tr>
<td>GLB nanoparticles</td>
<td>176.7433</td>
</tr>
<tr>
<td>Pure GLB</td>
<td>97.83333</td>
</tr>
</tbody>
</table>

Figure 7.10 % Decrease in BGL (Mean ± S.E.)
7.6 Conclusion:

GLB nanoparticles were prepared by nanoprecipitation, Placket Burman screening design helped in identifying the significant parameters that affected the response variables. All the predetermined independent variables except drug concentration were found to affect the dependent variables. The optimized formulation maintained the crystallinity of GLB and released almost 80% drug within 5 minutes. The *in vivo* studies confirmed that GLB nanocrystals showed a significantly better AUEC as compared to pure GLB. The improved formulation could offer improved drug delivery strategy which might allow concomitant use of GLB with food.