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Research Article

FORMULATION AND EVALUATION OF GLICLAZIDE NANOSUSPENSION FOR SOLUBILITY AND DISSOLUTION ENHANCEMENT

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ABSTRACT

Objectives: The present aim of this research was to develop and characterize a gliclazide-containing nanosuspension using various polymers.

Methods: There are many approaches to enhance the low solubility and dissolution rate of medications and drug substances. A gliclazide nanosuspension with Soluplus, hydroxypropyl methylcellulose, and polyvinylpyrrolidone was formulated using the high-pressure homogenization method.

Results: The physiochemical properties of the drug, polymers, and evaluation characterization of nanosuspension were carried out using Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry, X-ray diffraction (XRD), viscosity, zeta potential, particle size, and solubility of nanosuspension were also checked. FTIR study explained that characteristic peaks of gliclazide were observed in all prepared batches. XRD study indicates that the crystalline structure of the drug was preserved in nanosuspension. The particle size of the formulation decreased using a nanosuspension.

Conclusion: Drug solubility was pH dependent, and the effect of nanosuspension on solubility was more pronounced at low pH values. Nanosuspension bioavailability is also influenced by the preparation of the nanosuspension. It was concluded that the nanosuspension method seems to be a promising technique for increasing the solubility, dissolution, and bioavailability of poorly water-soluble drugs.

Keywords: BCS classification, Bioavailability, Dissolution, Nanosuspension, Solubility.

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INTRODUCTION

New chemical compounds generally face the problem of lower solubility and hence lead to lower absorption in the body [1-3]. It was calculated that nearly 40% of drugs are not easily soluble in water. Consequently, enhancing the solubility and dissolution rate of drugs that are not easily soluble in water is of utmost importance. Unfortunately, it is challenging to create drug products using compounds that are not easily dissolved in water through conventional methods. There are various methods used to increase the oral bioavailability of insoluble drugs, such as hydro trophy [4], complexation with cyclodextrin [5], solid dispersion, particle size reduction, emulsion, micellar dispersion [6], and addition of cosolvents, salt formation, and adjustment in pH [7]. In addition, nanosuspension is a novel technique used to increase the dissolution, solubility, and oral bioavailability of numerous poorly soluble drugs [8]. Nanosuspension may be defined as sub-micron colloidal dispersions comprised of drug nanocrystals, a stabilizing agent, such as surfactant, and some stabilizers, and a liquid dispersion medium. The media for dispersion can be solutions containing water, aqueous or non-aqueous media [9,10]. Gliclazide falls in the category of anti-diabetic drugs, which comes in the second generation of sulfonyl urea [11]. The main mechanism of action of gliclazide is binding with the ATP-sensitive potassium channel receptor and reducing the conductance of potassium and causing depolarization of the membrane, which leads to an increase in the intracellular calcium ion concentration and helps to induce secretion of insulin. Diabetes may be a chronic disorder, and various drugs having different mechanisms of action are available in the market [12-15]. Because of this nanosuspension technology, the absorption from absorption windows of the drugs can be increased due to a reduction in particle size, which improves bioavailability [16-22]. For the water-insoluble drug to increase the solubility and dissolution behavior, it can be converted into a nanosuspension using different concentrations of polymers, solvents, surfactants, and stabilizers. Nanosuspension of gliclazide may be suitable for dose titration therapy for age-appropriate patients and better patient convenience for diabetic treatment [23-26]. There are various methods for the formulation of nanosuspension formulations that can be accomplished in different ways, which consist of media milling, spray drying, nanoprecipitation, and homogenization [27]. The top-down disintegration method contributes to the production of nanometer-sized particles of drugs that are poorly soluble in water and typically results in agglomeration and crystal growth. As a result, stabilizers are frequently utilized in this method to stop the growth of crystals. For the purpose of stabilization, a common method is steric prevention and the electrostatic technique. Adsorption of polymers onto the drug particle surface results in electrostatic or steric stabilization by adsorbing molecules [28,29], and this is how this is accomplished. The stabilizing agents used in the preparation of nanosuspension can be polymers such as polyvinylpyrrolidone (PVP) K30, hydroxypropyl methylcellulose (HPMC), and surface-active agents (surfactants) commonly used include ionic sodium lauryl sulphate and non-ionic polysorbate. The formulation that contains inhibitors, which leads to crystal growth [30]. Thus, dissolution rate, solubility, and hence the bioavailability of the water-insoluble drugs can be increased by application of the nanosuspension technique. In order to accomplish this, Tween 80 was used as a stabilizing agent for the formulation of gliclazide nanosuspension in this research study. Using various tools, the medication and stabilizer were combined and homogenized. There are several benefits of nanosuspension, such as applicability to most drugs, reduced food effects, easy preparation, enhanced oral bioavailability, and sterile filtration is possible because of the reduced

range of particle sizes [31]. Gliclazide is widely used in the treatment of diabetes mellitus and has lower water solubility and bioavailability.

The main purpose of this study is to enhance the dissolution rate and solubility of gliclazide by nanosuspension using the high-pressure homogenization method and precipitation.

MATERIALS AND METHODS

Material

Gliclazide was obtained as a generous gift sample from JB Pharma Thane. HPMC, PVP, Soluplus, and all other ingredients were purchased from Merck. All other chemicals and reagents used were of analytical quality and grade.

Method description

The pure drug sample and all polymers were studied for organoleptic characteristics such as odor, color, and nature.

pH value

pH of the solution was determined using a pH meter. A 0.1% solution was prepared in distilled water, with a minimum amount of methanol (Table 1).

Determination of melting point

The drug's melting point was established using the capillary tube technique. The tube was filled with fine powder of gliclazide and tied to a thermometer, and it was placed in a fire. The temperature at which the powder was melted was noted down [32].

Determination of absorption maxima (λ) of gliclazide

A simple quantitative ultraviolet (UV) spectrophotometric method was used to identify a drug using a Shimadzu Spectrophotometer UV1800 (Shimadzu Corp, Japan). 10 mg of gliclazide was dissolved in a phosphate buffer, pH 7.4, and diluted to 100 mL with the buffer solution. 1 mL of the drug solution was diluted to 10 mL with the same solvent and detected between 200 nm and 400 nm. The maximum absorbance at 232 nm shown in the graph was considered a maximum of the drug [33].

Preparation of calibration curve

The calibration curve of gliclazide in phosphate buffer was carried out by the UV method.

Stock solution preparation

100 mg gliclazide in 100 mL 7.4 buffer solution. 10 mL was taken from this solution and added to 100 mL of phosphate buffer, pH 7.4. A standard graph was plotted using different concentrations (0-20 µg/mL). Then, the absorbance was measured spectrophotometrically at 232 nm.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of pure drug gliclazide, physical mixture, excipients, and optimized formulation were carried out in the range of 4000–400 cm^{-1} using the KBr pellet method (Jasco FTIR 410). The characteristic peak of the drug was observed and studied [34].

X-ray diffraction (XRD) analysis

X-ray diffracts of grams of pure drug and nanosuspension were determined using the Ultima X-ray diffractometer (Brucker D8, India). Standard run was carried out at 40 kV voltages and at a scanning rate of 0.02/min over a range of 1–40°

Differential scanning calorimetry (DSC)

It was determined using a DSC Q100 SYSTEM (TA Instruments, Delaware, USA), and the thermal properties of pure drug gliclazide, PM, and nanosuspension formulation were studied. A 5 mg sample was sealed in a pan and heated at a rate of 10°C/min under a nitrogen purge of 50 mL/min. The sample change in heat was evaluated and checked for any change in the temperature.

Formulation of nanosuspension

The nanoprecipitation method was used to create the gliclazide nanosuspension. Methanol was used to dissolve the pure medication, creating a homogenous organic solution. To create the aqueous phase, the stabilizer was dissolved in water. Using a syringe, the organic solution was gradually injected drop by drop into an aqueous phase while being mechanically stirred for 30 min at 1500 rpm. Following mechanical agitation, the formulation was homogenized using a lab homogenizer for 120 min at 1500 rpm.

Saturation solubility determination

The saturation solubility of drug gliclazide, physical mixture, and nanosuspension was determined at different pH levels, 4.6, 6.8, and 7.4. A surplus of every sample was mixed with an appropriate buffer and shaken constantly for 24 h at 50 rpm at 37°C in a water bath. After passing through a membrane filter, the samples were examined using a UV spectrophotometer set to 232 nm (Shimadzu). The solubility concentration for each formulation was determined using a calibration curve designed for the buffer solution used.

Characterization of nanosuspension

Total drug content

Total drug content of the formulation was studied using a UV-visible spectrophotometer at 232 nm. 1 mL of the prepared nanosuspension was taken and diluted with phosphate buffer 7.4.

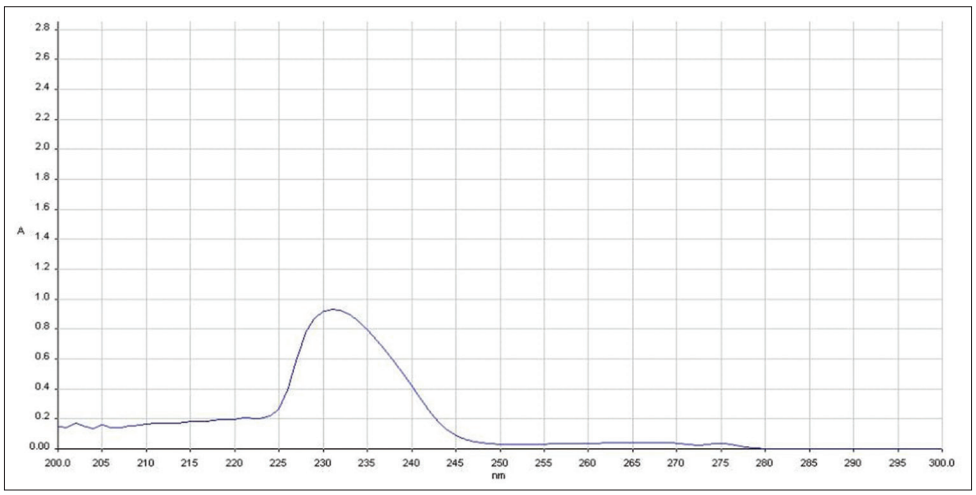

Fig. 1: Absorption spectra of gliclazide

Redispersibility

Formulation stored in vials was detected by tilting the vial bottle up and down using a hand till the sediment was uniformly dispersed in the aqueous, and the number of tilted times was noted.

Viscosity

The Brookfield Viscometer is widely used for measuring the viscosity of liquid formulations, including nanosuspensions. This device measures the resistance of a fluid to flow under a controlled shear rate. It provides a precise and accurate assessment of viscosity, allowing for the evaluation of the flow properties of the nanosuspension.

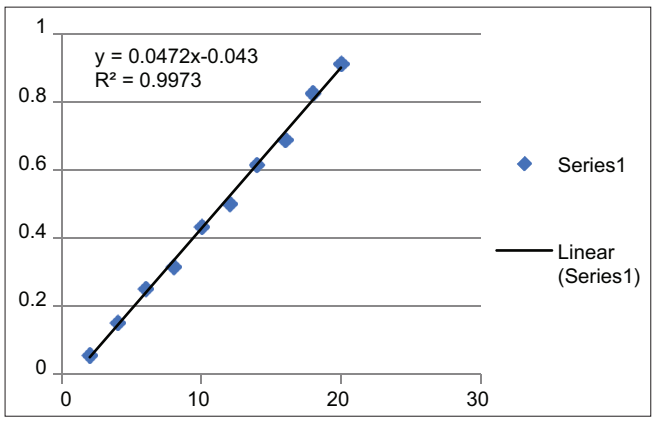

Fig. 2: Calibration curve of gliclazide using an ultraviolet spectrophotometer at 226 nm

Particle size distribution

Photon correlation spectroscopy (PCS) is used to calculate the polydispersibility index (PI). Saturated solubility, dissolution rate, and biological performance are all influenced by particle size and the PI. A Malvern Zetasizer was used to measure the diluted dispersion after 100 µL of the optimized nanosuspension was diluted to 5 mL with double-distilled water.

Zeta potential

Zetasizer (The Malvern Zetasizer ZS, UK) was used to determine the nanosuspension zeta potential. The prepared nanosuspension was stored in the electrophoresis cell after being diluted with water. At 25% C, each sample was measured 3 times, and the average values were used to gauge the response. A zeta potential of at least ±30 mV is necessary for the physically stable nanosuspension to be stabilized only by electro-stable repulsion.

Dissolution velocity

The entire formulation by the way of utilizing the USP category II dissolution equipment, dissolution medium 900 mL of 0.1N HCL, rotating speed was 50 rpm, Temperature °C, sampling withdrawing time was kept constant at 37 ± 0.5, followed by 1–8 h at programmed time interval aliquot samples (5 mL) were collected and replenished by the same quantity of fresh medium. The aliquot sample (5 mL) was filtered using the support of a 0.45 µm restricted membrane filter paper, and the filtrate was diluted properly through the fresh medium and was predictable using a UV-Vis spectrophotometer (model UV-2600 plus) at a wavelength of 232 nm.

***In vitro* drug release study**

A modified diffusion cell apparatus was used to take the nanosuspension. Using a dialysis bag with 10 mL of the nanosuspension in a

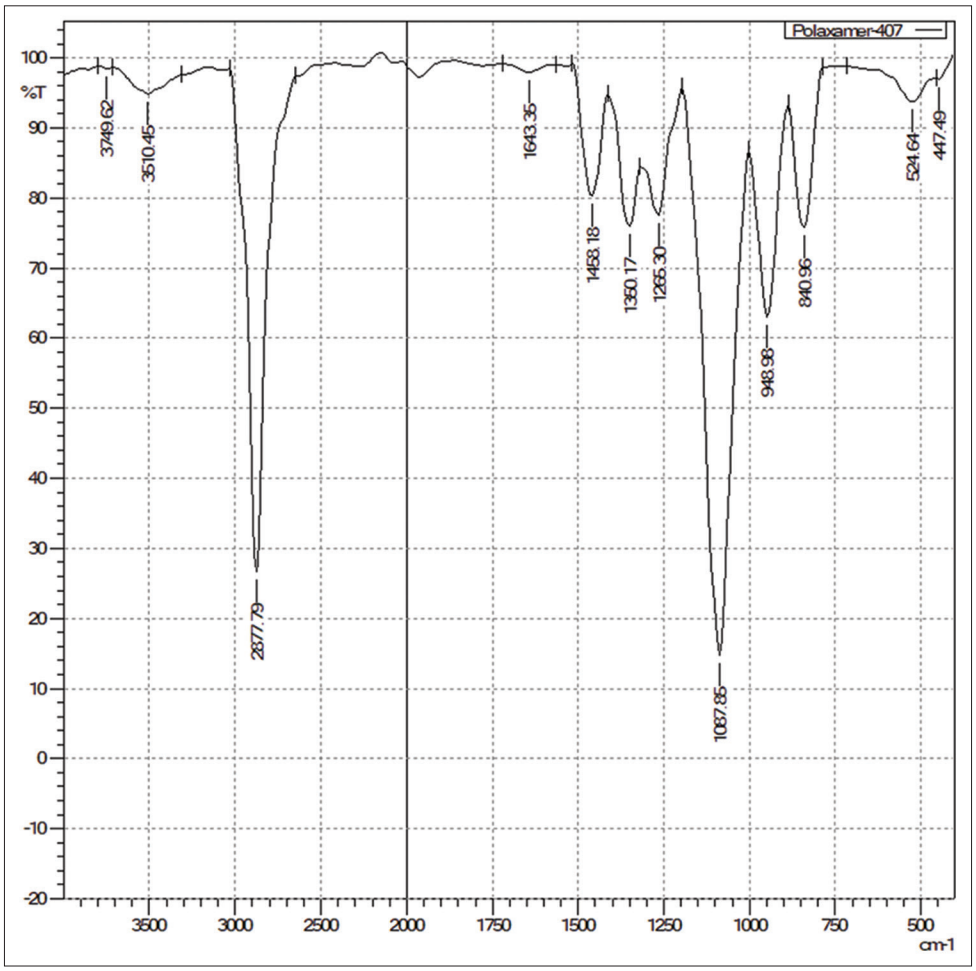

Fig. 3: Fourier transform infrared spectroscopy spectra of the drug and polymers

Table 1: Formulation of gliclazide nanosuspension

Formulation code	Drug (mg)	Types of polymers (mg/mL)			Ethanol (mL)	Water (mL)	Stirring speed rpm
		PVP K 30	HPMC	Soluplus			
F1	30	5	-	10	10	100	1500
F2	30	10	-	10	20	100	1500
F3	30	15	-	10	30	100	1500
F4	30	20	-	10	40	100	1500
F5	30	25	-	10	10	100	1500
F6	30	30	-	10	20	100	1500
F7	30	-	5	10	30	100	1500
F8	30	-	10	10	40	100	1500
F9	30	-	15	10	10	100	1500
F10	30	-	20	10	20	100	1500
F11	30	-	25	10	30	100	1500
F12	30	-	30	10	40	100	1500

PVP: Polyvinylpyrrolidone

Table 2: Solubility data of the drug

S. No.	Medium	Solubility
1.	Water	Insoluble
2.	Ethanol	Slightly soluble
3.	Acetone	Sparingly soluble
4.	Phosphate buffer 7.7	Soluble
5.	Dichloromethane	Soluble

Table 3: Calibration curve of gliclazide using a UV spectrophotometer at 226 nm

S. No.	Conc. (μg/mL)	Absorption
1.	2	0.057
2.	4	0.1532
3.	6	0.2512
4.	8	0.3168
5.	10	0.4355
6.	12	0.501
7.	14	0.616
8.	16	0.6911
9.	18	0.8255
10.	20	0.9129

Table 4: Total drug content of formulation

Batch code	Total drug content
F1	85±3.2
F2	87±2.3
F3	90±3.6
F4	92±1.4
F5	96±2.1

*Data given in mean±SD, n=3. SD: Standard deviation

Table 5: Redispersibility of different formulations

Formulation code	Redispersibility
F1	Medium
F2	Medium
F3	Fast
F4	Fast
F5	Very fast

water-jacketed beaker with 300 mL of phosphate buffer pH 7.4 at 37±1°C for 24 h, the drug release from the nanosuspension was measured. Samples were taken out every 24 h and replaced with a pH 7.4 phosphate buffer medium. After filtering the sample, UV spectroscopy was used to measure the drug content at 232 nm [35].

Table 6: Viscosity measurement of different formulations

Formulation code	Viscosity (mPas)
F 1	0.856±0.005
F 2	0.865±0.006
F 3	0.871±0.008
F 4	0.869±0.008
F 5	0.894±0.009

Data given in mean±SD, n=3. SD: Standard deviation

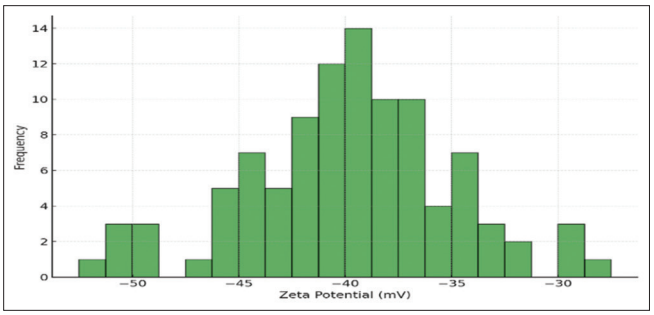

Fig. 4: Zeta potential distribution of gliclazide nanosuspension

Comparison of optimized formulation with marketed nanosuspension

The optimized nanosuspension formulation was compared with the marketed formulation, which has the same dose and drug release pattern.

Stability study

The optimized gliclazide nanosuspension stability was investigated by putting the formulation in glass vials and varying the temperature for 3 months at room temperature (25°C) and in a refrigerator (4°C). Samples were visually inspected after 3 months, and a Zetasizer was used to observe changes in the physiochemical characteristics and particle size distribution.

RESULTS

Description

The drug was found in crystalline powder, off white, and odorless.

pH value

It is weakly acidic; pH was found to be 3.6 using a digital pH meter.

Melting point determination

The melting point of gliclazide was found in the range of 167°C, which was determined using the capillary tube method.

Solubility study

Gliclazide is insoluble in water, slightly soluble in ethanol, sparingly soluble in acetone, and soluble in dichloromethane and a solution of buffer pH 7.4 (Table 2).

Determination of λ max

Gliclazide drug absorption spectra were scanned in the range 200–400 nm in phosphate buffer pH 7.4. The peak was shown at 232 nm, as shown in the figure 1.

Preparation of calibration curve of gliclazide

The calibration curve of gliclazide was performed in the concentration range 1–10 µg/mL. The calibration curve value was 0.997, which indicates excellent linearity data (Fig. 2 and Table 3).

Drug polymer interaction study

FTIR studies (Fig. 3) show the characteristics of all functional groups of pure drugs, gliclazide, polymers, and an optimized formulation of nanosuspension of gliclazide was identified. The spectrum of optimized nanosuspension (Fig. 4) showed the presence of C=C str (aro), indicating N-H (str) present, which represents S=O (str). Due to C=O (str), amide indicates C-N (str) present, and shows that the characteristic band of gliclazide was not altered by the addition of any other excipients after being formulated into suspension. Formulation does not change in its position and indicates no interaction between the excipients and the drug.

Total drug content

All formulations were studied for drug content. The total drug content was found in the range of 85–96%. The optimized formulation showed F5 showed 96% and the results are shown as Table 4:

Redispersibility

The optimized formulation showed a very fast redispersibility index (Table 5).

Viscosity

Result of viscosity of all prepared formulation was described in Table 6.

Particle size, Polydispersity, and zeta potential

The reduction of nanosuspension particle size is the primary objective of current research. As soon as the nanosuspension was formulated, estimates of the average particle size, polydispersity index (PDI), and zeta potential were made. Compared to the physical mixture and nanosuspension, the pure gliclazide had a larger particle size. The particle size distribution is assessed by measuring the PDI value. A narrow particle size distribution is indicated if the PDI value is <0.5. Narrow particle size distributions are confirmed by PDI values of <0.5 for all nanosuspension formulations. The zeta potential is used to measure the electric charge at the particle surface, which provides information about the suspension's physical stability between −25 and −45 mV; these values are regarded as strongly stable nanosuspension (Table 7).

DSC

As per the DSC thermogram (Fig. 5), the gliclazide demonstrates an endothermic peak at 214°C, whereas other ingredients that are used in the preparation of nanosuspension were observed to have abrupt endothermic peaks at 62°C, 197°C, and 213°C, which clearly state that the pure drug and other ingredients were there crystalline state. Gliclazide and other excipients are included in the optimized batch formulation, which showed a single endothermic peak at 199°C. It indicates that there is no crystalline form of gliclazide and that it dissolves or melts with the excipients.

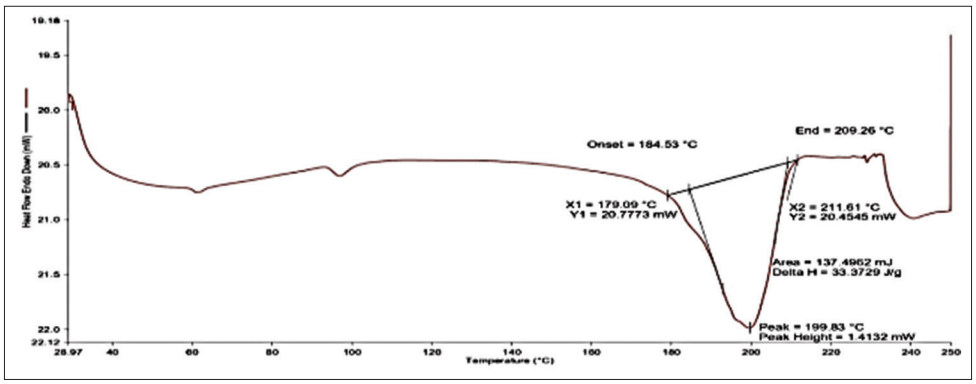

Fig. 5: Differential scanning calorimetry thermogram of gliclazide nanosuspension

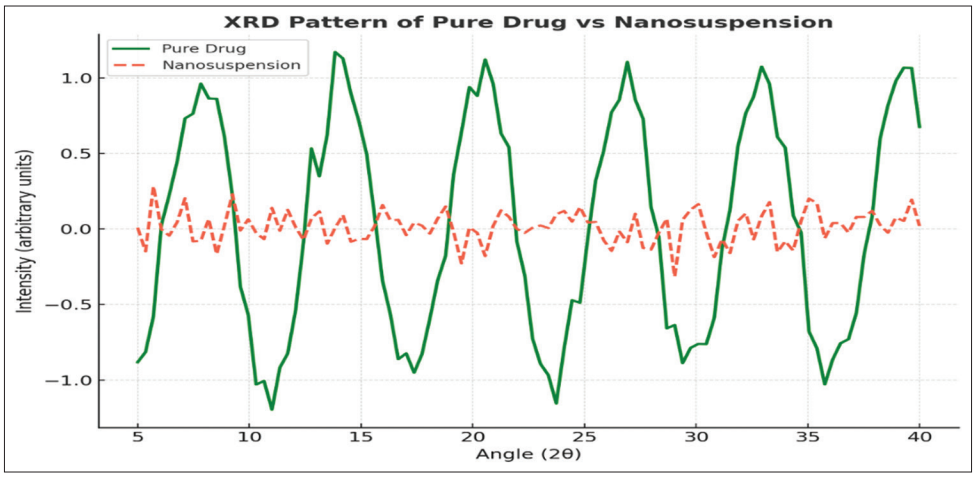

Fig. 6: XRD graph of gliclazide nanosuspension

Table 7: Particle size, polydispersity, and zeta potential

Batch code	Mean particle size (nm)	Polydispersibility index	Zeta potential
F1	0.421±0.012	0.420±0.03	−45±1.01
F2	0.460±0.015	0.482±0.04	−40±1.10
F3	0.401±0.010	0.487±0.06	−35±1.05
F4	0.489±0.018	0.486±0.06	−30±1.01
F5	0.298±0.08	0.230±0.10	−25±0.09

Data given in mean±SD, n=3. SD: Standard deviation

Table 8: Drug release of gliclazide nanosuspension

Time (min)	Pure drug release (%)	% release of optimized nanosuspension
0	0	0±0
5	10	19±1.15
10	20	42±1.02
15	30	60±1.23
20	40	70±2.01
30	50	85±1.50
45	60	93±0.82
60	70	99±1.20

Data given in mean±SD, n=3. SD: Standard deviation

XRD study

An XRD study was conducted to determine the nature of the compound that was formed during the precipitation process, which could be either amorphous or crystalline (Fig. 6). The analysis showed that the pure drug's XRD pattern, which had distinct peaks at particular diffraction angles, closely matched that of the crystalline form.

Drug release of nanosuspension

In vitro drug release of prepared formulation batches was found in the range of 93–99%. All the formulations were prepared with different concentrations of various polymers, from the optimum concentration of polymer, which showed maximum drug release, as shown in Table 8.

Stability studies of nanosuspension

A stability study was conducted for the optimized formulation for 3 months at 4°C. There was no change in physical appearance to the optimized nanosuspension F5. A thin layer of sediment was observed, and it disappeared with slight handshaking. The average particle size diameter was 0.0202 nm and 0.360 nm when it was stored at room temperature and in the refrigerator, and before the stability study, it was 0.200 nm. From this observation, it was clear that this optimized formulation was stable during the study period of 3 months.

DISCUSSION

During the FTIR study, it was clear that there was no chemical interaction between the drug and various excipients. Gliclazide is from a poorly water-soluble drug category, and this nanoprecipitation method was used for the preparation of a nanosuspension, which was able to increase the solubility and dissolution rate of the formulation. Particle size of the drug can be obtained in the nano range, by changing various surfactant and polymer concentrations, and agitated at 6000 rpm, and the time was kept constant at 12 h. The optimum concentration of different polymers and surfactants successfully showed an effect on particle size reduction. Formulation F5 showed the greatest drug release and drug content due to the optimal concentration of different polymers used in the study. For all the formulations, various evaluation parameters were done, such as particle size, PI, and zeta potential, which show rapid dissolution. The value of the optimized formulation was desirable, and it indicates that the prepared formulation was stable. Surface morphology was also studied, which showed that the prepared nanosuspension was spherical with a smooth surface. For all formulation batches, a drug release study was carried out, and among them, the F5 formulation was selected, which showed the highest % release of nanosuspension among other batches. Cumulative percentage drug release of nanosuspension with increased optimal concentration of the polymers. Optimized formulation F6 was compared with the marketed gliclazide tablet. The marketed formulation had a similar dose and drug release pattern to that of the optimized one. The outcome revealed that the optimized formulation release pattern was similar to that of the marketed one. The release kinetics confirmed that the optimized formulation follows zero-order kinetics. The stability study was also conducted as per the ICH guideline, which showed that the prepared nanosuspension is stable during the period of the stability study.

CONCLUSION

Several techniques were employed in this study to create the nanosuspension. The results of these studies unequivocally show that the formulation of the nanosuspension was successfully prepared. In addition, the nanosuspension zeta potential, particle size, and PI were measured. According to the study's findings, creating a nanosuspension improved the gliclazide permeability and solubility. When compared to raw gliclazide, the prepared nanosuspension's average particle size was noticeably smaller. The goal of the gliclazide formulation using the nanoprecipitation method was successfully accomplished, according to the study's findings, as the prepared optimized nanosuspension F5 demonstrated controlled drug release primarily because of the formation of nanosized particles. In conclusion, poorly water-soluble materials can have their solubility, dissolution, and permeability improved using nanosuspension technology.

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AUTHORS' CONTRIBUTIONS

All authors have contributed to designing the study as well as to the analysis and interpretation of data. Each of us played active roles in drafting, evaluating data, and revision of manuscript. All authors have consented to the submission of the manuscript to the journal and given final approval for the publication.

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CONFLICT OF INTEREST

No financial conflicts of interest are disclosed by the authors of this study.

ETHICAL APPROVALS

No human or animal volunteers or experiments are used in this study. Therefore, ethical approval is not required.

DATA AVAILABILITY

All data produced and analyzed during this work have been incorporated into this article.

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