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ORIGINAL ARTICLE

Formulation of Nanoparticles of Gliclazide For The Better Drug Delivery and Enhanced Bioavailability

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ABSTRACT
Gliclazide is having limitations such as low solubility, but it is used in the treatment of diabetes leading to lower oral bioavailability. Gliclazide conventional formulation despite having anti diabetic activity, its therapeutic activity was limited due to its limited and slow release in gastrointestinal tract. So the major objective was to formulate polymeric nanoparticles, which can increase solubility and drug release along with sustained release property of the drug. In the present study, it was proposed to develop nanotechnology-based systems, for selected poorly water-soluble drug gliclazide using PLGA as polymer (with different drug: polymer ratios) selected randomly and was expected to improve dissolution properties that may increase its bioavailability. The polymeric nanoparticles were subjected to particle size evaluation, drug content, entrapment efficiency and in vitro release studies. Nanoparticles with drug: polymer ratio of 1:1 has shown a particle size, drug loading and entrapment efficiency of respectively. Optimized batch in drug: polymer ratio of 1:1 has shown a particle size, drug loading and entrapment efficiency of respectively. In vitro drug release studies concluded that gliclazide nanoparticles released drug was following sustained release till 24 hr concluding its solubility enhancement.
Keywords: *Gliclazide, Polymeric nanoparticles.*

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INTRODUCTION

Diabetes mellitus describes a metabolic disorder of multiple etiologies distinct by chronic hyperglycemias with disturbances of protein, fat and carbohydrate metabolism resulting from defects in insulin action, insulin secretion, or both. It is a group of metabolic diseases in which a person has high blood sugar because the pancreas does not produce enough insulin or because cells do not respond to the insulin that is produced within the body. Diabetes mellitus may present with characteristic symptoms such as blurring of vision, polyuria, thirst and weight loss. In its most severe forms, a non–ketotic hyperosmolar or ketoacidosis state may develop and lead to coma and in absence of effective treatment, death [1-3].

The major difficulty associated with many newly developed pharmaceutical drugs are poor solubility in water and simultaneously in organic phase. The basic challenge associated with poorly soluble drugs is poor bioavailability absorption. In case of poor bioavailability after oral administration, parenteral administration can't solve problem. Available strategies for enhance the solubility of poorly soluble drugs include: the effect of pH or Salt form, formation of complexes, crystallization, solid dispersion, solubilization and aqueous mixture with an organic solvent. Recent drug delivery mainly focuses on nanotechnology based strategies of poorly water soluble drugs in order to improve their therapeutic performance [4].

Polymeric nanoparticles have acquired attraction for drug delivery systems in the last few decades owing to their ability to deliver the drug at the site of action in a controlled manner. Biodegradable nanoparticles were useful for developing controlled/sustained release and can be more compliant to patient [5]. Also biodegradable nanoparticles can provide constant rate of degradation, which can be

beneficial for sustained release approach. Poly (lactide-co-glycolide) (PLGA) can be selected as the polymer because it is used broadly in preparing various drugs, proteins and also used for the marketed products like micro particles. These biodegradable nanoparticles using PLGA that can deliver controlled drug delivery and also can reduce the serious systemic side effects caused by the drug administration. Surface morphology, particle size, size distribution, drug content has a significant effect on the controlled drug release from the hydrophobic drug [6-8].

So above characteristics help to formulate Gliclazide polymeric nanoparticles by encapsulating this hydrophobic drug inside PLGA. PLGA polymeric nanoparticles were prepared by nanoprecpitation technique using different polymer ratios, which provides best particle size, surface morphology, drug content etc. The prepared polymeric nanoparticles has sustained release property and achieve improvement in the solubility of Gliclazide which may help the drug to overcome its poor oral bioavailability that benefit in therapeutic activity, which has been lowered because of its poor pharmacokinetics [9].

MATERIALS AND METHODS

Purchases were as follows. Poly (lactide-co-glycolide) (PLGA), Gliclazide and Acetonitrile were purchased from Sigma-Aldrich (USA). Drug solutions were freshly prepared in Milliporewater. Organic solvents like polyvinyl alcohol (PVA), Methanol and Ethanol were purchased from Qualigens fine chemicals (Mumbai). Polaxomer 188 and Mannitol were obtained from Merck India. All chemicals were used without further purification. Gliclazide, PLGA, Acetonitrile, Polyvinyl alcohol (PVA) and all other chemicals were of analytical grade. Doubly distilled water was used throughout the study.

Preformulation studies

Preformulation studies provide the information needed to define the nature of drug and a frame work for the drug combination with pharmaceutical excipients in fabrication of dosage form. A thorough understanding of physico-chemical properties may ultimately give a rational for formulation design are support the need for molecular modification nearly conformed that there are no significant barriers to the compound development. The Preformulation studies like study of organoleptic properties and determination of λ max was carried out [10-12].

Preparation of gliclazide nanoparticles

The method used for the preparation of PLGA nanoparticles containing gliclazide is nanoprecpitation technique. Different ratios of drug: polymer (1:1, 1:2, 1:3, 1:4 and 1:5) was selected in order to optimize the best one and also to observe the effect of polymer on the formulation. Acetonitrile was used as organic solvent and PVA as surfactant in a fixed concentration of 0.5% w/v. Drug was dissolved in acetonitrile with varying polymer ratios (1:1, 1:2, 1:3, 1:4 and 1:5). Then it was followed by addition of aqueous surfactant polyvinyl alcohol using high-speed stabilizer Polaxomer 188 and was stirred continuously for 3 hr. Then the suspension was subjected to centrifugation for 30 minutes at 12000 rpm. Supernatant was removed and washed repeatedly three times and subjected to lyophilisation using 5% mannitol as cryoprotectant [13-16].

Fourier Transformed Infrared Spectroscopy (FTIR)

The FTIR spectra of pure gliclazide and formulation F1 were obtained using FTIR spectrometer (FTIR-8300 Shimadzu, Japan) by potassium bromide (KBr) pellet method. This study was employed to ascertain the compatibility between Gliclazide nanoparticles and selected excipients. The spectrum obtained was in between the wave number of 4000-400 cm^{-1}.

Solubility study

Solubility studies were carried out by preparing saturated solution of drug in water. An excess quantity of drug with approximately 2 ml of solvent was taken in vial with rubber stopper then the vial was shaking for 24 hr at room temperature. After 24 hr sample was centrifuged at 300 rpm for 20 min. Then supernetant liquid was pipette out from each sample followed by dilution with suitable solvent and the solubility was determined in the UV-Visible Spectrophotometer at 229.50 nm [17].

Differential scanning calorimetry study (DSC)

The DSC measurements were performed on a DSC-60 (Shimadzu), differential scanning calorimeter with a thermal analyzer. Accurately weighed samples (about 5–10 mg) were heated in hermetically sealed aluminum pans under a nitrogen atmosphere at the flow rate of 20 mL 1 min with a scanning rate of 15 °C 1 min from 60 to 250 °C. An empty aluminum pan was used as a reference.

Size measurement and Zeta potential analysis

The particle size and size distribution of Gliclazide nanoparticles were determined by Photon correlation spectroscopy using zeta sizer. Nanoparticles were diluted with filtered [0.22μm] ultra pure water. Zeta

potential of nanoparticles was measured by zeta sizer. The zeta sizer mainly consists of laser which is used to provide the light source to illuminate the particles within the sample. Zeta sizer software produces a frequency spectrum from which electrophorotic mobility hence zeta potential is calculated [18].

Entrapment efficiency

The entrapment efficiency was determined by taking 2 ml of nanosuspension centrifuged at 500 rpm for 30 minutes. The amount of Gliclazide loaded in nanoparticles was calculated as the difference between the total amounts of used to prepare the nanoparticles and was found in supernetant. The amount of free drug in supernetant was measured at 229.50 nm using UV-visible spectrophotometer after suitable dilution with 7.4 buffer.

$$\text{Percentage Drug Entrapment Efficiency} = \frac{W\ (\text{Initial Drug}) - w\ (\text{Free Drug})}{W\ (\text{Initial Drug})}$$

Scanning electron microscopy (SEM)

The pure drug powder of gliclazide was confirmed by direct deposition of powder as thin film on double-sided carbon tape, while SEM for the liquid of the selected formulation of the prepared nanosuspension was confirmed by the droplet evaporation technique and photographs were taken at different magnification [19]. A droplet of liquid was deposited on a double-sided carbon tape and dried at room temperature using a Vega/TESCAN scanning electron microscope operated with a secondary detector at different acceleration voltage and at different magnification [20].

Drug content

The actual quantity of Gliclazide nanoparticles formulation was measured using UV-visible spectroscopic method after dilution with methanol of 10 mg equivalent weight of formulation. The absorbance of sample measured at max of λ 229.50 nm. Theoretical quantity of drug [TDC] in nanoparticles was compared with actual quantity of drug [ADC].

$$\text{Drug Content} = \text{ADC/TDC X 100.}$$

***In-vitro* Release Studies**

The *In-vitro* drug release studies performed using diffusion technique. The membrane was soaked before use in distilled water for 4 hrs than rinsed with distilled water. Gliclazide nanoparticles dispersion was transferred into dialysis membrane bag, tied and placed in beaker containing 500 ml dissolution medium. The entire system was kept at 37.5 ± 50g with magnetic stirring [50 rpm]. At appropriate interval 5 ml of release medium was removed and 5 ml fresh medium was added to maintain sink condition. The amount of Gliclazide in the release medium was evaluated by UV spectrophotometer at 229.50 nm [21].

RESULTS AND DISCUSSION

Gliclazide is poorly soluble drug, which was belongs to class-II. Thus, it was challenging to enhance the solubility of Gliclazide in aqueous medium. Precipitation method has been employed to produce the Gliclazide nanoparticles [22].

Preformulation studies

Gliclazide was found to be white to off-white crystalline powder and odor less. The absorbance maxima of Gliclazide in 7.4 buffer was observed at 229.50 nm.

Table 1: Standard curve of Gliclazide

S. N.	Concentration	Absorbance
1.	0	0
2.	5	0.174
3.	10	0.377
4.	15	0.594
5.	20	0.76
6.	25	0.931
7.	30	1.013

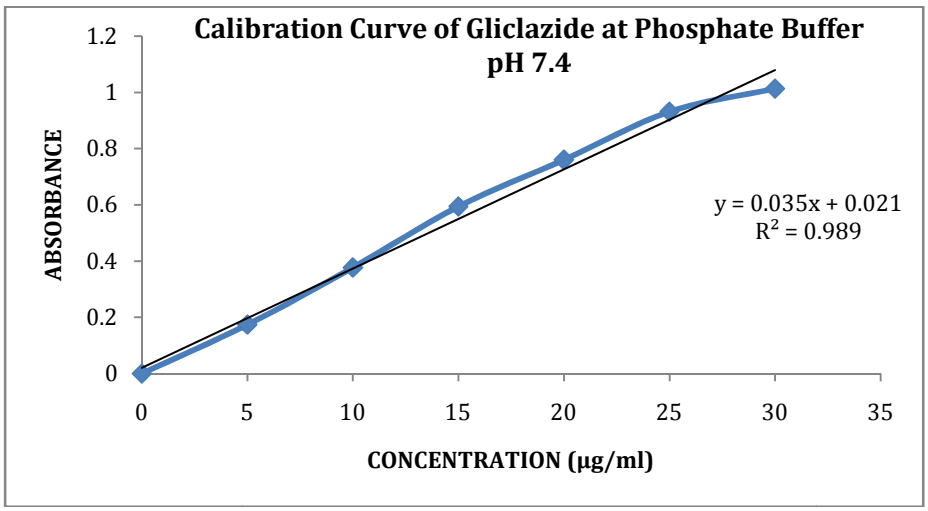

Figure 1: Calibration curve of Gliclazide

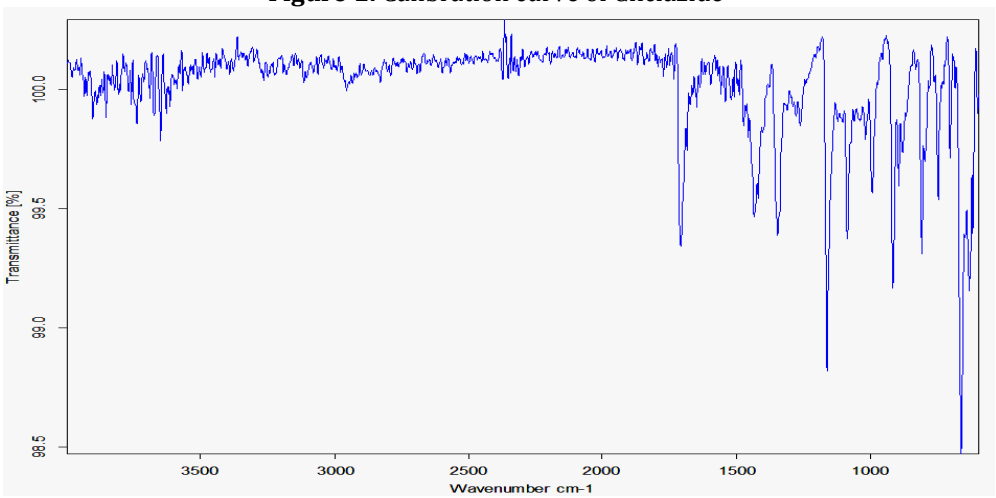

Figure 2: FTIR spectra of pure drug Gliclazide

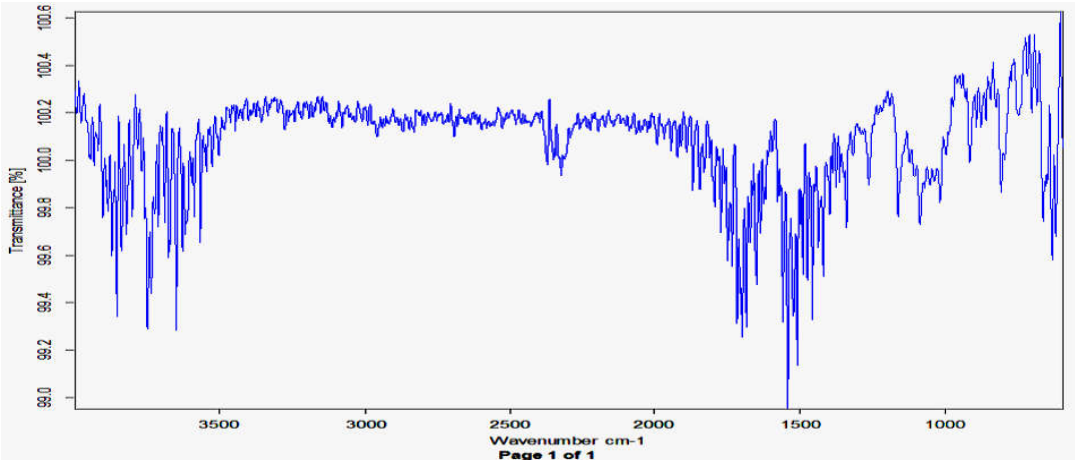

Figure 3: FTIR spectra of F1

FTIR Studies

FTIR Studies were conducted to determine the possible interactions between drug and excipients. FTIR Spectra of pure Gliclazide and drug with excipients show no chemical interaction drug and excipients. The FTIR Spectra were shown in **Figures 2** and **3**.

Melting Point

The melting of the pure drug was determined by capillary method. The drug was melted in the capillary tube using the burner and the melting point was determined by the thermometer.

Table 2: Melting point determination

Method (Capillary Tube Method)	Melting point (^{0}C)	Standard (^{0}C)
Gliclazide	180-182^{0}C	181^{0}C

DSC Studies

DSC Studies were used to characterize the physical state of drug in various formulations. Thermogram of pure Gliclazide and optimized formulation were shown in the **Figures 4**. Pure Gliclazide shows single sharp endotherm at 181^{0}C, which corresponds to the melting point. The decrease in melting point in optimized formulation indicated that decrease in crystallinity.

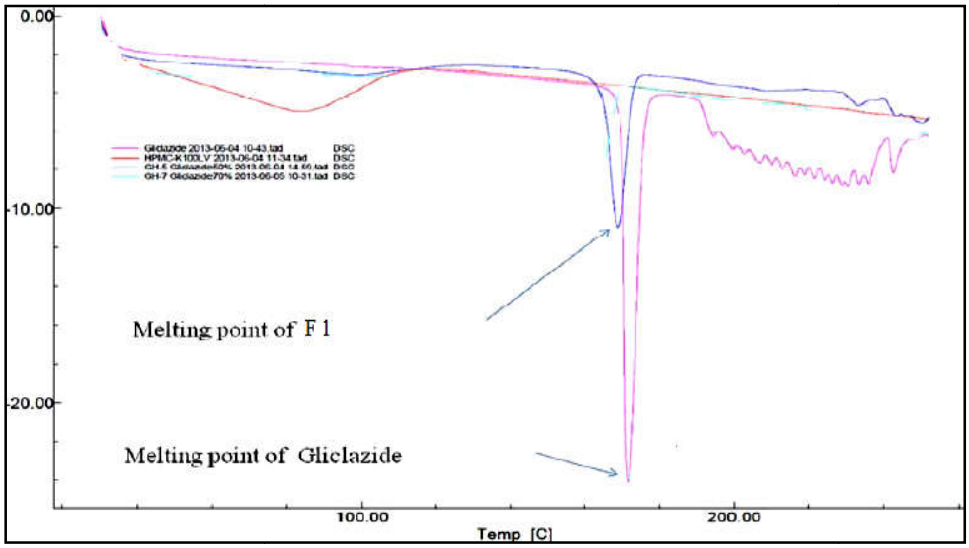

Figure 4: DSC thermogram of pure Gliclazide and optimized formulation F1

Particle Size and Zeta Potential

In this nano preparation the particle diameter was found to be in the range of 100-600 nm. The particle sizes of different formulations were shown in the table, which was clearly indicated that the optimized formulation had particle size of 138.8 nm. The zeta potential values of nanoparticles formulation found to be negative due to presence of carboxylic groups. Zeta potential gives certain information about the surface charge properties and further the long-term physical stability of the nanosuspensions. The obtained value for selected formulation indicates stable nanosuspension. The values of zeta potential and particle size were shown in **Table 3**.

Table 3: Particle size and zeta potential

Formulation	Particle Size	Zeta Potential
F1	135.2	-28.9
F2	309.1	-33.3
F3	443.7	-34.8
F4	484.5	-25.4
F5	342.8	-12.1

Scanning electron microscope

The SEM of pure Gliclazide is presented in **Figure 5** at 100x and 500x magnification. The particles of Gliclazide were large in size (from 50-350um) and has irregular shape and when the picture is closer at 500x and more of magnification it would illustrate the rough surface of Gliclazide particles. while the images of the SEM at different magnification for that of the selected formula of the nanosuspension (F1) is represent in **Figure 5** and it indicate uniform submicron sized particles and results also show nearly

spherical shaped nanoparticles and a size within the nano size and this micrograph was in agreement with those measured by particle size distribution.

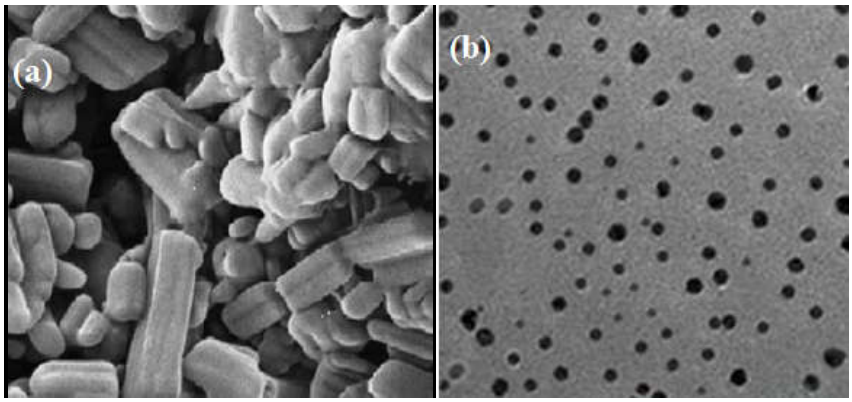

Figure 5: SEM of (a) Pure Gliclazide (b) Gliclazide nanosuspenion

Solubility determination

It has been found that the solubility of nanoparticles formulation was showing 10 folds increase in solubility when compared to pure drug. The increase in solubility in nanoparticles formulation may be due to increase in surface area. The solubility data was shown in Table 4.

Drug entrapment efficiency

The formulations showed entrapment efficiency in the range of 87-93%. Formulation F1 containing 1% Polaxomer 188 as stabilizer containing highest entrapment efficiency [92.45%]. The results have been shown in Table 4.

Drug Content

Drug content in percentage was calculated and the results were tabulated in **Table 4.** The formulation F1 had 98.08% drug content.

Table 4: Solubility, entrapment efficiency and drug content values of formulations

Formulation	Solubility [mg/ml]	Entrapment efficiency [%]	Drug content [%]
F1	23.17	91.36	98.08
F2	21.55	89.15	94.51
F3	19.16	88.68	92.17
F4	21.67	87.93	90.46
F5	20.51	92.45	91.64

***In-Vitro* dissolution studies**

The dissolution profile of Gliclazide prepared with Polaxomer as stabilizer and PLGA as polymer shows % drug release in 50mins, which was comparatively increase than original drug. Comparatives release of various formulations shown in **Figure 5**. Formulation based on Polaxomer 188 showed better release than other stabilizers.

Table 5: *In-vitro* release data

S.NO	Time	% Drug released				
		F1	F2	F3	F4	F5
1	10	29.61	24.32	21.71	23.01	24.16
2	20	55.83	48.99	33.56	36.47	47.82
3	30	80.09	65.10	53.62	61.71	66.71
4	40	86.32	74.01	74.91	76.70	78.95
5	50	95.11	83.23	78.83	78.83	91.46
6	60	97.69	94.11	82.23	85.91	95.11

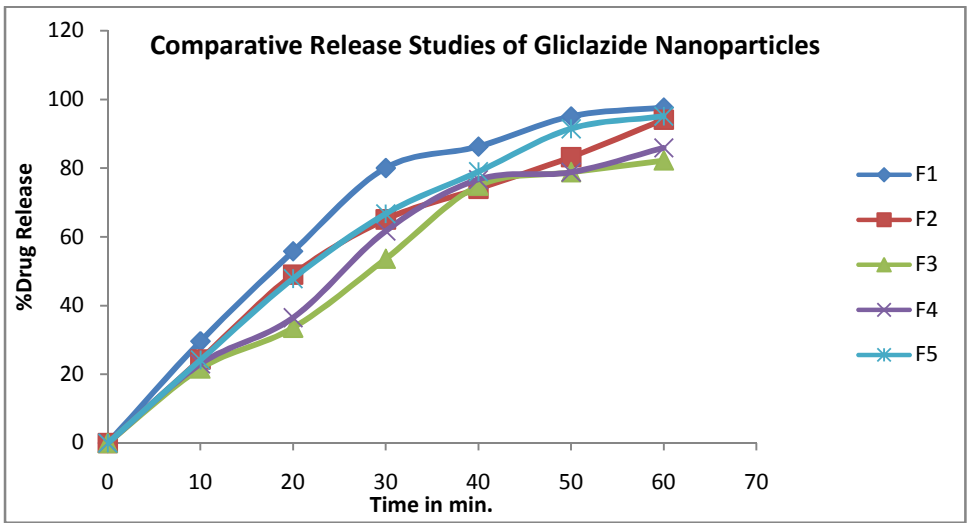

Figure 5: *In-vitro* release plot

CONCLUSION

It may conclude that nanoprecpitation method was successful in preparing the nanoparticles. The increase in surface area and decrease in crystallinity of nanosized particles result in enhanced solubility of Gliclazide. Nanoparticles obtained using PLGA as polymer and Polaxomer 188 as stabilizer in acetonitrile gave comparatively good results. Since, limited oral bioavailability of Gliclazide is due to poor dissolution hence, increasing in solubility and thereby the dissolution of Gliclazide in form of nanoparticles may enhance the oral bioavailability of Gliclazide.

CONFLICT OF INTEREST

None of the author has any conflict of interest in the context of this work.

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