

**IN VIVO EVALUATION OF HYPOGLYCEMIC ACTIVITY OF SOLID DISPERSIONS OF GLIBENCLAMIDE USING PREGELATINISED STARCH AS A SOLUBILITY ENHANCER**

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**ABSTRACT**

Current study was undertaken to investigate the aptness of pregelatinised starch to get better solubility and *In vitro* dissolution rate of the poorly water soluble drug glibenclamide. Physical mixtures, kneading mixtures and solid dispersions of glibenclamide & pregelatinised starch were prepared at drug carrier ratio 1:1, 1:3, 1:5, 1:7 and 1:9. These resulting systems were subjected to solubility analysis, *In vitro* dissolution, X-ray diffraction, differential scanning calorimetry and Infrared Fourier Transform Spectroscopy (FTIR) to characterize the resulting systems. All systems fashioned improvement of the solubility of glibenclamide, however solvent evaporation method was realistic to offer the superior solubility and dissolution rates. *In vitro* dissolution studies of solid dispersion of glibenclamide with pregelatinised starch at 1:9 drug carrier ratio discovered utmost dissolution (39.15 %) after 120 minutes in comparison with pure drug (3.27%). This improvement in *in-vitro* dissolution was accredited to amorphous form which is revealed by DSC and R-ray diffraction studies. *In vivo* evaluation of the selected solid dispersions significantly enhanced hypoglycemia attributed to improvement in superior gastrointestinal solubility of the drug in solid dispersions.

**KEYWORDS:** Glibenclamide, solid dispersions, dissolution improvement, hypoglycemia, solubility.

**INTRODUCTION**

The improvement of solubility or dissolution has been a challenging task for the investigators for the years. Numerous methods are available to improve these characteristics in water and in biological fluid at physiological pH. However, these methods possess their own drawbacks which limit their application in pharmaceutical field<sup>1-3</sup>. Virtually, 40 % of the new seeds currently being revealed are poorly water soluble drugs<sup>4</sup>. The most trendy and widely used techniques is solid dispersions<sup>3</sup>.

Despite of continual wakefulness in solid dispersion, very less number of different polymeric carriers has been investigated in past 40 years. In fact majorities of studies have been published so far report on the use of polyethyleneglycol or polyvinylpyrrolidone, hydroxyl-popyrilmethylalcohol, polysorbate alone or in combination with polymers or surfactants. Therefore, definitely there is need to investigate new carriers for solubility enhancement<sup>5</sup>.

Pregelatinised starch (PGS) is a starch that has been modified chemically or mechanically processed to rupture all or part of the starch granules<sup>6-7</sup>. Typically, In comparison to starch, grades of pregelatinised starch may be produced with enhanced flow and comparison characteristics such that the pregelatinised material may be used as a tablet binder in dry-compression processes. In such processes, pregelatinised is self lubricating. However, when it is used with other excipients it may be necessary to add a lubricant to a

formulation<sup>8</sup>. Fully pregelatinised starch swells rapidly in water to develop strong binding properties in wet granulation processes. They agglomerate powders most efficiently into granules with good flow and compression characteristics<sup>9-10</sup>.

PGS has not been well investigated for its solubility enhancement potentials. Thus taking the above view in consideration current study was undertaken to explore PGS for its solubility enhancement properties.

Glibenclamide (GLB) is a second-generation orally administered sulphonylurea derivative with potent hypoglycemic activity. It is used in treatment of non-insulin dependent diabetes. Its hypoglycemic effect is mainly attributed to beta cells and sensitization of the peripheral tissues to insulin<sup>11-12</sup>.

It is poorly water soluble and shows poor solubility in gastrointestinal fluids, which can give rise to variations in its dissolution rate and incomplete and/or unpredictable bioavailability which is investigated by several investigators<sup>13-16</sup>.

## MATERIALS AND METHODS

Glibenclamide and PGS (Starch 1500) were generously gifted by Lifeasia Pharma International Mumbai, India and Colorcon, Goa, India respectively. All other ingredients were used as such and were of analytical grade.

### Preparation of physical mixtures (PM) or kneading mixtures (KM) or solid dispersions (SD) with PGS

Solvent evaporation method was used to prepare the solid dispersions of GLB with PGS at drug carrier ratio 1: 1, 1:3, 1:5, 1:7 and 1:9. Briefly, the accurately weighed quantity of PGS was added to a solution of GLB (100mg) in chloroform. The solvent was evaporated at  $37 \pm 0.5$  °C with constant stirring using magnetic stirrer. The resulting residue was dried in oven till constant weight at 40 °C and was stored overnight in a desiccator. KM were prepared by thoroughly mixing weight quantities of GLB and PGS at above mentioned drug to carrier weight ratios placed in mortar and then the mixtures were kneaded with approximately 1.5 times their amount of ethanol (90%) for 20 minutes. KM were dried in oven at 40 °C until it reached uniform weight. PM were freshly prepared prior to analysis by thoroughly blending the accurately-weighed amount of the drug and PGS at above mentioned drug to carrier weight ratios. All these systems were pulverized and passed through mesh # 100 and were kept in desiccator for further study.

### Solubility analysis of the resulting systems

Simulated intestinal fluid (SIF) (pH 7.2) maintained at  $37 \pm 0.45$  °C temperature was used for determination of apparent solubility of resulting systems of GLB. A quantity equivalent to 10 mg of drug resulting systems was added to 5 ml of SIF in a conical flask with screw cap. The conical flasks were kept at a shaker at  $37 \pm 0.5$  °C for 24 h. Next morning, the solutions were filtered through 0.45 µm millipore filter and the filtrate was analyzed spectrophotometrically at 237 nm. The results are shown in table 1

### Dissolution rate studies

USP rotating basket apparatus (USP XXI) was used for performing dissolution studies. 900 ml of SIF was used as dissolution medium. It was maintained at  $37 \pm 0.5$  °C temperature and rotation speed was kept at 100 rpm. A quantity equivalent to 10 mg of drug of resulting systems was added to above medium. Three ml of dissolution medium was withdrawn at 5, 15, 30, 45, 60, 90 & 120 minutes intervals and filtered through 0.5 µm millipore filter and the filtrate was analyzed spectrophotometrically at 237 nm using SIF (pH 7.2) as blank. Three ml of SIF was added to dissolution medium after every determination to maintain the sink conditions. The experiments were performed in triplicate. The mean concentration of the drug was plotted against time. (fig.1)

### X-ray diffraction

X-ray diffraction patterns of the drugs and resulting systems were recorded using X-ray diffractometer (Punjab university PW3050/60: Goniometer). The experiments were carried out at 25 °C under the following conditions: voltage 40kV, current 30 mA, 2θ angle with scan step time of 10.33 s with specimen length of 10 mm. The results are depicted in fig. 2.

### Differential scanning calorimetry (DSC)

The DSC thermograms were recorded using a differential scanning calorimeter. Approximately 2-5 mg of each sample was heated in an open aluminum pan from 30-300 °C at a scanning rate of 10 °C/min under

a stream of nitrogen. DSC was performed at S. K. Patel college of Pharmaceutical education, Mehsana, Gujrat, India.

DSC thermograms of selected SD were obtained on a TA Inst 2000 MTDSC instrument. About 2-3 mg of sample was taken in one of the matched aluminium pan and heated with a continuous purge of argon (44 ml/min). The thermograms recorded for GLB and that of their selected SD are presented in fig. 3.

#### **FTIR spectroscopy**

The KBr disk sample preparation technique was used to obtain the IR spectra of the samples on an IR spectrophotometer (Shimadzu-FTIR-8400S). The obtained spectra were compared with those reported in official compendia. The FTIR spectra of samples of GLB are shown in fig. 4. Characteristic peaks attributable to functional groups present in the molecule of each drug were assigned to establish the identity. This study was performed at B.R.Nahata College of Pharmacy, Mandsaur, M.P., India.

#### **Hypoglycemic activity**

##### **Animals**

Albino rats of either sex weighing 100-150 gm kept at Animal House, B.R. Nahata College of Pharmacy, Mandsaur were used. The animals were housed under standard environmental conditions and had free access to standard pellet diet and water ad libitum. Twelve hours before the start of experimentation, the rats were deprived of food, but given free access to water. The experimentations were carried out according to Committee for the Purpose of Control and Supervision to Experiments on Animals (CPCSEA) guidelines and institutional Ethical Committee (B.R.Nahata College of Pharmacy) approved all the procedures. A fifteen days wash period was specified for next study. During experiment the animals were divided into two groups of five animals in each group. The blood was withdrawn by pricking the rat's tail. All the samples were administered with the glass syringe and micro suction canula no 18. Weight of animals was approximately 100 gm. Aqua Check were used for estimation of blood glucose level. 0.5 mg dose was used to produce hypoglycemia.

##### **Grouping**

Group I: Glibenclamide (0.5 mg/kg)

Group II: GLB: PGS SD 1:9 (5 mg/kg)

##### **Determination of Hypoglycemic Activity**

Test samples were administered using oral gastric gavages to the fasted animals. The blood glucose concentration of the animals was measured at the beginning of the study and the measurements were repeated at 1 hr, 2 hrs, 3 hrs, 4 hrs and 6 hrs<sup>17-18</sup>.

## **RESULTS AND DISCUSSIONS**

### **Solubility analysis**

The results of solubility studies are depicted in table 1. Solubility of pure GLB was reported 8.62 µg/mg indicating that it is practically insoluble in water. There was observed remarkable increase in solubility of drug in resulting systems. This increase was function of concentration of PGS. PM at 1:9 ratio produced three times increase in solubility in comparison to pure GLB, although kneading method was also reported to enhance solubility but it was less effective in comparisons to PM. Among all resulting systems solvent evaporation was most effective method to improve the solubility of the drug. Approximately 11 times improvement in solubility was reported in SD at drug carrier ratio 1:9 in comparison to pure drug.

### **Dissolution studies**

*In vitro* dissolution profile of resulting systems is shown in fig. 1. *In vitro* dissolution profile of the resulting systems of GLB produced higher dissolution in comparison with pure drug. Among all systems solvent evaporation technique was most effective in improving the dissolution. Superior results were obtained with SD at drug carrier ratio 1:9. The % drug dissolved after 5 minutes was 0.42 % and 14.26 % for pure drug and GLB: PGS SD 1:9 respectively. The % drug dissolved for GLB: PGS SD 1:9 was 39.15% after 120 minutes which was 12 times more in comparisons with pure drug (3.27 %). Solubility analysis and dissolution studies formed basis for selecting GLB: PGS SD 1:9 for further study. Thus, GLB: PGS SD 1:9 were subjected to X-ray diffraction studies, DSC, FTIR and *in vivo* evaluation.

**XRD of GLB: PGS: SD 1:9**

X-ray diffraction of GLB displayed four major peaks at  $2\theta$  11.68 (3337), 18.91(4614), 20.96(4281) and 23.03(3387) and are marked as 1, 2, 3 and 4 in fig. 2.

These major peaks in GLB: PGS SD 1:9 changed to 11.89 (152), 18.96(815), 20.97(1001) and 23.22(772) with reduction in intensity of 95.44 %, 82.33 %, 76.61 % and 77.20 % respectively which is indicative of less crystallinity of GLB or presence of amorphous form of the drug.(table 2)

**DSC studies of GLB: PGS SD1: 9**

DSC is very effective analytical method to study the drug carrier interaction. DSC studies of GLB PGS SD1: 9 reported shift of characteristic melting endotherm from 177.34 °C to 170.92 °C and existence of new melting endotherm at 294.85 °C. These reports suggested possibilities of interactions in SD of GLB. (Fig. 3)

**FTIR of GLB**

FTIR spectra of GLB reported characteristic peaks of aromatic C-H stretching, aromatic C-H bending, C-H bending methyl group, Secondary amine N-H stretching, C=O stretching, Asymmetric S(=O)<sub>2</sub> stretching, Symmetric S(=O)<sub>2</sub> stretching and Aryl benzene (Chloro benzene) at 3047, 896, 1340, 3315, 1714, 1340, 1155 and 1024 respectively.

**FTIR of GLB: PGS SD 1:9**

FTIR of GLB: PGS SD 1:9 reported absence of characteristic peaks of aromatic C-H stretching in given range of 3100-3000 instead this range a peak was seen at 2937. This result in association with DSC forms the basis of possible interaction between drug and carrier. All other characteristic peaks for GLB did not show any significant changes. (Fig. 4 and table 3)

**Hypoglycemic activity of GLB**

Hypoglycemic activity of GLB was determined and compared with solid dispersions of GLB prepared with PGS. GLB was able to reduce the blood glucose level to 62.0 mg/dl after 4 hrs thereafter slight increase in blood glucose level was observed. GLB:PGS SD 1:9 produced better reduction in blood glucose level (56.8±1.158 mg/dl ) after 4hr when compared with GLB alone attributed to better solubility of the drug in the prepared SD (table 4).

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**Table 1: Solubility studies of glibenclamide in resulting systems**

Resulting systems of glibenclamide/ weight ratios	Solubility (µg/ml) at 1:1 ratio	Solubility (µg/ml) at 1:3 ratio	Solubility (µg/ml) at 1:5 ratio	Solubility (µg/ml) at 1:7 ratio	Solubility (µg/ml) at 1:9 ratio
Glibenclamide	8.62				
PM-GLB-PGS	25.15	26.45	27.53	31.65	32.46
KM-GLB-PGS	31.51	32.85	34.42	26.52	28.09
SD-GLB-PGS	52.74	52.99	53.33	88.09	93.50

GLB-glibenclamide, GG-Gum Ghatii, PM-Physical mixture, KM-Kneading mixtures, SD-solid dispersions

**Table 2: XRD results and % reduction of intensity of peaks for glibenclamide and corresponding systems prepared using different carriers**

S.No.	Major Peaks Glibenclamide	Major Peaks GLB: PGS: SD 1:9	% reduction of intensity of peaks
1.	11.68 (3337)	11.89 (152)	95.44
2.	18.91(4614)	18.96(815)	82.33
3.	20.96(4281)	20.97(1001)	76.61
4.	23.03(3387)	23.22(772)	77.20

**Table 3: Functional groups and corresponding IR peaks of glibenclamide**

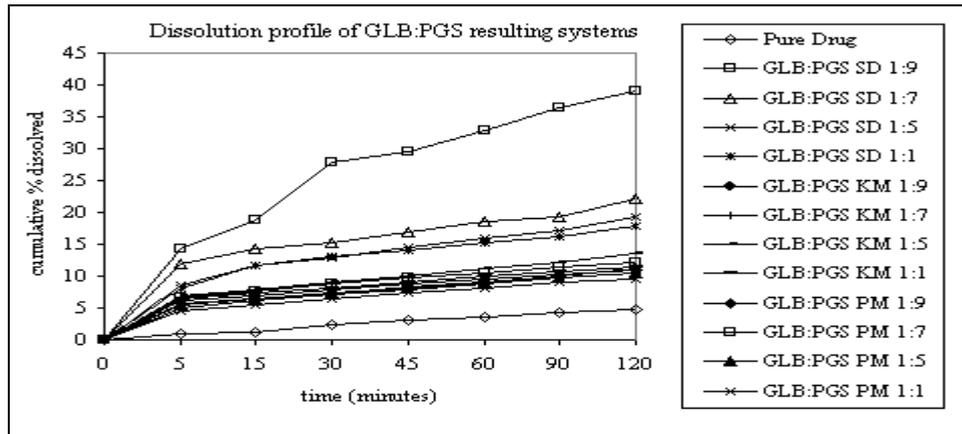
Group	Reported Values	Observed Values for GLB	GLB: PGS SD 1:9
Aromatic C-H stretching	3100-3000	3047	2927
Aromatic C-H bending	900-675	896	833
C-H bending methyl group	1450-1350	1340	1340
Secondary amine N-H stretching	3350-3310	3315	3317
C=O stretching	1870-1540	1714	1708
Asymmetric S(=O) <sub>2</sub> stretching	1350-1300	1340	1340
Symmetric S(=O) <sub>2</sub> stretching	1160-1170	1155	1151
Aryl benzene (Chloro benzene)	1096-1089	1024	1024

**Table 4: Hypoglycemic activity of glibenclamide and selected solid dispersions of glibenclamide as determined by one way ANOVA using post test Dunnett Test**

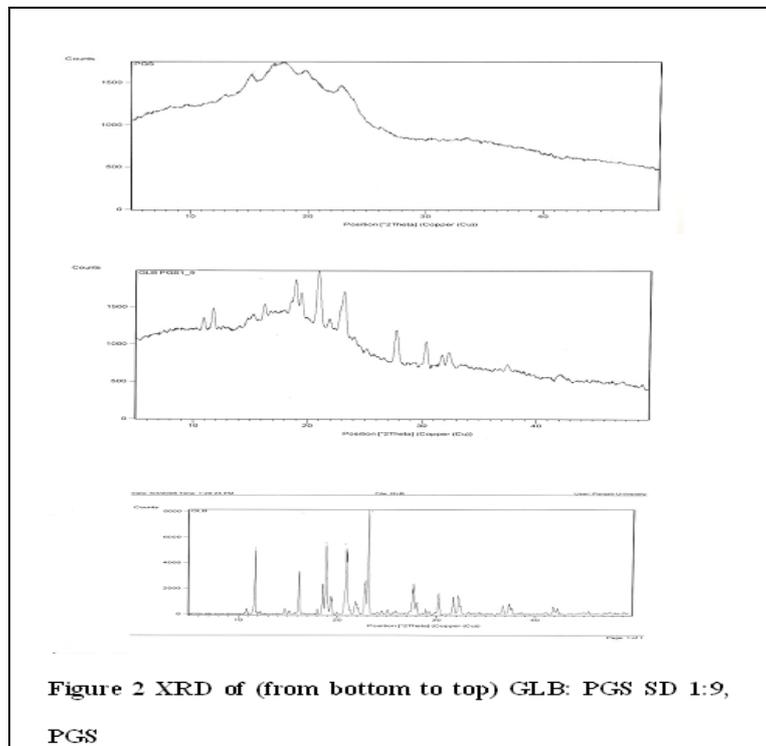
Group	0hr mg/dl	1 hr (mg/dl)	2hrs (mg/dl)	3hrs (mg/dl)	4hrs (mg/dl)	6hrs (mg/dl)
Group I: Glibenclamide (0.5 mg/kg)	93.4±2.441	77.0±1.871	69.0±1.463	64.2±1.068	62.0±1.581	69.8±1.428
Group II: GLB:PGS SD 1:9 (5 mg/kg)	88.4±1.503	72.0±1.304*	67.8±1.241*	62.8±0.583*	56.8±1.158*	58.4±1.077**

Values are expressed as mean ± SEM, n = 5 rats in one group.

\* Significant (p>0.05), \*\*significant (p<0.01) compared to Group I (Glibenclamide)



**Figure 1: Dissolution profile of GLB: PGS Solid dispersions**



**Figure 2 XRD of (from bottom to top) GLB: PGS SD 1:9, PGS**

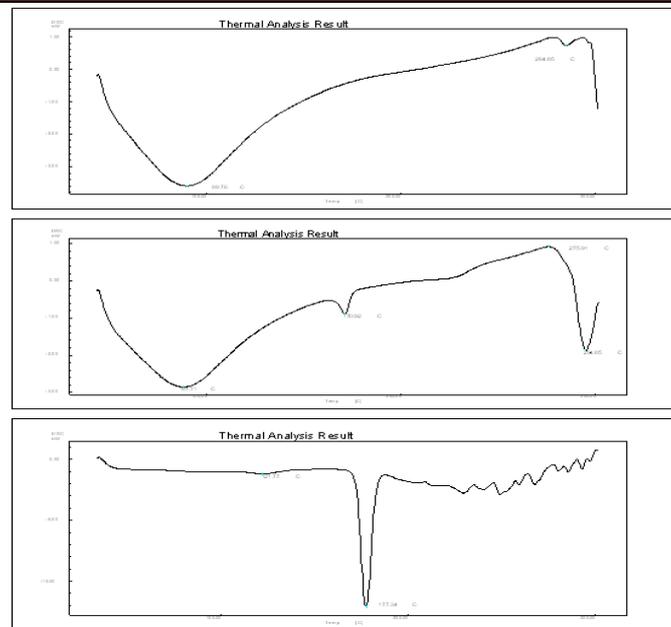


Figure 3 DSC of (from bottom to top) GLB, GLB:PGS SD1: 9, PGS

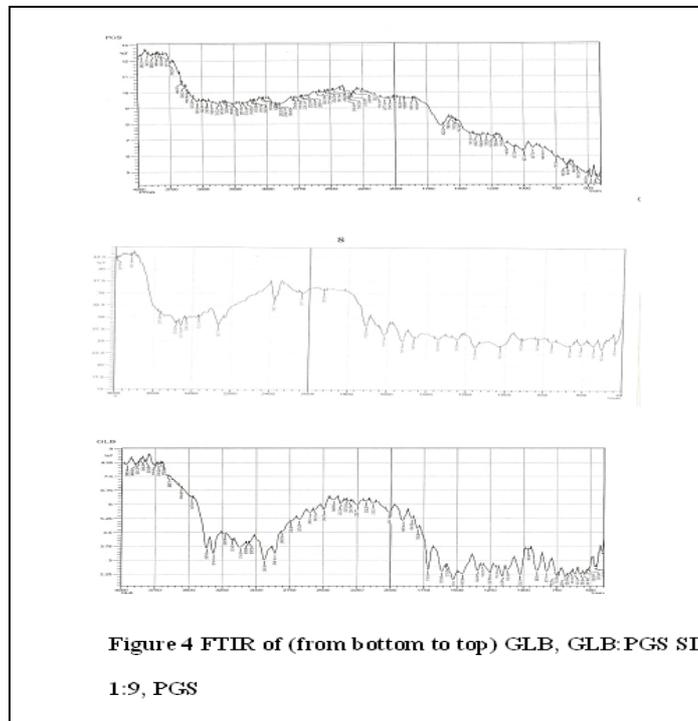


Figure 4 FTIR of (from bottom to top) GLB, GLB:PGS SD 1:9, PGS

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