Solubility is an important criterion for drug efficacy, independent of route of administration. It also poses a major challenge for pharmaceutical industries, which are developing new pharmaceutical products, since 40% of the active substances being identified are either insoluble or poorly soluble in aqueous media (1). A limiting factor for in-vivo performance of poorly water soluble drugs, following oral administration, is their resistance to being wetted and being dissolved into the fluid in the gastrointestinal tract. Increasing the dissolution rate of poorly water soluble drugs is thus important for optimizing bioavailability (2, 3).

The role of solubility enhancement is an attempt to shift the classification of a drug (II → I) in order to eliminate the problems associated with dissolution-limited compounds. Over the last 10 years, nanoparticle (NP) engineering processes have been developed and reported for enhancement of solubility of poorly aqueous soluble drugs. In this approach, poorly water soluble compounds are formulated as nanometer sized drug particles (4). According to Muller, NPs are solid colloidal particles ranging in size from 1 to 1000 nm (1 µm). They have the advantage of having an even greater surface area, and being characterized, unlike micronized drugs, by an increase in saturation solubility.

GB is a second-generation sulfonylurea oral hypoglycemic agent used in the treatment of non insulin dependent diabetes mellitus. It causes hypoglycemia by stimulating release of insulin from pancreatic β cells and by increasing the sensitivity of peripheral tissue to insulin (5). It has a history of low bioavailability, which is attributed to poor dissolution (6-8). Several attempts for increasing dissolution and bioavailability of GB have been made, such as micronization (9), molecular dispersion (10), incorporation of surfactants (11), inclusion complexation with cyclodextrin (12, 13), crystal modification (14, 15), glass formation (16, 17) and coprecipitation (18).

Eudragit L100 polymer is commonly used for enteric coating and also for preparation of controlled

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**Keywords**: nanoparticles, solvent displacement, glibenclamide, Eudragit L100, alloxan
release dosage forms (19). Eudragit L100 is also used in preparation of nanosuspensions for controlled delivery of ketoprofen (20) and also in microparticles as a carrier for enhancing oral bioavailability of carbamazepine (21).

The aim of this study was to formulate and evaluate NPs containing GB prepared by Eudragit L100 using solvent displacement to achieve a better release profile suitable for per oral administration with enhanced efficacy than previous GB delivery.

EXPERIMENTAL

Materials
GB and EUDRAGIT® L100 were received as gift sample from Cadila Healthcare (India) and Röhm Gmbh & Co, KG (Germany), respectively. Pluronic® F-68 was generously gifted by BASF (USA), Alloxan was procured from Spectrochem Pvt. Ltd. (India). Glucose estimation kit was purchased from Erba Diagnostics Mannheim GmbH (Germany). All other chemicals used, were of suitable analytical grade.

Preparation of NPs by solvent displacement method
GB enriched NPs containing the polymers were formulated by solvent displacement technique (22). The crystalline drug was dissolved in a mixture of 9 mL of acetone and methanol (2 : 1) and then the polymer was added and completely dissolved in this organic phase. The organic solution containing drug and polymer was slowly introduced, drop by drop, to 30 mL of aqueous phase containing surfactant, under moderate magnetic stirring, which yielded aqueous turbid dispersion. Seventy mL of water was subsequently added to this dispersion with continuous stirring for 30 min. Additional evaporation process was introduced to remove organic solvent from the colloidal dispersion, which was carried out using a rotary evaporator (STRIKE 102, Stereoglass, Italy) under reduced pressure. Mannitol (5%) was added as cryoprotectant in this nanodispersion and then the formulation was freeze dried. Specifications of different batches and schematic representation of the method are shown in Table 1.

Physiochemical evaluation
Particle size, zeta potential and polydispersity index
To evaluate particle size, zeta potential and polydispersity index (PI), freeze dried NPs were reconstituted in distilled water. The size of NPs was determined by Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK) based on dynamic light scattering technique. Zeta potential, an indicator of surface charge, which determines particle stability in dispersion, was also measured using the principle of electrophoretic mobility in an electric field. The PI which is a dimensionless number indicating the width of the size distribution, was also measured.

Encapsulation efficiency and drug loading capacity
For the determination of encapsulation efficiency accurately weighed NPs (10 mg) were added to 10 mL of distilled water and after the equilibrium solubility was attained, clear supernatant after centrifugation was filtered and 1 mL of the filtrate was mixed with 4 mL of methanolic HCl. Resulting sample was analyzed on UV visible spectrophotometer at 300 nm. The encapsulation efficiency was determined by using the following formula (23):

\[
\text{Encapsulation efficiency (\%) } = \left[ 1 - \frac{\text{Drug in supernatant liquid}}{\text{Total drug added}} \right] \times 100
\]

For the determination of drug loading capacity, NPs (5 mg) were dissolved in 5 mL of methanolic HCl and the solution was filtered through 0.2 µm fil-

<table>
<thead>
<tr>
<th>Formulation no. (GBS0-GBS9)</th>
<th>Drug (mg)</th>
<th>Polymer (mg)</th>
<th>Surfactant (w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBS0</td>
<td>25</td>
<td>100</td>
<td>1% of PF-68</td>
</tr>
<tr>
<td>GBS1</td>
<td>50</td>
<td>100</td>
<td>1% of PF-68</td>
</tr>
<tr>
<td>GBS2</td>
<td>50</td>
<td>50</td>
<td>1% of PF-68</td>
</tr>
<tr>
<td>GBS3</td>
<td>100</td>
<td>50</td>
<td>1% of PF-68</td>
</tr>
<tr>
<td>GBS4</td>
<td>50</td>
<td>50</td>
<td>0.25% of PF-68</td>
</tr>
<tr>
<td>GBS5</td>
<td>50</td>
<td>50</td>
<td>0.5% of PF-68</td>
</tr>
<tr>
<td>GBS6</td>
<td>50</td>
<td>50</td>
<td>2% of PF-68</td>
</tr>
</tbody>
</table>

GB = GB, PF = Pluronic F, PVA = Polyvinyl alcohol; Volume of aqueous phase for each batch = 30 + 70 mL
ter (Axiva syringe filter). GB concentration in the sample was determined using UV visible spectrophotometer at 300 nm. The percentage drug loading capacity was determined using the following formula (24):

\[
\% \text{ Drug loading} = \left( \frac{\text{Mass of GB in NP}}{\text{Mass of NP recovered}} \right) \times 100
\]

Saturation solubility test

Solubility of GB and freeze dried NPs of GB were obtained by adding an excess (2 g) of the sample to 10 mL of water in a glass container at room temperature. The samples were kept on water bath shaker for 24 h to ensure saturation. After the equilibrium solubility was attained, clear supernatant was filtered and 1 mL of the filtrate was mixed with 4 mL of methanol HCl. Resulting sample was analyzed on UV-vis spectrophotometer at 300 nm.

In vitro release study

The release of GB from the NPs was studied using a United States Pharmacopoeia (USP) XXIII 8-station dissolution rate test apparatus with a rotating paddle stirrer at 50 rpm and 37°C ± 0.5°C in phosphate buffer (pH 7.4). A sample of NPs equivalent to 5 mg of GB was used in each test. Samples of dissolution fluid were withdrawn through a filter (0.45 µm, Millipore Millex-HN) at different time intervals and were assayed at 300 nm for GB content using a double-beam UV spectrophotometer. The drug release experiments were conducted in triplicate (n = 3).

DSC characterization

Phase transition behavior of GB loaded freeze dried NPs were analyzed by the differential scanning calorimeter (DSC Q10, TA Instruments, USA). As a control, the pure GB, Eudragit L100, Mannitol, Pluronic F-68 and Eudragit L100 coated NPs of GB were analyzed by DSC. Approximately 4-5 mg of each sample was sealed in a standard aluminum pan with lid. The temperature range of measurement was 20–250°C and the heat flow rate was set to 10°C per min.

Morphology

Morphological analysis of NPs in suspension was performed using transmission electron microscopy (Hitachi, H-7500, Japan). Samples of the nanosuspension (5–10 µL) were dropped onto Formvar-coated copper grids and dried. Digital Micrograph and Soft Imaging Viewer software were used to perform the image capture and analysis, including particle sizing.

In vivo study (alloxan-induced diabetic rabbit model)

Development of diabetes in rabbits was done by injection (ip) of alloxan (140–150 mg/kg; 3% solution in saline water) (25). Rabbits developed diabetes after 72 h. The rabbits with the non-fasting PGL of ≥ 300 mg/dL were considered as diabetic and selected for further pharmacological studies (26). In-vivo evaluation studies were conducted on (1) control, (2) pure GB, (3) GB nanosuspension in diabetic rabbits, by measuring serum glucose levels following their oral administration at a dose equivalent to 2 mg/kg of GB. The products were administered orally in the morning followed by overnight fasting. No food or liquid other than water was allowed during the experimental period. After the zero-hour blood sample was collected, the product in the study was administered orally. Blood samples (0.5 mL) were collected from marginal vein of rabbits through a catheter inserted in the vein to heparinized centrifuge tubes at 1 h intervals up to 10 h after administration. Serum glucose concentrations were determined by a known oxidase-peroxidase method (27) as described below, employing a glucose estimation kit on Auto-analyzer. Blood samples collected were allowed to clot without any anticoagulant and were centrifuged immediately at 3500 rpm for 20 min to separate the serum. To the serum (0.01 mL) and standard (0.01 mL) in separate clean, dry test tubes, enzyme reagent (1 mL) was added, mixed well, and incubated at 37°C for 10 min. The solutions were diluted to 5 mL with distilled water, and the absorbance of the pink-colored solutions was measured in a spectrophotometer at 505 nm using a reagent blank. Serum glucose levels (mg/100 mL) and percentage reduction in serum glucose levels were calculated. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSEA), Govt. of India (Registration No. 0436). Statistical analysis

All the results were expressed as the mean ± SEM. The data of all groups were analyzed using one-way ANOVA by Dunnett’s t-test using SigmaStat 3.5. In all the tests, the criterion for statistical significance was p < 0.05.

RESULTS AND DISCUSSION

The importance of enhanced drug incorporation efficiency in NPs has been emphasized earlier, and since a high NPs recovery is required for reduc-
ing manufacturing costs and its size and morphology are important prerequisite for quality control and biodistribution (28), it was necessary to study the influence of processing parameters on NP preparation. The selection of optimal formulation in our study was, therefore, based on that which provided a combination of good morphology (in terms of particle size, PI), high drug loading, high encapsulation efficiency and zeta potential.

**Effect of drug: polymer ratio**

Various drug: polymer ratio tried were 1:4, 1:2, 1:1, and 2:1 (Table 2). Particle size increased with increasing drug: polymer ratio. When the particle sizes were examined, it was seen that the particle size increases with the decreasing polymer amount. As some researchers pointed out, particle sizes were also observed to be proportional with dispersed phase viscosities (29-32). But the smallest particle size was observed with formulations having drug: polymer ratio 1:1 (GBS2). This could be attributed to the amount of surfactant enough to maintain the stability of NPs at equal molar ratio of drug and polymer and coalescence of droplets did not occur at this ratio.

The higher PI value of GBS0 formulation means a broad particle size distribution resulting from the biggest difference between the viscosities of its dispersed phase and dispersing medium compared with the other formulations (33). Higher value of zeta potential indicating maximum stability was also observed in the same formulation. High zeta potential (either negative or positive) requires higher energy for bringing two particles in contact with each other, i.e., it possesses high energy barrier between particles. An increase in GB concentration with respect to polymer showed increased drug loading and also revealed an increase in encapsulation efficiency. Maximum drug loading and encapsulation was found in formulation having drug: polymer ratio of 2:1 (GBS3).

### Table 2. Effect of different formulations prepared by solvent displacement method on particle size, polydispersity index, zeta potential, encapsulation efficiency and drug loading capacity.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Mean particle diameter (nm)</th>
<th>PI</th>
<th>Zeta potential (mV)</th>
<th>Encapsulation efficiency*</th>
<th>Drug loading capacity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBS0</td>
<td>1086.33</td>
<td>1.15</td>
<td>-16.07</td>
<td>53.97 ± 0.77</td>
<td>21.78 ± 1.02</td>
</tr>
<tr>
<td>GBS1</td>
<td>1237</td>
<td>0.87</td>
<td>-11</td>
<td>60.98 ± 0.73</td>
<td>29.38 ± 2.41</td>
</tr>
<tr>
<td>GBS2</td>
<td>704.67</td>
<td>0.56</td>
<td>-27.8</td>
<td>71.27 ± 1.37</td>
<td>32.67 ± 0.73</td>
</tr>
<tr>
<td>GBS3</td>
<td>1632</td>
<td>0.37</td>
<td>-15.27</td>
<td>74.97 ± 1.69</td>
<td>35.85 ± 1.32</td>
</tr>
<tr>
<td>GBS4</td>
<td>1012.33</td>
<td>0.41</td>
<td>-5.133</td>
<td>60.23 ± 0.94</td>
<td>17.47 ± 0.92</td>
</tr>
<tr>
<td>GBS5</td>
<td>858.47</td>
<td>0.51</td>
<td>-20.67</td>
<td>63.46 ± 1.22</td>
<td>26.83 ± 0.66</td>
</tr>
<tr>
<td>GBS6</td>
<td>808.15</td>
<td>0.81</td>
<td>-28.33</td>
<td>73.57 ± 1.76</td>
<td>33.20 ± 0.93</td>
</tr>
</tbody>
</table>

* Values shown as the mean ± SEM

![Figure 1. Saturation solubility of different NPs (GBS0-9, GBE0-9) and pure drug; **p < 0.01, analyzed by ANOVA followed by Dunnett’s test, compared with pure drug](image-url)
Effect of surfactant concentration

The type of compound employed for stabilization has a pronounced effect on particle size (Table 2). The mean NP size was found to decrease sharply with an increase in concentration of Pluronic F-68 (34) up to 1% w/v. Higher surfactant concentrations reduce the surface tension and facilitate particle partition. The decrease in the particle size is accompanied by a rapid, tremendous increase in the surface area. Thus, the process of primary coverage of the newer surfaces competes with the agglomeration of the uncovered surfaces. Hence, an increase in the surfactant concentration in the primary dispersion results in rapid coverage of the newly formed particle surfaces. There was an optimum concentration, above which an increase in surfactant concentration did not result in a decrease in particle size due to saturation point (35).

The broadness of the size distribution observed at higher surfactant concentrations could be due to the higher viscosity of the continuous phase which disperses the stirring energy (36). Thus, the PI value increased with increasing surfactant concentrations. Stability (high zeta potential either positive or negative) increased with increasing concentrations of surfactant. Thus, higher stability was observed with formulations having 2% w/v surfactant (GBS6). An increase in concentration of surfactant resulted in a slight increase in encapsulation efficiency and loading capacity (37).

Saturation solubility test

NPS (NPs) systems have the advantage of having an even greater surface area, and being characterized, unlike micronized drugs, by an increase in saturation solubility (38). There was a very significant increase (p < 0.01) in saturation solubility (Fig. 1) of different formulations when compared with saturation solubility of pure drug. The saturation solubility \( C_s \) increases with decreasing particle size according to the Ostwald Freundlich equation:

\[
\log \frac{C_s}{C} = \frac{2s}{2.303} \frac{V}{RT} \frac{r_1}{r}
\]

where \( C_s \) = solubility, \( C \) = solubility of the solid consisting of large particles, \( s \) = interfacial tension substance, \( V \) = molar volume of the particle material, \( R \) = gas constant, \( T \) = absolute temperature, \( r_1 \) = density of the solid, and \( r \) = radius.
The dissolution profile of drug release studies of different batches (GBS 0-6) prepared by solvent displacement method are summarized in Figures 2(A) and 2(B). The effect of different parameters on drug release was also evaluated. The percent cumulative release of GB decreased with increasing polymer concentration. The increase in the polymer matrix at higher concentrations results in an increased diffusion path length (39). This may decrease the overall drug release from the polymer matrix. Effect of drug : polymer ratio on the release profile of different formulations (GBS 0-3) prepared by both methods are shown in Figure 2(A). However, GBS2 (drug : polymer, 1 : 1) showed high drug release which could be due to small particle size found in these formulations, resulting in increased surface area.

Incorporation of surfactant during preparation of NPs also affects drug release from NPs, as well as the concentration of surfactant incorporated in NPs. The effect of concentration of surfactants are shown in Figure 2(B).

Drug loaded NPs showed a biphasic pattern: an immediate release ("burst effect") followed by a slower release profile. This initial fast release is probably due to the fraction of the drug which is adsorbed or weakly bound to surface area of NPs (40). A comparison of release profile of formulations prepared by solvent displacement method with a marketed product Daonil® [Fig. 2(A) and (B)] showed a significant increase in drug release with NPs. This was due to an increase in saturation solubility after making NPs of GB.

DSC characterization

DSC is a fast and reliable method to understand the polymorphic transitions, to screen drug excipient compatibility and providing maximum information about possible interactions. The DSC heating curves were recorded as a plot of enthalpy (m/w) vs. temperature (°C). The results are shown in Figure 3.

GB showed melting endotherm at 172.75°C. The thermogram of Eudragit L100 showed three peaks at 52.14, 104.96 and 212.65°C, but sharp peak only at 52.14°C. Pluronic F-68 and mannitol showed melting thermogram at 55.76°C and 168.26°C, respectively.

Thermogram of GB loaded NPs showed not one but multiple endotherms. This indicates the presence of more than one polymorphic modifica-

### Table 3. Comparative effect of formulations on different parameters in alloxan-induced diabetic rabbits.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>$T_{\text{min}}$ (h)</th>
<th>$C_{\text{min}}$ (% decrease in SGL)</th>
<th>$AUC_{0-10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.83 ± 0.87</td>
<td>12.59 ± 1.74*</td>
<td>941.36 ± 18.59*</td>
</tr>
<tr>
<td>GB</td>
<td>7.83 ± 0.48</td>
<td>30.94 ± 1.53</td>
<td>781.43 ± 9.73</td>
</tr>
<tr>
<td>Nanosuspension</td>
<td>8.17 ± 0.40</td>
<td>53.13 ± 1.67*</td>
<td>201.80 ± 13.13*</td>
</tr>
</tbody>
</table>

*p < 0.01, analyzed by ANOVA followed by Dunnett’s test, compared with GB treated group

Figure 3. Different thermograms of GB NPs (A), pure GB (B), Eudragit L100 (C), Mannitol (D), Pluronic F-68 (E)
Development and characterization of nanoparticles of glibenclamide by solvent displacement method

Melting endotherm of drug was found absent in thermogram of NP. It seems that during the formulation of NPs, the drug has changed from crystalline phase to amorphous phase, which may be responsible for higher solubility observed with NPs with respect to pure drug.

Morphology of the prepared nanosuspension was examined by TEM at different magnifications. The results show that GB loaded NPs were predominantly spherical. The distinct, porous nature and spherical shape of the NPs is evident from their TEM photographs (Fig. 4(A) (B)). NPs appeared to be considerably smaller when viewed with TEM, as compared to the average particle size observed with Zetasizer. This apparent discrepancy between the two results can be explained by the dehydration of the NPs during sample preparation for TEM imaging (41).

In vivo study (alloxan-induced diabetic model)

Diabetic control rabbits did not show any significant changes in serum glucose level (SGL) over a period of experiments. However, as the time progressed, a slight decrease in SGL was observed in diabetic control. The decrease may be attributed to the fasting effect on SGL and Devarajan (42) supported it. GB treated rabbits showed t_{min} at 7.83 h with C_{min} 30.94% decrease in SGL of basal level. Nanosuspension treated rabbits showed t_{min} 8.17 and C_{min} of 53.13% decrease in SGL of basal level, compared with plain GB (Fig. 5.). Nanosuspension showed a very significant decrease (p < 0.01) in SGL, t_{min} and AUC (area under the curve) when compared to GB tested group (Table 3).

Morphology

Figure 4. TEM of GB NPs (5000x A, 100000x B)

Figure 5. Comparative effect of formulations on SGL in alloxan-induced diabetic rabbits
CONCLUSION

It was shown that it is possible to obtain a GB loaded NPs prepared by solvent displacement method, having drug : polymer ratio of 1 : 1, and stabilized by 1% w/v Pluronic F-68. They had small mean particle diameter and high zeta potential. They possessed good encapsulation efficiency and drug loading capacity and showed very significant change in saturation solubility in comparison of pure drug. Developed NPs revealed a decreased $t_{\text{min}}$ and enhanced bioavailability and hence superior activity as compared to plain GB in alloxan-induced diabetic rabbit model. The developed NPs could reduce dose frequency, decrease side effects, and improve patient compliance.

REFERENCES


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